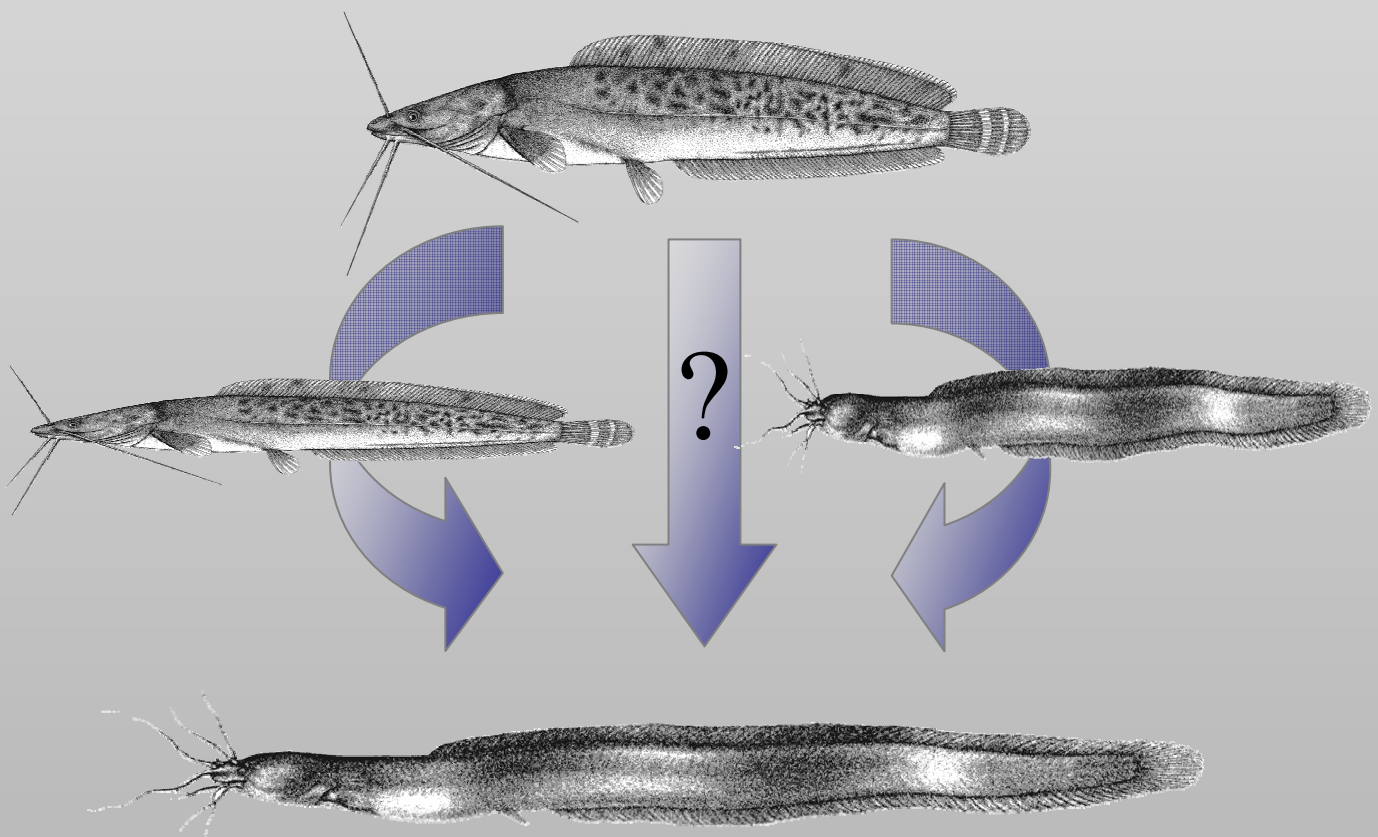# Taxonomy and evolutionary morphology of African catfishes (Clariidae), roads to anguilliformity

I – Text

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STIJN DEVAERE

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Nothing has such power to broaden the mind as the ability to investigate systematically and truly all that comes under thy observation in life.

Marcus Aurelius

All truths are easy to understand once they are discovered; the point is to discover them.

Galileo Galilei

Contents

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PART I

General Introduction

I.1 - History and Aims

I.1.1 History

This thesis, "Taxonomy and Evolutionary morphology of African catfishes (Clariidae): the road to anguilliformity", presents a taxonomic and systematic study of the anguilliform air–breathing catfish (Clariidae, Siluriformes). The subsequent phylogenetic study focuses on the evolutionary road towards body elongation and emphasizes on the polarisation and the morphological co–evolution of this elongation.

The study presented in this thesis was performed at the research group of Evolutionary Morphology of Vertebrates at the Ghent University. Since the seventies, research has focused on the detailed morphology of the vertebrate "Bauplan", more specific that in Teleostei. The holistic approach, with attention for both hard and soft tissue, on macroscopic and microscopic level, has been continuing ever since. Since the nineties, research mainly focused on the head morphology of siluriform representatives, with special attention to the morphology, functionality, ontogeny, systematics and adaptations in the feeding apparatus. The research on the African catfish family, the Clariidae, has since been in close association with the Royal Museum of Central Africa (KMMA/MRAC). This museum houses a large collection of Central African fishes, including one of the largest collections of Clariidae in the world. The original point of interest involved the morphology and especially the ontogeny of *Clarias gariepinus*. Subsequent research focused on two anguilliform species, *Gymnallabes typus* and *Channallabes apus*. Besides many other differences, these anguilliform species showed extreme hypertrophied jaw muscles. The functional morphological implications of this enlargement were tackled in a FWO funded project: "Ecomorphology of the feeding apparatus in catfishes (Clariidae)", a collaboration with the university of Antwerp (Laboratory for Functional Morphology) and the Royal Museum of Central Africa (KMMA/MRAC). One of the major problems encountered in this project was the absence of correct and unambiguous species demarcations, especially of the anguilliform species, compromising any conclusions. It is this very basic problem that led to this present study.

Why taxonomy and systematics (see III.3)? When we look around we can only be impressed by the huge diversity of the living world and the unique nature of each of its components. No two individuals of a sexually reproducing population are identical, nor are

any two populations, species, or higher taxa. This unique nature of each individual, every population and species leads to a tremendous diversity (MAYR, 1982; MAYR and ASHLOCK, 1991). Grasping this diversity is one of the most interesting and challenging aspects of nature (MAYR, 1982; WILSON, 1988). In this context, taxonomy, defined as the theory and practice of classifying organisms (MAYR and ASHLOCK, 1991), is the oldest of the biological disciplines but still far from completed. Taxonomy is at the same time the most elementary and most inclusive part of zoology. It is the most elementary discipline, since animals cannot be discussed or treated in a scientific way until some clarification of its taxonomic status has been achieved, in other words it makes the diversity accessible to other biological disciplines and must remain the basis (WILSON, 2000). But, simultaneously it is also most inclusive, because (together with systematics) in its various branches it gathers, utilises, summarises, and implements everything that is known about animals, whether morphological, physiological or ecological (SIMPSON, 1945; MAYR and ASHLOCK, 1991). Additionally, the importance of taxonomy and systematics lies not only in making the information on diversity accessible to other biological disciplines, but an accurate understanding of alpha-level taxonomy is nevertheless crucial if we want to formulate effective and appropriate management measures to protect and preserve the biodiversity as a whole (KOTTELAT, 1995), so that both taxonomy and systematics have their part in the protection of the environment.

Why Phylogeny? As phylogeny is an expression of the evolutionary relationships within a group of animals with the purpose to illustrate which taxa (e.g., species, genera, etc) are most closely related to which, and also, to present the evidence that we have to support that relationship, it will form a solid base from which insight in the origin, evolutionary life history and the morphological and ecological evolution of the Clariidae can be gained. It is only when a robust and reliable phylogenetic hypothesis is formed that many questions can be answered. As stated by WEITZMAN and WEITZMAN (1982), the current distribution of fishes of a given geographical area can best be explained only when our systematic and phylogenetic knowledge on that fish fauna reaches a high level of sophistication. So, a recurrent problem for biogeographic studies is the lack of a suitable phylogenetic framework. Although the number of siluriform fossil records is not insignificant and reasonably complete, the paleobiogeography and origin of catfishes are not well understood. The paleobiogeographical history of the Siluriformes can only be settled with a thorough knowledge of the phylogeny of the group (GAYET and MEUNIER, 2003). Only when a clear phylogenetic study is present, it is possible to discuss the macroevolution of complex morphological systems in different taxa (e. g. Clariidae). Only then an interpretation can

be done of adaptations, as for example body elongation and co–occurring morphological changes.

Why Clariidae? On the African continent, compared to some other parts of the world, an important amount of work has already been done on freshwater biodiversity (STIASSNY, 1996). A tremendous amount of information has been compiled in the CLOFFA (Check-list of the freshwater fishes of Africa) (DAGET et al., 1986). Since this publication however, numerous changes have been introduced into African fish systematics and updated accounts for many groups are needed. Besides this pan–African work, several more local faunal surveys give us a more complete and detailed view on some well–defined regions: West Africa (LÉVÊQUE et al., 1990, 1992), South Africa (SKELTON, 1993a), coastal central Africa (TEUGELS et al., in press),…. For the remaining parts of Africa, in most cases, we must still refer to some out of date works (e.g. BOULENGER, 1909, 1911, 1915, 1916). And besides some well documented families (e.g. Mastacembelidae), most African fish families are still insufficiently studied (LUNDBERG et al., 2000). The African clariids can certainly be added to this list.

The freshwater clariids are one of the 37 catfish families within the Siluriformes. Although they occur in Syria, southern Turkey and large parts of Southeast Asia, their diversity is the largest in Africa (TEUGELS, 1996; TEUGELS and ADRIAENS, 2003). This richness is demonstrated by the presence of 12 genera with up to 74 species (TEUGELS, 1996; TEUGELS and ADRIAENS, 2003). Some of the generalised, fusiform species, such as *Clarias gariepinus* show a large geographic distribution, whereas the anguilliform species occur in a small area, occupying a more specialized, burrowing niche. They can only be found in swampy areas in Nilo-Sudan (Niger delta), Lower Guinea and the Zaire (Congo River basin) ichthyological provinces (POLL, 1957a; ROBERTS, 1975; TEUGELS, 1986; TEUGELS et al., 1990). The most important characteristic of clariid catfishes is the presence of a unique suprabranchial organ, formed by arborescent structures from the second and fourth gill arches (GREENWOOD, 1961; TEUGELS and ADRIAENS, 2003). Also unique in these clariids is the extreme variation in body shape, ranging between fusiform and completely anguilliform morphs (PELLEGRIN, 1927). It is the latter group of anguilliform genera that makes the clariid family so special among teleost fishes, because of the evolutionary transformation of fusiform species into extreme anguilliform species, within one family.

Although a large amount of scientific effort was already put into this family, rather few publications have been made on the anguilliform clariids. As will be clear throughout this work, for most of the studied species no ecological, behavioural or reproductive data are available. The present knowledge of many species is limited to the fact that we only know

that indeed "they exist". The reasons for this absence are numerous. The main focus has been on the economically more important clariid species, such as those of the genera *Clarias* and *Heterobranchus* (SPATARU et al., 1987; TEUGELS et al., 1990; LEGENDRE et al., 1992; PONCIN et al., 1998, 2002). The cryptic life style of the anguilliform clariids, i.e. living submerged in the mud, makes that they are not caught in the usual biodiversity expeditions, and are therefore not included in most faunal surveys. Subsequently, anguilliform clariid taxonomy is poorly understood and only a few keys for identification exist.

I.1.2 Aims

This thesis can largely be subdivided in two major "sections": (1) a descriptive, taxonomic and systematic section, and (2) a phylogenetic section.

The taxonomic–systematic section tries to give some answers to the following questions:

1. How many different species can be distinguished in the group of the anguilliform morphs in the Clariidae?
2. What are the diagnostic features of each species?
3. In what does each species differ from the others, based on an extended morphological comparison?
4. Where do these species naturally occur?
5. How can the different species be easily recognised (key to the Clariidae)?

The following fundamental questions describe the aims of the phylogenetic section of this study:

6. What clades can be distinguished in the African Clariidae?
7. When and how did anguilliformity arise in the Clariidae?
8. What is the evolutionary polarisation of body elongation?
9. What morphological co-evolution occurs with body elongation?
10. How can the road towards anguilliformity be described from a biogeographical point of view?

I.1.3 Structure of this thesis

This thesis is divided into seven parts. Part two gives an overview of earlier and present classifications of the Clariidae, Siluriformes and Ostariophysii. The third part treats the material used in this study. Although a list of the methods used is given in this part, the methods are discussed separately in detail in each separate chapter. This optimises the comprehensibility of each chapter and leaves it as a complete entity. This, of course, brings some unavoidable recapitulations, which are anyway reduced to a necessary minimum.

Part four deals with an evaluation at the species level of the anguilliform representatives, using the extended Morphological Species Concept (see III.3). Further, in this part the validity of the different taxa studied is discussed. All recognised species are described in detail, where each description includes a list of the type material, a species diagnosis, the known geographical distribution and an illustration of each species. These results are obtained through a holistic approach including morphological, biometric, osteological and myological data and this on a macroscopic and microscopic level. Finally, an identification key for all valid species is provided. In other words, Part four tries to give an answer to the questions one through five, as listed in the aims (I.1.2). Most species are described separately, except for the species in the *Channallabes* genus. For this genus, there is opted to work from a geographic angle (Congo and central and southern West Coastal Equatorial freshwater ecoregion). However, this means that the first key to the species of *Channallabes* (VI.5.1.b) includes some species that will be described for the first time in the following chapter (VI.5.2.a).

Part five combines the results of part four, together with additional (obtained) data on the Clariidae in a molecular and combined (morphological and molecular) phylogenetic analyses, in order to provide an answer to questions six through ten (I.1.2).

Part six contains a general discussion on several divergent topics, related to the taxonomy and phylogeny of the anguilliform Clariids, the Clariidae and biodiversity in general. Part seven sums up and concludes the different results.

PART II

Taxonomic overview

II.1 - Catfishes, a group of specialised Ostariophysi

The Clariidae are a family of catfishes (order Siluriformes) belonging to the Ostariophysi, a taxon of the Teleostei, the most successful group of fish (and vertebrates).

II.1.1 - Ostariophysi

The Ostariophysi (etymology: *osteon* = bone, and *fysis* = bladder) comprise a group of five orders, 59 families, 960 genera and about 6 507 species. The Ostariophysi include the Characiformes, e.g. the piranhas and the most popular aquarium fish or tetra's; the Gymnotiformes, e.g. the electric eel; the Cypriniformes, with the large family of Cyprinidae; the Gonorynchiformes, e.g. the milkfish and the Siluriformes or catfishes (NELSON, 1994; TEUGELS, 1996). The remarkable, newly proposed fossil ostariophysan order Sorbininardiformes is not yet included (TAVERNE, 1999).

The Ostariophysi contain both monotypic families, as well as one of the largest families of all, the Cyprinidae (± 2010 species). The Ostariophysi represent 25% of all known teleost and 64% of all freshwater fishes. They can be found world wide (NELSON, 1994).

II.1.1.a The Ostariophysi within the Teleostei

The general idea, that was long generally accepted, is that the Ostariophysi were seen as somewhat derived teleosts belonging to the Euteleostei and, thus, not closely related with the Clupeiformes (LECOINTRE, 1995). However, this view was severely disputed in the last ten years. Several studies support the sister-group relationship between ostariophysans and Clupeiformes, and, so the exclusion of the Ostariophysi from the Euteleostei (LECOINTRE, 1995). A somewhat basal teleostean classification is presented by ARRATIA (1997) (**Fig. II.1-1**).

```
TELEOSTEI
        - some basal teleostean fossil †
        - SUPERCOHORT Elopomorpha
                                        -ORDER Elopiformes
                                        -ORDER Albuliformes
                                        -ORDER Anguilliformes
        -SUPERCOHORT Osteoglossocephala
                -COHORT Osteoglossomorpha
```

-ORDER Osteoglossiformes

-COHORT Clupeocephala

-SUBCOHORT Ostarioclupeomorpha

-SUPERORDER Clupeomorpha

-ORDER Ellimmichthyiformes †

-ORDER Clupeiformes

-SUPERORDER Ostariophysi

-SERIES Anatophysi

-ORDER Gonorynchiformes

-SERIES Otophysi

-ORDER Cypriniformes

-CLADE Characiphysi

-ORDER Characiformes

-CLADE Siluriphysi

-ORDER Siluriformes

-ORDER Gymnotiformes

-SUBCOHORT Euteleostei

Fig. II.1- 1 Teleostean classification (after Arratia, 1997)

II.1.1.b Ostariophysan characteristics

This is a topic which has already been discussed to a great extent (REGAN, 1911a, b; GREENWOOD et al., 1979; ROBERTS, 1973; FINK and FINK, 1981, 1996; GAYET, 1986, 1993). It is therefore redundant to discuss all the characters which are recognised as ostariophysan synapomorphies into detail in this taxonomical placement. However, I want to give a list of some of the more general features, frequently mentioned as being typically ostariophysan. This may not be seen as either a complete list nor as a list with the most important characteristics, just as an anthology of the diversity of characters common in the Ostariophysi.

As mentioned above, Ostariophysi possess a specialised set of anterior vertebrae, which in combination with the swimbladder have formed a sensory organ for the perception of sound or differences in pressure, *i.e.,* the Weberian apparatus (**Fig. II.1-2**). In the Anotophysi, the differentiation is much less than in the Otophysi, where a true chain of ossicles interconnects the swimbladder with the labyrinth organ (GAYET and CHARDON, 1987).

In relation to this Weberian apparatus connection, the swimbladder is subdivided in generalised ostariophysans, in an anterior part (the camera aerea weberiana) and a posterior part (the functional buoyancy organ). The specialisation for the perception of vibrations is believed to be one of the major adaptive characters at the base of their wide ecological and evolutionary diversity (GOSLINE, 1971; ROBERTS, 1975).
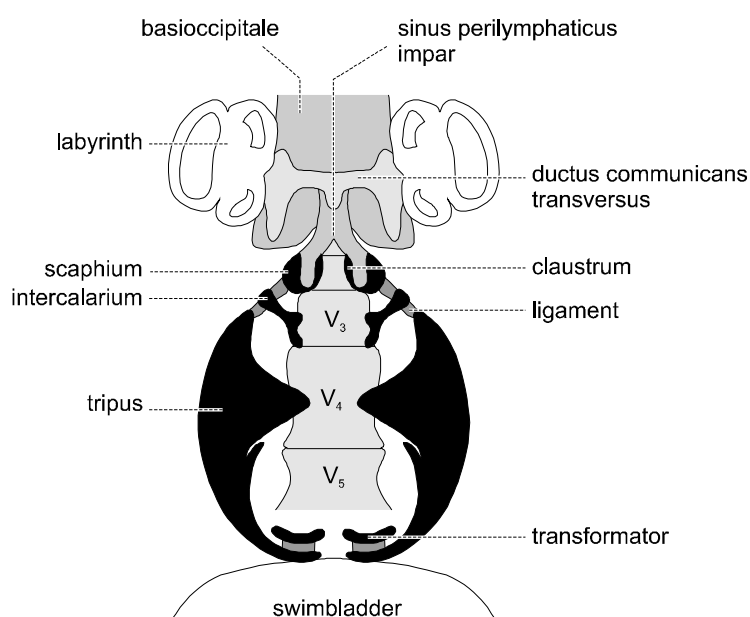
Fig. II.1- 2 Scheme of the Weberian apparatus (Adriaens,1998)

Another ostariophysan feature is the presence of a certain alarm substance or 'Schreckstoff' (GOSLINE, 1971; NELSON, 1994). This substance is a kind of pheromone, which induces a wide range of fright reactions in ostariophysans, when released in the water (PFEIFFER, 1977). The reaction may be both intraspecifically and interspecifically. This fright reaction mechanism involves two crucial components: (1) the production of 'Schreckstoff' in alarm substance cells and (2) the behavioural response to the detection of such a substance.

Another feature, frequently mentioned as typical ostariophysan, involves the morphology of the caudal skeleton. Ostariophysans, together with Clupeomorpha, have a so-called pleurostylar type of caudal skeleton, instead of a so-called stegural type of higher teleosts (GOSLINE, 1971). The major difference involves what part of the ural vertebrae takes part in the formation of the supportive structure for most of the hypurals.

The presence of nuptial tubercles has been observed in some Characiformes (Parodontinae, Lebiasinidae and Characidae), in most Cypriniformes and some Siluriformes (Mochokidae, Sisoridae and Astroblepidae) (ROBERTS, 1973). They are absent in the Gymnotiformes (FINK and FINK, 1981). The tubercles may range from simple aggregations of non-keratinised epidermal cells to large structures, consisting of several layers of fully keratinised cells. In *Phoxinus* (Cyprinidae), up to nine different morphotypes of nuptial tubercles could be recognised (CHEN and ARRATIA, 1996). The so-called unculi refer to those types of tubercles which bear unicellular, horny projections. The distribution of tubercles is frequently found on top of the skull, but may occur at the ventro-lateral side or ventral side of the skull, as well as above the scales (CHEN and ARRATIA, 1996).

II.1.1.c Ostariophysan affinities

Throughout the twentieth century, several extensive and less extensive surveys dealt with the relationships within the Ostariophysi, several of these are chronologically presented below (REGAN 1911a, b; ROSEN and GREENWOOD, 1970; GOSLINE, 1971; ROBERTS, 1973; GREENWOOD et al., 1979; FINK and FINK, 1981, 1996). None of these researches rely on a cladistic approach, thus although a lot of work has been done on the phylogeny of the Ostariophysans, questions on the reliability of these phenetic works remain.

REGAN (1911a, b) stated that the Ostariophysi were united because of one common feature: the presence of the Weberian apparatus and that further this group contains very divergent forms and appearances (REGAN, 1911b). Even in the presence of the Weberian apparatus a lot of variation occurs; in the members of the Anatophysi (Gonorynchiformes), this specialisation is less developed than in the Otophysi (Cypriniformes, Characiformes, Gymnotiformes and Siluriformes). According to REGAN (1911a, b), the Ostariophysi comprised the suborders Cyprinoidea and the Siluroidea (**Plate II.1-1A**). The Characiformes, belonging to the Cyprinoidea, were regarded as the least specialised group. Both groups show some of the typical malacopterygian features: an abdominally positioned pectoral fin, with the pelvic fins placed well behind them and a ductus pneumaticus connecting the swimbladder to the gut.

In their paper on teleostean phylogeny, GREENWOOD et al. (1979) could not ascertain ostariophysan affinities with other major groups, although they already insinuated the possible common ancestry of their 'ostariophysans' and the Gonorhynchiformes. According to them, starting from the pholidophoroid lineage, one of three divisions gave rise to a salmonoid stock, from which evolved the Gonorhynchiformes. The latter were classified as an order of the protacanthopterygians. During that same period, the Ostariophysi might have split off. Still according to GREENWOOD et al. (1979) however, the clupeomorphs showed no affinity with the ostariophysans, although they were not able to give a plausible position of that group. In correspondence with REGAN (1911a, b), the Ostariophysi comprised two groups, now raised at ordo level (Cypriniformes and Siluriformes) (**Plate II.1-1B**).

A close relationship between the group, until then referred to as the Ostariophysi, and the Gonorhynchiformes, was demonstrated by ROSEN and GREENWOOD (1970), based on evidence of the caudal skeleton, the presence of a fright reaction mechanism, swimbladder morphology, presence of nuptial tubercles and a striking similarity in the mouth opening mechanism in *Phractolaemus ansorgii* (Gonorhynchiformes, Phractolaemidae) and *Bivibranchia* (Characiformes, Hemiodontidae). This resulted in the

subdivision of the Ostariophysi in the Anotophysi (Gonorhynchiformes) and the Otophysi (Ostariophysi *s.s.*) (ROSEN and GREENWOOD, 1970) (**Plate II.1-1C**).

Based on the comparison of adult morphology, GOSLINE (1971) grouped the Gonorhynchiformes (his Gonorhynchoidei) within the 'Clupeiformes', as a reflection of the affinities with the 'Elopoidei' and the 'Clupeoidei' (**Plate II.1-1D**). Caudal fin anatomy, however, again suggested a relation between the Gonorhynchiformes (his Gonorhynchoidei) and the Ostariophysi (his Cypriniformes), as well as with the Clupeiformes (his Clupeoidei), *i.e.,* the presence of a pleurostyl (see below). On the other hand, TAVERNE (1974) suggested that the two features used by ROSEN and GREENWOOD (1970), claiming the Gonorhynchiformes as the primitive sister group of the Ostariophysi, were invalid (*i.e.,* a primitive state of the Weberian apparatus and the caudal skeleton in Gonorhynchiformes). It was again suggested that the Gonorhynchiformes were derived from the Protacanthopterygii, more specifically some primitive Pattersonellidae.

The first detailed survey of the ostariophysan interrelationships was given by ROBERTS (1973). He stated that the morphology of the caudal skeleton supports a relationship between Clupeomorpha and Ostariophysi, and between Clupeomorpha and Gonorhynchiformes, as well as between Gonorhynchiformes and Ostariophysi. ROBERTS (1973) objected to the taxonomic value of the parallelism of the protrusion mechanism between *Phractolaemus* and *Bivibranchia* (see ROSEN and GREENWOOD, 1970), because they do demonstrate both morphological and functional differences. Additionally, ROBERTS (1973) opposed against the notion that characins and cyprinids would be more closely related to each other than to the catfishes, consequently raising the latter to the same level as the former two (**Plate II.1-1E**). In this survey Cypriniformes than correspond with the Otophysi of ROSEN and GREENWOOD (1970), containing the three suborders: (1) Characoidei, (2) Cyprinoidei and (3) Siluroidei.

An even more extensive, detailed comparison of ostariophysan groups has led to the classification of ostariophysans as used today. FINK and FINK (1981) state five hypotheses, resulting in a new cladistic shift (**Plate II.1-1F**):

1. all five ostariophysan groups represent a monophyletic lineage: (1) Gonorhynchiformes, (2) Cypriniformes, (3) Characiformes, (4) Siluroidei and (5) Gymnotoidei;
2. the Siluroidei (catfishes) and Gymnotoidei (knife fishes) constitute a monophyletic group: the Siluriformes;
3. the Siluriformes are the sister group of the Characiformes, which comprise the Characiphysi;

4. the Characiphysi and Cypriniformes are sister groups and are referred to as the Otophysi;

5. the Gonorhynchiformes form the sister group of the Otophysi, constituting the Ostariophysi

More recently, a revision of the characters described in that paper has been made (FINK and FINK, 1996), resulting in raising of the Siluroidei and Gymnotoidei to the ordo level, both being grouped within the Siluriphysi (**Plate II.1-1G**). Recent, molecular phylogenetic works tend to support the phenetic works (*e.g.* Alves–Gomes, 1999).

II.1.1.d Ostariophysan zoogeography

The Ostariophysi show a global distribution. More in detail we see that the Siluriformes show a cosmopolitan distribution, whereas the Gymnotiformes are confined to Central and South America, and the largest diversity of the Cypriniformes lies in southeastern Asia. The Characiformes occur in Africa, North, Central and South America; the Gonorhynchiformes occur only in Africa, tropical and subtropical Indian and Pacific oceans (NELSON, 1994). This means that only in Africa Siluriformes, Characiformes and Cypriniformes co-occur. The majority of the ostariophysans can be categorised as primary freshwater fishes (obligatory freshwater fishes). Secondary fresh water fishes (almost exclusively in fresh water but tolerate sea water well enough to disperse through it) can be found among Cypriniformes (Cyprinidae), Characiformes and Siluriformes (*e.g.*, Clariidae, Siluridae, Claroteidae, Pangasiidae, Loricariidae), whereas peripheral species (living in both sea and fresh water and use the sea as their highway of dispersal) have been observed in Gonorhynchiformes (Chanidae, Gonorhynchidae) and Siluriformes (Aspredinidae, Ariidae and Plotosidae) only (ROBERTS, 1975; NELSON, 1994; TEUGELS, 1996).

Concerning the origin of the ostariophysans and the starting point to reconstruct the ostariophysan evolutionary dispersal, it was generally agreed upon that they did not have an early Gondwanaland origin, as they are absent in Madagascar and the freshwaters of Ausrtalia (BRIGGS, 1979). But on the exact location were the origin must have been, no ambiguity can be found. According to DARLINGTON (1957) and BRIGGS (1979), early ostariophysans emerged in Asia. Alternatively, a South American origin was proposed by CHARDON (1967a) and NOVACEK and MARSHALL (1976). In the scenario of BRIGGS, gonorhynchiform ancestors gave rise to an ancestral characiform group, from which the ancestral, and thus Asian, cypriniforms and siluriforms were derived. Subsequent dispersal occurred to Europe, through which they entered Africa, and through the Bering Street, up to North America. A possible route of Siluriformes to North America through Europe and

Greenland, during the Upper Jurassic, has been proposed as well (BRIGGS, 1979). The absence of the Cypriniformes in South America would then be explained by the fact that by the time they had reached Africa, the continent was already separated completely from South America (around the Middle Cretaceous, 90 million years ago).

This point of view is recently indirectly questioned by DIOGO (2005), who states that Siluriformes have a pre–Gondwana origin (see II.3.2c) and by this also the ostariophysans.

II.1.2 - Siluriformes

The catfishes, or Siluriformes, with 34 families, about 437 genera and more than 2700 species (DE PINNA, 1996; FERRARIS and de PINNA, 1999; TEUGELS, 2003, All Catfish Species Inventory project[1]), represent about one third of all freshwater fishes. They can be considered as one of the economically most important groups of fresh and brackish fishes in the world (TEUGELS, 1996), as it is shown by the "Asian-American economical catfish war" nowadays going on (e.g. ROY, 2003: in Diogo, 2005). Catfish are characterised, as their name slightly indicates, by the presence of barbels surrounding the snout region. They have a wide geographical distribution and are found in Africa, Eurasia, South-east Asia, Japan, Australia, North, Central and South America, with fossil evidence even being reported in Antarctica (GRANDE and EASTMAN, 1986).

II.1.2.a Some catfishes specialisations

A survey of the most striking morphological specialisations in some catfishes has been given by ALEXANDER (1965). In many cases, catfishes are considered to be adapted to a benthic and nocturnal life style. In general, catfishes possess broad, dorso-ventrally flattened skulls with small eyes, coupled to a reorganisation of cranial structures. The reduction in visual input is compensated by input through the Weberian apparatus and the oral barbels.

In catfishes, the Weberian apparatus is more specialised (reduced and encapsulated) than in other ostariophysans. The swimbladder only consists of one compartment anymore, (the camera aerea weberiana, see II.2.1.c), which has become almost completely enclosed by the extended parapophyses of the fourth and fifth vertebrae (CHARDON, 1967b). The swimbladder remains uncovered laterally, where it comes to lie close to the surface of the body, in an area with little body musculature. Consequently, this region of the body wall

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[1] http://clade.acnatsci.org/allcatfish/ACSI/taxa/Families.html

functions as a kind of tympanum, transporting vibrations through the swimbladder and the Weberian apparatus to the inner ear. The reduction of the swimbladder, however, implicates the loss of buoyancy. The benthic behaviour may then be considered as one of the consequences of the specialisation of the Weberian apparatus, or the reduction of the swimbladder may be seen as a logic consequence of a benthic behaviour, thereby enabling the specialisation of the Weberian apparatus (ALEXANDER, 1964). Another specialisation in the catfish Weberian apparatus involves the increased fusion between vertebral centra, resulting in the formation of the vertebral complex (ADRIAENS, 1998).

The loss of the buoyancy function of the swimbladder has provided another functional shift in some catfishes. At least two different sound producing mechanisms, involving the swimbladder, have been found (*e.g.* in Pimelodidae: contractions of the muscles on the swimbladder produce pulsations; *e.g.* in Mochokidae: through the elastic spring mechanism, the broad tip of the flexible, anterior part of the 4th parapophysis is responsible for the sound production) (ALEXANDER, 1965). Yet another more simple method for sound production involves the pectoral spines. Most catfishes posses pectoral spines, which can be locked in the pectoral girdle as a defence mechanism (see below). The base of the spine is frequently thickened, bearing several ridges of different sizes (TILAK, 1963; GOEL, 1966; KAATZ, 1997). The movements, based on the muscular control for pectoral spine locking, enable the production of sound by stridulation of the spine base in the pectoral socket. In catfishes, this stridulation is believed to have evolved as a result of predation pressure (disturbance stridulation), towards intraspecific communication in, for example, Callichthyidae (courtship stridulation) (KAATZ, 1997). The effectiveness of sound production, as an intraspecific communication, can easily be related to the presence of a highly specialised sound perception apparatus: the Weberian apparatus. In Sisoridae, stridulation may even occur with the dorsal spine (ALEXANDER, 1965).

Since the eyes are reduced and the fact that catfishes are frequently nocturnal, prey detection is improved by specialised sensory apparatuses. A specialised palatine-maxillary mechanism has enabled the application of the maxillary oral barbels as active probes. Electroreception has been considered as one of the features suggesting Siluriphysi monophyly (FINK and FINK, 1996). Gymnotiformes are characterised by the potential of creating weak electric fields for prey detection and communication, or even prey inactivation and predator repulsion (up to 600 V in *Electrophorus electricus*, Electrophoridae) (ALVES-GOMES et al., 1995). In Gymnotiformes, for the reception, specialised tuberous electroreceptors can be distinguished, whereas Siluriformes have

ampullary organs (HOPKINS, 1983; ARRATIA and HUAQUIN, 1995). Ampullary organs[2] have been found in Clariidae, Heteropneustidae, Bagridae, Ictaluridae, Plotosidae and Pimelodidae (ARRATIA and HUAQUIN, 1995). The presence of an organ, generating electric pulses for predation and predator avoidance, has been observed in one genus of catfishes, *i.e.,* in *Malapterurus* (Malapteruridae). Here, the electric organ is believed to have a myoblastic origin, corresponding to the superficial part of the obliquus inferioris muscle of the hypaxials (HOWES, 1985).

Another adaptive feature, which facilitated a great distribution and diversity in catfishes, involves the potential to perform aerial respiration (ROBERTS, 1975). Air breathing has been observed in other ostariophysans as well, but catfishes are the only ostariophysans that developed specialised accessory breathing organs. Several existing structures have become involved in air breathing (*e.g.,* the stomach in Loricariidae and Trichomycteridae, the intestine of Callichthyidae, the swimbladder of Pangasiidae) (BROWMAN and KRAMER, 1985), as well as *de novo* differentiations of existing structures have occurred (*e.g.,* paired diverticula of the branchial chamber in Heteropneustidae, transformations of branchial structures to an arborescent breathing organ in Clariidae) (OLSON et al., 1990).

Defence mechanisms in catfishes show some diversity as well. As mentioned, pectoral spines, as well as dorsal fin spines, can be erected and locked, making it extremely difficult for predators to manipulate and swallow them. The locking mechanisms are based on a friction lock, as explained by ALEXANDER (1965).
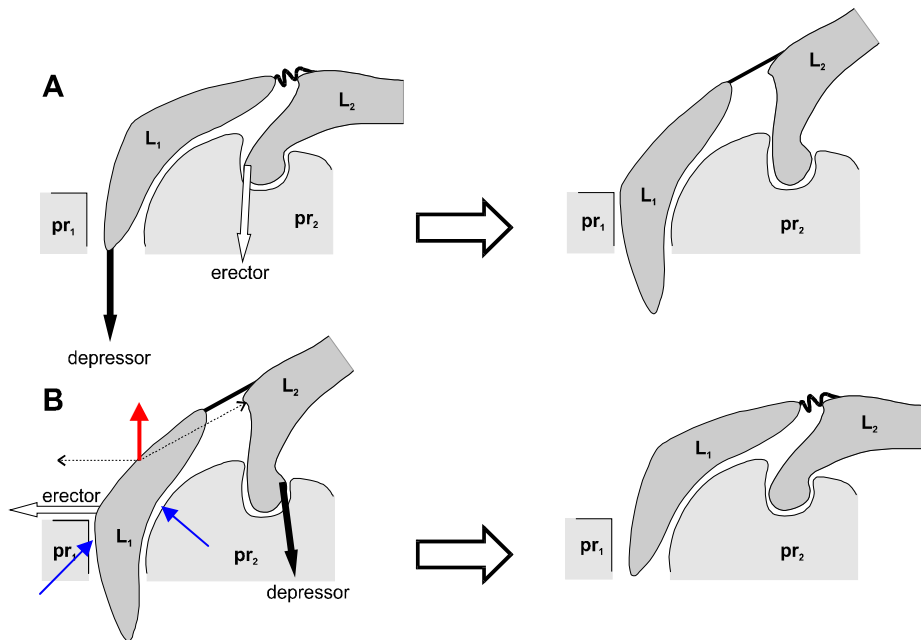
Fig. II.1- 3 Dorsal fin spine locking mechanism through dorsal fin erector and depressor muscles: A. spine erection, B. spine adduction, blue arrows indicate actual position of the friction lock (L_{1-2} = dorsal fin lepidotrichs 1 and 2, pr_{1-2} = proximal radials 1 and 2) (Adriaens, 1998)

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[2] They are found on the whole external surface of the body, but are most abundant on the head (ARRATIA and HUAQUIN, 1995)

In the dorsal spine locking mechanism, a reduced anterior fin ray enables the locking of the spine, the latter corresponds to the enlarged second fin ray (**Fig. II.1-3**). In order to withstand large forces of a biting predator, not only does the mechanism have to hold, also the strength of the pectoral spine, the pectoral girdle and the attachment of the girdle to the skull are determining factors. All of them show an increased strength (TILAK, 1963d; SCHAEFER, 1984). In some lineages, the effectiveness of the spines as an anti-predator mechanism has been improved: (1) large scutes, covering the body, enhance the fortification of the dorsal and pectoral fin spines (as comparable to the situation in the stickleback), or (2) the integumentary sheath surrounding the spine produces toxic substances (*e.g.,* in Ictaluridae, Ariidae, Heteropneustidae, Plotosidae) (BIRKHEAD, 1972; BURGESS, 1989).

Further, the most characteristic of Siluriformes osteological features are: symplectic, subopercular, basihyal, and intermuscular bones absent; parietals probably present but fused to supraoccipital; entopterygoid very much reduced; preopercle and interopercle relatively small; posttemporal probably fused to supracleithrum but thought by some to be present as a separate element in many families; prevomer usually toothed; dorsal- and anal fin pterygiophores lacking middle radial ossification ('baseost') (as is also true for gymnotiforms), distal radial ('epibaseost') also absent in Siluridae; adipose fin usually present; spinelike (=spinous) rays often present at the front of the dorsal and pectoral fins (referred to as spines in taxa descriptions) (the dorsal fin of most catfishes technically has two spines - the first being very short and is part of a locking mechanism for the second spine, only the latter is usually referred to in the taxa descriptions) (NELSON, 1994).

II.1.2.b Affinities between catfishes

Looking closer at the phylogenetic relationships within catfishes and the many, many papers published on that subject (*e.g.* CHARDON, 1968; HOWES, 1985; STRAUSS, 1985; ARRATIA, 1987; MO, 1991; DE PINNA, 1993; DE PINNA and VARI, 1995; TEUGELS, 1996; HE, 1997; DIOGO, 2005), it can be concluded that a lot has been done, but since no unanimity is reached yet, a lot has to be done. Moreover, many of the early works are phenetic, only the last decade cladistic works have been presented.

Since it is not the purpose of this dissertation to give an historic and complete overview of the existing hypotheses of siluriform phylogeny, I chose to give the most recent phylogenetic view (DIOGO, 2005) and compare it with some other recent phylogenies; this to show some of the discordance that still exists today. The presented phylogeny of DIOGO (2005) is based on a large set of morphological characters (osteological and myological, 440 characters).

As in general, the Diplomystidae are accepted to represent the most plesiomorphic condition in the Siluriformes (**Plate II.1-2: 1**). ARRATIA (1987) gave a detailed description of the morphology of several representatives of this family. Several features could be distinguished, which were shared with primitive teleosts (toothed, non-reduced maxillary bone and the lapillus smaller or equally sized than the asteriscus), and which were plesiomorphic catfish features (caudal fin ray number 9/9 and presence of maxillary barbels only). Some features were shared with other generalised catfishes (*e.g.*, body scaleless, pectoral and dorsal fin spines present, adipose fin present, fifth vertebra fused to the vertebral complex of the Weberian apparatus), whereas others were autapomorphies (*e.g.*, skin completely covered with papillae, hyomandibular articulation with the pteroticum, prooticum, sphenoticum and pterosphenoid, more than one row of maxillary teeth, double-headed palatine, …) (ARRATIA and HUAQUIN, 1995; ARRATIA, 1987).

The Loricarioidea are considered as the sister-group of all the remaining non-diplomystid catfishes (**Plate II.1-2: 2**). DE PINNA (1993) finds equally strong support for the monophyly of that same superfamily, however the above mentioned, sister-group relationship is not shown in his results. Next, respectively the Cetopsidae and the Siluridae are split off from the other Siluriformes (**Plate II.1-2: 3,4**). The isolated position of the Cetopsidae was also recognised by DE PINNA (1993), although he found that this family was the sister-group of all non-diplomystid families (so including the Loricarioidea). Again, according to DE PINNA (1993), the Siluridae are in an unresolved clade with the Clariidae, Plotosidae, Malapteruridae, Auchenoglanididae and Chacidae, comprising the infraorder Silurimorpha

The remaining taxa are divided into two clades. The first clade strongly groups the families Ariidae, Schilbidae, Pangasiidae, Ictaluridae, Cranoglanididae, Austroglanididae and Claroteidae (**Plate II.1-2: Clade a**). This grouping was also found by DE PINNA (1993) (Infraorder Bagromorpha), except for the Ictaluridae and the Cranoglanididae. Within this big clade we find the sister-group relationship between *Ancharius* and the remaining Ariidae, which has been questioned by many authors, such as, *e.g.*, MO (1991), but is supported by DE PINNA (1993). The valid status of the Austroglanididae, as was first suggested by MO (1991), is supported. Furthermore, the monophyly of the Schilbidae is presented in this work; again this has been questioned by many authors, but supported in the unpublished thesis of DE PINNA (1993). The Ariidae, which are often associated with the Mochokidae, Doradidae and Auchenipteridae, are in DIOGO (2005) in close relation to the Claroteidae (**Plate II.1-2: 5**), including the Auchenoglanidinae. This is in contrast to the findings of DE PINNA.

The second clade groups all the other families, although very weakly supported (**Plate II.1-2: Clade b**). However, within this large clade, the Malapteruridae, Mochokidae, Auchenipteridae, Doradidae, Bagridae, Pimelodidae form a strong clade (**Plate II.1-2: 6**). According to DE PINNA (1993) the latter two families are incorporated into a different clade (the Infraorder Bagromorpha (see above)), but, nevertheless, a similar close relation could be found. The Malapteruridae (*Malapterurus*) are placed in a clade together with the African Mochokidae and the Neotropical Doradidae and Auchenipteridae, while the Bagridae and the Pimelodidae are groups in another clade. The Bagridae appear as a monophyletic clade, as defined by MO (1991), but contrarily to DE PINNA (1993). The second subdivision includes the Chacidae, Clariidae, Plotosidae, Amblycipitidae, Akysidae, Sisoridae, Erethistidae, Aspredinidae and Amphiliidae (**Plate II.1-2: 7**), with the latter being the sister-group of all the former. This sister–group relation has been questioned by HE (1997) and HE et al. (1999). In this large clade, the Chacidae, Clariidae and Plotosidae are grouped (**Plate II.1-2: 8**), whereas *Heteropneustes* is included in the Clariidae. This confirms the suggestions of CHARDON (1968) and DE PINNA (1993), but contradicts the results presented among others by TEUGELS and ADRIAENS (2003). The latter would not only mean that the Heteropneustidae should be a valid family, but also the Uegitglanidae should be considered a separate catfish family and no longer a synonym of the Clariidae, as suggested in DE PINNA (1993), since the Uegitglanidae are the sister–group of all Clariidae and Heteropneustidae. The basis for the sister-group relationship relies on the considerable difference of the air-breathing organ in Heteropneustidae compared to that found in Clariidae (see GRAHAM, 1997). The phylogenetic position of the remaining families (Amblycipitidae, Akysidae, Sisoridae, Erethistidae and Aspredinidae) is not fully resolved (**Plate II.1-2: 9**). Although, this is fully resolved in DE PINNA (1996), where the amblycipitids appear as the sister-group of all the remaining sisoroids, the author recognised that the sister-group relationship between the Amblycipitidae and the Akysidae was not considerably weaker and so caution needs to be taken concerning the relationships of these families.

II.1.2.c Siluriform zoogeography

The general idea of the biogeography of catfishes is formulated by GAYET and MEUNIER (2003). They state that the Siluriformes are likely to have originated in Gondwana. (1) The presence of the Ictaluridae in North America is then being explained by a Paleocene dispersal from South America. (2) The appearance of several groups in Asia is explained by the Eocene collision of India with this continent and (3) the European silurids are found

only after the Eocene. Currently, however, several arguments have been formulated that undermine this general idea. This new hypothesis is based on several findings.

With respect to the European silurids, fossils are found from the late Cretaceous in Europe, which is much older than the Paleocene in GAYET and MEUNIER (2003) (DIOGO, 2005). When incorporating some questioned fossil record of ariid otoliths, the oldest fossil is set to the late Campanian, which is much older than the Paleocene. Further evidence for an older origin of the silurids is the strongly supported clade of the Neotropical aspredinids and the Asian sisoriods (FERRARIS, 1989; CHEN, 1994; DE PINNA, 1993, 1996, 1998), besides many other Old World and New World apomorphic monophyletic groups in the Siluriformes suggested in DIOGO (2005). All these above mentioned groupings would be very unlikely outside an older "Pangean hypothesis". One other point pointing towards an older origin of the catfishes is that the actual worldwide distribution of these fishes already indicates a markedly old origin of the Siluriformes (see LUNDBERG, 1998). This appears as still more complex at a paleobiogeographic level with for example clariid fossils reported from Europe, a continent where this family is nowadays absent. The overall analysis of all these point thus strongly indicates a significantly older catfish origin than mostly suggested (DIOGO, 2005). A seemingly more plausible, parsimonious reading of all this data would be, thus, to hypothesise that catfishes originate in a time where South America, Africa, but also North America, Europe and Asia were still associated in a Pangean continent. The origin would be confined to the South American side. The catfish would then have dispersed from South America to Africa and later to Asia and Europe (DIOGO, 2005). Such a Pangean origin of the Siluriformes would subsequently incorporate that the origin of the Ostariophysi is older than commonly accepted. A Pangean origin of the otophysans was recently proposed in SAITOH et al. (2003).

II.1.3 - Clariidae

The Clariidae are a group of specialised catfishes (TILAK, 1963). The family comprises 12 African genera with 74 species, and two endemic Asian genera (*Horaglanis* and *Encheloclarias*) with six species, as well as about 16 Asian *Clarias* species (SKELTON and TEUGELS, 1991; TEUGELS, 1996; NG, 2003). They naturally occur in Africa, Asia Minor, the Indian subcontinent and South-east Asia (GREENWOOD, 1961; TEUGELS, 1996). They inhabit freshwater rivers and lakes, although they have been observed to enter brackish water as well (ROBERTS, 1975; BURGESS, 1989). The oldest remains of clariids come from the Eocene in Egypt. The first records from Central West Africa (Dem. Rep. Congo) are from the

Pliocene (GAYET and MEUNIER, 2003). More generalized species, like *Clarias gariepinus* (Burchell, 1822) and *Heterobranchus longifilis* (Valenciennes, 1840) have a wide distribution in Africa, whereas that of the highly specialized anguilliform (eel-like) species is restricted to Central West Africa (BOULENGER, 1911; POLL, 1957a; TEUGELS, 1986; TEUGELS et al., 1990; TEUGELS et al., 1992; SKELTON, 1993). Some species (*e.g.* the African *Clarias gariepinus* and the Asian *C. batrachus*) are of great economic importance as food fish and have been introduced, generally without thorough consideration, for aquacultural purposes in other parts of the world, where they might represent a considerable threat to the biodiversity of local species (TEUGELS and ADRIAENS, 2003).

II.1.3.a Some clariid specialisations

Generalised clariids can be recognised by the heavy, dorso-ventrally flattened head, bearing four pairs of oral barbels. The eyes are generally very small. The upper and lower jaws bear patches of small, villiform teeth. The clariids have, as their commonly used names, "air-breathing" or "walking catfish" suggest, several adaptations to

Fig. II.1- 4 The suprabranchial organ in a specimen of *Clarias gariepinus* from Kenya. Upper side of the head (left side of the figure) and body removed (Photo, D. Adriaens)

improve their dispersal abilities. The posterior part of the branchial cavity houses the suprabranchial organ, *i.e.,* the arborescent, air–breathing organ (**Fig. II.1-4**). Their ability to perform aerial respiration enables them to migrate through deoxygenated swamps and pools, as well as migrate over land. Besides, the terrestrial migration is facilitated by the specialised locomotion pattern, in which pectoral spines and undulatory body movements are used. These adaptations lie at the base of the vast distribution of certain species, as for example the African species *Clarias gariepinus*, which has an almost Pan-African distribution and even occurs into the Middle East (TEUGELS, 1986).

II.1.3.b Clariid composition

In the Clariidae the existence of a range between fusiform and anguilliform genera has been noted (PELLEGRIN, 1927) (**Plate II.1-3A-K**). This tendency makes the clariid family unique among teleost fishes, *i.e.* an evolutionary transformation of a fusiform body plan towards anguilliform body plan within one family. Although this has been observed in other families of teleosts and even amphibians and reptiles (LANDE, 1978), it is never as extreme as within the Clariidae. Together with the elongated body, a whole set of morphological changes are observed, such as reduction and loss of the adipose fin, continuous unpaired fins, reduction of paired fins, reduction of the skull bones, reduction of the eyes and hypertrophied jaw muscles (DEVAERE et al., 2001, **IV.5.1.a**). This trend thus not only involved the striking transformation of both paired and unpaired fins, but also the cranial morphology. Species of the genus *Heterobranchus* Geoffrey St.-Hilaire, 1809, (**Plate II.1-3A**) recognized by a large, robust body, a large adipose fin and a strongly ossified head, have the most fusiform body. Without implying an anagenetic shape transformation, the evolutionary trend can be illustrated through *Dinotopterus* (**Plate II.1-3B**) to *Clarias (Dinotopteroides)* in the reduction of the adipose fin and the consequent elongation of the dorsal fin. In other *Clarias* species, the adipose fin is completely lost, its space being taken by the dorsal fin (TEUGELS, 1986) (**Plate II.1-3C**). In some *Clariallabes*, the body becomes more elongated, which is coupled to the elongations of median fins (**Plate II.1-3F**). In *C. platyprosopos*, the median fins are still separated from the caudal fin, whereas in *C. petricola*, they have already fused (GREENWOOD, 1956; JUBB, 1964; SKELTON and TEUGELS, 1991). Elongation of the body continues through *Platyclarias, Platyallabes, Gymnallabes*, reaching its extreme in *Channallabes* and *Dolichallabes* (**Plate II.1-3G-K**) (BOULENGER, 1907; POLL, 1942a, 1957a, 1977; GREENWOOD, 1956, 1961).

The bulk of clariid literature deals with the few economically important species. However, clariids are a highly diversified and specious group. In TEUGELS and ADRIAENS (2003) a recent survey of the different genera is given. Since the main genera of interest for this dissertation are the eel–like representatives, only these will be described here. All anguilliform and intermediate genera are endemic to Africa, more specific to the central part of West Africa. The results and suggested taxonomical shift of this study are not yet enclosed here.

Platyallabes Poll, 1977

POLL (1977) described this species as *Gymnallabes tihoni* Poll, 1944. This species is only known from the Pool Malebo in Dem. Rep. Congo. Although it resembles the other anguilliform clariids (lateral sides of the skull not ossified, hypertrophied jaw muscles, confluent impaired fins), it differs in the absence of the suprabranchial organ and well-developed paired fins. (**see IV.1**)

Platyclarias Poll, 1977

As many other, more elongated clariids, this genus is monotypic, including only *Platyclarias machadoi* Poll, 1977. This species is known from the Cuango River in Angola. The body is eel-like, the lateral bones are to a great extent reduced. Atypical with respect to the other intermediate and anguilliform morphs are the well-developed paired fins and the caudal, dorsal and anal fins are not confluent. (**see IV.2**)

Gymnallabes Günther, 1867

Species of this anguilliform clariid genus have dorsal and anal fins that are confluent with the caudal fin. Pectoral fins are always present, pelvic fins may be absent. The head is short and its lateral side is not protected by bones, but filled with greatly enlarged jaw muscles. Three valid, nominal species are known. *Gymnallabes typus* Günther, 1867 (type species), *G. alvarezi* Roman, 1970 and *G. nops* Roberts and Stewart, 1976. (**see IV.3**)

Dolichallabes Poll, 1942

Dolichallabes microphthalmus Poll, 1942 is the only species in this genus. It is only known from the Congo Basin. It can be diagnosed by an extremely elongated body, greatly reduced eyes, lateral sides of the head not covered with bone and a large outgrowth of the external jaw muscles. (**see IV.4**)

Channallabes Günther 1973

Only one species is designated to this genus; *Channallabes apus* (Günther, 1873). It has an even more anguilliform body than the *Gymnallabes* species. Pectoral and pelvic fins show a high amount of variation and this not only on an intraspecific, but also on an interpopulational level (ADRIAENS et al., 2002). (**see IV.5**)

II.1.3.c Affinities within the Clariidae

In the past, phylogenetic research on clariids has largely focused on the fusiform genera, and more specific on *Clarias* (*e.g.* TEUGELS, 1986, 1996; GRAHAM, 1997).

Anguilliform clariid taxonomy and phylogeny, however, is poorly understood and no reliable, updated keys are available. The only key incorporating the anguilliform clariids are those of POLL (*e.g.* 1977). The characters used in these keys, such as presence of paired fins, number of ribs and vertebrae are no longer discriminative and overlap between species. This is partially due to the limited number of specimens used in the original descriptions of the species (*G. typus*: n=1, *G. alvarezi*: n=1, *C. apus*: n=1, *D. microphthalmus*: n=7).

The first preliminary phylogenetic works on the clariids followed from the interest in the tendency towards anguilliformity. BOULENGER (1908) argued that "the Clariidae are of special interest from an evolutionary point of view." He lined the known genera in an orthogenetic series, going from the more typical catfish to the more anguilliform, with several links in the chain connecting the two extremes; *Clarias – Allabenchelys – Clariallabes – Gymnallabes – Channallabes*. PELLEGRIN (1927) added to this elongation trend the disappearance of the paired fins. He presented an orthogenetic evolution starting from *Heterobranchus* and ending with *Channallabes* (**Fig. II.1-5**) based on the anguilliform body shape, but mainly on the disappearance of the paired fins.

Fig. II.1- 5 Scheme of the orthogenetic evolution in the Clariidae [After Pellgrin (1927)]

DAVID (1935) added other characters such as the structure of the suspensorium, head skeleton and confirms the evolutionary trend proposed by BOULENGER (1908) and PELLEGRIN (1927). However, she recognised three groups within the family, with each group subjected to an identical orthogenesis. These three groups descended from a common ancestor or derived one out of the other. The first group contained *Heterobranchus, Dinotopterus* and her subgenus *Clarias* (*Heterobranchoides*), subsequently changed to *Clarias* (*Clarias*) (DAVID 1935) and included the species presently arranged under *Clarias* (*Clarias*) and *C.* (*Dinotopteroides*) by TEUGELS (1986). The second group included her two other subgenera in *Clarias, C. (Clarias)*, subsequently changed in *Clarias (Clarioides)* (DAVID 1935) and *C. (Allabenchelys)*. This includes most of the species presently arranged in the other subgenera of *Clarias* and the species originally described in the genus *Allabenchelys* but transferred to *Clariallabes* by TEUGELS (1986). Finally, the third group comprised *Clariallabes, Channallabes* and *Gymnallabes*.

Already in 1942, POLL clearly stated that the orthogenetic series as described by BOULENGER (1908) and PELLEGRIN (1927) and partially followed in DAVID (1935), is not a natural one. POLL stated that *Heterobranchus* and *Dinotopterus* should not be considered as the ancestors of *Clarias*. He split up *Clarias* into two lineages: the first contained the small-sized species with a completely ossified cranium and belonging to the subgenus *Clarioides*; and the second comprised the large-sized species that definitely do not include the ancestor of the *Clarioides* group. The *Clarioides* group gave rise to the *Allabenchelys* group and the *Clariallabes* group. *Dolichallabes*, *Gymnallabes* and *Channallabes,* still according to POLL (1942) were independently derived from the *Clarioides* group. A schematic illustration of clariid phylogeny as described by POLL (1942b) is given in **Plate II.1-4A**.

In a new synopsis for clariid genera, POLL (1977) considers the tendency towards an anguilliform body an evolutionary trend linked to an evolutionary regression of several characters such as fins, skull, air-breathing organ or caudal skeleton. In each of the eel-like genera this regression reaches a different degree in each of the characters involved, resulting in an overlap that allow no statement on the relationships between species and as a result, on their generic placement. The species (end-products) of the different polyphyletic radiations differ in one or another character, demonstrating their independent origin.

A first real attempt to apply cladistics in clariid phylogeny was made by TEUGELS et al. (1990) in their systematic revision of the genus *Heterobranchus*. GRAHAM (1997) presents a tentative cladistic arrangement for the clariid genera and subgenera "derived from the genus *Clarias* " only based on the air-breathing organ. Data on the organ are largely taken from GREENWOOD (1961). Five genera (*Bathyclarias, Uegitglanis, Platyclarias, Platyallabes* and *Encheloclarias*) are not included and the subgeneric division of *Clarias* is that given by DAVID (1935) (**Plate II.1-4B**).

Finally in the last ten years, a multidisciplinary, cladistic approach including both the classical morphological and the more recent genetic methods was introduced in the study of clariid relationships. This leads to a number of papers. TEUGELS et al. (1992) demonstrated using allozymes that *Clarias gariepinus* and *C. anguillaris*, both belonging to *Clarias (Clarias)* were more closely related to *Heterobranchus longifilis* than to *Clarias ebriensis,* the latter belonging to *Clarias (Anguilloclarias)*. This was subsequently for the first time, it was suggested that *Clarias* apparently is not a monophyletic genus. This was acceded by the results (again using allozymes) of ROGNON et al. (1998). AGNÈSE and TEUGELS (2001a), studying the cytochrome b gene in the mitochondrial DNA of the four known *Heterobranchus* species, four *Clarias* species (*C. gariepinus, C. anguillaris, C. buettikoferi*

and *C. ebriensis*) and *Channallabes apus*, clearly demonstrated the monophyly of the genus *Heterobranchus* and the paraphyly of *Clarias*. Interestingly, *Channallabes apus* showed to be genetically closely related to *Clarias buettikoferi*, a *Clarias (Clarioides)* species. This result tends to confirm the observations made by POLL (1942). Finally, AGNÈSE and TEUGELS (2001b) studying sequences of part of the cytochrome b, demonstrated that the *Bathyclarias* species flock endemic to Lake Malawi (East Africa), descends from *Clarias gariepinus*. In their analysis *Clarias (Dinotopteroides)* and *Heterobranchus* species showed to be closely related to this group, while species from other *Clarias* subgenera were distantly set, demonstrating once again the paraphyletic nature of *Clarias*.

The last, most up to date, phylogenies are by TEUGELS and ADRIAENS (2003) and AGNÈSE and TEUGELS (in press). The former study not only gives a, morphology based phylogeny, dealing with the most clariid representatives ever used, it also sums up the most important (syn)apomorphic characteristics, using all available sources known. This resulted in a tentative cladogram, shown in **Plate II.1-4C**, as this study did not involve a parsimony analysis on a large data matrix. This research shows that a lot of work has been done, but that still a lot needs to be done, to clarify all the relationships between the different taxa. The next sections summarize the results of this study.

A first clear lineage comprises the species of *Heterobranchus*, *Dinotopterus*, *Clarias (Dinotopteroides)*, *Clarias (Clarias)* and *Bathyclarias*. Both morphological and genetical research tends to confirm this existence, where the monophyletic nature of this lineage is supported by several synapomorphies. All species included have elongated neural spines in the caudal part of the body. Those genera and species showing a clearly marked adipose fin, the elongated neural spines are entering and supporting the fin. In those lacking a notable adipose fin, the last neural spines are still elongated, reaching the dorsal part of the body. All the species included in this lineage also display a very long, heavily ossified head. The branchial apparatus is well developed with long gill arches provided with numerous slender gill rakers. Within this lineage, the *Heterobranchus* species form a monophyletic group, with *Dinotopterus* considered as its sister-group. Synapomorphies include the clearly marked adipose fin and the degree of extension of the elongated neural spines. *Clarias (Clarias)* and *Bathyclarias* also form a monophyletic group. The *Bathyclarias* species flock descended from *C. gariepinus* and the species display numerous derived characters (developed as adaptations to the particular lacustrine conditions in Lake Malawi). Finally, molecular data shows *Clarias (Dinotopteroides)* as the sister group of *(Clarias) (Clarias)* and *Bathyclarias* (AGNÈSE and TEUGELS, 2001b).

Another separate lineage tends to be supported by molecular evidence. This lineage includes the Asian *Clarias* and is considered as the sister-group of all the other clariids (AGNÈSE and TEUGELS, 2001a). One of the synapomorphies uniting them could well be the clearly marked pit organs on the body, seen as big white spots showing a regular pattern on the flanks. Based on zoogeographical evidence, *Encheloclarias* and *Horaglanis* probably descended independently from Asian *Clarias*.

TEUGELS and ADRIAENS (2003) also show that, besides the two well documented lineages (see above), the relationships of the remaining clariids are at present largely unresolved. However, according to TEUGELS and ADRIAENS (2003) preliminary morphological and genetic evidence tend to confirm, at least in part, the ideas of POLL (1942). According to this author, *Clarias (Clarioides)* species [= *C. (Clarioides)*, *C. (Anguilloclarias)* and in part *C. (Platycephaloides) sensu* TEUGELS, 1986] are ancestral forms for *Clarias (Allabenchelys)* [= *C. (Brevicephaloides)* and *Clariallabes sensu* TEUGELS, 1986], *Clariallabes* and the complex formed by *Gymnallabes, Dolichallabes* and *Channallabes*. Cytochrome b sequences of the mitochondrial DNA clearly demonstrated that *C. (Clarioides) sensu* TEUGELS (1986) and *C. (Anguilloclarias)* are monophyletic taxa (AGNÈSE and TEUGELS, in press). Interestingly, the former appeared as the sister group of *Channallabes* and the latter as the sister group of *Gymnallabes*.

Finally, based on the position of *Channallabes* and *Gymnallabes* in the preliminary tree obtained from the genetic study, TEUGELS and ADRIAENS (2003) tentatively tried to complete the cladogram with the remaining genera. *Tanganikallabes* and *Platyallabes* share with *Gymnallabes* a narrow skull roof and tubular fourth infraorbitals. These features are to some extent also present in *Dolichallabes*, but other characters suggest that this genus is more related to *Channallabes*. Finally, *Platyallabes* and *Gymnallabes* are in this group diagnosed by the confluency of dorsal, caudal and anal fins. *Platyallabes* is distinguished by the short distance between the occipital process and the dorsal fin origin. *Channallabes* shares with *Dolichallabes* the highly increased number of dorsal and anal fin rays in relation to number of vertebrae and a similar pattern of hyomandibular interdigitations with the neurocranium. The position of the remaining subgenera of *Clarias, Clariallabes, Uegitglanis, Xenoclarias* and *Platyclarias* is presently unknown.

PART III

Material and Methods

III.1 - Material

A total of 547 specimens have been studied, which initially comprised 5 genera and 7 nominal species, known at the start of this study. Of the African anguilliform taxa, all available type material has been examined. Most material available in several museums all over the world has been studied in this work. Besides this museum material, material was also collected during two expeditions to Gabon (1999, 2000). Three large regions have been sampled. The first region is situated in the North of Gabon, close to the Equatorial Guinea and Cameroon border, in the Woleu/Ntem River system. A total of seven sites were sampled in the precincts of Oyem. The second region is situated around Makokou, Ivindo River system where three different sites were sampled. The next sampling area was situated in the Ogowe River system, were the two sampling sites were situated in the vicinity of Franceville. The last sampling area was in the vicinity of Ntchouo, River Mbessy, across the border in the Republic of the Congo. All sampling sites were characterized by shallow, muddy, still water (**Plate III.1-1A**). Most specimens were caught by local fishermen using fyke nets and fish hooks. All these specimens are deposited and catalogued in the collection of the Royal Museum of Central Africa (MRAC/KMMA), Tervuren, Belgium.

All anguilliform clariids occur in Central West–Africa (**Plate III.1-1B**). Most of them have a small distribution, except for *Channallabes apus*, which occurs in large parts of the Congo Basin. Although for most species all museum material available is included in this study, in the huge number of *C. apus* specimens present in museums, a selection was made, so to include the largest possible and complete geographic representation.

The majority of the examined African Clariidae specimens, and especially the anguilliform specimens, are housed at the collection of the Royal Museum of Central Africa (MRAC/KMMA) in Tervuren (Belgium). Further, additional specimens are housed in several other institutions all over the world. A complete list of the museum and institution abbreviations used in this work is given below. I follow LEVITON et al. (1985) for the museum acronyms, except for the Instituto de Biologia Aplicada in Barcelona, which was not included in the work of LEVITON et al.

BMNH: British Museum (Natural History), Department of Zoology, Cromwell Road, London SW7 5BD, England.

Instituto de Biologia aplicada, Colegio La Salle, Passeig Bonanova 8, 08022, Barcelona

MCZ: Harvard University, Museum of Comparative Zoology, Cambridge, Massachusetts 02138, U.S.A.

MHNG: Musée d'Histoire Naturelle, Route de Malagnou, Case Postale 284, CH–1211 Genève 6, Switzerland.

MNHN: Musée National d'Histoire Naturelle, 43 Rue Cuvier, 75231 Paris V, France.

MRAC/KMMA: Musée Royal de l'Afrique Centrale / Koninklijk Museum voor Midden Afrika, Afdeling Vertebraten, Laboratorium Ichthyologie, Leuvensesteenweg 13, B-3080 Tervuren, Belgium.

NMW: Naturhistorisches Museum, Postfach 417, Burgring 1, A-1014 Wien 1, Austria.

ZMB: Universität Humboldt, Museum fur Naturkunde, Invalidenstrasse 43, 104 Berlin, Germany.

ZMUU: Uppsala Universitat, Zoologisk Museet, Villavägen 9, S-751 36 Uppsala, Sweden.

What follows is a list of all the specimens used for the species demarcation and the morphological study. These include all 11 anguilliform clariid species currently recognised (see enclosed **CD-Rom, Clariidae.xls**). A second listing includes all specimens used for the phylogenetic analyses. These include several other catfish taxa (clariids and non–clariids) as outgroups, with some of these species also used for morphological comparison. A justification for each outgroup is given. The anguilliform specimens used for the morphological phylogenetic analysis are indicated in the first list by the underlined collection numbers.

List I – Anguilliform species

In this list 11 species are included, as is stated throughout this thesis; these comprise two morphs; anguilliform and intermediate. The first two species in this listing are included in the latter, all the rest are considered as anguilliform morphs.

Platyallabes tihoni **(Poll, 1944). Dem. Rep. Congo**. Kingabwa, Stanley pool, MRAC 13307 (holotype); Kinsuka, MRAC 73-68-P-143, MRAC 138698-699 (n=2), 125345-349 (n=4), MRAC 73-22-P-3127 (n=3); Bulu, Luozi, BMNH 1976.5.21.30-39 (n=9), MCZ 50239 (n=13); Inga, MCZ 88947, MCZ 50537 (n=15); Tadi, Kibunzi, MCZ 50297 (n=5).

Platyclarias machadoi **Poll, 1977. Angola**. Cuango, Cafunfo, Borio River, MRAC 78-6-P-1345, 181 mm SL (holotype), MRAC 78-6-P-1348-364, 78-6-P-1346, 78-6-P-1366-1367 (76-180 mm SL) (21 paratypes).

Gymnallabes typus **Günther, 1867. Nigeria.** Old Calabar, BMNH 1866.12.4 (n=2) (Syntypes); Umu-Eze Amambra, MRAC 84-16-P-1-2 (n=1); Riv. Sombreiro, East of Erema, MRAC 91-067-P0134 (n=1); Niger Delta, MRAC 97-030-P-0001-0010 (n=10); lake Odediginni, Agudama, Yenagoa, MRAC 92-083-P-0035-0036 (n=2); Okaka, Epie Creek, Between Nun an Rashi Riv, MRAC 97-085-P-0001-0004 (n=4); Riv Sombreiro, Odiemerenyi, Ahoada, MRAC 91-

067-P-0135-0136 (n=2); New Calabar, Choba, MRAC 91-105-P-1 (n=1); Rumuji Swamps, MRAC 86-10-P-72 (n=1); Oshika, MRAC 84-28-P-28, MRAC 84-28-P-25 (n=2); River Cron, Itu, MRAC 88-36-P-10 (n=1); Between Sapele and War, Niger Delta, MRAC 74-29-P-600 (n=1); Muoha, New Calabar, MRAC 91-10-P-478 (n=1); Biseni, taylor Creek, MRAC 91-01-P278 (n=1); Ossomari, BMNH 1902.11.10.119 (n=1).

Gymnallabes nops **Roberts and Stewart, 1976**. **Dem. Rep. Congo**. Tadi, Kibunzi, Congo River, MCZ 50298, 57 mm SL (holotype).

Dolichallabes microphthalmus **Poll, 1942**. **Dem. Rep. Congo**. Kunungu, MRAC 44655, adult male, 229 mm SL (holotype), MRAC 44656-659 (n=3) (196-210 mm SL) and 62407, 188 mm SL (paratypes), MRAC 57662, 196 mm SL, MRAC 18850, 90 mm SL; Boende swamps, MRAC 101843, 149 mm SL, MRAC 176123-124 (n=1), 68 mm SL; Bokuma, MRAC 79093, 134 mm SL, MRAC 93774, 66 mm SL; Bokuma – Tchuapa, MRAC 79258-260 (n=3) (85-126 mm SL); Ndwa (Boloko), MRAC 78808-810 (n=3) (99-110 mm SL); Inonge, MRAC 96672, 110 mm SL; Maylimbe, Tshela, MRAC 66721, 97 mm SL.

Channallabes apus **(Günther, 1873)**. **Angola**. Ambriz, BMNH 1873.7.28.16 (holotype). **Dem. Rep. Congo**. Bokalakala, MRAC 175247-270 (n=10); Kinshasa, MRAC 97-056-P-0001-0003 (n=2); Bumba, MRAC 88-25-P-2192-227 (n=36); Boma, MRAC 939; Riv. Lula, Bushimaie, MRAC 153505; Kelé, MRAC 1491; Stanleyville, MRAC 30893-30900 (n=8), MRAC 88-01-P-1976-1992 (n=17); Riv. Ruki, Eala, MRAC 14747-49 (n=3); Lake Tumba swamp area, MRAC 46299; Katanga, MRAC 39480; Riv Botota, Keseki, MRAC 67763-77 (n=15); Mwilambongo, MRAC 72886-887 (n=2); Dekese, Riv. Lofu, Anga, MRAC 153352; Yangambi, MRAC 68700; Riv. Oubangui, Imfondo, MNHN 1922-0029; Loango, MNHN 1924-0079, MNHN 1924-0080; Sangha, MNHN 1925-0137; Mogende, MNHN 1926-0155-59 (n=5); Riv Congo, MNHN, 1937-0124-25; Stanleypool, Bamu, MNHN 1958-0111; Boloko, Riv. Likouala, MNHN 1962-0401 (n=7); Mossaka, Riv. Likouala, MNHN 1963-0402 (n=2); Riv. Loadjili, Songolo, MNHN 1967-0143 (n=6); Mangala, BMNH 1896.3.9.17; Riv. Lebuzi, Kaka Muno, BMNH 1912.4.1411-12 (n=2); Lower Congo, BMNH 1887.1.13.8-9 (n=2); Stanley Falls, BMNH 1889.11.20.5; New Antwerp, Upper Congo, BMNH 1899.2.20.16; Siala-Ntoto Swamps, BMNH 99.11.27.92; Bangyville, Ubangi, BMNH 1907.12.26.34; Kashi, Lulua, MHNG 1248.3; Banana, NMW 47240-42; Mollunda, NMW 47245 (n=4), NMW 47246. **Congo**. Yangala Youbi, MNHN 1967-0146; Djembo, Kouilou, MNHN 1967-0147; Cayo, MNHN 1989-0527; Riv. Nanga, between Boukou-Zassi and Kouilou swamp area, MRAC 90-57-P2315; Sintou, Riv. Kibombo, Kouilou, MNHN 1967-0144; Riv. Loadjili, Songolo, Pointe Noire, MNHN 1967-0145 (n=6); Riv. Youbi, Noumbi. **Angola**. Caungula, Mabete, Riv. Uamba, MRAC 162088; Riv. Camuconda, Tchimenji, MRAC 162089, MRAC 162090-094 (n=5), MRAC 162095-100 (n=6); Riv Ganga-Ludchimo, MRAC162083-086 (n=4).

Channallabes sanghaensis **Devaere (in press). Pop. Rep. Congo**. in the vicinity of Ntchouo, River Mbessy (0° 46'N-14° 19'E) MRAC A4-31-P-171-183, 114 mm SL (holotype), MRAC A4-31-P-171-183 (n=12), 114-221mm SL (paratypes).

Channallabes alvarezi **(Roman, 1970). Equatorial Guinea**. Rio Kie, close to Ebebiyin, 317.6 mm SL. **Gabon.** Aben Lang, Metui (1° 29'N-11° 36'E), MRAC A4-31-P-1-13, A4-31-P-14-18, A4-31-P-55-60, A4-31-P-74-75, A4-31-P-76-77 (n=38), 193-383 mm SL; Ebeigne, Woleu River (1° 28'N-11° 36'E), MRAC A4-31-P-19, MRAC A4-31-P-21, A4-31-P-24, A4-31-P-26, A4-31-P-30, A4-31-P-31, A4-31-P-32, A4-31-P-67-72, A4-31-P-90-93 (n=18), 202-375 mm SL; Assok Ngomo, Woleu River (1° 41'N-11° 39'E), MRAC A4-31-P-20, A4-31-P-25, A4-31-P-27-28, A4-31-P-29 (n=5), 225-342 mm SL; Okoallissis, Otolo, Otagna, Woleu River (1° 31'N-11° 31'E), MRAC A4-31-P-22-23 (n=2), 301-412 mm SL; Zogongone, close to Oyem (1° 34'N-11° 31'E), MRAC A4-31-P-47-54, A4-31-P-73 (n=9), 160-398 mm SL; Mbenga, close to Oyem (1° 37'N-11° 41'E), MRAC A4-31-P-61-63, A4-31-P-74-75 (n=5), 221-345 mm SL; Oyem (1°36'N-11°34'E), MRAC A4-31-P-64-65, A4-31-P-66, A4-31-P-94, A4-31-P-1-95-9613, A4-31-P-97, A4-31-P-98 (n=8), 238-413 mm SL.

Channallabes longicaudatus **(Pappenheim, 1911). Spanish Guinea, South Cameroon**. In der Mabelle, ZMB 18401, 220 mm SL (holotype). **Gabon.** Makokou, River Ivindo (0° 33'N-12° 51'E) MRAC A4-31-P-99-105, A4-31-P-137-151, A4-31-P-152-157, A4-31-P-159-162, A4-31-P-163-164 (n=34), 95-295 mm SL; Etakaniabe, River Liboumba (0° 31'N-12° 59'E) MRAC A4-31-P-106-131, A4-31-P132-136 (n=31), 140-284 mm SL; Iyoko, Makokou, (0°32'N-12° 54'E), MRAC A4-31-P-158 (n=1), 102 mm SL.

Channallabes ogooensis **Devaere (in press). Gabon**. Moanda (1° 33'S-13° 16'E), MRAC A4-31-P-170, 150 mm SL (holotype); Malima, River Kahjaka Kanjaka(1° 40'N-13° 20'E), MRAC A4-31-P-165-169 (n=5); 109-244 mm SL.

Channallabes teugelsi **Devaere (in press). Pop. Rep. Congo**. Magogo, 1km from Lékoli, Komono-Sibiti road, Rep. Congo (2° 36'S-13° 38'E), MRAC 78-22-P-1046, 80 mm SL; Zanaga, Lésala, River Ogowe, (2° 50'S-13° 50'E), MRAC 78-22-P-1047-050 (n=4), 87-1445 mm SL; Ndengué, Moungoundou-Ndziba-Ndziba road (2° 40'S-12° 41'E), MRAC 78-22-P-1051 (n=1), 51 mm SL. **Gabon.** Loa Loa, M'Passa, Makokou (0° 30'N-12° 46'E), MRAC 75-24-P-683-693 (n=11), 31-97 mm SL.

List II specimens used for the phylogenetic research

Extra information (coordinates of location, collection number, used literature, collector and date of collection) on the outgroup (not elongated clariids) is given in the enclosed CD–rom (**outgroup.doc**)

Taxon	Location	Research
Genus *Clarias*		
Subgenus *Dinopteroides*		
Clarias ngamensis	– Okavango, Botswana	Molecular
	– Mpika-stream (Tanganika)	Molecular
	– Shabunda (Dem. Rep. Congo)	Morphological
Subgenus *Clarias*		
Clarias gariepinus	– Lab raised (KULeuven)	Molecular
	– Lab raised (KULeuven)	Morphological
Subgenus *Platycephaloides*		
Clarias platycephalus	– Ebeigne, (North Gabon)	Molecular
	– Nyame Pende (East Gabon)	Morphological
Clarias jaensis	– Oyem (North Gabon)	Molecular
	– Ebeigne (North Gabon)	Morphological
Clarias stappersii	– Mofwe (Zambia)	Molecular
	– Elisabethville (Dem. Rep. Congo)	Morphological
Subgenus *Claroides*		
Clarias buthupogon	– Zambia	Molecular
	– Masendula (Dem. Rep. Congo)	Morphological
Subgenus *Anguilloclarias*		
Clarias pachynema	– Ebeigne (North Gabon)	Molecular
	– Masendula (Dem. Rep. Congo)	Morphological
Clarias submarginatus	– Abenelang (North Gabon)	Molecular
Clarias theodorae	– Zambia	Molecular
	– Botswana	Molecular
	– Luapula (Zambia)	Morphological
Subgenus *Brevicephaloides*		
Clarias camerunensis	– Ebeigne (North Gabon)	Molecular
	– Verie (North–East Gabon)	Morphological
Asian *Clarias*		
Clarias fuscus	– China	Morphological

Genus *Heterobranchus*

Heterobranchus isopterus	– Bia river (Ivory Coast)	Molecular
Heterobranchus longifilis	–Nile (Egypt)	Morphological

Genus *Clariallabes*

Clariallabes longicauda	– Mefange (North Gabon)	Molecular
	– Makokou (North–East Gabon)	Molecular
	– North Gabon	Morphological

Genus *Dinotopterus*

Dinotopterus cunningtoni	– Nsumbu Island (Tanganyika)	Morphological

Genus *Tanganikallabes*

Tanganikallabes mortiauxi	– Cape Kachese harbour (Malawi)	Molecular
	– Lake Tanganyika (Malawi)	Morphological

Genus *Uegitglanis*

Uegitglanis zammaranoi	– Uegit (Somalia)	Morphological

Genus *Bathyclarias*

Bathyclarias longibarbis	– lake Malawi	Morphological

Genus *Channallabes*

Channallabes alvarezi	– Abenelang (North Gabon)	Molecular
	– Zogongone (North Gabon)	Molecular
	– Abenelang (North Gabon)	Morphological
Channallabes sanghaensis	– Ntchouo (Pop. Rep. Congo)	Molecular
	– Ntchouo (Pop. Rep. Congo)	Morphological
Channallabes apus	– Kinshasa (Dem. Rep. Congo)	Molecular
	– Kinshasa (Dem. Rep. Congo)	Morphological

	– Stanleyville (Dem. Rep. Congo)	Morphological
	– Kesiki (Dem. Rep. Congo)	Morphological
	– Tchimenji (Dem. Rep. Congo)	Morphological
Channallabes	– Etakaniabe (North–East Gabon)	Molecular
longicaudatus	– Makokou (North–East Gabon)	Morphological
Channallabes ogooensis	– Malima (South Gabon)	Morphological
Channallabes teugelsi	– Zanaga (North-East Gabon)	Morphological
	– Lésala (Pop. Rep. Congo)	Morphological

Genus *Gymnallabes*

Gymnallabes typus	– Niger delta (Nigeria)	Molecular
	– Niger delta (Nigeria)	Morphological
Gymnallabes nops	– Tadi (Dem. Rep. Congo)	Morphological

Genus *Dolichallabes*

Dolichallabes	– Kunungu (Dem. Rep. Congo)	Morphological
microphthalmus	– Bokuma (Dem. Rep. Congo)	Morphological

Genus *Platyallabes*

Platyallabes tihoni	– Kinsuka (Dem. Rep. Congo)	Morphological

Genus *Platyclarias*

Platyclarias machadoi	– Cuango (Angola)	Morphological

OUTGROUPS

Heteropneustes fossilis	– cultured in Hungary	Molecular
	– Aquarium specimen	Morphological
Diplomystes chilensis	– Santiago (Chile)	Morphological
Pangasianodon *hypophthalmus*	– Aquarium store	Molecular
Kryptopterus bicirrhis	– Aquarium store	Molecular

Ictalurus punctatus   – Data obtained from Genbank[1]   Molecular

Clupea harengus   – Data obtained from Genbank   Molecular

Cyprinus carpio   – Data obtained from Genbank   Molecular

All non–anguilliform clariids are chosen such as to give a good synopsis of the family. The choice of the *Clarias* species is based on the recognition of different subgenera in TEUGELS (1986), an additional Asian *Clarias* (*C. fuscus*) is added to the morphological analysis.

Heteropneustes fossilis is chosen as the closest outgroup to the clariids. The Heteropneustidae are generally considered as the closest sister group of Clariidae (e.g. TEUGELS and ADRIAENS, 2004; this present study), although this is not always accepted in all phylogenetic studies (DE PINNA, 1993; DIOGO, 2005). According to these studies, *Heteropneustes* is to be considered a member of the Clariidae, with *Uegitglanis* being the sister group of *Heteropneustes* and all other clariids (DIOGO, 2005). The problematic relation of *Uegitglanis* with respect to other clariids is among other things related to the absence of the synapomorphic suprabranchial organs (CHARDON, 1968; TEUGELS and ADRIAENS, 2003).

The South American catfish family Diplomystidae is generally considered as the most primitive living family of the Siluriformes (ARRATIA, 1987, FINK and FINK, 1996, DIOGO, 2005). Therefore we included *Diplomystes chilensis* as one of the outgroups in the morphological analyses. Furthermore, we included *Pangasianodon hypophthalmus* (Pangasiidae), *Kryptopterus bicirrhis* (Siluridae) and *Ictalurus punctatus* (Ictaluridae) as some additional Siluriformes representatives.

Finally we included *Clupea harengus* (Clupeiformes) and *Cyprinus carpio* (Cypriniformes). *Cyprinus carpio* is included in the Cypriniformes, *Clupea harengus* is a member of the order Clupeiformes, comprised in the Clupeomorpha. This latter is the sister taxa of the Ostariophysi (**Fig II.1.1**). Both these taxa will thus help to basally root our molecular trees.

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[1] http://www.ebi.ac.uk/cgi-bin/sva/sva.pl

III.2 - Methods

What follows is a list of the methods used in this thesis. Although this section contains some additional information (biometric measurements and meristic counts), it mainly refers to the specific sections where more information in detail can be found. This will optimise the comprehensibility of each section separately and leaves each section as a complete entity. This, of course, brings some unavoidable recapitulations, which are anyway reduced to a necessary minimum.

III.2.1 Preparation of living specimens

III.2.1.a Anaesthesia and Sacrifice

Prior to fixation, the specimens were anaesthetized by submerging them in a 0.01% water solution MS222 (3–Aminobenzoic acid methanesulfonate salt – SIGMA Chemical Co) and killed by an overdose of MS222, in accordance with the Belgian law in the protection of laboratory animals (KB d.d. November 14th, 1993).

III.2.1.b Fixation

Fixation was done using 4% buffered formalin solution at neutral pH. Formaldehyde is a widely used fixative, when dealing with larger specimens. Using this fixative allowed us to use the fixed specimens for serial sections in a later stage.

III.2.2 Morphological descriptions

Different procedures were used for studying the detailed morphology of the anguilliform clariid. The osteology could best be studied, using *in toto* cleared and stained material, however, serial sections proved to be very useful for checking internal structures, indistinct ossifications, attachments of ligaments or connection between bones. The cranial musculature is studied through dissections, but again some details (distinguishing separate muscle bundles, tendons, …) required serial sections.

III.2.2.a *In toto* clearing and staining

The most satisfying results were obtained using the procedure according to HANKEN and WASSERSUG (1981) for *in toto* clearing and staining. This procedure allows the differential staining of cartilage and bone. In the original protocol, trypsine was used as the clearing agent. The clearing activity of trypsine, however, was very slow, as it took a long time to clear individuals, up to several months for larger specimens. Therefore, different concentrations (up to 10%) of the more aggressive potassium hydroxide (KOH) were used. Although more aggressive, potassium hydroxide gave equally good results also on smaller specimens and this in a reasonable short time.

Staining was done using alizarine red S for bone and alcian blue for cartilage (**Table III.2-1** In the protocol of HANKEN and WASSERSUG (1981), the cartilage staining precedes that of bone. However, the alcian blue 8GX has to be dissolved in ethanol and glacial acetic acid. The latter is very acidic, which thus induces decalcification of the bone. As alizarine red S actually binds onto calcified matrix of bone, true ossifications may thus be masked after being decalcified during the cartilage staining. To avoid this problem, several specimens only were stained with alizarine red S only.

Table III.2 - 1: Composition of the bone and cartilage stainings

Product	Ratio
BONE STAINING	
Alizarine red S	15mg
KOH	0.5g
Aqua dest.	100ml
CARTILAGE STAINING	
Ethanol (96%)	40ml
Glacial acetic acid	10 ml
Alcian blue 8GX	7.5 mg

III.2.2.b Dissections

The osteology and myology were studied by means of dissections, using an Olympus SZX9 stereoscopic microscope, equipped with a camera lucida and a digital camera (Colorview 8). Osteological data was obtained through cleared and stained material. The discrimination of fibre orientation and insertion of the musculature was improved by using an iodium solution, which was sprayed over the muscles until fibres became apparent (BOCK and SHEAR, 1972).

III.2.2.c Serial sections

The use of serial sections was crucial for studying the detailed morphology of the cranial area, this for discriminating exact insertion sites of muscles, positions of tendons and ligaments, types of ossifications, etc. For this study, specimens were embedded in Technovit 7100. Sections of 5 µm were cut using a Reichert–Jung "Polycut" microtome, whereas staining was done with toluidin blue. The sections were all mounted on glass slides and covered. They were studied using a Polyvar light microscope (**Table III.2-2**).

Table III.2 - 2: list of all the specimens used for serial sectioning

species	collection number
Channallabes teugelsi	8-22-P-1047-050
Gymnallabes typus	97-030-0009
Platyclarias machadoi	78-6-P-1348-364
Platyallabes tihoni	73-22-P-3127
Dolichallabes microphthalmus	79258-260
Channallabes alvarezi	A4-31-P-78-89

III.2.3 Visualisation

III.2.3.a Camera lucida

Camera lucida drawings of different views of the cranium, caudal skeleton, etc. were made during stereomicroscopic observation on a Olympus SZX. Attention was paid to the orientations of the different views of the skull, in order to standardize observations in the different species.

III.2.3.b CT–scanning (IV.3.1 *G. nops*)

A high-resolution desktop X-ray microtomography instrument [Skyscan-1072, Belgium (www.skyscan.be)] was used to visualise the holotype of *Gymnallabes nops*. The CT–scanning was performed in the Department Biomedical Sciences, University of Antwerp (RUCA).

III.2.3.c Radiographs

The morphology of the vertebrae and the several meristic counts (total number of vertebrae, number of ribs, number of dorsal and anal fin rays) were made on each specimen using the radiographs. These were made with a MPG 65 generator and a RSN 620

X-ray-tube (General Electric), the ideal settings were a focus distance of 1m, an exposure time of 10msec, with a power of 42 kilovoltage and a number of 320 milli-amperes. All radiographs were taken at research group: Interne geneeskunde en klinische biologie van de grote huisdieren.

III.2.4 Biometric and Morphometric analysis

In the Clariidae literature some detailed definitions for measurements and meristics were available (TEUGELS, 1986). For some other measurements a brief description is given in some other systematic studies (BODEN et al., 1997; VAN DEN BERGH and TEUGELS, 1998; ANSEAUME and TEUGELS, 1999). Therefore a more complete description of the meristic and morphometric characters examined is given below. The definitions follow those given by DEVAERE et al. (2004) (**IV.4**), with some additional measurements. All measurements were taken point-to-point using digital callipers to 0.1 mm (digital ruler, Mauser), interfaced directly with a computer. Measurements of bilaterally paired structures were taken on the left side.

III.2.4.a Biometric measurements

The numbers correspond to the numbers on **Plate III.2.1A**

1°. *Total length* (TL): from anterior border of the snout to posterior end of caudal fin.

2°. *Standard length* (SL): from anterior border of the snout to caudal-fin base.

3°. *Preanal length* (PaL): from the anterior border of the snout to the anus

4°. *Prepelvic length* (PPvL): from the anterior border of the snout to the to dorsal edge of pelvic-fin base

5°. *Prepectoral length* (PPcL): from the anterior border of the snout to the to dorsal edge of pectoral-fin base

6°. *Predorsal length* (PdL): from the anterior border of the snout to the to anterior edge of dorsal-fin base

7°. *Distance between the occipital process and the dorsal fin* (SPDFL): from the caudal tip of the supraoccipital process to the anterior edge of dorsal-fin base

8°. *Pelvic fin length* (PvFL): from the base of the median pectoral-fin rays to the distal edge of the pelvic fin

9°. *Pectoral fin length* (PcFL): from the base of the median pectoral-fin rays to the distal edge of the pectoral fin

10°. *Pectoral spine length* (PcSL): from the base to the posterior end of the pectoral spine

11°. *Caudal peduncle depth* (CPD): height of the caudal fin base

12°. *Body depth at anus* (ABD): height of dorsal fin not included

13°. *Maxillary barbel length* (MxB): base to distal tip of the maxillary barbel

14°. *External mandibular barbel length* (EmnB): base to distal tip of the external mandibular barbel

15°. *Internal mandibular barbel length* (ImnB): base to distal tip of the internal mandibular barbel

16°. *Nasal barbel length* (NB): base to distal tip of the nasal barbel

17°. *Interpelvic distance* (IpvD): distance between left and right pelvic fin base

18°. *Interpectoral distance* (IpcD): distance between left and right pectoral fin base

19°. *Skull length* (SkL): from the anterior border of the snout to the caudal tip of the supraoccipital process

20°. *Preorbital length* (PoL): from the tip of the premaxillae to rostral border of the eye

21°. *Supraoccipital process length* (SpL): base to caudal tip of the supraoccipital process

22°. *Posterior fontanel length* (PF)

23°. *Anterior fontanel length* (AF)

24°. Skull width (SkW): maximum width of the skull (at the level of the pectoral fins)

25°. *Supraoccipital process width* (SpW): width of the supraoccipital process base

26°. *Interorbital distance* (IoD): minimum distance between the two orbits

27°. *Anterior nostril interdistance* (ANID): minimum distance between the left and right anterior nostril

28°. *Posterior nostril interdistance* (PNID): minimum distance between the left and right posterior nostril

29°. *Rostral skull width* (RSkW): measured at the level of the maxillary barbel

30°. *Orbital skull width* (OSkW): measured at the level of the orbits

31°. *Skull height* (SkH): maximum skull height (at the level of the caudal tip of the supraoccipital)

32°. *Eye diameter* (ED): distance between the anterior and posterior edges of the eye

33°. *Snout height* (SnH): measured at the level of the anterior nostrils

34°. *Orbital skull height* (OskH): measured at the level of the orbits

35°. *Prehyoid length* (PhL): measured from tip of premaxillae to hyoid skinfold:

36°. *Internal mandibular interdistance* (ImnID): minimum distance between the left and right internal mandibular barbels

37°. *External mandibular interdistance* (EmnID): minimum distance between the left and right external mandibular barbels

38°. *Mouth width* (MW): maximum width of the mouth opening

39°. *Skull roof width* (SkR): minimal skull roof width

III.2.4.b Meristic

Apart from the external biometric measurements, a set of meristic counts are used. Visualisation of these characteristics occured through X–rays and CT–scanning (**see III.2.3.b, c).**The different meristic counts are illustrated in **Plate III.2.1B**

1°. *Total number of vertebrae* (TV): the sum of the precaudal (abdominal) and caudal vertebrae number (including the vertebrae incorporated in the Weberian apparatus).

2°. *Total number of ribs* (RB).

3°. *Number of non-rib bearing precaudal vertebrae* (nRPCV): number of vertebrae from the last vertebra bearing ribs (not included) to the first vertebra (not included) whose haemal spine supports pterygiophore of the first anal spine.

4°. *Number of caudal vertebrae* (CV): number of vertebrae from first anal pterygiophore supporting vertebra to last vertebra (the last vertebra is defined here as the the complex, supporting the hypural bones and the parhypural bone)

5°. *Number of dorsal fin rays* (DFR): the number of soft fin rays up to the first caudal fin ray (dorsally).

6°. *Number of anal fin rays* (AFR): the number of soft fin rays up to the first caudal fin ray (ventrally).

III.2.5 Statistical analyses

All measurements, except the total (TL) and standard length (SL), are expressed as ratios of a reference length. Most body and paired fin measurements and the head length are expressed as a percentage of the standard length. (see **III.2.4.a** for a detailed definition of these measurements). Measurements taken on the head are all expressed as a percentage of the skull length (SkL).

For each species, a descriptive table of the biometrics is given including for each length variable the following descriptive statistics: the minimum (min.), the maximum (max.), the number of specimens examined (n), the mean and the standard deviation (see **Table IV.3-2**).

In addition, for each species a descriptive table of the meristics is given including for each meristic variable the following descriptive statistics: minimum (min.), maximum (max.), the number of specimens examined (n) and the mode (mode = is defined as the

measure of central tendency, the *mode* of a sample is the value which occurs most frequently in the sample) (see **Table IV.3-2**).

A test of normality was performed, based on the Kolmogorov-Smirnov test, using Statistica 6.0. A significant result indicated a significant deviation from the normal distribution (see **IV.5.1.b**). Therefore, the Mann-Whitney U tests have been performed to statistically confirm the discriminative power of some "most important" meristic and length variables (see **IV.5.1.b**). A Principal Component Analysis was used as the multivariate analysis. The complete method of using Principal Components Analysis and especially the method used by BODEN et al. (1997) is thoroughly explained in **IV.5.1.b**.

III.2.6 Molecular–phylogenetic analyses

Most molecular–phylogenetic research on Clariidae has been focussing on mtDNA genomic sequences (e.g. cytochrome b) (Agnèse and Teugels, 2001a, in press). Although, these genes have proven their utility to reflect higher–level affinities, independent genetic markers are needed as an additional source of data on genetic evolution. For this reason, ribosomal DNA–genes are used. rDNA proved to be extremely valuable for revealing phylogenetic relationships and for studying genetic variability and divergence between species, this because of its attractive properties such as secondary structures, differential rates of evolution between different regions, … (Gebri, 1985). More specifically, 18S, 5.8S, 28S (low mutation speed, coding, suitable at family level) and Internal Transcribed Spacers (high mutation speed, non-coding, suitable at lower taxonomical level) are used in this research. The combination of both must allow us to retrieve phylogenetic affinities at all levels.

The methods of DNA extraction, PCR amplification, sequencing reactions, sequence alignment and the construction of datasets can be found and are explained in **V.1**.

Additional to MrBayes (Bayesian inference algorithms) and PAUP 4.0b10 (neighbour–joining, maximum–parsimony, maximum–likelihood algorithms), the program POY was used. When using sequence data in a phylogenetic analysis, it is common practice to apply the following two-step procedure. First create a multiple alignment to resolve positional correspondences of residues and sizes and positions of indels. This can be done manually and/or by using programs such as CLUSTAL W. Next the resulting multiple alignment is used for evaluating and searching trees, using regular programs for phylogenetic analysis

such as PAUP. In spite of the popularity of this two–step approach, however, alignment on the one hand and tree evaluation and tree search on the other hand are better not separated in a phylogenetic analysis and therefore POY was used, which combines the two steps in one program. The different phylogenetic programs and methods used are all explained in more detail in **V.1** and **V.2**.

III.3 - Some Terminologies used

Systematic: the study of biological diversity and of the evolutionary relationships among organisms (SIMPSON, 1961 in WINSTON, 1999). It also includes the study of the process of evolution and phylogeny.

Taxonomy: by the above mentioned definition of systematics, taxonomy is a subdivision of systematics, consisting of three associated activities: identification (assigning a specimen to a previously classified and named group), classification (ordering organisms into groups based on perceived similarities or differences) and nomenclature (naming groups of organisms according to the rules developed for the process) (WINSTON, 1999).

Phylogeny: the genealogical relationships among a set of taxa; sometimes, the process of evolutionary diversification (SCHUH, 2000).

Cladistics: grouping by synapomorphy through the application of the parsimony criterion (SCHUH, 2000)

Species concept: At present at least 22 species concepts are still in use, many of which are notably overlapping (MAYDEN, 1997). According to HULL (1997), three groups of concepts can be recognized: (1) Similarity requiring species concept; (2) Evolutionary theory committed species concepts; and (3) Hennig's Phylogenetic systematics connected species concepts.

The most important concept in the first group is the Morphological Species Concept, which defines species as easily recognized kinds of organisms, characterised by easy to determine phenotypic traits, or as stated by SHULL (1923): simple gross observations.

The most important species concept of the second group and perhaps also the most important of all species concepts is the Biological Species Concept. This concept sees species as a group of actually or potentially interbreeding natural populations which are reproductively isolated from other such groups (MAYR, 1942). Two of the most important characteristics of this concept are that it defines species by distinctness (reproductive gap) rather than by differences (morphological) and that a species can be seen as a genetic unit, a gene pool, whereas the individual is a temporary vessel holding a portion of the gene pool for a short time. The most important points of criticism are that this concept is difficult or impossible to apply as a taxonomic tool and that this concept is clearly non–

dimensional. This last criticism is answered in the <u>Evolutionary Species Concept</u>, which can be seen as an extension of the Biological Species Concept through time (HULL, 1997).

The <u>Phylogenetic Species Concept</u> is an example of the third group. It can best be defined as a species definition which seeks to delineate species as the smallest aggregate population or lineage which can be united by synapomorphic characters. In other words species are defined as the smallest diagnosable monophyletic group. Often this is coupled to the <u>Cladistic Species Concept</u>, which considers a species as a set of organisms (an evolutionary lineage) between two branch points or between one branch point and an extinction event or a modern population (RIDLEY, 1993).

Choosing the best concept is a difficult and ambiguous task. In the most complete taxonomic works the Biological Species Concept has been used. However, it is obvious that it is impossible to directly apply the Biological Species Concept on preserved museum specimens. But, as stated by MAYR (1970) the biological species is, in most cases, also a morphological species (except for sibling species). The morphological species concept, which is based on the degree of morphological difference, presents a good working hypothesis for groups of which the α-taxonomy is poorly known and in many cases it is the only approach available (VREVEN, 2001). Within this work the Morphological Species Concept was used as practical approach and morphological, meristical, … differences were used to define species, so that this species concept can be called the Extended Morphological Species Concept. There is indeed an enormous difference between basing a species concept entirely on morphology (e.g. Typological Species Concept) or using morphological evidence as an inference in the application of the Biological Species Concept. We indeed look at the same but from a different background. A degree of genetic relationship is generally also reflected in a degree of physical resemblance (except for sexual dimorphism and several others...). Therefore, morphological evidence can still be used, in addition to other categories of characters or facts, to determine whether a population can be considered as a different species or not.

However, when we look at the results and the consequences of the phylogenetic analysis (VI.1), we look at the different taxa from a more cladistic point of view. Therefore we use a combined Cladistic/Phylogenetic Species Concept.

PART IV

Morphology and species demarcation

of the anguilliform clariids

IV.1 - The genus *Platyallabes*

IV.1.1 Morphology and spatial constraints in a dorso-ventrally flattened skull, with a revised species description of *Platyallabes tihoni* (Poll, 1944).

ABSTRACT

This study details the morphology of *Platyallabes tihoni* (Poll, 1944) as part of a complete revision of the anguilliform clariids. The overall body form of air-breathing clariids ranges from fusiform to anguilliform genera (BOULENGER, 1911; PELLEGRIN, 1927). Although *P. tihoni* has the typical external morphological features of other elongate clariids, this study shows that it occupies an intermediate position between fusiform and anguilliform taxa with regards to its cranial and postcranial morphology. The main morphological similarities of anguilliform species are a narrow skull roof with a high level of interdigitation between the bones, the reduced canal bones, a connection between the neurocranium and suspensorium via several processes, an extended tooth patch on the lower jaw and the hypertrophied jaw muscle complex. Shared features typical for the fusiform species are a low coronoid process on the lower jaw and the posteroventral orientation of the opercular process on the hyomandibula. *Platyallabes tihoni* shows a series of unique features: an anterior fontanel situated entirely between the frontals, two tooth plates on the prevomer, a reduced height of the suspensorium, the absence of an anterior bony plate on the hyomandibula, a horizontal position of the sphenotic and pterotic and a toothed entopterygoid. Many of these unique characters are linked to the spatial constraints associated with an extremely flattened skull of this species.

INTRODUCTION

The Clariidae are one of the more than 30 families within the Siluriformes (DE PINNA, 1998; TEUGELS, 2003), with diversity of the family largest in Africa, where 12 genera and up to 74 species are known (TEUGELS, 1986). Clariids also occur in Syria, southern Turkey and parts of Southeast Asia (TEUGELS, 1986). Some of the generalised, fusiform species, such as *Clarias gariepinus* (BURCHELL, 1822) show a broad geographic distribution, whereas the anguilliform species occur only in Lower Guinea ichthyological province and the Congo River basin (BOULENGER, 1911; POLL, 1957b; TEUGELS, 1986; TEUGELS et al., 1990; SKELTON, 1993), where they occupy a more specialized, burrowing niche.

Clariids are characterized externally by long dorsal and anal fins, the presence of four pairs of barbels, a dorsoventrally flattened head, and a unique form of suprabranchial organ[1] formed from the second and fourth gill arches (GREENWOOD, 1961; VANDEWALLE and CHARDON, 1991; TEUGELS and ADRIAENS, 2003).

The anatomy of the anguilliform clariids has barely been studied in detail to date. *Platyallabes tihoni* has been referred to in only a few papers (POLL, 1944, 1957b, 1977), which include descriptions of external morphology and to a limited degree a superficial cranial study. The aims of this paper are: (1) to provide a detailed description of the cranium and (2) describe the myology of the skull, (3) discuss the postcranial characteristics, (4) provide a redescription of *P. tihoni* and (5) compare *P. tihoni* with other clariid species. For this comparison we use *Clarias gariepinus* (Burchell, 1822) as a fusiform representative and *Gymnallabes typus* (Günther, 1867), *Dolichallabes microphthalmus* (Poll, 1942) and *Channallabes apus* (Günther, 1873) as anguilliform species.

MATERIALS AND METHODS

The specimens examined in this study were obtained from the American Museum of Natural History (AMNH), the Museum of Comparative Zoology, Harvard University (MCZ), the Natural History Museum (BMNH) and the Royal Museum for Central Africa (MRAC) (Tervuren, Belgium). These specimens include the holotype of *Platyallabes tihoni* (MRAC 13307) (see list below). Two specimens (MRAC 125345-349, SL: 156mm and 14473-68-P-144,

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[1] Based on the current knowledge on clariid phylogeny, it can be assumed that the absence of a suprabranchial organ is a derived condition (AGNÈSE and TEUGELS, in press). With the current absence of a good knowledge of the phylogenetic position of *Uegitglanis* this remains the most plausible hypothesis up till now.

SL: 295mm) were cleared and stained for osteological examination following the procedure of TAYLOR and VAN DYKE (1985). One specimen (MRAC 125345-349, SL: 136mm) was used to study the external morphology as well as the myology by means of dissection and muscle fibre staining (BOCK and SHEAR, 1972). One specimen (MRAC 73-22-P-3127, SL: 139) was serially sectioned after embedding in Technovit 7100 (Kulzer). The resultant 5 μm thick sections were stained with Toluidin Blue. Terminology of bones follows ADRIAENS and VERRAES (1997a, b, 1998) and ADRIAENS et al. (1997), except for the pelvic girdle elements for which we use ARRATIA (2003). Cranial myology terminology follows WINTERBOTTOM (1974) and ADRIAENS and VERRAES (1996, 1997c, d).

Measurements were taken point-to-point using digital calipers to 0.1 mm (Digital ruler, Mauser), interfaced directly with a computer, on 55 specimens of *P. tihoni*. Measurements follow TEUGELS (1986), with some additions. The morphology of the vertebrae and the following meristic counts were made on each specimen using the radiographs: total number of vertebrae, number of ribs, and number of dorsal and anal fin rays.

A list of the species examined for comparison is given in a separate section (see below). For *Gymnallabes typus* and *Channallabes apus*, we refer to respectively CABUY et al. (1999) and DEVAERE et al. (2001) (**IV.5.1.a**), but many additional specimens have been examined for this study. The museum abbreviations are listed in LEVITON et al. (1985).

RESULTS

External morphology

Platyallabes tihoni has a long, dorsoventrally flattened body, with only caudal tip laterally compressed. The specimens show an even light-brownish colour (**Plate IV.1-1A**).

One of the most striking features is the extremely flattened head with bulging jaw muscles. The mouth is surrounded by fleshy lips, which are fused at the corners of the mouth. *Platyallabes tihoni* shows a distinct prognation of the lower jaw (**Plate IV.1-1B**) and the eyes are clearly visible[2]. The dorsal fin originates very close to the tip of the supraoccipital process and forms a continuous fin-fold with the anal and caudal fins. Both pectoral and pelvic fins are typically present (pelvic fins were absent however, in one specimen (MCZ 50239)). The pectoral fin has a large spine anteriorly.

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[2] except for two specimens

Cranial skeleton

Neurocranium

Platyallabes tihoni has a remarkably narrow skull roof (orbito-temporal region), though the total skull width is distinctly broad (**Plate IV.1-1B**).

Ethmoid region: the tubular nasal bone of *P. tihoni* is the anterior most bone of the supraorbital portion of the laterosensory canal system. The nasal bears small lateral plate-like extensions, as observed in some other clariids (Cabuy et al., 1999).

The large, forked mesethmoid (**Plate IV.1-1B**) is connected to the frontals posteriorly via large interdigitations. There is a limited constriction of he mesethmoid behind the rounded, anterior wings. Further, the posterior border does not reach the anterior limit of the anterior fontanel, which as a consequence is completely surrounded by the frontals. The partially open supraorbital canal passes between the lateral ethmoid and the mesethmoid and forms a distinct boundary between the bones. The lateral ethmoid (**Plate IV.1-1b**) bears a large, laterally pointed process but lacks any articulation with the infraorbitals and as a consequence also lacks an articular ridge. The arrow-shaped prevomer runs along the mesethmoid ventrally and interdigitates posteriorly with the parasphenoid via a pointed process. The prevomer bears contralateral tooth plates (**Plate IV.1-2B**).

Orbital region (**Plate IV.1-1B**): the circumorbital series involves four infraorbitals and an antorbital. The antorbital is a small bone located at the base of the nasal barbel and situated dorsal to the anterior tip of the autopalatine. The tubular lacrimal (first infraorbital) lies at the level of the olfactory organ. The reduced, tubular second through fourth infraorbitals surround the eye. The fourth infraorbital lies dorsal to the adductor mandibulae complex posterolateral of the lateral ethmoid and is sometimes divided into two parts. The infraorbital canal splits within the fourth infraorbital and ends in a lateral pore (I_6 in Adriaens et al., 1997).

The narrow frontals are the largest bones of the skull roof (**Plate IV.1-1B**). The two frontals, slightly concave in the middle of their lateral margins, are strongly fused, with a barely visible median seam. These bones are anteriorly separated by the anterior fontanel, with a short anterior area of contact between these bones behind the mesethmoid. The frontal bones have two lateral plates visible on a ventral view slightly extending beyond the orbitosphenoid bone margins (**Plate IV.1-2B**). The anastomosis of the infraorbital and the supraorbital canal is situated on the anterior part of the frontal. The lateral wall of the central portion of the skull is formed by the orbitosphenoid and pterosphenoid, which connect ventrally to the dermal parasphenoid. The latter bone forms the skull floor up to

the temporal region and has an elongated process extending to the occipital region (**Plate IV.1-2B**).

Temporal region (**Plate IV.1-1B**): the sphenotic interdigitates anteromedially with the frontal and posteriorly with the pterotic. The latter interdigitates medially with the parieto-supraoccipital and the frontals and posteriorly with the posttemporo-cleithrum. The sphenotic and pterotic are horizontally aligned, with a slight ventrolateral curvature and no lateral plate. Both bones form the only firm connection between the neurocranium and the suspensorium. The sphenotic has a plate-like and a spiny process anterolaterally that extend ventrally to interdigitate with the hyomandibula. Posterolaterally, a cartilaginous facet on the pterotic and sphenotic articulates with the hyomandibula. The pterotic also has one spiny and one plate-like process. Ventrally, the brain cavity is enclosed by the paired perichondral prootics (**Plate IV.1-2B**).

Occipital region (**Plate IV.1-1B**): the complex parieto-supraoccipital is situated on the posteromedial portion of the skull roof and is characterized by a short, pointed posteromedial process. This bone encloses the posterior fontanel in the posterior part of the bone. The dermal posttemporo-supracleithrum connects to the pectoral girdle, the parapophyses of the fourth vertebra (by means of the transscapular process) and to the pterotic anteriorly. The occipital region of the skull is ventrally bordered by the unpaired basioccipital and paired exoccipitals (**Plate IV.1-2B**). *Platyallabes tihoni* lacks epioccipitals.

Upper jaw (**Plate IV.1-1B, IV.1-2A,B**): the premaxilla are plate-like bones supporting the nasal sac ventrally. The anterior, larger ventral portion of each premaxilla is covered with several rows of posteriorly-directed teeth and the posterior half bears a small process. The maxilla forms a cup-like bone, which encloses the base of the maxillary barbel and bears two articulatory facets for articulation with the palatine.

Splanchnocranium

Lower jaw (**Plate IV.1-2C,D**): the long lower jaw consists of two components: the dento-splenio-mentomeckelium and the angulo-splenio-articulo-retroarticular complexes. The coronoid process on the lower jaw is indistinct and dorsal surface of the anterior part of the lower jaw is covered with a large tooth patch of posteriorly-curved teeth that extends almost to the coronoid process. The two processes on the retroarticular process are highly asymmetrical and lie completely medial to the quadrate (**Plate IV.1-2C,D**).

Suspensorium (**Plate IV.1-2C,D**): as in most siluriforms, the suspensorium consists of the hyomandibula, quadrate, preopercle, metapterygoid and entopterygoid. The hyomandibula suspends the suspensorium from the neurocranium in the area of the

sphenotic and pterotic. The hyomandibula connects to the sphenotic through one broad, anterior process and two pointed, posterior processes and to the pterotic by one pointed and one broader process. These secure the neurocranial-hyomandibula articulation. In between these two sets of processes lies a short articulatory facet is. No bony process is present at the anterior margin of the hyomandibula. The opercular process of the hyomandibula is posteroventrally orientated. Medially, the hyomandibula bears a ridge for the attachment of the hyomandibula-ceratohyal ligament. The quadrate interdigitates with the metapterygoid, but no direct contact is present between the quadrate and the entopterygoid. Anteroventrally, the quadrate has a well-developed articulatory facet, articulating with the angular complex. The small metapterygoid connects with the quadrate through a synchondrosis and a bony interdigitation, and is dorsally bordered by the quadrate and the large entopterygoid. The plate-like entopterygoid lies anterodorsally to the metapterygoid. The entopterygoid bears several teeth on its ventral margin (**Plate IV.1-2C**). Anteriorly, the entopterygoid is connected ligamentously to the prevomer, the palatine and the lateral ethmoid, thus corresponding to a sesamoid 'entopterygoid type 4' (ARRATIA, 1992). The cylindrical autopalatine (**Plate IV.1-2B**) extends ventrally from the lateral ethmoid, with which it articulates through a well-developed articular facet. Both ends of the autopalatine are cartilaginous and it articulates anteriorly with the maxillary, thus being part of the palatine-maxillary mechanism.

Hyoid arches (**Plate IV.1-2A**): the hyoid arch consists of paired anterior and posterior ceratohyals and paired ventral and dorsal hypohyals. The hyoid arch articulates ventrally with nine branchiostegal rays. The parurohyal lies in between the two hyoid arches and bears two lateral processes and one medial process. The parurohyal is connected to the hypohyal by means of two separate paruro-hypohyal ligaments (**Plate IV.1-4C**).

Branchial arches: the branchial morphology resembles that of *Clarias gariepinus* (ADRIAENS and VERRAES, 1998) with the exception of the low number of gill rakers (nine or fewer).

Opercular series (**Plate IV.1-2C,D**): the opercle is a pointed, slender, somewhat triangular, dorsoventrally compressed bone, bearing a large articulatory facet for its articulation with the hyomandibula. The posterior part of the bone bears a horizontal ridge for the attachment of the opercular levator muscle. The opercle is ligamentously attached to the anteriorly positioned interopercle. The interopercle is a long, flat bone, ligamentously attached to the angular complex of the lower jaw. The preopercular bone is incorporated in the suspensorium and surrounds the dorsal part of the preoperculo-mandibular canal. The posterior part of the canal extends through a series of four or five 'open' tubular suprapreopercular bones (**Plate IV.1-1B**).

Cranial myology

Muscles of the lower jaw (**Plate IV.1-3**)

Adductor mandibulae muscle: this complex forms an enormous, jaw-closing muscle covering most of the lateral surface of the head of *Platyallabes tihoni*. It consists, as in other clariids, of an external A_2A_3'-part and an internal A_3", that are separated by the levator arcus palatini (ADRIAENS and VERRAES, 1996).

The bipennate A_2A_3' forms the largest part of the complex (**Plate IV.1-3A,B,C**). It is divided into a dorsal A_2A_3'α-part and a ventral A_2A_3'ß-part, both of which are attached to an aponeurosis connected to the angular complex of the lower jaw close to the coronoid process. The directions of the muscle fibres of the A_2A_3' has a range of almost 160°. The A_2A_3'α is attached to the frontal, the sphenotic, the pterotic, the parieto-supraoccipital and the suprapreopercular series and is covered by the fourth infraorbital. The ventral A_2A_3'ß is posteriorly connected to the hyomandibula, the quadrate and the preopercle. The A_2A_3' covers the levator arcus palatini, the dilatator and adductor operculi and part of the levator operculi.

The A_3" lies between the levator arcus palatini and the medially-situated retractor tentaculi (**Plate IV.1-3C**). The horizontally-orientated muscle fibres originate on the medial side of the suspensorium, more specifically on the hyomandibula and the anterodorsal rim of the quadrate, on the frontal, on the sphenotic and on the pterosphenoid and insert on the angular complex of the lower jaw medial to the A_2A_3' tendons.

Intermandibular muscle: this muscle is a short, solid muscle with transverse fibres, lying over the mandibular symphysis connecting the contralateral lower jaws (**Plate IV.1-4A,B**). The intermandibularis is bordered posteriorly by the interconnecting cartilage of the left and right bases of the internal-mandibular barbels, thus separating it from the protractor hyoidei muscle.

Suspensorial muscles

Levator arcus palatini muscle (**Plate IV.1-3C**): this thin muscle sheet connects the ventrolateral side of the skull roof with the suspensorium. This muscle complex consists of two separate parts (pars rostralis and pars caudalis) and a large complex of aponeuroses. The muscle complex originates on the posterolateral surface of the lateral ethmoid, the ventrolateral side of the frontal up to the sphenotic and inserts mostly tendinously on the anterodorsal border of the interdigitation between the quadrate and the hyomandibula. The levator arcus palatini runs medially from the eye. The levator arcus palatini pars

rostralis is located in the anterodorsal part of the complex. It runs from the lateral ethmoid and the frontal and attaches through a medial aponeurosis on the suspensorium. The levator arcus palatini pars caudalis lies more posteriorly and runs between the medial and lateral aponeuroses. The posterior part of the pars caudalis muscle inserts directly on the suspensorium. The predominant fibre direction in the muscle is dorsoventrally orientated, with the anterior fibres showing a more a horizontal radiation anteriorly. This complex morphology appears to be two separate muscles.

Adductor arcus palatini muscle (**Plate IV.1-3F**): the adductor arcus palatini connects the skull floor and the dorsal rim of the suspensorium. This muscle has the most medial position of all cranial muscles, lining the mouth cavity dorsolaterally. On the neurocranium, it inserts primarily on the parasphenoid, but also on the orbitosphenoid and pterosphenoid. The adductor arcus palatini attaches on the suspensorium (dorsal rim of the hyomandibula, the quadrate and the entopterygoid). The fibres run in a transverse plane with the anterior fibres more obliquely orientated.

Opercular muscles

Three opercular muscles could be discerned. The dilatator operculi is situated most anteriorly, partially covering the adductor operculi, with the levator operculi most posteriorly.

Dilatator operculi muscle (**Plate IV.1-3C**): the flattened dilatator operculi starts from halfway up the ventrolateral surface of the frontal between the two aponeuroses of the levator arcus palatini and the ventrolateral part of the sphenotic and the pterotic (**Plate IV.1-3C**). It inserts through a large tendon on the lateral surface of the dorsal process of the opercle close to the articulation with the hyomandibula. This tendon runs along the anteroventral side of the dilatator operculi.

Adductor operculi muscle (**Plate IV.1-3D,E**): this shorter and more robust muscle connects the posterior side of the suspensorium with the opercle. The adductor operculi originates on the posterodorsal part of the hyomandibula and the posteroventral part of the pterotic and inserts on the dorsal process of the opercle and thus lateral to the opercular articulation with the hyomandibula, in the region medial to the insertion of the dilatator operculi. The predominantly dorsoventrally directed fibres also attach to the connective tissue covering the dorsolateral surface of the remnant suprabranchial cavity. The posterior part lies against the posteriorly-situated levator operculi.

Levator operculi muscle (**Plate IV.1-3D,E**): the levator operculi is the largest and most robust of the three opercle muscles. It connects the posterior most part of the neurocranium with the opercle. This muscle originates on the posteroventral part of the

posttemporo-supracleithrum, the suprapreopercles and the connective tissue covering the suprabranchial cavity. Ventrally, the levator operculi attaches to nearly all of the complete dorsal side on a ridge of the opercular bone.

Maxillary barbel muscles

Retractor tentaculi muscle (**Plate IV.1-3D**): the retractor is a bundle-like muscle running from the maxillary bone to the suspensorium between the A_3" and the adductor arcus palatini. The fibres in general all follow the same anteroposterior direction. Anteriorly, the retractor lies medial to the eye and lateral to the autopalatine. It inserts on the anterior side of the hyomandibula and the quadrate. The muscle is divided into a dorsal and ventral part, separated by an aponeurosis, and attaches to the posterodorsal side of the maxillary through a short tendon.

Extensor tentaculi muscle (**Plate IV.1-3E**): The extensor tentaculi extends from the ventral and ventrolateral surface of the lateroethmoid, the ventral side of the frontal and the lateral side of the orbitosphenoid to the autopalatine. The fibres, organised in two separate bundles, attach to the autopalatine posterior from the articulatory facet and enclose the posterior end of the autopalatine.

Hyoid muscles

Protractor hyoidei muscle (**Plate IV.1-4A,B**): the protractor hyoidei connects the hyoid bars with the lower jaw and is divided in a ventral and dorsal part, each of which originate on the ventrolateral side of the anterior ceratohyal and inserts on both complexes of the lower jaw. The ventral part is V-shaped (**Plate IV.1-4A**); with the left and right halves attached medially by a fascia at the plane through the mandibular symphysis. The insertion of the muscle on the mandibula occurs over the entire antero-ventral surface of the lower jaw and on the bases of the mandibular barbels. Several fields of fibres can be distinguished interconnecting different parts of the barbel bases. The dorsal part of the protractor inserts on the medial surface of both lower jaws (**Plate IV.1-4B**) and is divided into a medial and a lateral part. The medial parts attach postero-medially to the dental complexes of the lower jaw through a tendon and are separate. The lateral subparts attach to the ventrolateral surface of the dental complexes through a double tendon system.

Hyohyoidei inferior muscle (**Plate IV.1-4B**): this muscle inserts on the ventral side of the anterior ceratohyal, medial to the insertions of the protractor hyoidei, and to the dorsal and ventral hypohyals. The contralateral fibres are medially connected to a fascia. Posteriorly the muscle also attaches to the bases of the branchiostegal rays.

Hyohyoideus abductor muscle (**Plate IV.1-4C**): the hyohyoideus abductor links the anterior tip of the hyoid bars with the first contralateral branchiostegal rays. The muscles originate from the anterior face of these branchiostegal rays and insert onto the ventral hypohyal of the opposite side by means of a double tendon.

Hyohyoidei adductores muscles (**Plate IV.1-4C**): the hyohyoidei adductores muscles lie between successive branchiostegal rays, starting from the first ray and attaching onto the medial surface of the opercle.

Sternohyoideus muscle (**Plate IV.1-4D**): this muscle connects the pectoral girdle to the parurohyal, which, in turn, is ligamentously connected to the ventral hypohyals of the hyoid bars. Posteriorly, the sternohyoideus inserts on the cleithrum on both its dorsal and ventral surfaces. Anteriorly, both muscle heads attach onto the double forked parurohyal. On the anterolateral surfaces, two contralateral tendineus plates lie on top of the muscle. No myocommata were observed.

Postcranial skeleton

The total number of vertebrae in *Platyallabes tihoni* (58-490mm SL) ranges from 63 to 83 (mode: 78). The numbers of precaudal vertebrae varies from 16 to 20 (mode: 17) and of caudal vertebrae from 47 to 66 (mode: 63). *Platyallabes tihoni* has from four to seven pairs of ribs. A notable feature of the vertebrae is the presence of a large foramen at the bases of the parapophyses of the precaudal vertebrae (**Plate IV.1-5A**).

The pectoral fins of *P. tihoni* have non-serrated spines and 11 branched fin rays that articulate with two proximal radials. The pectoral girdle has a distinct fenestra between the scapulo-coracoid and the cleithrum. Furthermore, the cleithrum lacks an anterior process but has a distinct anterior rim ventrally, onto which the sternohyoideus is attached. The pelvic fins each carry six, branched fin rays which articulate with the broad and flat basipterygium of the pelvic girdle. Anteriorly an internal and external anterior process can be distinguished (**Plate IV.1-5B**). The number of dorsal and ventral fin rays varies respectively from 125 to 139 and from 114 to 129.

The morphology of the caudal skeleton of *P. tihoni* shows some variation but always consists of the parhypural, five hypurals and an urostyl, but various fusion patterns can be discerned. POLL (1977) reported a total fusion of all hypurals and the epural; however, our observations show that hypurals 3 and 4 appear fused, as well as the urostyl and hypural 5. Dorsally of the latter complex lies the broadly tipped epural. The neural arches of the second and third preural vertebrae are spiny, not elongated and do not support fin rays. The haemal arch of the second preural vertebra is elongated, broadly tipped and supports fin rays, the haemal arch of the third preural vertebra is not elongated, spiny and does not

support rays (**Plate IV.1-5C**). At the level of the non-supporting arches pterygiophores support the fin rays.

Species description

The proportional measurements and counts are given in **Table IV.1-1**. *Platyallabes tihoni* is characterized by elongated, dorsoventrally flattened body; with only the caudal tip transversely flattened (**Plate IV.1-1A**). Degree of anguilliformity as expressed by ratio of total length to body height (POLL, 1942a) between 15.6 and 33.9, with average of 24.7 ± 4.4, with postanal length of 65.3% up to 81.4% of SL.

Head length 7.4-17.2% of SL; and skull width 62.9-82.5% of head length. Skull roof width (exposed) 11.5-40.1% of orbital skull width. Skull roof appears very small, due to dorsomedial outgrowth of hypertrophied adductor mandibulae complex, but remains visible. Eyes clearly visible, in contrast to situation in some other anguilliform clariids (CABUY et al., 1999; DEVAERE et al., 2001 (**IV.5.1.a**)). Mouth width almost equals interorbital distance. Fleshy lips fused at mouth corners. Lower jaw distinctly prognathous (**Plate IV.1-1**).

Unpaired fins form continuous fin fold. Dorsal fin origin very close to skull; with small distance between dorsal fin origin and occipital process on parieto-supraoccipital (ADRIAENS et al., 2002: figure 1). Distance only 2.2-6.6% of SL.

Both pectoral fins always present. Pectoral fin length 43.3-82.0% of head length. Pelvic fins present in all except for one specimen (MCZ 50239). Pelvic fin length 31.2-71.2% of head length. Pectoral fins always preceded by non-serrated spine (59.2-95.1% of pectoral fin length). The pectoral fins have 11 fin rays; the pelvic fins carry six fin rays. Branchiostegal rays eight to ten.

Teeth present on dentary, premaxilla, prevomer and on entopterygoid, with latter unique within clariids (see below). All teeth pointed and curved. Prevomer with two separate tooth plates.

Alcohol preserved specimens show even, light-brownish colour over whole body, nostrils, barbels and fins.

Platyallabes tihoni lacks usual components of suprabranchial organ, with arborescent organs and fan-like covers completely absent. Remnant of small suprabranchial cavity apparently present.

DISCUSSION

The anatomy and taxonomy of the more extremely elongate clariids is poorly known. This is the case for *Platyallabes tihoni*, which was only been cited in several papers by POLL (e.g. 1944, 1957b, 1977), dealing mainly with morphometric and meristic features and some gross osteological characters. These were all based solely on the holotype.

As a result, a complete description of *P. tihoni* was necessary, as is a comparison with several other representatives of the clariid family, in order to determine their affinity and the systematic position of *P. tihoni*.

Morphology and comparison with other clariids

Platyallabes tihoni has previously been described as an anguilliform clariid (POLL, 1944, 1957b, 1977), with this character as one of the primary reasons for its original placement within the genus *Gymnallabes* (POLL, 1944, 1957b). Our own observations show that *P. tihoni* has an intermediate position with characters of both anguilliform and fusiform Bauplan.

The reduced skull and narrow skull roof is comparable to that of the anguilliform clariid species (CABUY et al., 1999; DEVAERE et al., 2001, 2004 (**IV.5.1a, IV.4**)). Also typical for anguilliform species is the extreme reduction of all infraorbitals and nasal bones, as they become tubular but still enclosing limited portions of the infraorbital canal of the lateral line system. A comparable reduction occurs in the suprapreopercles and the praeoperculo-mandibular canal. Although the primitive catfish family Diplomystidae, has tubular infraorbitals and suprapreopercles (ARRATIA, 1987), many clariids possess plate-like infraorbitals and suprapreopercles. Based on the widespread assumption that Heteropneustidae are the sister group of Clariidae, the presence of plate-like bones is to be considered plesiomorphic for Clariidae (Heteropneustidae have plate-like bones). However, following the phylogeny suggested by DE PINNA (1993) and DIOGO (2005), *Heteropneustes* is to be considered a member of the Clariidae, with *Uegitglanis* being the sister group of *Heteropneustes* and all other clariids. The blind and cave-dwelling *Uegitglanis* bears tubular infraorbitals and suprapreopercles (DAVID, 1936). This would imply that the plesiomorphic condition of Clariidae would be tubular bones, a retained plesiomorphic character of Siluriformes in general. The problematic relation of *Uegitglanis* with respect to other clariids is among other things because of the absence of the synapomorphic suprabranchial organs (CHARDON, 1968; TEUGELS and ADRIAENS, 2003). The absence of the typical arborescent organs in Heteropneustidae also makes the phylogenetic position of this genus problematic (DIOGO, 2005; TEUGELS and ADRIAENS, 2003). Further

knowledge on the true nature of the respiratory organs, as well as morphological and molecular data might elucidate the true relationships, and thus the status of the character of tubular bones.

The higher level of interdigitation of, among others, the skull roof bones is another correspondence with the anguilliform species and contributes to the solidity of the skull; additionally *P. tihoni* even shows a fusion of the frontals (**Plate IV.1-1B**). Furthermore the infraorbital-supraorbital canal anastomosis is situated in the anterior part of the frontals (**Plate IV.1-1B**) and no longer in the sphenotic, the latter being the case in the fusiform species and in *Diplomystis chilensis* (ADRIAENS et al., 1997; ARRATIA, 1987). The lateral ethmoid has no contact with the second infraorbital bone; this is also seen in *Gymnallabes typus* (CABUY et al., 1999). The sphenotic and pterotic lack a lateral plate, as is the case in all elongated species, except for *Platyclarias machadoi* (**IV.2**). The hyomandibula has a high number of anterior and posterior processes (respectively two and three) (**Plate IV.1-2C,D**), comparable to the number in anguilliform species. The lower jaw also shows osteological similarities: the robust tooth battery that runs close up to the coronoid process (**Plate IV.1-2C,D**) and the low number of gill rakers (up to nine).

Also the general muscular morphology of *P. tihoni* resembles that of the anguilliform species and shows the same insertion sites for the different muscles. A first clear example is the hypertrophied jaw muscle and more specifically the A_2A_3'-part. Compared to *C. gariepinus* additional insertion sites are found on the frontal, pterotic and posttemporo-supracleithrum. Besides this, there is also a large increase in the range of muscle fibre directions, up to almost 160°. This is still less than that found in most extremely elongated species (*C. gariepinus*: 105°, *Clariallabes* sp.: 140°, *G. typus*: 150°, *C. apus*: 180°). As in *C. apus* and *G. typus*, the ventral part of the protractor hyoidei is V-shaped. Consequently, the fascia, connecting the two contralateral parts of that muscle, does not overlap with the anterior side of the hyohyoideus inferior. Furthermore, the two contralateral parts of the dorsal part of the protractor hyoidei do not contact each other either through direct muscle contact or through tendons (**Plate IV.1-4B**), comparable to the situation in *G. typus* (CABUY et al., 1999).

Besides these character state similarities to the anguilliform clariids, *Platyallabes tihoni* shares a list of synplesiomorphies with the fusiform representatives, *e.g.* the highly reduced coronoid process.

Special traits of *Platyallabes tihoni*

The intermediate degree of anguilliformity of *P. tihoni* is reflected in the number of vertebrae, as this number can give an indication of the degree of anguilliformity. The

mode of the total number of vertebrae of *P. tihoni* is 78. The vertebral counts for the extremely anguilliform representatives are higher, e.g. *Dolichallabes microphthalmus* (n=11): 106, *Channallabes apus* (n=98): 104 and *Gymnallabes typus* (n=26): 87, while the number of vertebrae found in a fusiform member of the family, *Clarias gariepinus* (n=47): 56-63 (TEUGELS, 1986) is clearly lower.

Besides the above mentioned characters that exhibit a resemblance with the anguilliform or fusiform representatives, *P. tihoni* shows a large number of unique features. The fused frontals show a restricted concavity along their lateral sides (**Plate IV.1-1B**), whereas the width remains the same along the whole length in anguilliform species (CABUY et al., 1999; DEVAERE et al., 2001 (**IV.5.1.a**)) or broadens in the middle in most fusiform representatives (ADRIAENS and VERRAES, 1998). These lateral plates slightly pass the orbitosphenoid outlines (**Plate IV.1-2B**); a situation otherwise only seen in *Diplomystis chilensis* (ARRATIA, 1987) but that does not correspond with the broader plates in the fusiform clariids or the smaller extensions in the anguilliform species. The suprapreopercular bones are reduced to a set of tubular bones. This lack of any plate-like extension on the dorsal suprapreopercular is unique within the clariids, as is the higher number of bones (up to five). Both the sphenotic and pterotic, although lacking a lateral plate, exhibit a more or less horizontal position. Also, on the low suspensorium some unique features can be observed: on the anterior margin of the hyomandibula there is no bony outgrowth present, an absence only viewed elsewhere in *Heteropneustes fossilis* (sister group of the Clariidae) (SRINIVASACHAR, 1958) and *Platyclarias machadoi* (**IV.2**). The prevomer shows in all specimens two tooth plates, clearly separated from each other, a situation seen in other juvenile clariids, but never in adults (the strongly fused frontals, with a hardly visible seam, indicate that we are dealing with adult specimens of *P. tihoni*) (CABUY et al., 1999). The feature most remarkable in *P. tihoni* is the dentigerous entopterygoid (**Plate IV.1-2C,D**). This characteristic has not been observed elsewhere in the Clariidae, except on one side in one *G. typus* specimen. This was at that point considered as an aberrant condition (CABUY et al., 1999). Moreover, a toothed entopterygoid generally does not occur in catfishes, with the exception of some ariids (ARRATIA, 1992). These teeth could possibly be linked to a more specialized diet. The retroarticular process on the lower jaw is extremely asymmetric, lying completely on the medial side of the angular bone. Although this process is never fully symmetrical in the other clariids, it is never asymmetrical to this degree. A last feature of *P. tihoni* is the absence of a suprabranchial organ, together with a reduced suprabranchial cavity. Such a situation can also be seen in *Uegitglanis, Bathyclarias* and *Xenoclarias*. In *Xenoclarias* and *Bathyclarias,* the reduction is considered to be related to living in deep waters (CHARDON,

1968; POLL, 1977; ANSEAUME and TEUGELS, 1999; AGNÈSE and TEUGELS, 2001b; TEUGELS and ADRIAENS, 2003). Phylogenetically, this should consequently be considered as a secondary reduction, a hypothesis also supported by genetic evidence (AGNÈSE and TEUGELS, 2001b).

On the muscular level, we see that there is a unique system of two tendons for the insertion of the lateral parts of the pars dorsalis of the protractor hyoidei onto the lower jaw. A similar system with two tendons is seen on the hyohyoideus abductor (**Plate IV.1-4B,C**). A last remarkable myological difference is the very complex morphology of the levator arcus palatini (see results) (**Plate IV.1-3C**).

These unique features are not limited to cranial characteristics; post-cranially there are also some characters that are typical for *P. tihoni*. The cleithral bone has no anterior process; once more a condition only previously observed in *H. fossilis*. The same is true for the very large parapophyseal foramina present in the precaudal vertebrae (**Plate IV.1-5A**). *Platyallabes tihoni* has a very low number of ribs (four to seven, mode: five), which is the lowest number observed among examined clariids: *C. apus*: 10-17, *G. typus*: seven-14, *D. microphthalmus*: six-nine, *C. gariepinus*: 12 (CABUY et al., 1999).

This list of above mentioned autapomorphies can help to indicate the validity of the species and genus, while the synapomorphies help to interpret the phylogenetic situation within the clariids and especially within the anguilliform genera.

Functional implications of the extremely depressed skull

The flattened skull can be expected to be related to spatial changes in the surrounding structures, in all three dimensions of the Bauplan framework, since the spatial constraints within an integrated design imply that trade-offs will occur (BAREL, 1984).

Most of these characters are situated in the splanchnocranium and the articulation with the neurocranium. As mentioned above, the sphenotic and pterotic bones are in a more or less horizontal orientation. Consequently the interdigitation with and position of the suspensorium will also be in a more horizontal, lateral plane. This results in a dorso-ventral reduction in skull height. The orientation of these above-mentioned bones is in contrast with the tendency within the anguilliform clariids, in which the sphenotic and pterotic are in a more vertical position, which could be beneficial to accommodate the hypertrophic jaw muscles (CABUY et al., 1999; DEVAERE et al., 2001 (**IV.5.1.a**)). Despite this different orientation, *P. tihoni* exhibits large jaw muscles and this with similar insertion sites as within the anguilliform species (CABUY et al., 1999; DEVAERE et al., 2001 (**IV.5.1.a**)). The consequently more elevated position of the adductor mandibulae complex could explain the extensive bulging of these jaw muscles. More posterior on the suspensorium, the opercular process of the hyomandibula has a more posterior orientation. This means that

the dorso-ventrally compressed opercle is positioned more posterior to the suspensorium instead of ventral to it. All this leads to a decrease of the branchial cavity volume, which can have some consequences for feeding and respiratory efficiency (ADRIAENS and VERRAES, 1997d). Also the absence of the suprabranchial organ can be considered as a consequence of arising spatial constraints in the trend towards a miniaturisation of the skull in the anguilliform clariids (DEVAERE et al., 2001 (**IV.5.1.a**)). Additionally, the substantial dorso-ventral compression of the skull of *P. tihoni* will have generated a competition for space for both gill-apparatus and the suprabranchial apparatus. The combination of both constraints apparently favoured the loss of the suprabranchial organ.

The medial position of the retroarticular process in *P. tihoni*, with respect to the articulation head of the quadrate, is unique for clariids. This condition presumably is a simple consequence of the topographic relation between lower jaw and suspensorium, the plane of mandibular movements and the fact that this suspensorium has become tilted extremely, to an almost horizontal position (**Plate IV.1-6A**). Because the axis of rotation of the lower jaw has to run through both lower jaw articulations (in order to avoid dislocations), a horizontal tilting of the suspensorium will result in a medial shift of the articulation on the quadrate. Consequently, the post-articulation part of the lower jaw, i.e. the retroarticular process, becomes situated ventro-medially to the quadrate head (**Plate IV.1-6B**).

COMPARATIVE MATERIAL EXAMINED

Platyallabes tihoni (Poll, 1944). Democratic Republic of Congo. Kingabwa, Stanley pool, MRAC 13307 (Holotype); Kinsuka, MRAC 73-68-P-143, MRAC 138698-699 (n=2), 125345-349 (n=4), MRAC 73-22-P-3127 (n=3); Bulu, Luozi, BMNH 1976.5.21.30-39 (n=9), MCZ 50239 (n=13); Inga, MCZ 88947, MCZ 50537 (n=15); Tadi, Kibunzi, MCZ 50297 (n=5).

Channallabes apus (Günther, 1873). Angola. Ambriz, BMNH 1873.7.28.16 (Holotype); Democratic Repuplic of Congo. Bokalakala, MRAC 175247-270 (n=10); Kinshasa, MRAC 97-056-P-0001-0003 (n=2); Bumba, MRAC 88-25-P-2192-227 (n=36); Boma, MRAC 939; River Lula, Bushimaie, MRAC 153505; Kelé, MRAC 1491; Stanleyville, MRAC 30893-30900 (n=8), MRAC 88-01-P-1976-1992 (n=17); River Ruki, Eala, MRAC 14747-49 (n=3); Lake Tumba swamp area, MRAC 46299; Katanga, MRAC 39480; River Botota, keseki, MRAC 67763-77 (n=15); Mwilambongo, MRAC 72886-887 (n=2); Dekese, River Lofu, Anga, MRAC 153352; Yangambi, MRAC 68700; River Oubangui, Imfondo, MNHN 1922-0029; Loango, MNHN 1924-0079, MNHN 1924-0080; Sangha, MNHN 1925-0137; Mogende, MNHN 1926-0155-59 (n=5); River Congo, MNHN, 1937-0124-25; Stanleypool, Bamu, MNHN 1958-0111; Boloko, River

Likouala, MNHN 1962-0401 (n=7); Mossaka, River Likouala, MNHN 1963-0402 (n=2); River Loadjili, Songolo, MNHN 1967-0143 (n=6); Mangala, BMNH 1896.3.9.17; River Lebuzi, Kaka Muno, BMNH 1912.4.14.11-12 (n=2); Lower Congo, BMNH 1887.1.13.8-9 (n=2); Stanley Falls, BMNH 1889.11.20.5; New Antwerp, Upper Congo, BMNH 1899.2.20.16; Siala-Ntoto Swamps, BMNH 99.11.27.92; Bangyville, Ubangi, BMNH 1907.12.26.34; Kashi, Lulua, MHNG 1248.3; Banana, NMW 47240-42; Mollunda, NMW 47245 (n=4), NMW 47246. Congo. Yangala Youbi, MNHN 1967-0146; Djembo, Kouilou, MNHN 1967-0147; Cayo, MNHN 1989-0527; River Nanga, between Boukou-Zassi and Kouilou swamp area, MRAC 90-57-P2315; Sintou, River Kibombo, Kouilou, MNHN 1967-0144; River Loadjili, Songolo, Pointe Noire, MNHN 1967-0145 (n=6); River Youbi, Noumbi. Angola. Caungula, Mabete, River Uamba, MRAC 162088; River Camuconda, Tchimenji, MRAC 162089, MRAC 162090-094 (n=5), MRAC 162095-100 (n=6); River Ganga-Ludchimo, MRAC162083-086 (n=4).

Gymnallabes typus Günther, 1867. Nigeria. Old Calabar, BMNH 1866.12.4 (n=2) (Syntypes); Other specimens, Nigeria. Umu-Eze Amambra, MRAC 84-16-P-1-2; Riv. Sombreiro, East of Erema, MRAC 91-067-P0134; Niger Delta, MRAC 97-030-P-0001-0010 (n=10); lake Odediginni, Agudama, Yenagoa, MRAC 92-083-P-0035-0036; Okaka, Epie Creek, Between Nun an Rashi Riv, MRAC 97-085-P-0001-0004 (n=4); Riv Sombreiro, Odiemerenyi, Ahoada, MRAC 91-067-P-0135-0136; New Calabar, Choba, MRAC 91-105-P-1; Rumuji Swamps, MRAC 86-10-P-72; Oshika, MRAC 84-28-P-28, MRAC 84-28-P-25; Riv. Cron, Itu, MRAC 88-36-P-10; Between Sapele and War, Niger Delta, MRAC 74-29-P-600; Muoha, New Calabar, MRAC 91-10-P-478; Biseni, taylor Creek, MRAC 91-01-P278; Ossomari, BMNH 1902.11.10.119. Cameroun. Riv. Kom, Ntem, Aboulou, MRAC 73-18-P-3307-309.

Clarias gariepinus (Burchell, 1822). Artificially cultivated specimens (n=10), Ghent University.

Heteropneustes fossilis (Bloch, 1794). Aquarium specimen, AMNH 172276 SW.

Heterobranchus longifilis Valenciennes, 1840. Egypt. Nile, AMNH 3054 SW.

IV.2 - The genus *Platyclarias*

IV.2.1 Morphology of the cranial system of *Platyclarias machadoi* Poll, 1977: interdependencies of skull flattening and suspensorial structure in Clariidae

ABSRTACT

The African catfish *Platyclarias machadoi* stands out from other clariid species by its extremely dorso-ventrally flattened skull. This paper focuses on the spatial constraints and consequent functional interdependencies in this very low skull (average skull height of $27.5\% \pm 3.0\%$ skull length); such as the medial position of the retroarticular process on the lower jaw, the horizontal position of the suspensorium, the lateral tilting of the pterygoid bones and the anterior part of the quadrate with regard to the suspensorial transverse long axis. These characteristics can be presumed to have major influences on the capacity of suspensorial adduction, influencing the feeding and respiratory mechanisms. A comparison with other clariid species showed that one of the apomorphies is the presence of an extra muscle (musculus adductor mandibulae A_3'' pars levator tendinis) in the adductor mandibulae complex. This extra muscle lifts the tendon complex of the adductor mandibulae, resulting in a higher moment on the lower jaw. Some comments on the original species/genus descriptions are given.

Key Words: Catfish, *Platyclarias machadoi*, osteology, myology

INTRODUCTION

The freshwater clariids are one of the 37 catfish taxa in the Siluriformes (SABAJ et al., 2004). Although they occur in Syria, southern Turkey and large parts of Southeast Asia, their diversity is the largest in Africa (TEUGELS, 1996). This African richness is demonstrated by the presence of 12 genera with up to 74 species (TEUGELS, 1996). Clariid catfishes are characterized by an elongate body, the presence of four barbels, long dorsal and anal fins, and especially by the autapomorphic presence of a suprabranchial organ, formed by arborescent structures from the second and fourth gill arches (GREENWOOD, 1961; TEUGELS and ADRIAENS, 2003).

Unique for these clariids is the presence of an evolutionary range between fusiform and anguilliform representatives, with *Heterobranchus* at one end and *Dolichallabes* at the other end (PELLEGRIN, 1927), and with *Platyclarias* as one of the intermediate morphs. Although this trend towards anguilliformity has been observed in other taxa of teleosts, and even amphibians and sauropsidans (LANDE, 1978), it is never as extreme as within the Clariidae. Together with this tendency, a whole set of morphological changes have been observed, such as decrease and loss of the adipose fin, continuous unpaired fins, reduction of paired fins, reduction of the eyes, reduction of the skull bones and hypertrophied jaw muscles (DEVAERE et al., 2001 (**IV.5.1.a**)). Another characteristic in clariids is a dorso-ventrally flattened skull. Even though all clariid representatives have a flattened head, it is quite extreme in *Platyclarias machadoi* (still not as extreme as in for example the Asian and non-clariid catfish species *Chaca chaca* (HAMILTON, 1822)) (TILAK, 1971). Such extensive flattening of the skull imposes substantial changes in those physical parameters that are crucial for the head kinematics during feeding and respiration. Trade offs can especially be suspected at the level of the suprabranchial organ and the hypertrophied jaw muscles, which will undoubtedly have an impact on the functioning of the respiratory and feeding apparatuses.

As with all other economically less important representatives of the clariid taxa, the morphology of *Platyclarias machadoi* is poorly understood. This also accounts for the taxonomy and systematics. POLL (1977) only provides some characteristic, external morphological features, as well as some gross osteological characters. A detailed morphological study is thus required before any inferences can be made with respect to spatial constraints. As a result, this study focuses on morphological (gross morphology and histology) and biometrical data (metric and meristic) of the cranial bones and muscles.

This data serves the aims of this paper to identify possible spatial constraints and consequent functional implications of an extremely dorsoventrally flattened skull, which

may interfere with respiration and feeding. The observations in *P. machadoi* are compared with that of representative species of non-anguilliform and anguilliform clariids, where an evaluation of species-specific traits for *P. machadoi* is subsequently done. The species used for comparison are *Clarias gariepinus* (Burchell, 1822), as a non-anguilliform morph, *Gymnallabes typus* Günther, 1867 as an anguilliform morph, and *Platyallabes tihoni* (Poll, 1944) as an intermediate morph.

MATERIALS AND METHODS

The material examined in this study was obtained from the Royal Museum for Central Africa (MRAC) (Tervuren, Belgium). These specimens include the holotype and the 21 paratypes of *Platyclarias machadoi* (MRAC 13307). The specimens were all collected in Upper Cuango, Cafunfo, Borio River, Angola. One specimen (MRAC 78-6-P-1348-364 (paratype 14), SL: 123mm) was cleared and stained following the procedure of TAYLOR and VAN DYKE (1985), for osteological examination. One specimen (MRAC 78-6-P-1348-364 (paratype 16), SL: 113mm) was used to study the external morphology as well as the myology by means of dissection and muscle fibre staining (BOCK and SHEAR, 1972). Furthermore one specimen (MRAC 78-6-P-1348-364 (paratype 17), SL: 119mm) was used for serial sectioning after embedding in Technovit 7100 (Kulzer). The obtained 5 μm thick sections were stained with Toluidin Blue (ADRIAENS, 1998). Sections were studied using a Leitz Diaplan light microscope. This helped to better visualize the attachment areas of the muscles, the contact area of the bones and the angles between the different suspensorial bones, adductor arcus palatine and the neurocranium. Because of the assumed homology of the bones with those of *Clarias gariepinus,* for the terminology and the nomenclature we refer to ADRIAENS and VERRAES (1997a, b, 1998), for pelvic girdle we use ARRATIA (2003), whereas that of the cranial myology follows (WINTERBOTTOM, 1974; ADRIAENS and VERRAES, 1996; ADRIAENS and VERRAES, 1997c, d). Drawings of both cleared and dissected material were made using a stereoscopic microscope (Wild M5) with a camera lucida. We compared this material with that of seven other clariid species, as well as one heteropneustid species (which is presumed to be the sistergroup of clariids) (TEUGELS and ADRIAENS, 2003): *Clarias gariepinus* (TEUGELS, 1986)*, Heterobranchus longifilis* Valenciennes, 1840 (DAVID, 1935) and *Heteropneustes fossilis* (Bloch, 1794) (DAVID, 1935) as representatives of the fusiform genera; *Gymnallabes typus* (CABUY et al., 1999)*, Channallabes apus* (Günther, 1873) (DEVAERE et al., 2001 (**IV.5.1.a**)) and *Dolichallabes microphthalmus* Poll, 1942b (DEVAERE et al., 2004 (**IV.4**)) as anguilliform representatives and *Platyallabes tihoni* as a more

intermediate representative. Between brackets are the references from which the measurements were obtained. Other data were obtained in this study.

Furthermore, 38 measurements were taken point-to-point using digital callipers to 0.1 mm (Digital ruler, Mauser), interfaced directly with a computer, on a set of 22 specimens of *Platyclarias machadoi*. Measurements terminology follows that of DEVAERE et al. (2004) (**IV.4**): total length (TL); standard length (SL); preanal length (PaL); anal fin length (AFL); dorsal fin length (DFL); prepelvic length (PPvL); prepectoral length (PPcL); predorsal length (PdL); distance between the occipital process and the dorsal fin (SPDFL); pelvic fin length (PvFL), pectoral fin length (PcFL); pectoral spine length (PcSL); caudal peduncle depth (CPD); body depth at anus (ABD); maxillary barbel length (MxB); external mandibular barbel length (EmnB); internal mandibular barbel length (ImnB); nasal barbel length (NB); interpelvic distance (IpvD); interpectoral distance (IpcD); skull length (SkL); preorbital length (PoL); skull width (SkW); supraoccipital process length (SpL); supraoccipital process width (SpW); interorbital distance (IoD); anterior nostril interdistance (ANID); posterior nostril interdistance (PNID); rostral skull width (RSkW); orbital skull width (OskW); skull height (SkH); eye diameter (ED); snout height (SnH); prehyoid length (PhL); internal mandibular interdistance (ImnID); external mandibular interdistance (EmnID); mouth width (MW) and skull roof width (SkR). The morphology of the vertebrae and the following meristic counts were made on each specimen using the radiographies made with a MPG 65 generator and a RSN 620 X-ray-tube (General Electric) (42kV, 320Ma, 10msec, focus distance: 1m): total number of vertebrae (TV), number of ribs (RB).

COMPARATIVE MATERIAL EXAMINED

Museum abbreviations are listed in LEVITON et al. (1985).

Platyallabes tihoni. Dem. Rep. Congo. Kingabwa, Stanley pool, MRAC 13307 (Holotype); Kinsuka, MRAC 73-68-P-143, MRAC 138698-699 (n=2), 125345-349 (n=4), MRAC 73-22-P-3127 (n=3); Bulu, Luozi, BMNH 1976.5.21.30-39 (n=9), MCZ 50239 (n=13); Inga, MCZ 88947, MCZ 50537 (n=15); Tadi, Kibunzi, MCZ 50297 (n=5).

Channallabes apus. Angola. Ambriz, BMNH 1873.7.28.16 (Holotype); Other specimens, Dem. Rep. Congo. Bokalakala, MRAC 175247-270 (n=10); Kinshasa, MRAC 97-056-P-0001-0003 (n=2); Bumba, MRAC 88-25-P-2192-227 (n=36); Boma, MRAC 939; Riv. Lula, Bushimaie, MRAC 153505; Kelé, MRAC 1491; Stanleyville, MRAC 30893-30900 (n=8), MRAC 88-01-P-1976-1992 (n=17); Riv. Ruki, Eala, MRAC 14747-49 (n=3); Lake Tumba swamp area, MRAC 46299; Katanga, MRAC 39480; Riv Botota, keseki, MRAC 67763-77 (n=15); Mwilambongo, MRAC 72886-887 (n=2); Dekese, Riv. Lofu, Anga, MRAC 153352; Yangambi, MRAC 68700;

Riv. Oubangui, Imfondo, MNHN 1922-0029; Loango, MNHN 1924-0079, MNHN 1924-0080; Sangha, MNHN 1925-0137; Mogende, MNHN 1926-0155-59 (n=5); Riv Congo, MNHN, 1937-0124-25; Stanleypool, Bamu, MNHN 1958-0111; Boloko, Riv. Likouala, MNHN 1962-0401 (n=7); Mossaka, Riv. Likouala, MNHN 1963-0402 (n=2); Riv. Loadjili, Songolo, MNHN 1967-0143 (n=6); Mangala, BMNH 1896.3.9.17; Riv. Lebuzi, Kaka Muno, BMNH 1912.4.1411-12 (n=2); Lower Congo, BMNH 1887.1.13.8-9 (n=2); Stanley Falls, BMNH 1889.11.20.5; New Antwerp, Upper Congo, BMNH 1899.2.20.16; Siala-Ntoto Swamps, BMNH 99.11.27.92; Bangyville, Ubangi, BMNH 1907.12.26.34; Kashi, Lulua, MHNG 1248.3; Banana, NMW 47240-42; Mollunda, NMW 47245 (n=4), NMW 47246. **Congo.** Yangala Youbi, MNHN 1967-0146; Djembo, Kouilou, MNHN 1967-0147; Cayo, MNHN 1989-0527; Riv. Nanga, between Boukou-Zassi and Kouilou swamp area, MRAC 90-57-P2315; Sintou, Riv. Kibombo, Kouilou, MNHN 1967-0144; Riv. Loadjili, Songolo, Pointe Noire, MNHN 1967-0145 (n=6); Riv. Youbi, Noumbi. **Angola.** Caungula, Mabete, Riv. Uamba, MRAC 162088; Riv. Camuconda, Tchimenji, MRAC 162089, MRAC 162090-094 (n=5), MRAC 162095-100 (n=6); Riv Ganga-Ludchimo, MRAC162083-086 (n=4).

Gymnallabes typus. **Nigeria.** Old Calabar, BMNH 1866.12.4 (n=2) (Syntypes); Other specimens, Nigeria. Umu-Eze Amambra, MRAC 84-16-P-1-2; Riv. Sombreiro, East of Erema, MRAC 91-067-P0134; Niger Delta, MRAC 97-030-P-0001-0010 (n=10); lake Odediginni, Agudama, Yenagoa, MRAC 92-083-P-0035-0036; Okaka, Epie Creek, Between Nun an Rashi Riv, MRAC 97-085-P-0001-0004 (n=4); Riv Sombreiro, Odiemerenyi, Ahoada, MRAC 91-067-P-0135-0136; New Calabar, Choba, MRAC 91-105-P-1; Rumuji Swamps, MRAC 86-10-P-72; Oshika, MRAC 84-28-P-28, MRAC 84-28-P-25; Riv. Cron, Itu, MRAC 88-36-P-10; Between Sapele and War, Niger Delta, MRAC 74-29-P-600; Muoha, New Calabar, MRAC 91-10-P-478; Biseni, taylor Creek, MRAC 91-01-P278; Ossomari, BMNH 1902.11.10.119. **Cameroun.** Riv. Kom, Ntem, Aboulou, MRAC 73-18-P-3307-309.

Dolichallabes microphthalmus. **Dem. Rep. Congo.** Kunungu, MRAC 44655, adult male, 229 mm SL (holotype), MRAC 44656-659 (n=3) (196-210 mm SL) and 62407, 188 mm SL (paratypes), MRAC 57662, 196 mm SL, MRAC 18850, 90 mm SL; Boende swamps, MRAC 101843, 149 mm SL, MRAC 176123-124 (n=1), 68 mm SL; Bokuma, MRAC 79093, 134 mm SL, MRAC 93774, 66 mm SL; Bokuma – Tchuapa, MRAC 79258-260 (n=3) (85-126 mm SL); Ndwa (Boloko), MRAC 78808-810 (n=3) (99-110 mm SL); Inonge, MRAC 96672, 110 mm SL; Maylimbe, Tshela, MRAC 66721, 97 mm SL.

Clarias gariepinus. Artificially cultivated specimens (n=10), Ghent University.

Heterobranchus longifilis. **Egypt.** Nile, AMNH 3054 SW.

Heteropneustes fossilis. Aquarium specimen, AMNH 172276 SW.

RESULTS

External morphology

Platyclarias machadoi has a dorso-ventrally flattened body. The body is elongated, ovoid behind the head and laterally compressed caudally. The preserved specimens of *Platyclarias machadoi* show an even somewhat dark-brownish colour, with a slightly lighter ventral side (**Plate IV.2-1A**).

Even more striking than the flattening of the body, is the extremely dorso-ventrally compressed head. Although the jaw muscles are clearly visible, they are not bulging. The triangular skull roof is the only bony part of the skull that is distinctly visible without removing the skin. On top of the skull roof there is a well-defined, lightly-coloured stain (otherwise only seen in *Gymnallabes alvarezi*, IV.5.2), although not always sharply delimited. The eyes are always clearly visible in all specimens.

When removing the skin on the head, many hidden fat particles emerge. The lower lip slightly extends beyond the upper lip.

The unpaired fins (dorsal, anal and caudal) are not fused. The dorsal fin originates far behind the supraoccipital process of the parieto-supraoccipital bone. Also the anal fin originates far behind the head (**Plate IV.2-1A**). The paired fins are always present. The large pectoral fins are preceded by a large, non-serrated pectoral spine. Also the pelvic fins are distinctly present.

Cranial skeleton

Neurocranium

In general, the skull of *Platyclarias machadoi* is reduced. The skull roof shows a limited constriction in the orbito-ethmoid region. The overall skull has a wide appearance and this is mainly due to the horizontal position of the suspensorium (**Plate IV.2-1B**).

Ethmoid region: the nasal bone bears a distinct expansion on the lateral side and a small plate on the medial side. The nasal bone covers the nasal sac and encloses the rostral end of the supraorbital canal of the lateral line system, which splits up in this bone. The nasal bone is medially enclosed by the mesethmoid. It is attached to all surrounding bones by connective tissue, allowing the nasal cavity to expand.

The solid mesethmoid shows a moderate constriction behind the two firm, rostral wings. It interdigitates caudally with the frontals and laterally makes contact with the lateroethmoid. The supraorbital canal runs between the latero- and mesethmoid and forms rostrally a clear superficial separation of these two bones. The mesethmoid forms the short anterior border of the anterior fontanel. The lateroethmoid shows a distinct,

laterally pointed process. Although there is no direct contact between the lateroethmoid and the infraorbital II, this latter bone shows a limited articulation ridge and there is a strong connective tissue link between the two. Ventro-laterally, the lateroethmoid shows a clear articulatory facet for the articulation with the rod-shaped autopalatine. Ventrally, the premaxillaries are supported by the mesethmoid. The arrow-shaped prevomer adjoins the mesethmoid and interdigitates caudally with the parasphenoid through a variable number of pointed spines (one to three). The prevomer carries one continuous large tooth plate (**Plate IV.2-2B**).

Orbital region: the largest bones of the neurocranium are the contra-lateral frontals. Rostrally, they interdigitate with the latero- and mesethmoid. On the caudal side, each frontal makes contact with the sphenotic, pterotic and the parieto-supraoccipital. The two frontals broaden somewhat caudally. Both halves are connected to each other, except on their rostral side, where they enclose the anterior fontanel. Ventrally, the frontals show two lateral flanges, expanding clearly beyond the orbitosphenoid outlines. The anastomosis of the supraorbital and infraorbital canals of the lateral line system is situated at the boundary of the frontals and the sphenotics.

The lateral walls of the neurocranium are formed by the orbitosphenoid and the pterosphenoid, which both connect to the ventrally situated parasphenoid. This bone reaches backward and upward to the temporal region, covering a major part of the skull floor. It bears one elongated and several short processes, reaching occipital region (**Plate IV.2-2B**).

Lateral of the skull roof lie the circumorbital series, comprising four infraorbitals and the antorbital bone. Except for infraorbital IV and to some extent infraorbital III, all other circumorbital bones are tubular. The small antorbital bone is the most rostrally situated bone in the series, lying at the rostral tip of the autopalatine, close to the base of the nasal barbel. The tubular lacrimal lies at the level of the olfactory organ. The infraorbitals II to IV surround the eye. The fourth infraorbital is the largest and bears a supraorbital outgrowth bordering the eye postero-dorsally. It is situated laterally against the frontals, from which it is rather widely separated by connective tissue.

Temporal region: The sphenotic of *Platyclarias machadoi* interdigitates rostro-medially with the frontals and caudally with the pterotic. The latter interdigitates rostrally over a small length with the frontal, medially to the parieto-supraoccipital and caudally to the posttemporo-supracleithrum. Both sphenotic and pterotic bones take a more or less horizontal position, with some downward curving, especially at the level of the sphenotic. Both bones form the only connection between the neurocranium and the suspensorium, by means of a set of processes and an articulatory ridge. Anterolaterally on the sphenotic and

posterolaterally on the pterotic one large process can be distinguished. Between these two processes a distinct cartilaginous ridge, articulating with the hyomandibula, can be found on both bones. Ventrally, this region is covered by the paired prootic.

Occipital region: The parieto-supraoccipital complex is situated in the medio-caudal part of the skull roof, rostrally enclosed by the frontals and medially by the pterotic bones. This bone is characterised by a large, somewhat pointed process on the caudal edge. The parieto-supraoccipital encloses the posterior fontanel, which lies in the caudal part of this bone. The separation between the two parietal halves is still partially visible on one specimen (MRAC 78-6-P-1348-364, paratype 14), from the medio-rostral border to the posterior fontanel. The posttemporo-supracleithrum is rostrally attached to the pterotic and connects caudo-ventrally to the pectoral girdle. Furthermore, *Platyclarias machadoi* shows two clear epioccipitals. Ventrally, the unpaired basioccipital and a paired exoccipital border this region.

Splanchnocranium

Maxillary bones: The premaxillaries are plate-shaped bones, ventrally supporting the nasal sac. Almost the complete ventral surface of this bone is covered with posteriorly directed teeth. There is only a limited caudal outgrowth of the plate. The maxillaries form a cup-like bone. They enclose the bases of the maxillary barbels and each bear two articulatory facets for the articulation with the autopalatine.

Mandibula: The long lower jaw consists of two parts: the os dento-splenio-mentomeckelium and the os angulo-articulo-retroarticulare (**Plate IV.2-2C,D**). The coronoid process, which is situated at the interdigitation between the two above mentioned mandibular bone complexes, is distinct. The tooth battery runs close up to the coronoid process. The retroarticular process is highly asymmetrical and situated completely medial from the quadrate. Caudally of the mandibular bone complex up to two small, tubular splenials can be discerned. The lower jaw complex embeds the rostral end of the preoperculo-mandibular canal of the lateral line system and shows five pores for branches of this canal. The first three pores lie in the os dento-splenio-mentomeckelium, the fourth is situated on the border of both bone complexes, while the fifth one is located on the caudal end of the lower jaw.

Palatine: The rod-like autopalatine has two cartilaginous ends. Anteriorly, it articulates with the maxillary part. Furthermore, the palatine lies ventrolaterally from the lateroethmoid and articulates with it through a central, elliptic articulation facet.

Suspensorium: As in most Siluriformes, the suspensorium consists of the hyomandibula, quadrate, entopterygoid, metapterygoid and the preopercle (**Plates IV.2-1B, IV.2-2C,D**).

In *Platyclarias machadoi*, the suspensorium takes a remarkable horizontal position. The hyomandibula connects the suspensorium to the neurocranium, through a set of interdigitating processes. At the level of the sphenotic, this connection occurs through one long, pointed process. Also at the level of the pterotic, only one process is present. In between these processes lies the articulation ridge, close to the border of the sphenotic and pterotic bones. At the anterior margin of the hyomandibula, no distinct bulgy outgrowth can be discerned. On both sides, the hyomandibula bears a ridge; on the medial side, for the attachment of the ligamentum hyomandibula-ceratohyale, on the lateral side for the insertion of several muscles (levator arcus palatini, A_3'' part of the adductor mandibular complex and the retractor tentaculi). The opercular process of the hyomandibula is caudo-ventrally orientated. The dorsal edge of the hyomandibula and the quadrate shows almost a straight line, with little indentation. Rostrally, the quadrate only makes contact with the metapterygoid and has no direct contact with the entopterygoid, as these two are separated by the metapterygoid. At the ventro-rostral side of the quadrate a broad, flat slightly rounded surfaced articulatory facet, for the articulation with the mandibula, is present. The metapterygoid connects caudally to the quadrate through a synchondrosis, and an interdigitation zone and is rostrodorsally enclosed by the larger entopterygoid. Anteriorly, the entopterygoid is connected ligamentously to the prevomer, the palatine and the lateral ethmoid, thus corresponding to a sesamoid 'entopterygoid type 4' (ARRATIA, 1992).

Hyoid arches: the hyoid arch consists of two ceratohyals (anterior and posterior) and two hypohyals (ventral and dorsal) (**Plate IV.2-2A**). Ventrocaudally, the hyoid arch articulates with eight (up to nine) branchiostegal rays. The parurohyal lies in between the two hyoid arches and bears two caudo-lateral processes and one large, broad, forked, caudal process (**Plate IV.2-2A**). The parurohyal is connected to the ventral hypohyal by means of two separate paruro-hypohyal ligaments (**Plate IV.2-5C**).

Branchial arches: The branchial morphology resembles that of *Clarias gariepinus* (ADRIAENS and VERRAES, 1998). One exception is the low number of gill rakers (up to seven).

Opercular: the main opercular bone is the opercle; this triangular, long, flattened bone shows a large articulatory facet for the hyomandibula on its rostro-dorsal side (**Plate IV.2-1B**). The opercular muscles attach on the distinct ridge on the medial side of the posterior part. The opercle and the interopercle are ligamentously connected to each other (**Plate IV.2-2B**). The interopercle, on its turn is also ligamentously attached to the lower jaw. The interopercle is a long, flat bone, situated medially of the suspensorium. As already mentioned, the preopercle is incorporated into the suspensorium. This bone surrounds a part of the preoperculo-mandibular canal. The caudal part of this canal is guided by up to

two suprapreopercular bones. The most proximal suprapreopercular bone has a plate-like expansion, while the second bone, when present, is tubular (**Plate IV.2-1B**).

Cranial myology

Muscles of the lower jaw (**Plate IV.2-3**)

M. adductor mandibulae: This complex forms a large jaw-closing muscle, covering a large part of the laterodorsal side of the head in *Platyclarias machadoi*. It consists, as in other clariids, of an external A_2A_3'-part and an internal A_3", which are separated by the levator arcus palatini (ADRIAENS and VERRAES, 1996).

The bipennate A_2A_3' forms the biggest part of the complex (**Plate IV.2-3A,B,C**). This part covers the A_3'' completely, as well as the levator arcus palatini and the adductor and dilatator operculi. It can be divided in a dorsal A_2A_3'α-part and a ventral A_2A_3'ß-part. Both these parts attach to the lower jaw through an aponeurosis, which inserts on the angular complex, close to the coronoid process. The A_2A_3'α-part originates at the frontal, the sphenotic, the pterotic, the suprapreopercular series, and even the posttemporo-supracleithrum. This part is dorsorostrally covered by the fourth infraorbital. The more ventrally situated A_2A_3'ß connects the lower jaw to the suspensorium. More specifically it originates from the angular-complex and inserts on the hyomandibula, the quadrate and the preopercle. The muscle fiber direction of the A_2A_3' has a range of almost 160°. Medially, a tendon originates caudally at the levator arcus palatini and has its insertion on the most anteriorly situated tendon of this muscle (**Plate IV.2-4**)

The deeper part of the adductor mandibulae, the A_3", is connected to the neurocranium and the suspensorium as well. More specific, the horizontally situated muscles fibers originate both directly and through a tendon at the angular-complex and attach to the medial side of the suspensorium, on the hyomandibula and the rostrodorsal rim of the quadrate and furthermore to the frontal, the sphenotic and the pterosphenoid. The A_3" lies medially from the levator arcus palatini and laterally of the retractor tentaculi (**Plate IV.2-3c**). In between this A_3" and the more laterally situated levator arcus palatini runs an extra muscle. This muscle is situated close to the anterior border of the levator arcus palatini, where it originates on the lateral ethmoid and the frontal and inserts on the large tendon complex of the jaw muscle and more posterior on a medial tendon of the A_2A_3'. Until now, such a separate muscle could not be observed in other clariids.

M. intermandibularis: This compact muscle covers the mandibular symphysis, ventral on the rostral end of the mandibula (**Plate IV.2-5A**). The intermandibular muscle is separated from the protractor hyoidei through the wide interconnecting tissue of the left and right mandibular barbel base.

Suspensorial muscles

M. levator arcus palatini: This thin muscle sheet has a complex morphology, consisting of a muscle part and a complex of aponeuroses (**Plates IV.2-3B,C; IV.2-4**). This muscle is situated between to two parts of the adductor mandibulae complex and runs medially from the eye. There is a predominantly dorsoventral fiber direction, with more horizontal fibers anteriorly. This muscle originates on the caudolateral side of the lateral ethmoid, the ventrolateral side of the frontal back to the sphenotic and inserts at the dorsal side of the corpus of the hyomandibula and the quadrate. Although this muscle originates for a large part directly on the neurocranium, the attachment at the sphenotic is through an aponeurosis.

M. adductor arcus palatini: The adductor arcus palatini connects the skull floor and the mediodorsal rim of the suspensorium. This muscle takes the most medial position of all cranial muscles, lining the mouth cavity dorsolaterally. On the neurocranium, it originates mostly at the parasphenoid, but also at the orbitosphenoid and the pterosphenoid. On the suspensorium, the adductor arcus palatini inserts on the dorsal rim of the hyomandibula, the quadrate, the metapterygoid and the entopterygoid. The fibers run in a transversal plane, with the rostral fibers more obliquely orientated (**Plate IV.2-3E,F**).

Opercular muscles

Of the three opercular muscles that were discerned, the dilatator operculi is the one most rostrally situated, partially covering the adductor operculi. Caudally lies the levator operculi.

M. dilatator operculi (**Plate IV.2-3C**): The flattened dilatator operculi originates from halfway up the ventrolateral side of the frontal, medial to the levator arcus palatini, up to the ventrolateral part of the sphenotic (**Plate IV.2-3C**). It inserts through a large tendon on the lateral side of the dorsal process of the opercle. This long tendon runs along the rostroventral side of the muscle.

M. adductor operculi (**Plate IV.2-3D,E**): this shortest muscle of the three opercular muscles connects the posterior side of the suspensorium with the opercle. The adductor operculi originates at the dorsocaudal part of the hyomandibula and the most ventrocaudal part of the pterotic and inserts at the dorsal process of the opercle, medial to the insertion of the dilatator operculi, but still lateral to the processus opercularis. The caudal part abuts the posteriorly situated levator operculi. It is also attached to the connective tissue covering the dorso-lateral side of the deformed suprabranchial cavity. The fiber direction is predominantly dorso-ventral.

M. levator operculi (**Plate IV.2-D,E**): The levator operculi is the most caudally situated and robust of the three opercle muscle. It connects the most posterior part of the neurocranium with the opercle, originating at the ventro-caudal part of the pterotic and the ventrorostral side of the posttemporo-supracleithrum, the suprapreopercular bones and the connective tissue covering the suprabranchial cavity. Ventrally, the levator operculi inserts on almost the complete dorsal side of a ridge of the opercular bone.

Maxillary barbel muscles

M. retractor tentaculi (**Plate IV.2-3E**): the retractor is a large bundle of fibers running from the suspensorium to the maxillary bone. It originates on the rostral side of the hyomandibula and the quadrate. The fibers are all inserted on the centrally situated tendon, which runs from the postero-dorsal side of the maxillary bone up to halfway into the muscle. The fibers follow an oblique rostro-caudal direction, dorsally and ventrally of the tendon in a way that a feathered appearance results. The muscle lies medially of the A_3" and laterally of the adductor arcus palatini and extensor tentaculi. Furthermore, the retractor lies laterally of the autopalatine. On the cross sections two bundles are distinguished.

M. extensor tentaculi (**Plate IV.2-3E**): This muscle is the antagonist of the above mentioned muscle, abducting the maxillary barbel. The origin of the extensor tentaculi covers the ventral and ventrolateral side of the lateroethmoid, the rostroventral side of the frontal and the lateral side of the orbitosphenoid and runs to the autopalatine. Several, differently oriented, bundles of fibers attach to the upper half of the autopalatine, caudally of the articulatory facet. They consequently enclose the caudal end of the autopalatine.

Hyoid muscles

M. protractor hyoidei (**Plate IV.2-5A,B**): The protractor hyoidei connects the lower jaw with both hyoid bars. It can be divided into a larger ventral and smaller dorsal part. They both insert on the ventral side of the anterior ceratohyal. The ventral part originates at the whole length of the ventral side of the lower jaw and at the bases of the mandibular barbels. The ventral part is rather U-shaped (**Plate IV.2-5A**); left and right half are attached to each other by a fascia at the level of the mandibular symphysis. Several fields of fibres can be distinguished interconnecting different parts of the bases of the mandibular barbels. There is a large gap between the rostral part of this muscle and the posteriorly lying hyohyoideus inferior. A smaller gap is discerned between the mediorostral part of the protractor hyoidei and the intermandibularis. The dorsal part originates at the

medial side of both mandibulae (**Plate IV.2-5B**). This part can be further divided into two subunits, a medial and a lateral one. The lateral subunit originates tendinously at the lateroventral side of the mandibula, while the larger medial subunit originates caudomedially on the mandibula, through aponeuroses and not contacting each other but running up to the intermandibularis. Both parts insert on the ventrolateral side of the anterior ceratohyal. The protractor hyoidei pars dorsalis lies completely laterally of the hyohyoidei inferiores.

M. hyohyoideus inferior (**Plate IV.2-5B**): This massive muscle covers the ventral side of the contralateral anterior ceratohyal and parts of both ventral hypohyals. The muscle inserts on the ventral side of the anterior ceratohyal, medial from the insertions of the protractor hyoidei, and at the ventral hypohyals. Caudally, it is also attached to the bases of the branchiostegal rays. The contralateral fibers originate from a fascia.

Mm. hyohyoidei adductores (**Plate IV.2-5C**): The hyohyoidei adductores muscles form a series of sheets between successive branchiostegal rays, starting from the first one and ending onto the medial side of the opercular bone.

M. hyohyoideus abductor (**Plate IV.2-5C**): The hyohyoideus abductor links the rostral tip of the hyoid bars with the first contralateral branchiostegal rays. The muscles originate tendinously onto the ventral hypohyal of the opposite side and insert on the medial face of this branchiostegal ray.

M. sternohyoideus (**Plate IV.2-5D**): This muscle forms a broad fiber mass, connecting the pectoral girdle to the hyoid bars through the parurohyal. Posteriorly, the sternohyoideus originates at the cleithrum. On the rostral side, both muscle-heads insert into the double forked parurohyal. No myocommata could be observed.

Postcranial skeleton

The total number of vertebrae in the studied specimens of *Platyclarias machadoi* (80.5-181mm SL) ranges from 65 up to 71 (mode: 70). The number of precaudal vertebrae varies from 19 to 22 (mode: 20) and the number of caudal vertebrae from 44 to 50 (mode: 50). *Platyclarias machadoi* has 9 up to 11 pairs of ribs.

The pectoral fins of *Platyclarias machadoi* have 11 branched fin rays that articulate with two proximal radials. The fins are preceded by non-serrated spines. The pectoral girdle shows a robust bond between the scapulo–scapulo-coracoid and the cleithrum bones, with one small, lateral fenestra. Furthermore, the cleithrum shows an anterior process (**Plate IV.2-1B**). The pelvic fins each carry six, branched fin rays, which articulate with the broad and flat basipterygium of the pelvic girdle. Rostrally, an internal and external anterior process can be distinguished (**Plate IV.2-6A**). The dorsal and anal fins are clearly

separated from the caudal fin. The number of dorsal and anal fin rays varies respectively around 77-87 and 69-80.

The morphology of the caudal endoskeleton of *Platyclarias machadoi* shows some intraspecific variation but always consists of the parhypural, five hypuralia, an epurale and an urostyl. Different fusion patterns can be discerned; even though POLL (1977) previously stated that no fusions within the hypurals are present, and only the fifth hypural and the urostyl show a fusion. However, our observations show that hypural four and five are fused, with or without a fusion of the urostyl. Dorsally of the hypurals and the urostyl lies the broadly tipped epural. Only the haemal spine of the second preural vertebra is unpaired, elongated and broadly tipped. The remainder (on the neural side of the second and third preural vertebra and on the haemal side of the third preural vertebra) shows slender elongate paired neural and haemal spines. No pterygiophores are observed. **Plate IV.2-6B** shows an additional fusion of the third and fourth preural vertebrae.

DISCUSSION

Spatial constraints and functional implications of the low skull

Platyclarias machadoi has an extremely flattened skull (average skull height of 27.5% ± 3.0% skull length), compared to that of other clariids (*Platyallabes tihoni*: 34.6% ± 6.9%, *Gymnallabes typus*: 40% ± 5.8%) (**Table IV.2-1**). This can be expected to impose spatial constraints on the surrounding structures, in all three dimensions of the 'construction' framework (BAREL, 1984).

One striking similarity with *Platyallabes tihoni* is the medial position of the retroarticular process on the lower jaw, presumably the simple consequence of the topographic relation between lower jaw, the plane of mandibular movements and the fact that the suspensorium is extremely tilted, up to an almost horizontal position. Another resemblance is the caudal orientation of the opercular process on the hyomandibula (instead of caudoventrally), giving the opercle a more posterior position with respect to the suspensorium. Also the dorso-ventrally compressed suspensorium is a shared characteristic (DEVAERE et al., in press (**IV.1**)).

The most impressive spatial reorganisation of the skull elements in *Platyclarias machadoi*, however, is the almost completely horizontal position of the suspensorium. Only *Platyallabes tihoni* shows a comparable horizontal position, as can be shown when superimposing the cross sections of the neurocranium and suspensorium at the level their articulation (**Plate IV.2-6C**). It could be argued that these sections only represent one phase in the dynamic system of the suspensorium, during abduction or adduction.

However, as the axis through the articulatory facet at the neurocranium lies in the line with the axis through the hyomandibula, this gives us a good indication of the position of the suspensorium at rest. Apparently, in the species studied, three levels of tilting can be observed: ± 30° in *Clarias gariepinus* (angle of axis through suspensorium with respect to mediosagittal plane), ±40°- 46° in *Clariallabes longicauda* and *Gymnallabes typus*, and 69°-76° in *Platyallabes tihoni* and *Platyclarias machadoi* (**Plate IV.2-7B** (hyo-med)).

It can be expected that this horizontal tilting will also have a substantial influence on the morphology of the anterior part of the suspensorium. Variation in the morphology and especially orientation of the plate-like entopterygoid, metapterygoid and the anterior part of the quadrate can be observed. As judged from the histological sections, it becomes clear that the entopterygoid plate has a comparable horizontal orientation in *Clarias gariepinus, Gymnallabes typus, Platyallabes tihoni* and *Platyclarias machadoi*, with respect to the mediosagittal plane (**Plate IV.2-8**, entopterygoid). However, compared to the orientation of the hyomandibula (at the level of the articulation with the neurocranium), some differences become clear too (**Plate IV.2-7C**). In *Clarias gariepinus* the anterior part of the suspensorium shows an altered orientation with its dorsal margin being rotated medially (negative values in **Plate IV.2-7C** (ento-susp)). In *Platyclarias machadoi* and *Platyallabes tihoni* on the other hand, the anterior part shows a slightly comparable, lateral shift in orientation (positive values) instead. For *Gymnallabes typus*, there is a small medial change in orientation visible.

Different angles at different levels of the suspensorium (with respect to the mediosagittal plane) can be expected to have different influences on the adduction forces exerted by the adductor arcus palatini onto the suspensorium. As this muscle inserts almost onto the complete dorsal margin of the suspensorium, and as the transverse axis through the suspensorium at all levels changes (**Plate IV.2-7C**), the orientation of the force exerted onto this bony rim with respect to this transverse axis will change as well. In other words, the direction of the contraction force may not always be in line with the long axis through the bony plates and torsion and shear forces may thus be generated. Since the anterior part of the suspensorium comprises plate-like, thin bones, such shear forces are a potential danger for fracturing the bones. The histological sections confirm that indeed the line of action of the adductor arcus palatini is more in line with the transverse long axis through the suspensorial bones that are thin (entopterygoid, metapterygoid, anterior part of quadrate) but less in-line with that of the posterior, thick bones (posterior part of quadrate and hyomandibula) (**Plates IV.2-6C, IV.2-7D**). Fracturing of the suspensorium during ad- and abduction is thus avoided.

In a general situation, suspensorial abductions will generate an increase of the orobranchial cavity. However, in a dorsoventrally flattened skull with an almost horizontally situated suspensorium, the opposite may actually occur.

In a more vertically situated suspensorium, the adduction of this suspensorium results in a decrease of the branchial cavity volume. Since a more vertical suspensorium enlarges the adduction component of the adductor arcus palatini (large angle between suspensorium and line of action of adductor arcus palatini) (**Plate IV.2-7E**). This implies a less efficient adduction in both *Platyclarias machadoi* and *Platyallabes tihoni*, which could be partially overruled by a larger depression of the hyoid bars. Since, in the dorsoventrally flattened head of *Platyclarias machadoi* and *Platyallabes tihoni* there is a relatively large cranial floor, as is the case in all platybasic benthic catfishes (ADRIAENS and VERRAES, 1997b); a depression of these bars will result in a substantial volume increase in these two species (for a certain overall skull size).

Apart from these conformities with *Platyallabes tihoni*, *Platyclarias machadoi* does show some differences. Surprisingly, these differences involve conditions, which at first sight are essential to maintain a well functioning feeding and respiration mechanism. One difference is the less hypertrophied jaw muscle in *Platyclarias machadoi*, although a hypertrophied jaw muscle complex can be expected as the suspensorium takes a more or less horizontal position (see above). This horizontal tilting can be seen as a disadvantage to fit in the large adductor mandibulae complex, unless the large muscles would be allowed bulge. This bulging would allow jaw muscles with a similar physiological cross section and insertion sites as in other clariid representatives, as is the case in *Platyallabes tihoni* (DEVAERE et al., in press (**IV.1**)). In *Platyclarias machadoi* however, the jaw muscles are not bulging, although the same insertion sites are present, thus resulting in a smaller muscle (**Plate IV.2-3A,B**). Furthermore, the histological sections show no substantial medial increase of the muscle volume. However, even though smaller jaw muscles would suggest reduced biting forces, the spatial relationships need to be known first, before any conclusion can be drawn (HERREL et al., 2002). Herein lies a possible functional explanation for the unique extra muscle bundle, laterally from the A_3''-part and medially from the rostral part of the levator arcus palatine. This muscle links the neurocranium to the tendon complex of the mandibular muscle complex and caudally to a medial tendon of the A_2A_3'. This would mean that during contraction a lifting of the mandibular muscle tendons would occur, thereby increasing its angle of insertion on the lower jaw. Consequently, this would result in a higher moment generated by the adductor mandibulae on the lower jaw. Within a spatially constrained, flattened 'construction' this muscular rearrangement is an advantageous solution to increase biting force, without the need to increase muscle

volume. A comparable situation has been observed in gobiids (not in relation to a dorsoventrally flattened skull, though), where the Aω is assumed to change the position of the A3-tendon, and thus increasing the moment during biting (DECLEYRE et al., 1990). Because of the plausible function of this muscle bundle, the name 'pars levator tendinis' is suggested (**Plate IV.2-4**). The other dissimilarity is that both gill apparatus and suprabranchial organ are well developed in *Platyclarias machadoi*. In contrast, in *Platyallabes tihoni* there is no evidence of the suprabranchial organ. The absence of this organ in *Platyallabes tihoni* could be explained by the decreased branchial cavity (DEVAERE et al., in press (**IV.1**)). Apparently, different strategies with respect to spatial organisation and constraints exist in *Platyclarias machadoi*.

Comparison with other Clariidae

Even though variation exists with respect to relative skull height in clariids, the extreme situation in *Platyclarias machadoi* seems to be unique (see above). Some features can be given that corroborate the distinct nature of this species, and are thus diagnostic for both the genus and the species. Evidence comes from osteological, myological and biometric (both metric and meristic) evidence of the cranial and postcranial system. For a comparison, we used representatives of the three morphotypes, with respect to the degree of body elongation, in clariids: fusiform, intermediate and anguilliform. The following species are used: *Clarias gariepinus* (fusiform morphotype), *Gymnallabes typus* and *Channallabes apus* (anguilliform morphotype) and *Platyallabes tihoni* (intermediate morphotype). The proportional measurements and counts of *Platyclarias machadoi* and some other clariid representatives are given in **Table IV.2-1**.

Comparison with the fusiform morphotype

Platyclarias machadoi shares a list of symplesiomorphies with *Clarias gariepinus*. They show a similar lateral plate on the frontals, the sphenotics and the pterotics. They also share a low number of processes present on the sphenotic (1 process) and pterotic (1 process) for the connection with the hyomandibula. Also on the muscular level some resemblances exist. The ventral part of the protractor hyoidei, has a distinct U-shape, in contrast to the more V-shaped protractor of the anguilliform species. A postcranial similarity shared only with *Clarias gariepinus* is that no continuous fin fold is present.

Comparison with the intermediate morphotype

Platyclarias machadoi and *Platyallabes tihoni* show a reduced skull morphology, with the exception of some canal bones retaining a plate-like extension (for example, the nasal, infraorbital IV and the proximal suprapreopercle).

Also postcranially, the similarity with *Platyallabes tihoni* is reflected in a number of features. The most obvious is the comparable mode of the total number of vertebrae: 70 for *Platyclarias machadoi* and 78 for *Platyallabes tihoni* (n=33). The number in the *Gymnallabes typus, Channallabes apus* and *Dolichallabes microphthalmus* is substantially higher (*Gymnallabes typus* (n=26): 87, *Channallabes apus* (n=98): 104 and *Dolichallabes microphthalmus*: 106 (DEVAERE et al., 2004) (**IV.4**)), while the number in the *Clarias* species is clearly lower (*Clarias gariepinus*: 56-63; *Clarias ngamensis*: 56-60; *Clarias platycephalus*: 59-63 (TEUGELS, 1986)).

Comparison with the anguilliform morphotype

The general skull morphology of *P. machadoi* resembles that of the anguilliform species: there is a narrow skull roof, with partially reduced circumorbital and suprapreopercular bones, which are clearly separated from each other.

In general, the morphology of the cranial musculature of *Platyclarias machadoi* resembles that of *Gymnallabes typus* and *Channallabes apus* and shows the same origin and insertion sites for the different muscles. Besides this, there is also an increase in the range of muscle fiber directions of the jaw muscle, as seen in these anguilliform clariids (DEVAERE et al., in press (**IV.1**)). The two contralateral parts of the pars medialis of the dorsal part of the protractor hyoidei, however, do not make contact, as is the case *Gymnallabes typus* and *Channallabes apus* (CABUY et al., 1999; DEVAERE et al., 2001 (**IV.5.1.a**)).

Some comments on the genus/species description by POLL (1977)

Based on the morphological and biometrical data gathered in this study, the description of POLL (1977) proved to insufficiently demarcate *Platyclarias machadoi* from some other clariids species (of which a substantially larger number of specimens was studied by us). This allowed us to formulate some new diagnostic characters, which will be essential for future systematic studies on clariids. Updated ranges and morphological features are listed below.

The dorsal fin starts far behind the skull, reflected in a large distance measured between the dorsal fin origin and the occipital process on the parieto-supraoccipitale, (**Table IV.2-1**, SPDFL). This distance is 10.1-17.2% of the standard length. The predorsal

length in *Platyclarias machadoi* ranges between 28.2 and 33.5% (%SL) (30.8 ± 1.6), which is highest in all clariids studied (**Table IV.2-1**). Also the anal fin originates far from the snout, the preanal length ranges between 37.9% and 43.9% of the standard length. The pectoral fins are always preceded by a large, non-serrated pectoral spine (24.8-53.1% of pectoral fin length).

The total number of vertebrae ranges from 65 to 71, with 44 to 50 caudal vertebrae. Both ranges are the lowest compared to the anguilliform clariids (*Dolichallabes microphthalmus, Channallabes apus, Gymnallabes typus, Gymnallabes nops, Gymnallabes alvarezi*). Additionally the number appears very stable, which is not the case in some elongated clariid representatives where higher vertebral counts and a larger intraspecific variation is reported (CABUY et al., 1999; DEVAERE et al., 2001, 2004, in press (**IV.5.1.a, IV.4, IV.1**)).

One of the most diagnostic characters is the presence of a lightly coloured stain on the skull roof, at the level of the parieto-supraoccipital, a feature only seen in *Gymnallabes alvarezi* Roman, 1970 (this species is under taxonomic revision, **IV.5-2**)[3]. After removing the skin, a high amount of subcutaneous fat can be observed in *Platyclarias machadoi*, which is never observed in other clariids.

The absence of any additional outgrowth on the anterior bony plate of the hyomandibula and the dorsal edge of the hyomandibula, as well as the fact that the quadrate form almost a straight line with only a weak indentation, are autapomorphic characters. Further, the interopercle lies medial to the suspensorium, while in all clariids studied so far it lies in a lateral position (DEVAERE et al., 2001, 2004 (**IV.5.1.a, IV.4**)). The medial position of the retroarticular processes of the lower jaws, with respect to the quadrates, is shared only with *Platyallabes tihoni* (DEVAERE et al., in press (**IV.1**)).

Many of the apomorphic features of *Platyclarias machadoi* involve muscular characteristics. The large jaw muscle complex, shows the presence of an extra muscle bundle, laterally from the A_3''-part and medially from the rostral part of the levator arcus palatine, the 'pars levator tendinis' (**Plate IV.2-4**). Until now, such a separate muscle could not be observed in other clariids. This poses a difficult question for homology. Until this can be resolved, the most plausible explanation for now would be to consider this new muscle as a *de novo* splitting off of the A_3''-part fibers. In a lateral view, the dorsal $A_2A_3'\alpha$-part of the adductor mandibulae complex runs from the mandible up to the posttemporo-supracleithrum, which is far more caudal than the ventral part. This results in a dorsal part of the A_2A_3' clearly distinguished from the ventral $A_2A_3'\beta$.

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[3] has been transferred to *Channallabes alvarezi*

The morphology of the hyoid musculature of *Platyclarias machadoi* is especially characterized by muscle fiber reorientations. The gap between the intermandibularis and the mediorostral part of the protractor hyoidei pars ventralis is larger than in all other clariids studied. Apparently, this is caused by the caudally subsided structure of the interconnecting bases of the internal mandibular barbels. Because of the more caudal position of the hyohyoideus inferior, as well as its rostro-caudal compression, a large gap exists between it and the protractor hyoidei. Concerning the hyohyoideus abductor, in other clariids the two contralateral parts overlap or touch each other at the level of the tendinous insertion onto the ventral hypohyal of the opposite side. In *Platyclarias machadoi,* however, a large gap remains between the two parts, causing the crossing of the tendons to occur more rostrally.

This paper shows that the constraints of a very low skull in *Platyclarias machadoi* result in some structural interdependencies. These are: the medial position of the retroarticular process on the lower jaw, the horizontal position of the suspensorium, the lateral tilting of the pterygoid bones and the anterior part of the quadrate with regard to the suspensorial transverse long axis. Besides some other unique traits, these characteristics help to refine the genus/species description.

IV.3 - The genus *Gymnallabes*

IV.3.1 Holotype skeletal morphology of *Gymnallabes nops* Roberts and Stewart, 1976, using micro CT-scanning

ABSTRACT

One of the major problems in the anguilliform Clariidae is the taxonomical validity and the systematic position of *Gymnallabes nops*. This vagueness is largely due to the fact that the description of this species is based on the holotype, and no additional specimens are known. This also means that past studies on this holotype were limited to non-invasive research, such as external morphology and x-rays. These methods, however, yield only a limited number of valuable characters. In this study a high-resolution desktop X-ray microtomography instrument (CT-scan) was used. This enables us to confirm the results of the radiographies but also to perform a detailed osteological study, without damaging the holotype. The osteological survey showed similarities with other anguilliform clariids, in particular with *Platyallabes tihoni* and *Gymnallabes typus*. Besides these traits, *G. nops* shows a set of characters, revealed in this study, unique among the clariids, such as a reduction of the infraorbital bones in number and size, a large anterior outgrowth of the opercle, a clearly visible epiphyseal bridge and the typical positioning of entopterygoid and metapterygoid bones. These extra results may help to clarify the validity and the phylogenetic position of *G. nops*.

Key words: X-ray scanning, *Gymnallabes nops*, Holotype, Clariidae, Siluriformes

The freshwater Clariidae is one of the 37 catfish families known within the Siluriformes order (SABAJ et al., 2004). Their diversity is the largest on the African continent. Besides in Africa, they can also be found in Syria, southern Turkey and some parts of Southeast Asia (TEUGELS, 2003). Although some of the more generalized, fusiform species, in particular *Clarias gariepinus* (Burchell, 1822), show a large distribution, the occupation of the anguilliform species is a more specialized, burrowing niche (ADRIAENS et al., 2001). They occur only in Equatorial Central and West Africa (BOULENGER, 1911; POLL, 1957b; TEUGELS et al., 1990; TEUGELS, 2003).

Clariid catfishes are characterized by an elongate body, long dorsal and anal fins, the presence of four pairs of barbels, and especially by the unique presence of a suprabranchial organ, formed by arborescent structures from the second and fourth gill arches (GREENWOOD, 1961; TEUGELS and ADRIAENS, 2003).

The anatomy of the anguilliform clariids is poorly known. Only a few studies have superficially described the cranial morphology into detail (POLL, 1957a, 1977; CABUY et al., 1999; DEVAERE et al., 2001 (**IV.5.1.a**)). This is certainly the case for *Gymnallabes nops*, on which, to this moment, no morphological research has been done. The fact that the holotype is the only found representative of *G. nops* can be considered as the main reason for this deficiency, as this allows only a narrow field of non-invasive studies that can be performed on such unique material. Thanks to the use of computerized microtomography (μCT), we obtained 3D information of the complete skeleton in a non-destructive manner. The objectives of this paper are therefore: (1) to give a detailed description of the cranium and the most relevant post-cranial structures; (2) to use this new information for the comparison with other anguilliform clariids; (3) to redescribe *G. nops* based on characters, found in this study and (4) to provide diagnostic characters for this species

MATERIALS AND METHODS

Material examined

The holotype of *Gymnallabes nops* Roberts and Stewart, 1976 (MCZ 50.298) (**Plate IV.3-1A**) was obtained from the Museum of Comparative Zoology, Harvard University (MCZ). The specimen was collected in Tadi, the lower Congo-River basin (5° 14'S, 13° 56'E). The sample site is a 2m deep, backwater with boulders, with a silty or sandy bottom (ROBERTS and STEWART, 1976).

Comparative material examined

Museum abbreviations are listed in Leviton et al. (1985).

Channallabes apus. Angola. Ambriz, BMNH 1873.7.28.16 (holotype); Other specimens, Dem. Rep. Congo. Bokalakala, MRAC 175247-270 (n=10); Kinshasa, MRAC 97-056-P-0001-0003 (n=2); Bumba, MRAC 88-25-P-2192-227 (n=36); Boma, MRAC 939; Riv. Lula, Bushimaie, MRAC 153505; Kelé, MRAC 1491; Stanleyville, MRAC 30893-30900 (n=8), MRAC 88-01-P-1976-1992 (n=17); Riv. Ruki, Eala, MRAC 14747-49 (n=3); Lake Tumba swamp area, MRAC 46299; Katanga, MRAC 39480; Riv Botota, keseki, MRAC 67763-77 (n=15); Mwilambongo, MRAC 72886-887 (n=2); Dekese, Riv. Lofu, Anga, MRAC 153352; Yangambi, MRAC 68700; Riv. Oubangui, Imfondo, MNHN 1922-0029; Loango, MNHN 1924-0079, MNHN 1924-0080; Sangha, MNHN 1925-0137; Mogende, MNHN 1926-0155-59 (n=5); Riv Congo, MNHN, 1937-0124-25; Stanleypool, Bamu, MNHN 1958-0111; Boloko, Riv. Likouala, MNHN 1962-0401 (n=7); Mossaka, Riv. Likouala, MNHN 1963-0402 (n=2); Riv. Loadjili, Songolo, MNHN 1967-0143 (n=6); Mangala, BMNH 1896.3.9.17; Riv. Lebuzi, Kaka Muno, BMNH 1912.4.1411-12 (n=2); Lower Congo, BMNH 1887.1.13.8-9 (n=2); Stanley Falls, BMNH 1889.11.20.5; New Antwerp, Upper Congo, BMNH 1899.2.20.16; Siala-Ntoto Swamps, BMNH 99.11.27.92; Bangyville, Ubangi, BMNH 1907.12.26.34; Kashi, Lulua, MHNG 1248.3; Banana, NMW 47240-42; Mollunda, NMW 47245 (n=4), NMW 47246. Congo. Yangala Youbi, MNHN 1967-0146; Djembo, Kouilou, MNHN 1967-0147; Cayo, MNHN 1989-0527; Riv. Nanga, between Boukou-Zassi and Kouilou swamp area, MRAC 90-57-P2315; Sintou, Riv. Kibombo, Kouilou, MNHN 1967-0144; Riv. Loadjili, Songolo, Pointe Noire, MNHN 1967-0145 (n=6); Riv. Youbi, Noumbi. Angola. Caungula, Mabete, Riv. Uamba, MRAC 162088; Riv. Camuconda, Tchimenji, MRAC 162089, MRAC 162090-094 (n=5), MRAC 162095-100 (n=6); Riv Ganga-Ludchimo, MRAC162083-086 (n=4).

Dolichallabes microphthalmus. Dem. Rep. Congo. Kunungu, MRAC 44655, adult male, 229 mm SL (holotype), MRAC 44656-659 (n=3) (196-210 mm SL) and 62407, 188 mm SL (paratypes), MRAC 57662, 196 mm SL, MRAC 18850, 90 mm SL; Boende swamps, MRAC 101843, 149 mm SL, MRAC 176123-124 (n=1), 68 mm SL; Bokuma, MRAC 79093, 134 mm SL, MRAC 93774, 66 mm SL; Bokuma – Tchuapa, MRAC 79258-260 (n=3) (85-126 mm SL); Ndwa (Boloko), MRAC 78808-810 (n=3) (99-110 mm SL); Inonge, MRAC 96672, 110 mm SL; Maylimbe, Tshela, MRAC 66721, 97 mm SL.

Gymnallabes alvarezi. Rep. Congo. Zanaga, Lésala, MRAC 8-22-P-1047-050, 141 mm SL.

Gymnallabes typus. Nigeria. Old Calabar, BMNH 1866.12.4 (n=2) (Syntypes); Other specimens, Nigeria. Umu-Eze Amambra, MRAC 84-16-P-1-2; Riv. Sombreiro, East of Erema, MRAC 91-067-P0134; Niger Delta, MRAC 97-030-P-0001-0010 (n=10); lake Odediginni, Agudama, Yenagoa, MRAC 92-083-P-0035-0036; Okaka, Epie Creek, Between Nun an Rashi

Riv, MRAC 97-085-P-0001-0004 (n=4); Riv Sombreiro, Odiemerenyi, Ahoada, MRAC 91-067-P-0135-0136; New Calabar, Choba, MRAC 91-105-P-1; Rumuji Swamps, MRAC 86-10-P-72; Oshika, MRAC 84-28-P-28, MRAC 84-28-P-25; Riv. Cron, Itu, MRAC 88-36-P-10; Between Sapele and War, Niger Delta, MRAC 74-29-P-600; Muoha, New Calabar, MRAC 91-10-P-478; Biseni, taylor Creek, MRAC 91-01-P278; Ossomari, BMNH 1902.11.10.119. Cameroun. Riv. Kom, Ntem, Aboulou, MRAC 73-18-P-3307-309.

Platyclarias machadoi. *Angola. Cuango, Cafunfo, Riv. Borio, MRAC 78-6-P-1345, adult female, 181 mm SL (holotype), MRAC 78-6-P-1346-1367 (n=21) (76.0-146 mm SL).*

Platyallabes tihoni. Dem. Rep. Congo. Kingabwa, Stanley pool, MRAC 13307 (Holotype); Kinsuka, MRAC 73-68-P-143, MRAC 138698-699 (n=2), 125345-349 (n=4), MRAC 73-22-P-3127 (n=3); Bulu, Luozi, BMNH 1976.5.21.30-39 (n=9), MCZ 50239 (n=13); Inga, MCZ 88947, MCZ 50537 (n=15); Tadi, Kibunzi, MCZ 50297 (n=5).

Measurements

All measurements were made on the holotype using a high-resolution desktop X-ray microtomography instrument [Skyscan-1072, Belgium (www.skyscan.be)]. A Tungsten air-cooled micro-focus X-rays tube with 9-micron spot size and a maximum voltage of 80 kV was used as a source. A 12-bit 1 mega-pixel low-noise CCD camera was applied as a detector. During acquisition both the X-ray source and the camera remained motionless while the object was rotated around its vertical axis. The system allows achieving 9-micron resolution but measured resolution depends on the size of the object and on the contrast. Biggest field of view possible for this device was 20 mm. The investigated specimen was too big for one acquisition so it was scanned 5 times and then reconstructed separately and assembled in one model afterwards. As our fish was fixated for a time in a decalcifying medium, the observed contrast was much lower than for ordinary fresh fish bones. That constrained us to use lower energies to improve contrast for soft tissues. The sample was scanned with 40 kV X-ray tube voltage, 0.9-degree rotation step and approximately 10 sec exposure time per individual shadow projection. After the shadow images were collected they were reconstructed into virtual cross-section using Feldkamp cone-beam algorithm. These cross-sections are the analogues of histological slices. The advantage is that the specimen remained intact although the resolution is lower than in classical histology. Another important advantage is that micro-tomography provides isotropic resolution thus allowing to build 3D-models (see following illustrations). 3D models presented in this paper were created with ANT software supplied by Skyscan with the instrument. (ANT software, Skyscan, Belgium) (POSTNOV et al., 2002; DE CLERCK et al., 2003).

Furthermore, a set of 33 metric measurements were taken point-to-point using digital callipers to 0.1 mm (Digital ruler, Mauser), interfaced directly with a computer. Measurements terminology follows that of DEVAERE et al. (2004) (**IV.4**): total length (TL); standard length (SL); preanal length (PaL); anal fin length (AFL); dorsal fin length (DFL); prepelvic length (PPvL); prepectoral length (PPcL); predorsal length (PdL); distance between the occipital process and the dorsal fin (SPDFL); pelvic fin length (PvFL), pectoral fin length (PcFL); pectoral spine length (PcSL); caudal peduncle depth (CPD); body depth at anus (ABD); maxillary barbel length (MxB); external mandibular barbel length (EmnB); internal mandibular barbel length (ImnB); nasal barbel length (NB); interpelvic distance (IpvD); interpectoral distance (IpcD); skull length (SkL); postorbital length (PoL); skull width (SkW); supraoccipital process length (SpL); supraoccipital process width (SpW); interorbital distance (IoD); anterior nostril interdistance (ANID); posterior nostril interdistance (PNID); rostral skull width (RSkW); orbital skull width (OskW); skull height (SkH); eye diameter (ED); snout height (SnH); prehyoid length (PhL); internal mandibular interdistance (ImnID); external mandibular interdistance (EmnID); mouth width (MW) and skull roof width (SkR). The following meristic counts were made, using the radiographies made with a Bennett X-ray (25-50kV, 25-150 Ma, 1/30-20 sec, focus distance: 0.3-0.6 m): total number of vertebrae (TV), number of ribs (RB).

We compared the holotype with a large sample of four other anguilliform species; *Platyallabes tihoni* (Poll, 1944), *Gymnallabes typus* Günther, 1867, *Dolichallabes microphthalmus* Poll, 1942, *Channallabes apus* (Günther, 1873) and *Platyclarias machadoi* Poll, 1977.

Analyses

The morphometric data and the meristic counts were submitted to a Principal Component Analysis (BOOKSTEIN et al., 1985). Biometric data were log-transformed (BOOKSTEIN et al., 1985). The first principal component was not used since it is considered as a size factor, whereas the other components are considered as shape factors, largely independent of size (TEUGELS et al., 1999). Different combinations of components were used to give the plot that expressed the most variation.

RESULTS

Neurocranium

Ethmoid region: The nasal bone (**Plates IV.3-1B, IV.3-2A**), covering the nasal sac and enclosing the anterior-most part of the supraorbital canal, is reduced to a partially open,

elongated bone, with little to no lateral plate extensions present. The supraorbital canal appears to split in this bone, as a lateral pore can be distinguished.

The mesethmoid (**Plates IV.3-1B, IV.3-2A**) shows a substantial constriction caudally from the two rostral wings. It is sutured to the frontals, by means of large interdigitations. The main part of the lateral ethmoid is covered by the frontal. Ventrally, the mesethmoid supports the premaxillary bones. Although very restricted, the mesethmoid makes contact with the anterior fontanel. The lateral ethmoid shows a long, pointed lateral processes (**Plates IV.3-1B, IV.3-2**). Anteriorly, the supraorbital canal leaves the neurocranium where the mesethmoid and lateral ethmoid suture. At this point the supraorbital canal is covered by the rostral extension of the frontal. Since there is no second infraorbital bone (see below), the lateral ethmoid shows only an articulation surface for the autopalatine. The arrow-shaped prevomer interconnects with the parasphenoid through one short interdigitating spine. It carries one tooth plate, which shows a constriction in the middle (**Plate IV.3-7A**).

Orbital region: The circumorbital series is highly reduced, both in shape and the number of bones. Of the whole set of five bones present in most other clariids (CABUY et al., 1999; DEVAERE et al., 2001 (**IV.5.1.a**)), only the infraorbital IV and the antorbital are present in *Gymnallabes nops* (**Plates IV.3-1B, IV.3-2A**) (for discussion on the nomenclature of the circumorbital bones in clariids, we refer to ADRIAENS et al. (1997)). The antorbital bone is very small, lying dorso-posteriorly of the base of the maxillary barbel, close to the maxillary bone and dorsal to the rostral end of the autopalatine. The infraorbital IV is reduced to a more or less completely open, canal bone. This makes that the infraorbital canal runs almost completely unprotected along the lateral side of the head. This infraorbital bone has the most dorsal position of the neurocranial bones, elevated by the hypertrophied jaw adductor muscles.

The frontals (**Plates IV.3-1B, IV.3-2A**) form the largest bones of the skull roof. The two contralateral parts are lying closely against each other, leaving a clear joint. The frontals enclose the anterior fontanel entirely, except for the anterior most border contacting the mesethmoid. The anterior fontanel separates the two frontals along the rostral half. Within this fontanel, the bony epiphyseal bridge is clearly visible; with the anterior part of the postpineal fontanel being exposed. The infraorbital canal exits the skull roof at the level of the frontals, close to the connection between the latter bone and the lateral ethmoid. The lateral side of the skull is formed by the orbitosphenoid and pterosphenoid (**Plates IV.3-2A,B, IV.3-3A**), which are ventrally connected to the parasphenoid (**Plate IV.3-2B**). This latter bone runs from the rostral end of the orbital region up to the occipital region; it bears one elongated process.

Temporal region: The skull roof in this region is formed by the frontals, the sphenotic and the pterotic (**Plate IV.3-1B**). The sphenotic (**Plates IV.3-1B, IV.3-2A**) interdigitates rostro-medially with the frontal and caudally with the pterotic. The pterotic (**Plates IV.3-1B, IV.3-2A**), in turn, contacts the parieto-supraoccipital on the medial side and the posttemporo-supracleithrum caudally. Both the sphenotic and pterotic lack lateral plates. They form a firm connection between the neurocranium and the suspensorium through a set of interdigitating processes. On the anterior side of the sphenotic one large and one small process are present. On the posterior side of the pterotic, two additional processes are present. In the pterotic, however, the largest process is situated most externally to the hyomandibula, which is opposite for the sphenotic spines. Ventrally, the neurocranium is formed by the paired prootics (**Plate IV.3-2B**).

Occipital region: The neurocranium is caudally bordered by the parieto-supraoccipital (**Plates IV.3-1B, IV.3-2A**), bearing a medial, long, caudally pointed spine. This bony complex encloses the posterior fontanel, which is very small and lies in the caudal half of the parieto-supraoccipital. The posttemporo-supracleithrum (**Plates IV.3-1B, IV.3-2A**) rostrally makes contact with the pterotic. Further, it is connected to the pectoral girdle and to the parapophysis of the fourth vertebra, through a solid transscapular process. Ventrally, this region of the skull consists of the exoccipitals and the basioccipitals (**Plate IV.3-2B**).

Maxillary bones: The premaxillaries (**Plates IV.3-1B, IV.3-2A,B**) are large, toothed plate-like bones. The anterior part of the premaxillaries bears several rows of slightly curved teeth, while the posterior, somewhat smaller, part remains toothless. The maxilla is a rod-like bone (**Plates IV.3-1B, IV.3-2A, IV.3-3A**). It has a little indentation, in which the base of the barbel is enclosed. The proximal part of the maxilla is broader and bears two articulatory facets for the articulation with the autopalatine.

Splanchnocranium

Lower jaw: The lower jaw consists of two parts: the dentary and the angular complexes (**Plates IV.3-2A, IV.3-3A**). The coronoid process on the lower jaw is distinct and firm. The anterior part of the lower jaw is covered with a large tooth battery, with slightly curved teeth, which run close up to the top of the coronoid process. The lower jaw shows five pores for branches of the preopercular-mandibular canal. The well-developed retroarticular process lies mostly ventral from the articulation facet of the quadrate. The lower jaw embeds the rostral end of the preoperculo-mandibular canal of the lateral line system. The first three pores (PM1-3) lie in the dento-splenio-mentomeckelium complex,

the fourth (PM4) is situated on the border of both bone complexes, while the fifth one (PM5) is located on the caudal end of the lower jaw.

Suspensorium: The suspensorium consists of the hyomandibula, quadrate, entopterygoid, metapterygoid and the preopercle (**Plates IV.3-1B, IV.3-3B**). This latter bone is mentioned here since it is fused to the suspensorium and forms one functional unit. The hyomandibula connects to the sphenotic and the pterotic through a set of processes. At the level of the sphenotic, a plate-like process is present anteriorly, followed by a smaller, pointed process. On the caudal side of the articulation ridge, three processes are present, rostrally to caudally, in size increasing for the interdigitation with the pterotic. Between these two sets of processes an articulation ridge is present, making contact only with the sphenotic bone. On the lateral side, a clear ridge is present, inclosing a foramen. On the rostral side of the hyomandibula no plate-like outgrowth is present. The opercular process is ventro-caudally oriented. The quadrate ventro-laterally contacts with the lower jaw through a well-developed articulation head. It makes a clear contact with the complete caudal side of the metapterygoid bone; dorsally this occurs through an interdigitation zone, ventrally through a synchondrosis. On the other hand no contact between the quadrate and the entopterygoid can be observed, as they are completely separated by the metapterygoid. The last bone is the preopercle (see below), which is completely incorporated in the suspensorium. This bone surrounds a part of the preoperculo-mandibular canal, and shows two pores (PM5-6).

The autopalatine bone (**Plates IV.3-1B, IV.3-2A, IV.3-3A**) shows a clear concave medial side, with a rostral and caudal cartilaginous tip. The autopalatine lies ventrolateral to the lateral ethmoid and shows a clear and well-pronounced articulation facet at the articulation site with the lateral ethmoid. On the rostral side it articulates with the maxillary bone, thus being part of the palatine-maxillary mechanism. A single, small, tubular, splenial bone (Adriaens et al., 1997a) lies laterally to the articulation between lower jaw and the quadrate (**Plates IV.3-2A, IV.3-3A**).

Hyoid arches: The hyoid arch consists of two anterior and posterior ceratohyals and two ventral hypohyals (**Plates IV.3-2A, IV.3-3A**). On the scans there was no evidence of the dorsal hypohyals, this could be due to decalcification, since in other clariids the dorsal hypohyals are present (Cabuy et al., 1999; Devaere et al., 2001, 2004 (**IV.5.1.a, IV.4**)). Ventrally, the hyoid arch articulates with nine branchiostegal rays. The first six branchiostegal rays articulate with the ventral rim of the anterior ceratohyal, the following ray is placed at the small cartilaginous region between the anterior and posterior ceratohyal. The last two rays are situated on the posterior ceratohyal. The parurohyal lies

in between the two hyoid arches and bears two caudo-lateral processes and one very small, spiny, caudal process (**Plate IV.3-3A**).

Branchial arches: The "Bauplan" of the branchial basket corresponds to the general clariid situation. Only the size and the number form somewhat an exception to this general situation. The gill rakers in *Gymnallabes nops* are not only small, but also occur in small numbers. For a detailed description of this general configuration in clariids, we refer to ADRIAENS and VERRAES (1998).

Opercular series: The opercular bone is a triangular, caudally, pointed structure (**Plate IV.3-2A**). On the rostro-dorsal side it bears a large articulatory facet for the articulation with the hyomandibula. Caudally to this facet, a large process is present. On the medial side of the caudal part, a ridge is present, presumably for the attachment of the levator operculi muscle. On the rostro-ventral side of the articulation facet, a large, more plate-like, extension is present, ending on a border at the contact zone with the interopercular bone. This latter bone is situated between the opercular bone and the lower jaw (**Plate IV.3-2A**). On the medial side, the interopercle bears a marked concavity enclosing the caudal tip of the posterior ceratohyal. Laterally, on top of the jaw muscles, two suprapreopercular (**Plates IV.3-2A, IV.3-3A**) bones are present. The larger, dorsally situated suprapreopercular bone shows a triangular shape. The ventral one is reduced to a tubular bone, enclosing the preopercular canal. As mentioned above, the praeopercular bone is incorporated in the suspensorium (**Plate IV.3-2A**).

Postcranial Skeleton

Vertebrae: The total number of vertebrae is 62 (**Plate IV.3-4A**) (including those comprised by the Weberian apparatus). There are 18 precaudal vertebrae, of which five carry ribs. The dorsal fin comprises a total of 75 fin rays; 59 fin rays are present in the anal fin. A little foramen can be found at the bases of the parapophyses of the first precaudal vertebrae.

Pectoral girdle: The pectoral girdle comprises a scapulo-scapulo-coracoid and a cleithral bone (**Plates IV.3-2B, IV.3-3A**), which are strongly sutured to each other. Ventrally, the two contralateral parts are strongly connected to each other. In the caudal part, this occurs through several large processes. A clear fenestra is present between the right cleithral and scapulo-scapulo-coracoid bone, but completely absent in the left part (**Plate IV.3-3A**). The cleithral bone shows a clear anterior process, as well as a ridge on its rostro-ventral side. On the scapulo-scapulo-coracoid, a distinct insertion ridge is present for the attachment of the pectoral muscles. The pectoral fin has a non-serrated spine and eight fin rays that articulate with the two radials present.

Pelvic girdle: Although the pelvic girdle is highly decalcified, the basipterygium shows two clear processes (internal and external anterior process: see ARRATIA, 2003). No more than four pelvic fin rays can be discerned.

Caudal skeleton: The caudal skeleton (**Plate IV.3-4B**) consists of the parhypural, five hypurals, urostyl and uroneural, which are all fused in a single bony plate. Dorsally to this fusion lies the broad tipped, epural. The neural spine of the second preural vertebra is elongated, broadly tipped and supports the caudal fin rays; the neural spine of the third preural vertebra is also elongated but ends with a spiny tip and does not support the dorsal fin rays. This supporting function is performed by a pterygiophore. Both haemal spines of the second and third preural vertebrae are elongated, broad and support the anal fin rays. Anteriorly, the dorsal and anal fin rays are supported by pterygiophores.

DISCUSSION

Original description

In the original description of ROBERTS and STEWART (1976), containing some metric and meristic data, the holotype of *Gymnallabes nops* was included in the genus *Gymnallabes* Günther, 1867. At that time this genus included *Gymnallabes typus* Günther, 1867; *G. alvarezi* Roman, 1970 and *G. tihoni* (Poll, 1944) of which the latter was later transferred to *Platyallabes* Poll, 1977. Although the largest similarity was observed between *G. nops* and *Platyallabes tihoni*, the authors pointed out that a comprehensive set of differences existed. Most of them, however, proved to be invalid, as this study indicates, simply by taking more specimens of *P. tihoni* into account (see below).

The authors gave as a first striking difference the lack of eyes in *G. nops*. This is not unique and also occurs in some specimens of *Platyallabes tihoni* (MCZ 88947, MCZ 50297). A second character used was the observation that the pectoral and pelvic fins did not extend respectively beyond the origin of the dorsal and anal fin, in contrast to the situation in *P. tihoni*. In our dataset (**Table IV.3-1**) a large variation on the length of both pectoral and pelvic fins in *P. tihoni* was observed, a variation already shown in several other anguilliform clariids (ADRIAENS et al., 2002). Paired fins not reaching or reaching further than the origin of the respectively dorsal and anal fin could both be found. Also the length of the innermost pelvic fin ray in *P. tihoni* shows a lot of variation and is not always extremely reduced, as stated in the original description. Further, it was stated that *G. nops* has a higher abdominal body depth and caudal peduncle depth, shorter pectoral spine and a smaller skull roof width, but all these lie within the ranges of *P. tihoni* (**Table IV.3-1**). The distance between the occipital process and the dorsal fin was given as a last metric

difference. This was stated to be double in *G. nops* compared to *P. tihoni*. Since a small distance is one of the distinctive characters of *P. tihoni* (DEVAERE et al., in press (**IV.1**)), the distance is larger in *G. nops* but not twice that of *P. tihoni* (**Table IV.3-1**).

Since metric and meristic data of *Gymnallabes nops* overlap, mostly lying within the range of *Platyallabes tihoni,* the question raises if *G. nops* is still a valid species. Furthermore, since *Platyallabes* has become a different, monotypic genus, the question raises if *G. nops* is designated to the proper genus. A discussion on shared characters follows, based on the obtained morphological characters.

Diagnosis

Principal Components Analysis

For the analysis, we examined 110 specimens of the wide area around the type locality of *G. nops*. This region includes the lower Congo stream up to Kinshasa, Southern West Coastal Equatorial, and the Kasai region (THIEME et al., in press). These include specimens of *Channallabes apus* (n = 29), *Platyallabes tihoni* (n = 39), *Platyclarias machadoi,* (n = 21), *Gymnallabes alvarezi* (n = 1)[4] with type material of *Gymnallabes nops, Platyallabes tihoni* and *Platyclarias machadoi*. We also included the specimens of *Gymnallabes typus* (n = 19), from the Niger delta, Northern and Central West Coastal Equatorial Freshwater Ecoregion (THIEME et al., in press) (includes *G. typus* types).

Plate IV.3-5 plots the second principal component derived from a PCA on the covariance matrix for 25 measurements against the first principal component derived from a correlation matrix of 5 meristic characters. The dominant characters for the second principal component are the distance between the occipital process and the dorsal fin, the caudal peduncle depth and the barbel lengths; while for the first component, the total number of vertebrae and the number of ribs are the two most important. This reveals the presence of several groups that do not overlap. In this plot *Gymnallabes nops* takes a separate position, apart from other groups and type material, indicating that *G. nops* is indeed a valid species.

Diagnosis

Gymnallabes nops differs from all other anguilliform clariids by the combination of following characters: a large rostral outgrowth of the hypertrophied adductor mandibulae complex; a reduced infraorbital series, both in number and size; the absence of the medial expansions of the frontals plates, leaving the epiphyseal bridge clearly exposed; the

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[4] has been transferred to *Channallabes alvarezi*

entopterygoid situated in a completely rostral position of the metapterygoid, a short posterior process on the prevomer and a large rostro-ventral process on the opercle.

Although the absence of the eyes is not unique, it has some typical consequences; the available orbital space is taken by an outgrowth of the hypertrophied adductor mandibulae complex, covering the lateral and dorsal side of the skull (**Plate IV.3-6A,B**). Since no discrimination between soft tissues is possible on radiographies or CT-scan, we have no certainty that nothing of the visual sense organ is developed. It cannot be overruled that the eyes could have developed up to a certain embryonic stage, but remain invisible as they are covered by the integument. A comparable situation has been observed in *Caecomastacembelus brichardi* (Poll, 1973). The only certainty that we have obtained through the CT-scans is that the foramen for the optic nerve is clearly present between the orbitosphenoid and the pterosphenoid (**Plate IV.3-2B**), which could indicate the passage of an optic nerve and thus a certain level of development of the visual sense organ itself.

As in other anguilliform species, as well as in *Uegitglanis* and the primitive catfish family Diplomystidae (DAVID, 1936; ARRATIA, 1987), the plesiomorphic state of a reduced series of circumorbital bones occurs in *G. nops*. However, this reduction in *G. nops* not only involves a reduction of the plate-like expansions on each of the infraorbital bones, but is also reflected in a reduction of the number of infraorbital bones. Only the first and last one are still present, namely the antorbital and infraorbital IV (**Plates IV.3-1B, IV.3-2A**). Those two are the most dorsally situated bones and are also the only circumorbital bones present in *Dolichallabes microphthalmus* (DEVAERE et al., 2004 (**IV.4**)). As was stated in DEVAERE et al. (2004) (**IV.4**) this could be due to a heterochronic process, although for *G. nops* some other reasons for this reduction could be suggested. Since *G. nops* shows no evidence of eyes, a protecting series of bones around the orbital region is not required. This, however, leaves the infraorbital canal unprotected. Although a discontinuous infraorbital canal does sometimes occur (WEBB, 1989), this seems not to be the case here. There is no evidence of a discontinuous canal in clariids (ADRIAENS et al., 1997; CABUY et al., 1999; DEVAERE et al., 2001 (**IV.5.1.a**)) Furthermore, the absence of the infraorbital bones II and III could also be linked to the subsequent outgrowth of the jaw muscle complex (**Plate IV.3-6A,B**). Another explanation is that the reduced number of infraorbital bones in the reconstruction is an artifact due to decalcification of those small bones, which makes them indistinguishable for CT-scanning. But as other small bones are still present (cf. nasal and splenial bones) this seems very unlikely.

Furthermore, in *G. nops* the plates on the frontals, medially overgrowing the anterior fontanel are absent, leaving the epiphyseal bridge clearly visible (the cartilaginous bridge, however, is still enclosed by a bony case formed by the frontals) (ADRIAENS and VERRAES,

1998). The typical posterior process on the prevomer is rather short, in contrast to the long process present in *Gymnallabes typus* and *Channallabes apus* (DEVAERE et al., 2001 (**IV.5.1.a**)) (**Plate IV.3-7A,B,C**). Also, on the prevomer a single tooth plate is present, as is frequently the case in other adult clariids. In *Gymnallabes nops*, however, the tooth plate shows a constriction in the middle, almost dividing the tooth plates in two (**Plate IV.3-7A**). Additionally, the configuration of the entopterygoid and the metapterygoid is typical in *G. nops*. The entopterygoid lies in a completely rostral position of the metapterygoid, with no dorsal or ventral contact zone (**Plate IV.3-3B**). The entopterygoid and metapterygoid lie somewhat separate from each other but remain attached through a sheet of connective tissue (**Plate IV.3-3B**). A last unique trait is the large rostro-ventral process on the opercle (**Plate IV.3-2A**). In other anguilliform clariids the extension anterior of the articulation facet of the opercle with the hyomandibula is shorter.

Redescription of *Gymnallabes nops*

The proportional measurements and counts are given in Table 1. *Gymnallabes nops* is characterized by an elongated, in cross-section round, body with a dorso-ventrally flattened head. The degree of anguilliformity can be expressed as the ratio of the total length to the body height (POLL, 1942a). This ratio, in the holotype of *G. nops*, is 15.2 with a postanal length of 59.7% of the standard length.

The most striking character is that *Gymnallabes nops* show no external evidence of the presence of eyes (**Plate IV.3-1A**). The orbital region is occupied by an anterior outgrowth of the hypertrophied adductor mandibulae complex. The dorsal outgrowth of this muscle complex makes that the skull roof is largely covered (**Plate IV.3-6A,B**). The in alcohol preserved holotype of *G. nops* shows an evenly light brownish/pinkish coloration, but not a total depigmentation as was stated in the original description. Both paired and impaired fins and the nostrils show an even lighter brown color. Only near the barbels, no pigmentation can be observed and the cartilage present in the barbels is visible through the skin (**Plate IV.3-1A**).

The skull length is 15.1% of the standard length. The skull width is 68.7% of the skull length. Two large jaw muscles, slightly bulging, further characterize the head. This makes that only a small part of the skull roof is visible (11.3% of the skull length).

In the skull roof, both anterior and posterior fontanel are present. The anterior fontanel can be located by the presence of double set of pores of the supraorbital canal (S4). Behind these two pores, also the next pair of pores (S5) of the supraorbital canal are clearly visible.

The unpaired fins form one continuous finfold. The dorsal fin originates at a considerable distance of the supraoccipital process (SPDFL is 8.7% of SL). The paired fins are both well developed. The pectoral fins have a length of 9.4% of the standard length. Also the pectoral spine is clearly visible and is 53.8% of the total pectoral fin length. The pelvic fins are 5.6% of the standard length.

The rostrally situated lips are fused in the mouth corners, upper and lower lips are of equal length.

Comparison with other clariids

For the comparison with other anguilliform representatives, we used the two species to which *Gymnallabes nops* shows the greatest affinity: *Platyallabes tihoni* and *Gymnallabes typus*, as well as two other elongated species: *Channallabes apus* and *Dolichallabes microphthalmus*. In this paper we use *G. typus* as the only representative of the genus *Gymnallabes* to compare with *G. nops*. This due to the questionable generic placement of *Gymnallabes alvarezi* (Roman, 1970) (research on the systematic position of *G. alvarezi* is in progress, **IV.5.2.a**)[5]. *G. nops* is characterized by a reduced skull, the presence of hypertrophied jaw muscles and the narrow part of the skull roof, in between the jaw muscles (**Plate IV.3-6A,B**). This corresponds to the general cranial morphology of anguilliform species (CABUY et al., 1999; DEVAERE et al., 2001 (**IV.5.1.a**)). For the comparison of the general morphology of *G. typus, C. apus* and *D. microphthalmus* we refer respectively to CABUY et al. (1999), DEVAERE et al. (2001, 2004) (**IV.5.1.a; IV.4**) and *P. tihoni* (see **IV.4**).

Besides a whole set of symplesiomorphic characters with the other anguilliform species, *G. nops* shows several character states that differ only with *P. tihoni*. Such characters involve the clearly constriction of the mesethmoid bone, caudally from the two rostral wings (comparable to most anguilliform species, except for *Platyallabes tihoni* were the constriction is less pronounced). *G. nops* has the general anguilliform lower jaw morphology with a tooth battery running up to a distinct coronoid process (**Plate IV.3-2A**) (DEVAERE et al., 2001, 2004 (**IV.5.1.a, IV.4**)). Only *Platyallabes tihoni* shows a less developed coronoid process. Also the fact that the sphenotic serves as the only articulation of the neurocranium with the suspensorium is in common with all anguilliform clariids except for *P. tihoni* were also the pterotic is involved in the articulation. Further at the level of the suspensorium the interdigitation with the neurocranium occurs through two sets of processes, two anterior and three posterior processes, increasing in size from

[5] has been transferred to *Channallabes alvarezi*

rostral to caudal. This configuration also viewed in *Gymnallabes typus* and in some specimens of *Channallabes apus* (**Plate IV.3-3B,C,D**).

Next to the above mentioned differences, *G. nops* shows a set of similarities only with *P. tihoni*. The premaxilla, which is supported by the mesethmoid, has a small, posterior, plate-like extension, as only viewed in *P. tihoni*. Two processes are present on the sphenotic and pterotic for the interdigitation with the suspensorium (**Plate IV.3-2A**). On the caudal side of the parieto-supraoccipital bone, which embeds a very small posterior fontanel, a somewhat short, pointed process is present. Further, no bony plate is present on the rostral side of the hyomandibula. A last similarity is that the opercular process is ventro-caudally orientated, comparable to the position in *P. tihoni*. The number of pre-caudal and total number of vertebrae of *G. nops* lies within the range of that in *P. tihoni*. The total number of vertebrae in *Gymnallabes typus* is higher than that in *G. nops* (**Table IV.3-1**). Since the number of specimens on which gill rakers, dorsal and anal fin rays have been counted is small, no conclusive results can be given.

Geographic distribution

The type locality of *Gymnallabes nops* is near Tadi, about 50 km downstream from Luozi, along the lower stream of the Congo River, Democratic Republic of Congo (lat 5° 14'S, long 13° 56'). This is situated in the Lower Congo Rapids freshwater Ecoregion (THIEME et al., in press). *Platyallabes tihoni* occurs mostly in the same freshwater ecoregion, but also in the vicinity of Stanley Pool (Kinshsa region) and more downstream on the lower Congo. In contrast, *Gymnallabes typus* occurs nowhere near those localities or the Congo River. Most *G. typus* can be found near the Niger delta and in Cameroon. Even when incorporating the localities of the questionable *Gymnallabes alvarezi*, there is still no presence of other species of the *Gymnallabes* genus in the Congo basin. The similar biogeographical distribution of *G. nops* with *P. tihoni* and clear allopatry with other *Gymnallabes* species can be an additional indication of the wrong systematic position of *G. nops*, besides several synapomorphies with *P. tihoni* (**IV.1**).

The above mentioned results show that *Gymnallabes nops* can indeed be considered a valid species based on both metric and meristic measurements. This is additionally shown through a set of synapomorphies based on the osteology revealed by CT scanning. These morphological results, however, fail to unambiguously determine the correct taxonomic position. Further research on other clariids (including the other *Gymnallabes* species) will hopefully bring more clarity.

IV.3.2 *Gymnallabes typus*

A morphological study of *Gymnallabes typus* is given in Cabuy et al. (1999). Although this study discusses the cranial morphology of *G. typus* in great detail, it was not the aim of this article to include a diagnostic survey. Therefore, it is not the intension of this chapter to give a detailed enumeration of the cranial morphology of *G. typus* again for which we refer to Cabuy et al. (1999), but merely to give a list of the most diagnostic characteristics. These are based on the results obtained in this study and comprise the data of 33 specimens.

MATERIAL

Type material. Syntypes: BMNH 1866.12.4.1–2 (n = 2), 154 and 162 mm SL, Old calabar, West Africa.

Non-type material examined: Total of 31 specimens. Nigeria. Umu-Eze Amambra, MRAC 84-16-P-1-2 (n=1); Riv. Sombreiro, East of Erema, MRAC 91-067-P0134 (n=1); Niger Delta, MRAC 97-030-P-0001-0010 (n=10); lake Odediginni, Agudama, Yenagoa, MRAC 92-083-P-0035-0036 (n=2); Okaka, Epie Creek, Between Nun an Rashi Riv, MRAC 97-085-P-0001-0004 (n=4); Riv Sombreiro, Odiemerenyi, Ahoada, MRAC 91-067-P-0135-0136 (n=2); New Calabar, Choba, MRAC 91-105-P-1 (n=1); Rumuji Swamps, MRAC 86-10-P-72 (n=1); Oshika, MRAC 84-28-P-28, MRAC 84-28-P-25 (n=2); River Cron, Itu, MRAC 88-36-P-10 (n=1); Between Sapele and War, Niger Delta, MRAC 74-29-P-600 (n=1); Muoha, New Calabar, MRAC 91-10-P-478 (n=1); Biseni, taylor Creek, MRAC 91-01-P278 (n=1); Ossomari, BMNH 1902.11.10.119 (n=1).

DIFFERENTIAL DIAGNOSIS

Gymnallabes typus differs from all other anguilliform species by the presence of extremely well developed skin folds bordering the side wall of the mouth (clearly visible from above).

DESCRIPTION

Measurements and meristic counts for syntypes and additional specimens are given in
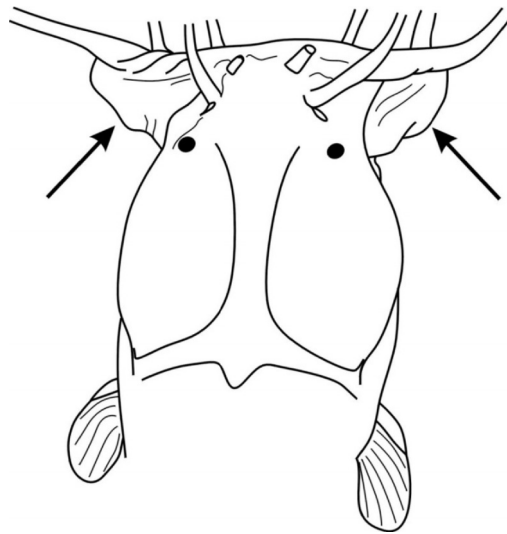
Fig. IV.3–1 Illustration of the head, arrows indicate skin folds

Table IV.3-2. Standard length ranges from 44 to 259 mm. *G. typus* has an elongated body (ABD up to 4.6-7.8% of SL), with a preanal length of 29.2% up to 37.7% of SL. Distance between the supraoccipital process and dorsal fin short, 5.2-11.2% SL (mean 8.3%). Small skull length (11.1-18.0% SL). Skull width ranges between 52.1 and 69.6% (mean: 60.3%) of the skull length. Skull roof remains visible (4.0-44.1% of maximal skull width), although in some cases the contralateral jaw muscles almost make contact with a width of. Eyes are always visible and bordered by small infraorbital bones (hard to observe externally). The lower lip reaches the upper lip. Maxillary barbel connected at base to large skin folds that border mouth opening. When the mouth is closed, these skin flaps are folded and conspicuous from a dorsal view (**Fig. IV.3-1**).

Unpaired fins form a continuous finfold. Pectorals always present (length 3.8-8.5% SL), always preceded by a firm pectoral spine (pectoral spine length 1.8-3.8% SL). Both sides of the pectoral spine serrated, with outer side more irregularly serrated. Also the pelvic fins are always present (length 1.7-6.8% SL). Number of vertebrae in *G. typus* lies between 78-98 (mode = 84). Number of ribs 6-12 (mode = 9). Dorsal fin with 96-109 rays, anal fin supported by 83-94 rays.

Gymnallabes typus has a more or less reduced skull and narrow skull roof. The high rate of reduction is shown in the total absence of a lateral plate on the frontal, as from a ventral view no lateral plates can be observed. This is only observed in *D. microphthalmus* as well. Another indication for the large extent of reduction is the absence of any plate-like outgrowth on the posttemporo-supracleithrum, pterotic and sphenotic. Furthermore, we see that the nasal, infraorbital and suprapreopercular bones are extremely tubular. Several suprapreopercular bones may be present. The epiotic is present. The posterior border of the mesethmoid shows no indentation, placing the anterior fontanel in the frontals. The entopterygoid makes contact with the metapterygoid on its complete rostro-

dorsal side and also with its ventral side. On the other hand, no contact is present between the entopterygoid and the quadrate. Although CABUY et al. (1999) mentioned the presence of teeth on the entopterygoid, this has never been observed in any other *G. typus* specimen. A toothed entypterygoid is only observed in *P. tihoni* (see **IV.1**). The first dorsal fin pterygiophore is situated between the fifth and sixth post–Weberian vertebrae and has a clear lateral process.

COLOR

In life, uniformly light brown. Preserved specimens may be darker brown, with a slightly paler ventral prepelvic region.

DISTRIBUTION

The natural range of *Gymnallabes typus* is restricted to Nigeria (the Niger Delta and Old Calabar (type location)) and possibly western Cameroon (Cross River) and Benin (Lower Oueme).

IV.4 - The genus *Dolichallabes*

IV.4.1 Redescription of *Dolichallabes microphthalmus* Poll, 1942 (Siluriformes, Clariidae)

ABSTRACT

As a part of the general revision of anguilliform clariid genera and species, the status of *Dolichallabes microphthalmus* Poll, 1942 is reviewed, based on external morphology and osteology of all available museum specimens. *Dolichallabes microphthalmus*, the most elongate species within the Clariidae, has been redescribed. Compared to *C. apus* and *G. typus, D. microphthalmus* is characterized by, in addition to some meristic differences, an elongate body, reduced skull ossification, with (1) one elongate fontanel (2) antorbital and infraorbital IV the only circumorbital bones present, (3) only one or two suprapreopercular bones on each side, (4) and a sphenotic bearing only one process. Osteological evidence suggests that *D. microphthalmus* could be considered a paedomorphic clariid.

INTRODUCTION

Clariidae is one of the 35 catfish families within the Siluriformes; they occur naturally in freshwaters in Africa, Middle East and South-East Asia (TEUGELS, 1996). Their diversity is largest in Africa where 12 genera are known, including 74 species (TEUGELS, 1996).

Clariid catfishes are characterized by an elongate body, the presence of four barbels, long dorsal and anal fins, and especially by the unique presence of a suprabranchial organ, formed by arborescent structures from the second and fourth gill arches (GREENWOOD, 1961; TEUGELS and ADRIAENS, 2003)

Within the Clariidae the presence of a range between fusiform and anguilliform genera has been noted (PELLEGRIN, 1927). Although this has been observed in other families of teleosts, amphibians and reptiles (LANDE, 1978), it is never so extreme as within the Clariidae. Together with the elongated body, a whole set of morphological changes are observed, such as decrease and loss of the adipose fin, continuous unpaired fins, reduction of paired fins, reduction of the skull bones, reduction of the eyes and hypertrophied jaw muscles. The genus *Heterobranchus* Geoffrey St.-Hilaire, 1809, recognized by a large, robust body, a large adipose fin and a strongly ossified head, has the most fusiform body, while the genus *Dolichallabes* Poll, 1942, with an extreme anguilliform body, continuous unpaired fins and reduced skull, is the most anguilliform within the clariids. In this study *D. microphthalmus* is compared to two other anguilliform species. *Channallabes* Günther, 1873 is a monotypic genus, *C. apus* Günther, 1873 is found in the Congo river basin up to Kisangani, in North Angola and in the Kouilou region in Congo-Brazzaville. The genus *Gymnallabes* Günther, 1867 currently comprises three species: *G. alvarezi* Roman, 1970 (Ogooué river system and Equatorial Guinea), *G. nops* Roberts and Stewart, 1976 (Lower Congo stream) and *G. typus* Günther, 1867 (Niger delta, Nigeria and Cameroon). The phylogenetic relationships of these, phenetically similar, genera is not known, but is the subject of a research in progress.

Presently, anguilliform clariid taxonomy is poorly understood and no reliable, updated keys are available. The only keys incorporating the anguilliform clariids are those of POLL (*e.g.* 1977). The characters used in this key, such as presence of paired fins, number of ribs and vertebrae are no longer discriminative and overlap among species. This is partially due to the limited number of specimens used in the original descriptions of the species (*G. typus*: n=1, *G. alvarezi*: n=1, *C. apus*: n=1, *D. microphthalmus*: n=7). Preliminarily research shows that the above-mentioned species should be valid, but that inferences on a higher taxonomical level are inconclusive at this point (*e.g. G. alvarezi* is phenetically and cladistically very similar to *G. typus* and *C. apus*). The current redescription is based on

the type material and 14 specimens from the Lower and Middle Congo Basin. This expands the size range (66-210mm SL) considerably, compared to that given in POLL (1942b).

The objectives of this paper are (1) to demonstrate that *Dolichallabes microphthalmus* is a valid species differing from other anguilliform representatives *Gymnallabes typus* and *Channallabes apus*; (2) to redescribe *D. microphthalmus* based on a substantially expanded data set (compared to the original description) and (3) to provide diagnostic characters for this species.

MATERIALS AND METHODS

This study is based on all known museum material of *D. microphthalmus*. All 19 specimens are housed in the Royal Museum for Central Africa (MRAC) (Tervuren, Belgium). These include the holotype (MRAC 44655) and the four paratypes (MRAC 44656-659 (n=3) and 62407). Two of these specimens (MRAC 62407 and 79260) are cleared and stained following the procedure of TAYLOR and VAN DYKE (1985), for osteological examination. Since the taxonomic status at the genus level is indistinct, as already mentioned, we compared this material to a large sample of two species, *G. typus* and *C. apus*, which are according to POLL (1977), morphologically the closest relatives of the former species (list of specimens is given in the section 'Comparative material examined'). In this paper we use *G. typus* as the only representative of the genus *Gymnallabes*. This due to the questionable generic placement of *G. alvarezi* (Roman, 1970) (research on the systematic position of *G. alvarezi* in progress)[6], and furthermore we do not take into account the, one specimen based, description of the albino[7] and eyeless species *G. nops* (Roberts and Stewart, 1976), of which only the holotype is known.

Measurements

On each specimen 36 measurements were taken point-to-point using digital callipers to 0.1 mm (digital ruler, Mauser), interfaced directly with a computer.

Measurements terminology follows that of TEUGELS (1986), with some additions: total length (TL); standard length (SL); preanal length (PaL); prepelvic length (PPvL); prepectoral length (PPcL); predorsal length (PdL); distance between the occipital process and the dorsal fin (SPDFL); pelvic fin length (PvFL), pectoral fin length (PcFL); pectoral

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[6] has been transferred to *Channallabes alvarezi*

[7] this according to the original description, but IV.3.1 shows that this is not the case

spine length (PcSL); caudal peduncle depth (CPD); body depth at anus (ABD); maxillary barbel length (MxB); external mandibular barbel length (EmnB); internal mandibular barbel length (ImnB); nasal barbel length (NB); interpelvic distance (IpvD); interpectoral distance (IpcD); skull length (SkL); preorbital length (PoL): measured from the tip of the premaxillae to rostral border of the eye; skull width (SkW); supraoccipital process length (SpL); supraoccipital process width (SpW), interorbital distance (IoD); anterior nostril interdistance (ANID); posterior nostril interdistance (PNID); rostral skull width (RSkW): measured at the level of the maxillary barbel; orbital skull width (OskW): measured at the level of the eyes; skull height (SkH); eye diameter (ED); snout height (SnH); prehyoid length (PhL): measured from tip of premaxillae to hyoid skinfold; internal mandibular interdistance (ImnID); external mandibular interdistance (EmnID); mouth width (MW) and skull roof width (SkR): minimal width. For the analyses, measurements involving the paired fins are not considered due to their high level of intraspecific variation in their absence or presence in the anguilliform catfishes (ADRIAENS et al., 2002). For discussion on the nomenclature of the circumorbital bones in clariids, we refer to ADRIAENS et al. (1997).

The following meristic counts were made on each specimen with a MPG 65 generator and a RSN 620 X-ray-tube (General Electric) (42kV, 320Ma, 10msec, focus distance: 1m): total number of vertebrae (TV), number of ribs (RB). Holotype values are given in parentheses. Institutional abbreviations follow LEVITON et al. (1985).

DOLICHALLABES MICROPHTHALMUS Poll, 1942

Specimens Examined

Dem. Rep. Congo. Kunungu, MRAC 44655, adult male, 229 mm SL (holotype), MRAC 44656-659 (n=3) (196-210 mm SL) and 62407, 188 mm SL (paratypes), MRAC 57662, 196 mm SL, MRAC 18850, 90 mm SL; Boende swamps, MRAC 101843, 149 mm SL, MRAC 176123-124 (n=1), 68 mm SL; Bokuma, MRAC 79093, 134 mm SL, MRAC 93774, 66 mm SL; Bokuma – Tchuapa, MRAC 79258-260 (n=3) (85-126 mm SL); Ndwa (Boloko), MRAC 78808-810 (n=3) (99-110 mm SL); Inonge, MRAC 96672, 110 mm SL; Maylimbe, Tshela, MRAC 66721, 97 mm SL.

Diagnosis

Dolichallabes microphthalmus can be distinguished from *C. apus* and *G. typus*, by the presence of one elongate fontanel; a reduced set of circumorbital bones (only antorbital and infraorbital IV are present); the presence of at most two suprapreopercular bones and the sphenotic bearing only one process. *Dolichallabes microphthalmus* can be further

distinguished from *C. apus* by a narrow skull roof, a small distance between supraoccipital process and the dorsal fin, low number of ribs (6-9 instead of 10-17) (**Plate IV.4-1B**) and by a high number of pre-caudal rib less vertebrae (10-12 instead of 1-8); *D. microphthalmus* can be further distinguished from *G. typus* by a very elongate body (SL/ABD: 18-31 instead of 12.6-22), a narrow and short supraoccipital process, a large distance between the supraoccipital process and the dorsal fin and a high number of vertebrae (95-116 instead of 78-86) (**Plate IV.4-1A**).

Description

Proportional measurements and counts given in **Table IV.4-1**. *Dolichallabes microphthalmus* characterized by very elongate body (**Plate IV.4-1C**) (ABD up to 3.4-5.8% of SL). Skull length to SL ratio very small. Skull width 45.5-62.5% of skull length. Very narrow skull roof, width 25-40% of maximal skull width. Large specimens with skull roof nearly hidden by large, dorsomedial outgrowth of hypertrophied adductor mandibulae complex (pars A_2A_3'). In smaller specimens, adductor mandibulae situated lateral to skull roof (at level of lateral ethmoid and frontal), leaving skull roof clearly visible. Eyes small.

Mouth width equals or exceeds interorbital distance. Lower lip equals or overgrows upper lip. Distinct tube-like anterior nostrils are present.

Unpaired fins continuous. Pectorals always present, although in some specimens extremely reduced. Length 10- 41% of SkL. Pelvic fins present, though very small, in only two specimens (MRAC 78808-810 and MRAC 66721); in the other specimens no evidence of pelvic fins. Supraoccipital process-dorsal fin distance small. Vertebrae 95-114 (mode = 109) (112). Ribs 6-9 (mode = 8) (6). Branchiostegal rays 10 (10). Dorsal fin (156). Anal fin (136).

Teeth present on premaxilla and prevomer. On premaxilla, tooth plates width 30% of tooth plates length. Prevomer tooth plate shows two backwardly-curved wings, as in most other clariids (CABUY et al., 1999; DEVAERE et al., 2001 (**IV.5.1.a**)).

The coloration of the alcohol preserved specimens mainly brown, with continuous transition from darker dorsal side to lighter ventral side. Unpaired fins light brown. Pectorals and nares lack any colour. The barbels have light-brown coloration except for darker bases.

In the skull roof, both anterior and posterior fontanel elongate and continuous with each other (**Plate IV.4-2B**), resulting in typical longitudinal furrow on dorsomedial skull roof. Left and right frontals do not come in contact, apart from the ossification surrounding the epiphysial bridge.

Extreme reduction of circumorbital series. Except for infraorbital IV and antorbital, all bones of that series as well as the lacrimal (1st infraorbital) absent. Infraorbital IV reduced to tube-like bone and only remaining protection for infraorbital canal. This is the most reduced situation observed within anguilliform clariids[8].

Suprapreopercular bones, enclosing proximal part of preoperculo-mandibular canal, also reduced. One or two suprapreopercular bones present, with dorsal one always bearing plate-like extension and ventral ones tubular.

Sphenotic of *D. microphthalmus* only has small cartilaginous articulatory ridge for hyomandibula and one distinct process that descend to fix latter at its rostral margin. Pterotic bears two or three processes, which lock posterior hyomandibular processes.

Distribution

Dolichallabes microphthalmus was originally described from Kunungu, close to the central Congo River. Furthermore this species is found in the same region around Bolobo and in the swamp areas near to Boende, Bokuma (Tshuapa) and Ingonge, in the Ruki Basin; it has also been collected around Maylimbe, Tshela region, on the Lower Congo Basin (**Plate IV.4-2C**).

DISCUSSION

According to POLL (1942b, 1977), *Dolichallabes* is diagnosed from other anguilliform species by a very elongate body; small skull roof, covered with muscles; eyes hardly visible, covered with skin; small pectoral fins and the absence of pelvic fins; 106 vertebrae and 6 pairs of ribs. Based on this study, Poll's diagnostic characters do not delineate *D. microphthalmus* unambiguously.

The two best characteristics for diagnosing *D. microphthalmus* (**Plate IV.4-2B**), are (1) the presence of a single large fontanel in the skull roof, compared to two smaller fontanels (anterior and posterior) in other clariids (see below) and (2) infraorbital IV and antorbital as the only two bones present in the circumorbital series.

The situation of both characters in *D. microphthalmus* appears to correspond to that of larval *Clarias* (ADRIAENS and VERRAES, 1998). During the early ontogeny of the skull roof in *C. gariepinus* (Burchell, 1822), a large fontanel is enclosed by the mesethmoid, the frontals and the parieto-supraoccipital bones. Later on, the configuration as observed in *D. microphthalmus* is reached, where the frontals contact each other anteriorly and at the

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[8] comparable to the situation in *Gymnallabes nops*

level of the epiphysial bridge. In *C. gariepinus*, as well as in all other clariids that have been examined, the large fontanel becomes subdivided as the posterior part of the frontals and anterior part of the parieto-supraoccipital contact each other at the midline (DAVID, 1935; POLL, 1977). Consequently, an anterior fontanel is bordered by the paired frontals, whereas a posterior fontanel is enclosed by the unpaired parieto-supraoccipital bone (whose anterior halves subdividing the initial large fontanel have completely fused).

During that same period after hatching the different bones of the circumorbital series are formed. Again in *C. gariepinus*, it is shown that the lacrimal (infraorbital I) and the antorbital are the first bones of that series formed, followed by infraorbital II. In the next stage infraorbital III and IV are the last bones that are formed (ADRIAENS and VERRAES, 1998). In *C. gariepinus* there is an antero-posterior developmental sequence.

When we compare the extreme reduction in the morphology of the circumorbital bones in *D. microphthalmus* to those of *C. apus* and *G. typus*, only a tubular infraorbital IV and antorbital are present in *D. microphthalmus*. Within *G. typus* and *C. apus* all infraorbital bones (II-IV), the antorbital and the lacrimal, are still present, although very small compared to most other clariids (CABUY et al., 1999; DEVAERE et al., 2001 (**IV.5.1.a**)). In both species, the infraorbitals are reduced to tubular bones protecting the infraorbital canal, except for the infraorbital IV in *C. apus* where a lamina is still present. As a consequence the eye and especially the infraorbital canal may no longer be sufficiently supported and protected. The reduction of the eye could then be considered as being advantageous when proper protection is lacking. However, both characters could be a consequence of the extensive jaw muscle hypertrophy. These similarities of the diagnostic features of *D. microphthalmus* with early stages of *C. gariepinus* suggest that *D. microphthalmus* could be a paedomorphic clariid. However, it has to be noted that both traits seem to be the result of different heterochronic processes, since only one of them (the neurocranial fontanel) strictly follows the ontogenetic sequence of *Clarias*.

A remarkable feature within the Clariidae is the presence of a continuous series of morphotypes between fusiform and anguilliform genera. The elongation of the body coexists with a whole set of morphological changes, *e.g.*: continuous median fins, reduction of the paired fins, reduction of the eyes, reduction of several skull bones and hypertrophied jaw muscles. *Dolichallabes microphthalmus* can be considered as being the most anguilliform clariid from a group showing similar adaptations. Therefore it is important to look from this point of view at the different characteristics these anguilliform clariids have in common, as well as look for additionally characters in which they differ.

The high level of variation in the paired fins is not only present in *D. microphthalmus*, but also in other anguilliform clariids (*e.g. G. alvarezi* and especially *C. apus*). This

intraspecific level of variation represents a unique example of morphological variability at a micro-evolutionary level. In these species, fin reduction is related to body elongation and also coupled to girdle reduction, as observed in *C. apus* where the loss of pelvic fins is accompanied by the loss of the pelvic girdle (ADRIAENS et al., 2002). This makes the absence of paired fins no longer diagnostic for distinguishing the anguilliform clariid species. Additionally, pelvic fin loss has been reported for non-anguilliform clariids as well (POLL, 1941). Furthermore, the loss of fins in clariids may be related to a highly specialized fossorial life, as is observed in other vertebrates by O'REILLY et al. (1997).

Another adaptation to this fossorial life style is a reduced eye (WITHERS, 1981), since the utility of eyes is questionable in a burrowing life-style. Reduced eyes are also present in the other anguilliform species (CABUY et al., 1999; DEVAERE et al., 2001 (**IV.5.1.a**)) and some mastacembelid species (POLL, 1973). A reduced eye size is a transformation related to the benthic and nocturnal life-style of Siluriformes, as is also reflected in the general morphology of the chondrocranium (DAVID and POLL, 1937; ADRIAENS and VERRAES, 1997a, b).

The reduction of several skull bones (*e.g.* infraorbitals, frontals) and a hypertrophy of the adductor-mandibulae complex can be linked, since the reduction of these bones facilitates a more extensive growth and expansion in volume (*e.g.* during contraction) of the jaw muscles (CABUY et al., 1999). The reduction of the skull roof is especially marked in the orbitotemporal and otoccipital region (DEVAERE et al., 2001 (**IV.5.1.a**)). The orbitotemporal region of the skull in *D. microphthalmus* and *G. typus* is narrower, compared to that of *C. apus* (**Plate IV.4-2A,B**) (POLL, 1942b; CABUY et al., 1999).

The number of suprapreopercular bones is also reduced in contrast to *C. apus* and *G. typus,* where up to three suprapreopercular bones are present. At the level of the sphenotic there is also a reduction in the number of processes and the size of the articulation ridge.

COMPARATIVE MATERIAL EXAMINED

Channallabes apus. Angola. Ambriz, BMNH 1873.7.28.16 (Holotype); Other specimens, Dem. Rep. Congo. Bokalakala, MRAC 175247-270 (n=10); Kinshasa, MRAC 97-056-P-0001-0003 (n=2); Bumba, MRAC 88-25-P-2192-227 (n=36); Boma, MRAC 939; Riv. Lula, Bushimaie, MRAC 153505; Kelé, MRAC 1491; Stanleyville, MRAC 30893-30900 (n=8), MRAC 88-01-P-1976-1992 (n=17); Riv. Ruki, Eala, MRAC 14747-49 (n=3); Lake Tumba swamp area, MRAC 46299; Katanga, MRAC 39480; Riv Botota, keseki, MRAC 67763-77 (n=15); Mwilambongo, MRAC 72886-887 (n=2); Dekese, Riv. Lofu, Anga, MRAC 153352; Yangambi, MRAC 68700; Riv. Oubangui, Imfondo, MNHN 1922-0029; Loango, MNHN 1924-0079, MNHN 1924-0080;

Sangha, MNHN 1925-0137; Mogende, MNHN 1926-0155-59; Riv Congo, MNHN, 1937-0124-25; Stanleypool, Bamu, MNHN 1958-0111; Boloko, Riv. Likouala, MNHN 1962-0401 (n=7); Mossaka, Riv. Likouala, MNHN 1963-0402 (n=2); Riv. Loadjili, Songolo, MNHN 1967-0143 (n=6); Mangala, BMNH 1896.3.9.17; Riv. Lebuzi, Kaka Muno, BMNH 1912.4.1411-12 (n=2); Lower Congo, BMNH 1887.1.13.8-9 (n=2); Stanley Falls, BMNH 1889.11.20.5; New Antwerp, Upper Congo, BMNH 1899.2.20.16; Siala-Ntoto Swamps, BMNH 99.11.27.92; Bangyville, Ubangi, BMNH 1907.12.26.34; Kashi, Lulua, MHNG 1248.3; Banana, NMW 47240-42; Mollunda, NMW 47245 (n=4), NMW 47246. Congo. Yangala Youbi, MNHN 1967-0146; Djembo, Kouilou, MNHN 1967-0147; Cayo, MNHN 1989-0527; Riv. Nanga, between Boukou-Zassi and Kouilou swamp area, MRAC 90-57-P2315; Sintou, Riv. Kibombo, Kouilou, MNHN 1967-0144; Riv. Loadjili, Songolo, Pointe Noire, MNHN 1967-0145 (n=6); Riv. Youbi, Noumbi.Angola. Caungula, Mabete, Riv. Uamba, MRAC 162088; Riv. Camuconda, Tchimenji, MRAC 162089, MRAC 162090-094 (n=5), MRAC 162095-100 (n=6); Riv Ganga-Ludchimo, MRAC162083-086 (n=4)

Gymnallabes typus. Nigeria. Old Calabar, BMNH 1866.12.4 (n=2) (Syntypes); Other specimens, Nigeria. Umu-Eze Amambra, MRAC 84-16-P-1-2; Riv. Sombreiro, East of Erema, MRAC 91-067-P0134; Niger Delta, MRAC 97-030-P-0001-0010 (n=10); lake Odediginni, Agudama, Yenagoa, MRAC 92-083-P-0035-0036; Okaka, Epie Creek, Between Nun an Rashi Riv, MRAC 97-085-P-0001-0004 (n=4); Riv Sombreiro, Odiemerenyi, Ahoada, MRAC 91-067-P-0135-0136; New Calabar, Choba, MRAC 91-105-P-1; Rumuji Swamps, MRAC 86-10-P-72; Oshika, MRAC 84-28-P-28, MRAC 84-28-P-25; Riv. Cron, Itu, MRAC 88-36-P-10; Between Sapele and War, Niger Delta, MRAC 74-29-P-600; Muoha, New Calabar, MRAC 91-10-P-478; Biseni, taylor Creek, MRAC 91-01-P278; Ossomari, BMNH 1902.11.10.119. Cameroun. Riv. Kom, Ntem, Aboulou, MRAC 73-18-P-3307-309.

IV.5 - The genus *Channallabes*

IV.5.1 - The Congo River specimens

IV.5.1.a Cranial morphology of the anguilliform clariid *Channallabes apus* (Günther, 1873) (Teleostei: Siluriformes): are adaptations related to powerful biting?

ABSTRACT

Within the clariids (air-breathing catfish), a complete range of fusiform to anguilliform species can be observed. This study deals with the cranial morphology of *Channallabes apus*, an extreme anguilliform (eel-like) species, compared to the likewise anguilliform *Gymnallabes typus* and the more fusiform *Clarias gariepinus*. The overall morphology of the head of *C. apus* shows a hypertrophied adductor mandibulae complex, with the corresponding substantial narrowing of the neurocranium, seen in the frontals, the sphenotics, the pterotics and the posttemporo-supracleithrums, as well as the reduction and displacement of the eyes and some canal bones, such as the infraorbitals and the suprapreopercles. The presence of a hypertrophied muscle complex leads to the assumption that a more powerful bite may occur. This implies that adaptations can be expected in several parts of the skull: on the lower jaw of *C. apus* a higher coronoid process is found; on the suspensorium, two sets of three processes are present on the hyomandibula, indicating a stronger connection to the neurocranium. Several of the observed features, such as the elongation of the body, the reduction of the eyes, the increase in vertebrae number, limblessness and the increasing rigidity of the skull, may be related to a process of miniaturization[9].

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[9] The evolution of extremely small body size within a lineage. Extremely small size is identified as the size class at which important physiological or ecological functions are affected (Rieppel, 1996)

INTRODUCTION

Catfishes belonging to the family Clariidae are found all over Africa, as well as in the Middle East and parts of Asia (GREENWOOD, 1961; TEUGELS, 1996). More generalized species, like *Clarias gariepinus* (Burchell, 1822) and *Heterobranchus longifilis* Valenciennes, 1840 have a wide distribution in Africa, whereas that of the highly specialized anguilliform (eel-like) species is restricted to Central and West Africa (BOULENGER, 1911; POLL, 1957a; TEUGELS, 1986; TEUGELS et al., 1990; TEUGELS et al., 1992; SKELTON, 1993). It is the latter group of anguilliform genera that makes the clariid family unique among teleost fishes, *i.e.* an evolutionary transformation exists of fusiform species into anguilliform species, within one family (PELLEGRIN, 1927). Initially, it was suggested that a gradual trend can be observed in this family, in which the genus *Heterobranchus* Geoffrey St-Hilaire, 1809 could be placed at the one end and the anguilliform *Dolichallabes* Poll, 1943 at the other end. This trend not only involved the striking transformation of both paired and unpaired fins, but also the cranial morphology.

Consequently, a basic morphological description of the skull in several of these anguilliform species was made (DAVID, 1935; POLL, 1942b, 1957b, 1977). These studies demonstrated the presence of a hypertrophied adductor mandibulae complex in all the anguilliform genera, *i.e. Platyallabes* Poll, 1977*, Platyclarias* Poll, 1977*, Gymnallabes* Günther, 1867*, Channallabes* (Günther, 1873) and *Dolichallabes*, which could be related to the reduction of several cranial bones. However, a detailed morphological description of these species is still lacking. Previous research has focused on the cranial morphology of *Gymnallabes typus* Günther, 1867, in which special adaptations to a powerful closure of the mouth were observed (CABUY et al., 1999).

Although at first sight, the Clariidae appear to demonstrate a gradual speciation, which involved an increasing anguilliformity (eel-like shape) coupled to a hypertrophy of the adductor mandibulae complex, arguments can be made to support the hypothesis that anguilliformity evolved several times, as well as the fact that anguilliformity is not related to adductor mandibulae morphology. A polyphyletic origin of anguilliformity, involving the reduction of paired fins and elongation of the dorsal and anal fin, has already been suggested (PELLEGRIN, 1927; POLL, 1977). The hypothesis that anguilliformity should not be coupled to adductor mandibulae hypertrophy is, for example supported by the large muscle complex in the non-anguilliform *Tanganikallabes* Poll, 1943. In order to investigate the true nature of the cranial adaptations in those clariid species, the detailed morphology of all those species needs to be studied, thus enabling the discrimination of possible adaptive trends. As part of this research, the cranial morphology of *Gymnallabes typus* has

been studied, as a representative with extremely large adductor muscles. On the other hand, *Channallabes apus* (Günther, 1873) is known as one of the most anguilliform species (PELLEGRIN, 1927), where the adductor muscle is not as strongly developed as in *Gymnallabes* (POLL, 1942b; POLL, 1957a). For that reason, we made a detailed morphological study of the skull of *Channallabes apus*, paying special attention to adaptations for powerful closure of the mouth.

MATERIAL AND METHODS

The material examined in the present study was obtained from the Koninklijk Museum voor Midden-Afrika/ Musée Royal de l'Afrique centrale (KMMA), Tervuren, Belgium, as well as from aquarium import. Museum specimens of *Channallabes apus* (KMMA 175247-270) were collected in the Mbole River, (Democratic Republic Congo). For comparison, specimens of *Clarias gariepinus* and *Gymnallabes typus* were used. Specimens of *G. typus* originate from the Nyanga River (Gabon) (KMMA 179111-113), the Odeginni lake (Nigeria) (KMMA 92-083-P-0035-0036), and the Sombreiro River (Nigeria) (KMMA 91-067-0135-0136).

Descriptions of the cranial morphology of *C. gariepinus* and *G. typus* have already been published (ADRIAENS and VERRAES, 1996, 1997a–e, 1998; ADRIAENS et al., 1997; CABUY et al., 1999).

The osteology was studied using a total of four specimens of *C. apus*, measuring between 210 and 302 mm standard length (SL). They were cleared and stained according to a modified protocol of HANKEN and WASSERSUG (1981) (trypsine being replaced by a 5% KOH-solution). Three specimens, measuring between 236 and 245 mm SL, were used to study the external morphology as well as the myology by means of dissection and muscle fibre staining (BOCK and SHEAR, 1972). Drawings were made with a stereoscopic microscope (Wild M5) with a camera lucida.

Specimens of *C. gariepinus* measured between 126 and 136 mm SL (a total of 4 specimens), whereas the three specimens of *G. typus* measured between 217 and 239 mm total length.

Terminology of the cranial skeleton follows ADRIAENS and VERRAES (1998), whereas that of the cranial myology follows WINTERBOTTOM (1974).

External morphology

The preserved specimens of *Channallabes apus* showed a darkish brown skin, with the dorsal side being darkest. The degree of anguilliformity can be expressed as ratio of the total length and body height (POLL, 1942a). In the specimens examined, this ratio lies between 18.6 and 23.6 for *C. apus*, between 15.4 and 19.6 for *G. typus*, and between 7 and 9 for *C. gariepinus*. The head length (definition measurements: TEUGELS, 1986) only comprised between 8.0 and 9.1% of the standard length in *C. apus*, between 10.0% and 11.7% of the total length in *G. typus*, and between 26.6% and 35.0% of the standard length in *C. gariepinus* (TEUGELS, 1986; CABUY et al., 1999).

Although not bulging, as in *Gymnallabes typus*, the position of the adductor mandibulae complex can be distinguished as it is hardly covered by bones (**Plate IV.5-1A,B**). Also compared to *G. typus*, the snout appears to be more pointed, due to the fact that the skin folds surrounding the barbel bases are markedly smaller in *C. apus* (CABUY et al., 1999: Fig. 1). The eyes are largely reduced, compared to *C. gariepinus*, but less than in *G. typus*. The dorsal fin originates close to the supraoccipital process of the head, and forms a continuous finfold, with the anal and caudal fins. No external traces of pectoral or pelvic fins could be discerned.

Cranial skeleton

Neurocranium

The narrow neurocranium of *Channallabes apus* consists of a rigid complex of dermal, endo- and perichondral bones (**Plate IV.5-1C**). The orbito-temporal region of the skull is markedly less broad than that of *Clarias gariepinus* (**Plate IV.5-1D**), but broader than that of *Gymnallabes typus* (CABUY et al., 1999: Fig. 3A).

Ethmoid region: The slender, forked mesethmoid bone supports the underlying premaxillary bones and partially encloses the tubular nasal bone. From the latter bone, the supraorbital canal continues in-between the lateral ethmoid and the mesethmoid bones into the frontal bones. Three configurations could be discerned in *C. apus* specimens: (1) the anterior portion of the supraorbital canal not being covered by bone; (2) the anterior portion being covered by a lamellar outgrowth of the mesethmoid; and (3) being covered by a lamellar outgrowth of the lateral ethmoid. As in other clariids, the mesethmoid forms the anterior border of the anterior fontanel. The lateral ethmoid bears a pointed, lateral process, which has lost the connection with the second infraorbital bone, compared to *C. gariepinus*. Ventrally, the lateral ethmoid articulates with the large

palatine. The axe-shaped prevomer carries a large, semilunar tooth plate and shows the elongated interdigitation with the parasphenoid, as observed in other clariids.

Orbital region: The circumorbital series in *C. apus* comprises the antorbital bone and a series of four infraorbital bones, as in *C. gariepinus*. Most of these bones appeared to be tubulous, which is in contrast with *C. gariepinus* (**Plate IV.5-1C,D**). Only infraorbital bone IV contains a plate-like extension, although much reduced compared to *C. gariepinus*. The antorbital is a very small bone, lying at the base of the nasal barbel, onto the rostral tip of the palatine. In contrast to *C. gariepinus*, the antorbital did not enclose the rostral part of the infraorbital canal in *C. apus*. The tubulous lacrimal lies at the level of the olfactory organ, whereas the eye is bordered ventrally by the second and third infraorbitals. The plate-like fourth infraorbital only borders the eye caudally, as the supraorbital process of *C. gariepinus* is lacking. In one specimen, this infraorbital bone was subdivided into two plate-like ossicles. From this infraorbital bone, the infraorbital canal continues into the frontal bone, instead of the dermosphenotic bone as in *C. gariepinus* as well as in most teleosts (DAGET, 1964; GOSLINE, 1975). The latter was also observed in *G. typus*, as well as in some other specialized siluriform (LUNDBERG, 1982). The dermosphenotic bone, however, is strongly reduced, compared to *C. gariepinus*. The skull roof of the orbito-temporal region is formed by the large frontal bones, which enclose the anterior fontanel. The lateral wall of the skull is constituted by the orbitosphenoid and pterosphenoid (synonym pleurosphenoid, DAGET, 1964) bones, which are connected to the parasphenoid ventrally.

Temporal region: The skull wall at this level is formed by the sphenotic and pterotic bone complexes, which form the only firm connection between neurocranium and suspensorium. Special adaptations for a firm connection could be observed, as both bone complexes formed distinct processes, which interdigitate with corresponding processes on the suspensorium (**Plates IV.5-2b, IV.5-3A**). The lateral plates of both the sphenotic and pterotic bones are lacking, as was observed in *G. typus*, but were much more elaborated in *C. gariepinus*. Ventrally, the brain and labyrinth organ are covered by the parasphenoid and the prootic bones.

Occipital region: The caudal part of the skull roof is formed by the parieto-supraoccipital bone (a fusion of the two bones has been suggested in FINK and FINK (1996), enclosing a central fontanel (**Plate IV.5-1C,D**)). The latter bears a strongly pointed supraoccipital process, comparable to the one in *G. typus*, but much more slender than that in *C. gariepinus*. The narrow pterotic bones interconnect the parieto-supraoccipital bone with the posttemporo-supracleithral bone, which in turn connects to the pectoral girdle, as well as the parapophyses of the fourth vertebra by means of a solid transscapular

process. Ventrally, in occipital region of the skull consists of the exoccipitals, the basioccipitals and small epiotics; the latter however are substantially larger in *C. apus* and *G. typus*, compared to *C. gariepinus*.

Splanchnocranium

Maxillary bones: The premaxillaries are large, plate-like bones bearing a large number of teeth (**Plate IV.5-3A**). The surface of the latter bone has substantially increased, compared to *C. gariepinus*. In comparison to *G. typus*, the dentate surface is larger, as dentition ranges closer to the posterior border (Cabuy et al., 1999: Fig. 4A). The maxillary bone forms a cup-like bone, which encloses the base of the maxillary barbel and bears two articulatory facets for the articulation with the palatine.

Mandibula: As in other clariids, the lower jaw consists of two parts: the os dento-splenio-mentomeckelium and the os angulo-splenio-articulo-retroarticulare (**Plates IV.5-2A, IV.5-3B,C**). The posterior dental teeth are attached more posteriorly, closer to the base of the coronoid process, which is hardly the case in *C. gariepinus*. This posterior margin of the tooth series reaches even further in *G. typus* than in *C. apus* (Cabuy et al., 1999: Fig. 6C). The coronoid process, which is situated at the interdigitation between the two mandibular bone complexes, is strikingly larger in *C. apus* than in *C. gariepinus*, which is also the case for *G. typus*. Medially, the Meckel's cartilage lies partially exposed, with the caudal part being covered by the sesamoid coronomeckelian bone. Caudal to the mandibula, a small tubulous ossicle, referred to as the splenial, could be discerned.

Palatine: The rod-like palatine lies ventrally against the lateral ethmoid, with which it articulates. The articulatory facet on the palatine is oval shaped, in contrast to the slender facet in *C. gariepinus* (Adriaens and Verraes, 1998: Fig. 20C). Anteriorly, the palatine articulates with the modified maxillary bone, being part of the palatine-maxillary mechanism. As in other clariids, both the anterior and posterior tip of the palatine remain cartilaginous.

Hyoid arch: The hyoid arch consists of the two ceratohyals (anterior and posterior) and the two hypohyals (ventral and dorsal) (**Plate IV.5-4A**). The general morphology of the hyoid arch and its components strongly resembles that of *C. gariepinus* (Adriaens and Verraes, 1998: Fig. 22A). Ventrally, the hyoid arch articulates with eight branchiostegal rays (exceptionally nine). The anterior six (or seven in the case of nine rays) articulate with a ventral rim onto the anterior ceratohyal. The following ray articulates with a ventral cartilaginous region, separating the anterior and posterior ceratohyal, whereas the posterior ray articulates with the posterior ceratohyal (**Plate IV.5-3A**). To the latter ceratohyal, the ligamenta hyomandibulo-ceratohyale and angulo-ceratohyale are attached.

The parurohyal (**Plate IV.5-2A**) resembles that of other clariids, bearing two lateral and one medial processes. The parurohyal is connected to the ventral hypohyal through the ligamentum parurohyalo-hypohyale. In contrast to *C. gariepinus,* this ligament is odd in *C. apus*.

Suspensorium: As in most siluriforms, the suspensorium consists of the hyomandibula, the quadrate, the metapterygoid, the entopterygoid and the preopercle (**Plate IV.5-4B,C**). The hyomandibula suspends the suspensorium with the neurocranium, in relation to which some modifications can be discerned as several anterior and posterior processes have formed. In-between these two sets of processes, lies the slender and short articulatory facet of the hyomandibula. The anterior ones fit into similar processes of the sphenotic, whereas the posterior ones fit into gaps onto the pterotic (**Plate IV.5-2B**). At the anterior margin of the perichondral part, the hyomandibula bears a large, bony plate, as was also observed in other clariids. Ventrally, the hyomandibula interdigitates with the preopercula, thereby bordering the foramen of the hyomandibular truncus (**Plate IV.5-4B,C**). The opercular process of the hyomandibula is markedly directed ventrally, comparable to the situation in *G. typus*, but more ventrally than in *C. gariepinus*. Medially, the hyomandibula bears a bony ridge for the attachment of the ligamentum hyomandibulo-ceratohyale. The quadrate is comparable to that of other clariids, except for the rostral part. In *C. apus*, the quadrate interdigitates with both the entopterygoid and the metapterygoid, whereas in other clariids, the connection with the entopterygoid is absent. This quadrate-entopterygoid suture spans almost over three quarters of the suspensorial width at that level, which is consequently reflected in the absence of the dorsal, plate-like extension of the metapterygoid, observed in other clariids. Medially, a non-ossified symplectic cartilage can be observed, in-between the quadrate and hyomandibular bones (**Plate IV.5-4C**). The articulatory facet, for the articulation with the mandibula, is markedly larger in *C. apus*, compared to *C. gariepinus*. As already mentioned, the metapterygoid is small in *C. apus*, as it mainly consists of the perichondral part. As in other clariids, the connection with the quadrate is through a synchondrosis, whereas that with the entopterygoid is ligamentous and sutures are lacking. The plate-like entopterygoid strongly interdigitates with the quadrate, a feature observed in some other clariids (*e.g. Clariallabes, Heterobranchus*) (DAVID, 1935). Anteriorly, the entopterygoid is connected ligamentously to the prevomer, the palatine and the lateral ethmoid, thus corresponding to an 'entopterygoid type 4' (ARRATIA, 1992).

Branchial arches: The "Bauplan" (terminology: VERRAES, 1981) of the branchial basket corresponds to the general clariid situation (**Plate IV.5-5**), with the exception of the number and size of the gill rakers. These gill rakers are smaller, both in size and number in

C. apus, compared to *C. gariepinus* and even compared with *G. typus*. For a detailed description of this general configuration, we refer to ADRIAENS and VERRAES (1998).

Opercular series

The opercular bone is a pointed, triangular bone, bearing a large articulator facet for articulation with the suspensorium (**Plate IV.5-3A**). Dorsal to this articulator facet, the opercular bone bears a plate-like process, onto which some of the opercular muscles are attached (see below). The posterior part of this bone consists of a horizontal ridge (for the attachment of the opercular levator muscle), as well as a small ventral plate. As in most other teleosts, the opercular is ligamentously connected to the interopercular bone (DAGET, 1964). The latter bone is comparable to that of other clariids, including the ligamentous connection with the lower jaw. As already mentioned, the preopercular bone is firmly connected to the suspensorium, as it encloses the preoperculo-mandibular canal (**Plate IV.5-4B,C**). The caudal part of the latter canal is enclosed in a series of small suprapreopercular bones, of which the proximal ones are tubulous. The number of suprapreopercular bones ranged from two to three, with the proximal one always bearing a plate-like extension. As in *G. typus*, the surface covered by the suprapreopercular bones is much reduced, compared to the situation in *C. gariepinus*. From the distal suprapreopercular bone, the preopercular canal continues into the pterotic bone.

Cranial myology

Muscles of the lower jaw

M. adductor mandibulae: The adductor mandibulae of *C. apus* is an enormous muscle complex, covering almost the entire lateral side of the skull (**Plate IV.5-6A,B**). It consists, as in other clariids studied, of an external A_2A_3'-part and an internal A_3''-part (terminology: VETTER, 1878), which are separated by the levator arcus palatini (ADRIAENS and VERRAES, 1996). The bipinnate A_2A_3' muscle forms the largest part of the complex, with its tendon inserting onto the lower jaw, at the level of the coronoid process. The fibres of the dorsal $A_2A_3'\alpha$ run more vertically, whereas those of the ventral $A_2A_3'\beta$ lie more horizontally. Anteriorly, the dorsal part of the adductor complex is attached directly to the infraorbitale IV, the frontal, the sphenotic, the pterotic, the suprapreopercle bones and the posttemporo-supracleithrum. The ventral $A_2A_3'\beta$ inserts onto the lateral side of the suspensorium: the quadrate, the preopercle and the hyomandibula. The A_2A_3' covers the levator arcus palatini, the dilatator operculi and the adductor operculi. The complete

covering of the latter two muscles could also be observed in *G. typus*, but was only partial in *C. gariepinus*.

The deeper part of the complex, the A$_3$", is linked to the neurocranium and the suspensorium (**Plate IV.5-6C**). As in other clariids, the A$_3$" can be divided in a lateral *pars superficialis* and a medial *pars profunda*. The *pars superficialis* forms the largest part, but with a smaller tendon compared to the other one. The insertion occurs onto the frontal, the sphenotic and the pterosphenoid, as well as onto the caudal part of the quadrate and hyomandibular membranous plate. The *pars profunda* is completely separated of the *pars superficialis*, as is the case in both *C. gariepinus* and *G. typus*. The vertically directed fibres originate on the hyomandibular membranous plate, inserting tendinously onto the antero-medial side of the angular-complex.

M. intermandibularis: This muscle is a short, broad muscle covering the mandibular symphysis, ventral on the rostral ending of the mandibula (**Plate IV.5-7A**). As in other clariids, the intermandibular muscle is separated from the protractor hyoidei through the interconnecting part of the left and right mandibular barbel base.

Suspensorial muscles

M. levator arcus palatini: As in other clariids, the levator arcus palatini is a thin muscle sheet, interconnecting the lateroventral ridge of the neurocranium with the suspensorium, thereby separating the two parts of the adductor mandibulae complex (**Plate IV.5-6C**). Compared to other clariids, this levator is much reduced in *C. apus*, although in all of them, the origin is spread over the lateroethmoid and the frontal bones. It is constituted of a tendinous part for more than three quarters of its length (in *C. gariepinus*, this is only half of the muscle)(ADRIAENS and VERRAES, 1997d: Fig. 7B). Compared to *C. gariepinus* and *G. typus*, however, the levator muscle has become much smaller. As a result, the posteriormost fibers originate from the frontal bone, at the level of the mandibular articulation (whereas in the other species these fibres lie well posterior to that articulation). The long tendon inserts onto the anterior perichondral part of the hyomandula.

M. adductor palatini: The adductor arcus palatini connects the neurocranium with the dorsal rim of the suspensorium (**Plate IV.5-6D**). The muscle fibres originate mainly on the lateral side of the parasphenoid but also on the orbito- and pterosphenoid. The insertion on the suspensorium is spread over the quadrate and hyomandibular membranous plates, as well as the entopterygoid. Due to the direct connection between the entopterygoid and the quadrate, the insertion onto the metapterygoid is lost in *C. apus*.

Opercular muscles

M. dilatator operculi: The flattened dilatator operculi originates from the frontal, the sphenotic, the pterotic and the dorsal part of the hyomandibula. The fibres attach through a long and narrow tendon to the dorsal processus of the opercle, close to the articulation with the hyomandibula and lateral to the insertion of the adductor operculi. Whereas in *C. gariepinus* and in *G. typus* the anterior dilatator fibres contact the posterior fibres of the levator arcus palatini, both are well separated in *C. apus*, as a result of the narrowing of the latter muscle. The dilatator seems to positioned (or tilted) more posteriorly as well, as it no longer covers the caudal part of the $A_3"$ and it inserts onto the pterotic, which is not the case in *C. gariepinus* or in *G. typus*.

M. adductor operculi: The adductor operculi connects the dorsocaudal ridge of the hyomandibula to the opercle. It is attached to the connective tissue covering the suprabranchial cavity, as well as to the suprapreopercular bone and the ventral side of the pterotic. This muscle attaches musculously to the dorsal process of the opercular bone, where the fibers join the dilatator operculi tendon.

M. levator operculi: The levator operculi is the largest, caudally situated opercular muscle, which connects the opercle with the neurocranium. The muscle originates from the rostral ridge of the postemporo-supracleithrum, the caudolateral rim of the pterotic, the suprapreopercular bone and the connective tissue covering the dorsoventral side of the suprabranchial cavity. Ventrally, the levator inserts on the dorsal side of the opercle.

Maxillary barbel muscles

M. retractor tentaculi: The retractor tentaculi enables the retraction of the maxillary bone, as it connects the latter bone to the suspensorium. It runs in-between the $A_3"$ and the adductor arcus palatini. The muscle originates from the quadrate and hyomandibular membranous plate, and insert through a long tendon on the posterodorsal side of the maxillary bone (**Plate IV.5-6C**).

M. extensor tentaculi: The extensor tentaculi connects the ventral and ventrolateral side of the lateroethmoid, the ventral side of the frontal and the lateral side of the orbito- and pterosphenoid to the autopalatinum. All fibres insert musculously on the autopalatinum, caudal to its articulatory facet. In *C. gariepinus* the extensor tentaculi consists of a medial part connected to the metapterygoid and a lateral part connected to the neurocranium (ADRIAENS and VERRAES, 1997e).

Hyoid muscles

M. protractor hyoidei: The hyoid bars are connected with the lower jaw through the large, compact protractor hyoidei (**Plate IV.5-7A**). As in other clariids, a ventral and a dorsal part could be discerned. Comparable to the situation in *G. typus*, the left and right dorsal parts are separated and attached to the lower jaw through an aponeurosis, thus differing from *C. gariepinus* (ADRIAENS and VERRAES, 1997c: Fig. 6B). However, in *C. apus*, the aponeuroses are markedly larger than in *G. typus* (CABUY et al., 1999: Fig. 8B). Both the ventral and dorsal parts originate on the ventrolateral side of the anterior ceratohyal. In relation to the mandibular barbel control, several fields can be discerned in the ventral part of the hyoid protractor (DIOGO, *pers. comm.*).

M. hyohyoideus inferior: The hyohyoideus inferior is a massive muscle, covering the ventral side of the contralateral anterior ceratohyals and ventral hypohyals (**Plate IV.5-7A,B**). Caudo-laterally, it is also connected to the bases of the branchiostegal rays, this in contrast to *G. typus* and *C. gariepinus*. A subdivision into a *pars caudalis* and a *pars rostralis*, as observed in *G. typus* is absent in *C. apus*.

M. hyohyoideus abductor: The hyohyoideus abductor links the rostral tip of the hyoidbar with the first contralateral branchiostegal rays (**Plate IV.5-7A-C**). The muscles originate from the rostral face of this branchiostegal ray, and insert tendinously onto the ventral hypohyal of the opposite side, as observed in other clariids.

Mm. hyohyoidei adductores: The hyohyoidei adductor muscles form a series of sheets between consecutive branchiostegal rays, starting from the first one and ending onto the medial side of the opercular bone (**Plate IV.5-7A-C**).

M. sternohyoideus: The sternohyoideus forms a broad muscle between the pectoral girdle and the hyoidbars, by means of the parurohyal (**Plate IV.5-7A-D**). As in *C. gariepinus*, both the medial fibres of the middle myomere, as well as those of the caudal myomere attach to the cleithrum (ADRIAENS and VERRAES, 1997c). Anteriorly, the muscle heads fit into the double-forked parurohyal bone, which in turn is ligamentously connected to the ventral hypohyals.

DISCUSSION

Compared with a generalized clariid species, *Clarias gariepinus*, the overall morphology of the skull in *Channallabes apus* shows the hypertrophied adductor mandibulae complex, with a corresponding, substantial narrowing of the neurocranium. In general, this corresponds to the shape changes observed in other anguilliform clariids, like *Gymnallabes typus* (CABUY et al., 1999). The narrowing of the skull provides additional space for the

hypertrophied muscle, thereby taking into account the spatial constraints within the design. Additional space is also provided by the reduction of the eyes, as well as the reduction of the infraorbital bones (BAREL, 1984). A large adductor mandibulae complex may be seen as an adaptation to powerful biting. However, simply enlarging the muscle will not be sufficient: structural adaptations for coping with the related increase in mechanical load have to be provided in order to avoid damaging the skull. As a consequence of the reinforcement of the mouth closing apparatus, special adaptations for mouth opening may be present as well.

A hypertrophied adductor mandibulae complex

Hypertrophy of the adductor mandibulae complex has been observed in several species of the Clariidae, especially in the extreme anguilliform species *Gymnallabes typus* and *Dolichallabes microphthalmus* (POLL, 1942, 1957b, 1977). Hypertrophy of these mouth closing muscles, however, has also been noted in other teleosts, such as the Mastacembelidae (TRAVERS, 1984) and Synbranchidae (LIEM, 1980), as well as in other vertebrates (*e.g.* the sexual dimorphism in primate fossils, reflected in the presence of a sagittal ridge on the skull) (BENTON, 1997).

The hypertrophy of the mandibular adductor muscle does not involve an increase in its complexity, as it closely resembles that of *Clarias gariepinus* (ADRIAENS and VERRAES, 1996). In contrast to the extreme complex adductor mandibulae in bagrids, only an A_2A_3' and two A_3"-parts could be discerned (DIOGO et al., 1999). The major part of the adductor mandibulae complex is formed by the large A_2A_3', which covers the other parts of the adductor completely, as well as other muscles (*e.g.* the levator arcus palatini and dilatator operculi) and skeletal elements (*e.g.* the suspensorium) (**Plate IV.5-6A**). Although in *C. gariepinus*, it is only the dorsal fibres of the dorsal part of A_2A_3' that are markedly elongated, in *C. apus*, as well as in *G. typus*, the whole dorsal part reaches substantially more posteriorly. Consequently, in the latter two species, the A_2A_3' borders against the levator operculi, thus completely covering the dilatator and adductor operculi muscles. As a result of the increased height (relative to the length), the range of fibre directions is much increased in the anguilliform species, *G. typus* and *C. apus*, compared to *C. gariepinus*. In *Clariallabes*, which represents an intermediate configuration, an increase in the height can already be observed, compared to *C. gariepinus* (CABUY et al., 1999). This increase also implies that the rostralmost insertion of the A_2A_3' shifted more anteriorly, resulting in the fact that the anterior fibres of the dorsal part of the A_2A_3' are directed more vertically.

The medial part of the complex, the A_3" comprises a superficial and a deeper muscle (*i.e.* the *pars superficialis* and *pars profunda*, resp.) (**Plate IV.5-6C**). The same subdivision, as well as the fact that A_2A_3' and A_3" are separated by a levator arcus palatini, can be observed in all other clariids studied (ADRIAENS and VERRAES, 1996; CABUY et al., 1999). The subdivision of the complex by the levator arcus palatini can also be observed in many other teleosts (WINTERBOTTOM, 1974). Compared to *C. gariepinus*, the superficial part is much larger in *C. apus*, as well as in *G. typus*. In both species, a dorsal set of fibres seems to be added, which inserts onto the dorsal part of the A_3" tendon. In *C. gariepinus*, the tendon continues into the dorsal most fibers, whereas in *C. apus* and *G. typus*, it continues into the medial fibers (**Plate IV.5-6C**). In analogy to the A_2A_3' muscle, this implies that the range of fiber directions is also increased in this part of the A_3".

The morphology of the deeper part of A_3" seems to be less variable in clariids, as in *C. apus*, *G. typus* and *C. gariepinus*, the muscle is a small bundle with dorso-ventrally directed fibers.

Spatial constraints within the integrated design

The spatial constraints within an integrated design imply that trade-offs will occur (BAREL, 1984). The expansion of the adductor mandibulae complex can thus be expected to be related to spatial changes in the surrounding structures, in all three dimensions of the "Bauplan" framework.

The results of this paper do not provide evidence that allows directional interpretations of causalities and consequences. The assumptions made in the following discussion have to be considered as a reflection of what may be the logic behind the spatial interactions that must have occurred in the cranial Bauplan.

The reflections are based on the assumption that the driving force in terms of fitness must have been related to the trophic advantage of larger jaw muscles. Other adaptations can be regarded in this context, without necessarily knowing the exact causal/morphogenic relationship.

Relations to the expansion of the adductor mandibulae complex in the latero-medial direction, are manifested in the narrowing of the central part of the neurocranium, as well as the reduction and displacement of canal bones. Compared to *C. gariepinus*, both the skull roof and the skull floor are much more narrow in *C. apus*, although less narrow than in *G. typus*. The reduction of the skull roof appears to be concentrated in the orbito-temporal and oticoccipital regions. This largely involves the reduction of the lateral plate-like extensions of the frontals, the sphenotics, the pterotics and the posttemporo-supracleithrals (**Plate IV.5-1C**) (CABUY et al., 1999: Fig.3). Whereas in *G. typus* the

complete lateral parts, extending beyond the lateral walls of the skull, are absent, a small rim can still be distinguished in *C. apus*. The narrowing in the ethmoid region is less pronounced, as a comparable constriction can be observed in all three, non-generalized species (*C. melas, C. apus* and *G. typus*). In *G. typus*, which shows the most constricted skull roof, the narrowing of the skull starts in the lateral ethmoid and stops in the frontal, at the level of the contact with the posterior infraorbitals (CABUY et al., 1999: Fig. 3A). In the three, above mentioned species, the forked tip of the mesethmoid and the premaxillaries are of comparable shape, although markedly more narrow than that of *C. gariepinus*. However, the overall increasing skull narrowing is coupled to a caudal extension of the premaxillaries. Whereas in *G. typus*, this only involves the extension of the edentulous part, in *C. apus* the tooth battery is distinctly expanded caudally (**Plate IV.5-2C**).

In the dorso-ventral direction (as well as the antero-posterior direction), the muscle hypertrophy is coupled to the separation and reduction of the dermal, plate-like bones covering the adductor mandibulae complex in *C. gariepinus*. A broad range in the degree of such separations and reductions have frequently been observed in many other clariid species (including the genus *Clarias*), and is used as an important taxonomic character (POLL, 1957a; CAROLL, 1988; TEUGELS, 1986). The impact of the adductor mandibulae complex enlargement is manifested both anteriorly and posteriorly. Anteriorly, the eye has been substantially reduced, coupled to the fact that it has been shifted slightly anteriorly. Also, the infraorbital series is much reduced. The anterior ones are tube-like, whereas the posterior ones have lost the posterior plate-like extension, thus providing space for the expanded muscle. As a consequence, the eye can no longer be supported by the second infraorbital, which is the case in *C. gariepinus*. However, due to substantial reduction in eye size, this is no longer necessary. In *G. typus*, which has the smallest eyes and the largest adductor complex, the infraorbitals are the most reduced, as all of them are tubulous (CABUY et al., 1999: Fig. 3A). Posteriorly, the relation to the muscle hypertrophy is situated at the level of the suprapreopercular bones. The shape changes are analogous to those in the infraorbitals: (1) the suprapreopercular bones have shifted away from the muscle (thus caudally in this case), (2) the distal bones have become tubulous, and (3) the proximal one has only lost that part of the plate-like extension close to the adductor mandibulae complex (**Plate IV.5-1C**). As a result of the rostral and caudal displacement of the infraorbital and suprapreopercular bones, respectively, the corresponding lateral line canals have shifted as well. The infraorbital canal no longer exits the dermosphenotic bone but leaves the frontal bone. The branch to the preopercular canal is displaced caudally, coupled to an elongation of the pterotic bone, and the canal is directed caudally (in

contrast to latero-rostrally in *C. gariepinus*). The same trend can be observed in the other clariids studied.

In the antero-posterior direction, the accommodation for the enlarged adductor muscle is related to the reduction and displacement of the above mentioned infraorbitals and suprapreopercular bones. Other substantial skeletal transformations or displacements appeared not to be essential for this accommodation, as the insertion site of the adductor muscle only became expanded caudally, onto the lateral surface of the suspensorium (**Plate IV.5-6**). However, this expansion appears to be coupled to the position of the levator operculi muscle. Whereas in *C. gariepinus* both muscles are well separated, with increasing adductor mandibulae size, both approach each other increasingly (CABUY et al., 1999: Fig. 9). In *Clariallabes*, both muscles still remain separated, whereas in *Channallabes* they almost touch each other. In *Gymnallabes*, the genus with the largest adductor mandibulae, these muscles contact each other completely.

Apparently, the spatial impact of the adductor mandibulae complex on the cranial "Bauplan" is extensive. The question that can be raised here is at what cost this has occurred. For some of the observed changes, it can be strongly suggested that they will have involved sacrifices of certain structures and/or apparatuses. (1) The reduction of the eye size, as well as its dorsal displacement, will surely influence the visual capacities. (2) Reduction of the size of the lateral bony plates implies the loss of protection of the underlying parts. (3) Narrowing of the skull may involve a reduction in strength of the braincase. (4) A caudal shift of the neurocranial insertion of the levator operculi will most certainly imply an alteration in efficiency of certain related mechanisms, especially that of the opercular mouth opening mechanism.

Catfishes in general have small eyes as an adaptation to a nocturnal and benthic life style (ALEXANDER, 1965; TEUGELS, 1996; ADRIAENS and VERRAES, 1997b). As a compensation, several other sensory organs are well developed, in order to survive in the murky waters. Catfishes have well developed oral barbels, which support both taste and tactile buds, and which can be moved in a controlled manner (ALEXANDER, 1965; SINGH, 1967; GHIOT, 1978; LONG and HUANG, 1995). Other sensory organs have become specialized as well: Weberian apparatus (CHARDON, 1968), the lateral line system (ARRATIA and HUAQUIN, 1995; ADRIAENS et al., 1997), and the ampullary organs for electrodetection (JAKUBOWSKI, 1987; BRETSCHNEIDER et al., 1991; WHITEHEAD et al., 1999). The importance of the latter sensory organs, and compared to the unimportance of visual observation is clearly shown in blind catfish (NORMAN, 1926; WEISEL and MCLAURY, 1964; HAUSER, 1976; LUNDBERG and PY-DANIEL, 1994; UFERMANN, 1998). However, in most of these blind catfishes, the blindness is related to a

cryptic life style (LUNDBERG, 1982). This presumably is also the case in *Channallabes apus*, as well as in other anguilliform clariids, which live in murky swamps (DAVID and POLL, 1937; MATTHES, 1964). However, the cryptic behaviour is more extreme in these species, as they live burrowed in the mud, in-between roots of trees (*pers. observ.*) (MENON, 1951; MATTHES, 1964).

One consequence of the dorsal expansion of the adductor mandibulae complex, is the fact that the dermal bones covering it (as observed in *Clarias gariepinus*) have to be reduced and thus can no longer cover and protect that muscle complex. This, however, is not favourable for a burrowing species, as burrowing behaviour is frequently associated with cranial reinforcements (RIEPPEL, 1996). It thus appears that in the anguilliform clariids, the resulting benefit of the enlargement of the adductor mandibulae may have overruled the reduced protection. Although the lateral bones have become much reduced in *Channallabes*, they still perform an important function, *i.e.* enclosing and protecting the infraorbital and preopercular canal.

In the anguilliform clariids, the skull becomes markedly narrow, especially in the orbito-temporal and otical region. This will most certainly have an impact on strength of that part of the braincase. It can thus be suspected that some special, reinforcing adaptations may be present. This appears to be the case in *Channallabes*, as well as in *Gymnallabes*. Compared to *Clarias* and *Clariallabes*, the degree of interdigitation of the sutures of the cranial roof bones is substantially more elaborated (**Plate IV.5-1C**) (CABUY et al., 1999: Fig. 3). This is not the case, however, for the skull floor bones. The central, supporting beam, formed by the prevomer, parasphenoid and basioccipital is firm, although, due to the reduction in width, the number of interdigitating processes in-between these bones has decreased. A reinforcement could also be observed in the skull wall, especially at the level of the pterosphenoid. In a *Clarias gariepinus* specimen with a skull length of 35.8 mm, this perichondral bone is small, only covering the ventral part of the taenia marginalis (ADRIAENS and VERRAES, 1998: Fig. 19E). It thereby interdigitates ventrally with the parasphenoid along half of its length. In *Channallabes* and *Gymnallabes*, however, the pterosphenoid is more heavily ossified and connected to the parasphenoid along the whole of its length, even in specimens with a much smaller skull (skull length 22 mm).

As mentioned, the caudal expansion of the adductor mandibulae complex can be linked to the caudal shift of the levator operculi insertion on the neurocranium. As a result, the fibers of this levator are directed much more oblique in *Channallabes* and *Gymnallabes*, compared to *Clarias* (**Plate IV.5-6**) (CABUY et al., 1999: Fig. 9). Also, in the former two species, the opercular bone has become much more reduced, as well as tilted more in a

clock-wise direction. This has been suggested to be advantageous as in this way, the contraction force of the levator is presumably more efficiently transformed into a mandibular depression (CABUY et al., 1999).

Although several arguments suggest an importance of the adductor mandibulae hypertrophy as a fitness advantage, the true nature of the resulting increase in performance of feeding or otherwise remains to be studied.

Adaptations to powerful biting or simply the result of miniaturisation?

The presence of a hypertrophied adductor mandibulae complex immediately leads to the assumption that powerful biting may occur. If this would be so in *Channallabes*, some other adaptations can be expected to be found, related to a powerful mouth closure. Powerful biting implies a substantial increase in mechanical loading on several skeletal elements composing the biting apparatus. Those elements where adaptations can be expected are: (1) the lower jaw, (2) the upper jaw, (3) the suspensorium, (4) the neurocranium (especially at the level of adductor muscle insertion and articulation with the suspensorium). As already stated by CABUY et al. (1999), the increasing cross section area of the adductor muscle may require special adaptations for a more powerful mouth opening. Such adaptations, found in the protractor hyoidei, the levator operculi and the sternohyoideus, as observed in *Gymnallabes*, could also be observed in *Channallabes* (**Plate IV.5-8A**) (CABUY et al., 1999: Fig. 10).

As observed in *Gymnallabes*, comparable adaptations can be observed in the lower jaw in *Channallabes* (CABUY et al., 1999). The mandibulary tooth battery in *Channallabes* covers a larger surface of the lower jaw (relative to the total surface) compared to *Clarias*, as in the former the posterior teeth are situated at the anterior base of the coronoid process. This configuration is comparable to that of *Clariallabes*, but less pronounced than in *Gymnallabes* where teeth can be found on this coronoid process. The lower jaw is also markedly more slender in *Clarias* than in *Channallabes* and *Gymnallabes*. Another aspect that can be related to a more powerful bite is the size of the coronoid process. Compared to *Clarias*, this process is substantially higher. As discussed by CABUY et al. (1999), this is advantageous for a powerful elevation of the lower jaw. During biting, the exertion of an asymmetrical load onto the contralateral mandibular bars will frequently occur. Consequently, the larger the contraction force of the adductor, the larger the differential loading on left and right mandibular bars may be. The observed increased symphysial surface in *Channallabes*, as well as *Gymnallabes* may thus prevent the dislocation of these bars.

The upper jaws in clariids, as well as in most other siluriforms consists of the premaxillaries and the prevomeral tooth plate. As already mentioned, the only marked difference in the premaxillaries of *Channallabes* and *Gymnallabes* involved the narrowing and distal expansion. However, whether this transformation is linked to the narrowing of the skull or to the powerful bite remains unclear at present. The prevomeral tooth plates in *Channallabes* and *Gymnallabes* appear to be fused in the middle, which they are not in *Clarias gariepinus* specimens of a comparable skull length (fusion in larger specimens). This implies that in the former two species, teeth are present in the middle, which they are not in *C. gariepinus*.

As in most other teleosts, the suspensorium in clariids articulates with the neurocranium (at the level of the sphenotic and the pterotic), and suspends the lower jaw, the hyoid bar and the opercular bone. A difference, however, is the loss of the anterior articulation with the neurocranium, as the palatine has become isolated in catfishes (ARRATIA and SCHULTZE, 1991; ARRATIA, 1992). This implies that the only firm connection between the suspensorium and the neurocranium occurs at the level of the hyomandibula, and consequently that all forces exerted onto the suspensorium will have to be resisted at that point. As the adductor mandibulae inserts both on the neurocranium as well as on the suspensorium, an alternating loading of this suspensorium-neurocranium articulation may be expected during contraction (**Plate IV.5-8B**). In *Channallabes apus*, the hyomandibula bears a narrow articulatory ridge, which would be extremely disadvantageous, if it were not that both anterior and posterior to it, a series of well developed processes are present. As in other anguilliform clariids, these processes fit in-between comparable processes onto the sphenotic and the pterotic (CABUY et al., 1999). A disarticulation of the suspensorium during adductor mandibulae contraction is thus prevented.

Concerning the adaptations of the neurocranium, the presence of the processes of the sphenotic and the pterotic can be explained in analogy with the above mentioned adaptations in the suspensorium. The adaptations to the increased insertion site of the adductor mandibulae are already discussed above, as the reduced lateral skull bones have enabled the increase in attachment surface for the adductor.

As mentioned above, the Clariidae represent a group of species, in which a trend to an increasing anguilliformity is noted, which may have occurred more than once (BOULENGER, 1908; PELLEGRIN, 1927). The elongation of the body is a process which has been observed in fishes, early tetrapods (amphibians and reptiles) and some mammals (LANDE, 1978; CAROLL, 1988). Together with elongation, a whole set of morphological changes has been observed, *e.g.* increase of the number or size of vertebrae, limblessness, reduction of the eyes,

increasing rigidity of the skull, all of which are regarded as an adaptation to a fossorial habit (WITHERS, 1981). All of these traits appear to be present in the anguilliform clariids as well. The increasing number of vertebrae has been demonstrated by CABUY et al. (1999). Limblessness can be observed in *Channallabes* and *Dolichallabes* (POLL, 1942a, 1957a). The reduction of the eyes has been mentioned above. Concerning the rigidity of the skull, this generally involves the braincase only, as this part needs to be reinforced due to reductions in other parts of the skull. This can be observed, for example, in fossorial reptiles: the skull transformations involve a decrease of the skull diameter and the upper temporal arch is lost (RIEPPEL, 1996). Analogies of both these transformations can also be observed in the anguilliform clariids: the narrowing of the skull and the reduction of the lateral skull bones (see above). It has also been stated that "in lizards, miniaturisation is generally correlated with body elongation and limb reduction" (RIEPPEL, 1996). Compared to *Clarias* and *Heterobranchus*, the skulls of the anguilliform species are very small, and morphological transformations related to miniaturization are not excludible. Several cranial transformations, related to the miniaturization in lizards, involved the mouth closing apparatus. It was observed that, due to the reduced size, the size between the lower jaw and the neurocranium becomes reduced, especially in fossorial species. Hence, the jaw adductor musculature becomes obliquely oriented in those species, in order to allow the fibres to be sufficiently long (RIEPPEL, 1996). The elongation of the adductor muscle in the anguilliform clariids, as well as the increase in volume, may thus simply be an adaptation to enable an adequate biting force in the miniature skull. The burrowing habit of these species supports this hypothesis.

This miniaturization hypothesis, however, has to be taken cautiously. Within the clariids, it is a fact that the degree of cranial reductions and mandibular adductor hypertrophy is not correlated with the degree of anguilliformity. For example, the species *Tanganikallabes mortiauxi* is not anguilliform, but has a markedly reduced neurocranium with a large adductor mandibulae (POLL, 1943). On the other hand, *Channallabes* is more anguilliform than *Gymnallabes*, although the reduction of cranial bones is less pronounced in the former. As stated by RIEPPEL (1996), however, the cranial transformations in miniature lizards are coupled to the fossorial behaviour and not only to the degree of miniaturization. Thus it may be likely that the relation between cranial adaptations and anguilliformity in Clariidae is more complex, and depends largely on the burrowing habits.

IV.5.1.b A survey of the anguilliform Clariidae of the Congo River basin, with the description of a new species

ABSTRACT

A survey of anguilliform clariids from the Congo River basin, including the Upper and Lower Congo Rapids, Lower and Central Congo, Sudanic Congo, Sangha, Tumba, Pool Malebo (Stanley Pool) and Kasai freshwater ecoregions supports the existence of six species in five genera, of which one is new to science. 30 biometric features, both metric and meristic descriptive characters, have been studied on 304 specimens.

Channallabes sanghaensis sp. n. can be recognized by the combination of following characters: a large foramen on the fourth post-Weberian vertebra, two large lateral processes on the second dorsal fin ray pterygiophore, a small supraorbital process on infraorbital IV, not reaching the rostral border of the eye, a fenestra between the scapulo-scapulo-coracoid and the cleithrum and an interdigitation zone between the quadrate and the entopterygoid. Furthermore, *C. sanghaensis* shows serrations on both sides of the pectoral spine. The number of vertebrae ranges between 86-89; number of dorsal and anal fin rays respectively between 121-125 and 104-124.

INTRODUCTION

The freshwater clariids are one of the 37 catfish families within the Siluriformes (SABAJ et al., 2004). Although they occur in Syria, southern Turkey and large parts of Southeast Asia, their diversity is the largest in Africa (TEUGELS, 1996; TEUGELS and ADRIAENS, 2003). This richness is demonstrated by the presence of 12 genera with up to 74 species (TEUGELS, 1996; TEUGELS and ADRIAENS, 2003). Some of the generalised, fusiform species show a large geographic distribution, whereas the anguilliform species occur in a small area, occupying a more specialized, burrowing niche. They can only be found in swampy areas in the Nilo-Sudan (Niger delta), Lower Guinea and the Zaire (Congo River basin) ichthyological province (POLL, 1957B; ROBERTS, 1975; TEUGELS, 1986; TEUGELS et al., 1990).

Clariid catfishes are characterized by an elongate body, the presence of four barbels, long dorsal and anal fins, and especially by the unique presence of a suprabranchial organ, formed by arborescent structures from the second and fourth gill arches (GREENWOOD, 1961; TEUGELS and ADRIAENS, 2003). Unique in these clariids is the extreme variation in body shape ranging between fusiform and completely anguilliform genera (PELLEGRIN, 1927). Although this has been observed in other families of teleosts, amphibians and reptiles (LANDE, 1978), it is never as extreme as in the Clariidae. Together with the elongated body, a whole set of morphological changes are observed, such as decrease and loss of the adipose fin, continuity of unpaired fins, reduction of paired fins, reduction of the skull bones, reduction of the eyes and hypertrophy of the jaw muscle complex (CABUY et al., 1999; DEVAERE et al., 2001, 2004 (**IV.5.1.a, IV.4**)).

Presently, anguilliform clariid taxonomy is poorly understood. The only keys incorporating the anguilliform clariids are those of POLL (e.g. 1977). The characters used in this key, such as presence of paired fins, number of ribs and vertebrae are no longer discriminative and overlap among species. This is partially due to the limited number of specimens used in the original descriptions of the species (e.g. *Gymnallabes typus*: n=1, *Channallabes apus*: n=1, *Dolichallabes microphthalmus*: n=7), so that intraspecific variation was not recognized.

As a part of an ongoing revision of the alpha-level taxonomy of the African catfishes, material from the Congo River basin housed in several musea was studied and revealed the presence of a group specimens clearly differing from all clariid species currently known. The study of this material led to the description of a new species given herein.

MATERIAL AND METHODS

304 anguilliform clariids originating from the Upper, the Central and Lower Congo Rapids, Lower and Central Congo, Sudanic Congo (Oubangui), Sangha, Tumba, Pool Malebo (Stanley Pool) and Kasai freshwater ecoregion (THIEME et al., in press) have been examined. Also the holotype of *Channallabes apus* from the neighbouring Cuanza freshwater ecoregion is included (BMNH 1873.7.28: 16, 135 mm SL, Interior of Ambriz, Angola (7° 50'Z-13° 06'E)). Details of their origin are given in the list of specimens examined. Institutional abbreviations are listed in LEVITON et al. (1985).

For this study, we used available museum material from the Royal Museum of Central Africa (MRAC); British Museum of Natural History; Museum of comparative Zoology, Harvard; Musée National d'Histoire Naturelle, Paris; Musée d'Histoire Naturelle de Genève and the Naturhistorisches Museum, Vienna. Furthermore, some specimens were collected in an expedition to Gabon and the border of the Republic of the Congo (2000). The area sampled in that expedition was situated in the vicinity of Onga, across the border in the Republic of the Congo. All sampling sites were characterised by shallow, muddy, still water. The specimens were caught by local fisherman, using fyke traps and fish hooks. All these specimens are deposited in the collection of the Africa Museum (MRAC), Tervuren, Belgium.

For all specimens, 36 measurements were taken point to point using a digital callipers with an accuracy of +/- 0.1mm following DEVAERE et al. (2004) (**IV.4**). Measurements of bilaterally paired structures were taken on the left side. Not all specimens were preserved well enough to make all meristic counts. Six specimens were cleared and stained following TAYLOR and VAN DYKE (1985).

The data obtained was submitted to a Principal Components Analysis, using Statistica 6.0 (StatSoft Inc.). Morphometric and meristic data were independently submitted to a PCA. The different PCA's were performed according to BODEN et al. (1997). Qualitative and absence/presence characteristics were not included in the analyses but help to further identify and differentiate the species.

RESULTS

In the intitial analysis all anguilliform clariids from the Congo River basin are included, inclusive type material of *Platyclarias machadoi* Poll, 1977*, Platyallabes tihoni* (Poll, 1944)*, Gymnallabes nops* Roberts and Stewart, 1976*, Dolichallabes miscrophthalmus* Poll, 1942 and *Channallabes apus* (Günther, 1873).

The PCA's were performed, using the covariance matrix for 28 log transformed measurements and correlation matrix of 5 meristic counts. **Plate IV.5-9A** combines the second factor scores for the measurements PCA with the first factor scores for the meristic PCA. The first principal component proved to be an overall size factor and was thus omitted (BOOKSTEIN et al., 1985). We obtain three clear groups. One group, located in the upper left quadrant, contains the type material of *Platyallabes tihoni*. Closely located to that group is the holotype of *Gymnallabes nops*, indicating the questionable systematic status of this species (see discussion). Type material of both species and additional specimens all come from the Lower Congo Rapids, the Lower Congo and the Pool Malebo freshwater ecoregions. A second group located in the upper right corner, contains all type material from *Platyclarias machadoi*. All specimens come from the Kasai freshwater ecoregion. The group, in the lower right quadrant, contains the type material from *Dolichallabes microphthalmus* and *Channallabes apus*. The factor loadings are shown in **Table IV.5-1**. The dominant characters for the second principal component for the metric characters are the distance between the origin of the dorsal fin and the occipital process and the caudal peduncle depth; while for the first principal component of the meristic counts, total number of vertebrae and the number of precaudal vertebrae are the most important.

The group in the lower right quadrant of **Plate IV.5-9A**, clustering around the type material of *Dolichallabes microphthalmus* and *Channallabes apus* was then isolated for further analysis. We performed another PCA on meristic and log-transformed metric data for the remaining specimens. The results are combined in a single plot (**Plate IV.5-9B**). Again three groups can be recognized. The first group (lower left quadrant) includes the type material of *Dolichallabes microphthalmus*, all coming from the Sudanic Congo (Oubangui) freshwater ecoregion. The second group in the upper left corner does not contain any type material and include all the specimens from the Sangha freshwater ecoregion included in this study. The third group includes the type material of *Channallabes apus* and contains specimens from all ecoregions, except the Sangha (see above). The factor loadings are shown in **Table IV.5-2**. The dominant characters for the second principal component for the metric characters are the length and width of the occipital spine, width of the skull roof, mouth width and the snout height; while for the first principal component of the meristic counts, total number of vertebrae, the number of caudal vertebrae and the number of precaudal vertebrae are the most important. This indicates that body elongation occurs through the addition of both caudal and pre-caudal vertebrae. The distinct population of specimens found in the Sangha freshwater ecoregion is sufficiently distinct that we consider this population as a new species.

Next, we turn to the remaining large group on the right of **Plate IV.5-9B**, including the type material of *Channallabes apus*. Again, a combined PCA was plotted (**Plate IV.5-10A**). A subdivision can be observed, splitting the remaining specimens into two groups. The group in the upper part of the plot includes the type material of *Channallabes apus* (group I). The most important factor for recognizing these groups are the total number of vertebrae and the number of caudal vertebrae. In **Plate IV.5-10B**, the total number of vertebrae is then plotted against the SL. Since the total number of vertebrae could be counted on a larger dataset, more specimens (n= 123) could be included. The two groups can again be separated, group I is again the group including the holotype of *Channallabes apus*, while group II includes the specimens with a low number of vertebrae. To test whether these groups are significantly different, some basic statistics was carried out. The Kolmogorov-Smirnov test showed that group I was not normally distributed ($p<0.05$). Therefore, a non-parametric Mann-Whitney U-test was performed which showed a p-level of $1.5e^{-15}$ ($p<0.05$), rejecting the null-hypothesis of equal means. This shows that the two groups are significant different. All plots were tested for growth allometry and showed that the observed differences were not related to this allometry.

Key to the species of Channallabes

1a Small supraorbital process on infraorbital IV, not reaching the rostral border of the eye (**Plate IV.5-11A**); interdigitation between entopterygoid and quadrate.............2

b Large, well-pronounced supraorbital process present on infraorbital IV, reaching the rostral border of the eye (**Plate IV.5-11B**); no contact between entopterygoid and quadrate 3

2a Fenestra present between the cleithrum and scapulo–scapulo-coracoid, large foramina at the bases of the parapophyses of the first post-Weberian vertebrae (2nd to 10th) (**Plate IV.5-12A**), second dorsal fin pterygiophore with two large lateral processes (**Plate IV.5-12C**), and the combination of a small spine width (13.5–18.6 % SkL) and large mouth width (27.9–38.4% SkL)....................................*C. sanghaensis* sp. n.

b No fenestra present between the cleithrum and scapulo–scapulo-coracoid, small foramina at the bases of the parapophyses of the first post-Weberian vertebrae (2nd to 10th) (**Plate IV.5-12B**), second dorsal fin pterygiophore with two small lateral processes (**Plate IV.5-12D**), and the combination of a broad spine width (12.1–45 % SkL) and narrow mouth width (13.9–36.9% SkL) ....*C. apus*

3a   Spot present on skull roof between anterior and posterior fontanel, low number of dorsal (98-116) and anal (75-105) fin rays...3

b   No spot present on skull roof, high number of dorsal (118-160) and anal (105-155) fin rays...*C. alvarezi*

4a   Serrations only frequently on the innermost side of the pectoral spine.....*C. ogooensis*

b   Serrations only on the outermost side of the pectoral spine ....*C. teugelsi*

c   Serrations on both sides of the pectoral spine.............................*C. longicaudatus*

Channallabes sanghaensis n. sp.

Holotype: MRAC A4-31-P-171-183, 114 mm SL, in the vicinity of Ntchouo, River Mbessy, Republic of the Congo (0° 46'N-14° 19'E), S. Devaere, D. Adriaens and A. Herrel, September 2000. (**Plate IV.5-13A**)

Paratype: Total of 12 specimens. S. Devaere, D. Adriaens and A. Herrel, September 2000. 12 specimens, 114-221mm SL, MRAC A4-31-P-171-183, in the vicinity of Ntchouo, River Mbessy, Republic of the Congo (0° 46'S-14° 32'E).

Differential diagnosis: *Channallabes sanghaensis* differs from *C. alvarezi, C. longicaudatus, C. teugelsi* and *C. ogooensis* in having a small supraorbital process on infraorbital IV, not reaching the rostral border of the eye and in the presence of an interdigitation zone between the quadrate and the entopterygoid. *C. sanghaensis* can be distinguished from *C. apus* in the presence of a fenestra between the scapulo–scapulo-coracoid and the cleithrum, the presence of large foramen at the bases of the parapophyses of the first post-Weberian vertebrae (2nd to 10th) (**Plate IV.5-12A**), the presence of two large lateral processes on the second dorsal fin ray pterygiophore (**Plate IV.5-12C**) and the combination of a small spine width (13.5–18.6 % SkL) and large mouth width (27.9–38.4% SkL).

Description: Measurements and meristic counts for holotype and paratypes are given in **Table IV.5-3**. The standard length ranges from 114 to 221 mm. *C. sanghaensis* has an elongated body (**Plate IV.5-13A**) (ABD 4.6-6.4% of SL, mean: 5.6%), with a preanal length of 27.5% up to 33.2% of SL (mean: 30.1%). Due to the extreme elongation of the body, there is a very small skull length (10.0-12.1% SL). Skull width is 58.2-71.5% (mean: 64.2%) of skull length. The very narrow skull roof, width 16.5-24.4% of maximal skull width, remains clearly visible between the bulging jaw muscles. Although the eyes are small, they remain clearly visible. Tube-like anterior nostrils are present, although small.

The fleshy dorsal, anal and caudal fins form a continuous finfold. The pectorals fins are always present, length 4.0-5.3% of SL and are always preceded by a pectoral spine with a

length of 2.3-3.3% of SL. The pectoral spine is serrated on both sides. Pelvic fins present in only one specimen; in the other specimens no evidence of pelvic fins. The total number of vertebrae is 86-89 (mode = 87), ribs 12-14 (mode = 13). Branchiostegal rays 8, dorsal fin rays 121-125, anal fin rays 104-124.

The skull in *C. sanghaensis* shows reduced ossifications and a narrow skull roof. This reduction is shown in the reduced lateral plate of the frontal, as from a ventral view this lateral plate is wider than the outlines of the orbitosphenoid (**Plate IV.5-13B**). A plate-like outgrowth is present on the posttemporo-supracleithral bone. The posterior border of the mesethmoid is indented, which makes that the anterior part of the anterior fontanel lies within the mesethmoid (**Plate IV.5-13C**). One suprapreopercular bone is present. On the prevomer, one long posterior process is present. The entopterygoid rostro-dorsally encloses the metapterygoid. For the interdigitation with the neurocranium, the hyomandibula has two sets of three processes. Oral teeth are present on dentary, premaxilla and prevomer.

Color: Alcohol preserved specimens gradually fade from dark brown on the dorsal side to whitish brown on the ventral side. Both sides are separated by a white line, representing the lateral line. The skin on the jaw muscles shows a somewhat paler brownish colour than the surrounding skin of the head. The barbels and nostrils have a similar coloration.

Distribution: Currently known from the Congo River system, in the region of in the vicinity of Ntchouo (**Plate IV.5-14**).

Etymology: From the Sangha freshwater ecoregion (THIEME et al., in press) where the species was found.

DISCUSSION

Based on the species diagnosis, the new species should be included in the genus *Channallabes*. This genus is characterized by reduced infraorbital and suprapreopercular bones, with small plate-like extensions on the posterior most bone in both series; small lateral plates of the frontals are present and the first dorsal fin pterygiophore is situated posterior to the sixth post-Weberian vertebrae.

Besides this new species, ten other anguilliform clariids are known, designated to five genera. The species presented can be clearly discerned from each one of the other anguilliform species. *Platyallabes tihoni* (Poll, 1944) has a very small distance between the origin of the dorsal fin and the supraoccipital process (2.2-6.6% of SL vs 7.8-11.5% of SL). *Platyclarias machadoi* Poll, 1977 has an extremely flattened skull (SkH 22.9-37.1% of

SkL)[10]. *Dolichallabes microphthalmus* Poll, 1942 is characterized by the presence of only one fontanel on the skull roof (DEVAERE et al., 2004 (**IV.4**)). *Gymnallabes nops* Roberts and Stewart, 1976 shows a large reduction in the infraorbital series in size and number (two instead of five) (DEVAERE et al., in press (**IV.3.1**)). *Gymnallabes typus* GÜNTHER, 1867 is characterized by well developed skin folds, bordering the side wall of the mouth (CABUY et al., 1999: Fig.1).

The remaining five anguilliform species are all in the genus *Channallabes*. The differences present between "the Congo basin" species (*C. apus, C. sanghaensis*) and the "Lower Guinea" species (*C. alvarezi, C. longicaudatus, C. teugelsi, C.ogooensis*) are reflected in both genetic (JANSEN et al., submitted (**V.1**)) and morphological (cfr. Key to the species of *Channallabes* in DEVAERE et al., submitted (**IV.5.2**)) differences. The differences of the "Congo basin" species compared to the "Lower Guinea" species are expressed in the presence of a small supraorbital process on infraorbital IV, not reaching the rostral border of the eye; the presence of an articulation process on infraorbital II, which never makes contact with the lateral ethmoid and the presence of an interdigitation zone between the quadrate and the entopterygoid. Finally, the differences of *C. sanghaensis* compared to *C. apus* are the presence of two clear, lateral processes on the second dorsal fin pterygiophore and the presence of a large foramen at the bases of the parapophyses of the first post-Weberian vertebrae. Although this foramen is larger than in most other anguilliform clariids, it is not as large as in *Platyallabes tihoni*, where it is one of the diagnostic features for the genus and species (DEVAERE et al., in press (**IV.1**)).

Although **Plate IV.5-9B** and **IV.5-10A** show two clear groups, which were tested significantly different, no new species is currently recognized. There is no evidence for size-related changes of the meristics in the anguilliform clariids species studied here. Moreover, in fishes in general, several authors, as for instance LANDRUM and DARK (1968), have reported on the independence of the total vertebrae number from the length of the fish within the same species. Thus, the apparent different growth series in **Plate IV.5-10A** cannot be considered as such. Besides the difference in total vertebra number, no other differences (morphometric, osteological, …) were found between these groups, indicating the large similarity of these two groups. This shows that morphologically similar (external morphology) specimens in one species can obtain an elongated body through two ways; with numerous small vertebrae or less, but larger vertebrae (polymorphic species).

Plate IV.5-9A also shows the great similarity between *Platyallabes tihoni* and *Gymnallabes nops*. This large similarity, as well as morphological correspondences, was

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[10] small overlap with *C. sanghaensis*

already shown by DEVAERE et al. (in press) (**IV.3.1**), this similarity is based on both meristic and osteological characteristics. The additional metric data, presented in this paper, could be an extra argument for a systematic shift of *Gymnallabes nops* to the *Platyallabes* genus; however, further data is required.

The geographic distribution shows *C. apus* and *C. sanghaensis* occur in two different parts of the Congo River system (**Plate IV.5-14**). While *C. sanghaensis* only occurs in the border region with Gabon, close to the watershed of the Southern West Coastal Equatorial (Ogowe and Ivindo River), *C. apus* is found in the largest part of the Congo basin, from the mouth up to the Upper Congo Rapids. The group with the low number of vertebrae (Group II in **Plate IV.5-10B**) is only found in the Kasai, Upper Congo Rapids, Sudanic Congo, Central Congo and Lower Congo freshwater Ecoregions.

ADDITIONAL MATERIAL EXAMINED

Museum abbreviations are listed in LEVITON et al. (1985).

Channallabes apus. Angola. Ambriz, BMNH 1873.7.28.16 (holotype); Dem. Rep. Congo. Bokalakala, MRAC 175247-270 (n=10); Kinshasa, MRAC 97-056-P-0001-0003 (n=2); Bumba, MRAC 88-25-P-2192-227 (n=36); Boma, MRAC 939; Riv. Lula, Bushimaie, MRAC 153505; Kelé, MRAC 1491; Stanleyville, MRAC 30893-30900 (n=8), MRAC 88-01-P-1976-1992 (n=17); Riv. Ruki, Eala, MRAC 14747-49 (n=3); Lake Tumba swamp area, MRAC 46299; Katanga, MRAC 39480; Riv Botota, keseki, MRAC 67763-77 (n=15); Mwilambongo, MRAC 72886-887 (n=2); Dekese, Riv. Lofu, Anga, MRAC 153352; Yangambi, MRAC 68700; Riv. Oubangui, Imfondo, MNHN 1922-0029; Loango, MNHN 1924-0079, MNHN 1924-0080; Sangha, MNHN 1925-0137; Mogende, MNHN 1926-0155-59 (n=5); Riv Congo, MNHN, 1937-0124-25; Stanleypool, Bamu, MNHN 1958-0111; Boloko, Riv. Likouala, MNHN 1962-0401 (n=7); Mossaka, Riv. Likouala, MNHN 1963-0402 (n=2); Riv. Loadjili, Songolo, MNHN 1967-0143 (n=6); Mangala, BMNH 1896.3.9.17; Riv. Lebuzi, Kaka Muno, BMNH 1912.4.1411-12 (n=2); Lower Congo, BMNH 1887.1.13.8-9 (n=2); Stanley Falls, BMNH 1889.11.20.5; New Antwerp, Upper Congo, BMNH 1899.2.20.16; Siala-Ntoto Swamps, BMNH 99.11.27.92; Bangyville, Ubangi, BMNH 1907.12.26.34; Kashi, Lulua, MHNG 1248.3; Banana, NMW 47240-42; Mollunda, NMW 47245 (n=4), NMW 47246. Congo. Yangala Youbi, MNHN 1967-0146; Djembo, Kouilou, MNHN 1967-0147; Cayo, MNHN 1989-0527; Riv. Nanga, between Boukou-Zassi and Kouilou swamp area, MRAC 90-57-P2315; Sintou, Riv. Kibombo, Kouilou, MNHN 1967-0144; Riv. Loadjili, Songolo, Pointe Noire, MNHN 1967-0145 (n=6); Riv. Youbi, Noumbi. Angola. Caungula, Mabete, Riv. Uamba, MRAC 162088; Riv. Camuconda, Tchimenji, MRAC 162089,

MRAC 162090-094 (n=5), MRAC 162095-100 (n=6); Riv Ganga-Ludchimo, MRAC162083-086 (n=4).

Platyallabes tihoni. Dem. Rep. Congo. Kingabwa, Stanley pool, MRAC 13307 (holotype); Kinsuka, MRAC 73-68-P-143, MRAC 138698-699 (n=2), 125345-349 (n=4), MRAC 73-22-P-3127 (n=3); Bulu, Luozi, BMNH 1976.5.21.30-39 (n=9), MCZ 50239 (n=13); Inga, MCZ 88947, MCZ 50537 (n=15); Tadi, Kibunzi, MCZ 50297 (n=5).

Platyclarias machadoi. Angola. Cuango, Cafunfo, Borio River, MRAC 78-6-P-1345, 181 mm SL (holotype), MRAC 78-6-P-1348-364, 78-6-P-1346, 78-6-P-1366-1367 (76-180 mm SL) (21 paratypes).

Dolichallabes microphthalmus. Dem. Rep. Congo. Kunungu, MRAC 44655, adult male, 229 mm SL (holotype), MRAC 44656-659 (n=3) (196-210 mm SL) and 62407, 188 mm SL (paratypes), MRAC 57662, 196 mm SL, MRAC 18850, 90 mm SL; Boende swamps, MRAC 101843, 149 mm SL, MRAC 176123-124 (n=1), 68 mm SL; Bokuma, MRAC 79093, 134 mm SL, MRAC 93774, 66 mm SL; Bokuma – Tchuapa, MRAC 79258-260 (n=3) (85-126 mm SL); Ndwa (Boloko), MRAC 78808-810 (n=3) (99-110 mm SL); Inonge, MRAC 96672, 110 mm SL; Maylimbe, Tshela, MRAC 66721, 97 mm SL.

Gymnallabes nops. Dem. Rep. Congo. Tadi, Kibunzi, Congo River, MCZ 50298, 57 mm SL (holotype).

IV.5.2 - The non-Congo River Specimens

IV.5.2.a A survey of the anguilliform Clariidae of the Central and Southern West Coastal Equatorial freshwater ecoregions (Gabon and Republic of the Congo); with the description of two new species, including a new, complete key of the African Clariidae genera

ABSTRACT

A survey of anguilliform African clariids from the central and southern West Coastal Equatorial freshwater ecoregion supports the existence of four species, of which two are new to science. 30 morphometric features, as well as several meristic and descriptive characters have been studied on 174 specimens. *Channallabes ogooensis* sp. n. can be recognized by the combination of following characters: serrations on both side of the pectoral spine, with on the inner side more frequent and more distinct serrations; number of vertebrae between 84-97; number of dorsal and anal fin rays respectively between 100-113 and 85-102; a pale spot on the skull roof in between the position of the anterior and posterior fontanel; absence of an epiotic; overall skull moderately reduced in size and several bones show plate-like outgrowths (sphenotic and pterotic) and the posterior border of the mesethmoid is indented, which makes that the anterior part of the anterior fontanel lies within the mesethmoid. *Channallabes teugelsi* sp. n. can be recognized by a low number of vertebrae (70-82); serrations only on the outer side of the pectoral spine; a number of dorsal and anal fin rays respectively between 99-109 and 90-100 and a clear, a pale spot on the skull roof in between the position of the anterior and posterior fontanel. Furthermore, this study presents a clear diagnose of three genera, *Channallabes*, *Gymnallabes* and *Clariallabes* and arguments the generic reassignment of two anguilliform species. Consequently, a revised key of all African clariid genera is given.

INTRODUCTION

Few anguilliform clariids of the central and southern West Coastal Equatorial freshwater ecoregions are present in museum collections. The few specimens present have been categorized in three species (*Gymnallabes typus, G. alvarezi* and *Channallabes apus*). A closer look shows that most of these are wrongly determined. A sampling in Gabon in 1999 and 2000 showed variation in the anguilliform clariids caught, indicating that plausibly several species were present. These anguilliform clariids have received considerable attention recently because of their remarkable variation, especially in their postcranial skeleton (ADRIAENS et al., 2002; DE SCHEPPER et al., 2004). The lack of an unambiguous determination of the species caused that no clear species demarcation could be made. This became evident when following the currently most complete, available key from POLL (1977) (see below). Therefore a complete screening of all the anguilliform clariids is needed.

The freshwater clariids are one of the 37 catfish families within the Siluriformes (http://clade.acnatsci.org/allcatfish/ACSI/taxa/Families.html). Although they occur in Syria, southern Turkey and large parts of Southeast Asia, their diversity is greatest in Africa, where there are 12 genera and as many as 74 species (TEUGELS, 1996; TEUGELS and ADRIAENS, 2003). Some of the generalized, fusiform species, such as *Clarias gariepinus* (Burchell, 1822) show a large geographic distribution, whereas the anguilliform species occur in a small area. They can only be found in swampy areas in Nilo-Sudan (Niger delta), Lower Guinea and the Zaire (Congo River basin) ichthyological province (POLL, 1957b; ROBERTS, 1975; TEUGELS, 1986; TEUGELS et al., 1990).

Clariid catfishes have an elongate body, four barbels, long dorsal and anal fins, and especially by the unique presence of a suprabranchial organ, formed by arborisation of the second and fourth gill arches (GREENWOOD, 1961; TEUGELS and ADRIAENS, 2003). Unique in these clariids is the extreme variation in body shape ranging between fusiform and completely anguilliform genera (PELLEGRIN, 1927). Although this has been observed in other families of teleosts, amphibians and reptiles (LANDE, 1978), it is never as extreme as in the Clariidae. Together with the elongated body, a whole set of morphological changes are observed, such as decrease and loss of the adipose fin, continuity of unpaired fins, reduction of paired fins, reduction of the skull bones, reduction of the eyes and hypertrophy of the jaw muscle complex (CABUY et al., 1999; DEVAERE et al., 2001, 2004) (**IV.5.1.a, IV.4**).

Presently, anguilliform clariid taxonomy is poorly understood. The only keys incorporating the anguilliform clariids are those of POLL (e.g. 1977). The characters used in

this key, such as presence of paired fins, number of ribs and vertebrae are no longer discriminative and overlap among species. This is partially due to the limited number of specimens used in the original descriptions of the species (e.g. *Gymnallabes typus*: n=1, *Channallabes apus*: n=1, *Dolichallabes microphthalmus*: n=7), so that intraspecific variation was not recognized.

The areas of interest for this study are the central and southern West Coastal Equatorial freshwater ecoregions (THIEME et al., in press), most specimens come from the Woleu/Ntem and the Ivindo/Ogowe basins.

Because correct identifications of the species are difficult, a systematic survey, with both morphological analyses was performed. This was based on all available material, including type-specimens, museum specimens and newly collected fishes of our region of interest.

MATERIAL AND METHODS

One hundred seventy four anguilliform clariids originating from the central and southern West Coastal Equatorial freshwater ecoregion have been examined. Details of their origin are given in the list of specimens examined. Institutional abbreviations are listed in LEVITON et al. (1985).

For this study, we used available museum material but most specimens were collected from different expeditions to Gabon (1999, 2000). Three large regions have been sampled. The first region is situated in the North of Gabon, close to the Equatorial Guinea and Cameroon border, in the Woleu/Ntem River system. A total of seven sites were sampled in the precincts of Oyem. The second region is situated around Makokou, Ivindo River system where three different sites were sampled. The last sampling area was situated in the Ogowe River system, were the two sampling sites were situated in the vicinity of Franceville. All sampling sites were characterized by shallow, muddy, still water. Most specimens were caught using fyke nets and fish hooks. All these specimens are deposited in the collection of the Africa Museum (MRAC), Tervuren, Belgium.

For all specimens, 36 measurements were taken point to point using a digital callipers with an accuracy of +/- 0.1mm following DEVAERE et al. (2004) (**IV.4**). Measurements of bilaterally paired structures were taken on the left side. Abbreviations are given in appendix 1. Not all specimens were preserved well enough to make all meristic counts. Seven specimens were cleared and stained following TAYLOR and VAN DYKE (1985).

Data obtained were subjected to two Principal Component Analyses, using Statistica 6.0 (StatSoft Inc.) with morphometric and meristic data treated separately. The different

PCA's were performed according to BODEN et al. (1997). Qualitative and absence/presence characteristics were not included in the analyses but help to further identify and divergence the species.

In addition we have examined some type material from outside the region: syntypes *Gymnallabes typus*: 2 specimens, BMNH 1866.12.4.1-2, 139-150 mm SL, West Africa, probably Old Calabar, Nigeria. Holotype *Channallabes apus*: BMNH 1873.7.28: 16, 135 mm SL, Interior of Ambriz, Angola (7° 50'Z-13° 06'E).

RESULTS

In JANSEN et al. (submitted) (**V.1**) the genetic relationships of the African clariids, based on ribosomal gene (5.8S) and spacer (ITS1, ITS2) sequence data are presented. These data show a clear separation between the specimens of the Woleu and Ivindo River systems (North and East Gabon) and the specimens of the Congo River system. This shows that the anguilliform clariid ichthyofauna from both systems is different (see below) and that the lower Guinea specimens form one natural group. Also shown in this tree is the clear separation of the Woleu and the Ivindo/Ogowe systems. Because morphological characteristics allow us to distinguish several species in the genetically more homogeneous groups (eastern and northern populations) of the Woleu and Ivindo River systems (see below); the genetically distinct Congo River specimens must also be considered as another species. This means that the Woleu and Ivindo River Systems specimens can be analysed separately from the Congo River system specimens, with this paper focusing on the former group.

For the metric and meristic analyses, we used 137 specimens originating from the Woleu/Ntem, Ivindo and Ogowe River systems, including the type material of *Gymnallabes alvarezi* Roman, 1970 (type locality: Rio Kie, close to Ebebiyin, Equatorial Guinea) and *Clariallabes longicaudatus* Pappenheim, 1911 (type locality: Mabelle, South Cameroon). The latter species was placed in synonymy with *G. typus* Günther, 1867 (POLL, 1967). Also in this analysis are two of the 17 specimens, known in musea from the studied area, that were originally identified as *G. alvarezi*. In the rest of the results and discussion, these specimens will be referred to as group III specimens. This low number (two of 17) in this combined metric/meristic analysis is due to the low number of well-preserved specimens. Only on a few specimens some counts could be made. Finally, the analysis included the type material of two closely related anguilliform species, *Channallabes apus* (type locality: Ambriz, Angola) and *Gymnallabes typus* (type locality: Old Calabar, Nigeria). The PCA's

were performed, using the covariance matrix for 30 log transformed measurements and correlation matrix of 5 meristic counts.

We combined the first factor scores for the meristic PCA with the second factor scores for the measurements PCA (**Plate IV.5-15A**). The first principal component proved to be an overall size factor and was thus omitted (BOOKSTEIN et al., 1985). In this combined plot, three distinct groups could be distinguished: (1) Group I, all the specimens from the Woleu/Ntem system; (2) group II, including most specimens of the Ivindo and Ogowe systems; and (3) group III, with the specimens from Zanaga-Ogowe, Lésala, Rep. Congo and Loa Loa beu M'Passa, Makokou, Gabon. The subdivision of the northern (Woleu/Ntem) and eastern (Ivindo/Ogowe) population was also supported by genetic evidence (JANSEN et al., submitted (**V.1**)). Group I encloses the type material of *G. alvarezi*. Additionally, we see that the specimens of the Ogowe lie separate from the Ivindo specimens, as well as from the type material of *C. longicaudatus* (which clusters with the Ivindo specimens). All three groups lie clearly separated from the type material of *C. apus* and *G. typus*. The factor loadings are shown in **Table IV.5-4**.

An additional osteological analysis on Group II, combining the Ivindo and Ogowe specimens, shows some clear differences. The Ogowe specimens show the presence of an epiotic, while no epiotic can be found in the Ivindo specimens. Both have clear serrations on the inner side of the pectoral spine, whereas the outer side of the pectoral spine is only clearly serrated in the Ivindo population. In the Ogowe population some serrations on the outer side can be observed, although never as numerous and well-defined as in the Ivindo group (**Plate IV.5-15B**). The overall skull is less reduced in the Ogowe specimens. Several bones show plate-like outgrowths (sphenotic and pterotic), which is more elaborate compared to that of the Ivindo specimens. Furthermore, in the Ogowe specimens the posterior border of the mesethmoid is indented, thus bordering the anterior part of the anterior fontanel. This is not the case in the Ivindo specimens, where the fontanel is completely enclosed in the frontals.

Because not all meristic variables could be counted on most of the specimens in "group III" (see above), only two specimens could be analysed, complicating any conclusions on this group. Therefore some additional counts (number of dorsal and ventral fin rays) were performed which could be counted on a larger subset of this group and on a representative subset of the total dataset including all different regions and all type material (**Plate IV.5-16A**). Group I has a high number of both dorsal and anal fin rays (resp. 110-160 and 101-155), as well as a high number of vertebrae (96-105). The second group has a lower number of both dorsal and anal fin rays (resp. 96-116 and 75-105) and vertebrae (82-91), not distinguishing the Ivindo from Ogowe specimens. The specimens of group III show an

even lower vertebrae number (70-82). The type material of *G. alvarezi* is plotted in group I, while the type of *C. longicaudatus* clusters in the group of the Ivindo and Ogowe specimens (group II).

Group I, including all the specimens from the Wolue/Ntem system, coincide with the holotype of *G. alvarezi* Roman, 1970. We can thus conclude that this is a homogenous group representing this species. This consequently means that group III, in the left lower corner in **Plate IV.5-16A**, can no longer be identified as *G. alvarezi*, which was the case in the museum collections. The data here show that group III is sufficiently distinct and consequently, it should be considered as representing a separate species. Group II, which assembles all the specimens of the Ivindo, groups with the type of *Clariallabes longicaudatus,* a junior synonymy of *G. typus,* and should consequently also be seen as a separate species. This synonymy is thus no longer confirmed here (**Plates IV.5-15A, IV.5-16A**) and the species should be reinstated. Finally, the group with the Ogowe specimens should also be considered as a new species. This group is different from the specimens in the '*longicaudatus*-cluster' based on mainly osteological characteristics. All plots were tested for growth allometry and showed that the found differences were not related to this allometry.

A new diagnosis, description and clear systematic position is given for *C. alvarezi, C. longicaudatus, C. ogooensis* and *C. teugelsi*. The argumentation for the generic transfer is given in the discussion section.

Group	River Systems	Species
Group I	Wolue/Ntem	*Channallabes alvarezi*
Group II	Ivindo	*Channallabes longicaudatus*
Group II	Ogowe	*Channallabes ogooensis* sp. n.
Group III	Ivindo/Ogowe	*Channallabes teugelsi* sp. n.

Key to the species of Channallabes

1a   Large, well-pronounced supraorbital process present on infraorbital IV (**Plate IV.5-16B**), reaching the rostral border of the eye; fenestra between scapulo-coracoid and cleithrum present; no contact between entopterygoid and quadrate, in Gabon and Pop. Rep. Congo...2

b   Small supraorbital process on infraorbital IV (**Plate IV.5-16C**), not reaching the rostral

border of the eye; fenestra between scapulo-coracoid and cleithrum absent; interdigitation between entopterygoid and quadrate..................................*C. apus*

2a   Spot present on skull roof between anterior and posterior fontanel, low number of dorsal (98-116) and anal (75-105) fin rays..3

b   No spot present on skull roof, high number of dorsal (118-160) and anal (105-155) fin rays..*C. alvarezi*

3a   Serrations only frequently on the innermost side of the pectoral spine (**Plate IV.5-15B**) ....*C. ogooensis* sp. n.

b   Serrations only on the outermost side of the pectoral spine (**Plate IV.5-15B**) ....*C. teugelsi* sp. n.

c   Serrations on both sides of the pectoral spine (**Plate IV.5-15B**).........*C. longicaudatus*

Channallabes alvarezi (Roman, 1970)

Gymnallabes alvarezi Roman, 1970

Holotype: Instituto de Biologia aplicada, Barcelona, 317.6 mm SL; Equatorial Guinea: Rio Kie, close to Ebebiyin, Roman, 1970.

Nontype material examined: Total of 84 specimens. D. Adriaens, June 1999 and S. Devaere, D. Adriaens and A. Herrel, September 2000. 38 specimens, 193-383 mm SL, MRAC A4-31-P-1-13, A4-31-P-14-18, A4-31-P-55-60, A4-31-P-76-77, A4-31-P-78-89 Aben Lang, Metui, Gabon (1° 29'N-11° 36'E) – 17 specimens, 202-375 mm SL, MRAC A4-31-P-19, MRAC A4-31-P-21, A4-31-P-24, A4-31-P-26, A4-31-P-30, A4-31-P-31, A4-31-P-32, A4-31-P-67-72, A4-31-P-90-93, Ebeigne, Woleu River, Gabon (1° 28'N-11° 36'E) – 5 specimens, 225-342 mm SL, MRAC A4-31-P-20, A4-31-P-25, A4-31-P-27-28, A4-31-P-29, Assok Ngomo, Woleu River, Gabon (1° 41'N-11° 39'E) – 2 specimens, 301-412 mm SL, MRAC A4-31-P-22-23, Okoallissis, Otolo, Otagna, Woleu River, Gabon (1° 31'N-11° 31'E) – 9 specimens, 160-398 mm SL, MRAC A4-31-P-47-54, A4-31-P-73, Zogongone, close to Oyem, Gabon (1° 34'N-11° 31'E) – 5 specimens, 221-345 mm SL, MRAC A4-31-P-61-63, A4-31-P-74-75, Mbenga, close to Oyem, Gabon (1° 37'N-11° 41'E) – 8 specimens, 238-413 mm SL, MRAC A4-31-P-64-65, A4-31-P-66, A4-31-P-94, A4-31-P-1-95-96, A4-31-P-97, A4-31-P-98, Oyem, Gabon (1°36'N-11°34'E).

Differential diagnosis: *Channallabes alvarezi* can be recognized by the combination of following characters: a high number of vertebrae (92-105), no serrations on the pectoral spine and postcranially, the lateral line system is clearly visible as a white dotted line along the lateral flank of the body. It differs from all other *Channallabes* except *C. apus* in not having a pale spot on the skull roof, in a high number of dorsal and anal fin rays (resp. 118-160 vs 98-116 and 105-155 vs 75-105). It can be distinguished from *C. apus,* in the absence of an interdigitating area between entopterygoid and quadrate and in the presence of a large, well-pronounced supraorbital process, reaching the rostral border of the eye, on the fourth infraorbital bone (**Plate IV.5-16C**).

Description: Measurements and meristic counts for holotype and additional specimens are given in **Table IV.5-5**. The standard length ranges from 150 to 413 mm. *C. alvarezi* has a very elongated body (ABD up to 3.2-6.8% of SL), with a preanal length of 17.7% up to 38.8% of SL (**Plate IV.5-17A**). Due to the extreme elongation of the body, there is a very small skull length (5.8-13.8% SL). Skull width is 55.6-90.7% (mean: 64.6%) of skull length. The very narrow skull roof, width 12.0-31.1% of maximal skull width, remains clearly visible between the bulging jaw muscles. Although the eyes are small, they remain visible. Tube-like anterior nostrils are present, although small. Upper lip slightly extends beyond lower lip.

The fleshy, unpaired fins are continuous. The pectorals fins are always present, length 2.5-7.2% of SL, and are always preceded by a small pectoral spine with a length of 2.9-7.1% of SL. The pectoral spine is not serrated. The pectoral fins have seven branched fin rays. Pelvic fins present in only three specimens; in the other specimens (n = 82) no evidence of pelvic fins. The total number of vertebrae is 92-105 (mode = 102), ribs 12-14 (mode = 14), branchiostegal rays 8, dorsal fin rays 118-160, anal fin rays 105-155.

The skull in *C. alvarezi* shows reduced ossifications and narrow skull roof. This reduction is shown in the reduced lateral plate of the frontal, as from a ventral view this lateral plate is limited to the outlines of the orbitosphenoid (**Plate IV.5-17B**). A comparable reduced plate-like outgrowth is present on the posttemporo-supracleithral bone. The epiotic is always absent. One or two suprapreopercular bones are present. On the prevomer, one or two posterior processes are present. The contact of the entopterygoid with the metapterygoid is limited to the rostro-dorsal side of the latter. Posteriorly on the hyomandibula, three caudally increasing processes are present for the interdigitation with the neurocranium (DEVAERE et al., 2001 (**IV.5.1.a**); Fig. 6). Oral teeth are present on dentary, premaxilla and prevomer.

Color: Alcohol preserved specimens gradually fade from dark brown on the dorsal side to whitish brown on the ventral side. Both sides are separated by the white dotted line,

representing the lateral line. The skin on the jaw muscles shows a paler brownish color than the surrounding skin of the head. The barbels have a darkish-brown coloration. *C. alvarezi* shows no distinct pale region on the skull roof.

Distribution: Currently known from the Woleu/Ntem River system in the region of Oyem and Equatorial Guinea, rio Kie (holotype) (**Plate IV.5-18**).

Etymology: Named after Sr. Mario Álvarez (ROMAN, 1970).

Channallabes longicaudatus (Pappenheim, 1911)

Clariallabes longicaudatus Pappenheim, 1911

Holotype: ZMB 18401, Zoologisches Museum, Humboldt Universitat (ZMHU), Berlin, 220 mm SL; in der Mabelle, Spanish Guinea, South Cameroon, Pappenheim, 1911.

Nontype material examined: Total of 67 specimens, S. Devaere, D. Adriaens and A. Herrel, September 2000. 34 specimens, 95-295 mm SL, MRAC A4-31-P-99-105, A4-31-P-137-151, A4-31-P-152-157, A4-31-P-159-162, A4-31-P-163-164, Makokou, River Ivindo, Gabon (0° 33'N-12° 51'E) – 31 specimens, MRAC A4-31-P-106-131, A4-31-P132-136, Etakaniabe, River Liboumba, Gabon (0° 31'N-12° 59'E) – 1 specimen, MRAC A4-31-P-158, Iyoko, Makokou, Gabon (0°32'N-12° 54'E).

Differential diagnosis: It differs from *C. alvarezi*, *C. ogooensis* and *C. teugelsi* in having clear serrations on both sides of the pectoral spine. *C. longicaudatus* can be distinguished from *C. alvarezi* in the low number of dorsal fin rays (resp. 98-116 vs 118-160 and 75-105 vs 105-155) and the presence of a pale spot on the skull roof. Further, it differs from *C. apus* in the absence of an interdigitation area between entopterygoid and quadrate and in a large, well-pronounced supraorbital process, reaching the rostral border of the eye, on the fourth infraorbital bone (**Plate IV.5-16A**). Furthermore, *C. longicaudatus* can be discerned by the presence of an epiotic and a straight posterior edge of the mesethmoid, leaving the anterior fontanel completely enclosed by the frontal bones.

Description: Measurements and meristic counts for holotype and additional specimens are given in **Table IV.5-6**. The standard length ranges from 95 to 295 mm. *C. longicaudatus* has an elongated body (**Plate IV.5-19A**) (ABD up to 4.0-9.8% of SL), with a preanal length of 27.3% up to 43.0% of SL. Very small skull length to SL ratio (8.7-15.9%). The large skull width ranges between 64.4 and 79.6% (mean: 71.5%) of the skull length. The skull roof always exposed with a width of 13.3-42.0% of maximal skull width. Eyes always visible. Whitish tube-like anterior nostrils are strikingly visible. Lower lip clearly shorter than the upper lip.

Unpaired fins forming a continuous finfold. Pectorals always present (length 5.0-9.7% of SL) always preceded by a firm pectoral spine (pectoral spine length 2.9-6.8% of SL). Both

sides of the pectoral spine are serrated. The pectoral fins have nine branched fin rays. Pelvic fins mostly present except in one specimen, intraspecific variation at this level has already been described by ADRIAENS et al. (2002). The number of vertebrae in *C. longicaudatus* lies between 84-91 (mode = 88). The number of ribs 12-14 (mode = 13), branchiostegal rays 8-9, dorsal fin rays 98-116 and anal fin rays 75-105.

Channallabes longicaudatus has a reduced skull and narrow skull roof. This reduction is shown in the lateral plate present on the frontal, which from a ventral view is wider than the outlines of the orbitosphenoid (**Plate IV.5-17C**). No further plate-like outgrowths can be seen. There is an epiotic and only one suprapreopercular bone is present. The entopterygoid makes contact with the metapterygoid on the complete rostro-dorsal side and partially on the ventral side. On the hyomandibular – neurocranium connection, three processes increasing in size caudally, are present. Further, the posterior border of the mesethmoid has a straight line. Teeth are present on dentary, premaxilla and prevomer.

Color: Alcohol preserved specimens have a light brown color, gradually fading to a paler ventral side. In the middle there is an undefined paler line. *C. longicaudatus* shows a well-defined pale spot on the skull roof, due to a lighter thinning of the neurocranium in that area.

Distribution: Currently known from the Ivindo River system. This species occurs in the Makokou region, Gabon (**Plate IV.5–18**).

Etymology: From the latin words "Longus" (long) and "Cauda" (tail).

Channallabes ogooensis sp. n.

Holotype: MRAC A4-31-P-170, 150 mm SL, Moanda, Gabon (1° 33'S-13° 16'E), S. Devaere, D. Adriaens and A. Herrel, September 2000.

Nontype material examined: Total of five specimens, 109-244 mm SL, MRAC A4-31-P-165-169, Malima, River Kahjaka Kanjaka, Gabon (1° 40'N-13° 20'E), S. Devaere, D. Adriaens and A. Herrel, September 2000.

Differential diagnosis: *Channallabes ogooensis* differs from *C. longicaudatus* and *C. teugelsi* by serrations on both sides of the pectoral spine, with those on the inner side more frequent and more distinct. *C. ogooensis* can be distinguished from *C. alvarezi* in the low number of dorsal and anal fin rays (resp.100-113 vs 118-160 and 85-102 vs 105-155) and the presence of a pale spot on the skull roof. Further, it differs from *C. apus*, in the absence of an interdigitation area between entopterygoid and quadrate and in a large, well-pronounced supraorbital process, reaching the rostral border of the eye, on the fourth infraorbital bone (**Plate IV.5-16B**).

Channallabes ogooensis can be recognized by the absence of an epiotic. Several skull bones show plate-like outgrowths (sphenotic and pterotic) giving the overall skull a less reduced appearance. The posterior border of the mesethmoid is indented, such that the anterior part of the anterior fontanel is bordered by the mesethmoid.

Description: Measurements and meristic counts for holotype and additional specimens are given in **Table IV.5-7**. Standard length ranges from 109 to 223 mm. *C. ogooensis* has an elongated body (ABD up to 5.7-7.5% of SL), with a preanal length of 28.4% up to 36.2% of SL (**Plate IV.5-19B**). Very small skull length (12.0-13.8% SL). Skull width ranges between 68.4 and 73.4% (mean: 71.1%) of the skull length. Skull roof remains always clearly visible with a width of 16.1-32.9% of maximal skull width. Eyes are always visible. The whitish tube-like anterior nostrils are clearly visible. The lower lip clearly shorter than the upper lip.

Unpaired fins form a continuous finfold. Pectorals always present (length 6.4-11.1% SL), always preceded by a firm pectoral spine (pectoral spine length 4.4-7.1% SL). Both sides of the pectoral spine serrated, with outer side more irregularly serrated. Pectoral fins have eight branched fin rays. Pelvic fins always absent. Number of vertebrae in *C. ogooensis* lies between 84-87 (mode = 84). Number of ribs 12-13 (mode = 12), branchiostegal rays 8-9, dorsal fin rays 100-113 and anal fin rays 85-102.

Channallabes ogooensis has a more or less reduced skull and narrow skull roof. A less extensive reduction (compared to *C. alvarezi, C. longicaudatus* and *C. teugelsi)* is shown in the presence of a lateral plate on the frontal: from a ventral view this lateral plate is wider than the outlines of the orbitosphenoid (**Plate IV.5-17C**), as well as the presence of a limited plate-like outgrowth on the posttemporo-supracleithrum, pterygoid and sphenotic. The epiotic is always absent. Only one suprapreopercular bone is present. The posterior border of the mesethmoid shows a clear indentation, placing the anterior part of the anterior fontanel in the mesethmoid. The entopterygoid makes contact with the metapterygoid on its complete rostro-dorsal side and also partially restricted with the ventral side. The hyomandibular – neurocranium connection comprises two sets of processes. Oral teeth present on dentary, premaxilla, and prevomer.

Color: Alcohol preserved specimens have a brown color, gradually fading to a paler ventral side. In the middle there is an indefinite paler line, which connects the different pores of the lateral line system. *C. ogooensis* shows a well-defined pale spot on the skull roof, due to a lighter coloration of the neurocranium in that area.

Distribution: Currently known from the Ogowe River system. The specimens appear in the Franceville region, Gabon (**Plate IV.5-18**).

Etymology: From the River Ogowe where the species was found.

Channallabes teugelsi sp. n.

Holotype: MRAC 78-22-P-1046, 80 mm SL, Magogo, 1km from Lékoli, Komono-Sibiti road, Rep. Congo (2° 36'S-13° 38'E), W. Wachters, July 1978.

Nontype material examined: Total of 16 specimens. 4 specimens, 87-144.6 mm SL, MRAC 78-22-P-1047-050, Zanaga, Lésala, River Ogowe, Rep. Congo, (2° 50'S-13° 50'E), W. Wachters, July 1978 – 1 specimen, 51 mm SL, MRAC 78-22-P-1051, Ndengué, Moungoundou-Ndziba-Ndziba road, Rep. Congo (2° 40'S-12° 41'E), W. Wachters, July 1978 – 11 specimens, 31-97 mm SL, MRAC 75-24-P-683-693, Loa Loa, M'Passa, Makokou, Gabon (0° 30'N-12° 46'E), A. Heymer, November 1974.

Differential diagnosis: *Channallabes teugelsi* differs from *C. longicaudatus* and *C. ogooensis* by serrations on the outer side of the pectoral spine. *C. teugelsi* can be distinguished from *C. alvarezi* in the low number of dorsal and anal fin rays (resp. 99-109 vs 118-160 and 90-100 vs 105-155) and the presence of a pale spot on the skull roof. Further, it differs from *C. apus*, in the presence of an interdigitation between entopterygoid and quadrate and in a large, well-pronounced supraorbital process, reaching the rostral border of the eye, on the fourth infraorbital bone (**Plate IV.5-16C**). *C. teugelsi* can be recognized by the combination of following characters: a number of vertebrae that ranges between 70-82 and a lower lip that reaches almost equally far as the upper lip.

Description: Measurements and meristic counts for holotype and additional specimens are given in **Table IV.5-8**. The standard length ranges from 31 to 145 mm. *C. teugelsi* has an elongated body, but not as extreme as the other three described species (**Plate IV.5-19C**), ABD up to 6.0-11.6% of SL. The postanal length ranges from 59.4% to 70.2% SL, indicating a smaller tail section than in *C. alvarezi, C. longicaudatus* and *C. ogooensis*. The skull length lies between 12.2 and 27.0% SL. Skull width 45.4-71.5% (mean: 57.8%) of skull length. The narrow skull roof, width 14.9-57.8% of maximal skull width, remains clearly visible.

Although eyes small, they remain visible. Whitish tube-like anterior nostrils are clearly present. Lower lip reaches almost as the upper lip.

Unpaired fins form a continuous finfold. Pectorals and pelvics are present in most specimens. The pectoral fin length ranges between 4.5 and 11.3% SL, sometimes preceded by a small pectoral spine with a length of 2.9-4.6% SL. The pelvic length totals 3.8-7.0% SL. In four specimens (MRAC 8-22-P-1047-050) no evidence of pelvic fins is found. Only the outer side of the pectoral spine is serrated. The pectoral fins have nine branched fin rays. Total number of vertebrae between 70-82 (mode = 72), number of ribs between 10-12, number of branchiostegal rays 8, dorsal fin rays 99-109, anal fin rays 90-100.

Channallabes teugelsi has a reduced skull and narrow skull roof. From a ventral view the lateral plate is wider than the orbitosphenoid (**Plate IV.5-17C**). There is distinct plate-like outgrowth on the posttemporo-supracleithrum. Epiotic always present. Always two suprapreopercular bones are present. Two posterior processes are present on the prevomer. The contact of the entopterygoid with the metapterygoid is situated over the rostro-dorsal side and runs up to a limited part of the ventral side of this metapterygoid. Two extended processes, on the hyomandibula, are present for the interdigitation with neurocranium, at the level of the pterotic. Oral teeth present on dentary, premaxilla and prevomer.

Color: Alcohol preserved specimens are equally light brown along the whole body. *C. teugelsi* shows a clear whitish spot on the skull roof, between the anterior and posterior fontanel, due to a lighter coloration of the neurocranium in that area.

Distribution: Currently known from the Ivindo River system in the region of Makokou, Gabon and the Ogowe River system in the region of Zanaga, Ndengué, Magogo, Rep. Congo (**Plate IV.5-18**).

Etymology: Named after Prof. Dr. Guy Teugels, who worked on several aspect of the African catfish family. He is renown for his systematic research, especially of the genus *Clarias*.

DISCUSSION

Based on all four species diagnoses, all species should be included in the genus *Channallabes* and no longer in *Gymnallabes* and *Clariallabes*, corresponding to the genera to which type material of *C. alvarezi* and *C. longicaudatus* were originally assigned[11]. This taxonomic transfer is especially based on several osteological characteristics. What follows is the comparison of these three genera, with special attention to some discriminating characters (**Table IV.5-9**).

Because *Gymnallabes* is poorly described and all of the original diagnostic characters (GÜNTHER, 1867; POLL, 1957b) are subject to large inter- and intraspecific variation and no longer help to unambiguously diagnose the genus *Gymnallabes*, new characteristics are provided to support the generic diagnostic difference of *Gymnallabes* and *Channallabes* (**Table IV.5-9**). In *Channallabes*, the infraorbital bones are reduced to canal bearing bones with a small plate-like outgrowth on the posterior-most infraorbitals. In *Gymnallabes*, however, all infraorbital bones are extremely reduced to small tubular bones. Another

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[11] The phylogenetic hypotheses in **V.2.1** confirm the validity of both species, however, this involves some taxonomical implications (see **VI.1**)

difference with *Gymnallabes* is the presence of a supraorbital process on infraorbital IV in *Channallabes* (**Plate IV.5-16B,C**). Also the suprapreopercular bones are extremely reduced in *Gymnallabes*, while in *Channallabes* the distal one bears plate-like extensions. In a ventral view, the lateral plates of the frontals slightly exceed or equal the boundaries of the orbitosphenoid (**Plate IV.5-17B,C**), while in *Gymnallabes* this lateral plate is completely absent. The first dorsal fin pterygiophore is situated posterior to the sixth post-Weberian vertebrae, while in *Gymnallabes* the dorsal fin originates more anteriorly.

The complex genus *Clariallabes* Boulenger, 1900 is characterized by an elongated body, especially the tail region, that is somewhat intermediate between that of most species of *Clarias* and the extremely anguilliform species (post anal length in *Clariallabes* between 50-65% of SL). Furthermore, species of *Clariallabes* have a dorsal and anal fin that are not or only partially fused to the caudal fin, still leaving the different fins clearly visible (Teugels et al., in press). In addition to these diagnostic characteristics, other differences between *Clariallabes* and *Channallabes* are given here (**Table IV.5-9**). While in *Channallabes* the infraorbital bones are reduced, with small plate-like extensions, larger plates occur on these bones in *Clariallabes*, especially on the postero-ventral one. The same counts for the suprapreopercular bones. Also, in *Clariallabes*, the lateral plates of the frontals are much wider than in *Channallabes*. In addition to these expansions, lateral plates are present on the sphenotic and pterotic, which is not the case in *Channallabes*. Also in *Clariallabes*, the first dorsal fin pterygiophore is situated more anteriorly to the sixth post-Weberian vertebrae, while in *Channallabes*, the dorsal fin origin is situated more posteriorly.

In addition to the two new species, six other anguilliform clariids are known, designated to five genera. The species presented here are clearly different from each one of the other. *Platyallabes tihoni* (Poll, 1944) can be recognized by a very small distance between the origin of the dorsal fin and the supraoccipital process (2.2-6.6% of SL vs 6.6-17.6% of SL) (DEVAERE et al., in press (**IV.1**)). *Platyclarias machadoi* Poll, 1977 has an extremely flattened skull. *Dolichallabes microphthalmus* Poll, 1942 is characterized by the presence of only one fontanel on the skull roof (DEVAERE et al., 2004) (**IV.4**). *Gymnallabes nops* Roberts and Stewart, 1976 shows a large reduction in the infraorbital series in size and number (two instead of five) (DEVAERE et al., in press (**IV.3.1**)). *Gymnallabes typus* Günther, 1867 is characterized by well developed skin folds, bordering the side wall of the mouth. A last anguilliform species is *C. apus*. The differences present between *C. apus* of the Congo basin and the 'Gabon' specimens are not only reflected in genetic differences (JANSEN et al., submitted (**V.1**)), but also in some osteological differences. Although in *C. apus*, an articulation process on infraorbital II is present, it never makes contact with the

lateral ethmoid. This is in contrast with the clear contact found in the 'Gabon' specimens. Another difference is that in *C. apus,* an interdigitation zone between the entopterygoid and the quadrate is present (DEVAERE et al., 2001) (**IV.5.1.a**), while no contact is observed in the 'Gabon' specimens. A last difference is the absence of a fenestra in the pectoral girdle between the scapulo-coracoid and the cleithrum in *C. apus* (present in all "Gabon" species).

Measurements and meristic counts for the anguilliform clariids were only rarely reported in the literature (POLL, 1942a, b, 1957b, 1967, 1977; ROBERTS and STEWART, 1976; ROMAN, 1970). These were all based on samples that were too limited in number to demonstrate the full morphological variability of the species. In clariid classification and in anguilliform clariids in particular, a character which shows little variation in one species can be very variable in other species. Due to this high level of variation, the new species diagnoses all include the range of intraspecific variation of the measurements and meristic counts. In clariids, intraspecific variation is not only restricted to size ranges, but is observed at the structural level as well. Striking examples have already been demonstrated in the paired fins (ADRIAENS et al., 2002) and the caudal skeleton (DE SCHEPPER et al., 2004). This makes it very difficult to define diagnostic characters that includes all members and excludes all non-members of all species. Therefore different combinations of character types (measurements, counts, osteological, …) have to be used.

The distinction between *C. alvarezi* (group I in **Plate IV.5-15A**) and the other species is clear and based on several types of characteristics. Additionally, the validity of *C. alvarezi* was already noticed by other studies (DE SCHEPPER et al., 2004). Furthermore, *C. longicaudatus* and *C. ogooensis* (Group II in **Plate IV.5-15A**) can easily be distinguished from the rest. Separating the two is more difficult, because no clear metric and meristic differences can be found, even though the combined plot already gives an indication of the existing differences. Similar osteological differences, which help to discern other species can be found between these two species, for instance, the absence/presence of the epiotic (see above). As a last one, *C. teugelsi* (Group III in **Plate IV.5-15A**) is separated in the combined plot, mainly based on the meristic characters (with the problem of the low number of used specimens). However, a plot of the number of vertebrae against the number of dorsal fin rays shows that *C. teugelsi n. sp.* differs from the other species by a low number of vertebrae (70-82 versus 84-105) (**Plate IV.5-16A**). Although these specimens have a low SL, there is no evidence for size-related changes of the meristics in the anguilliform clariids species studied here. Not only in anguilliform fishes, but also in general in fishes, several authors, as for instance LANDRUM and DARK (1968), have reported on the independence of the total vertebrae number from the length of the fish.

The geographic distribution shows that the four species occur in the two large river systems: the Woleu/Ntem and the Ivindo/Ogowe from Gabon. *C. alvarezi* is the only species occurring in the Woleu/Ntem system. The other three species are located in the Ivindo/Ogowe system. While *C. teugelsi* can be found in the Ogowe and Ivindo, *C. longicaudatus* is only situated in the latter, and *C. ogooensis* is only found in the Ogowe River (upstream from the joining of the two large rivers). According to Thieme et al. (in press) these two locations are situated in two different freshwater ecoregions, respectively the central West Coastal Equatorial and the southern West Coastal Equatorial. The northern tributaries of the Ogowe River (Abanga, Okano, and Ivindo) are included in the central West Coastal Equatorial freshwater ecoregion because their fauna is more similar to that of other rivers in the Central West Coastal Equatorial ecoregion than to the mainstem Ogooué (Thieme et al., in press). Since the Eoocene, the African continent has experienced multiple phases of climate changes, oscillating between the so-called greenhouse and icehouse (see Séranne, 1999). This resulted in a fluctuating pattern of expanding savannah and reduction of forest patches (refugia), and vice versa. These refugia are situated in southern Cameroon, southwestern Nigeria, central Gabon (divided around the Woleu, Ivindo and Ogooue) and various spots in the Congo basin (Haffer, 1982; Maley, 1987). Interestingly, when we compared the location where anguilliform species occur (*C. alvarezi*: North Gabon, Woleu, *C. longicaudatus*: East Gabon, Ivindo; *C. ogooensis*: South-East Gabon, Ogowe; *C. teugelsi*: Gabon and Pop. Rep. de Congo) with the suggested location of the refugia during the phases of vegetation retraction, we see a close match. In these refugia, rapid isolation could become a fact and, as a consequence, speciation could easily occur, helping to explain the presence of three different species in these different areas. The only species not following this pattern is *C. teugelsi*. *C. teugelsi*, as already mentioned, occurs in both central and southern West Coastal Equatorial freshwater ecoregions. Both ecoregions are well defined with a reasonable percentage of endemic fish species (20–25%) (Thieme et al., in press). Although the distribution is spread over two ecoregions, all findings are in the watershed of one river crossing the boundary of the two mentioned ecoregions, viz. the Ivindo. The evolutionary history of the Ivindo River fauna, including how it arose still remains a question, but according to Lévêque (1997), during the climatic fluctuations of the Pleistocene, the central West Coast Equatorial freshwater ecoregion may have been a refuge from which the ichthyofauna and other aquatic-dependent taxa of the whole West Coast Equatorial (or Lower Guinea) bioregion could disperse again. This could explain the distribution pattern of *C. teugelsi*.

The apparent split distribution of *C. teugelsi* could also be linked to its specialized, burrowing lifestyle that it shares with the other anguilliform species. Its preference for swamps and flood plains, submerged in the mud (even in artificial, acidic manioc ponds), undoubtedly explains its absence in many collections when sampling has been done in rivers and lakes of these regions. Unless sampling is focusing on these niches, thereby largely relying on local fishermen and their traditional ways of fishing, very few specimens can be caught. As a consequence, the distribution of *C. teugelsi* may well be continuous for the Ogowe and Ivindo. Additional targeted sampling would be required to verify this.

Consequent to the reassignment of several species in different genera, presented in this paper and to the modification of several intraspecific variable discriminating characteristics into new unambiguously characters in several other genera (*e.g.* DEVAERE et al., 2004) (**IV.4**), a new key to the genera of the African Clariidae is presented here[12].

Key to the genera of the African Clariidae:

1a   Adipose fin absent or very short (less than 25% SL); more than 50 dorsal fin rays ……………...2

b   Large adipose fin (24–33% SL); less than 50 dorsal fin rays ………….………....*Heterobranchus*

2a   Unpaired fins not continuous or partially fused with the caudal.…………….………….…......3

b   Unpaired fins and caudal form continuous fin fold..……………………....……………..……….10

3a   Eyes present, sometimes small; skull roof exposed in between jaw muscles……..…………..…..4

b   Eyes absent; skull roof invisible………………….……..........…….……….…*Uegitglanis*

4a   Eyes lateral, part of lateral border of the skull; dorsal and caudal fins clearly separated (gap 10% of SL); lateral dermal skull bones large but separated…………………………………....……...5

b   Eyes laterodorsal or dorsal, no part of lateral border of the skull; dorsal and caudal fins not apart or slightly separated (gap max 5% of SL); lateral dermal skull bones continuous or little spaced or are reduced in size …………………………...…………………………………....……...6

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[12] Additional information obtained from Poll (1957a, 1977)

5a   Number of dorsal fin rays (maximally 54); large number of elongated neural spines (9–12); suprapreopercular and posttemporo-supracleithrum make contact; no serrations on pectoral spine... ….………..……………………………………………………………....*Dinotopterus*

b   Dorsal fin rays (more than 59); low number of elongated neural spines (5–8); gap between suprapreopercular and posttemporo-supracleithrum; serrations on outer side of pectoral spine …......………….…………………………………………....………………..*Bathyclarias*

6a   Suprabranchial organ with developed arborescent structures.................………………....……7

b   Suprabranchial organ absent or vestigial and incomplete………………………………..………….8

7a   Head short (11–26% SL); lateral head bones separated; body elongate; distance between anus-caudal fin origin between 50–65% SL………...………..………..…….......................*Clariallabes*

b   Head more elongated (20–34% SL); lateral head bones in contact (often fused in larger specimens); body less elongate; distance between anus-caudal fin origin maximally 50% SL…………….……………….…………………………………………………......*Clarias*

8a   Skull length 14–19% SL; skull roof 15–36.5% skull width; infraorbital IV and suprapreopercular reduced and well separated, number of vertebrae 59–71……………………………..………..9

b   Skull length 20–21% SL; skull roof more than 50% skull width; large infraorbital IV and suprapreopercular and close to each other , number of vertebrae 51–52…………….....*Xenoclarias*

9a   Extremely dorsoventrally flattened skull (skull height 22.9–37.1% of skull length); abdominal depth 4.1–6.5% of SL; skull roof 26.0–36.5% of skull width; number of vertebrae 65–71; number of ribs 9–11……………………………...…….……..*Platyclarias*

b   No dorsoventrally flattened skull (skull height 50% of skull length); abdominal depth 16% of SL; skull roof 15% of skull width; number of vertebrae 59; number of ribs 8 …………..

…………………………..……………………………………………*Tanganikallabes*

10a Suprabranchial organ with arborescent organ present but more or less reduced, large distance between the occipital process and the dorsal fin (5.2–17.1% of SL)...…..........................………11

b Suprabranchial organ lacks arborescent organs and fan-like covers completely; small distance between the occipital process and the dorsal fin (2.2–6.6% of SL)………………...…*Platyallabes*

11a Extremely reduced infraorbital series (tubular bones); no pale spot on skull roof…...................…12

b Slightly reduced infraorbital series (plate-like extensions present); with or without pale spot on skull roof………….………………………………….…………….…..….*Channallabes*

12a One longitudinal fontanel present; high number of vertebrae (95–116); skull length 6–10% of SL…………………………….……………….……………………......*Dolichallabes*

b Two fontanels present; low number of vertebrae (62–86); skull length 11–18% of SL…………………………………...……………………….…..…………*Gymnallabes*

PART V

Phylogenetic Analyses

V.1 - Molecular Phylogeny

V.1.1 Phylogenetic and biogeographic analysis of air-breathing catfish (Siluriformes: Clariidae) inferred from ribosomal gene and spacer sequences, with an emphasis on anguilliformity

ABSTRACT

The catfish family Clariidae comprises species in which the body shape ranges from fusiform to anguilliform. Recent studies have shown that the evolution involving body elongation is the result of convergent evolution. This study aims to investigate the phylogeny of African representatives of the Clariidae, in order to find an answer to questions regarding the evolution towards anguilliformity, as well as its paleobiogeographical background. Sequences of 28 specimens were analyzed using the neighbor-joining, maximum-likelihood, maximum-parsimony, and Bayesian inference algorithms and the parsimony algorithm in POY. The study yields phylogenetic hypotheses showing well-supported clades. A first clade shows a close relationship between *Clariallabes longicauda*, *Clarias camerunensis*, *Channallabes alvarez*i and *Channallabes longicaudatus*. Second, *Channallabes sanghaensis*, *Clarias platycephalus* and *Clarias jaensis* form a monophyletic group. *Clarias gariepinus*, *Clarias ngamensis*, *Dinotopterus cunningtoni* and *Heterobranchus isopterus* cluster together in a very well supported group, strongly corroborated by previous studies as well. Anguilliformity appears to involve homoplastic transformations of the body plan, occurring at least four times and originating from a fusiform *Clarias*-like ancestor. Anguilliformity could have evolved in different lowland forest refugia in Central West Africa, where a selective pressure could have favored the arise of eel-like burrowers, better adapted to living in rain forest flood plains and swamps. After subsequent expansions of the forest patches, the fusiform taxa could have spread again using their adaptations to disperse in large rivers and to cross land bridges between them, whereas the specialized anguilliforms taxa would have been more restricted to their moist habitats.

INTRODUCTION

Representatives of the catfish family Clariidae can be found all over Africa, the Middle East and parts of Asia (GREENWOOD, 1961; TEUGELS, 2003). They have a suprabranchial organ, permitting them to extract oxygen directly from the atmosphere (GREENWOOD, 1961; Graham, 1997). This adaptation and their thick skin with mucous pores could explain their distribution in swamps, flood plains and periodically dry pools (MATTHES, 1964; BURGESS, 1989; TEUGELS, 1986). The family consists of fifteen genera, 12 endemic to Africa and two endemic to Asia, comprising a total of 93 species (SABAJ et al., 2004). *Clarias* is the only genus occuring on both African and Asian continents and can be divided into seven subgenera (TEUGELS, 2003). This family presents a unique example among Teleostei since it is the only one where an evolutionary transformation from fusiform to anguilliform species has been observed at the species level (PELLEGRIN, 1927). Initially, anguilliformity was considered to have evolved gradually in this family, in a series starting with *Heterobranchus* Geoffrey St. Hilaire, 1809 and ending with the extreme anguilliform *Dolichallabes* Poll, 1942 (BOULENGER, 1908; PELLEGRIN, 1927; DAVID, 1935). This intuitional gradualism was first doubted by POLL (1942, 1977). More recent studies on clariid phylogeny, based on molecular data (AGNÈSE and TEUGELS, 2001a,b), a review of the literature on morphological data (TEUGELS and ADRIAENS, 2003), and mitochondrial DNA (AGNÈSE and TEUGELS, in press) all confirm the polyphyletic nature of anguilliformity, and the monophyletic origin of the family, although the interrelations of different species and genera of Clariidae are not yet resolved. *Bathyclarias* species appear to have evolved recently from *Clarias gariepinus* ancestors in Lake Malawi (AGNÈSE and TEUGELS, 2001a). A close relationship between *Heterobranchus* and *Clarias gariepinus* is suggested by LEGENDRE et al. (1992), TEUGELS et al. (1992) and TEUGELS and ADRIAENS (2003). Specimens previously considered as *Channallabes apus* have been assigned to six new species, of which three are new to science and two rehabilitated nominal taxa (*Channallabes apus*, *C. ogooensis*, *C. longicaudatus*, *C. sanghaensis*, *C. alvarezi*, *C. teugelsi*) (Devaere et al, submitted)(**IV.5.1.a, IV.5.2.a**). The validity of these recent changes, which were based on morphology, is tested in this study.

In this paper we are using molecular data and try to solve part of the issues involved in the problematic taxonomy of the family Clariidae. The monophyly of several (sub)genera have been investigated, with special attention being paid to the genesis of anguilliformity and the biogeographical patterns behind it.

DNA extraction, PCR Amplification, and Sequencing Reactions

The origins of the samples used in this study (28 new samples, 3 Genbank sequences) are listed in **table V.1-1**. The Gabon specimens were collected during expeditions in 1999 and 2000 to assess the complete species richness in the Gabon area. Several samples of different regions of what was then still known as *C. apus* were included in this analysis. Tissue was isolated from the body musculature and anal fin clippings and total DNA was prepared according to the protocol of the Puregene™ DNA isolation kit type D-5000A (Gentra Systems, Inc., BIOzym, Landgraaf, The Netherlands). The complete region of the ribosomal spacers (ITS1 and ITS2) and the ribosomal 18S, 5.8S and part of the 28S genes was amplified using the polymerase chain reaction (PCR) with Qiagen DNA polymerase (Westburg, Leusden, The Netherlands). Eukaryote-specific external primers complementary to the 5'-terminus of the 18S rDNA gene (5'-TYCCTGGTTGATYYTGCCAG-3') and the 5'-terminus of the 28S rDNA gene (5'-CCG CTG AAT TTA AGC-3') were used to amplify the entire 18S-ITS1-5.8S-ITS2 and part of the 28S region. As a new reverse primer we used (5'-GCT TAA ATT CAG CGG-3'). Internal primers were used as described in literature (WEEKERS et al., 1994; SAMRAOUI et al., 2003). PCR amplifications, purification of the PCR products, and DNA sequencing was done according to standard procedures (SAMRAOUI et al., 2003). External and internal primers in conserved regions of the 18S and 5.8S rDNA were used for sequencing (WEEKERS et al., 1994; SAMRAOUI et al., 2003).

Sequence Alignment and the Construction of Datasets

Saturation was analyzed by plotting the absolute number of transitions and transversions against patristic distance values.

The DNA sequences covering the complete 18S-ITS1-5.8S-ITS2-28S (partial) region was aligned with CLUSTALW 1.64b (THOMPSON et al., 1994) using default settings, resulting in an initial dataset. A second dataset was created by fine-tuning the alignment of the initial dataset based on secondary structural information, using GeneDoc 2.6.002 (NICHOLAS et al., 1997) (alignment: see enclosed **CD Rom, alignment**). The alignment of the 18S gene region was manually optimized with published 18S rDNA sequences based on the conservation of both primary sequence data and inferred secondary structural features (the rRNA WWW Server: "http://www-rrna.uia.ac.be/ssu/index.html") (The Ribosomal Database Project: "http://rdp.cme.msu.edu/download/SSU_rRNA/alignments/"). The small and highly conserved 5.8S gene region and the small portion of the 28S gene were easy to align, and

were used to position the highly variable ITS1 and ITS2 regions. The boundaries of the ITS1 and ITS2 were determined by comparison of the aligned dataset with ITS sequences of fish taxa available in the EMBL databank (e.g. *Cyprinus carpio* - Cyprinidae). The ITS regions were manually optimized based on conservation of both primary sequence data and inferred secondary structural features. The secondary structures of the ITS1 and ITS2 regions were predicted using the Mfold webserver for nucleic acid folding and hybridization prediction (ZUKER, 2003) (http://www.bioinfo.rpi.edu/applications/mfold), and compared with published data (FRITZ et al., 1994; MAY and COLEMAN, 1997; MORGAN and BLAIR, 1998).

Sequence and Phylogenetic Analyses

The Akaike Information Criterion (AIC) in MODELTEST 3.6 (POSADA and CRANDALL, 1998; POSADA and BUCKLEY, 2004) were used to select an appropriate substitution model of DNA evolution. The dataset was analyzed with the Bayesian inference algorithm (MrBayes, version 3.0b4; HUELSENBECK and RONQUIST, 2001), and the neighbour-joining (NJ), maximum-parsimony (MP) and the maximum-likelihood (ML) algorithms in PAUP* 4.0b10 (SWOFFORD, 2003) to resolve the phylogenetic relationships. The model with corresponding nucleotide frequencies, substitution rates and types, and Ti/Tv ratios was selected by MODELTEST 3.6 (POSADA and CRANDALL, 1998; POSADA and BUCKLEY, 2004), and used for NJ, MP and ML algorithms in PAUP*, and in the Bayesian analysis.

Pairwise sequence divergence data between taxa were computed for the complete 18S-ITS1-5.8S-ITS2-28S(partial) region. Absolute distance values and distances based on a maximum-likelihood distance matrix (PAUP*), with appropriate parameters for the DNA evolution model (MODELTEST) were calculated (data not shown).

After selection of the appropriate substitution model of DNA evolution with corresponding parameters that best fitted the data, the ML analysis was conducted in PAUP*. Heuristic search settings were stepwise taxon addition, TBR branch swapping, MulTrees option in effect, no steepest descent, and rearrangements limited to 10,000. The nonparametric-bootstrap analysis with 100 replicates was used to assess the reliability of individual branches in the phylogenetic trees obtained by heuristic search with stepwise sequence addition (FELSENSTEIN, 1985).

Minimum-evolution analysis was performed with PAUP* by application of the selected ML substitution model to the NJ algorithm. The nonparametric bootstrap analysis used 1000 replicates to assess the reliability of individual branches in the phylogenetic tree.

The Bayesian analysis was performed with MrBayes version 3.0b4 (HUELSENBECK and RONQUIST, 2001) specifying the appropriate model structure for each partition (18S, ITS1, 5.8S, ITS2) in the dataset. The appropriate mixed models for the heterogeneous data were

specified, thus applying site-specific models of rate variation for each partition. In a first analysis the length of the Bayesian run was tested in order to be certain of convergence. The Markov Chain Monte Carlo process was set so that four chains ran simultaneously for 5,000,000 generations, with trees being sampled every 100 generations for a total of 50,000 trees in the initial sample. For the final analysis five independent Bayesian runs where performed in order to confirm that there was adequate convergence and mixing. Each MCMC process started from random starting points and was set so that four chains ran simultaneously for 1,000,000 generations, with trees sampled every 100 generations for a total of 10,000 trees in the initial sample. Variation in the ML scores in the samples was examined by inspecting the MrBayes-logfile, and the position where the ML scores stopped improving was determined. The portion of the trees before the position (tree number) where the ML score stopped improving dramatically and only fluctuated around a plateau, was discarded. The posterior probability of the phylogeny and its branches was determined for all those trees in the plateau phase with nearly the best ML scores. The Bayesian estimates of posterior probability and bootstrap analyses were included to assess support.

Two different types of parsimony analysis were conducted. First, equally weighted MP analyses were performed with PAUP*. Heuristic search settings were: stepwise taxon addition, tree bisection-reconnection branch swapping, multiple trees retained, no steepest descent, rearrangements limited to 10,000, and accelerated transformation. Treating gaps as characters as in SWOFFORD (1996), or LUTZONI et al. (2001) would have provided more information from these sites, but we treated gaps as missing data so that the MP analysis could be directly compared to the ML analyses. The nonparametric-bootstrap analysis used 1,000 replicates to assess the reliability of individual branches in the phylogenetic trees obtained by heuristic search with stepwise sequence addition (Felsenstein, 1985). Trees were displayed with TREEVIEW 1.6.6 (PAGE, 1996).

A second parsimony analysis was done under the optimality criterion of parsimony with equal weights (i.e., gaps, transitions, and transversions all given a weight of 1). The parsimony analysis was conducted using direct optimization (WHEELER, 1996) and iterative pass (WHEELER, 2003a) as implemented in the program POY (WHEELER et al., 2002) and run on the American Museum of Natural History Parallel Computing Cluster. The analysis began by generating 30 random addition sequences (RAS) per random replicate for 5 replicates. These 150 RAS were improved with TBR branch swapping during the searches, an additional round of TBR branch swapping and tree fusing (GOLOBOFF, 1999) at the end. These random replicates resulted in one most parsimonious tree. The resulting tree was submitted to POY for further tree searching using the commands iterative pass (WHEELER, 2003a) and 'exact' (WHEELER et al., 2002). This second step of the analysis began by tree fusing (GOLOBOFF,

1999) the submitted topology, and it was followed by an additional round of tree fusing and TBR branch swapping to reduce heuristics in the first-step analysis.

The length of the resulting implied alignments (WHEELER, 2003b) were verified in NONA (GOLOBOFF, 1998) and WinClada (NIXON, 2000). To estimate the "robustness" of the clades recovered in the phylogenetic hypothesis, bootstrap percentages (200 replications, 10 random addition sequences per replicate) were calculated in NONA based on the resulting implied alignment. Character evolution on the recovered topology was examined using NONA and WinClada.

Divergence Time Estimation

Many methods have been proposed for phylogenetic dating (e.g. BRITTON et al. 2002; THORNE and KISHINO, 2002; YANG and YODER, 2003). Here the program r8s (SANDERSON, 2002; 2003) was used to do the dating. R8s performs a semiparametric rate smoothing, using a penalized likelihood approach applied to the distances inferred from the ML tree with branch lengths. It combines a model-based likelihood approach with a roughness penalty that prevents too much rate variation across the tree. A cross-validation procedure is done to obtain a smoothing parameter that specifies the size of the roughness parameter. Cross validation was performed for the trees with branch lengths, obtained by multiplying the per site values as reported by PAUP* with the number of sites. This procedure provides an objective method for model selection and choice of optimal smoothing value (SANDERSON, 2002, 2003). Comparison of 7 independent dating analyses (ML trees with smoothing factors 0.63, 1.00, 1.58, 2.51, 3.98, 6.31, 10.00), using optimal (= lowest) and sub-optimal smoothing factors, was used as a measure of confidence; average node date and confidence intervals were calculated (DUMONT et al., 2005).

We used two reference fossils, the first appearance of the African Clariidae in the Lower Eocene and the first appearance of *Heterobranchus* in the Miocene, to estimate divergence times (data obtained from GAYET and MEUNIER, 2003, **Table 3**). All fossil calibration points were used simultaneously, using the "fixage" command for the Miocene *Heterobranchus* group, and the "constrain" command with "minage" and "maxage" limits for the Eocene Clariidae of r8s. Alternatively, we tested dating each with one fossil as calibration point using the "fixage" command, and the other one as a constraint specifying minimum ages using the "constrain" command.

RESULTS

Sequence Analysis and Alignments

The transitions or tranversions were plotted against all pair wise distances. All plotting indicated relatively linear relationships between substitution rates and genetic distances (not shown). This suggested that transition and transversion were not saturated.

Length of ribosomal genes (18S, 5.8S) and spacers (ITS1, ITS2) are given in **Table V.1-2**. The length of the 18S and 5.8 genes showed little variation (1869-1870 bp in the ingroup, 1836-1882 bp in the outgroup and 156-157 bp in the ingroup, 157-158 bp in the outgroup, respectively). Length variation in the spacer regions was much higher (ITS1: ingroup: 454-567, outgroup: 368-409; ITS2: ingroup: 384-490, outgroup: 310-386). The GC% shows the same pattern of variation, being higher in spacer regions than in the genes (**Table V.1-2**). The GC percentages for the spacer regions were almost equal, indicating both regions are balanced. The sequences of representatives of *Clarias ngamensis* originating from different geographical locations were almost identical. ITS1 showed a slight difference in GC percentage in *C. ngamensis*, whereas in *C. theodorae* the length and GC percentage varied considerably between the two specimens. The two *C. pachynema* specimens differed even more.

Phylogenetic Analysis

A first dataset, including the 18S sequences only, was created, consisting of two new (*Kryptopterus bicirrhis* - Siluridae and *Pangasianodon hypophthalmus* - Pangasiidae) and three Genbank sequences (*Clupea harengus* - Clupeidae, *Cyprinus carpio* - Cyprinidae, and *Ictalurus punctatus* - Ictaluridae) as an outgroup, and 20 taxa as an ingroup. Phylogenetic analyses using four methods, NJ, MP, ML and Bayesian, showed a rather consistent topology (**Plate V.1-1**). The studied catfish (*Kryptopterus* + *Pangasianodon* + *Heteropneustes* + Clariidae) are a monophyletic group, well separated from the other three outgroup specimens (BS= 100, 99, 86, 100 for NJ, MP, ML, MrB resp.). *Heteropneustes* is the sistergroup of the included Clariidae. The 18S gene tree (**Plate V.1-1, Table V.1-2**) and combined 18S+5.8S gene tree (not shown) didn't show enough variation to resolve relationships within the ingroup, as could be expected since 18S is known to be suited for relationships on higher taxonomic levels (WEEKERS et al, 1994, 2002). However, the outgroup containing *Clupea harengus*, *Cyprinus carpio* and *Ictalurus punctatus* seemed to be well chosen, they could be used with confidence as outgroup in the analysis of the ITS1-5.8S-ITS2 dataset. The ingroup of African Clariidae itself is monophyletic (BS= 100, 89, 89, 100) (not shown). This is confirmed by all analyses (NJ, MP,

ML, MrB) of both the 18S-dataset and the dataset containing the complete sequences (18S-ITS1-5.8S-ITS2-28S).

The second dataset contained the ITS1, 5.8S and ITS2 sequences of all ingroup and four outgroup taxa, one being a Genbank sequence (*Cyprinus carpio*), others being newly sequenced (*Kryptopterus bicirrhis*, *Pangasianodon hypophthalmus* and *Heteropneustes fossilis*). The phylogenetic analysis was performed using the five different methods (**Plates V.1-2, V.1-3**). In each of the figures bootstrap values are indicated, except for the Bayesian analysis, where posterior probabilities are shown.

Heteropneustes fossilis is a close relative to the family of Clariidae (BS= 100, 100, 100, 89, 100) (resp. NJ, MP, POY, ML, MrB), as could be expected, but does not belong to it (TEUGELS and ADRIAENS, 2003; DIOGO, 2005).

What follows is an overview of the different clusters, which can be discerned in the analyses. Of each clade the fixed taxa are summed up and besides these, the different taxa that were not restrained to one specific cluster are discussed with each different clusters to which they belong.

The first cluster (1a) is present in all methods (BS= 44, 47, 100, 52, 97), with a strong support by two methods (MrB and POY), showing a sistergroup relationship between *Clarias buthupogon-Channallabes apus* and *Clarias pachynema-Clarias submarginatus*. Cluster 1b unites the two specimens of *Clarias ngamensis* with *Clarias gariepinus*, *Dinotopterus cunningtoni* and *Heterobranchus isopterus*. This clade is well supported by all methods (BS= 72, 79, 100, 84, 100).

In cluster 2a *Clarias camerunensis* is the sistergroup of two specimens of *Clariallabes longicauda*, and two specimens of *Channallabes alvarezi* always cluster together. The most basal taxon is *C. stapersii* in MP, ML and MrB. In NJ, however, it is the sistergroup to the *C. theodorae* specimens. *Channallabes longicaudatus* appears in the clade, twice as a sistergroup of the *Ch. alvarezi* specimens (MP, MrB), once as an outgroup of the rest of the third clade (NJ, where *C. stappersi* is not included in the cluster), once as the sistergroup of the subclade of *C. camerunensis* and *C. longicauda* (ML) and once (POY), *Clarias stappersi* is the closest relative of *Ch. longicaudatus*, forming the sistergroup of *Ch. alvarezi*. We can find a monophyletic group (cluster 2b) consisting of two specimens of *Channallabes sanghaensis* and *Clarias platycephalus* in the POY analysis. However, one of the two *Ch. sanghaensis* specimens is most basal in the cluster in the NJ, MP, ML and MrB trees. The NJ, ML, MP and MrB analyses all show, contrary to the POY tree, *Clarias jaensis* as an outgroup of *Clarias platycephalus* and one of the *Ch. sanghaensis* specimens (BS= 30, 90, 93, 100). The two representatives of *C. theodorae* from very distant locations always cluster together, but differ a bit in position, once as the sistergroup of clusters 1a, 1b and

Tanganikallabes mortiauxi (ML, MrB), once switched in position with *Tanganikallabes*, ending up as the sistergroup of cluster 1a (POY). In the MP tree, they are the sistergroup of clusters 1b, 2a and 2b. In NJ they form a sistergroup with *C. stappersi*. The positions of *C. stappersi*, *C. jaensis* and *Tanganikallabes mortiauxi* remain uncertain. The two specimens of *C. pachynema* do not cluster together in NJ, ML, MP and MrB. The representative from Ebeigne, Northern Gabon (*C. pachynema 2*) is always a member of cluster 1a, whereas the other one, from Mopia, Southern Gabon, errs throughout the phylogeny, as a sistergroup of all taxa of the ingroup except 1a, of 2a + 2b in MP, of 1a + 1b + *C. theodorae* in ML or of 1a + 1b + *C. theodorae* + *T. mortiauxi* in MrB.

In POY, the most primitive taxon of the studied ingroup appears to be *Gymnallabes typus*, a situation that is not supported by the other four analyses, where it clusters with 1b (NJ, MP, ML, MrB).

Dating Analysis

Application of the two available fossil calibration points in the penalized likelihood procedure applied to the ML tree, provided us with a range of data. Initial results were obtained with the default settings for dating analysis in the r8s program, with cross validation function enforced. The rate smoothing parameters with optimal (= lowest) and sub-optimal cross validation scores were selected, and the dating procedure was then repeated. The result of the time divergence estimation is shown in **Figure 5**, and age estimates for all internal nodes are shown in **Table 4**. The analyses using the ML tree topology and different smoothing factors, resulting from optimal and sub-optimal cross validation scores, yielded small deviations in age estimates. The use of several reference fossils is expected to reduce variation due to error. The age constraints imposed by the two fossils in different parts of the tree are likely to restrict variation caused by a variety of other factors.

DISCUSSION

Sequence variation

The length and GC% of the 18S and 5.8S genes of the studied Clariidae fall within the range of known sequences available in Genbank/EMBL. The same goes for the length and GC content of the spacers. A balanced GC% between ITS1 and ITS2 is considered to be a general property, as suggested by TORRES et al. (1990). The GC% is quite high, and could be due to the fact that these animals occur in the tropics: higher GC content can be an adaptation to warmer climates, as has been shown in tetrapods (BERNARDI et al, 1988).

Phylogenetic analysis

The 18S dataset and complete sequence analysis show that the included African Clariidae form a monophyletic group, well separated from the outgroup. *Heteropneustes* is the sistergroup of the included African Clariidae. The ingroup is divided into two groups by the Bayesian analysis, with very low support (BP = 8). The NJ, ML and MP analyses show the same groups, but within a polytomy. Information in the 18S region was thus not sufficient to resolve the terminal nodes.

The analyses of the ITS1-5.8S-ITS2 dataset shows that *Heteropneustes* is again the sistergroup of the included Clariidae, which confirms the results of TEUGELS and ADRIAENS (2003).

According to the POY analysis, the most basal taxon within the Clariidae is *Gymnallabes typus*, an eel-like species occurring in the Niger delta. In all other analyses, however, it is basal to cluster 1b, *i.e.* the one containing *C. gariepinus*.

Clade 1a, comprising *C. pachynema 2* and *C. submarginatus* on the one hand, and *C. buthupogon* and *Ch. apus* on the other hand, is found in every analysis. *Clarias pachynema*, *C. submarginatus*, but also *C. theodorae* all belong to the *Clarias* (*Anguilloclarias*) subgenus sensu Teugels (1986), which is thus paraphyletic. In TEUGELS and ADRIAENS (2003), *Clarias* (*Anguilloclarias)* was considered the sisterclade of *Gymnallabes*, but is not found in any of the trees presented here.

The study of AGNÈSE and TEUGELS (in press), using Cyb, did not yield a close relationship between *C. theodorae* (see below) and *C. pachynema*. Agnèse and TEUGELS used four representatives of the subgenus *Clarioides*, which formed a monophyletic group. TEUGELS and ADRIAENS (2003) and AGNÈSE and TEUGELS (in press) both show a close relationship between the *Clarioides* species and *Channallabes apus*. In the latter study, only species (*Ch. apus*) was used. In our study, however, several species were used, of which only one forms the sistergroup of the *Clarioides* species (*i.e.* the Congo-population, the type species of this genus). A close relationship between *Clarioides* and *Clariallabes s.l.*, as suggested by POLL (1942a), could not be corroborated.

Cluster 1b is a very interesting one, uniting two specimens of *C. ngamensis* with *C. gariepinus*, *Dinotopterus* and *Heterobranchus isopterus*. This clade is supported by the whole–sequence analysis as well. The close relationship between *Clarias (Clarias)*, comprising *C. gariepinus* and *C. anguillaris*, *Clarias (Dinotopteroides),* comprising *C. ngamensis*, and *Heterobranchus* was previously suggested by TEUGELS and ADRIAENS (2003). It has also been shown that *Clarias (Clarias)* is the ancestral group of *Bathyclarias*, as a result of a recent speciation event in Lake Malawi (AGNÈSE and TEUGELS, 2001b). The close

relationship between *Clarias (Clarias)* and *Heterobranchus* was also confirmed by LEGENDRE et al. (1992), TEUGELS et al., (1992) and AGNÈSE and TEUGELS (in press). Finally, Agnèse and TEUGELS (in press), TEUGELS and ADRIAENS (2003) and GRAHAM (1997) also found a sistergroup relationship between *Heterobranchus* and *Dinotopterus*. This clade is thus supported both by general morphology, morphology of the suprabranchial organ, cytochrome b and nuclear genetic analyses.

The two *C. theodorae* specimens (one from Zambia and one from Botswana) cluster together. They seem to be related to cluster 2a and 2b in NJ, to *Tanganikallabes mortiauxi* and cluster 1b in MrB, to cluster 1a and 1b in ML, to clusters 1b, 2a and 2b in MP and to cluster 1a in POY. *Tanganikallabes* was considered part of a trichotomy with *Gymnallabes* and *Clariallabes* by GRAHAM (1997). This relationship can't be confirmed here, neither that between *Clariallabes* and *Gymnallabes*. The position of *C. pachynema 1* is too variable to derive any conclusions from. It appears the species is a complex of at least two subgroups, which deserves further study.

In the next group (cluster 2a), we see two monophyletic groups. In the first subclade, *Clariallabes longicauda* is the closest relative of *C. camerunensis* (subgenus *Clarias (Brevicephaloides)*). This is in contrast to what has previously been suggested: POLL (1942a) placed *Clariallabes* with *Clarias (Clarioides)*; GRAHAM (1997) considered it within a polytomy with *Channallabes* and *Dolichallabes*, whereas AGNÈSE and TEUGELS (in press) saw a close relationship between the *Brevicephaloides* and the *Platycephaloides* species, as commented on above. Also in contrast with our findings, according to AGNÈSE and TEUGELS (in press), *C. jaensis* forms the sistergroup of *Clariallabes*, with that clade being nested within representatives of both the *Brevicephaloides* and *Platycephaloides* subgenera.

The second subclade unites two monophyletic groups according to the POY-analysis, the first one comprising two *Ch. alvarezi* specimens (supporting their designation to a new species), and the second one comprising *Clarias stappersi* and *Channallabes longicaudatus*. The latter is also recently being recognized as a separate species (DEVAERE et al., submitted). In the other analyses, the second subgroup consists either of the two *Ch. alvarezi* specimens (NJ, with *Ch. longicaudatus* as most basal taxon in the whole cluster) or of these two with *Ch. longicaudatus* as a sistergroup (MP, MrB). In ML, *Ch. longicaudatus* clusters with *C. camerunensis* and the *Ch. longicauda* specimens. Again, it seems that another lineage of anguilliform species (*Ch. longicaudatus* and *Ch. alvarezi*) has a close relationship with a non-anguilliform *Clarias* species, this time a representative of the polyphyletic subgenus *Platycephaloides* (see above).

The last cluster (2b) unites taxa coming from the Congo region, *i.e. Clarias platycephalus* with two specimens of *Channallabes sanghaensis*. Remarkably, even though

both *Ch. sanghaensis* specimens come from the same region, one of the specimens seems to be more closely related to *C. platycephalus* than to the other *Ch. sanghaensis* specimen. Even more surprising is that in the NJ, MP, ML and MrB trees, the second *Ch. sanghaensis* forms the sistertaxon of the latter two with *Clarias jaensis*. Both *C. jaensis* and *C. platycephalus* do belong to the subgenus *Platycephaloides*, together with C. *stappersi*, which implies a polyphyletic nature of this subgenus sensu TEUGELS (1986). In AGNÈSE and TEUGELS (in press), *C. jaensis* forms a monophyletic group together with *Clariallabes longicauda* and *Clarias (Brevicephaloides)* (with a.o. *C. camerunensis*), which is not confirmed in this study.

The splitting of the original "*Channallabes apus*" into six species*, i.e. Channallabes apus, Ch. longicaudatus, Ch. alvarezi, Ch. sanghaensis, Ch. ogooensis* and *Ch. teugelsi* was originally based on morphological data (DEVAERE et al, submitted) and is confirmed by these molecular analyses, at least for those species included in this study. However, this genus now turns out to be polyphyletic (see **VI.1**).

Even though the clades mentioned above are well defined and largely supported by most topologies, the mutual relationships of the clades is less straight forward. It is even so that the topologies of the ML and Bayesian analysis seem to yield an opposite result to that of the POY, MP and NJ analysis. The bootstrap supports for the more basal nodes are very low in all analyses, except POY. This may be the result of rapid speciation events that occurred in a very short interval of time. When we look at the consensus data of the NJ, MP, ML and MrB analyses, the basal nodes all collapse (**Plate V.1-4**). In this case both topologies show a consistent pattern, with, most importantly, the same terminal clades.

Biogeography, Divergence Time Estimation, and morphological evolution of anguilliform taxa

The divergence time estimation, based on ML distances, is shown in **Plate V.1-5**. Stem and crown ages are given in **Table V.1-4**. Fossil data for Clariidae, or at least for taxa with a close affinity with the Clariidae, are available from the Eocene in Egypt; 34-56 MYA (GAYET and MEUNIER, 2003). According to our analyses the African Clariidae used in this study have emerged between 56 MYA (crown node age) and 123 MYA (stem node age). Two hypothetical scenarios for the timing of speciation events are envisaged here.

A late radiation would have occurred around the K/T crisis. Rapid speciation of the Clariidae during this event could explain the ambiguity and low bootstrap support of the nodes connecting the terminal clusters.

However, since the currently available data from the fossil record does not allow a more accurate estimation of the origin of oldest clariids (*i.e.* between 34-56 MYA), the split between Clariidae and the monotypic Heteropneustidae, and thus the origin of the Clariidae, may have occurred somewhere between 123 and 56 MYA. This, however, would predate the origin with 70 MYA years based on the estimate based on mtDNA (Angèse & Teugels, in press). Still, a possible origin of the Clariidae as early as 123 MYA would be possible, taking into consideration that in our analysis two basal clariid taxa are not included. Phylogenetic analyses, based on morphology, suggested a most basal position of the African, monotypic *Uegitglanis* within the Clariidae (DE PINNA, 1993; DIOGO, 2005). Recent molecular analysis have shown that Asian *Clarias* species are the sistergroup of all African clariids (AGNÈSE and TEUGELS, in press). Both taxa were not used in this study because of unavailability of tissue (*Uegitglanis*), whereas Asian clariids were not used in this study because of the focus on the phylogeny of African, anguilliform taxa. Subsequently, the period between 123 and 56 MYA may have included the speciation towards those basal groups (the Asian clariids also show a substantial number of species) (TEUGELS, 2003). The basal position of the blind *Uegitglanis* could, however, be questioned, because of its cave-dwelling lifestyle, and associated (derived) morphological traits (DAVID, 1936).

If the latter hypothesis would be correct, and the origin of Clariidae and Heteropneustidae is to be situated during the early Cretacious, the extant *Heterobranchus fossilis* may thus be the sole survivor of an old clade that could have gone extinct, e.g. at the K/T boundary. The fact that *Heteropneustes fossilis* has a morphology resembling that of the generalized clariids quite strikingly (according to some authors, it should even be considered a clariid, *e.g.* DE PINNA, 1993; DIOGO, 2005; SRINIVASACHAR, 1958), the origin of the first Clariidae could very well have been closer to the stem node than the crown node, and thus quite earlier than the K/T-boundary. To test the validity of these two hypothesis, a phylogenetic analysis including all the Asian representatives and *Uegitglanis* will have to be performed.

The basal position of *Gymnallabes typus* according to the POY analysis seems to indicate an early split towards anguilliformity. It seems, however, very unlikely that anguilliformity would be the plesiomorphic condition for the clariid family as the outgroup taxa are non-anguilliform and as this would imply a secondary reduction of body length over and over again. As a consequence, the most logical explanation would be that the lineage towards *Gymnallabes typus*, as represented here, only reflects one branch of evolution towards anguilliformity and that sister taxa within the lineage are missing (only one specimen from the Niger basin was used) or have become extinct. Other studies place

Gymnallabes close to *Channallabes* (POLL, 1942), *Clariallabes* and *Tanganikallabes* (GRAHAM, 1997); or close to *Platyallabes*, *Tanganikallabes* and *Clarias (Anguilloclarias)* (TEUGELS and ADRIAENS, 2003). According to AGNÈSE and TEUGELS (in press), *G. typus* is nested within a clade representing the *Clarias (Anguilloclarias)* subgenus.

If *G. typus* indeed is the result of a first and early split towards anguilliformity, that would imply that within the clariid family, body elongation occurred at least four times: (1) the branch to *G. typus*, (2) the branch of *Ch. apus* in cluster 1a, (3) the branch of *Ch. sanghaensis*, and (4) the branch to *Ch. alvarezi* and *Ch. longicaudatus*. The branch of *Clariallabes longicauda* is a special case, since the latter is morphologically intermediate between fusiforms and anguilliforms: infraorbitalia and suprapraeopercularia are slightly reduced, the jaw muscles are slightly hypertrophied and, as could be anticipated, their length is intermediate (BOULENGER, 1902). However, it does not seem to be an evolutionary intermediate, as fully elongated anguilliforms in this analysis never emanated from this lineage (more *Clariallabes* species would have to be included to support this). The taxonomic position of *Clariallabes longicauda* has thus always been a problematic puzzle. Originally, it was described by BOULENGER (1902) as *Allabenchelys longicauda*, distinct from *Clariallabes*, and intermediate between *Clarias* and *Clariallabes s.s.* DAVID (1935) placed it in the genus *Clarias*, *i.e.* in *Clarias (Allabenchelys)*, but since TEUGELS (1986) it is transferred to *Clariallabes*. Here it seems it has a close relationship with *Clarias camerunensis*, but the exact relationship with the other *Clariallabes* remains uncertain.

If this convergent evolution towards anguilliformity did occur as represented here, there must have been a very high selective pressure on the non-anguilliform ancestral taxa, for which body elongation was highly advantageous. Previous studies and field observations have indicated that anguilliform species of Clariidae are well adapted to survive in swampy areas where they can burrow and even hunt in the mud (DEVAERE et al, 2001). Oscillations through time between expansion and contraction of such rainforests with their seasonally flooded swamps, could have been this selective pressure. Forest retractions undoubtedly will have forced some species, inhabiting small rivers and brooks, into these smaller patches of rainforest, thus increasing competition in those habitats on small geographical scales. In his refuge theory, HAFFER (1982) proposed that some forest areas subsisted permanently during phases of lowland forest contraction during the Pleistocene. These areas, which have been proposed on the basis of various data sets, would have acted as refuges for forest species (HAMILTON, 1981; GRUBB, 1982; COLYN et al., 1991; SOSEF, 1994; MALEY, 1996). Haffer states that during the Pleistocene, shifts to drier climates left only small remnants of those forests (southern Cameroon, southwestern Nigeria, central Gabon and various spots in the Congo basin), separated by savanna-like

vegetation. In these refugia, rapid isolation could become a fact and, as a consequence, speciation could easily occur. An example of how speciation could happen as an answer to fluctations of dry and wet areas in the Congo basin is given for guenons in KINGDON (1989). However, our divergence time estimation indicates that the different anguilliform species would have radiated in roughly four periods: 37.6 MYA (node C), 27.3 MYA (node l), 21.0 MYA (node G), 13-14 MYA (nodes D, F, H). These all fall somewhere between the Eocene (54-38 millions of years ago) and the Miocene (23.8 to 5.32 millions of years ago). Similar climatic shifts as in the Pleistocene, with an impact on radiation events in hominoids, are known to have occurred through the Eocene, Oligocene and Miocene (LEWIN, 1998). Correspondingly, savanna's could well have been a barrier for those clariid species that are specialized in living in muddy soils, whereas non-anguilliform taxa are known to be able to migrate to a larger degree, through and between river systems and even over land (*e.g.* *Clarias gariepinus*, also known as "walking catfishes"; BURGESS, 1989). The anguilliform taxa, on the other hand, were bound to their habitats, which could explain why they are currently still restricted to relatively small areas in the Central Western African rain forests. Additional support for this hypothesis is provided when we plot the current distributions of these eel-like taxa: they seem to co-occur with those the ancient refuges (**Plate V.1-6**).

V.2 - Combined, morphological and molecular phylogeny

V.2.1 Phylogeny of the African catfish family Clariidae (Siluriformes) based on morphological and combined analyses: the road to anguilliformity

ABSTRACT

Clariids represent a unique range between fusiform and anguilliform morphs. Although this has been observed in other families of teleosts or amphibians and reptiles, it is never as extreme as in the Clariidae. Although originally the Clariidae were thought to have undergone an anagenetic evolution, more recent studies provide evidence that supports the hypothesis that anguilliformity evolved several times through a process of cladogenesis. In this study, it is shown that the morphological phylogenetic analysis mainly gives a reflection of the cranial evolution in the Clariidae despite the use of 18 postcranial characters (of a total of 53). A combined morphological and molecular phylogenetic analysis rather suggests the derived nature of body elongation. The corresponding morphological changes that co-occur with this elongation can be regarded as an extreme case of convergent evolution on the genus level. The demonstrated clariid paleobiogeography seems to be well explained by the principles of the refugia theory.

Key words: biogeography, body elongation, molecular, morphology

ACKNOWLEDGEMENTS

INTRODUCTION

The freshwater clariids are one of the 37 catfish families within the Siluriformes (SABAJ et al., 2004). Although they occur in Syria, southern Turkey and large parts of Southeast Asia, their diversity is the largest in Africa (TEUGELS, 1996; TEUGELS and ADRIAENS, 2003). This richness is demonstrated by the presence of 12 genera with over 90 species (TEUGELS and ADRIAENS, 2003; SABAJ et al., 2004). Some of the generalised, fusiform species, such as *Clarias gariepinus* (Burchell, 1922) show a large geographic distribution, whereas the anguilliform species occur in a small area, occupying a more specialized, burrowing niche. They can only be found in swampy areas in Nilo-Sudan (Niger delta), Lower Guinea and the Zaire (Congo River basin) ichthyological province (POLL, 1957a; ROBERTS, 1975; TEUGELS, 1986; TEUGELS et al., 1990).

Clariid catfishes are characterized by an elongate body, long dorsal and anal fins, the presence of four pairs of barbels, and especially by the unique presence of a suprabranchial organ, formed by arborescent structures from the second and fourth gill arches (GREENWOOD, 1961; TEUGELS and ADRIAENS, 2003).

Unique for these clariids is the presence of a range between fusiform and anguilliform morphs, representatives of with *Heterobranchus* Geoffrey St.-Hilaire, 1809 at one end and *Dolichallabes* Poll, 1942 at the other end (PELLEGRIN, 1927). Although this has been observed in other families of teleosts, amphibians and reptiles (LANDE, 1978), it is never as extreme as within the Clariidae. Together with the elongated body, a whole set of morphological changes are observed, such as decrease and loss of the adipose fin, continuous unpaired fins, reduction of paired fins, reduction of the eyes, reduction of the skull bones and hypertrophied jaw muscles (DEVAERE et al., 2001) (**IV.5.1.a**).

Originally, the Clariidae were thought to have undergone an anagenic evolution, which involved transformations towards an increasing anguilliformity (eel-like shape), coupled to a hypertrophy of the adductor mandibulae complex. This idea was first doubted by POLL (1977). And recent phylogenetic studies provide evidence that supports the hypothesis that anguilliformity evolved several times (GRAHAM, 1997; TEUGELS and ADRIAENS, 2003; AGNÈSE and TEUGELS, in press).

In this paper, the evolutionary path towards anguilliformity for several representatives of the Clariidae is discussed, using morphological and molecular data plotted on a phylogenetic framework.

MATERIALS AND METHODS

Preparations for the morphological study

Osteological examinations were done primarily on specimens cleared and counter-stained for bone and cartilage following the method of HANKEN and WASSERSUG (1981). To supplement cleared and stained specimens, certain species were also X-ray radiographed or examined as dry skeletal preparations.

Material examined

Outgroups are listed first, followed by the clariid species. Taxa are listed, corresponding to their body elongation, in three arbitrary groups. The taxa were chosen as such, in order to get a large degree of overlap with the molecular analysis in JANSEN et al. (submitted) (**V.1**). With respect to the recently revised taxonomy we refer to DEVAERE et al. (submitted) (**IV.5-1B, IV.5-2**). The morphological analysis presented here, contains more anguilliform species, since tissue samples of most of these species are not available. Values between brackets indicate the number of species used. All specimens are adults (based on SL). As a representative of the most primitive catfish family (Diplomystidae), *Diplomystes chilensis* (Molina, 1782) was used for the morphological analysis (ARRATIA, 1987; FINK and FINK, 1996). The morphological characters are based on the descriptions by ARRATIA (1987). The outgroup for both morphological and combined analyses is *Heteropneustes fossilis* (Bloch, 1974). The Heteropneustidae are considered to be very closely related to the Clariidae (DE PINNA, 1993; DIOGO, 2005).

Outgroups: Diplomystidae: *Diplomystes chilensis* (1) (based on ARRATIA, 1987). Heteropneustidae: *Heteropneustes fossilis* AMNH 178876 SW.

Ingroup: 22 anguilliform representatives: *Channallabes apus* (Günther, 1873) (MRAC 97-056-P-0001-0003, 88-25-P-2192-227, 88-01-P-1976-1992, 67763-777, 162095-100) (5) (represented as one OTU), *Channallabes sanghaensis* Devaere et al., in press (**IV.5-2**) (MRAC A4-31-P-171-183) (1), *Channallabes alvarezi* (Roman, 1980) (MRAC A4-31-P-1-13) (1), *Channallabes longicaudatus* (Pappenheim, 1911) (MRAC A4-31-P-163-164) (1), *Channallabes ogooensis* Devaere et al., in press (**IV.5-2**) (MRAC A4-31-P-165-169) (1), *Channallabes teugelsi* Devaere et al., in press (**IV.5-2**) (MRAC 78-22-P-1047-1050, 75-24-P-683-693) (2), *Gymnallabes typus* Günther, 1867 (MRAC 97-030-P-0010) (1), *Gymnallabes nops* Roberts and Stewart, 1976 (MCZ 50298) (1), *Dolichallabes microphthalmus* Poll, 1942 (MRAC 79258-260, 62407) (2), *Platyclarias machadoi* Poll, 1977 (MRAC 78-6-P-1348-364) (2) and *Platyallabes tihoni* Poll, 1977 (MRAC 125345-349) (2).

Intermediate representatives: *Clariallabes longicauda* (Boulenger, 1902) (MRAC 77-32-P-85) (1) and *Tanganikallabes mortiauxi* Poll, 1943 (MRAC 130952-970) (1).

16 fusiform representatives: *Dinotopterus cunningtoni* Boulenger, 1906 (MRAC dried specimen) (1), *Bathyclarias longibarbis* (Worthington, 1933) (based on A ₙ ₛ ₑ ₐ ᵤ ₘ ₑ and T ₑ ᵤ ₉ ₑ ₗ ₛ, 1999) (1), *Heterobranchus longifilis* Valenciennes, 1840 (AMNH 30545W)) (1), *Clarias fuscus* (Lacepède, 1803) (AMNH 10371) (1), *Clarias gariepinus* (based on A ᴅ ʀ ɪ ₐ ₑ ₙ ₛ and V ₑ ʀ ʀ ₐ ₑ ₛ, 1998) (1), *Clarias platycephalus* Boulenger, 1902 (MRAC 98-029-P-0912) (1), *Clarias stappersii* Boulenger, 1915 (MRAC MRAC P83367) (1), *Clarias ngamensis* Castelnau, 1861 (MRAC P47430) (1), *Clarias jaensis* Boulenger, 1909 (MRAC A0-049-P-120) (1), *Clarias theodorae* Weber, 1898 (MRAC 94-019-P-1002) (1), *Clarias pachynema* Boulenger, 1903 (MRAC 90-029-P-393-394) (1), *Clarias camerunensis* Lönnberg, 1895 (MRAC A0-048-P-1826-1827) (1) and *Clarias buthupogon* Sauvage, 1879 (MRAC 90-029-P-230-231) (1).

Molecular data

The molecular characters analyzed in this study were taken directly from the study of J ₐ ₙ ₛ ₑ ₙ et al. (submitted) (**V.1**).

Phylogenetic analysis

For the morphological phylogenetic analysis, a Maximum-Parsimony analysis was performed using NONA (G ₒ ₗ ₒ ᵦ ₒ ꜰ ꜰ, 1998). Equally parsimonious trees were obtained using the heuristic search algorithm with sequences added randomly (100 replicates with 10 trees held per replicate) and tree-bisection-reconnection (TBR) branch swapping. To estimate the robustness of the clades recovered in the phylogenetic hypotheses, Bremer supports (B ʀ ₑ ₘ ₑ ʀ, 1988, 1995) and bootstrap percentages (200 replications, 10 random addition sequences per replicate) were calculated in NONA based on the resulting implied alignment.

For the combined phylogenetic analysis, 1767 aligned base pairs from the ITS1-5.8S-ITS2 region were simultaneously analyzed with the 54 morphological characters, under the optimality criterion of parsimony with equal weights (i.e., gaps, transitions, and transversions all given a weight of 1). The parsimony analysis was conducted using direct optimization (W ʜ ₑ ₑ ₗ ₑ ʀ, 1996) and iterative pass (W ʜ ₑ ₑ ₗ ₑ ʀ, 2003a) as implemented in the program POY and run on the American Museum of Natural History Parallel Computing Cluster.

The analyses began by generating 30 random addition sequences (RAS) per random replicate for 5 replicates. These 150 RAS were improved with TBR branch swapping during the searches, an additional round of TBR branch swapping and tree fusing (G ₒ ₗ ₒ ᵦ ₒ ꜰ ꜰ, 1999)

at the end. These random replicates resulted in two equally most parsimonious trees. The resulting trees were submitted to POY for further tree searching using the commands iterative pass (WHEELER, 2003a) and exact. This second step of each of the analyses began by tree fusing (GOLOBOFF, 1999) the submitted topologies, and it was followed by an additional round of tree fusing and TBR branch swapping to reduce heuristics in the first-step analysis.

The length of the resulting implied alignments (WHEELER, 2003b) were verified in NONA (GOLOBOFF, 1998) and WinClada (NIXON, 2002). To estimate the robustness of the clades recovered in the phylogenetic hypotheses, Bremer supports (BREMER, 1988, 1995) and bootstrap percentages (200 replications, 10 random addition sequences per replicate) were calculated in NONA based on the resulting implied alignment. Character evolution on the recovered topologies was examined using NONA and WinClada.

RESULTS

For the morphological analysis, the characters are grouped according to anatomical complex: the neurocranium, jaws and hyoid (0-27), suspensorium (28-33), pectoral girdle (34-36), axial skeleton (37-44), and caudal skeleton (45-52). The character state (0-5) is listed as an integer in parenthese after each respective character state description. Six characters are considered additive, based on ontogenetic evidence in clariids and higher level phylogenetic evidence in Siluriformes (0, 12, 14, 28, 31, 32).

0. Form of the nasal bone. In *Diplomystes*, *Heterobranchus*, *Dolichallabes* and both *Gymnallabes* species, the nasal bone is tubular (0). In all other clariids examined, the nasal shows a plate-like extension (2), in *Channallabes sanghaensis* and *Tanganikallabes* this extension is only present on the lateral side of the supraorbital canal (1). *C. apus* shows intraspecific variation (1, 2).

1. Posterior margin of the mesethmoid (Plate V.2–1A,B). *Diplomystes*, all *Clarias*, except *C. buthupogon* and *C. theodorae*, *Platyclarias*, *Dinotopterus*, *Bathyclarias*, *Gymnallabes nops* and *C. ogooensis, C. alvarezi* and *C. teugelsi* have an indented mesethmoid (0) (**Plate V.2–1A**). All the rest shows a non-indented mesethmoid (1) (**Plate V.2–1B**).

2. Rostral projection on the frontal (Plate V.2–1C,D). Except for *Diplomystes* and *Bathyclarias* (0), all show a rostral projection on the frontal bone (1) (**Plate V.2–1C**).

3. Extent of the lateral wings of the frontal (Plate V.2–2A,B,C). *Diplomystes*, *Platyallabes* and *C. alvarezi* have a frontal that reaches as far as the orbitophenoid (0) (**Plate V.2–2A**). *Heterobranchus*, *Clariallabes*, *Dinotopterus*, *Bathyclarias* and all *Clarias*

have a frontal that is much broader than the orbitosphenoid (1) (**Plate V.2-2B**). In *Heteropneustes*, *Platyclarias*, *Gymnallabes* nops and all *Channallabes*, except *C. alvarezi*, the lateral plates are a little broader than the orbitosphenoid (2) (**Plate V.2-2C**). In *Dolichallabes* and *Gymnallabes* typus, no lateral plate is visible (3) (Fig. 4 in CABUY et al., 1999). Again, *C. apus* shows intraspecific variation (0, 2).

4. Lateral plate of the sphenotic (Plate V.2-1D). A lateral plate on the sphenotic is absent in *Diplomystes*, *Platyallabes*, *Dolichallabes*, *Gymnallabes*, *Tanganikallabes* and all *Channallabes* (0). All other show a clear lateral plate (1).

5. Lateral plate of the pterotic (Plate V.2-1D). Same division and character state distribution as in character 4, absent (0), present (1).

6. Lateral plate of the posttemporo-supracleithrum (Plate V.2-1C,D). No lateral plate on the posttemporo-supracleithrum is present in *Diplomystes*, *Platyallabes*, *Dolichallabes*, *Tanganikallabes*, *Gymnallabes nops* and *C. apus* (0). All other taxa show a lateral plate (1). *C. alvarezi* shows intraspecific variation (0, 1).

7. Posterior branch of the temporal canal in the posttemporo-supracleithrum. *Diplomystes* and *Heteropneustes* have a posterior branch (0). All clariids lack the posterior branch (1).

8. Shape of the supraoccipital process (**Plate V.2-1C**). Except for the rounded process in *C. fuscus* and *gariepinus* (1), all other taxa show a pointed process (0).

9. Position of the posterior tip of the supraoccipital spine relative to the Weberian neural spines. In *Diplomystes* and *Heteropneustes*, the posterior tip of the supraoccipital process does not reach the Weberian neural spines (0). In all clariids, the posterior tip does reach the Weberian neural spines (1).

10. Prevomeral tooth plates (Plate V.2-1D). In *Diplomystes*, *Heteropneustes*, *Platyallabes* and *Dolichallabes* the two tooth plates remain separated in the adult stage (0). In all other taxa, the tooth plates become fused (1).

11. Posterior process of the prevomer for the interdigitation with the parasphenoid. *Diplomystes* shows two, small rounded posterior processes on the prevomer (0). *Heterobranchus*, *Dinotopterus*, *Bathyclarias* and all *Clarias* species have two long processes (1). *Heteropneustes*, *Clariallabes* and all anguilliform taxa, except *Platyclarias* have one long process (2). *Platyclarias* shows intraspecific variation (one long process or three long processes) (2, 3).

12. Place of neurocranial articulation with the hyomandibula. In *Diplomystes*, the articulation of the hyomandibula occurs through the pterosphenoid, sphenotic and the pterotic (0). In *Heteropneustes*, *Dinotopterus, Bathyclarias, Clariallabes, Platyclarias, Dolichallabes, Gymnallabes, Channallabes* and *Clarias gariepinus*, the sphenotic and

pterotic articulate with the hyomandibula (1). In *Heterobranchus*, all *Clarias*, except *C. gariepinus* and *Tanganikallabes*, the articulation occurs at the level of the sphenotic only (2). *Platyallabes* shows intraspecific variation (1, 2).

13. Epiotic. In *Diplomystes*, *Heterobranchus*, *Clarias gariepinus* and *C. fuscus*, *Clariallabes*, *Dolichallabes*, *Platyclarias*, *Gymnallabes typus*, *Channallabes longicaudatus* and *C. teugelsi* the epiotic is present (0). In *Heteropneustes*, *Platyallabes*, *Bathyclarias*, *Dinotopterus*, *Gymnallabes nops*, most *Clarias* and *Channallabes* species, this bone is absent(1). *C. apus* shows intraspecific variation (0, 1).

14. Number of infraorbital bones. *Diplomystes* has seven infraorbital bones (0). *Heteropneustes* has five infraorbitals (1). All clariids, have four infraorbitals (2), except for *Dolichallabes* and *Gymnallabes nops*, where only two infraorbital bones are present (3).

15. Articulatory process on infraorbital III. No articulatory process is present in *Diplomystes*, *Heteropneustes*, *Platyallabes*, *Tanganikallabes* and *Gymnallabes typus* (0). One process is present, without contact with the lateroethmoid in *Dinotopterus*, *Bathyclarias*, *C. pachynema* and *C.* jaensis and most *Channallabes*, except *C. sanghaensis* (1). Contact is present in *Heterobranchus*, *Clariallabes*, *Platyclarias*, *C. sanghaensis* and most *Clarias,* except *C. pachynema* and *C. jaensis* (2). *C. apus* shows intraspecific variation (1, 2).

16. Shape of the posteroventral infraorbital bone (Plate V.2–1C). In *Diplomystes*, *Heteropneustes*, *Platyallabes*, *Tanganikallabes*, *C. pachynema*, *G. typus* and all *Channallabes* species, the postero-ventral infraorbital bone is tubular (0). In all other clariid taxa included, this infraorbital is plate-like (1).

17. Shape of the posterodorsal infraorbital bone. The shape of the postero-dorsal infraorbital in *Diplomystes*, *Platyallabes*, *Dolichallabes*, *Gymnallabes* and *Tanganikallabes* is tubular (0). In *Dinotopterus*, *Channallabes apus* and *C. sanghaensis* and *Clarias stappersii*, *C. pachynema*, *C. theodorae*, *C. platycephalus,* the postero-dorsal infraorbital is plate-like with a small supraorbital process (1) (**Plate V.2-2D**). *Heteropneustes*, *Heterobranchus*, *Clariallabes*, *Platyclarias*, *Bathyclarias* and all other *Clarias* and *Channallabes* species have a plate–like infraorbital with a large supraorbital process (2) (**Plate V.2-2E**).

18. Position of the suprapreopercular bones. Except for *Bathyclarias* and *C. stappersii*, where the suprapreopercular series are well in front, contacting the posterodorsal infraorbital bone (2) and *Heteropneustes, Heterobranchus* and all other *Clarias* species, where the suprapreopercular series is well in front but no contact with the posterodorsal infraorbital bone (1), all other taxa have a posteriorly shifted

suprapreopercular series and consequently widely separated from the posterodorsal infraorbital bone (0).

19. Place of entrance of the infraorbital canal in the neurocranium. In *Diplomystes*, *Heteropneustes*, *Heterobranchus*, *Clariallabes*, *Dinotopterus*, *Bathyclarias* and all *Clarias* species, the infraorbital canal enters the neurocranium at the level of the sphenotic (0). In all other clariids, the canal enters at the level of the frontal (1).

20. Position of the infraorbital-supraorbital canal anastomosis. The position of the infraorbital-supraorbital canal anastomosis shows a similar partitioning as character 19: in the sphenotic (0), frontal (1).

21. Shape of the suprapreopercular bone (Plate V.2-1C,D). Except for *Diplomystes* and *Platyallabes* (tubular) (0), all other taxa have a plate-like suprapreopercular bone (1).

22. Number of suprapreopercular bones (Plate V.2-1C,D). *Diplomystes*, *Platyallabes*, *Platyclarias*, *Dolichallabes*, *Gymnallabes* and *Tanganikallabes* have more than one suprapreopercular bone (0). All other taxa have only one suprapreopercular bone (1). *C. apus* en *C. alvarezi* show intraspecific variation (0, 1).

23. Extent of the tooth battery on the lower jaw (Plate V.2-2F,G). The tooth battery of *Diplomystes*, *Heteropneustes*, *Clariallabes*, *C. stappersii*, C. *platycephalus*, *Tanganikallabes* and all anguilliform clariids runs up to the coronoid process (0) (**Plate V.2-2G**). With the other clariids, the tooth battery does not run that far (1) (**Plate V.2-2F**).

24. Coronoid process (Plate V.2-2F,G). The coronoid process in *Diplomystes*, *Heteropneustes*, *Clariallabes*, *Dolichallabes*, *Gymnallabes*, *Tanganikallabes*, all *Channallabes* species and *C. fuscus, theodorae* and *C. platycephalus* is well developed (0) (**Plate V.2-2F**). In all other taxa, the coronoid process is hardly distinguishable (1) (**Plate V.2-2G**).

25. Shape of the distal tip of the posterior process of the parurohyal. The caudal tip of the medial process of parurohyal is simple in *Diplomystes*, *Dolichallabes*, *Gymnallabes*, *Bathyclarias*, *C. pachynema*, *C. buthupogon*, *C. ngamensis*, *C. jaensis*, *C. camerunensis* and *C. sanghaensis*, *C. ogooensis*, *C. teugelsi* (0). Forked in *Heteropneustes*, *Heterobranchus*, *Platyclarias*, *C. gariepinus*, *C. fuscus*, *C. theodorae*, *C. platycephalus* and *C. alvarezi* (1). Flat in *Clariallabes*, *Tanganikallabes*, *C. stappersii* (2). *Platyallabes* shows intraspecific variation (0, 2).

26. Spot on the dorsal neurocranium (Plate V.2-3A). Only in *Platyclarias* and *C. teugelsi*, *C. longicaudatus*, *C. ogooensis* a clear spot is present on the skull roof (1).

27. Contralateral parts of the "pre-fontanel" supraoccipital bone fused. Only in *Dolichallabes,* the contralateral parts of the supraoccipital bone are not fused (1).

28. Entopterygoid-quadrate connection. There is no contact between the entopterygoid and the quadrate in *Diplomystes*, *Heteropneustes*, *Clariallabes*, *Platyclarias*, *Dolichallabes*, *Gymnallabes*, *Dinotopterus*, *Bathyclarias*, *C. fuscus*, *C. ngamensis*, *C. stappersii*, *C. platycephalus* and all *Channallabes* except *C. apus* and *C. sanghaensis* (0) (**Plate V.2-3C,D**). In *Heterobranchus*, *Tanganikallabes*, *Platyallabes* and all other *Clarias* species, there is a clear contact between the entopterygoid and the quadrate (1) (**Plate V.2-3B**). In *C. apus* and *C. sanghaensis*, there is even a clear interdigitation zone present between the two suspensorial bones (2) (**Plate V.2-3E**).

29. Entopterygoid-metapterygoid contact. In *Diplomystes,* the contact of the entopterygoid with the metapterygoid is limited to the dorsal side of the latter bone (0). To the rostro-dorsal margin in *Heteropneustes*, *Heterobranchus*, *Tanganikallabes*, *G. nops*, *C. alvarezi* and most *Clarias* species (1) (**Plate V.2-3B**). To the rostro-dorsal side, with a restricted contact with the ventral side in the other *Clarias* species (*C. stappersii* and *C. platycephalus*), *Platyclarias*, *Dolichallabes*, *Dinotopterus*, *Bathyclarias*, *Channallabes teugelsi*, *C. ogooensis, C. longicaudatus* (2) (**Plate V.2-3C**). With substantial ventral contact in *Platyallabes*, *Gymnallabes typus* (3) (**Plate V.2-3D**). The metapterygoid is complete dorsally enclosed by the entopterygoid in *C. sanghaensis* (4) (**Plate V.2-3E**). *C. apus* shows some intraspecific variation for this character (3,4).

30. Dorsal border of membranous plate of quadrate and hyomandibula (Plate V.2-1C,D). Except for the *Platyclarias*, *Bathyclarias* and *C. gariepinus*, where the border is straight (0), all the taxa have an indented dorsal border of the membranous plate of quadrate and hyomandibula (1).

31. Rostral process on the hyomandibula (Plate V.2-1C,D). The rostral process on the hyomandibula is absent in *Diplomystes*, *Heteropneustes*, *Clariallabes*, *Platyclarias*, *Platyallabes, G. nops* and one *Clarias*: *C. theodorae* (0). A small rostral process is present in *Heterobranchus*, *Dolichallabes*, *Bathyclarias*, *Channallabes sanghaensis*, Clarias *pachynema*, *C. buthupogon*, *C. camerunensis*, *C. stappersii* and *C. platycephalus* (1). In *Tanganikallabes*, *Dinotopterus*, *Clarias gariepinus*, *C. ngamensis*, *C. jaensis*, *C. ogooensis*, *C. longicaudatus*, *C. alvarezi*, *C. teugelsi*, a large process is present (2). *G. typus* and *C. apus* show intraspecific variation (1, 2)

32. Number of processes present on the anterior side of the hyomandibula (Plate IV.5-4B-C). *Diplomystes* shows no anteriorly extended process (0). *Heteropneustes*, *Platyclarias* and *Dolichallabes* have one process (1), *Heterobranchus*, *Clariallabes*, *Gymnallabes*, *Tanganikallabes*, *Dinotopterus*, *Bathyclarias,* all *Channallabes*, except *C. sanghaensis* and most *Clarias* taxa have two processes (2). *Platyallabes*, *C. sanghaensis*, *C.*

pachynema, *C. buthupogon*, *C. camerunensis* have three anteriorly extended processes on the hyomandibula (3). Again, *C. apus* shows some intraspecific variation (2, 3).

33. Number of processes present on the posterior side of the hyomandibula (Plate V.2-1 B,C) *Diplomystes*, together with *Heteropneustes*, *Heterobranchus*, *Dinotopterus*, *Bathyclarias*, *C. gariepinus*, *C. ngamensis* and *C. stappersii* show no posterior process (0). Only one process is present in *Platyclarias*, *Clarias jaensis* and *C. platycephalus* (1). *C. ogooensis* and *C. fuscus* have two processes, with a small anterior one (2). *Clariallabes*, *Platyallabes*, *Dolichallabes*, and *C. teugelsi* posses two extended posterior process (3). *Gymnallabes*, *Tanganikallabes*, *C. sanghaensis*, *C. longicaudatus*, *C. alvarezi* and *C. pachynema*, *C. buthupogon*, *C. camerunensis* and *C. theodorae* have three posterior processes caudally increasing in length (5). *C. apus* shows intraspecific variation and shows either two (3) or three (4) extended process.

34. Anterior process on the cleithral bone (Plate V.2-3F). An anterior process on the cleithral bone is absent in *Diplomystes*, *Heteropneustes*, *Platyallabes*, *Dinotopterus* and *C. fuscus* (0). All other clariids have a clear process on the anterior side of the cleithrum (1).

35. Fenestra between the scapula-coracoid and cleithral bone (Plate V.2-3F). Except for *Heteropneustes*, *C. apus* and *C. platycephalus* (1), all other taxa have a fenestra between the scapulocoracoid and the cleithrum (0).

36. Pectoral spine serrations. Serrations are only present on the posterior side in *Diplomystes*, *G. nops* and *C. alvarezi* (0). On the anterior side in *Heterobranchus*, *Heteropneustes*, *Dinotopterus*, *Bathyclarias*, *C. gariepinus*, *C. fuscus*, *C. ngamensis*, *C. camerunensis* (1). Present on both sides in *Clariallabes*, *G. typus*, *C. buthupogon*, *C. pachynema*, *C. stappersii*, *C. theodorae*, *C. sanghaensis*, *C. ogooensis*, *C. longicaudatus* (2). Serration are absent on both sides in *Platyclarias*, *Platyallabes* (3). Intraspecific variation present in *Tanganikallabes* (0, 2), *C. platycephalus* (1,3), *C. jaensis* (2, 3).

37. Size of the parapophyseal foramina on the first precaudal vertebrae. Small foramina in *Heterobranchus*, *Clariallabes*, *Dolichallabes*, *Gymnallabes*, *Dinotopterus*, *Bathyclarias* and *Clarias gariepinus*, *C. fuscus*, *C. stappersii*, *C. theodorae* and *C. platycephalus* (0) (**Plate V.2-4A**). Large foramina present in *Platyclarias*, all *Channallabes* species and *Clarias. pachynema*, *C. buthupogon*, *C. ngamensis*, *C. jaensis*, *C. camerunensis* (1) (**Plate V.2-4B**). Very large foramina are present in *Heteropneustes*, *Platyallabes*, *Tanganikallabes* (2) (**Plate V.2-4C**). *C. apus* shows intraspecific variation (0, 1).

38. Lateral process on the first dorsal fin pterygiophore. Only in *Heterobranchus*, *Platyallabes*, *Dinotopterus*, *G. typus* and *C. stappersii*, a lateral process on the first dorsal fin pterygiophore is present (1).

39. Lateral process on the second dorsal fin pterygiophore. In *Diplomystes*, *Platyclarias*, *Platyallabes*, *Dolichallabes Dinotopterus*, *C. alvarezi* and *C. longicaudatus*, a lateral process is absent on the second dorsal fin pterygiophore (0). *Heteropneustes* and all other clariid representatives show a lateral process on the second dorsal fin pterygiophore (1). *C. apus* and *C. teugelsi* show intraspecific variation.

40. Anterior process on the second dorsal fin pterygiophore. An anterior process is absent in *Diplomystes*, *Platyclarias*, *C. pachynema*, *C. buthupogon*, *C. jaensis*, *C. camerunensis* and in *C. longicaudatus* and *C. alvarezi* (0). For *Heteropneustes* and all other clariids studied in this analysis, the process is present (1). *C. apus* and *C. teugelsi* show intraspecific variation.

41. Position of the first dorsal fin pterygiophore. In *Diplomystes*, *Heteropneustes*, *Heterobranchus*, *Dolichallabes*, *G. nops* and *C. sanghaensis*, the dorsal fin originates between the sixth and eighth vertebra (0). In *Tanganikallabes*, *Dinotopterus*, *G. typus* and *C. stappersii*, *C. jaensis*, *C. theodorae*, *C. platycephalus*, the dorsal fin originates between the fifth and sixth vertebra (1). *Platyallabes*, *Bathyclarias* and *C. gariepinus*, *C. fuscus*, *C. pachynema*, *C. buthupogon*, *C. ngamensis*, the origin lies anterior to the fifth process (2), while in *C. alvarezi*, *C. ogooensis*, *C. longicaudatus* and *C. teugelsi* the origin lies posterior to the eighth vertebra (3). *C. apus* shows some intraspecific variation (0, 4).

42. Adipose fin. An adipose fin is only present in *Diplomystes*, *Heterobranchus*, *Dinotopterus* and *C. ngamensis* (0). All the other lack an adipose fin (1).

43. First post-Weberian vertebra with ribs. *Heteropneustes*, *Heterobranchus*, *Bathyclarias* always have ribs on both sides on the first post-Weberian vertebra (0). All other clariids lack such ribs (1). *Platyclarias*, *C. teugelsi* and *C. apus* show variation (0, 1) (**Plate V.2-4A,B,C**)

44. Length of the last precaudal neural spine. Only the neural spines in *Diplomystes*, *Heterobranchus*, *Bathyclarias* and *C. gariepinus* are elongated (1).

45. Neural spine of the second preural vertebra (Plate V.2-4D,E). The neural arches of *Diplomystes*, *Heteropneustes*, *Heterobranchus*, *Platyclarias*, *Tanganikallabes*, *Dinotopterus*, *Bathyclarias*, *G. nops* and all *Clarias* taxa, except *C. gariepinus*, are elongated and broadly tipped (0). *Platyallabes*, *C. gariepinus* and *C. apus* show non–elongated, spiny neural spine (1), while *Dolichallabes*, all other *Channallabes* species and *G. typus* have elongated and spiny neural spine (2).

46. Haemal spine of the second preural vertebra (Plate V.2-4D,E). All taxa, except *Dolichallabes* and *G. nops* (0), have elongated, broadly tipped haemal spines on the second preural vertebra (1). *C. apus* shows intraspecific variation (0, 1).

47. Neural spine of the thirth preural vertebra (Plate V.2-4D,E). *Dolichallabes* and *Platyallabes* have no elongated, spiny neural spines (0). The neural spines of *Heteropneustes*, *Heterobranchus*, *Dinotopterus*, *Bathyclarias*, *G. nops* and *C. gariepinus*, *C. fuscus*, *C. ngamensis* are elongated, broadly tipped (1). *Platyclarias*, *G. typus*, all *Channallabes* species, except *C. apus* and *C. pachynema*, *C. buthupogon*, *C. jaensis*, *C. camerunensis*, *C. theodorae*, *C. platycephalus*, on the other hand, have elongated, spiny neural spines on the third preural vertebra (2). Again, *C. apus* shows variation (0, 1)

48. Haemal spine of the third preural vertebra (Plate V.2-4D,E). *Platyallabes*, *Dolichallabes C. jaensis* and *C. camerunensis* have non-elongated, spiny arches (0), the haemal arches of the third preural vertebra in *Heteropneustes*, *Heterobranchus*, *Dinotopterus*, *Bathyclarias*, *C. gariepinus*, *C. fuscus* and *C. ngamensis* are elongated and broadly tipped (1). All the *Channallabes* species, *Platyclarias*, *G. typus*, *Tanganikallabes*, *C. pachynema*, *C. buthupogon*, *C. theodorae* and *C. plathycephalus* have elongated, spiny haemal spines (2). Again, *C. apus* shows intraspecific variation (0, 2).

49. Hypurapophyses on the parhypural. In contrast to *Diplomystes* and *Heteropneustes* (0), all clariids lack hypurapophyses on the parhypural (1).

50. Hypurals 1-2. The hypurals one and two are not fused in *Diplomystes*, *Heteropneustes*, *Platyallabes*, *Tanganikallabes*, *Dinotopterus*, *Bathyclarias*, *G. typus*, all *Clarias* and *Channallabes* species, except *C. longicaudatus* (0). In *Heteropneustes*, *Dolichallabes*, *G. nops*, *C. longicaudatus*, the two hypurals are fused (1). *Platyclarias* and *C. apus* show variation (0, 1).

51. Hypurals 3-4. Similar to character 50, the hypurals three and four are now not fused (0), with the only exception in *Platyclarias* (0).

52. Hypurals 4-5. Similar to character 50.

A matrix of character states for all taxa is summarized in **Table V.2-1**.

The morphological analysis resulted in two most parsimonious trees with a length of 237 steps, consistency indices (CI, KLUGE and FARRIS, 1969) of 35, and retention indices (RI, FARRIS, 1989) of 66. A strict consensus of both trees is presented in **Plate V.2-5**. The monophyly of the Clariidae studied here is confirmed with a reasonable support (**Plate V.2-5**). This node has a bootstrap support of 82 and a Bremer support of 5. Eight synapomorphic characters support this node: no posterior branch of the temporal canal in the posttemporo-supracleithrum (7-1), the posterior tip of the supraoccipital process reaches the Weberian neural spines (9-1), fused prevomeral tooth plates (10-1), the number of infraorbital bones is less than five (14-2,3), the entrance of the infraorbital canal and the infraorbital-supraorbital canal anastomosis lies in the frontal (19-1, 20-1)

anterior process on the cleithral bone is present (34-1) and no hypurapophyses are present on the parhypural (49-1).

The included species from the genus *Clarias*, together with *Heterobranchus longifilis, Bathyclarias sp* and *Dinotopterus cunningtoni* are separated from all anguilliform representatives. The node has a bootstrap support of 54 and a Bremer of 2. The nonhomoplasious character that supports this node is the presence of two long posterior processes on the prevomer (11-1).

The taxon with an intermediate body elongation, *Clariallabes longicauda*, also takes an intermediate position in the phylogenetic analysis. Although the bootstrap support of this node is just below 50 (44) with a Bremer support of 2, it is supported by a non homoplasious character: the presence of a very broad lateral plate on the frontal (3-1). *Tanganikallabes* is incorporated in the anguilliform taxa. Furthermore, we see that of those species where different populations were represented, these OTU's cluster together and are well supported (bootstrap more than 95 for the *D. microphthalmus, P. tihoni, C. teugelsi* and *P. machadoi* populations). Two distinct clades with *Channallabes* species are present. These two clades represent species from two different ichthyological regions, *C. apus* and *C. sanghaensis* occur in the Congo basin, while *C. alvarezi, C. teugelsi, C. longicaudatus* and *C. ogoouensis* all occur in the Lower Guinea. In the fusiform group, the close relation between *C. gariepinus* and *H. longifilis*, as indicated earlier in TEUGELS et al. (1992) and AGNÈSE and TEUGELS (2001a) is supported. This analysis shows the paraphyletic nature of the genera *Channallabes* and *Clarias*.

The combined analysis of all 1767 molecular and 54 morphological characters resulted in two most parsimonious trees with length of 2320 steps, consistency indices (CI, KLUGE and FARRIS, 1969) of 74 and retention indices (RI, FARRIS, 1989) of 78, when only informative characters were retained. A strict consensus of both trees is presented in **Plate V.2-6**. The monophyly of the Clariidae is strongly supported. On the other hand, *Clarias* again is paraphyletic, spread over several clades. *Channallabes* also shows to be paraphyletic, with a strongly supported "Gabon" clade, as suggested in another study (DEVAERE et al., submitted (**IV.5.2**)). The "Congo" species, however, turned out paraphyletic. This phylogeny shows the independent evolution of elongated body form in four clades, with similar levels of overall body elongation.

Evolutionary polarisation of body elongation

Looking closer at the topology with respect to anguilliformity in both analyses (morphological and combined), we get a different result. The morphological analysis suggests that body elongation is plesiomorphic in Clariidae, with a transition from anguilliform representatives to fusiforms, over an intermediate situation (*Clariallabes longicauda*). Only *Tanganikallabes mortiauxi* does not fit in this trend, although it has a more fusiform bodyform, it clusters in the anguilliform taxa. However, the outgroup taxa used in this analysis imply a reversal to a fusiform "Bauplan" (both *Diplomystes* and *Heteropneustes* are not elongated). Furthermore, all sister-group of the Clariidae and Heteropneustidae are fusiform (DE PINNA, 1993; DIOGO, 2005). It is known that an expansion of the Hox gene expression domains along the body axis, both accounts for elongation of the body as well as the generally co-occuring loss of limbs (COHN and TICKLE, 1999). The reverse process, on the other hand, is less unambiguously documented. A number of authors have discussed and disputed the loss of limbs and the possible reappearance of well-developed hind limbs among the primitive lineages of snakes (ZAHER and RIEPPEL, 1999; GREENE and CUNDALL, 2000; LEE et al., 2000). Furthermore, such a reversal does not correspond with parsimonious evolution. While many examples can be given of elongation processes in several groups (LANDE, 1978; CAROLL, 1988), none are found with this reversal.

An explanation for this strange topology, however, could be that it is biased by certain aspects of the morphology. Looking at the data matrix, it seems that the topology indeed is merely a reflection of the cranial evolution in Clariidae. Together with a more elongated body, a whole set of morphological characteristics co-occur that are mainly situated in the cranial region (CABUY et al., 1999; DEVAERE et al., 2001 (**IV.5.1.a**)). In general, the anguilliform genera have a more reduced skull, compared to the fusiform genera. This reduction includes many bones, such as the suprapreopercular and infraorbital bones. This reduced skull is considered plesiomorphic, as, for instance, within the Siluriformes only in *Heteropneustes, Uegitglanis, Heterobranchus* and *Clarias* an enlarged infraorbital IV, which is feebly or firmly attached to the neurocranium, is present (DIOGO, 2005). This, however, means that in the Clariidae the robust skull morphology is plesiomorphic, since the closest sister-group, Heteropneustidae, shows a robust skull (see below). The skull of the anguilliform taxa resembles that of the primitive *Diplomystes chilensis*, e.g. the absence of a sphenotic and pterotic lateral plate, the tubular shape of infraorbital III, both suprapreopercular and infraorbital series well. To test this importance of the cranial characters, a new analysis was done with the postcranial characters being extra weighted.

The resulting topology supports the idea that the morphological topology is a reflection of cranial evolution as it shows a quite reversed order (**Plate V.2-7**). In this analysis, anguilliformity is suggested to be apomorphic, which is to be expected in view of parsimony.

The ambiguous nature, with respect to parsimonious evolution of this morphology based topology could be tested, using the molecular topology presented in JANSEN et al. (submitted)(**V.1**). In this analysis, four clades can be distinguished, with a surprising distribution of anguilliform taxa. Body elongation would have arisen independently from three different *Clarias* lineages. Consequently, when considering the high number of shared morphological traits in anguilliform taxa (see above), this topology would suggest a high degree of homoplasic evolution.

Subsequently, the combined analysis of both morphological and molecular datasets might clear this up (**Plate V.2-6**). However, it seems that aspects of both separate analyses are supported. Anguilliformity still seems to be plesiomorphic, because of the position of *Gymnallabes typus* at a basal node. On the other hand, body elongation still is the result of parallel evolution, with the *Channallabes* taxa arising from three different *Clarias* ancestors.

The basal position of *G. typus* is rather bizarre, which may be explained. First, when mapping anguilliformity down the tree, the common ancestor of *G. typus* and all other clariids can not be determined unambiguously (**Plate V.2-8**). Additionally, incorporating the biogeography of the different OTU's, *G. typus* is the only representative from the Niger delta, whereas all other anguilliform taxa originate from the Congo basin and Lower Guinea ichthyological province. Second, for the reason mentioned above, it seems very unlikely that anguilliformity is plesiomorphic in clariids. In the analysis of AGNÈSE and TEUGELS (in press), based on cyb, *G. typus* groups with *Clarias ebriensis* and *Clarias pachynema*, where the former also occurs in the Niger delta (LÉVÊQUE et al., 1992). This could mean that *G. typus* could be considered as an anguilliform branch of an early West African lineage, with a non-anguilliform ancestor. Other studies each suggested alternative positions for *G. typus,* so at this moment no consensus can be reached (GRAHAM, 1997; TEUGELS and ADRIAENS, 2003).

Morphological co-evolution with body elongation

Even though the extent of body elongation may be unique, body elongation itself is not unique for Clariidae, but has occurred several times throughout vertebrate evolution, as in other teleosts, amphibians and reptiles (GANS, 1975; NELSON, 1994; LANDE, 1978). This elongation is observed to co-occur with a whole set of morphological changes, *e.g.*

increase of the number or size of vertebrae, limblessness, reduction of the eyes, and/or increasing rigidity of the skull.

In the clariids highly similar observations can be made. Elongated clariids have a significantly higher number of vertebrae (DEVAERE et al., 2001, 2004) (**IV.5.1.a, IV.5.4**). Although all Clariidae are diagnosed with small eyes, the reduction in anguilliform species is far greater, with in some species even the complete absence of eyes (DEVAERE et al., in press)(**IV.5.1.a**). It is further shown, that elongation in clariids is combined with a loss of paired fins (ADRIAENS et al., 2002). Although the general skull morphology in elongated clariids shows a reduction or loss of bones (e.g. lateral bones), there is an increased rigidity, because of the outgrowth of several interdigitation zones (DEVAERE et al., 2001) (**IV.5.1.a**). Many characters, linked to these morphological changes, also seem to determine the topology of the morphology based cladogram (e.g. characters 3, 4, 5, 11, 16, 18) and lead to the clustering of the elongated species. In the combined analysis, however, the correlated morphological changes appear to be the result of convergent evolution and this on a genus level. Similar convergent evolution in vertebrates has occurred several times on a higher taxonomical evolution (GANS, 1975; NELSON, 1994; LANDE, 1978). Further, the presence or absence of the paired fins appeared to differ on a population level, indicating the existence of a high level of phenotypic plasticity of traits that were generally considered as stable on a micro–evolutionary level, and even up to some degree on a macro–evolutionary scale (ADRIAENS et al., 2002).

Further, RIEPPEL (1996) stated that in lizards, body elongation and limb reduction are generally correlated with miniaturisation (RIEPPEL, 1996). Compared to species of *Dinotopterus*, *Clarias* and *Heterobranchus*, the skulls of the anguilliform species are very small and morphological transformations related to miniaturization are not excludible. When plotting the average skull length (% SL) on the combined tree (**Plate V.2-8**) of all the taxa, we see the clades that give rise to anguilliformity include those *Clarias* species with the lowest relative skull length. The clade including *Heterobranchus longifilis, Dinotopterus cunningtoni, Clarias gariepinus* and *Clarias ngamensis,* however, incorporates the species with the highest relative skull length (indicated with bold values and branches) (measurements from BOULENGER, 1906; TEUGELS, 1986; POLL, 1943).

Biogeography and evolution towards anguilliformity

The idea that the evolution towards anguilliformity in Clariidae may have occurred more than once has been raised since long (BOULENGER, 1908; PELLEGRIN, 1927). Our results show that anguilliformity may have evolved even at least four times, where it has to be emphasized that in this study, other anguilliform taxa as *Dolichallabes microphthalmus* are

not even included. This multiple origin of anguilliformity also seems to be supported by the paleobiogeography and life history data.

Since the Eocene, the African continent has experienced multiple phases of climate changes, oscillating between the so–called greenhouse and icehouse (see SÉRANNE, 1999). This resulted in a fluctuating pattern of expanding savannah and reduction of forest patches, and vice versa. The fragmentation of tropical lowland forests resulted in small forest patches, which are believed to have functioned as a sanctuary of species. This idea has been formulated in the refuge theory of HAFFER (1982), focussing on Pleistocene climatological events. Several authors, however, have argumented that these speciation events should not be restricted to Pleistocene climatological changes only (PRANCE, 1982). An example is the subsequent radiation and extinction of hominoids during Oligocene through the Miocene, linked to expansions and contractions of forest patches (LEWIN, 1998). This theory has been explanatory for many groups (HAMILTON, 1981; SOSEF, 1994; MALEY, 1996). Such refugia are situated in southern Cameroon, southwestern Nigeria, central Gabon (subdivided around the Woleu, Ivindu, Ogowe) and various spots in the Congo basin (HAFFER, 1982; MALEY, 1987). In these refugia, isolation could very easily occur and subsequent speciation events are to be expected. With respect to the Pleistocene climatic fluctuations, the forested West Coast Equatorial bioregion (Lower Guinea) is considered to have been a refuge for many species. Various Amphiliidae, Mochokidae, and Cyprinidae, in addition to some anguilliform fish (e.g. Mastacembilidae) (LÉVÊQUE, 1997). Especially interesting is when comparing the current distributions of anguilliform species with the distribution of the above mentioned refugia, there seems to be a close match: *G. typus*: Niger Delta; *C. apus*: Congo Basin; *C. alvarezi*: North Gabon, Woleu, *C. longicaudatus*: East Gabon, Ivindo; *C. ogooensis*: South-East Gabon, Ogowe; *C. teugelsi*: Gabon and Pop. Rep. de Congo; *D. microphthalmus, G. nops, P. tihoni*: Congo basin; *P. machadoi*: Cuango. This may all support the hypothesis that the refugia theory tends to explain clariid paleobiogeography.

Current knowledge on fossil data (**Table V.1-3**) only allows us to situate the origin of the Clariidae at the lower Eocene (34–56 my), as well as fossils of *Heterobranchus* of the lower Miocene are known (GAYET and MEUNIER, 2003). As such, speciation events in clariids, with respect to forest fragmentation and consequent body elongation can both have occurred during pre–Pleistocene and Pleistocene.

Coupling the life history of the clariids to this refugia theory gives us a more complete picture. It could be hypothesized that the ancestors of the Clariidae were restricted to the rivers of these refugia, where subsequently speciation could have occurred. Forest contraction can be expected to have forced other river–inhabiting species to retreat to

smaller rivers and brooks in those restricted areas, which could have triggered a selective pressure towards anguilliformity. Only those species adapted to leave those smaller rivers and brooks, and to colonise new niches, like swamps, could have escaped the increasing competition. This has been possible for small species, like *Barbus jae* and *Ctenopoma nana*, but also for species that have become elongated, as this is extremely advantageous for inhabiting swampy areas. Many morphological specialisations can be linked to this life style, such as elongation, limblessness, jaw muscle hypertrophy, reduction of the eyes, increasing rigidity of the skull. These are all regarded as an adaptation to a fossorial habit (WITHERS, 1981). The subsequent shift to a more humid climate would again initiate forest expansion, providing the possibility to disperse again to some degree. Less restricted dispersion would have been able for the fusiforms, as they have several adaptations which permit them to do so. First, they are known to inhabit and spread throughout larger river systems, which is not the case for the anguilliform taxa. Second, they are better adapted to migrate between river systems in dryer conditions (i.e. by 'walking' over land, facilitated by the suprabranchial organ) (GREENWOOD, 1961). The almost Pan African distribution of *Clarias gariepinus* demonstrates this (TEUGELS, 1986), as well as most anguilliform clariids currently still have a very limited distribution, restricted to the forested, swampy areas.

Some critical notes have to be formulated at the end of this discussion. At this moment no data is available to check the correspondence in the chronology between the origin of these refugia and the speciation events (of the clariids or other fishes in general). Although the refugia during the Pleistocene are well documented and numerously discussed in literature, the data on the refugia prior to that period are far scarcer. Secondly, combining data (morphological and molecular) into one dataset may have some shortfalls, which are discussed in **VI.3**

PART VI

General Discussion

As each chapter in part IV and V have there own specific discussion, I have opted to present in this part a general discussion on specific consequences and problems encountered in this thesis. These have been subdivided into three large parts.

Section VI.1 deals with the consequences of the combined phylogenetic analysis and focusses more specifically on the taxonomical implications of this analysis.

Section VI.2 discusses two major taxonomic problems.

Section VI.2 provides arguments on the different ways of dealing with morphological and molecular data in phylogenetic analyses.

VI.1 - Consequences of the generated phylogeny (based on the consensus tree of the combined analysis)

The phylogenetic results presented in this thesis have some far–reaching consequences on systematic level. Several of the genera and subgenera of which some species are included, turn out to be paraphyletic (as was already suggested by other studies). An overview of these groups and the possible impacts are given here.

We included 12 specimens, comprising ten species of *Clarias*, the largest genus in the Clariidae and also the largest group represented in this phylogenetic analysis. These specimens are spread over eight different monophyletic "clades" on the tree (**Plate VI.1-1: 1-8**). As a comparison, in AGNÈSE and TEUGELS (in press) 19 *Clarias* species were include in the analysis, of which 13 are African representatives. The latter are divided in seven monophyletic positions. The reason for this clearer situation (seven instead of eight) is the fact that only two anguilliform clariids are included instead of the six in our analysis. These have each their own origin in a *Clarias* clade and thereby break the *Clarias* monophyly even more. Looking closer now to the systematic structure of this genus, the following points of interest can be raised.

When we use the phenetically driven systematic revision of TEUGELS (1986), the species used can be spread over six subgenera (I–VI).

I. Of the first subgenus *Clarias* (*Dinotopteroides*) only one of the two species (*C. ngamensis*, **Plate VI.1-1: 5b**) is included in this analysis as well as that of AGNÈSE and TEUGELS (in press), so no decisions can be made on the validity of this subgenus.

II. Of the second subgenus (*Clarias*), we included only one of two species; *C. gariepinus* (**Plate VI.1-1: 5a**). In AGNÈSE and TEUGELS (in press), however, both representatives are included; *C. gariepinus* and *C. anguillaris* (**Plate VI.1-2: 1**). These turn out to form a

paraphyletic group. However, they form one clear grouping, when all *Bathyclarias* species are included. This close relation between *Clarias* (more specific with *C. gariepinus*) and *Bathyclarias* was already demonstrated by AGNÈSE and TEUGELS (2001b). They found that the *Bathyclarias* species originated in a non–dichotomous way from a *C. gariepinus* population.

III. We included three species (*C. stappersii*, *C. jaensis* and *C. platycephalus*) (**Plate VI.1-1: resp. 1, 3, 4**) of the subgenus *Platycephaloides*, with the first two also incorporated in Agnèse and TEUGELS (in press). These three species occur completely scattered over the tree and although in Agnèse and TEUGELS (in press) the two species group more together, they still form a paraphyletic group together with *Clariallabes longicauda* and *Clarias camerunensis* (**Plate VI.1-2: 2**).

IV. The fourth subgenus, *Clarioides*, is the only subgenus of which could be concluded that indeed it was a monophyletic genus (**Plate VI.1-2: 3**).

V. Although, e.g. in the molecular analyses, the species (*C. theodorae, C. pachynema* and *C. submarginatus*) (**Plate VI.1-1: resp. 6, 7**) of the *Anguilloclarias* subgenus closely group together in one clade they still form a paraphyletic grouping.

VI. Of the last subgenus (*Brevicephaloides*) only one species (*C. camerunensis*, **Plate VI.1-1: 2**) was included so no conclusions on the validity could be made. As is demonstrated here several times, the genus *Clarias* turned out to be clearly paraphyletic and this in both analyses mentioned.

Looking closer at the affinities postulated by TEUGELS (1986) and DAVID (1935), we see that some correspondence is present. Morphologically, species of the subgenus *Dinotopteroides* closely resemble those of the subgenus *Clarias* (TEUGELS, 1986). Our analysis gave a combined (morphological/genetic) proof of this relationship (**Plate VI.1-1: 5**). Most species of the subgenus *Platycephaloides* have been the subject of many discussions and taxonomical replacements, indicating the difficult positioning of these species (DAVID, 1935; TEUGELS, 1986). *C. platycephalus* has been associated with *C. gariepinus, C. submarginatus, C. camerunensis* and *C. buettikoferi* (*C. platycephalus* has been seen as a hybrid of the first two) (BOULENGER, 1902; DAVID, 1935), *C. jaensis* has been included in the subgenus *Clarioides* (DAVID, 1935) and *C. stappersi* at some point was considered as a junior synonym of *C. submarginatus*. The difficult relation between these three species and among all other clariids is clearly visualised in their scattered positions in our analysis (**Plate VI.1-1: resp. 1, 3, 4**). A close relation between the subgenera *Clarioides* and *Anguilloclarias* has several times been postulated in the past, e.g. most species currently assembled in *Anguilloclarias* used to be reported in *Clarioides* (DAVID, 1935). This close relationship was not only demonstrated in our combined analysis (**Plate**

VI.1-1: clade IIc) but also in the morphological analysis (**Plate IV.2 -5**). A last relationship that in some way turned up in our combined analysis is the closer affinity between *Clarias* (*Brevicephaloides*) and *Clariallabes* presented in DAVID (1935). In our analysis a close relationship is demonstrated between *Clariallabes longicauda* and *C. camerunensis*. However, again only one representative was included, so this close relationship may only be seen as an indication.

Taking into account the phylogenetic (cladistic) species concept, this paraphyly of *Clarias* has some consequences. A first one is regarding the nomenclature. The type species for the genus *Clarias* is *Silurus anguillaris* Linnaeus, 1758, synonym of *Clarias anguillaris*. This means that only the species in the subgenus *Clarias* are still entitled to the name *Clarias*; *C. anguillaris* and *C. gariepinus*. The latter only remains *Clarias* with the incorporation of all *Bathyclarias* species (see above), with the consequent replacement of the *Bathyclarias* genus. All other currently recognised *Clarias* species should then be transferred to other genera. In our analysis this would mean that seven new genera would need to be raised (in AGNÈSE and TEUGELS (in press): six).

Also, the other well represented genus, *Channallabes*, turned out to be paraphyletic. Interestingly, the two species from the Congo basin, *C. apus* and *C. sanghaensis* are included in the same clade (**Plate VI.1-1: Clade II**). The same counts for the two species included from the Lower Guinea (*C. alvarezi* and *C. longicaudatus*) (**Plate VI.1-1: Clade I**). This clearly marks the distinct geographic difference. A similar paraphyletic subdivision is seen in the morphological analysis (now also including *C. teugelsi*). The paraphyletic clustering of the *C. sanghaensis* specimens may not be the correct hypothesis. Indeed, some methodological error may have caused this artificial paraphyletic grouping, implying that the suggested "reversion" towards fusiformity in this clade is erroneous as well (**Plate VI.1-1: Clade IIa**).

This all will have some nomenclatural and systematic consequences. The type species for the genus is *Gymnallabes apus* Günther, 1873, which was latter transferred to *Channallabes*, thus being *C. apus* (Günther, 1873). This means, according to **Plate IV.1-1,** that only *C. apus* is still entitled to the name *Channallabes*. All other currently recognised *Channallabes* species should then be transferred to other genera. All the "Gabon" species can then be grouped into one but new genus (**Plate VI.1-1: Clade I**), while *C. sanghaensis* should be transferred in yet another new genus. Because of the distinct morphology of those taxa, the designation to new genera (with respect to the *Clarias*-sister taxa) can be advised.

Also *Gymnallabes* turned out to be paraphyletic. Based on morphological differences the type material *G. alvarezi* was transferred to *Channallabes* (*C. alvarezi*) and the included museum specimens were assigned to a new species *C. teugelsi* (**IV.5.2**). This transfer of all material previously included in *G. alvarezi* into another genus is confirmed in the combined phylogenetic analysis, since *G. typus* and *C. alvarezi* do not cluster. Although, the position of *G. typus* varies a lot in the different analysis, it never groups with *C. alvarezi*. However, due to the questionable, unresolved position of *G. nops*, *Gymnallabes* remains paraphyletic.

All these examples show that, together with this study, the unravelling of the family Clariidae has continued for a long time, but the terminus is not yet reached.

VI.2 - Encountered taxonomic problems

VI.2.1 Single specimen species

The biggest taxonomical problem encountered in this thesis was the correct placement and validity of *Gymnallabes nops*. This problem was largely caused by the fact that only one specimen is known of this species. Consequently, this means that the species was described based on only one specimen, with some subsequent problems.

One problem is the absence of any knowledge on intraspecific variation. When species are described on the basis of a single specimen taken from a single locality, the considerable morphological variability that exists in natural populations of a species is generally not recognized. So that old and young individuals (merely endpoints in the continuum), male and female individuals of the same population can be described as distinct species. This is like describing the sticklebacks over and over again by virtue of differences in paired fins and girdles. This intraspecific variation, however, is an important part in a complete and well–balanced species description, as stressed in this thesis. In a group, as variable as the eel–like clariids, this is certainly the case. This absence makes it hard to compare and situate *G. nops* to other clariids.

In the case of *G. nops* another problem is whether or not we are dealing with an abberant specimen. Since the most striking, unique characteristic is the absence of any external evidence of the visual sense organ. Although this can be a seen as a very strong diagnostic character, it can also be seen as aberrant characteristic. Furthermore, it is not a unique character; as it is not the only eyeless specimen in the Clariidae (e.g. IV.1, *Platyallabes tihoni*). Unfortunately, working with only one specimen for the description of a new species makes it is impossible to check if this main character is linked to abnormalities. As a result of the above mentioned problems, no strict conclusions could be made. *G. nops* remains enclosed in the genus *Gymnallabes*, since no complete set of arguments could be given to justify the replacement, only some indications. Merely some indications (how strong they may feel) do not give a justification to question previously made scientific intentions (TEUGELS, pers comm.).

However, this may be the perfect place to sum up the different possibilities and to put forward the most likely solution for the *G. nops* classification. The first possibility is to consider *G. nops* as a valid species; *e.g.* based on the reduction of the infraorbital bones in number and size, a large anterior outgrowth of the opercle, a clearly visible epiphyseal bridge and the typical positioning of entopterygoid and metapterygoid bones, and the

singular position on the PCA (**Plate IV.3-5**). The other possibility is to place it in synonymy with another species. Although again the question can be raised whether to place it in synonymy with *G. typus* or *P. tihoni*, several arguments favour the synonymy with *P. tihoni*. One of these arguments is the close resemblance between these two species, as already noticed in the original description of *G. nops* (Roberts and Stewart, 1976) and several new similarities (see **IV.3.1**). Moreover, as already mentioned in **IV.3.1** most of the differences found in this original description can be countered, indicating again the similarity between these two. Another argument is that *G. nops* co–occurs with *P. tihoni*. This all makes the assumption that *G. nops* is an aberrant *P. tihoni* specimen very plausible. However, besides these phenetic arguments, there is also some cladistic evidence. Looking at these phylogenetic results based on morphology alone, we see a close relation between *G. nops* and *D. microphthalmus*. This is mostly based on the reduced number of infraorbital bones, which only characterises those two species (and thus is not as variable as some other features of the elongated species). However, the weighted consensus tree gives a similar result, indicating that the postcranial characteristics are similar too. Also from a biogeographic view, a close relation seems very plausible. So, with the present knowledge, arguments tend to favour a close relationship between *G. nops* and *D. microphthalmus*. However, *G. nops* and *D. microphthalmus* have each their own set of unique characters, so there is no reason to place *G. nops* in synonymy with *D. microphthalmus*. From all this, it could be concluded that *G. nops* should be better transferred in the genus *Dolichallabes*.

Most single specimen based descriptions are therefore not likely to be accepted and are difficult to publish in peer reviewed journals, because of the above mentioned problems. For example, the description of *G. nops* is part of a survey of the fishes in the rapids area of the lower Congo River and is not published in peer reviewed journal. However, looking closer at the taxonomic data on fishes, we see that single specimens based species descriptions do occur in some exceptional cases, dealing mainly with two categories of fish.

These exceptional, single–specimen based, descriptions mostly diagnose deep sea species (recent: *Vanderhorstia paplilio*, a shrimp goby). Another clear example of this first category are the dragonfish. Dragonfish (Stomiidae, Stomiiformes) are so rare that scientists often have been forced to study and describe new species based on a single specimen. An extra problem in most deep sea species is that the larval stage is very different from the adult morphs, what has conducted to the description of the larvae and the adults as different species. High tech sampling methods made tracking and capturing

these fish somewhat easier, so that a single–specimen description diminishes in these fishes too. In a second category, these descriptions will remain. New fish fossil discoveries are rarely found in groups of similar species (although exceptions are known, e.g. Piscine Pompeii, an upper jurassic fossil fish assemblage), enforcing the scientist to describe the new species or even an entire new genus based on a single specimen (e.g. *Hensopon*, Pycnodontiformes) (KRIWIT, 2004).

VI.2.2 Sub-(species)

A second taxonomic problem encountered in this study concerns the taxonomic unit "subspecies". Concrete, this problem was encountered with the correct taxonomical assignment of the new "groups" found in Gabon (**IV.5.2**) The taxonomic unit "subspecies" is liable to a lot of discussion. This discussion focuses around the use and how to delimit species from subspecies. The controversy over the subspecies centres around two major questions. The first question wrestles about: "what is a subspecies or what should a subspecies be?" The second question is best formulated: is there a need, a use for this nomenclatural concept called "subspecies? (MONROE, 1982).

Several definitions have been given to clarify the term subspecies. MAYR and ASHLOCK (1991) define a subspecies as "an aggregate of phenotypically similar populations of a species inhabiting a geographic subdivision of the range of that species and differing taxonomically from other populations of the species." They point out that neither a nonarbitrary criterion is available to define the category of subspecies, nor that the subspecies is a unit of evolution except in those cases where it happens to coincide with a geographic or other genetic isolate. They thereby take an intermediate point of view between the two most different ways of approaching the subspecies concept (ROLÁN-ALVAREZ and ROLÁN, 1995). On the one hand we have those for whom the subspecies is an intermediate outcome during the process of speciation (ROLÁN-ALVAREZ and ROLÁN, 1995) and on the other hand we have those for whom the subspecies is a completely arbitrary taxonomical unit which should be abandoned (WILSON and BROWN, 1953; CRACRAFT, 1983).

Thé big problem in the definition of a subspecies is the delimitation of the geographic subdivision. Indeed, numerous forms of zoogeographical variation can be recognised, from discontinuous or allopatric variation to cases of continuous variation (MONROE, 1982; O'NEILL, 1982). MONROE (1982) states that the subspecies category loses its potential usefulness if applied to situations of such continuous variation. Therefore, he thinks that the subspecies category should not be used to describe populations, differing from each

other only through smooth (*i.e.* with all characters grading into each other along the cline) clines.

However, the practical (in taxonomic studies) approach of those theoretical considerations is not always easy. Situations where, in one species, several natural populations occur along such a continuous geographical variation are widespread. In those examples, the most geographically isolated populations of such species can eventually clearly differ from each other in a number of characteristics. However, when considering the entire series of the joining contiguous populations, these characters can show a continuous variation from the one state to the other between both most geographically isolated populations (MAYR and ASHLOCK, 1991). If no material is available from the contact zones, this variation can not be detected and falsely conclusions can be made regarding species or subspecies assignment.
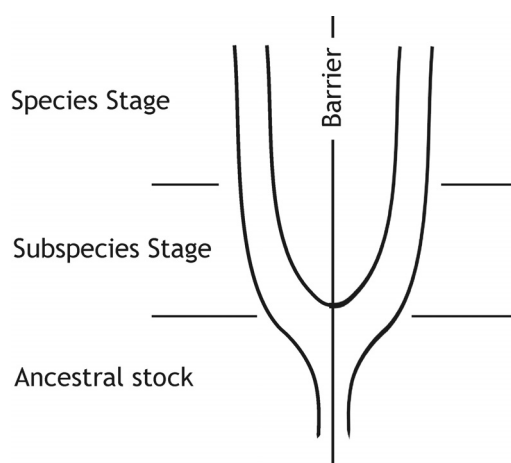
Fig. VI.2- 1: speciation process model (after Mayr and Ashlock, 1991)

Concerning the use of subspecies, it is mostly linked to the speciation process model (**Fig. VI.2-1**), keeping in mind that species do evolve, and almost always do so gradually (SIMPSON, 1961). It is so that among evolutionary species there cannot possibly be a general dichotomy between free interbreeding and no interbreeding. Every intermediate stage occurs, and there is no practically definable point in time when two intraspecific populations suddenly become separate species. MAYR and ASHLOCK (1991) referred to part of this intermediate stage as the subspecies stage (**Fig. VI.2-1**). Monroe's statements on the use of subspecies mentioned here is based on such thinking (MONROE, 1982). He states that the use of the subspecies provides a useful tool in a discussion of the evolutionary speciation process (model) involved. He couples this to a limitation of the use of subspecies to the following two situations: (1) *allopatric populations* where the definition of the populations is clear, distinct, and total (or nearly so); and (2) situations where *secondary contact* between distinct populations has occurred and the zone of intergradation is relatively narrow (Monroe, 1982).

A second point of view is already given in the first part of this discussion as one of the two large ways of approaching the subspecies concept. Namely, those for whom the subspecies is a completely arbitrary taxonomical unit which should be abandoned. The

main motivation for this abandoning is the fact that at this moment, there is a lack of consistency in the biological definition of the subspecies. This gives the taxonomist a major problem since it is impossible to conclude, based on the morphological characters, if you are dealing with a subspecies or a full species. Another argument for questioning the usefulness of this concept can be given. According to ZUSI (1982), a review of subspecies is not the most informative way to present the variation within a species, because it makes variation compartmental in an artificial way and obscures the synoptic comparison and interaction of character variations.

Consequently, many would like to see it disappear (e.g. READY and KULLANDER, Elimination of subspecies from fishbase, XI European congress of ichthyology, Tallinn 2004); WILSON and BROWN (1953) already long ago called for a serious, conscious consideration of the desirable abandonment of the subspecies trinomial. Instead, they suggest that for the study, analysis of geographical variation vernacular locality names or a brief statement of the range involved should be used. Informality and flexibility of the latter procedure were considered as among its most appealing characteristics.

Therefore, no subspecies have been designated in this work. Instead, detailed descriptions of the intraspecific variation and geographic distribution in each species are given. So, that a complete view on the variability of each species is provided.

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A discussion on the usefulness of morphological or/and molecular data for phylogeny purposes mostly ends up in defending the one or the other, with recently morphological data being generally under siege. Although this chapter tends to "defend" the use of morphological data for phylogeny, it also gives the advantages and disadvantages of both molecular and morphological data. Further, this part tends to give the possibilities on how to use both data (separate or combined), since that seems to be the most idealistic scenario.

We live in the age that more and more genes and genomes are being sequenced, the possibility that thousand even millions of informative, independently evolving molecular characters can be brought to bear on a given phylogenetic problem is quickly becoming realistic (ROKAS et al., 2003). Given the rate that new sequencing data are being added, and the rate at which new innovations continue to accelerate this process, it seems possible that in the not-too-distant future we will be able to have an accurate and well-supported phylogeny of most living species using molecular data alone. Furthermore, some even underline that morphological phylogenetics is so problematic that trees should no longer be reconstructed using morphological data and that unambiguous morphological characters should be merely mapped onto phylogenies established by molecular data to determine if they add further support to specific nodes (SCOTLAND et al., 2003). So why bother with morphology, in other words what are the advantages of morphological data justifying their use in the future?

There are a number of reasons to continue to reconstruct phylogenies using morphological data. The most compelling reason to continue collecting morphological data long into the future is to resolve the phylogenetic relationships of fossil taxa and their relationships to living taxa. The reconstructed Tree of Life must include fossil taxa. Considering all the species that have ever evolved, most are now extinct (>99% according to some estimates; NOVACEK and WHEELER, 1992), and many extinct groups were diverse, ecologically important, and very distinct from their closest living relatives. This makes it important to include them in phylogenetic analysis. The relationships of most fossil taxa can, however, only be determined through phylogenetic analysis of morphological data (despite impressive molecular studies of very recent fossil taxa). This is not merely important for their potential to help resolve these relationships but also to understand the rate and timing of macroevolutionary processes in both living and fossil taxa. Finally, the addition of fossil taxa seems to increase congruence between the molecular and

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morphological results in this case, suggesting that addition of fossil taxa is important for phylogenetic accuracy (WIENS, 2004).

The ability to study characters that reflect a variety of different genes can be seen as another major advantage of morphological data. When we study morphology, we can study different aspects of the body that are likely coded by many different genes. Contrarily, when we study DNA, we often have the technology and money to study only a single gene or a small number of genes. A potential problem with studying just one gene is that differences at different base pairs in the same gene may be related so that what we are calling separate characters may evolve in a correlated way. If we study traits coded by many genes, it is less likely that their evolution will be correlated. In this case, studies of morphology may be superior to our current studies of DNA. However, similar correlated characters can of course not be completely excluded in morphological data

Another obvious advantage is the fact that it is extremely less expensive to study morphology than DNA. This means that for the same cost we may be able to study more aspects of body structure than we can study from genes, assuming of course that morphological data yields a relevant insight in phylogeny.

Further, there are many taxa that are extant but may still be very difficult to include in molecular studies. For example, many fish species are only known from a limited number of specimens in museum and are preserved (i.e., formalin fixation), which makes obtaining molecular data very difficult (e.g. *Platyclarias machadoi*, only known from 22 specimens collected in 1967 and since then stored at the MRAC). Many of these species may never be collected again (i.e., because of limited distributions, difficulties reaching the habitats, …). Many species remain known from a single specimen that was collected decades ago (e.g. *Gymnallabes nops*). All this means that the only way that we may know anything about the relationships of these species is through phylogenetic analysis of morphology.

Until a stage is reached where all molecular phylogenies are reconstructed without error, it is still important to have rigorous, morphology-based phylogenies as a "reality check" for molecular results (e.g., HILLIS and WIENS, 2000). Of course, problems in individual molecular data sets can also be detected by comparison to other, independently evolving molecular data sets. But there may be cases where all molecular data sets may give the wrong answer (e.g., sequencing many different genes of a misidentified specimen). A typical set of morphological characters should focus on information from many different unlinked genes (HILLIS and WIENS, 2000), whereas the characters in a given molecular data set are often linked and inherited as a single unit.

The use of ontogenetic data is another not to neglect advantage of the use of morphological data. Using ontogenetic information gives the possibility to polarize characters, distinguishing phylogenetically informative data (apomorphies) from phylogenetic noise (plesiomorphies) in systematics. For this, ontogeny is seen as one of the phylogenetic methods besides the outgroup criterion.

Finally, it is important to note that we are very far from describing all the living species on earth, much less sequencing them. With some exceptions, new species are generally discovered, delimited, and described using morphological data.

On the other hand, molecular phylogeny also has a great number of "obvious" advantages, of which the most important ones are given here.

Perhaps the greatest advantage of molecular data is the extent of the data set. Because all heritable information of an organism is encoded in DNA, the set of morphological data is only a small subset of the molecular information.

When we study DNA, we are looking directly at the genetic material. We know that what we are looking at is inherited; DNA in modern organisms has been passed down ancestral organisms, so DNA should reflect ancestry and be reliable for studying phylogenetic relationships.

Although in principle, neither molecular nor morphological methods are limited by clade level in their application. In practice, however, few morphological characters are shared among major groups of organisms. In contrast, genetic information provides a phylogenetic record from very recent times to the origin of life (Hillis, 1987).

The genetic characters mostly represent heritable information. Molecular data are not or less confounded by environmental influences ("phenotypic plasticity") than are morphological data.

Although the output from molecular systematics has increased manyfold during the last decades, this has certainly not been accompanied by an equal decrease in output from morphologists. To a large extent, this is because molecular and morphological data, each are able to address questions and problems that cannot or less adequately be addressed by data from the other type. Although morphological studies have generally been successful in defining relationships up to the level of genera, studies which present a hypothesis of relationship above this level are somewhat more rare (although not absent, e.g. DE PINNA, 1993; DIOGO, 2005). This, however, is one of the strengths of molecular data, especially when analyzing longer segments of DNA (STEPIEN and KOCHER, 1997). So no single systematic data set can be expected to be informative at all phylogenetic levels simultaneously.

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Because of this complementarity, collaborations between morphological and molecular data often produce analysis that transcends the usefulness of separate studies. However, this raises a very important and difficulty problem how to bring these two together.

There has been much discussion in recent systematic literature about whether different data sets (not only morphological with molecular data, since conflicts among molecular studies are probably as common (HILLIS, 1987)) should be analysed separately or combined and analysed simultaneously. In the following paragraph arguments for both views are given. Summarizing, it comes down to this. Assuming that the goal is to discover the true phylogeny of the entities in question, arguments for (I) combining data are based on the notions that one should use the "total evidence" available (DE QUEIROZ et al., 1995; STEPIEN and KOCHER, 1997). On the other hand, if there is heterogeneity among data sets with respect to some property that affects phylogeny estimation, then combining the data can give misleading results and dealing with them separately (II) may be in favour.

II. The heterogeneity favouring the separate method can be of different types: a difference in rate of evolution, a difference in data set size and a too large independence between data sets (DE QUEIROZ et al., 1995). In the latter, non–independency within data sets does not necessarily imply functional or physical linkage, but only that characters within a data set are more likely to share some property relevant for phylogeny estimation than are characters in different data sets. Several methods of constructing a "consensus" cladogram of these separate data sets have been developed (strict consensus, majority consensus). These methods are however not uncriticised. MIYAMOTO (1985) states that these methods do not take into account the relative strengths of support for various groups by the different data and therefore don't give the best possible result.

I. Arguments for combining data can be divided into several categories (DE QUEIROZ et al., 1995):

1) A philosophical argument based on the idea of "total evidence", stating that conclusions based on all the relevant evidence are certainly to be preferred.

2) Combined analysis of all the data circumvents the need to choose among the various methods of consensus, a choice that is characterized as fundamentally arbitrary.

3) The difficulty of choosing a scheme to partition the data is no longer necessary. There are many ways to partition all the data and it is unclear how a particular scheme of partitioning can be justified. For example DNA sequence data might be partitioned into separate genes or by position in the codon, and morphological data

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might be partitioned into larval and adult, cranial versus post-cranial or soft tissue versus hard anatomy.

4) The fourth category involves criticism on the efficacy of separate (consensus) methods as a mean of producing phylogenetic hypotheses with descriptive and explanatory power. Miyamoto (1985) highlighted the fact that a consensus of trees produced by separate analysis of each data set can be less parsimonious than the tree(s) from a combined analysis of the data. The consensus approach fails to take into account the underlying evidential support for the trees from the separate analyses.

This combining method, however, has also some shortcomings. It is suggested that the phylogenetic signal of larger data sets will overwhelm that of smaller data sets in a combined analysis. Secondly, when the two separate datasets each give a different (contradictory) topology, it can be questioned whether the combined (average) analysis gives any surplus. To estimate this, however, this requires a good knowledge of which of the separate data sets gives the best result (and how "good" this result then is).

Again, the best (practical) approach to integrate molecular and morphological data may then be to link both methods (combined and separate) and to (1) perform separate analyses to identify areas of strongly supported incongruence between data sets (i.e., areas where combined analysis might be expected to fail); (2) perform a combined analysis; and (3) consider regions of the combined-data tree that are strongly contested by different data sets to be ambiguously resolved, until the source of error is identified, or if the source is unknown but a majority of independent data sets clearly supports for one hypothesis over another (WIENS, 2004). This approach may be advantageous relative to using a separate or combined analysis only. The approach outlined above, utilizes the results from combined analysis in those parts of the tree where combined analysis should succeed (i.e., no or weakly supported incongruence) and should treat the combined-data results as ambiguous in those sections of the tree where combined analysis might be expected to fail (i.e., strongly supported incongruence).

Apart from the many advantages of molecular data (some mentioned above), it is absolutely critical that systematists continue to be trained in morphological systematics as well, particularly for poorly known groups (HILLIS and WIENS, 2000). If students are trained exclusively in molecular techniques, the next generation of systematists may be incapable of identifying the species in their study groups, and phylogenetic progress in these groups may quickly "grind" to a halt (nonetheless the intensive amount of work that is put into

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recognising species through DNA). Quick and accurate identification of species in the field and laboratory based on morphological characters also is critical to many other areas of biology besides systematics (e.g., ecology, behavior, physiology; MADDISON, 1996), as well as applied sciences (e.g. aquaculture, agriculture, pest control, …).

PART VII

Conclusions and Summary

VII.1 - Conclusions and Summary

The present PhD thesis includes a systematic and phylogenetic study of the anguilliform air–breathing catfish (Clariidae, Siluriformes).

In the <u>introduction,</u> first an explanation is given about the reasons and needs for such a study and it is shown that, in broad outlines, this thesis encompasses a descriptive, taxonomic and systematic section, and a phylogenetic section.

The <u>second</u> part gives a taxonomic overview of the Clariidae and situates them within the Ostariophysi and Siluriformes. For each of these three taxonomic levels, a state of the art summary on the affinities, specialisations and zoogeography is given. In **II.1.3**, the attention is mainly focussed on the anguilliform taxa in the Clariidae.

The <u>third</u> part provides a detailed description of the material used and lists the ingroup and outgroup species in the present work. The methods are briefly explained in this part and are linked to the respective chapters were they are discussed more thoroughly. This part, however, does include a detailed description of the biometrics and meristics variables.

More than 500 specimens of 11 nominal anguilliform clariid species have been examined. All these specimens were obtained from nine musea (including all type material) or collected during two expeditions to Gabon. On each specimen a total of 39 biometric measurements were taken, together with six meristic counts (compiled together in several tables). Several specimens of each species have been used for dissections, *in toto* clearing and staining or serial sections. Visualisation occurred through microscope and camera lucida, CT–scanning or radiographs. The data was processed applying several statistical analyses, as well as multiple phylogenetic programs and methods were used to analyse the data.

In the <u>fourth</u> and <u>fifth</u> part the results obtained are presented and discussed in detail in the following order:

First, each of the eleven species recognised is described in detail, based on an exhaustive study. All nominal species are provided with a differential diagnosis, a description and a detailed geographic study. Each species description is followed by a proper discussion, which mainly deals with the most important characteristics. Most

species are described separately, except for the species in the *Channallabes* genus. For this genus, there is opted to work from a geographic angle (Congo and central and southern West Coastal Equatorial freshwater ecoregion). In this genus three new species and two rehabilitated species are described and their recognition is thoroughly motivated.

Finally, at the end of this descriptive part, practical determination keys are provided.

Secondly, several phylogenetic analyses were performed. These are split up into two large parts; (1) molecular and (2) combined, morphological and molecular analysis.

The systematic and phylogenetic study of the anguilliform Clariidae, as presented here, has given conclusive results on some of the questions, as mentioned in **I.1.2**, whereas some suggestions can be made, with respect to other questions. The following conclusions can be given, in correspondence with the questions posed in Chapter **I.1.2**.

1. Eleven species can be distinguished in the group of the elongated morphs in the Clariidae. These 11 species are spread over five different genera. The genera *Platyallabes, Platyclarias* and *Dolichallabes* are all monotypic and include respectively *P. tihoni, P. machadoi* and *D. microphthalmus*. The genus *Gymnallabes* comprises two, instead of the three previously recognised species, i.e. *G. typus* and *G. nops*. The genus *Channallabes* formerly comprised only one species. The extended morphological results presented in this study revealed the existence of three species, completely new to science (*C. sanghaensis, C. teugelsi* and *C. ogooensis*) and two rehabilitations (*C. alvarezi* and *C. longicaudatus*) which were previously both assigned to *Gymnallabes*. The recognition of all these species is thoroughly motivated.

2. A set of diagnostic features can be given for each of the eleven species. *P. tihoni* can be easily diagnosed by a small distance between the occipital process and the dorsal fin (2.2-6.6% of SL). *P. machadoi* is recognised by an extremely dorsoventrally flattened skull (skull height 22.9-37.1% of skull length). *D. microphthalmus* shows a unique single longitudinal fontanel in the skull roof. The most diagnostic character of *G. nops* is the reduction of the infraorbital bones in both number and size, while *G. typus* is unarguably characterized by well–developed skin folds bordering the side wall of the mouth. Distinguishing the species included in *Channallabes* is less univocal. *C. sanghaensis* and *C. apus* differ from *C. alvarezi, C. longicaudatus, C. teugelsi* and *C. ogooensis* in having a small supraorbital process on infraorbital IV, not reaching the rostral border of the eye. *C. alvarezi* can be separated from the latter three based on e.g. the absence of a spot on the skull. *C. teugelsi, C. longicaudatus* and *C. ogooensis*

differ from each other in the presence or absence and the place of serrations on the pectoral spine.

3. The detailed morphological study subsequently provided a list of synapomorphic characters; *P. tihoni* has among other things a horizontal position of the sphenotic and pterotic and a toothed entopterygoid. *P. machadoi* shows three long process on the prevomer and an extra muscle (musculus adductor mandibulae A_3'' pars levator tendinis) in the adductor mandibulae complex. Many of the unique characters in *P. tihoni* and *P. machadoi* are linked to the spatial constraints associated with an extremely flattened skull. *D. microphthalmus* shows a reduced skull ossification, with e.g. one elongated fontanel, and the antorbital and infraorbital IV the only circumorbital bones present; only *G. nops* shares this reduced number of circumorbital bones. *C. apus* and *C. sanghaensis* show a unique ento– and metapterygoid configuration.

4. All anguilliform taxa are found in Central West Africa. Except for *C. apus* and *C. teugelsi*, they all occur allopatric. *C. teugelsi* can be found in both Ivindo and Ogowe basin (resp. with *C. longicaudatus* and *C. ogooensis*) and *C. apus* is found in the large parts of the Lower and Central Congo basin, sympatrically with *P. tihoni, P. machadoi, G. nops* and *D. microphthalmus*.

5. Several internal, but mainly external characteristics have been combined in a key to the different genera in the African Clariidae. This practical tool is provided to identify all recognised genera in the African clariids (**IV.5.2**) and for the *Channallabes* species (**IV.5.1.b**).

6. All the different molecular phylogenetic analyses performed, distinguish four clusters, grouped in two clades. Cluster 1a contains *Clarias buthopogon, Clarias pachynema, Clarias submarginatus* and *Channallabes apus*, cluster 1b comprises *Clarias ngamensis, Clarias gariepinus, Dinotopterus cunningtoni* and *Heterobranchus isopterus*. The second large clade can again be divided in two clusters, cluster 2a groups *Clarias camerunensis, Clariallabes longicauda, Channallabes alvarezi* and *Channallabes longicauda*, except for the POY analysis this cluster also includes *Clarias stappersi*. The last cluster always includes *Clarias platycephalus, Channallabes sanghaensis* and again except for the POY analysis *Clarias jaensis*.

7. Our results show that anguilliformity may have evolved at least four times, where it has to be emphasized that in the molecular phylogenetic study, other anguilliform taxa, such as *Dolichallabes microphthalmus* are even not included. Fossil evidence shows that these elongation processes may have occurred starting from the Eocene till Pleistocene.

8. Even though *G. typus* takes a basal position in the combined analysis, it seems rather logical that anguilliformity is seen as a derived feature. The main support for this is, besides the molecular analyses, the fact that all sister-group of the Clariidae are fusiform and the well-documented pathway for achieving such an elongation through the expansion of the Hox gene expression domains along the body axis, while the opposite process is not so well-documented or generally accepted.

9. Together with the elongation in these clariids a whole set of morphological features can be observed: reduced eyes, loss of paired fins, an increased rigidity in the reduced skull morphology of elongated clariids and this through the outgrowth of several interdigitation zones. In the combined analysis, the correlated morphological changes appear to be the result of convergent evolution and this on a genus level. Moreover, many of the above mentioned features can be coupled to the fossorial life style of these anguilliform species. Also the fact that these may be related to miniaturization can not be excluded.

10. The multiple origin of anguilliformity (see above: four) also seems to be supported by paleobiogeographic data. When comparing the current distribution of anguilliform species, there seems to be a close match with the suggested location of the forest refugia during the phases of vegetation retraction in the Eocene till Pleistocene. Further, when coupling the life history of the clariids to this refugia theory it gives us a more complete picture.

In part <u>six</u>, a general discussion on specific consequences and problems encountered in this thesis is given. First, this part deals with the most important consequences of the combined phylogenetic analysis and looks more specifically at the taxonomical implications of this analysis. Secondly, this part also focusses on two major taxonomic problems; the dangers of using single specimen based descriptions and the usefulness of the taxonomic unit: "subspecies". As a last point of discussion, this part provides arguments on the different ways of dealing with morphological and molecular data in phylogenetic analyses.

VII.2 - Conclusies en Samenvatting

Deze doctoraatsverhandeling bevat een systematische en fylogenetische studie van de aalvormige, luchtademende katvissen (Clariidae, Siluriformes).

In de <u>inleiding</u> wordt in de eerste plaats de redenen en de noodzaak van dergelijke studies omschreven en wordt aangetoond dat dit proefschrift, in grote lijnen, uit enerzijds een beschrijvend, taxonomisch, systematisch en anderzijds een fylogenetisch deel bestaat.

Deel <u>twee</u> geeft een taxonomische situering van de Clariidae en plaatst deze binnen in de Ostariophysi en de Siluriformes. Voor elk van deze drie taxonomische niveaus wordt er een overzicht gegeven van de affiniteiten, de specialisaties en de zoogeografie. In **II.1.3** wordt de aandacht vooral gericht op de aalvormige taxa binnen de groep van de Clariidae.

Deel <u>drie</u> geeft een gedetailleerde beschrijving van het gebruikte materiaal en somt de gebruikte 'ingroup' en 'outgroup' species op. De gebruikte methodes worden heel kort uitgelegd in dit stuk en worden gelinkt aan de respectievelijke hoofdstukken waar ze dan meer in detail worden behandeld. Dit deel bevat echter wel al een gedetailleerde beschrijving van de gebruikte biometrische en meristieke variabelen.

Meer dan 500 specimens van de 11 aalvormige Clariidae zijn bestudeerd. Al deze specimens zijn verkregen uit negen musea (deze bevatten al het beschikbare type materiaal) of tijdens twee expedities naar Gabon. Van elk specimen zijn in totaal 39 biometrische en zes meristische gegevens verzameld (gesynthetiseerd in verschillende tabellen). Van elke soort zijn ook telkens enkele specimens gebruikt voor dissecties, *in toto* ophelderingen of seriële coupereeksen. De visualisatie van dit alles gebeurde via de microscoop en camera lucida, CT-scanning of radiografieën. Verschillende statistische analyses werden toegepast om de data te verwerken, alsook werden verscheidene fylogenetische programma's gebruikt om deze data te analyseren.

In deel <u>vier</u> en <u>vijf</u> worden de verkregen resultaten gedetailleerd voorgesteld en bediscussieerd en dit in de onderstaande volgorde:

Ten eerste wordt elk van de 11 herkende soorten in detail beschreven, gebaseerd op een uitgebreide studie. Alle nominale soorten zijn voorzien van een differentiële diagnose, een beschrijving en een gedetailleerde geografische studie. Elke soortsbeschrijving wordt gevolgd door een afzonderlijke discussie, die vooral de meest kenmerkende en belangijke

karakters behandeld. De meeste soorten worden apart behandeld, behalve de soorten binnen het genus *Channallabes*. Voor dit genus werd geopteerd om te werken vanuit een geografische hoek (Congo en de zuidelijke Equatoriale Westkust zoetwater ecoregio). In dit genus zijn drie nieuwe en twee gerehabiliteerde soorten beschreven, waarbij de voorgestelde taxonomische verschuivingen in dit hoofdstuk ook uitgebreid worden gemotiveerd.

Tenslotte, worden op het einde van dit beschrijvend deel enkele practische identificatiesleutels opgegeven.

Ten tweede worden verschillende fylogenetische analyses uitgevoerd. Deze worden opgesplits in twee grote delen: (1) een moleculaire en (2) een gecombineerde, morfologische en moleculaire analyse.

Deze huidige systematische en fylogenetische studie van de aalvormige Clariidae heeft enkele afdoende resultaten opgeleverd, die de vragen zoals opgesteld in **I.1.2,** van antwoord voorzien, terwijl een aantal suggesties kunnen gedaan worden bij het beantwoorden van enkele andere vragen. Hieropvolgend worden de conclusies gegeven in overeenkomst met de gestelde vragen uit **I.1.2 .**

1. Binnen de groep van de aalvormige taxa in de Clariidae kunnen 11 soorten onderscheiden worden. Deze 11 soorten worden ingedeeld in vijf verschillende genera. Drie van deze genera, *Platyallabes, Platyclarias* en *Dolichallabes* zijn monotypisch en bevatten respectievelijk *P. tihoni, P. machadoi* en *D. microphthalmus*. Het genus *Gymnallabes* bevat twee, in plaats van de drie voorheen erkende soorten; i.e. *G. typus* en *G. nops*. Het genus *Channallabes* bevatte voor dit proefwerk één enkele soort. De uitgebreide morfologische resultaten uit deze verhandeling tonen echter het bestaan aan van drie, voor de wetenschap, nieuwe soorten (*C. sanghaensis, C. teugelsi* en *C. ogooensis*) en twee gerehabiliteerde soorten (*C. alvarezi* en *C. longicaudatus*). De herkenning van al deze soorten wordt hier dan ook grondig gemotiveerd.

2. Voor elke soort wordt een set van diagnostische kenmerken gegeven. *P. tihoni* kan zeer eenvoudig onderscheiden worden door de korte afstand tussen de occipitaal processus en de origine van de dorsaal vin (2.2-6.6% SL). *P. machadoi* is te herkennen aan een extreme dorso-ventraal afgeplatte schedel (schedelhoogte 22.9-37.1% van de schedellengte). *D. microphthalmus* toont een unieke enkelvoudige longitudinale fontanel in het schedeldak. Het meest diagnostische kenmerk voor *G. nops* is de reductie in de infraorbitale beenderen, en dit zowel in aantal als grootte, terwijl *G. typus* ontegensprekelijk te determineren is aan de aanwezigheid van mondflappen. Het

onderscheid tussen de verschillende soorten binnen het genus *Channallabes* is echter niet zo éénduidig. *C. sanghaensis* en *C. apus* verschillen van *C. alvarezi, C. longicaudatus, C. teugelsi* en *C. ogooensis* in de aanwezigheid van een kleine supraorbitale processus op het infraorbitale IV, deze processus reikt niet tot de rostrale rand van het oog. *C. alvarezi* kan onderscheiden worden van deze laatste drie gebaseerd op de aan– of afwezigheid van een lichtgekleurde vlek op de kop. *C. teugelsi, C. longicaudatus* en *C. ogooensis* verschillen van elkaar in de aan– of afwezigheid en de positie van de tandjes op de pectorale stekel.

3. Uit de gedetailleerde morfologische studie volgt verder nog een lijst van synapomorfe kenmerken; *P. tihoni* heeft, naast nog andere kenmerken, een horizontaal gepositioneerd sphenoticum en pteroticum en een getand entopterygoid. *P. machadoi* heeft drie lange processussen op het praevomer en een extra spier (musculus adductor mandibulae A_3'' pars levator tendinis) als unieke kenmerken. Veel van deze kenmerken bij *P. tihoni* en *P. machadoi* zijn te linken aan de ruimtelijke beperkingen geassocieerd met de extreem afgeplatte schedel. *D. microphthalmus* toont een gereduceerde schedel ossificatie, met o.a. slechts één verlengde fontanel, en het antorbitale en infraorbital IV als de enige circumorbitale beenderen; enkel *G. nops* deelt dit gereduceerd aantal circumorbitale beenderen. *C. apus* en *C. sanghaensis* vertonen een unieke ento- en metapterygoid configuratie.

4. Alle aalvormige taxa komen voor in Centraal-West Afrika. Behalve *C. apus* en *C. teugelsi* komen alle soorten allopatrisch voor. *C. teugelsi* komt zowel voor in het Ogooué als Ivindu bekken (resp. met *C. longicaudatus* en *C. ogooensis*) en *C. apus* wordt aangetroffen in grote delen van het Beneden en Centraal Congo bekken en komt daar sympatrisch voor met *P. tihoni, P. machadoi, G. nops* en *D. microphthalmus*.

5. Op het einde van het beschrijvende deel werden verschillende inwendige, maar vooral uitwendige kenmerken gecombineerd in een sleutel naar de verschillende genera binnen de Afrikaanse Clariidae (**IV.5.2**) en naar de soorten binnen het genus *Channallabes* (**IV.5-1.b**).

6. In de verschillende moleculaire fylogenetische analyses worden vier clusters onderscheiden, die in twee grote clades worden gegroepeerd. Cluster 1a bevat *Clarias buthopogon, Clarias pachynema, Clarias submarginatus* en *Channallabes apus*. Cluster 1b groepeert *Clarias ngamensis, Clarias gariepinus, Dinotopterus cunningtoni* en *Heterobranchus isopterus*. De tweede grote clade kan op zijn beurt in twee cluster onderverdeeld worden. Cluster 2a brengt *Clarias camerunensis, Clariallabes longicauda, Channallabes alvarezi* en *Channallabes longicauda* samen, en behalve voor de POY analyse bevat deze cluster ook *Clarias stappersi*. De laatste cluster groepeert

altijd *Clarias platycephalus, Channallabes sanghaensis* en opnieuw met uitzondering van de POY analyse ook nog *Clarias jaensis*.

7. Onze resultaten tonen aan dat aalvormigheid minstens vier keer zou ontstaan zijn. Hiebij moet benadrukt worden dat in de moleculaire analyses sommige aalvormige taxa niet vertegenwoordigd werden, zoals bijvoorbeeld *D. microphthalmus*. Uit de fossiele evidentie blijkt dat dit verlengingsproces ergens vanaf het Eoceen tot het Pleistoceen zou kunnen hebben plaatsgevonden.

8. Alhoewel *G. typus* een basale plaats inneemt in de gecombineerde analyse, lijkt het toch logisch dat aalvormigheid een afgeleide eigenschap is. Naast de moleculaire analyses zijn de belangrijkste motivaties hiervoor het feit dat alle zustergroepen van de Clariidae fusiform zijn en de goed gedocumenteerde "pathway" voor de verlenging van de lichaams as via expansies van de Hox genen expressie domeinen, terwijl het omgekeerde proces minder gedocumenteerd is en niet zo algemeen aanvaard wordt.

9. Samen met de verlenging in deze Clariidae kan een ganse set van samen voorkomende morfologische eigenschappen aangetoond worden: gereduceerde ogen, verdwijnen van parige ledematen, een verhoogde versteving van de gereduceerde schedel morfologie en dit door de ontwikkeling van verschillende interdigitatie zones. Deze gecorreleerde morfologische veranderingen blijken, in de gecombineerde analyse, het resultaat te zijn van convergente evolutie en dit op het genus niveau. Veel van deze hierboven opgesomde eigenschappen kunnen tevens ook gekoppeld worden aan de gravende levensstijl van deze aalvormige soorten. Ook het feit dat deze gecorreleerd kunnen zijn aan het proces van miniaturisatie kan niet uitgesloten worden.

10. Het meermaals ontstaan van aalvormigheid (zie hierboven: vier) blijkt ook door de paleobiogeografie ondersteund te worden. Wanneer de huidige verspreiding van de aalvormige soorten vergeleken wordt met de voorgestelde plaats van de woud "refugia" tijdens fases van vegetatie inkrimping gedurende het Eoceen tot Pleistoceen, dan blijkt hiertussen een grote overeenkomst te bestaan. Een nog vollediger beeld wordt verkregen wanneer aan deze "refuge theory" de levensgeschiedenis wordt gekoppeld.

In deel <u>zes</u> volgt een algemene discussie m.b.t. enkele specifieke gevolgen en problemen ondervonden in dit proefschrift. Als eerste worden de meest belangrijke gevolgen van de gecombineerde fylogenetische analyse besproken. Hierbij wordt specifiek gekeken naar de taxonomische gevolgen van deze analyse. In een volgend deel wordt de aandacht gevestigd op twee grote taxonomische problemen; de gevaren van het formuleren van beschrijvingen op basis van één enkel specimen en het nut van de

taxonomische eenheid "subspecies". Als laatste onderdeel in deze algemene discussie worden de verschillende manieren om met morfologische en moleculaire data in fylogenetische analyses om te gaan van argumenten voorzien.

PART VIII

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VIII.1 - References

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APPENDIX I

Publication list Stijn Devaere

(As on 31 March, 2005)

I. IN INTERNATONAL JOURNALS (A1 PUBLICATIONS)

Devaere, S., Adriaens D., Verraes W. & Teugels, G.G. (2001) – Cranial morphology of the anguilliform clariid *Channallabes apus* (Günther, 1873) (teleostei, Siluriformes): adaptations related to a powerful biting. *Journal of Zoology* **255 (2)**: 235-250.

Adriaens, D., Devaere, S., Teugels, G.G. & Verraes, W. (2002) – Intraspecific variation in limblessness in catfishes, related to a specialized fossorial habit. *Biological Journal of the Linnean Society* **75**: 367-377.

Vandekerckhove, T. T. M., Watteyne, S., Bonne, W., Vanacker, D., Devaere, S., Rumes, B., Maelfait, J.-P., Gillis, M., Swings, J. G., Braig, H. R. & Mertens, J. (2003) – Evolutionary trends in feminization and intersexuality in woodlice (Crustacea, Isopoda) infected with *Wolbachia pipientis* (-Proteobacteria). *Belgian Journal of Zoology* 133 (1): 61-69.

Devaere, S., Teugels, G.G., Adriaens D., Huysentruyt F. &Verraes W. (2004) - Redescription of *Dolichallabes microphthalmus* (Poll, 1942) (Siluriformes, Clariidae). *Copeia* **2004**:108–115.

Huysentruyt F., D. Adriaens, G.G. Teugels, Devaere, S., Herrel, A., W. Verraes & Aerts, P. (2004) – Diet composition in relation to morphology in some African anguilliform clariid catfishes. *Belgian Journal of Zoology* **134 (1)**: 41-46.

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Devaere, S., D. Adriaens, G.G. Teugels, W. Verraes, N. De Clerq & A. Postnov – Holotype skeletal morphology of *Gymnallabes nops* (Roberts & Stewart, 1976), using micro CT-scanning. *Cybium* (**in press**).

Devaere, S., D. Adriaens, G.G. Teugels & W. Verraes – Detailed morphology of *Platyallabes tihoni* (Poll, 1944): spatial constraints in a dorso-ventrally flattened skull *Journal of Natural History* (**in press**).

Devaere, S., D. Adriaens, G.G. Teugels & W. Verraes – Morphology of the cranial system of *Platyclarias machadoi* Poll, 1977: interdependencies of skull flattening and suspensorial structure in clariidae *Zoomorphology* (**in press**).

De Schepper, N., D. Adriaens, S. Devaere & G.G. Teugels - Shape variation in the vertebrae of anguilliform Clariidae (Ostariophysi: Siluriformes): useful tool for taxonomy? *Proceedings of the PAFFA 3 meeting* (**in press**).

Huysentruyt F., D. Adriaens & Devaere, S. Early development and allometric growth in the armoured catfish *Corydoras aeneus. Animal biology* (**in press**).

II. CHAPTERS IN BOOKS

Devaere, S., D. Adriaens & G.G. Teugels – *Channallabes* (Günther, 1873). In: Fresh- and brackish water fishes of coastal Central Africa. G.G. Teugels, M.L.J. Stiassny & C.D. Hopkins (eds). IRD (Paris) & RMCA (Tervuren) (**in press**).

Adriaens, D., S. Devaere & G.G. Teugels –*Gymnallabes* Günther, 1867. In: Fresh- and brackish water fishes of coastal Central Africa. G.G. Teugels, M.L.J. Stiassny & C.D. Hopkins (eds). IRD (Paris) & RMCA (Tervuren) (**in press**).

Teugels G.G., D. Adriaens, S. Devaere & T. Musschoot – chapter 27 Clariidae. In: Fresh- and brackish water fishes of coastal Central Africa. G.G. Teugels, M.L.J. Stiassny & C.D. Hopkins (eds). IRD (Paris) & RMCA (Tervuren) (**in press**).

III. SUBMITTED

Devaere, S., D. Adriaens & W. Verraes – A survey of the anguilliform Clariidae of the Central and Southern West Coastal Equatorial freshwater ecoregions (Gabon and Republic

of the Congo); with the description of two new species, including a new, complete key of the African Clariidae genera. *Copeia*

Devaere, S., D. Adriaens & W. Verraes – A survey of the anguilliform Clariidae of the Congo River basin, with the description of two new species. *Belgian Journal of Zoology*

Devaere, S., G. Janssen, D. Adriaens & P. Weekers – A phylogeny of the African catfish family (Siluriformes, Clariidae) based on morphological and combined analyses: the road to anguilliformity. *Zoological journal of the Linnean Society*

Janssen, G., S. Devaere, P. Weekers & D. Adriaens – Phylogenetic and biogeographical analysis of African air–breathing catfish (Siluriformes: Clariidae): inferred from ribosomal genes and spacers sequences with emphasize on anguilliformity. *Molecular Phylogeny and Evolution*

IV. ABSTRACTS IN INTERNATONAL JOURNALS

Devaere S., Adriaens D., Verraes W. & Teugels G. G. (2001) **-** Cranial morphology of the anguilliform clariid *Channallabes apus* (Günther, 1873) (teleostei, Siluriformes): adaptations related to a powerful biting. *Journal of Morphology* **248 (3)**: 223.

Adriaens D., Devaere S., Verraes W., Teugels G. G., Herrel A. & Aerts P. (2001) – Structural Adaptations in Anguilliform Catfishes: Skulls and Fins. *Journal of Morphology* **248 (3)**: 2000.

Devaere S., Adriaens D., Teugels G. G., De Clerck N. & Postnov, A. (2004) – Holotype skeletal morphology of *Gymnallabes nops* Roberts & Stewart, 1976, using micro CT-scan. *Journal of Morphology* **260 (3).**

V. BOOKS

Editorial advisor for De geïllustreerde dierenencyclopedie voor de jeugd (The illustrated animal encyclopaedia), Tielt, Lannoo/Cantecleer, **2001**