Convergence and plasticity in the adaptive radiation of cichlid fishes

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Contents

Preface

> *“It would seem that here we have an experiment being conducted before our eyes on a scale unapproachable by man. Let one of the "new" biologists leave his laboratory and apply his methods to the fishes of Lanao; perhaps he might then make a real contribution to the study of evolution. By spending six months on the shores of the lake he could obtain with great ease all the material he could handle, as the Marinao fishermen bring in thousands of fish on market day, often many canoe loads of each of the commoner species. By studying several thousand fresh specimens of each of the ten most abundant species, and studying all the specimens obtainable of the rarer species and all the anomalous individuals, he could do much toward unravelling the phylogeny of the more puzzling forms and could perhaps place in their proper sequence the doubtful cases and those forms which seem to be examples of hybridism. With the foundation indicated, his statistical analysis of species would have real value and would throw light upon the evolution of so many species from one parent species.”*
>
> Albert W.C.T. Herre

In 1933, Albert Herre discussed the evolution of the cyprinid species flock of Lake Lanao in Indonesia, now basically extinct (Herre 1933). His main point, to which he refers to as “a problem in evolution”, is the question how one species can diversify into several ecologically differentiated species in the course of an adaptive radiation. Herre did not find a satisfying solution then, nor do we have a definite answer today, but considerable progress has been made in the last 80 years, and continues to be made. With this thesis, I hope to make a contribution to our understanding of the evolutionary processes involved in adaptive radiation. Although I learned of Herre’s article long after I started working on this topic, I basically took the approach he outlines in his closing paragraph: I spend about six month collecting fish at Lake Tanganyika, sifting through canoe loads of fish in search of rare species, studied more than a thousand specimens and am now hoping that my statistical analyses have real value and throw light upon the evolution of so many species from one parent species.

Moritz Muschick

Basel, October 2011

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This work would not have been possible without the help and guidance I received from many people. For this I am grateful and I would like to thank them here.

My family has supported me from my very early days on in the pursuit of a life as a biologist. To them this thesis is dedicated.

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Patrik Nosil took the burden on himself to critically examine this thesis, in spite of his immense workload at the time this evaluation was due.

Fieldwork at Lake Tanganyika would have been impossible without the help of Lawrence Makasa, Gilbert Sheltons, Ruben Shapola and Charity Muwene. And I would never have arrived there without the people who encouraged me along the way: Marta Barluenga, Birgit Dörges, Jürgen Heucke, Axel Meyer, Volker Petschik, Kathrin Lampert, Moritz Hilbrandt, Klaus Hantelmann and Harald Runte.

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Introduction

Investigation of speciation and the formation of biodiversity is central to evolutionary biology, which itself can be considered as the uniting discipline of life sciences. Ever since Charles Darwin and Alfred Wallace (1858) propelled our understanding about the importance of natural selection in the transformation of species, researchers endeavoured to use this intellectual foundation to explain larger patterns of biodiversity. One pattern emerging from the observation of phylogenetic relationships and ecological adaptations of species is the abundance of lineages, which are apparently rapidly diversifying, resulting in ecologically diverse clades of species (Schluter 2000). Most of the biodiversity we know is made up by such clades, being the result of so-called *adaptive radiations*. Phenotypic diversification and lineage accumulation in adaptive radiations have received considerable attention and great progress has been made in understanding these aspects (e.g. Glor 2010). Several groups of organisms played especially prominent in this research, including: Darwin's finches (Grant and Grant 2007), the replicated sets of ecomorphs of *Anolis* lizards on Caribbean islands (Losos 2009) or benthic-limnetic species pairs of threespine sticklebacks in postglacial lakes (McKinnon and Rundle 2002), several radiations on the Hawaiian archipelago, e.g. the silversword alliance (Baldwin and Sanderson 1998) and *Drosophila* and *Scaptomyza* fruitflies, or the East African cichlid fish flocks with their enormous species numbers (Salzburger 2009). Adaptive radiations can be triggered by what is called an *ecological opportunity*, i.e. a newly formed or colonized habitat lacking competing species or the formation of a key-innovation, a novel trait that allows for the invasion of a completely novel set of niches (Simpson 1953; Hunter 1998; Schluter 2000; Yoder *et al.* 2010). The radiation of East African cichlid fishes, and other groups of fishes, are hypothesized to have been triggered by a key-innovation, namely a reorganisation of the pharyngeal jaw apparatus (Liem 1973). The pharyngeal jaw apparatus is a second set of jaws in the throat of teleost fish, derived from the last branchial (or 'gill') arch. Liem's hypothesis attributes the evolutionary success of groups with certain pharyngeal jaw modifications to an increased versatility in exploiting resources. Furthermore, a functional and developmental decoupling from the oral jaws might increase the degrees of freedom for evolutionary change by modularization, possibly promoting adaptation and diversification (Liem 1973). Interestingly, the pharyngeal jaw is also used to produce sounds during mating, opening a possible route for ecological specializations to entail reproductive isolation. Although morphological descriptions of the pharyngeal jaw apparatus for many taxa of fishes abound in the literature, and studies with functional, biomechanical or ecological perspectives are numerous as well, as of yet no concise treatise about the evolutionary implications of the different aspects and characteristics of the pharyngeal jaw has been published. This gap I thrive to close with the first chapter of this thesis, entitled *"Pharyngeal jaws and their evolutionary, ecological and behavioural significance"*.

The course of adaptive radiations might be influenced by a phenomenon only little studied in this context so far. *Phenotypic plasticity*, the ability of a genotype to produce different phenotypes depending on environmental cues (West-Eberhard 2003), might increase a founding populations chance of persistence, if plastically produced phenotypes are better suited to the new environment (Yeh and Price 2004). Novel niches might also be invaded more quickly, since the phenotypic shift due to plasticity might place a population in the 'realm of attraction' of a peak on the adaptive landscape (Price *et al*. 2003). This peak represents the phenotypic optimum for use of the new niche, and its realm of attraction is the range of phenotypes in which directional selection is acting, driving adaptation towards the optimum. If plasticity is only exhibited in some directions in morphospace, but not in others (maybe due to developmental or genetic constraints) it has the potential of biasing evolutionary trajectories in adaptive radiations (Wund *et al.* 2008). To better understand if phenotypic plasticity in the pharyngeal jaw might have influenced the adaptive radiations of cichlids, I studied the Nicaraguan Midas cichlid in a common garden experiment. The Midas cichlid species complex comprises independent radiations in several crater lakes, with ecomorphologically convergent species (Barluenga and Meyer 2010) - the outcome predicted by the hypotheses outlined above. My demonstration of plasticity in the cichlids' pharyngeal jaw, reported in the second chapter ("*Adaptive phenotypic plasticity in the Midas cichlid fish pharyngeal jaw and its relevance in adaptive radiation*" (Muschick *et al.* 2011)), suggests it as a factor to be considered in answering the question of why there are so many cichlid species.

The concept of adaptive radiation is intimately related to ecological adaptation by means of natural selection (Schluter 2000). Thus, one would not be surprised if phenomena indicative of natural selection would be common in adaptive radiations. One of the strongest cases for the action of natural selection, since the birth of the idea, has been made with the argument of convergent evolution (McGhee 2007). If organisms independently evolve highly similar structures to similar ends, so the argument, natural selection is the most likely explanation. From the first mentioning of adaptive radiation, demonstration of convergence was integral as evidence of the actual adaptiveness of species' differences (Osborn 1902). Separation in time or by geography was, however, assumed to be necessary due to competitive exclusion (Osborn 1902). This principle, later formulated by Gause (1934), was questioned to be applicable to some communities of organisms, one of them being the cichlid species flocks of East Africa. Ernst Mayr (1984) asked:

> *"The coexistence of hundreds of closely related species in the same lake poses some fundamental questions concerning competition and resource utilization. To what extent, if any, is the existence of fish flocks in freshwater lakes in conflict with the concept of competitive exclusion?"*

This question is investigated in chapter 3 ("Convergent evolution within an adaptive radiation of cichlid fishes"), which is concerned with convergence within the cichlid radiation in Lake Tanganyika. This study is the largest comparative analysis of cichlid fishes to date and builds upon an extensive basis of different types of data — genetic, morphological and ecological — to accomplish a quantification of convergent evolution. The revealed abundance of ecomorphological convergence without geographical or chronological separation indeed seems to defy Gause's principle. Furthermore, it suggests the facility of coexistence of convergent species to be another key factor for the cichlids' species richness that has been previously overlooked.

The large overlap in morpho- and ecospace between subclades of Tanganyikan cichlids (called 'tribes') is not unique, but emerges as a common feature of adaptive radiations. This is exemplified by the adaptive radiation of Antarctic notothenioid fishes, the topic of chapter 4 ("*Parallel ecological diversification in Antarctic notothenioid fishes as evidence for adaptive radiation*"), comprising several families, which diversified in parallel along the benthic-pelagic axis. Thus, an adaptive radiation of fishes, taking place in a most different setting than the tropical, confined, freshwater environment in which cichlids diversified, nevertheless exhibits intriguing parallels in subclade overlap. Convergence might hence be a feature of radiations in general.

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Chapter 1

Pharyngeal jaws and their evolutionary, ecological and behavioural significance

Moritz Muschick and Walter Salzburger

MM reviewed the literature, drafted the manuscript and prepared the figures. WS received the invitation for this review from the *Journal of Fish Biology* editorial board and helped drafting the manuscript.

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Pharyngeal jaws and their evolutionary, ecological and behavioural significance

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Teleost fishes are the most diverse vertebrate group and comprise a stunning array of adaptations to secure food. Although less apparent than the sometimes extravagantly modified oral jaws, the pharyngeal jaw apparatus (PJA), a second set of jaws in the fishes' throat, is a trait of equal importance in fish ecology and behavior. It is used for food mastication and transportation, but also for sound production. Thus, adaptations in the pharyngeal jaws influence the evolution of fishes in multiple ways. Plasticity, allometry and genetic and constructional constraints are common in the teleosts' PJA and have an impact on morphological evolution and diversification. Here, the literature about the ecological and behavioral diversity mediated by the PJA, factors influencing its expression, as well as its importance in teleost evolution is reviewed. Furthermore, the questionable value of the PJA in systematics is discussed and peculiar modifications are highlighted.

INTRODUCTION

The origin of biodiversity is one of the central topics in evolutionary biology (Futuyma 1998; Grant and Grant 2007) and of great importance to related fields, such as conservation biology (Crandall *et al.* 2000). Teleost fishes have been heavily studied in this respect, due to their enormous species number and their diversity in ecological adaptations (Nelson 2006; Helfman 2009). Aside from overall body morphology, it is the trophic apparatus of fishes that prominently reflects the adaptation to distinct environments. The trophic apparatus of fishes consists of several components, including oral and pharyngeal jaws, gill raker structures, and the digestive tract. Their modifications constitute a large part of the morphological diversity to be found in fish (Helfman 2009). Modifications of the oral jaw apparatus, for example, allow for the exploitation of a vast range of food resources such as evasive prey fish, plankton, corals, stringy epilithic algae and even scales of other fishes. The diversity in functional morphology of the teleosts' pharyngeal jaw apparatus does not stand back. This structure involves various bones, it often has a diverse dentition, and – just as the oral jaws – muscles that intricately connect and operate this integrated system. Due to this large number of constituent parts - each of which is subject to evolutionary change - the pharyngeal jaw apparatus is used in very different ways by teleosts. Many studies in the last 150 years have furthered our knowledge about the morphological diversity and ecological consequences of the PJA. Evolutionary implications have been considered as well, since the role of ecology is now thought to be of utmost importance in diversification (Schluter 2000; Rundle and Nosil 2005). The famous radiations of cichlid fishes in East African Rift Lakes, for example, might be the result of diversification driven by ecological specialization (Salzburger 2009). If so, the PJA is likely to have had a huge influence, since species are well differentiated in PJA morphology as adaptation to their diverse food sources (Muschick *et al.* 2012). Independent adaptations in oral and pharyngeal jaws might have increased the number of attainable phenotypes and, thus, might have added to the evolutionary potential of cichlid fishes and other 'pharyngognath' teleosts (Liem 1973; Liem and Greenwood 1981). Similar scenarios might fit for other taxa, since labrids or cyprinids are very species-rich clades, too, and show an impressive diversity in their pharyngeal jaw morphology (Liem and Sanderson 1986; Mabuchi *et al.* 2007; Pasco-Viel *et al.* 2010).

This review is intended to provide an overview of the functional and morphological diversity in teleosts' pharyngeal jaws, its ecological consequences, and its developmental and genetic basis. Ways in which evolution in the PJA might trigger diversification are considered, as well as possible sources of evolutionary constraints. A synopsis of pharyngeal jaw diversity in adaptive radiations of fish in several lakes, and the abundance of convergently evolved morphologies provides evidence for its importance in diversification, but also calls into question the usefulness of PJA morphology in systematics.

THE PHARYNGEAL JAW APPARATUS OF TELEOSTS

The pharyngeal jaw apparatus (PJA) derives from bones, muscles and ligaments belonging to the branchial arches [Fig 1]. Of the seven visceral arches in a fish's head, the first forms the oral jaws, the second develops into the hyoid arch, and the remaining five make up the branchial basket. In its generalized form the PJA directly involves bones of the 2nd to 5th branchial arch: the fifth ceratobranchials, the second to fourth epibranchials and the second to fourth pharyngobranchials (Vandewalle *et al.* 2000) [Fig. 1-3]. Functionally relevant, however, are at least 15 other skeletal elements (Wainwright 2006). Muscles attaching and connecting pharyngeal jaw-bones are numerous and allow for sometimes intricate and versatile movements as well as for forceful bites in specialized species (Wainwright 2006). Movement of jaw-bones commonly takes place along a dorsal-ventral axis [Fig. 1(a)-(e)], but also anterior-posterior [Fig. 1(f)]. Even along a distal-proximal axis bones are shifted, at least in some species [Fig. 1(g),(h)]. The importance of muscles in PJA functioning and specialization is e.g. evidenced by the enormous differences in muscle mass found across labrid fishes (Wainwright *et al.* 2004). In the levator posterioris, an important muscle for LPJ adduction, 500 fold differences in mass have been measured between species, far more than in oral jaws (Wainwright *et al.* 2004). Tooth plates are found on (or fused to) the fifth ceratobranchial and different numbers of pharyngobranchials, which are referred to as lower pharyngeal jaw (LPJ) and upper pharyngeal jaw (UPJ), respectively. In Anabantoidei, a process of the parasphenoid reaches between the upper pharyngeals and bears teeth as well (Liem 1963). Comparing basal teleosts to more derived taxa, a pattern of reduction in the number of tooth bearing elements emerges (Vandewalle *et al.* 1994). While in primitive teleosts, e.g. elopomorphs, basically every part of the buccal cavity bears teeth, this is not the case in more derived teleosts. In cichlids or labrids, for example, dentition is generally restricted to the oral jaws and the pharyngeal jaw-bones in the rear of the buccal cavity, which are specialized for food manipulation (Vandewalle *et al.* 1994). In cyprinids teeth are only found on their lower pharyngeal jaw-bones.

Pharyngeal teeth may exhibit a great diversity in number and shapes, too. In the ancestral state, found in basal teleosts, teeth are numerous, small and pointed, with a single cusp (Vandewalle *et al.* 1994). This type of teeth is also encountered in derived teleosts, for example insectivorous cichlids, but many other tooth shapes are found in addition (Barel 1983). Teeth can be flattened, wide and robust (molariform) in molluscivorous species [Fig. 3(a)] or very thin and densely packed (villiform) in algae-eating species [Fig. 3(b), (d)]. Some piscivors exhibit two-cusped, hook-shaped pharyngeal teeth (Barel 1983), while species feeding on shrimps often show robust, single-pointed teeth [Fig. 3(c)]. In some species of pearlfish (Carapidae, Ophidiiformes) teeth have a somewhat phallic shape (Vandewalle *et al.* 1998). A single pharyngeal jaw may also contain different kinds of teeth. The flatfish *Cynoglossus zanzibarensis*, for example, exhibits differing dentition on two parts of its upper pharyngeal jaw (UPJ). Anteriorly, molariform teeth are present, while the posterior part is equipped with small and pointed teeth, probably serving a different function (Bürgin 1987). The hemiramphid Southeastern sub-nosed garfish *Arrhamphus sclerolepis krefftі* Günther 1866, comprises a veritable diversity of tooth shapes within its pharyngeal jaw apparatus, too, featuring conical uni- and tricuspid teeth, as well as spatula-shaped teeth (Tibbetts and Carseldine 2003).
Literature describing the pharyngeal apparatus from different perspectives in various taxa is abounding. Several reviews focus on variation in teleost PJA morphology and function and its relevance for feeding (Vandewalle and coauthors (1994), as well as Lauder (1983b) and Wainwright (2006)). Holstvoogd (1965), aiming to improve the systematics of teleosts, describes the arrangement of pharyngeal muscles in many different taxa; Hulsey et al. (2005) review pharyngeal jaw development within a broader context including oral jaws; the behavioral significance of the

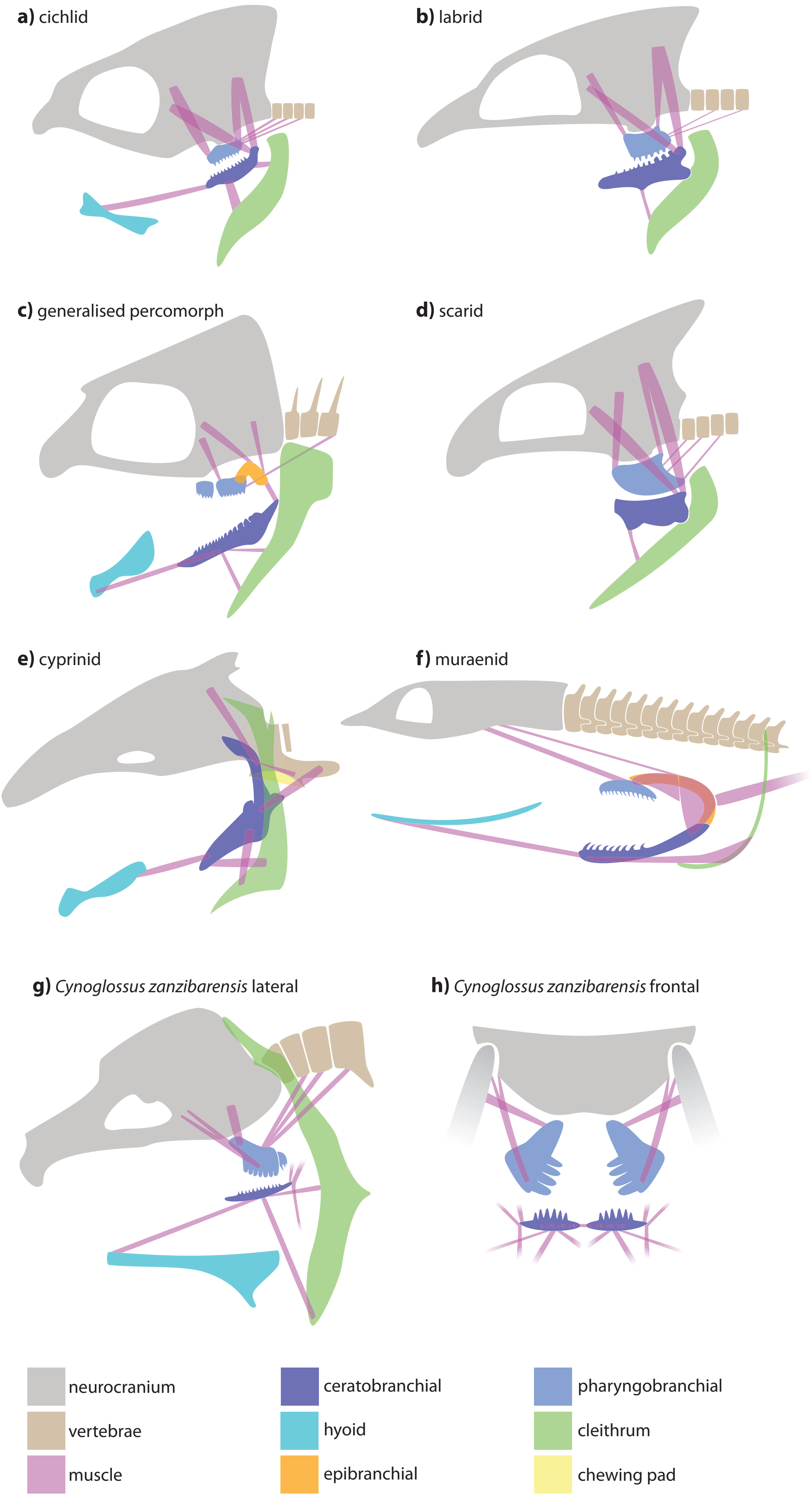

Figure 1 Examples of pharyngeal jaw apparatus construction in teleosts. (a,b) Cichlidae and labridae evolved a direct connection between the neurocranium and the lower pharyngeal jaw-bone, a muscular sling. (c) In generalised percomorpha the main biting action results from a depression of the upper pharyngeals via rotation of the epibranchials. (d) Scaridae, a subgroup of labridae, have evolved a massive "pharyngeal mill" able to crush pieces of coral. (e) Cyprinids have teeth on the lower pharyngeal jaw-bone only and direct the biting force against a ceratinized chewing pad. (f) Muraenidae have specialized PJAs which take prey out of the oral jaws and rake it into the pharynx. (g,h) In some flatfishes the upper pharyngeal jaw-bones act against each other. This way, according to their unusual body position, the axis of jaw movement is vertical. After Liem and Greenwood (1981; a,b,d); Lauder and Wainwright (1992; c); Sibbing (1991; e); Mehta and Wainwright (2007; f); Bürgin (1987; g,h).

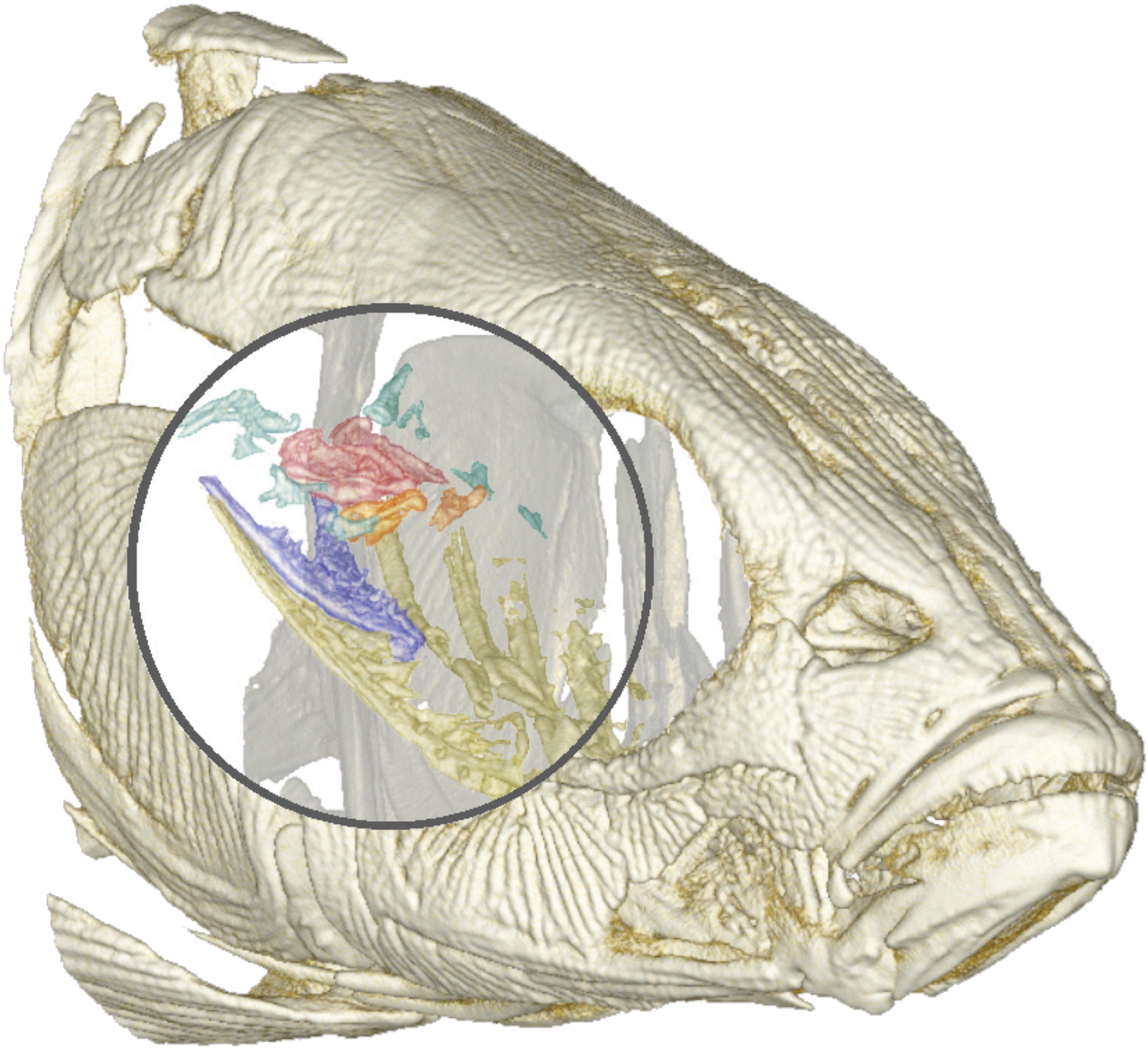

Figure 2 The pharyngeal jaw apparatus of a threespine stickleback (*Gasterosteus aculeatus*). CT-Scan reconstruction of the head with the bones involved in the PJA shown in colors: green: ceratobranchial 5 (lower pharyngeal jaw); red: pharyngobranchials 2 and 3, and yellow: pharyngobranchials 1 (both upper pharyngeal jaw); blue: epibranchials 1-4. Ventral part of the neurocranium removed for illustration purposes

PJA – mediated by sound production – is the topic of Rice and Lobel's review (2003). At archaeological and paleontological excavation sites pharyngeal teeth are often among the best preserved fish remains found (Rutte 1962; Eastman 1977; Stewart 2001) and can help identifying specimens to lower taxonomic levels than most bones (O'Connor 2000), because of the often species specific shape and size of these teeth. Instead of focusing on specific aspects of PJA function, ecological or behavioral relevance, development or evolution, and comparing across a range of taxa, some researchers go into greater detail for one or the other taxonomic group, for example: Embiotocidae (Liem 1986), Catostomidae (Eastman 1977), Cypriniformes (Pasco-Viel *et al.* 2010) Cyprinidae (Rutte 1962), Cichlidae (Liem 1973), Labridae (Liem and Sanderson 1986), Muraenidae (Mehta and Wainwright 2008), Clupeidae (Nelson 1967), Gobiidae (Parenti and Thomas 1998), Haemulidae (Wainwright 1989), or Soleidae/Cynoglossidae (Bürgin 1987). In the following, we attempt to provide a summary of the above-mentioned reviews as well as the – often very recent – primary literature on pharyngeal jaws. We present this information in an explicitly evolutionary context.

DEVELOPMENT

In order to interpret the mesmerizing variation found in the pharyngeal jaw apparatus across teleosts - or just in particularly diverse groups, such as cichlids or cyprinids - it is helpful to understand its development and genetic basis. The ontogenetic development of this trait's constituent bones and dentition is taking place over a large fraction of the organisms' total ontogeny, with some modifications being made as late as 100 days after fertilization of the eggs. Thus, the large number of factors in its development, which are amenable to change, might explain the apparent evolutionary malleability of this important trait.

Like most of the bones in a vertebrate head skeleton, those forming the PJA are derived from cranial neural crest (CNC) cells (Gans and Northcutt 1983). During early development, these cells migrate from the neural tube into the pharyngeal arches. The segmental patterning of the pharyngeal arches is brought about through nested and combinatorial expression of homeobox genes. The CNC cell populations then produce the cartilaginous precursors of later to be ossified bones.

The genetic network coordinating pharyngeal teeth development is apparently of ancient origin and might have, in a precursory form, already been present in the agnathan ancestors of jawed vertebrates (Fraser *et al.* 2009). Only later in evolution pharyngeal teeth became associated with novel jaws derived from pharyngeal arch bones, setting the stage for the highly specialized and derived constructions found in pharyngognath teleosts. The evolutionary legacy can still be seen in the development of, for example, cichlid pharyngeal jaws. In the Nile Tilapia *Oreochromis niloticus* (Linné 1758) two types of bones contribute to the formation of the PJA, dermal bone and cartilage bone (Patterson 1977; le Pabic *et al.* 2009). The constituents of the pharyngeal arches are first chondrified and later ossified (Ismail *et al.* 1982) and belong to the dermal bone type, while tooth plates are formed directly, without a cartilaginous precursor. The ossification of tooth bearing plates and their respective pharyngeal arch bones - to which they are later fused – is generally synchronized and starts around 5 days past fertilization (dpf) (le Pabic *et al.* 2009). Eight days past fertilization most of the PJA is ossified and larvae start to leave their mother's mouth temporarily and show feeding behavior (le Pabic *et al.* 2009). In cichlids, the fusion of the two fifth ceratobranchials to the lower pharyngeal jaw takes place much later in development (not present in 1 month old individuals of Tilapia (Ismail *et al.* 1982; le Pabic *et al.* 2009)). Neither is the diarthrosis of upper pharyngeal jaw elements and the pharyngeal apophysis on the ventral side of the neurocranium formed (le Pabic *et al.* 2009), another innovation deemed key to the efficacy of the PJA of pharyngognath teleosts (Liem 1973). Further PJA modifications take place even later, with the molariform dentition of the trophically polymorph Cuatro Cienegas cichlid *Herichthys minckleyi* (Kornfield and Taylor 1983) developing only after 100 dpf (Stephens and Hendrickson 2001). Notably, while most PJA elements ossify in parallel in cichlids, this is not the case in the zebrafish *Danio rerio* (Hamilton), a cyprinid. Here, the fifth ceratobranchials ossify around hatching (2-3 dpf) and are the first of the 74 ossified cranial elements to do so (Cubbage and Mabee 1996).

The genetic pathway of both the development of jaw-bones and teeth involve a number of genes and cofactors, of which several are shared. Most current knowledge has been gained from mutant screens generated in the laboratory, mainly in zebrafish (e.g. Piotrowski *et al.* 1996; Schilling *et al.* 1996), and, more recently, from studying the 'natural mutants' of the highly diverse East African cichlid species flocks (Albertson *et al.* 2003; Streelman *et al.* 2007; Kuraku and Meyer 2008; Fraser *et al.* 2009). Although few studies focus on the pharyngeal jaw apparatus specifically, most findings are probably relevant for the PJA as well, since conservation of the genetic pathways across the vertebrates has been found in several instances (Stock 2001). Major genes involved in the formation of jaw-bones belong to the family of bone morphogenetic proteins (*bmp*), most notably *bmp4* (Terai *et al.* 2002; Albertson and Kocher 2006), and distalless-like genes (*dlx*) (Depew *et al.* 2002; Borday-Birraux *et al.* 2006), which also interact. The Bmp4 protein is especially interesting here, since it was shown to be important in craniofacial development in many taxa and has been studied in Darwin's finches (Abzhanov *et al.* 2004) and cichlid fishes. Terai et al. (2002) detected differing patterns of evolution of the Bmp4 prodomain between lacustrine lineages of East African cichlids, which are highly diverse in their craniofacial morphology, and riverine species, which are more uniform. The authors suggest, that Bmp4 and its regulatory network might be key in the evolution of the exuberant morphological diversity of cichlids. Another possibility, how pharyngeal jaw diversity is produced, is by loss of *dlx* genes, or loss of their expression in certain tissues or developmental stages, in different lineages (Renz *et al.* 2011). Due to an additional round of whole genome duplication in the ancestors of teleosts, the members of the *dlx* gene family were present in several copies. Those might have differentially been lost, retained, or changed in emerging lineages, possibly influencing phenotypic diversity (Ohno 1970) and also evolvability (Carroll 2002) of the pharyngeal jaw apparatus.

Since the genes acting in the development of jaws are not exclusive and can be important in other developmental pathways, pleiotropic effects are likely, with interesting evolutionary implications (Franz-Odendaal and Hall 2006). In the blind cave-form of the Mexican Tetra *Astyanax mexicanus* (De Filippi 1853) oral-pharyngeal traits, like jaw size and taste bud number, are increased as an adaptive response to the cave-environment (Yamamoto *et al.* 2009). This increase is mediated by an overexpression of sonic hedgehog (*shh*) prior to 1 dpf in development (Yamamoto *et al.* 2009). However, the oral-pharyngeal traits are not the only ones affected: *shh* overexpression also leads to impaired eye development (Ekker *et al.* 1995; Yamamoto *et al.* 2004) leading to the typical eyeless cave-phenotype. *Astyanax*, being a member of the Characidae, does not have a derived pharyngeal jaw apparatus. However, Shh signaling has been found to have a conserved central function in the initiation of oral and pharyngeal dentition (Fraser *et al.* 2009). Thus, pleiotropic effects, via *shh* or other genes, might not be unusual in the development of trophic traits in fishes, and might constrain phenotypic evolution.

ALLOMETRY

During ontogeny of fish, not all body parts grow proportionally, resulting in adult shapes different from those of juveniles. This is a common adaptive feature of the trophic apparatus, since some

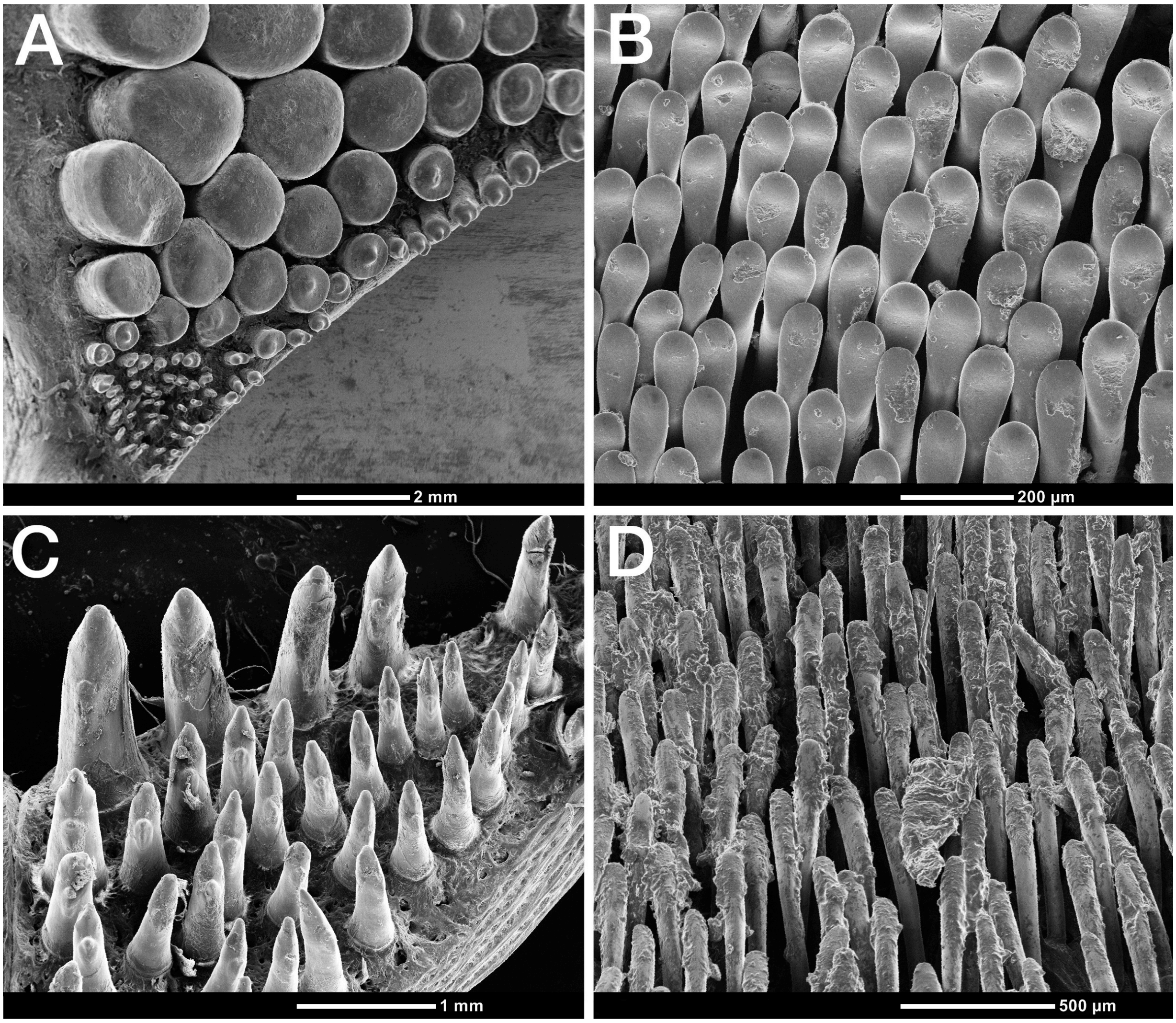

Figure 3 Diversity of pharyngeal jaw dentition in Lake Tanganyikan cichlids: (A) *Tylochromis polylepis*, (B) *Cyathopharynx furcifer*, (C) *Lamprologus lemairii*, and (D) *Oreochromis tanganyikae*. SEM micrographs of lower pharyngeal jaw-bones.

resources are only accessible for fish of a certain size. Once this size is reached, development might change its course and alter the trophic morphology to the adult version, allowing for efficient exploitation of the previously inaccessible resource. The cichlid *Lepidiolamprologus elongatus* (Boulenger 1898), for example, changes pharyngeal jaw shape allometrically, when switching from zooplanktivory to piscivory at a certain size (Hellig *et al.* 2010). The Mayan cichlid, *Cichlasoma urophthalmus* (Günther 1862), feeds opportunistically throughout its life, although hard-shelled prey items are only fed upon at later stages, when a more robust, molariform pharyngeal dentition is present. Pharyngeal jaw characters were the only ones found to show positive allometry throughout ontogeny of these fishes (Bergmann and Motta 2005). Individuals of the Shortfin Pompano *Trachinotus teraia* Cuvier 1832 (Carangidae) surpassing 120 mm of length develop bulky pharyngeal jaws suited for crushing bivalves, which from then on constitute a major part of the fish's diet (Francillon-Vieillot *et al.* 1994). Interestingly, those modifications do not resemble respective adaptations in other fish. Here, the teeth on the occlusal surface recede into the bone, which itself assumes the masticatory function (Francillon-Vieillot *et al.* 1994). The demonstration of allometry, however, is notoriously laborious, as fishes of the whole size range need to be examined. Distinguishing it from phenotypic plasticity (see below) is difficult too, since usually diet switch and change in morphology are coupled. Common garden experiments with differing feeding regimes are a good approach to tell apart phenotypic plasticity from genetically determined allometry, which should occur irrespective of diet.

PHENOTYPIC PLASTICITY

In many species and many traits the expression of the phenotype is not only determined by genotype, but influenced by environmental cues as well. This phenotypic plasticity is relevant for the persistence of populations in fluctuating or novel environments, for inter- and intraspecific ecological interactions, and may ultimately promote the evolution of new species (Pfennig *et al.* 2010).

Pharyngeal jaws have been found to be phenotypically plastic in many taxa. In *Astatoreochromis alluaudi* Pellegrin 1904, for example, a cichlid from the Lake Victoria region in East Africa, molariform PJs are induced by hard-shelled diet like snails (Greenwood 1965). If, however, fish are raised in snail-free environments (Greenwood 1965), or if strong, molluscivorous competitors are present (Hoogerhoud 1986), papilliform jaws are expressed. Plasticity in this species affects the structure of the lower pharyngeal jaw-bone (Huysseune *et al.* 1994) as well as its dentition (Huysseune 1995). Smits and colleagues (1996b) furthermore detected a volumetric increase in the PJA (including UPJ) leading to spatial and functional constraints onto many other structures in the head region of molluscivorous *A. alluaudi*. The plasticity of the PJA of *A. alluaudi* has even been discussed in the context of biological control of schistosomiasis, a serious tropical disease caused by an infection with trematodes. To fight schistosomiasis, molluscivorous cichlids were proposed as an agent to biologically control population sizes of snails, the intermediate hosts of *Schistosoma*. *A. alluaudi* first seemed to be a promising candidate species, but was later found ineffective in pond trials, in which less molluscivorous, yet opportunistically foraging morphs occurred in subsequent generations (Slootweg *et al.* 1994).

Phenotypic plasticity in the PJA has also been observed in the Nicaraguan Midas cichlid *Amphilophus citrinellus* (Günther 1864) (Muschick *et al.* 2011). *A. citrinellus* is trophically polymorphic and features papilliform and molariform pharyngeal jaw morphs, which are considered to represent optima in a trade-off in feeding performance (Meyer 1989). These morphs can be induced plastically by feeding food of differing hardness, for example snails with an intact shell and peeled snails (Muschick *et al.* 2011).

The case of pumpkinseed sunfish *Lepomis gibbosus* (Linné 1758) is similar to the one of *A. alluaudi*, in that populations occurring in lakes with a high abundance of snails exhibit strong pharyngeal jawbones and heavy levator posterioris muscles (Wainwright *et al.* 1991; Mittelbach *et al.* 1992). This correlation is probably due to phenotypic plasticity, since snail abundances vary over time rendering genetic differentiation as a cause unlikely (Mittelbach *et al.* 1992). Predicted effects have been demonstrated in feeding trials by supplementing one experimental group's diet with snails (Mittelbach *et al.* 1999). LPJ plasticity mediated by diet was also shown to be present in the orangespotted sunfish *Lepomis humilis* (Girard 1858) (Hegrenes 2001) and the shiner perch *Cymatogaster aggregata* Gibbons 1854 (Woods 2010).

The famous vertebrate model for the study of development, the zebrafish *Danio rerio*, exhibits differences in pharyngeal dentition if raised on different diets (Miller 1999). Whether these changes are induced by mechanical stimulation or nutrition is not established though. Small amounts of plasticity in the pharyngeal feeding muscles of Red Drum *Sciaenops ocellatus* (Linné 1766) (Sciaenidae), induced by hard-food diet, had negligible effect on feeding performance only (Ruehl and DeWitt 2007). Here, advantages due to structural changes were probably much less important than behavioral adaptation.

ASYMMETRY

Asymmetry of oral jaws is found in a number of fish species, e.g. flatfishes (Flüchter 1963; Friedman 2008) or some scale-eating cichlids from Lake Tanganyika (Hori 1993). This degree of asymmetry is not found in the pharyngeal jaws of either flatfishes (Bürgin 1987) or cichlids (MM, unpublished) and no other example of extensive asymmetry in PJAs has been reported to the authors' knowledge. However, relatively small yet significant amounts of variation due to asymmetry have been demonstrated in PJAs of Midas cichlids from Nicaragua (Klingenberg *et al.* 2002).

ECOLOGY & BEHAVIOR

MASTICATION

After uptake of food items, e.g. by suction, scraping or biting, many resources need to be further manipulated prior to transportation into the intestinal tract. Depending on the nature of the diet, different modes of processing the food are used, like crushing, lacerating, or piercing. Dentition and structure of the jawbones are often specialized for these actions. During pharyngeal biting, in a generalized teleost, the upper pharyngeals are depressed through a lever system involving a rotation of the connected epibranchials. This rotation is induced by a pull exerted by the fourth levator externus (LE4) muscle connected to the neurocranium. In a derived, 'pharyngognath' state, the LE4 no longer is connected to the epibranchial but to the lower pharyngeal jaw, thus forming a 'muscular sling'. The lower pharyngeal jaw is then directly adducted, also by the levator posterioris, against the upper pharyngeal jaws, which rest on the ventral side of the neurocranium (Vandewalle *et al.* 1994; Wainwright 2006). In labrids, biting seems to involve only the lower jaw adduction via the muscular sling, whereas other 'pharyngognath' lineages have retained the ancestral, generalized, upper jaw depression in addition to the muscular sling (Wainwright *et al.* 2012). In cyprinids, which lack an upper pharyngeal jaw, the biting force of the toothed fifth ceratobranchials is directed against a ceratinous chewing pad which rests on an area of fused neurocranial and vertebrae bone (Sibbing 1982).

Prey items possessing a resilient casing, like snails, mussels, crabs, certain seeds, etc. are rewarding food sources for those who can overcome their protection. To this end, the trophic apparatus has been adapted many times in the evolution of teleosts, with pharyngeal modification being apparently more common (Palmer 1979). In fishes, adaptations to durophagy (i.e. the inclusion of such protected resources in the diet) typically take the form of a 'molarization' of the teeth and the sometimes massive thickening of dentigerous bones (Liem 1973; Grubich 2003). The muscles, which adduct the tooth-bearing bones either directly or via a lever system, are often similarly hypertrophied for increased crushing power (Liem 1973; Lauder 1983a; Wainwright *et al.* 1991; Grubich 2003). According to Palmer (1979), there are nine marine teleost families of which some species use their oral jaws for mollusk crushing, while 19 families comprise species using their pharyngeal jaws. A peculiarity among species with a strong pharyngeal bite is the Zanzibar tonguesole *Cynoglossus zanzibarensis* Norman 1939, that probably uses only its UPJ with the two 3rd pharyngobranchials acting against each other to crush shells (Bürgin 1987). The fifth ceratobranchials are merely positioning the prey into this pharyngeal mill. Durophagous species living in freshwater are known from Centrarchidae (Lauder 1983a), Cichlidae (Liem 1973; Hulsey *et al.* 2008), Cyprinodontidae (Parenti 1984c) and Catostomidae (Eastman 1977). In addition, some cyprinids such as the Common carp *Cyprinus carpio* L. 1758 and Rudd *Scardinius erythrophthalmus* (L. 1758), which are thought to be omnivores, feature a molariform pharyngeal dentition (Pasco-Viel *et al.* 2010).

FOOD TRANSPORTATION

Transporting food items from the oral jaws or the buccal cavity via the pharynx towards the digestive tract is another important, most likely even the ancestral, function of the PJA (Vandewalle *et al.* 1994). Food items are moved towards the oesophagus by concerted anterior-posterior action of the UPJ and LPJ. Muscles displacing the pharyngeal jaws towards the mouth connect the UPJ to the neurocranium (e.g. Levator externus IV, Levator internus), and the LPJ to the hyoid or cleithrum (Rectus communis, Pharyngocleithralis externus) (Wainwright 2006; Mehta and Wainwright 2007). The pharyngeal jaws of higher teleosts are retracted by muscles connecting the UPJ to vertebrae (Retractor dorsalis) and the LPJ to the cleithrum (Pharyngocleithralis internus) (Holstvoogd 1965). In cyprinids, which exhibit a much more sophisticated PJA than other basal teleosts, the lower pharyngeal jaw is pulled backwards by retractor muscles, too. This retractor, however, is apparently not homologous to the retractor dorsalis of higher teleosts (Holstvoogd 1965).

The specialization of pharyngeal jaws for transportation is most stunning in moray eels (Muraenidae) where extremely mobile jaws are protracted into the buccal cavity and literally take the food item from the oral jaws to ratchet it towards the pharynx (Mehta and Wainwright 2007; Mehta and Wainwright 2008). Those jaws comprise strongly recurved teeth, providing excellent hold on the evasive prey. Interestingly, the lower pharyngeal jaw in muraenids is not a derivative of the fifth ceratobranchial, which is lacking, but of the fourth instead (Popta 1904; Nelson 1966; Mehta 2009), a situation similar to that in *Polypterus* (Gegenbaur 1898; Britz and Johnson 2003).

SOUND PRODUCTION

Many species of fish from a large number of different families are known to produce sounds during courtship, territorial behavior, predator-prey interactions or schooling (Amorim 2006; Kasumyan 2008; Helfman 2009). These sounds are produced using different organs, e.g. muscles attached to the swim bladder, specialized ligaments attached to the oral jaws, or by stridulation with pectoral girdle bones and pectoral fins (Demski *et al.* 1973; Amorim 2006; Kasumyan 2008). Another mechanism for sound production in fish involves the pharyngeal jaw apparatus (Darwin 1874). By rasping teeth stridulation sounds are produced which might get amplified by swim bladder-resonance (Burkenroad 1931; Moulton 1960; Rice and Lobel 2003). The role of the PJA in sound production is evidenced, for example, by differing functional capacities of involved muscles between males and females in the Malawi cichlid *Tramitichromis intermedius* (Trewavas 1935) (Rice and Lobel 2002). In this species only the male is known to produce sound and there is no apparent trophic differentiation between sexes (Lobel 1998; Ripley and Lobel 2004). Sound production using the PJA has been suggested for Rivulidae (Belote and Costa 2003), Cichlidae and Pomacentridae (Rice and Lobel 2003), Carangidae and Ephippidae (Burkenroad 1931), Haemulidae (Burkenroad 1931; Dobrin 1947), Anabantoidei (Kratochvil 1985), Acanthuridae (Knudson *et al.* 1948), Centrarchidae (Gerald 1971; Kratochvil 1985), and, interestingly, for the genus *Menthicirrus*, although most other members of the Sciaenidae produce sounds using their modified swimbladder (Burkenroad 1931; Schneider 1961). So far, evidence for the involvement of the PJA in sound production is rather circumstantial, and little is known about how exactly sounds might actually be produced with it.

Acoustic signaling, possibly involving the PJA, can be important in a range of behaviors. Sounds have been observed to be produced during schooling (Moulton 1960) and might be one way fish schools coordinate their concerted movements, although compelling evidence is lacking. Sounds produced during feeding, e.g. when manipulating the food with the pharyngeal jaws, can affect behavior of different receivers in different ways. Conspecifics might join the feeding individual in search of food. Predators might be drawn to the location of their feeding prey. And prey itself might try to evade or avoid the already feeding, but maybe not satisfied, predator.

During courtship and agonistic interactions sounds are produced, sometimes simultaneously with other typical behaviors like quivering. In the cichlid *Pseudotropheus zebra* specific types of vocalization have been recorded for male-male and female-female agonistic interactions, as well as male-female courtship behavior (Simoes *et al.* 2008). In the Lake Victoria cichlid *Pundamilia nyererei* (Witte-Maas and Witte 1985) sounds produced by males do not differ with context (Verzijden *et al.* 2010). The courtship sounds have been found to be species-specific in a few lake Malawi cichlids (Lobel 1998; Amorim *et al.* 2004; Amorim *et al.* 2008; Danley *et al.* 2012)

EVOLUTIONARY IMPORTANCE

The presence and malleability of pharyngeal jaws – both, on ecological and evolutionary timescales – probably had a large impact on the evolution of teleosts. Liem (1973) hypothesized that the derived form of the PJA found in labrids, cichlids, embiotocids and pomacentrids (the ‘pharyngognaths’) increases functional versatility and thereby might have triggered adaptive radiations in these groups. The highly integrated and derived ‘pharyngognath’ jaw might, hence, constitute an evolutionary key-innovation, giving access to new adaptive zones in which diversification might take place (Wainwright 2007). However, in a recent review, Wainwright (2006) reports no greater behavioral or functional versatility in derived labroid pharyngeal jaws compared to the generalized percomorph PJA - only a stronger and more efficient bite is asserted. Still, a forceful bite presumably extends the accessible range of food resources considerably and many members of the before mentioned groups have specialized on durophagy.

Due to the ample capabilities of the PJA in food processing, the functionally and developmentally decoupled oral jaws could be adapted for acquiring food (Liem and Osse 1975) and it might have been this increase in the degrees of freedom for adaptation to occur that led to the success of some of these taxa (Hulsey *et al.* 2006). Cichlids and some labrid groups (scarids and julidines) indeed comprise an impressive number of species featuring extremely diverse feeding modes. The adaptive radiation of East African cichlids is even regarded to be the prime example of vertebrate diversification (Salzburger and Meyer 2004). Plausible as this explanation might sound, studies testing the assumption of 'uncoupledness' and the apparent correlation with species richness cast some doubt on Liem's hypothesis: A study explicitly testing for rates of lineage diversification within the labrids by Alfaro and coworkers (2009) does not support the notion that the advent of the derived PJA structure triggered diversification, but attributes increased speciation rates to other factors, such as coloration and sexual selection. On the same line, of convergently evolved "pharyngognath" lineages only cichlids and labrids show an exceptional species richness, while four other clades do not (Wainwright *et al.* 2012). The assumption that oral and pharyngeal jaws are genetically and developmentally uncoupled might not hold true for the dentition in Lake Malawi cichlids, as Fraser and coworkers (2009) found evidence for the oral and pharyngeal dentitions to be genetically coupled. In Neotropical heroine cichlids, however, Hulsey and colleagues (2006) did find the two systems to be uncoupled. Clearly, more work is needed and is also imminent, since the genetic basis of these traits is revealed with modern genomic methods, as well as statistical comparative methods become more advanced and allow for powerful hypothesis testing.

The ability to fine-slice niche space by adaptation in the pharyngeal (and oral) jaws might facilitate ecological speciation (Rundle and Nosil 2005) and might be partly responsible for cichlids propensity to speciate. But to lead to speciation the ecological specialization needs to entail reproductive isolation. Several hypothetical scenarios can be imagined here: If one or more of the presumably few loci important in the determination of pharyngeal jaw shape and dentition is physically linked to loci determining traits involved in, for example, mate-choice, reproductive isolation might ensue divergent natural selection on the jaw determining loci. Sensory exploitation might play a central role as well, possibly linking diet or habitat preference and, subsequently, mate-coloration preference (Seehausen *et al.* 2008). If species-specific mating calls were indeed produced using the pharyngeal jaw, this would lead to interesting hypotheses about sexual selection acting on the PJA in cichlids and other taxa. If differently shaped pharyngeal jaws produce shape specific sounds and if females tend to prefer sounds of jaw shapes like their own, the stage would be set for trophic specialization of the pharyngeal jaw possibly leading to reproductive isolation (Lobel 1998; Rice and Lobel 2003). This way the PJA could act as a 'magic trait': certain kinds of divergent natural selection could lead to ecological specialization simultaneously entailing assortative mating, thus promoting speciation (Gavrilets 2004; Servedio *et al.* 2011). Here, again, much more work is needed to assess the plausibility and eventually the importance of this mechanism in the vast adaptive radiations of cichlids (Turner 2007; Salzburger 2009).

EVOLUTIONARY IMPLICATIONS OF PLASTICITY

Phenotypic plasticity was thought to counteract genetic evolution because it would realize adapted phenotypes while shielding variation in their heritable genetic basis from selection. Although proposed as an important factor in evolution already over a century ago (Baldwin 1896a; Baldwin 1896b), phenotypic plasticity only recently regained attention as a possible driving force of diversification (e.g. Crispo 2007; Pfennig *et al.* 2010; Thibert-Plante and Hendry 2010). Thus, it seems plausible that plasticity in the PJA of centrarchids and cichlids might have influenced the diversification of these clades. The ability to colonize a new habitat is often a prerequisite for allopatric speciation to occur. Phenotypic plasticity in trophic traits like the PJA boosts this ability and thereby positively influences the capacity to speciate. Although strong and low-cost phenotypic plasticity might yield well-adapted phenotypes, intermediate levels of it might place the phenotype not fully under a peak on the theoretical adaptive landscape (which would mean the phenotype would be perfectly adapted) but into its "realm of attraction" (Price *et al.* 2003). Then heritable genetic differences in trait expression could be selected for and might shift the population under the new peak (Waddington 1961; Price *et al.* 2003). Combined with the ecological speciation scenarios outlined above subsequent speciation would be imaginable (Muschick *et al.* 2011). However, the realization of phenotypic plasticity in pharyngeal jaws might be constraint by trade-offs with the branchial apparatus' function of breathing. Enlarged pharyngeal jaws might then not be expressed in low-oxygen environments, although the ability to feed on mollusks might, by itself, be advantageous (Binning *et al.* 2010).

CONVERGENCE

Convergence, the independent acquisition of similar traits by different lineages, is one of the strongest lines of evidence for the power of natural selection in evolution (McGhee 2007). Similar environmental circumstances might favor similar solutions to cope with them, resulting in also similar morphologies. An excellent example is the independent adaptation towards a predatory, aquatic lifestyle in the radiations of mammals (dolphin, porpoise), reptiles (ichthyosaur) and fishes (shark, swordfish). Convergence is common in the PJA, on several taxonomic levels and in several morphological aspects. Adaptations for durophagy in the PJA evolved convergently many times in teleosts as a whole (Grubich 2003; Wainwright 2006; Hulsey *et al.* 2008) as well as in smaller taxa like cichlids (Hulsey *et al.* 2008). Molecular phylogenetics revealed derived features, like the fusion of the fifth ceratobranchials in 'pharyngognaths', to have evolved at least two times independently (Mabuchi *et al.* 2007; see also "Taxonomical Issues"; Wainwright *et al.* 2012). This instance of convergence, together with both clades' species richness, has been interpreted as support for Liem's 'key-innovation' hypothesis (Mabuchi *et al.* 2007). However, other convergent lineages are considerably less species rich (Wainwright *et al.* 2012). In East Africa, cichlids are convergent in their lower pharyngeal jaw shape and dentition between lakes (Stiassny 1982) as well as within a single lake (Muschick *et al.* 2012), with implications for competition and species' coexistence. The abundance of convergent phenotypes is a strong indication on how very important for ecological specialization the PJA is. To conclusively interpret this phenomenon, however, it is necessary to learn about the genetic or developmental constrains limiting the number of possible morphologies, possibly a different explanation for convergence (Arendt and Reznick 2008).

PHARYNGEAL JAWS AND FISH SPECIES FLOCKS

With their astonishing diversity the cichlid species flocks of the East African Great Lakes are widely known among researchers and hobbyists alike. There have been many attempts to pin down the reasons why this taxon has produced so many species, while others have not. As outlined above, pharyngeal jaws have featured prominently in this discussion. But cichlids are not the only fish species flock, and not the only one with pharyngeal jaws being differentiated between species. In fact, fish species flocks are known from a wide taxonomical range and can be found in lakes across the world.

To learn more about the putative importance of pharyngeal jaws in the emergence of fish species flocks it might be informative to compare across systems and look at the ecomorphological diversity that can be found in each.

LAKE LANAO

Lake Lanao is a tropical lake at 700 m altitude in the Philippines and used to harbor a species flock of 18 endemic cyprinid species (Herre 1933). Sadly, due to anthropogenic influences and introduction of invasive fish species, only two of these species remain today (Villwock 1972; Ismail 2011). Since most of the type specimens have been destroyed in the Battle of Manila in February 1945, further investigation of adaptations in the PJA of those species, and their influence on diversification, is precluded. In Herre's original descriptions, however, some statements on the pharyngeal jaw teeth can be found (Herre 1924). Although pharyngeal teeth formulae are mentioned for nine species only, those comprise five different types already. Other comments describe different tooth sizes as well as shapes, like pointed, hooked, or cylindrical (Herre 1924). This indicates ecological differentiation of the species and renders plausible the idea, that diversification the Lake Lanao cyprinid species flock might have been influenced by adaptations in the pharyngeal jaw.

MALILI LAKES

The Malili Lake-system on Sulawesi, Indonesia, harbors an interesting radiation of sailfin silversides (*Telmatherina* spp., Atheriniformes). Resource specialization, conferred by adaptive shape differences in the PJA, has apparently initiated divergence of the two main lineages ('sharpfin' and 'roundfin' sailfin silversides) in Lake Matano (Roy *et al.* 2007). However, Pfaender *et al.* (2010) report less significant shape differences between trophic groups within the sharpfin-group. In contrast to what has been found in other adaptive radiations, the molluscivorous sharpfin *Telmatherina* do not exhibit adaptations for durophagy in their pharyngeal jaws (Pfaender *et al.* 2010). This is probably due to the small size of their prey, which is ingested as a whole.

LAKE TANA

The Ethopian Lake Tana is a shallow lake at high elevation and the source of the Blue Nile. Fifteen endemic, ecologically separated *Labeobarbus* (Cyprinidae) species of up to 100 cm in length occur there, of which eight are piscivorous (Nagelkerke and Sibbing 2000; de Graaf *et al.* 2008). They have no oral teeth, and food mastication is performed with the pharyngeal jaws. Although the attainable trophic specializations appear to be limited by the lack of oral teeth, the oral jaws are differentiated between species (de Graaf *et al.* 2008). They are adapted, for example, for suction feeding. The large palatal and sublingual organs, important for sorting small food items from debris, decrease the maximum prey size a cyprinid can ingest.

The Lake Tana barbs show considerable interspecific variation in several pharyngeal jaw dimensions like weight or symphysis length (Sibbing and Nagelkerke 2001; Dejen *et al.* 2006). One species, *L. gorgorensis* (Bini 1940), formerly known as 'carplike', has pharyngeal jaws indeed resembling those of the common carp *Cyprinus carpio*. The ceratobranchials are hypertrophied and their dentition is molariform (Nagelkerke *et al.* 1994). As would be expected, the diet was found to consist mainly of mollusks (Nagelkerke *et al.* 1994). The morphology of the piscivorous suction-feeder *L. acutirostris* (Bini 1940) is very different: relatively small pharyngeal jaws with lacerating-type teeth (Sibbing *et al.* 1998).

Like the endemic faunas of Lake Titicaca or Lake Lanao (Villwock 1972), the cyprinids of Lake Tana are endangered (Nagelkerke *et al.* 1995), although here the threat stems from overfishing by new and highly effective motorized commercial gillnet fishery (de Graaf *et al.* 2006) and less so from invasive species. Fortunately, and in contrast to the other lakes mentioned, no extinctions have been reported so far, rendering it the only known intact cyprinid species flock.

EAST AFRICAN RIFT LAKES

The East African Rift Lakes are among the oldest and largest lakes of the world (Schoen and Martens 2004). They harbor a unique fauna, of which cichlids are the most famous representatives. Cichlid fishes diversified into approximately 2000 species there, mainly in the three largest lakes Tanganyika, Malawi and Victoria (Fryer and Iles 1972; Turner *et al.* 2001). The ability of cichlids to adapt and specialize ecologically is believed to be one of the main factors responsible for this 'species explosion' (Salzburger 2009). In ecological specialization of cichlids the pharyngeal jaw apparatus features especially prominently. As Stiassny (1982), comparing the morphology of piscivorous cichlids of Malawi and Tanganyika, puts it: "It appears that throughout the cichlid radiation the full complement of perciform branchial muscles and bony elements of the PJA is retained and that no major changes occur in their spatial relationships to one another. However, within this configuration a seemingly endless spectrum of minor morphological variation is expressed. This is realized through differences in the relative size and robustness of the pharyngeal bones, the shape and distribution of their teeth, and through proportional changes in the various muscles coupled with slight differences in their sites of origin and insertion."

The correlation between ecology and jaw morphology suggests, that these cichlids are able to fine-slice the niche-space with adaptations in the pharyngeal jaws, probably in conjunction with oral jaw adaptations. On the other hand, the many instances of convergent evolution within the same habitat imply a lesser role for fine-slicing and competitive exclusion than previously thought. The Lake Tanganyikan cichlid species flock shows a remarkable diversity in shapes of lower pharyngeal jaw-bones, but also in dentition [Fig. 3](Fryer and Iles 1972; Muschick *et al.* 2012) making it a suitable system to study the genetics, development and functionality of different PJA adaptations.

LAKE TITICACA

In Lake Titicaca, the highest navigable lake in the world, 15 species of Killifish of the genus *Orestias* occur, which occupy different ecological niches (Lauzanne 1982; Parenti 1984b). They may have diversified through an adaptive radiation (Villwock 1986), but the Titicaca species do neither form a monophyletic flock (Parenti 1984a) nor are all of them endemic and it is not clear if the adaptation to different habitats played a role in the speciation process (Maldonado 2009). However, these species do exhibit different pharyngeal dentitions and some correlation with their trophic niche is apparent (Parenti 1984b). For example, *Orestias luteus* Valenciennes 1846, *O. crawfordi* Tchernavin 1944 and *O. incae* Garman 1895 all have a molariform dentition and feed predominantly on mollusks (Lauzanne 1982; Parenti 1984b; Maldonado *et al.* 2009), while the closely related *O. pentlandii* Valenciennes 1846 (Lüssen *et al.* 2003), feeding mainly on plankton (Parenti 1984b), has numerous small and pointed pharyngeal teeth (Lauzanne 1982). This resembles adaptations found in other species flocks, for example East African cichlids (Fryer and Iles 1972). Unfortunately, the native *Orestias* in Lake Titicaca are threatened by invasive species, and one, *O. cuvieri* Valenciennes 1846, probably went extinct already (Villwock 1972).

In most of the systems discussed above pharyngeal jaw specializations and adaptations are associated with species' trophic niches. Considering the species richness of some and the comparably young age of all of these groups, one might conclude, that pharyngeal jaws and their propensity to adapt have been very influential in the diversification of teleost. Although currently the extent of morphological diversity in the PJA in the different species flocks can not be compared directly, the impression is that the pharyngeal dentition has diverged in most systems, with tooth shapes, sizes and numbers often being very different between closely related species. The relative size of the pharyngeal jawbones has diverged, too. The shape of the jaw-bones, however, is most impressively differentiated between East African cichlid species, and much less in other systems. If that is the result of or the cause for the high species diversity in cichlids remains unknown.

TAXONOMICAL ISSUES [AS BOX]

THE "PHARYNGOGNATHS"

Shape and dentition of pharyngeal jaws have often been used in attempts to bring order into the confusing wealth of fish species. Predarwinian systematists like Cuvier (Cuvier and Valenciennes 1828-46) or Müller (1843) grouped some fish families by these traits, and later, phylogenetically oriented ichthyologists mainly kept these groupings (Rosen and Patterson 1990). From Müller's initial proposition of the "pharyngognathi acanthopterygii" quite a debate arose. First, Günther (1859-70) revised to "Acanthopterygii pharyngognathi" as a taxon uniting what nowadays would be Labridae, Embiotocidae, Gerreidae, and Chromides (=Cichlidae). The proposed synapomorphy of this group was a united (fused or sutured) lower pharyngeal jawbone. From then on for the next 120 years subsequent systematic hypotheses tugged apart this taxon, trusting the uniting, but not exclusive, character less (other taxa featuring a fused or sutured LPJ include: some Pleuronectidae (Bürgin 1987), Beloniformes (Rosen 1964; Stiassny and Jensen 1987; Tibbetts and Carseldine 2004), Cyprinodontidae (Rosen 1964), Gobiidae (Parenti and Thomas 1998), and Leiognathidae (James 1985)). This history has been reviewed in detail by Rosen and Patterson (1990) and Stiassny and Jensen (1987). Greenwood et al. (1966) grouped the 'pharyngognath' families similar to a later, molecular phylogenetic hypothesis (Mabuchi *et al.* 2007): Cichlidae sister to Pomacentridae and Embiotocidae, and apart from Labridae, which were placed together with Odacidae and Scaridae into Greenwood's suborder Labroidei. But Kaufman and Liem (1982) and Liem and Greenwood

(1981), using a more functional approach to phylogeny, restored the pharyngognaths as Labroidei joining the above groups and proposed them to be monophyletic. The proposed synapomorphies justifying this grouping were: "(1) united or fused fifth ceratobranchial resulting in the formation of one functional unit; (2) a true diarthrosis between upper pharyngeal jaw and the basicranium without an intervening part of the transversus dorsalis anterior muscle; and (3) the presence of an undivided sphincter oesophagi muscle forming a continuous sheet" (Kaufman and Liem 1982). This hypothesis, albeit with different intragroup relationships, was later supported by Stiassny's and Jensen's (1987) extensive cladistic analysis. Using a molecular approach, however, Streelman and Karl (1997) found the taxon to be polyphyletic with Cichlidae being the sister taxon of Labridae, both apart from the grouped Pomacentridae and Embiotocidae. This molecular phylogeny was based on one nuclear locus and used the phylogenetic algorithms available at the time. Much more data was generated for the study of Mabuchi and colleagues (2007), which used full mitochondrial genome sequences of many more species. Over the 10 years since Streelman and Karl's study algorithms for phylogenetic inference had been greatly improved to the end that the resulting phylogenetic hypothesis was more reliable than previous ones. Wainwright and coworkers extended the taxonomic sampling and used ten nuclear loci for phylogenetic inference, and were able to refute pharyngognath monophyly with great confidence (Wainwright *et al.* 2012). The main conclusion from these studies is that the derived "labroid" PJA must have arisen at least two times (more likely six to ten times) in teleost evolution (Streelman and Karl 1997; Mabuchi *et al.* 2007; Wainwright *et al.* 2012).

THE PHARYNGEAL APOPHYSIS IN THE SYSTEMATICS OF CICHLIDS

The species-rich radiations of East African cichlids have vexed systematists for a long time. Their close relatedness but, at the same time, rich morphological diversity confounds phylogenetic inference because many characters evolved homoplastically. Regan (1920) was the first to use the pharyngeal apophysis (PA) to infer associations between species of cichlids. Later, Greenwood (1978) revised Regan's classifications. If we compare Regan's and Greenwood's assignments - based on the relative involvement of basioccipital, parasphenoid and prootic in the PA – to modern, well established and supported molecular phylogenies it becomes clear that the structure of the pharyngeal apophysis does not provide good phylogenetic resolution. Instead, homoplasy seems to abound as is exemplified by the dispersal of these PA-informed groups across a, at tribe level most certainly correct, molecular phylogeny of Lake Tanganyikan cichlids (Salzburger *et al.* 2002).

In cichlids, the lower pharyngeal jaw has been found to be equally troublesome in systematic inference, for example due to large intraspecific variation and smooth morphoclines across taxa in Lake Victoria, which do not allow for distinctive groupings (Hoogerhoud 1984). Here, the shape and dentition are most often neatly adapted to the species' trophic niche, which leads to considerable convergence (e.g. Liem 1978; Stiassny 1982; Hulsey *et al.* 2008; Muschick *et al.* 2012). Phenotypic plasticity of cichlids' PJA has taxonomic implications, too, since the hypertrophy of the pharyngeal jaw has been used as a diagnostic character in distinguishing species and even genera in Lake Victoria cichlids (Hoogerhoud 1984). Later, an in-depth comparison between the intra- and interspecific adaptations to mollusk-crushing revealed – besides many similarities – differences due to constraints to plasticity within a species, which where overcome across species by genetic evolution (Smits *et al.* 1996a).

Although best documented in cichlids, the structure of PJA elements seems to be equally homoplasious in other taxa, for example some cyprinids (Zeng and Liu 2011) or muraenids (Mehta 2009; Reece *et al.* 2010).

All the above examples emphasize that great care must be taken when using pharyngeal jaw traits to infer phylogenetic associations on basically any taxonomic level, if one chooses to use them at all.

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Chapter 2

Adaptive phenotypic plasticity in the Midas cichlid fish pharyngeal jaw and its relevance in adaptive radiation

Moritz Muschick, Marta Barluenga, Walter Salzburger and Axel Meyer

MM participated in conceiving the study and the experimental design, ran the experiment, gathered the data, analyzed the data and drafted the manuscript. MB participated in conceiving the study and the experimental design and helped with gathering data and preparation of the manuscript. WS participated in conceiving the study and the experimental design and helped with preparation of the manuscript. AM participated in conceiving the study and the experimental design and helped with preparation of the manuscript.

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Adaptive phenotypic plasticity in the Midas cichlid fish pharyngeal jaw and its relevance in adaptive radiation

Moritz Muschick[1,2], Marta Barluenga[1,3], Walter Salzburger[1,2] and Axel Meyer[1*]

Abstract

Background: Phenotypic evolution and its role in the diversification of organisms is a central topic in evolutionary biology. A neglected factor during the modern evolutionary synthesis, adaptive phenotypic plasticity, more recently attracted the attention of many evolutionary biologists and is now recognized as an important ingredient in both population persistence and diversification. The traits and directions in which an ancestral source population displays phenotypic plasticity might partly determine the trajectories in morphospace, which are accessible for an adaptive radiation, starting from the colonization of a novel environment. In the case of repeated colonizations of similar environments from the same source population this "flexible stem" hypothesis predicts similar phenotypes to arise in repeated subsequent radiations. The Midas Cichlid (*Amphilophus* spp.) in Nicaragua has radiated in parallel in several crater-lakes seeded by populations originating from the Nicaraguan Great Lakes. Here, we tested phenotypic plasticity in the pharyngeal jaw of Midas Cichlids. The pharyngeal jaw apparatus of cichlids, a second set of jaws functionally decoupled from the oral ones, is known to mediate ecological specialization and often differs strongly between sister-species.

Results: We performed a common garden experiment raising three groups of Midas cichlids on food differing in hardness and calcium content. Analyzing the lower pharyngeal jaw-bones we find significant differences between diet groups qualitatively resembling the differences found between specialized species. Observed differences in pharyngeal jaw expression between groups were attributable to the diet's mechanical resistance, whereas surplus calcium in the diet was not found to be of importance.

Conclusions: The pharyngeal jaw apparatus of Midas Cichlids can be expressed plastically if stimulated mechanically during feeding. Since this trait is commonly differentiated - among other traits - between Midas Cichlid species, its plasticity might be an important factor in Midas Cichlid speciation. The prevalence of pharyngeal jaw differentiation across the Cichlidae further suggests that adaptive phenotypic plasticity in this trait could play an important role in cichlid speciation in general. We discuss several possibilities how the adaptive radiation of Midas Cichlids might have been influenced in this respect.

Background

Adaptive radiations arise through the rapid divergence of an ancestral species into a multitude of morphologically and ecologically differentiated taxa [1]. This process is assumed to be driven by divergent natural selection and ecological speciation where the adaptation to different niches eventually results in the evolution of reproductive isolation [2]. For example, specialization to certain food resources might lead to divergent habitat preferences, which in turn might isolate the populations reproductively [reviewed in [3]]. Specialization in diet is usually accompanied by morphological adaptations facilitating resource exploitation as has been shown in some textbook examples of adaptive radiation, *e.g.* the Darwin finches on the Galapagos Islands [4], the cichlid fishes in East African lakes [5-7], or the cosmopolitan tiger beetles [8].

Often, adaptive radiations are triggered by an altered adaptive landscape providing opportunity to invade

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previously not encountered ecological niches (*e.g.* after colonization of a new environment) or not accessible niches (*e.g.* after evolution of a 'key innovation') [9,10]. Recent studies showed that these adaptive peak shifts might happen rapidly [reviewed in [11]], and raise the question of how the adaptive morphological change drives the shift from one peak to another on the adaptive surface [12,13]. Mutation in coding and regulatory sequences and selection might not be sufficient to explain the rapidity of ecological adaptation seen in some instances [14]. Adaptation from standing genetic variation is also not likely to apply to all cases of adaptive radiations, particularly those with only a small number of founders [15]. Adaptive phenotypic plasticity might play a key role allowing populations to enter the 'realm of attraction' of a new adaptive peak, in which genetic assimilation occurs through directional selection favoring genotypes that produce even more extreme phenotypes than what would be possible by plastic response of the ancestral genotype alone [16,17]. Baldwin discussed this topic already in 1896 and described it as 'a new factor in evolution' [18,19]. Although its importance meanwhile became evident, phenotypic plasticity and genetic assimilation were dismissed as being unimportant during the modern evolutionary synthesis [20]. There has been a recent resurgence of interest in these phenomena [21-25], but the link to diversification is still little explored and under debate [26-28]. Not many investigations of phenotypic plasticity in model systems for speciation research, such as cichlid fishes, have been attempted (but see [29-33]).

The Neotropical Midas Cichlid species complex (*Amphilophus* spp.), is recognized among evolutionary biologists for its rapid phenotypic diversification and speciation [6,34]. This species complex has its center of its distribution in Nicaragua, and is comprised of an array of very young species that inhabit both the large Nicaraguan lakes, and several volcanic crater-lakes that contain small scale adaptive radiations [35,36]. The large Nicaraguan lakes, characterized by relatively turbid and shallow waters, have repeatedly acted as source populations for the colonization of nearby crater-lakes newly formed in the calderas of extinguished volcanoes. In these lakes the Midas cichlids encountered novel environmental conditions - *i.e.* presence of deeper zones and clearer water - and speciated *in situ* [34,35,37-41]. Crater-lake species have separated along depth and benthic-limnetic axes [34,35], with the open water column apparently being the first novel habitat invaded. Also, the Midas cichlid species have differentiated in their trophic adaptations. Usage of food sources like stonewort, *Aufwuchs*, evasive invertebrate prey, fish or snails differs species-specifically [39]. The Midas cichlids species, as well as other Neotropical and Old World cichlids, often differ in the relative degree of hypertrophy of a second set of jaws in the throat - the pharyngeal jaw - derived from branchial arch components and important for food mastication [reviewed in [42]]. Specialization for feeding on hard-shelled prey like snails, mussels, or crustaceans (durophagy) through this hypertrophy of the pharyngeal jaw apparatus (PJA) has been found to be a common axis of differentiation in crater-lake Midas cichlids as well as in other cichlid groups [5,31,32,34,42-44]. Its frequency and independency of acquisition across the phylogenetic tree suggests an important role of this adaptation in cichlid speciation [[5], [30], reviewed in [42]]

The Midas cichlid species in the crater lakes are often well differentiated in the trophic apparatus and only a few thousand years old [34-37]. The trophic polymorphism in the Midas crater-lake species could be derived from standing genetic variation, since the polymorphism is present in the large lakes, too [31,32,38,41]. However, the probably limited number of colonizing individuals would render a scenario of the evolution of trait divergence subsequent to colonization also plausible. This scenario is arguably more likely for remote crater-lakes with a monophyletic Midas cichlid assemblage, *e.g.* Lake Apoyo (see [34]). A plausible scenario could be that the divergence in the pharyngeal jaw apparatus in the crater lake Midas cichlid species might have been initiated by phenotypic plasticity in the ancestor. Reproductive isolation might then have occurred via habitat isolation through the heterogeneous distribution of snails in Nicaragua's volcanic crater-lakes, where densities appear to be dependent on depth and substrate type [45]. During times of low food availability otherwise opportunistic individuals adapted for durophagy might confine to areas of high snail density and thereby encounter mates non-randomly in respect to their pharyngeal jaw type [31,32,46,47]. If the ancestor of derived species was phenotypically plastic in ecologically relevant traits, this plasticity might have triggered the diversification. The "flexible stem" model, proposed by West-Eberhard [23], predicts that the directions in phenotypic space in which plasticity is expressed influence the trajectories of phenotypic evolution via genetic accommodation, similar to evolution along "genetic lines of least resistance" [48]. Therefore, it also predicts the outcomes of adaptive radiations seeded by the same ancestor and evolving in similar environments to be similar in terms of their phenotype composition.

In several cichlid fish species (family Cichlidae), plasticity in different traits has been demonstrated: Meyer experimentally induced changes in the oral jaw morphology in the Neotropical cichlid *Parachromis managuensis* by feeding different diets [30], a similar procedure was followed by Bouton and coworkers using the African cichlid *Neochromis greenwoodi* [49]. The Lake Victoria cichlid *Haplochromis pyrrhocephalus* was almost driven to extinction by the upsurge of the introduced, predatory Nile perch in the 1980s, but was able to adapt morphologically to the new environmental conditions of high predatory pressure and eutrophication in only two decades [50]. It has been interpreted that the speed and complexity of these morphological changes relied on a joined action of phenotypic plasticity and genetic change. The molluscivorous *Astatoreochromis alluaudi* naturally exhibits molariform pharyngeal jaws (i.e. stout, broad and strong jaw-bones with wide and flat teeth) [51]. However, when raised on soft artificial food under laboratory conditions [52], in natural conditions in lakes not inhabited by snails [51], or in lakes inhabited by snails but also with a molluscivorous competitor present [53], they develop less stout pharyngeal jaws with cuspid teeth (papilliform).

Specializations matter most during ecological "crunch times", when resource availability is low and opportunistic feeding is precluded [42,46]. The ability to exploit resources then at all or more efficiently than other species can, matters for the individual's survival. But specializations come with a trade-off. The specialization of being able to feed on particular diets especially efficiently often comes at the cost of being much less efficient when dealing with alternative diets. Apparently, such a trade-off exists in the Neotropical Midas Cichlid (*Amphilophus cf. citrinellus*) between two different types of pharyngeal jaws, molariform and papilliform. Individuals with papilliform lower pharyngeal jaws are more effective when dealing with soft food items [54]. Individuals with molariform jaws, on the other hand, can crack larger and harder snail shells and do this faster than papilliform individuals [54].

These cases of phenotypic plasticity, the basis of lacustrine cichlid radiations on trophic specialization [44,55,56] and the possible causal linkage of plasticity and diversification [23,30,31,57] call for examination of adaptive phenotypic plasticity in trophic traits in an adaptive radiation of cichlids comprising species differentiated in these traits. The lower pharyngeal jaw (LPJ) might constitute 'an ideal component of cichlid trophic morphology' to be investigated in this respect [43]. Preferably, the case in study should have a known and young history, involve colonization of new habitats and tests for plasticity in the ancestral or similar to the ancestral source population.

Here, we tested in a common garden experiment the developmental plasticity of the lower pharyngeal jaw of *Amphilophus citrinellus* (Günther, 1864) exposed to diets differing in hardness. Earlier work [31] had suggested that the species in this species complex are phenotypically plastic and that the abundance of molariform fish correlates with the abundance of their major prey item, hard-shelled snails.

The experiment was performed on a laboratory stock derived from the crater Lake Masaya, which was bred in captivity for several decades. Although Lake Masaya is a volcanic crater-lake, its *A. citrinellus* population is very close to the populations of the Lake Nicaragua - which is probably the ancestral source population of most crater-lake radiations - in terms of body shape [35] and phylogenetic relationships [36]. Furthermore, it has been suggested that Lake Masaya might have been colonized as recently as 450 years ago [58].

We investigated whether the development of pharyngeal jaws differed between three types of diets: (1) intact snails with shell, (2) peeled snails without shell, and (3) finely ground up whole snails frozen in pellets, from which fish could nibble off the thawed, soft outer layer when those were given into the water. We aimed to verify whether a hard diet could induce changes in the pharyngeal jaw of the fish, and whether the generation of robust pharyngeal jaws with stout teeth (molariform jaws) was determined by higher calcium content in the diet, or by mechanical stimulation of the jaws when crushing hard food items.

Our study finds that diet can induce changes on the trophic apparatus of the Midas cichlids, and that this changes are related to the mechanical stimulation of the jaws.

Results

Geometric morphometric analyses

The shape of the lower pharyngeal jaw differed significantly between the fish raised on a diet 'with shell' and the other two groups of fish as revealed by permutation testing of Procrustes distances (Table 1). The morphological differentiation measured by Procrustes distance was significant and similarly large between the 'with shell' and the two other groups (0.0175 and 0.0135, respectively). The distance between 'ground' and 'no shell' was considerably smaller (0.0067) and not significant. Depicting the between group changes along discriminant functions by warped outline drawings revealed that shape was altered most in functionally relevant regions of the LPJ, namely the posterior horns. In the 'with shell' group the horns (represented by landmarks

Table 1 Distances in LPJ shape

diet group comparison	procrustes distance	p value
'with shell' vs. 'no shell'	0.0175	<0.0001
'with shell' vs. 'ground'	0.0135	0.0026
'no shell' vs. 'ground'	0.0067	0.15

Distances between the group means in LPJ shape space for data regressed on body weight (Ln). Significance was assessed by permutation testing with 10000 permutations.

1, 2, 6 and 7) pointed more outward and were broader, and jaws were generally shorter along the anterior-posterior axis (Figure 1). Additionally, the posterior outline (represented by landmarks 3, 4 and 5) was less concave in the 'with shell' group as in the other groups. In the 'ground' group the posterior outline was as well less concave as in the 'no shell' group and the horns were directed outward slightly more, but horn width was smaller. The relative overlap on the first two principal components of shape variation between the treatment groups is illustrated in Figure 2.

Analyses of weights and lengths

Taking body weight as proxy for ontogenetic stage and correcting for it, measures not covered by the geometric morphometric shape analysis were investigated. The LPJ weight showed significant differences between groups with 'no shell' having the lightest, 'with shell' having the heaviest and 'ground' having intermediate jaws. The centroid size, *i.e.* the scaling factor from the size-removing step in the alignment of landmark configurations, was found to differ significantly between the 'shell' and the 'no shell' group and between the 'shell' and the 'ground' group. Differences were not significant between the 'ground' and the 'no shell' group (Table 2). The dimension not assessed by centroid size, the jaw height, showed no group differentiation if fish body weight was taken as covariate, but showed strong group differentiation when corrected for LPJ weight instead. In that case, the 'no shell' group had the highest, the 'with shell' group the most slender and the 'ground' group intermediate jaws relative to jaw weight. This points to an increase in bone density, moderate with high calcium diet and strong when mechanical impact acted also on the jaws during feeding.

The weight of the heavier of the fish's two largest otoliths - the sagittae - using fish body weight as covariate in an analysis of covariance, did not differ in the two

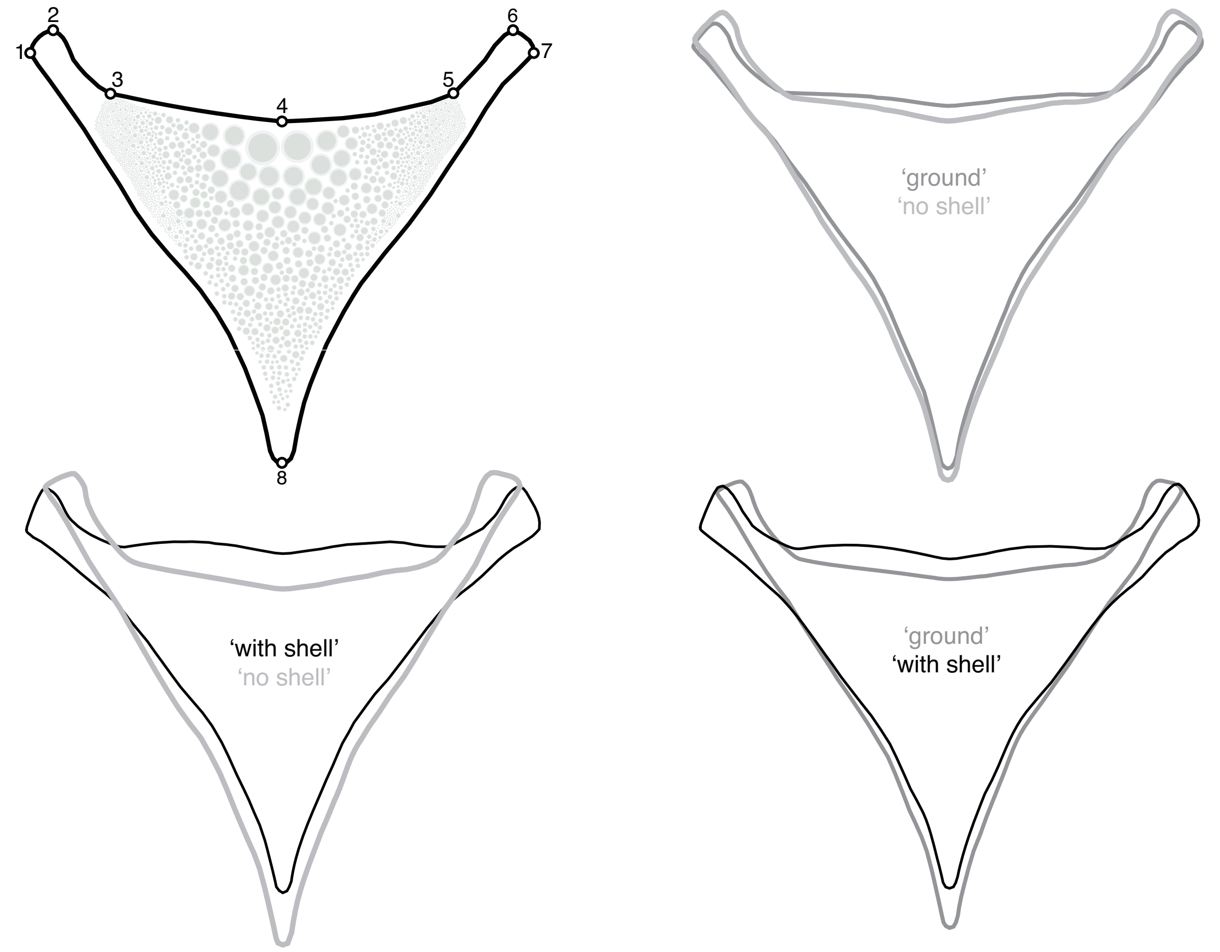

Figure 1 Induced shape differences. LPJ shape differences between the diet groups along pairwise discriminant functions depicted as interpolated outlines based on analysis of landmark coordinates. Landmark positions are shown in the upper left. Differences are exaggerated five times for illustration purposes.

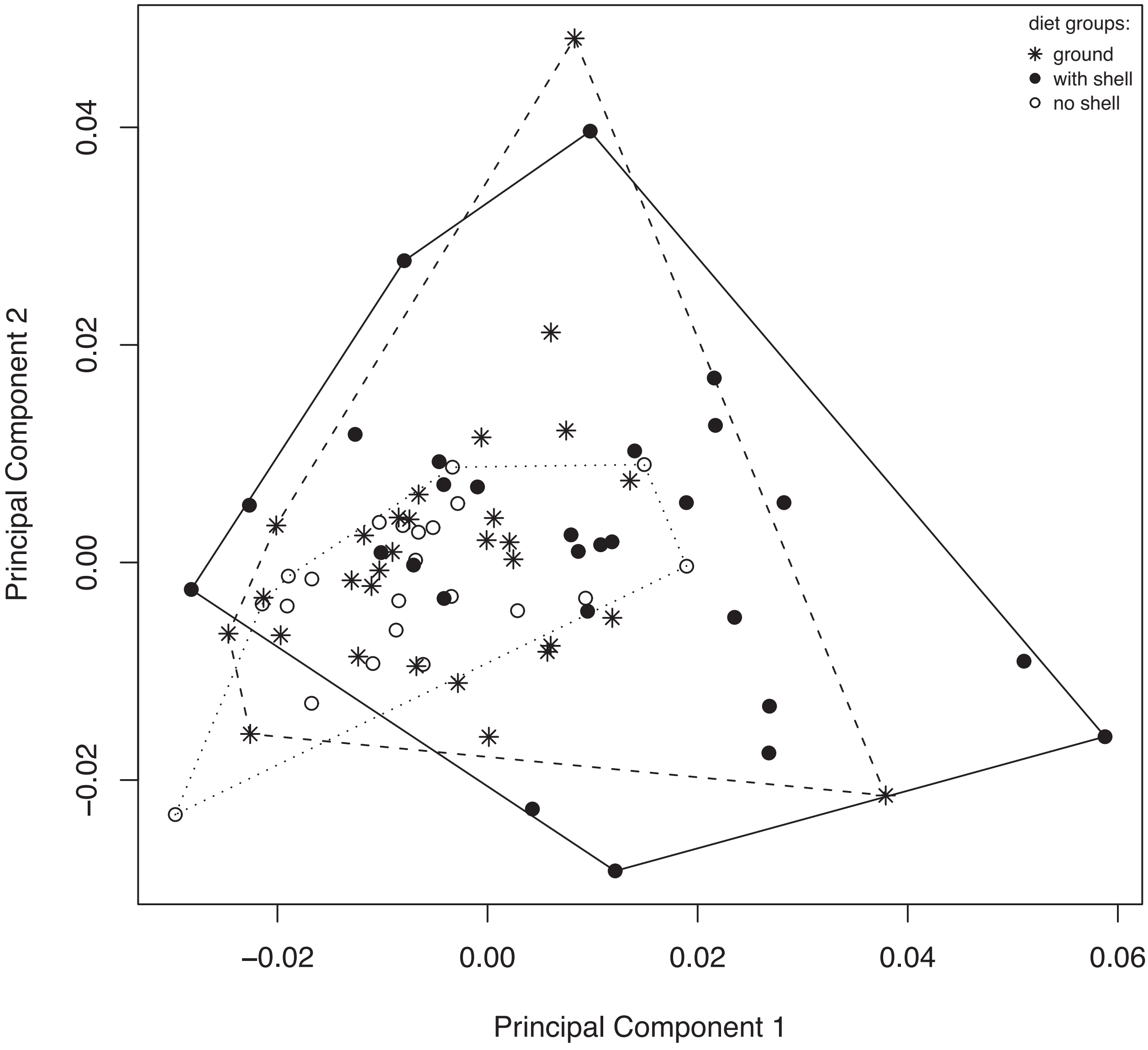

Figure 2 Morphological separation of treatment groups. Scatterplot for the first two axes derived from a principal component analysis (PCA) of LPJ landmark data. Percentage of variance explained by the axes is given in parentheses. Note that the large overlap of convex hulls of ‘ground’ and ‘with shell’ groups is mainly brought about by two extreme individuals in the ‘ground’ group.

high-calcium groups, but was significantly lower in the ‘no shell’ group (Table 2; Figure 3). Correcting for LPJ weight, the ‘with shell’ group had significantly lower relative sagitta weight, while ‘ground’ and ‘no shell’ did not differ (Table 2; Figure 3).

Discussion

Phenotypic plasticity has been hypothesized to be able to promote divergence only if it is not complete, *i.e.* sufficient to achieve the same fitness as if the trait was expressed constitutively [20]. A plastic response would be adaptive if it shifts the phenotype in the direction of a new peak on the adaptive surface, and non-adaptive or maladaptive responses to stressful environments would place the phenotype away from any optimum [59]. Here, we were able to induce an adaptive plastic response in the LPJ of *A. citrinellus* by feeding different diets. It qualitatively resembles interspecies differences found in nature, although less pronounced.

In our common garden experiment, the changes induced on the fish exposed to a hard shell diet - *i.e.* horns of the LPJ pointing more outwards, posterior outline less concave, LPJ relatively heavier and possibly increased bone density - mirror those identified as

Table 2 Group comparisons for morphometric data (non-geometric)

Trait	Factor	p value	WS vs. G	NS vs. G	NS vs. WS
LPJ centroid size	body weight (Ln)	<0.0001			
	diet group	<0.0001	0.036	0.06	<0.0001
	weight × group	0.77			
LPJ weight (Ln)	body weight (Ln)	<0.0001			
	diet group	<0.0001	0.018	<0.0001	<0.0001
	weight × group	0.08			
LPJ height (Ln)	body weight (Ln)	<0.0001			
	diet group	0.68	0.94	0.67	0.86
	weight × group	0.25			
Otolith weight (Ln)	body weight (Ln)	<0.0001			
	diet group	<0.0001	0.40	<0.0001	0.002
	weight × group	0.88			
LPJ height (Ln)	LPJ weight (Ln)	<0.0001			
	diet group	<0.0001	0.006	0.0007	<0.0001
	weight × group	0.68			
Otolith weight (Ln)	LPJ weight (Ln)	<0.0001			
	diet group	0.0044	0.004	0.71	0.07
	weight × group	0.15			

Results of ANOVAs for length and weight data using diet group and either body weight or LPJ weight as factors. Given are the *p*-values of the ANOVAs and the *p*-values from a subsequent Tukey honest significant difference-test for each group comparison. WS: 'with shell'-group; NS: 'no shell'-group; G: 'ground'-group

adaptations for mollusk crushing in several other cichlid sister-species pairs [42,43],very closely related species in the Midas cichlid complex in several crater lakes [34,37] and in constitutively expressed [60] or induced [61] phenotypes in other species. The expression of a relatively hypertrophied pharyngeal jaw due to durophagy resembling adaptations found in specialized molluscivorous fish, and the result that hypertrophication is much weaker when fish are fed with high-calcium, low-impact diet leads to the conclusion that the observed phenotypic plasticity is indeed adaptive. The trade-off in feeding performance between different phenotypes further evidences the adaptive nature of plasticity in this trait [54].

A surprising finding is that LPJ height did not differ between the experimental groups, since along this dimension divergence is commonly found in non-molluscivorous/molluscivorous species pairs [53]. A possible explanation would be that this trait behaves allometrically with larger and older molariform fishes expressing more re-growing molars thickening the LPJ. A longer common garden experiment might reveal plasticity in this trait as well. An alternative is that LPJ height is simply not plastic, and its evolution is solely governed by mutation and selection that might bring about developmental constraints. Structural constraints and the lack of phenotypic accommodation would be a possible explanation as well. Under this scenario, an increase in LPJ height would not be possible due to prohibitive spatial demands.

Several findings suggest that no specific and adaptive shape difference was induced by a high-calcium diet alone. Only small differences in shape were observed between 'no shell' and 'ground' groups, and those differences did not resemble known adaptations for durophagy. Furthermore, the comparisons including otolith weight show that calcium allocation is strongly biased towards the LPJ in the 'with shell' group but not in the 'ground' group. There, it appears to affect the skeleton evenly as indicated by the group comparison for sagittae weight when correcting for LPJ weight. This corroborates the finding that the mechanical impact on the LPJ during feeding triggers increased calcium allocation towards the jaw and suggests that a high-calcium diet leads to an unspecific increase in calcium deposition.

The sagittae, as well as the other otoliths, grow in small increments throughout the fish's life [62] and their weight is considered to reflect weight of the individual and availability of calcium during its life. However, Ichii and Mugiya [63] showed that fish raised on a calcium depleted diet did not show different bone densities after a period of 58 days, but were able to substitute the lacking dietary input of calcium by increasing uptake through the gills from the water. Farrell and Campana [64] observed that environmental availability of calcium does not affect its deposition on the otolith. These studies have background levels of calcium in both, supplied diet and water, which might differ from levels in our experiment, involve different species and their experiments were conducted significantly shorter. These differences in experimental setup might explain why in our study an effect of calcium availability on bone and otolith growth was observed as opposed to the other studies.

The effects of the mechanical impact were strong enough to exceed anticipated effects of a higher availability of calcium in the 'ground' diet due to facilitated uptake of minerals from the readily processed shells. 'With shell' fish regularly spat out shell fragments

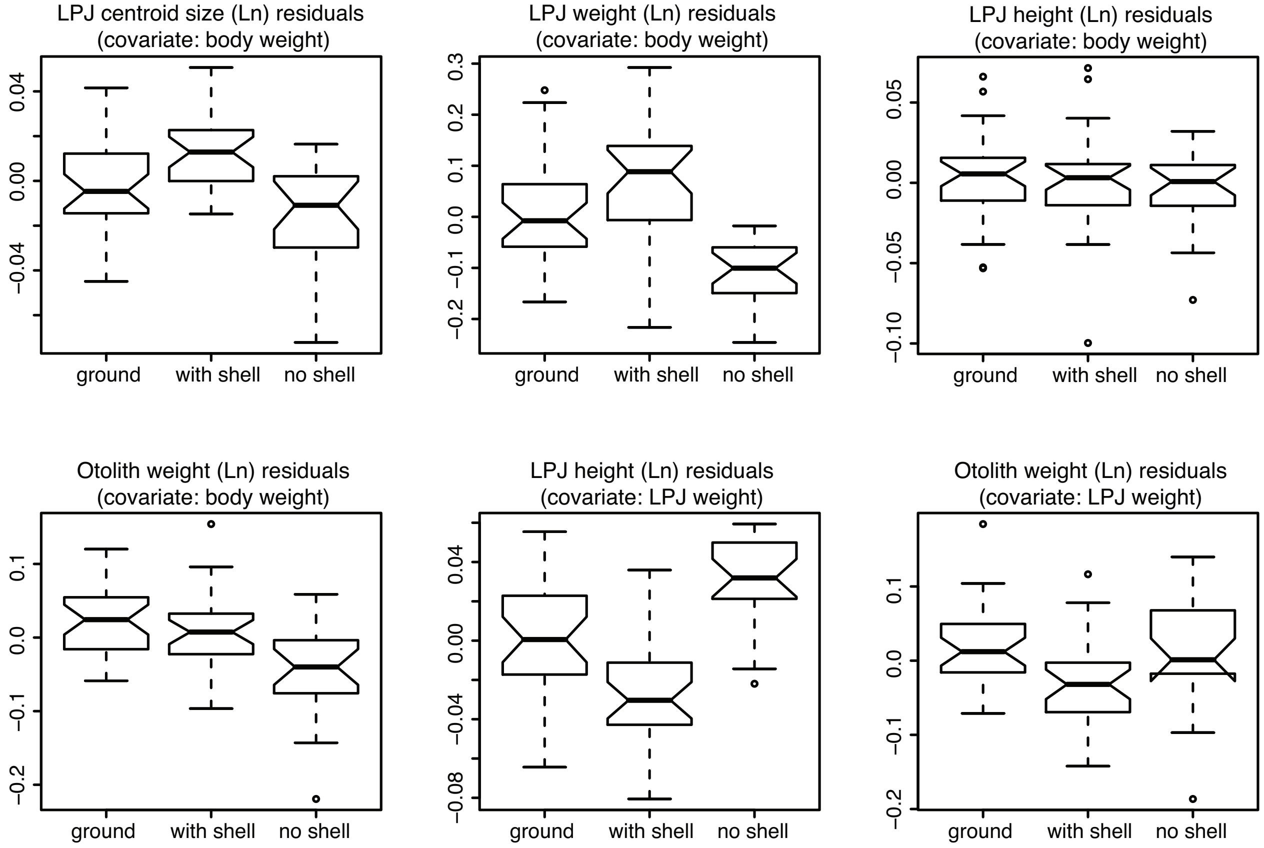

Figure 3 Character divergence between treatment groups. Diet group differentiation for regressed morphometric data from LPJs. Regression was either against body weight or LPJ weight. Significance levels are given in Table 2. Boxes range from the lower to the upper quartile and a bar indicates the median. The whiskers exceed the boxes by 1.5 times the inter-quartile-range of the lower or upper quartile, respectively. Notches are a rough proxy for confidence intervals of the median; if they do not overlap between two plots, the medians are most likely significantly different. They extend to +/- 1.58 inter-quartile-range divided by the square root of the number of observations from the median.

during mastication, and Hoogerhoud [53] reports snail shell pieces to pass the digestive tract of cichlids apparently unharmed. Such observations might explain the slight and non-significant shift towards relatively heavier otoliths in 'ground' fish when accounted for body weight. Several studies on phenotypic plasticity express concerns about the influence of diet quality on developmental differences between treatment groups, so that detrimental effects of a low-quality diet might be mistaken for (adaptive) phenotypic plasticity [33,47,65,66]. Here, we addressed these concerns with our feeding regime. Specifically, we are able to show that induced differences were not due to a lack of calcium in the diet. Even though the studied individuals descended from an inbred line, which has not been subject to artificial selection favoring plasticity in the pharyngeal jaw apparatus, ability to express this trait plastically persisted. This suggests that the plasticity of the LPJ in *A. citrinellus* might not be a trait under selection itself, but more likely an instance of a hidden reaction norm [20].

Similarly to the Midas Cichlid, other cichlid species show PJA adaptable or adapted to durophagy: in Neotropical cichlids non-molluscivorous and molluscivorous species, having papilliform and molariform LPJs respectively, often represent closely related sister species pairs [43]. The same trajectory of divergence has been found between trophic morphs of the same species, *Herichthys minckleyi*, occurring in the Cuatro Ciénegas basin, Mexico. Along the same axis allometric changes happen during the ontogeny of the Mayan Cichlid *Cichlasoma urophthalmus*, introduced in Southern Florida [67]. The presence of hypertrophied pharyngeal jaws is not restricted to cichlids, or even to freshwater fishes: members of the marine families Sciaenidae, Haemulidae and Carangidae express a similar type of PJA, allowing them to feed on hard-shelled prey. The phylogenetic relationship to species with non-hypertrophied pharyngeal jaws can be close, *e.g.* congeneric, in these cases as well [68].

The number of cases of closely related species or trophic morphs of a single species exhibiting such divergent morphologies, as well as their phylogenetic dispersal, is astonishing. This trajectory in morphospace might be similarly important as the well-known deep-bodied vs. elongated body trajectory found in many benthic-limnetic fish species pairs (*e.g.* [69-71], and those reviewed in [72]). Both phenotypic contrasts are usually accompanied by extensive diet and/or habitat preference differences, respectively. Such ecological diversification has been shown to be a major factor in empirically studied speciation events and its importance in speciation is well supported by theoretical models [34,73-75]. In the Midas Cichlid species complex, ecological diversification has been shown to occur along both axes, even in correlation [31], and probably led to speciation in several cases [34].

Phenotypic plasticity and rates of diversification

The importance of phenotypic plasticity in population divergence and speciation gained increasing attention in the last years [22,23,26,33,47,57,76-81]. Both studies focusing on single species and studies within a larger comparative framework investigated this link: Nylin & Wahlberg found support for a 'plasticity scenario' for the diversification of nymphaline butterflies during the Tertiary and argued that herbivorous taxa able to occupy several niches were more likely to diversify along with the angiosperm radiation [82]. In coastal San Diego a population of montane dark-eyed juncos (*Junco hyemalis*, Aves) was able to establish itself due to an adaptive plastic response in reproductive effort [83]. A recent review by Pfennig *et al.* [57] summarizes theoretical and empirical studies and diagnoses an important, but largely underappreciated, role of phenotypic plasticity in speciation and adaptive radiation. Comparing sister clade pairs - with one clade being known to include cases of resource polyphenism, while the other does not - Pfennig and McGee found evidence that resource polyphenism is associated with greater species richness in fishes and amphibians [28].

The role of phenotypic plasticity in population divergence appears to be at least twofold: (1) plasticity increases the probability of population persistence after colonization of a new environment, thus making its split from the ancestral population more likely [83,84], and (2) plasticity provides means of conquering other peaks on the adaptive landscape, possibly leading to assortative mating and speciation with parallel outcomes in repeated cases [12,14,17,23,33].

Theoretical investigations support these predictions. Probability of population persistence increases with plasticity while being dependent on the amount of environmental change and the costliness of plasticity [85]. At a moderate rate of environmental change and if plasticity is costly, high levels of plasticity are expected to lead to an increased probability of extinction while an intermediate level improves the ability of persistence [85]. Access to novel ecological niches is improved because an increase in epigenetic variability does facilitate the circumvention of adaptive valleys and smoothes the fitness landscape [13,86,87]. Using numerical simulations Thibert-Plante and Hendry [26] find plasticity to commence reduction in gene flow between populations in contrasting environments. To do so, plasticity must occur before dispersal but could then lead to reproductive isolation even prior to any adaptive genetic divergence.

Our demonstration of adaptive phenotypic plasticity in the LPJ of *A. cf. citrinellus* suggests that this could be a crucial factor in ecological speciation and adaptive radiation in the repeated *Amphilophus* crater-lake radiations and possibly in other cichlid clades as well [30,31]. The results of our experiment support the "flexible stem" hypothesis, in that the induced differences between treatment groups - more robust LPJs in the 'with-shell' group, less robust LPJs in groups fed soft food - resemble between-species differences in crater-lake radiations. However, we did not test for plasticity in the ancestor itself, nor in fish derived from the large Nicaraguan Lakes, but in a stock derived from Lake Masaya. The different history might have caused an alteration of the plastic response in experimental groups compared to the real ancestor. But since there is a considerable chance of the Lake Masaya *A. citrinellus* population being very young and since plasticity here seems not be lost easily (at least not over several generations), we suggest that our results endorse the "flexible stem" hypothesis for the Midas Cichlid assemblage. Because the induced plasticity does not reach the extent of morphological divergence found between species in nature we conclude, that the expectations from the "adaptive surface model" are fulfilled as well.

In which way exactly phenotypic plasticity and genetic accommodation in the pharyngeal jaw might abet diversification in the *Amphilophus* species complex remains speculative. A direct influence on the formation of reproductive isolation might be given through enhancement of habitat preference. If individuals expressing the same type of pharyngeal jaw have a higher chance of mating with each other, and gene flow between groups is hampered strongly enough, population subdivision might be initiated. The heterogeneous distribution of snails, if it is stable over time and patches are sufficiently large, might be the basis for habitat preference by jaw type. Alternatively, the hypothesized function of the pharyngeal jaw apparatus in sound production, *e.g.* during courtship, might bring about assortative mating according to jaw type if female sound preference is divergent as well [88].

However, even if phenotypic plasticity is less important in sympatric speciation scenarios it might still influence diversification in allopatry [reviewed in [89]]. By augmenting the probability of population persistence after colonization of a new environment, *e.g.* a crater-lake, and the possibility of genetic accommodation of plastic trait changes the likelihood of allopatric speciation between ancestral source population and the new colonizing population is increased. It remains unclear, whether or not the repeated endemic radiations of Midas cichlids in Nicaraguan crater-lakes are facilitated by phenotypic plasticity in the pharyngeal jaw or if the constitutively expressed differences in jaw shape between species are a secondary result of speciation driven by other factors. The best documented case of an in-crater-lake diversification, the origination of the Arrow cichlid *Amphilophus zaliosus* in Lake Apoyo, seems to have been driven by diverging habitat preferences with differences in pharyngeal jaw shape being probably secondary [34]. However, in other, less-well documented cases the hypothesis that adaptations in the pharyngeal jaw apparatus triggered divergence remains valid, but would need to be further investigated.

Conclusions

We demonstrated phenotypic plasticity in the pharyngeal jaw of the cichlid fish *Amphilophus citrinellus* that is due not to differences in nutritional composition of the diet, but brought about largely by the mode of feeding. This finding might suggest that plasticity plays an important role in diversification.

Future research on how a plastic reaction in one trait could impact the expression of other traits through correlated plastic responses might contribute to the understanding of parallelisms so often encountered in nature. For example, it seems the papilliform pharyngeal jaw type is correlated with fusiform limnetic body shape whereas the molariform jaw type is correlated with deeper, benthic body shape [31]. The extent to which this 'integration of plastic responses' [81] is determined, and by which factors, still remains to be elucidated. Also, what role a stage of fixed polymorphism plays in the process of diversification, whether it is an intermediate step [42] or a 'dead-end', remains to be investigated.

How adaptive phenotypic plasticity is mediated genetically is another important issue. In cichlids, the family of bone morphogenetic proteins (BMPs) is known to be involved in shaping bones of the oral and pharyngeal jaws [90] and might constitute good candidates, along with respective transcription factors and ligands, for the elucidation of the genetics of phenotypic plasticity in the PJA.

Cichlids, are a prime system for speciation research and have an important trophic trait expressed plastically, and therefore constitute a cogent group for investigating the role of adaptive phenotypic plasticity in diversification. Research combining experimental and field studies with modern tools of analysis, such as sensitive group assignment methods or gene expression quantification, will be most rewarding avenues of research to elucidate the link between plasticity and speciation

Methods

Common garden experiment

We divided fry of a single *Amphilophus citrinellus* brood from an inbred line into three similarly sized groups and fed them on diets differing in mechanical durability and calcium content. The three study groups of 30 *A. citrinellus* individuals each were kept under standardized laboratory conditions with 12 h daylight for a period of six month. The fish stock used (AM-stock at the University of Konstanz) derives from Lake Masaya, a volcanic crater-lake in Nicaragua. Originally, these fish came from the Berkeley stocks of George Barlow who gave some of these fish to the Steinhard Aquarium in San Francisco. In 2001 fish from there were brought to Konstanz and are the stock of *A. cf. citrinellus* that were used in these experiments. This fish stock has been bred in captivity on soft artificial food for several decades. Moreover, in Lake Masaya no snails occur and neither are cichlids with molariform pharyngeal jaws reported [54,91].

The fish groups were raised on different diets: (1) *Melanoides tuberculata* snails, laboratory grown, with intact bodies and intact or slightly damaged shells (in case the snail was deemed too large), (2) snail bodies, where the shells were manually removed, and (3) *M. tuberculata* with shell but ground to fine paste using mortar and pestle, which was given frozen in pieces to large to be swallowed as a whole. Food amount was adjusted to match group's estimated size gain. Fish were kept in one large tank (1.8 × 0.5 × 0.5 meter, 450 l) and perforated walls allowed water exchange between the compartments containing the three experimental groups. To counteract position bias, we swapped groups between compartments several times throughout the experiment.

Measurements & analyses

Fishes were sacrificed and weighed, and standard and total length were recorded. We excised LPJs and sagittae, and cleaned and dried them. LPJs and otoliths were weighed to the nearest milligram. LPJs were scanned on a standard desktop scanner. Coordinates of 8 landmarks were recorded for each LPJ using tpsDig 2.11 ([92], for landmark positions see Figure 1). Landmarks represented homologous, defined locations on the jaws outline. Their positioning followed Klingenberg *et al.* [93] with the exception of their landmarks 5 and 6 - instead the anterior tip was covered by our landmark 8.

Otherwise landmark position were the same, though differently numbered. Landmark arrangements were procrustes aligned, *i.e.* their positional, rotational and size information was removed from the dataset. However, size information was recorded in centroid size and was used for joint analysis with other data. Since the LPJ is a symmetrical structure we extracted the symmetric component of shape variation using MorphoJ [94]. We conducted discriminant function analyses (DFA) for each pair of groups to produce Figure 1. A canonical variates analyses (CVA) using residuals of a pooled-within-diet-groups regression on body weight (Ln) yielded mean shape distances and their significance levels were assessed by permutation testing (10.000 permutations).

Fish body weight, LPJ weight, height, and centroid size, and otolith weight were evaluated via analysis of variance (ANOVA) and group-pairwise differences of residuals means were assessed for significance using Tukey's honest significant difference-test. All these measures were Ln transformed prior to analysis. For otoliths the weight of the heavier sagitta was used, to minimize influence of preparation damage.

All statistical tests on length and weight data were performed using the R statistical environment [95].

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Authors' contributions

MM participated in conceiving the study and the experimental design, ran the experiment, gathered the data, analyzed the data and drafted the manuscript. MB participated in conceiving the study and the experimental design and helped with gathering data and preparation of the manuscript. WS participated in conceiving the study and the experimental design and helped with preparation of the manuscript. AM participated in conceiving the study and the experimental design and helped with preparation of the manuscript. All authors read and approved the final manuscript.

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MB was a postdoc in AM's laboratory when this study was conducted. She is an evolutionary ecologist interested in speciation and the origin of adaptive radiations.

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Chapter 3

Convergent evolution within an adaptive radiation of cichlid fishes

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MM and WS jointly conceived the study, drafted the manuscript and prepared the figures. MM gathered and analysed morphological and ecological data in a comparative framework. WS conducted the molecular phylogenetic analysis. AI acquired occurrence and abundance data and helped drafting the manuscript.

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Convergent Evolution within an Adaptive Radiation of Cichlid Fishes

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Summary

The recurrent evolution of convergent forms is a widespread phenomenon in adaptive radiations (e.g., [1–9]). For example, similar ecotypes of anoles lizards have evolved on different islands of the Caribbean [2, 6], benthic-limnetic species pairs of stickleback fish emerged repeatedly in postglacial lakes [1, 3], equivalent sets of spider ecomorphs have arisen on Hawaiian islands [7, 8], and a whole set of convergent species pairs of cichlid fishes evolved in East African Lakes Malawi and Tanganyika [10, 11]. In all these cases, convergent phenotypes originated in geographic isolation from each other. Recent theoretical models, however, predict that convergence should be common within species-rich communities [12, 13], such as species assemblages resulting from adaptive radiations. Here, we present the most extensive quantitative analysis to date of an adaptive radiation of cichlid fishes, discovering multiple instances of convergence in body and trophic morphology. Moreover, we show that convergent morphologies are associated with adaptations to specific habitats and resources and that Lake Tanganyika's cichlid communities are characterized by the sympatric occurrence of convergent forms. This prevalent coexistence of distantly related yet ecomorphologically similar species offers an explanation for the greatly elevated species numbers in cichlid species flocks.

Results and Discussion

Adaptive radiation, the rapid evolution of a multitude of species from a common ancestor as a consequence of their adaptation to various ecological niches, is thought to be responsible for much of the morphological and ecological diversity on earth [4, 9]. Interestingly, parallel adaptive radiations of the same group of organisms frequently produce convergent forms [1–9], which is commonly understood as the result of independent adaptations to similar ecological conditions [3, 4, 14, 15]. Convergence in morphology and behavior is typically observed between species that evolved in geographic isolation [2, 3, 7, 10]. Theoretical models, on the other hand, predict that convergence should also be common within species-rich communities [12, 13], thus challenging the standard ecological premises that closely related species should be ecologically similar [16, 17] and that two species cannot coexist in the same niche [18]. Such models suggest that there is an alternative strategy for enabling stable coexistence than to be sufficiently distinct: to be sufficiently similar. According to these models, convergent evolution actually appears to be characteristic in "species-saturated communities" [12] and to occur when the number of species exceeds the number of available niches [13], as is probably the case in the exceptionally diverse species flocks of cichlid fishes in the East African Great Lakes Victoria, Malawi, and Tanganyika.

Against this background we explore the cichlid fish assemblage of Lake Tanganyika (LT) (Figure 1A) and provide what is to date the most thorough examination of a cichlid adaptive radiation. Our integrative study combines molecular phylogenetic, geometric morphometric, and diet analyses in a data set of more than a thousand specimens from 71 species (see Table S1 available online and Experimental Procedures). Our morphological comparisons focus on two ecologically highly relevant characters, overall body shape and the shape of the lower pharyngeal jaw bone (LPJ). The LPJ is the central unit of the pharyngeal jaw apparatus, which is a second set of tooth-bearing jaws in the pharynx used to process food [11, 22] (Movie S1). Finally, we use carbon and nitrogen stable isotope ratios as proxy for trophic ecology—in combination with stomach and gut content analyses.

We first present a robust phylogenetic framework for the species flock (Figure 1B), which largely agrees with previous studies [19, 20]. When clustering the species according to body and LPJ shape, the phylogenetic structure vanishes (Figures 2A and 2C), indicating that the shape of these traits is largely uncoupled from the phylogenetic background of a species. All larger cichlid tribes are broken up into two or more body and LPJ shape clusters, and the different tribes overlap in morphospace (Figures S1A and S1B). A large fraction of the sister taxa are not each other's closest ally in the morphological cluster analyses, and the cluster trees based on shape data are incongruent with the molecular phylogeny (body shape: Δ -lnL = 2885.87; Δ tree length = 1059; $P_{SH} < 0.001$; $P_{KH} < 0.001$; LPJ shape: Δ -lnL = 3709.20; Δ tree length = 1484; $P_{SH} < 0.001$; $P_{KH} < 0.001$). Instead of correlating with phylogeny, species that are morphologically alike are, in general, more similar in trophic ecology (Figures 2 and S1). This integrated analysis leads to two main observations. First, species from distinct clades are grouped into the same morphoclusters, whereas sister-species are often quite distinct morphologically (Figure S2); this suggests prevalent convergence in body and LPJ shape within the cichlid species flock of LT. Second, there appears to be a strong link between (trophic) morphology and ecology in LT cichlids; this suggests that, just like in other cases of convergent evolution, natural selection is the driving force in the evolution of convergent forms [1, 5, 15, 23]. In the following, we provide examples for convergent species and quantify convergence in sympatry in the cichlid species flock of LT.

Perhaps the most striking case of convergent evolution within LT's cichlid assemblage involves *Neolamprologus prochilus* and the enigmatic "*Ctenochromis*" *benthicola* (Figure 3A and indicated in bold in Figures 1 and 2). Both species occur sympatrically and are similar to a degree that even local fishermen, who otherwise ably distinguish species, consider them as one. In line with this, geometric morphometric analyses cluster them together, they have similar stable isotope signatures (Figures 2 and S1), and they show the same

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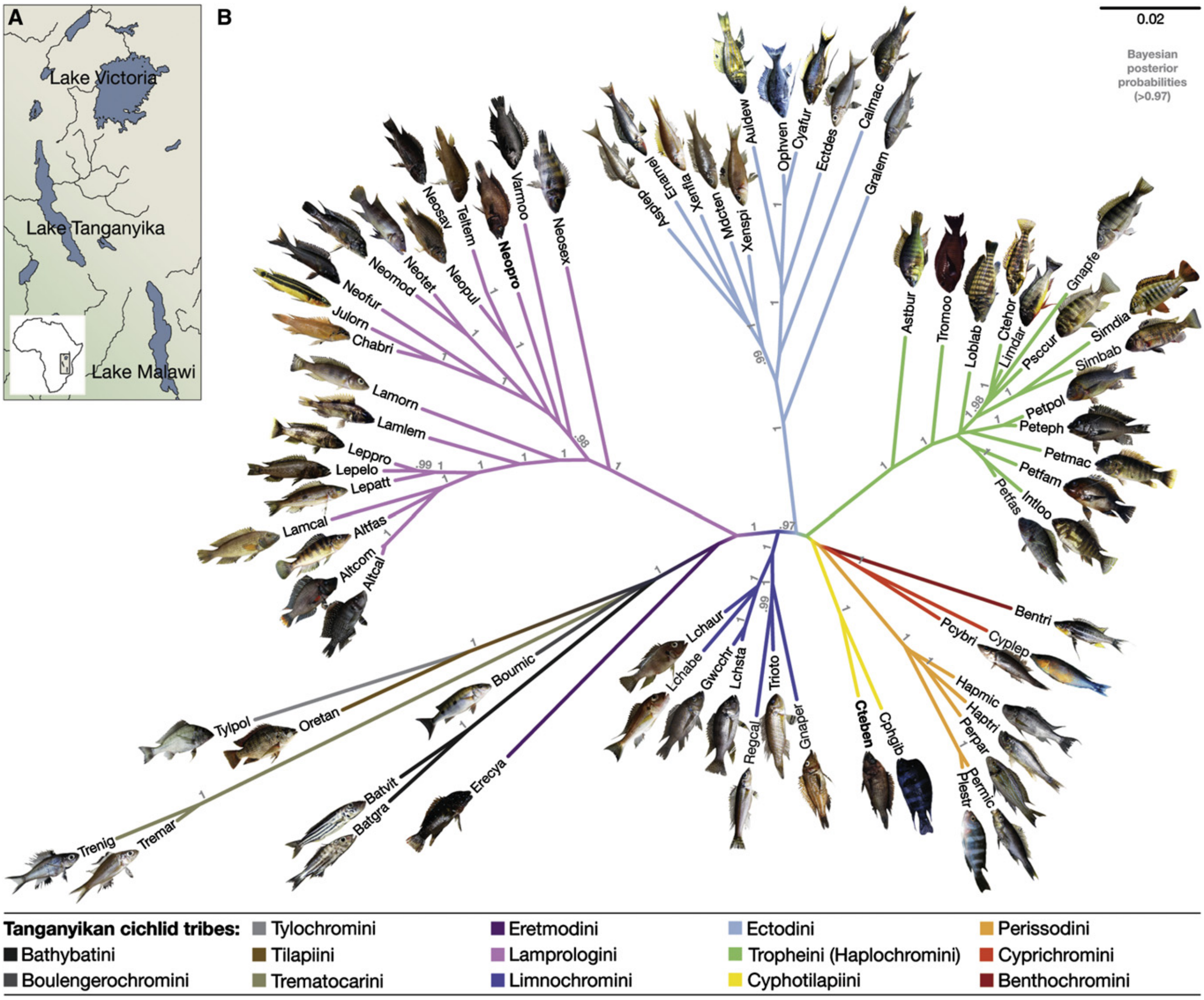

Figure 1. The Cichlid Species Flock of Lake Tanganyika

(A) Map of East Africa showing the three Great Lakes. Lake Tanganyika (LT) is the oldest lake in East Africa and, consequently, accommodates the genetically, morphologically, and ecologically most diverse cichlid species flock [11, 19].

(B) Maximum-likelihood phylogeny of the 71 Tanganyikan cichlid species in our core data set, based on two nuclear (*ednrb1*, *phpt1*) and one mitochondrial (ND2) marker (2,013 bp in total) and the GTR+G model of molecular evolution. Numbers above the branches depict Bayesian posterior probabilities >0.97. Full species names are given in Table S1; different colors denote the main cichlid lineages ("tribes"), some of which are likely to have undergone secondary subradiations [19–21]. Note that the cichlid adaptive radiations of Lakes Malawi and Victoria consist of one of these tribes only, the Haplochromini (the Tanganyikan representatives of which are often referred to as Tropheini) [21]. Our phylogeny confirms the monophyly of the tribes; at least seven genera are, however, paraphyletic, which already indicates convergence in traits used to classify them initially. For example, the putative haplochromine "*Ctenochromis*" *benthicola* (Cteben) emerges as a member of the Cyphotilapiini, whereas its congener, *C. horei* (Ctehor) remains within the Tropheini/Haplochromini. The other paraphyletic genera are *Gnathochromis* (Gna), *Lamprologus* (Lam), *Limnochromis* (Lch), *Neolamprologus* (Neo), *Perissodus* (Per), and *Petrochromis* (Pet). Images of the fishes were taken directly in the field.

stomach contents, namely remnants of the endemic shrimp *Limnocaridina* sp. (Figure 3A). Yet, whereas *N. prochilus* belongs to the Lamprologini, "*C.*" *benthicola*—formerly considered a Haplochromini and congener of *C. horei*—now emerges as a member of the Cyphotilapiini (Figure 1B). Pairwise genetic distances of 10.6% and 1.4% in the mitochondrial and nuclear DNA, respectively, suggest that the two species are separated by several million years of independent evolution, which lies in the range of the eye-catching convergent species pairs observed between Lakes Tanganyika and Malawi [10]. But cichlids do not only resemble other endemic cichlids. The rare *Baileychromis centropomoides*, for example, is very similar in overall body shape to an endemic *Lates* sp. (Figures 3B and S3).

To quantify convergence in the LT cichlid species flock, we plotted relative morphological distance against phylogenetic distance for each pair of species and compared it to simulations of trait evolution (Figure 4A). Applying a conservative threshold (see Experimental Procedures), we identify 122 and 132 species pairs that are convergent in body and LPJ shape, respectively, which is about five times more than predicted by the models. Importantly, more than three quarters of these convergent species pairs overlap in habitat and depth distribution (Table S2), and they show a significantly greater

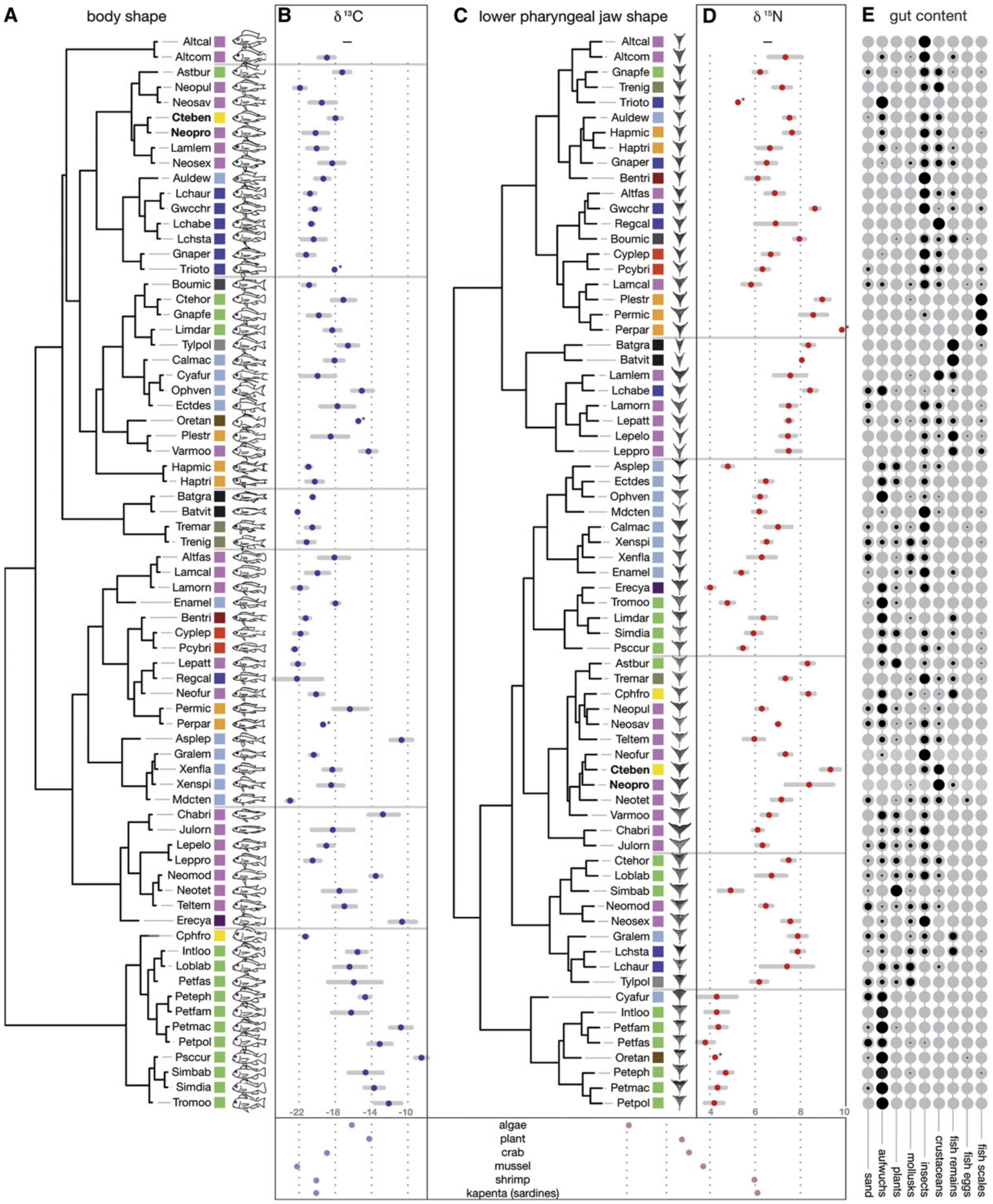

Figure 2. Ecomorphological Diversity in Cichlids from Lake Tanganyika

(A) Cluster analysis on the basis of 17 homologous landmarks on body shape.

(B) δ^{13}C stable isotope signatures.

(C) Cluster analysis on the basis of eight homologous and six sliding landmarks on the lower pharyngeal jaw bone.

(D) δ^{15}N stable isotope signatures.

(E) Results from the stomach and gut content analyses (in volume %).

Outlines in (A) are based on real photographs; images in (C) are taken from dissected LPJs (see Table S1 for details). The main morphoclusters are separated by gray lines, and the tribes are colored as in Figure 1. Colored dots in (B) and (D) represent average values; gray bars indicate 95% confidence limits of a t distribution. * marks species with too small a sample size, so that 95% confidence intervals were not calculated. The ratio between the rare isotope ^{13}C to ^{12}C (the δ^{13}C value) indicates the primary carbon source, which may vary between macrohabitats (e.g., benthic versus pelagic), whereas the δ^{15}N value (^{15}N to ^{14}N) serves as proxy for the relative trophic level of an organism. Accordingly, in LT cichlids, δ^{13}C values correlate with body shape clusters ($F = 2.66$, $p < 0.005$), whereas δ^{15}N values correlate with LPJ shape ($F = 4.03$, $p < 0.005$). Note that each trophic level is separated by approximately 3.4‰ in δ^{15}N from the one below. To facilitate comparisons, we also included average stable isotope values for some plant and animal species from LT (see box at the bottom).

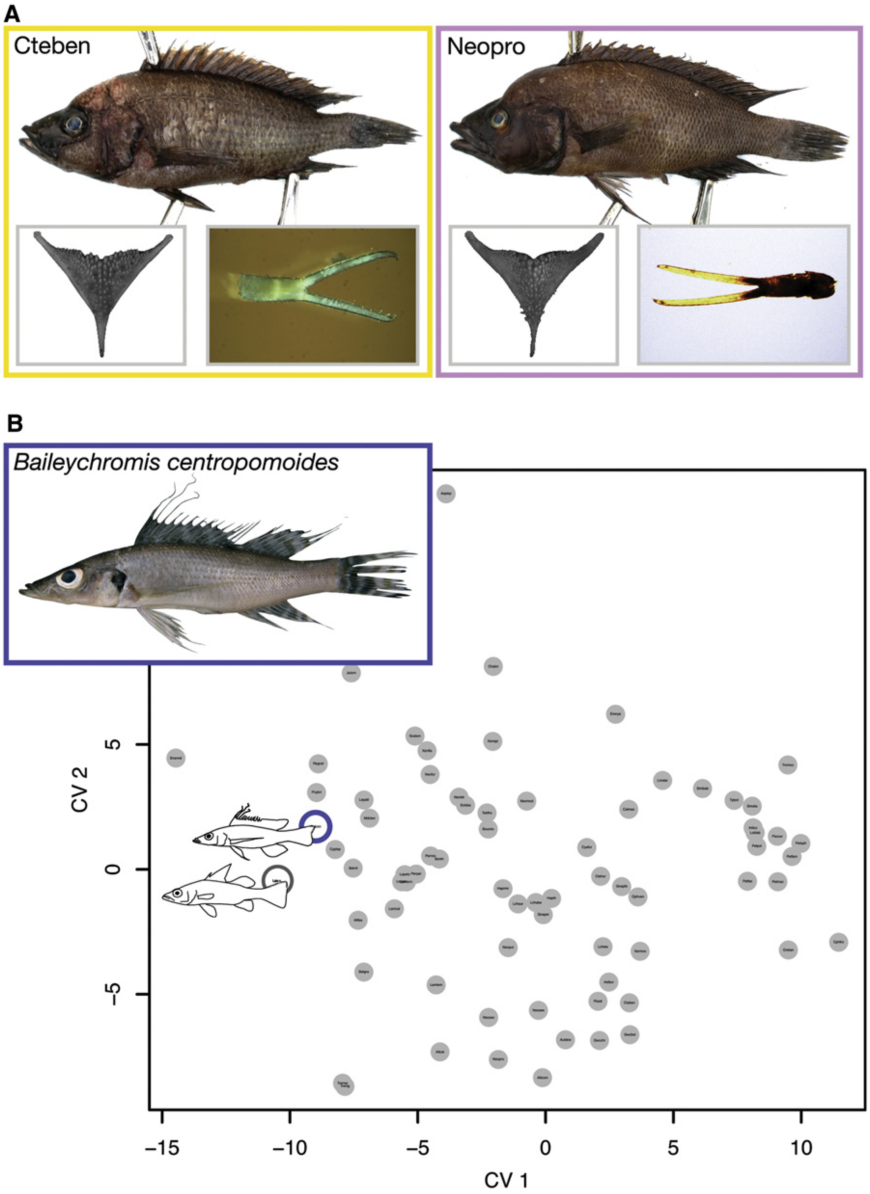

Figure 3. The Curious Cases of Convergent Evolution between "*Ctenochromis*" *benthicola* and *Neolamprologus prochilus* and between *Baileychromis centropomoides* and *Lates* sp.

(A) "*C.*" *benthicola* (Cteben) and *N. prochilus* (Neopro) are phylogenetically distinct (Figure 1) but show great similarities in morphology and in stable isotope signatures (Figure 2). For each species, the LPJ and a pincer of the freshwater shrimp *Limnocaridina* sp. (found in the stomach of the respective specimen) is shown.

(B) Canonical variates analysis showing that *B. centropomoides* is morphologically similar to *Lates* sp. endemic to LT (*B. centropomoides* shows the by far smallest Procrustes distance to *Lates*; see Figure S3). Each dot represents a species. Note that *Lates* used to be classified in the family Centropomidae until recently, which is where the species name for *Baileychromis* is derived from.

overlap in diet compared to random species pairs ($p < 0.05$ for body shape; $p < 0.0001$ for LPJ shape). These results demonstrate that cichlid communities within LT are characterized by the sympatric occurrence of convergent forms and that convergence is particularly prevalent in trophic morphology.

We then performed disparity-through-time (DTT) analyses to reconstruct convergent evolution along the evolutionary history of the species flock. The DTT analysis uncovers a large overlap in body morphology between the subclades emerging in the progress of the radiation (Figure 4B). The DTT plots on the basis of LPJ shape reveal that phases of larger subclade overlap are punctuated by a phase of neutral-like disparity. Overall, there is a strong signal of convergent evolution, which is unlikely to be explained by varying rates of speciation or of morphological evolution, because both have been shown to be rather constant in the cichlid adaptive radiation of LT [20, 25] (Figure S4). The DTT analyses thus suggest that convergent evolution in body and LPJ shape occurred throughout the time course of the radiation.

A large proportion of phenotypic differentiation in LT's cichlid assemblage occurred along only a few principal axes in morphospace (Figure 4C), which reflect adaptations to specific habitats and feeding regimes. For body shape, we detect divergence and convergence in the relative body height, which generally correlates with a pelagic or benthic lifestyle, respectively; the relative sizes of the head and trunk; the sizes of mouth and eye; and the position of the mouth. The divergent and convergent features of the LPJ involve its relative length and width (affecting lever ratios), the relative size and position of the posterior horns (important muscle attachment sites), and the shape of the toothed area. Interestingly, the DTT trajectory for LPJ shape largely coincides with the trajectory of the stable isotope data (Figure 4B), underpinning synchronized differentiation in both an important trophic character (the pharyngeal jaw apparatus) and the trophic niche (as approximated by stable isotopes). This once more confirms a strong link between morphology and ecology in LT cichlids.

In comparison with other renowned examples of adaptive radiation, the situation in LT is unique in its richness of convergent forms that evolved in situ and that coexist in the same habitats (Figures 2, 3, and 4). But what has triggered convergent evolution within the species flock of cichlids in LT? One possibility is that convergent evolution is a feature of advanced adaptive radiations, such as the LT cichlid species flock, which constitutes the relatively oldest cichlid radiation of the East African lakes. Representatives of distant lineages that independently adapt to the same habitat and the resources therein later in the radiation might then already be sufficiently distinct in certain life-history traits to enable coexistence. In the convergent species pair *N. prochilus* and "*C.*" *benthicola* (Figure 3), for example, the former is a substrate spawner, whereas the latter is a mouthbrooder. Convergence

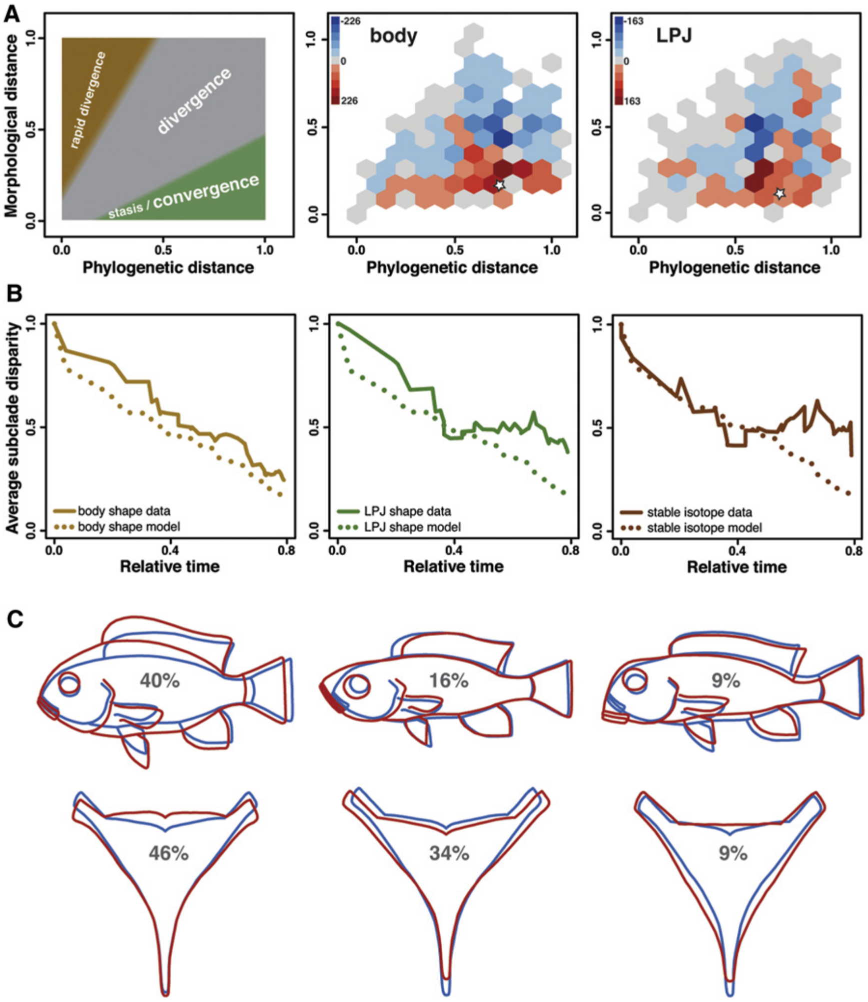

Figure 4. Convergence and Adaptive Disparity in the Cichlid Species Flock in Lake Tanganyika

(A) Pairwise distance-contrast plots showing the correlation between phylogenetic versus morphological distance. The expectation from neutral trait evolution (“divergence”) is a correlation between morphological and phylogenetic distance. Species pairs with small morphological yet large phylogenetic distance are indicative of stasis (in cases where there are no intermediate species with distinct morphologies) or convergent evolution [24]. To assess the prevalence of convergent evolution in body and jaw shape, we contrasted the positions occupied by all pairwise comparisons (n = 2,485) with those resulting from a Brownian motion model of trait evolution. We binned the data points into hexagons, the colors of which reflect the differential abundance of observed versus model comparisons. Different shades of blue indicate that our data contained fewer comparisons than expected from the model, whereas shades of red indicate that there were more pairwise comparisons in the data. The latter are predominant in the area indicative for convergence. The white asterisk marks the convergent species pair “*Ctenochromis*” *benthicola* and *Neolamprologus prochilus* (see Figure 3).

(B) Disparity-through-time (DTT) plots showing the average disparity retained in subclades (for body shape and LPJ shape and stable isotopes). Here, DTT plots inform about the time course of ecomorphological evolution. Moving along the phylogeny (from the root to the tips), the relative disparity of subclades is calculated at each internal node, averaged, and plotted against evolutionary time. The observed data is compared to a scenario of trait evolution estimated under a Brownian motion model (dotted line) on the same phylogeny. In order to avoid the effects of “tip overdispersion” due to missing terminal taxa, the most recent 20% of the plots were omitted.

(C) Shape changes along axes, which account for most of the divergence in the LT cichlid radiation. Axes are derived from evolutionary principal component analyses for body (first, second, and fourth axis) and LPJ shape (first, second, and third axis). The relative variance explained by each axis is given in percent.

(and niche overlap) would then be the product of secondary subradiations within the main Tanganyikan tribes [19, 20] superimposed upon each other—a stage that other adaptive radiations might not yet have reached. This scenario seems unlikely, though, given that our DTT analyses reveal a signal of convergence that is constantly high throughout the radiation (Figure 4B). Also, empirical studies comparing various adaptive radiations [26] and theoretical work [27] revealed that diversity appears to be greatest in radiations of intermediate ages and to actually decrease toward later stages. A second possibility is that convergent species initially emerged in isolation—e.g., when LT was temporarily split into separate basins during extremely low lake stands [28]—and only became admixed at a later stage of their evolution. Again, this does not seem to be compatible with our DTT and LTT analyses, which revealed that the signal of divergence and convergence is rather constant throughout the radiation and not restricted to certain periods—e.g., of lake level low stands—only.

That morphological differentiation resulted in convergence in LT might better be explained by the limited number of niches and, hence, adaptive zones (compared to the number of species) that cichlids can invade within the lake [29]. Alternatively, there might be a limit in the number of possible morphologies that cichlids can produce, due to some sort of developmental or genetic constraint [14]. The main morphoclusters in body and LPJ shape (Figure 2) might reflect such constraints. Perhaps it is also a combination of the finite number of niches and morphologies that explains convergence within the adaptive radiation of LT cichlids.

In any case, convergence in ecologically relevant traits within a single radiation is compatible with predictions made by current population ecology theory [12, 13]. It seems that self-organized similarity does not only play an important role in the maintenance of diversity, for example of plankton [30], but also in the rapid formation of organismal diversity via convergent evolution. Because resources are jointly used by several ecomorphologically similar and co-occurring cichlid species from distinct clades in LT, species numbers are maximized without increasing overall disparity. A key to the cichlid problem (i.e., why are there so many species?) might thus lie in the frequent occurrence of convergent evolution—not only between lakes but especially within a single lake and in adaptively relevant traits such as the LPJ. The question is now whether divergence via convergence is a more general pattern of diversification in species-rich communities. It would thus be of great interest to extend the kind of integrative analysis implemented in this study to other adaptive radiations and, especially, to the cichlid adaptive radiations in Lakes Malawi and Victoria. Even more so, because a recent comparison across 46 cichlid adaptive radiations [31] suggests that the LT radiation is an outlier from an otherwise more general trend in cichlid radiations, which appear to be triggered by both ecological opportunity and sexual selection.

Experimental Procedures

Sampling

Sampling was performed under permission from the Department of Fisheries, Lake Tanganyika Research Unit, Mpulungu, Zambia. In total, we sampled more than 1,000 specimens for this study (see Supplemental Experimental Procedures and Table S1 for further details).

Phylogenetic Analyses

We analyzed one mitochondrial (ND2) and two nuclear (*ednrb1*, *phpt*) markers (see Supplemental Experimental Procedures and Table S1 for GenBank accession numbers used in this study). We relied on maximum likelihood and Bayesian methods for phylogenetic analysis using PAUP*, MRBAYES, and the BEAST package. The appropriate model of molecular evolution for the heuristic tree searches in PAUP* was determined with JMODELTEST; MRBAYES was run for ten million generations with a burn-in of 10%; data were partitioned in BEAST. We first analyzed our core data set combining the mitochondrial and nuclear DNA sequences in 71 taxa, then the core data set including *Baileychromis centropomoides*, and, finally, a mitochondrial data set including the ND2 sequences of 180 taxa (i.e., ca. 90% of all Tanganyika species). Trees derived from the latter analysis were used for lineage-through-time plots. For incongruence testing, we applied the Kishino-Hasegawa (KH) and the Shimodaira-Hasegawa (SH) test implemented in PAUP*.

Geometric Morphometric and Morphological Analyses

We assessed the body shape of 1,049 individuals using landmark-based geometric morphometrics. xy coordinates of 17 landmarks, distributed across the whole fish body (see Figure S5A), and the scale of each picture were recorded using TPSDIG [32]. Aligned Procrustes coordinates were used for a pooled-within-species regression of shape against centroid size in MORPHOJ 1.02d [33]. Species averages were then used for principal component analysis (PCA), for disparity-through-time analyses, and for the calculation of pairwise distances between species. For LPJ assessment we recorded coordinates of eight true landmarks and 20 semilandmarks describing the outline of the bone (Figure S5B). We then clustered the species according to similarity in body and LPJ shape, using agglomerative hierarchical clustering in R.

Stomach and Gut Content Analyses

Contents were removed from the intestinal tracts of 506 specimens and separated up into one or more of the following categories: sand, aufwuchs (algae), plant material, mollusks, insects (imagines and larvae), crustaceans, fish (remains), fish eggs, and fish scales. We determined volume (in %) and weight (in μg) of each category.

Stable Isotope Analysis

White muscle tissue from 727 specimens (see Table S1) was dried, pulverized, and analyzed on an elemental analyzer (Thermo Finnigan) coupled to a Finnigan Delta V Advantage Isotope Ratio Mass Spectrometer (IRMS).

Pairwise Distance-Contrast Plots

To estimate the extent of convergence, we compared the phylogenetic distance to the morphological distance of each species pair [24]. The morphological distance was calculated as Euclidean distance from the pooled-within-species regressions of shape against centroid size using R's dist() function. In total, we had 2,485 species comparisons; therefore, we used hexagonal binning (x = 10 bins) to overcome overplotting. We also simulated neutral trait evolution on the phylogeny, using Brownian motion and Ornstein-Uhlenbeck models. Species comparisons that we derived from these simulations were then compared to our actual data by subtracting the binning counts of the simulations from those of the data. We tested for statistical significance of the difference of pointwise means between simulations and data (each 1/10 of the x axis) by bootstrapping (1,000 replications).

Disparity-through-Time Analysis

DTT analyses were performed according to Harmon et al. [34], comparing the observed data to a scenario of trait evolution estimated under a Brownian motion model. Positive deviations of the data from the simulations indicate a higher overlap in morphospace among subclades than would be expected under neutral evolution.

Evolutionary PCA

We estimated the ancestral character states for body and LPJ shape at each node in the phylogeny and calculated the extent and the direction of shape change along each branch. These branchwise estimates were then subjected to PCA to find the axes of greatest evolutionary divergence. All evolutionary PCAs were performed in MORPHOJ.

Supplemental Information

Supplemental Information includes five figures, two tables, Supplemental Experimental Procedures, and one movie and can be found with this article online at http://dx.doi.org/10.1016/j.cub.2012.10.048.

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Supplemental information for chapter 3

Current Biology, Volume *22*

Supplemental Information

Convergent Evolution within an Adaptive Radiation of Cichlid Fishes

Moritz Muschick, Adrian Indermaur, and Walter Salzburger

Supplemental Inventory

Supplemental Figures and Tables:

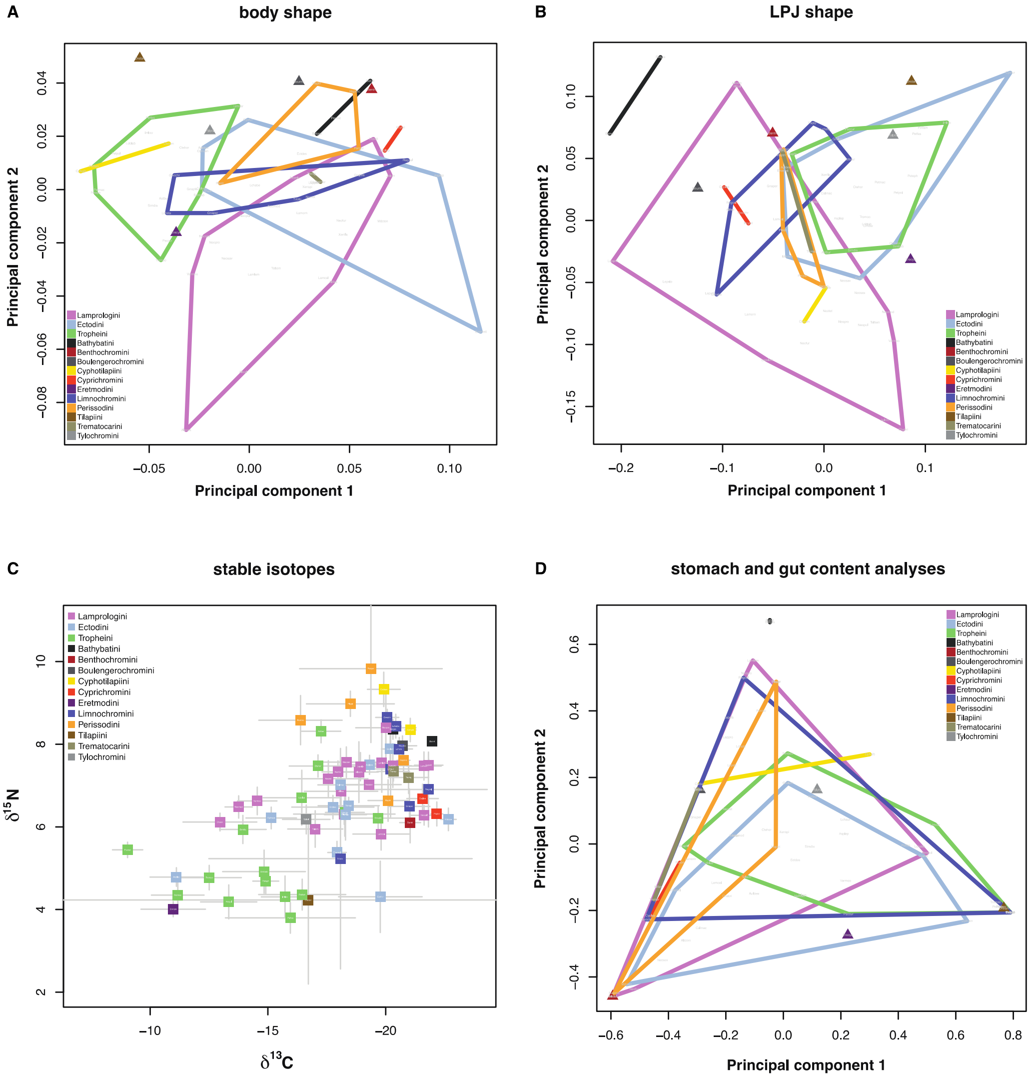


Figure S1. Morphometric Analysis of Lake Tanganyika Cichlid Fishes

Principal component analysis (PCA) of body shape (A) and LPJ shape (B) on the basis of the residuals from regression on centroid size from procrustes aligned landmarks showing a large overlap between tribes (see also [S1]). (C) Plot of stable isotope data (δ^{15}N *versus* δ^{13}C) for Lake Tanganyika cichlids. (D) Principal component analysis (PCA) of stomach and gut contents showing that the tribes largely overlap in resource use.

Filled triangles in (A, B, D) represent tribes for which only one species was analyzed; grey bars in (C) indicate t-based 95% confidence intervals.

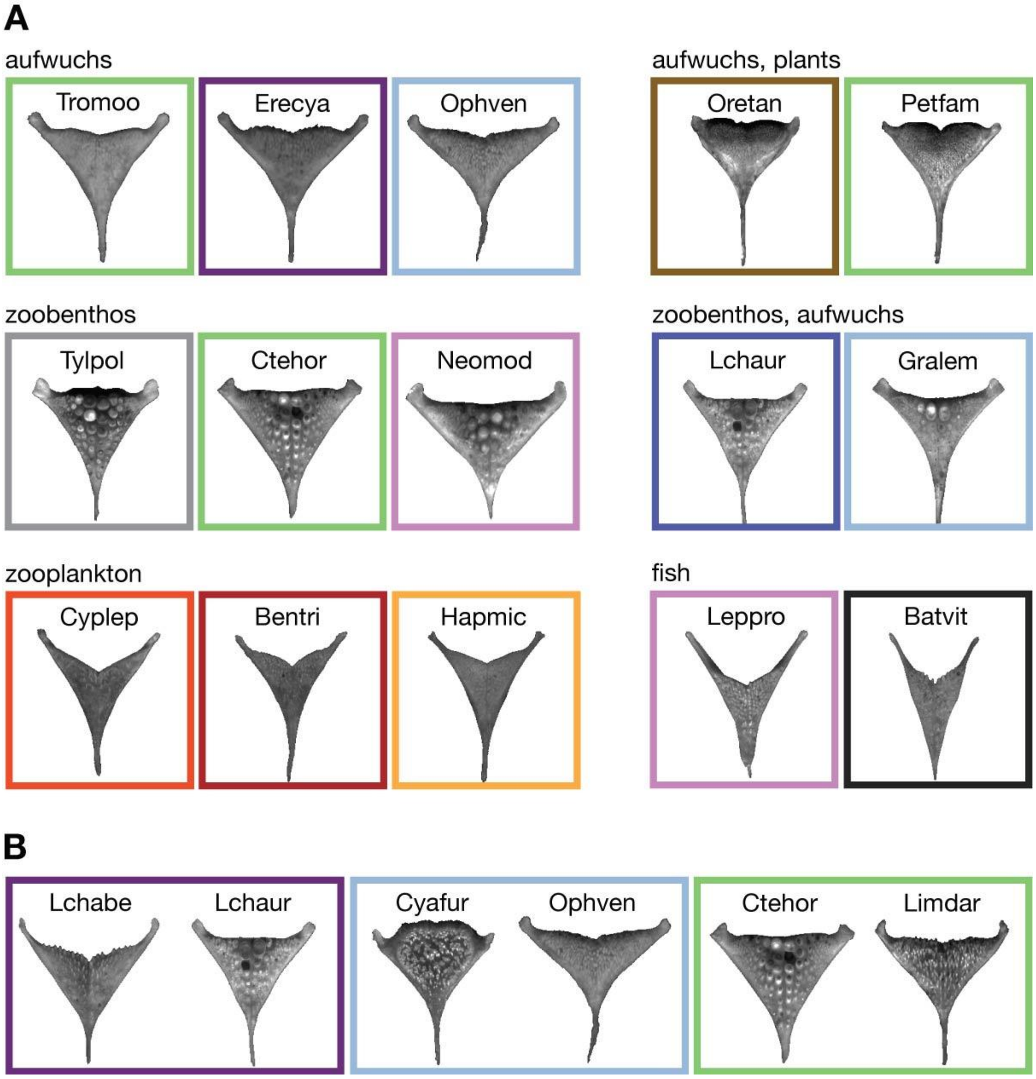


Figure S2. Convergence in Lake Tanganyika Cichlids
(A) Cichlid communities with convergent LPJs. The species in each panel belong to the same LPJ shape cluster (Figure 2C) and occur sympatrically (except for Bentri).
(B) Examples of three sister-species pairs with distinct LPJs. Colors refer to tribes (see Figure 1).

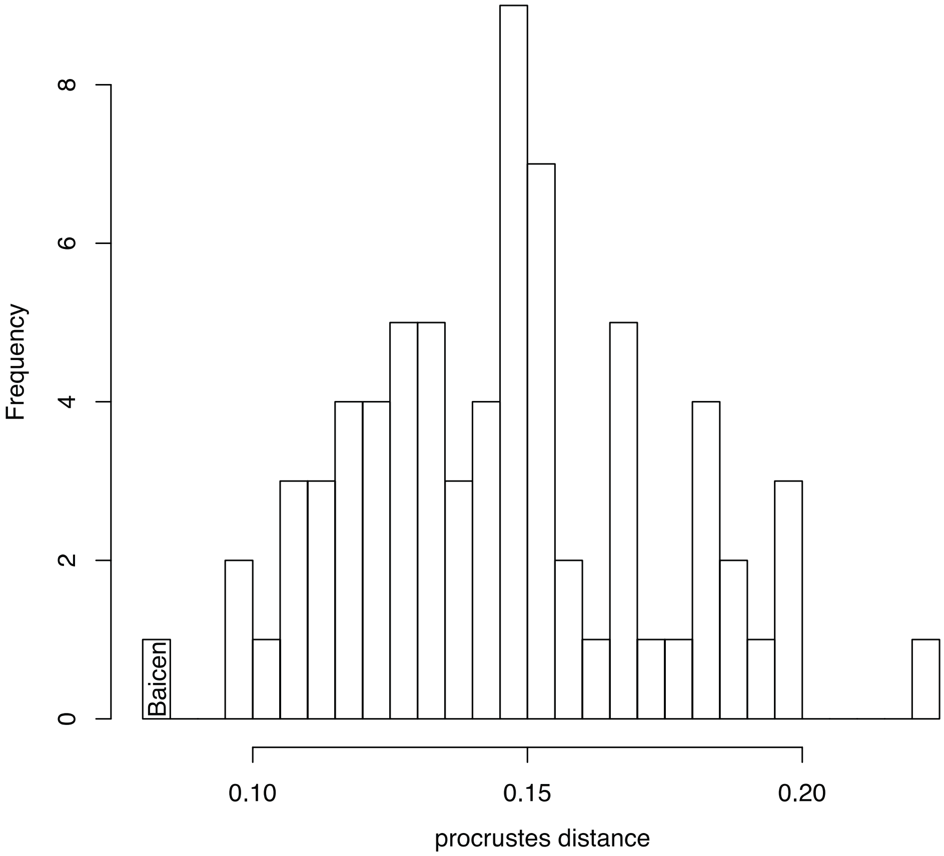


Figure S3. Similarity between a Cichlid and *Lates stappersi*
Frequency plot showing the procrustes distance based on body shape for each cichlid species in our core data set (plus *Baileychromis centropomoides*, Baicen) to *Lates stappersi*. Baicen shows the by far smallest distance of all cichlids examined.

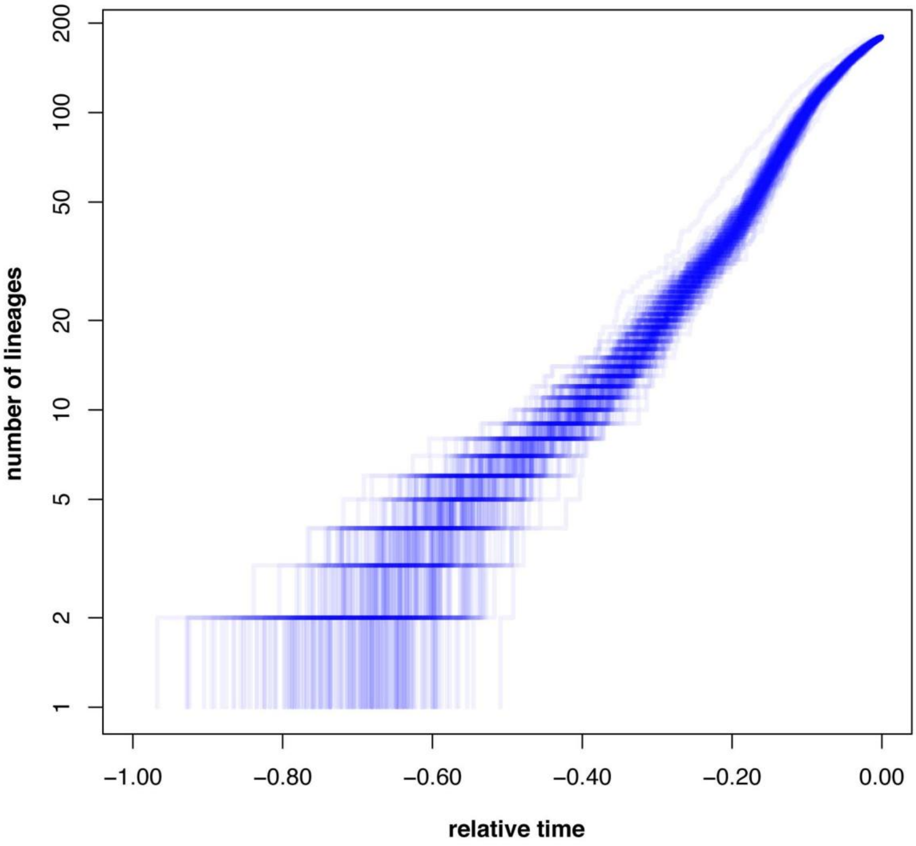


Figure S4. Lineage-through-Time Plot on the Basis of 180 Species of Lake Tanganyika Cichlids

From the posterior tree distribution, 200 trees were sampled and lineage through time (LTT) plotted individually to illustrate variance due to phylogenetic uncertainty.

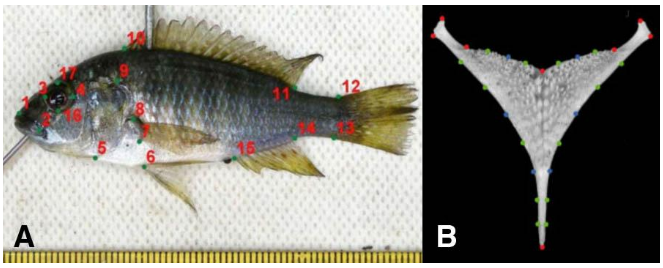


Figure S5. Distribution of Landmarks for the Morphometric Analyses of Overall Body Shape and LPJ Shape
Distribution for (A) overall body shape and (B) LPJ shape. Landmarks were treated differently in statistical analyses according to their color (see below for details).

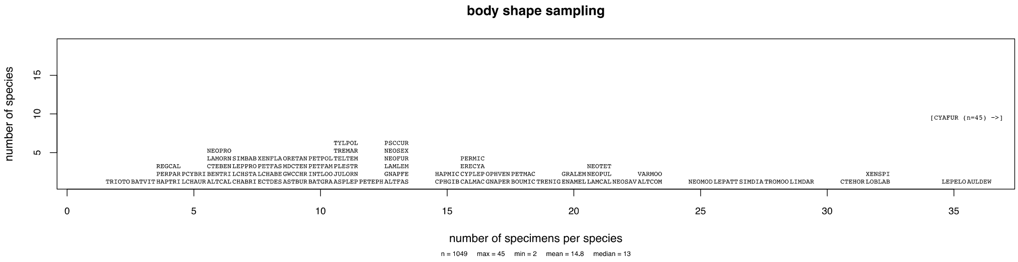
Table S1. List of Specimens Used in This Study

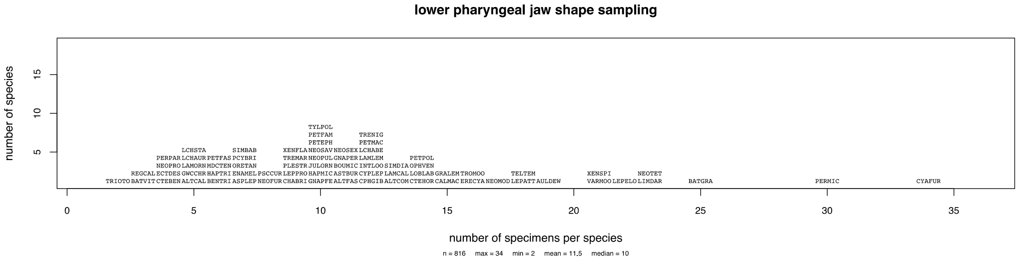
(A) Core dataset consisting of 71 species.

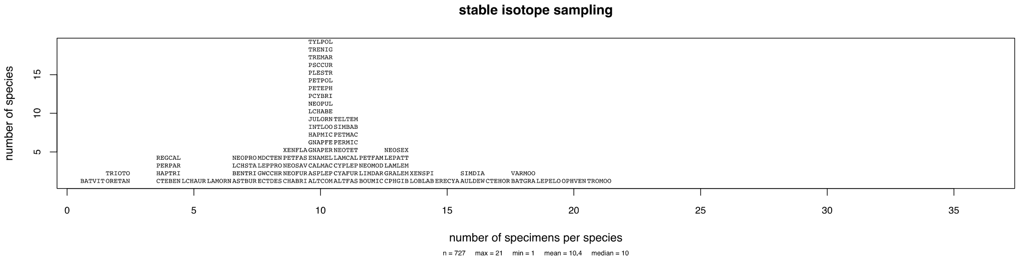
Taxonomic information					Number of specimens				GenBank accession numbers		
TID	**Taxon name**	**tribe**	**fish image**	**LPJ**	N_{body}	N_{LPJ}	N_{SIA}	N_{SGCA}	**ND2**	***ednrb***	***phpt***
Altcal	*Altolamprologus calvus*	Lamprologini			6	5	-	2	EF462256	JF900248	JF900177
Altcom	*Altolamprologus compressiceps*	Lamprologini			23	13	10	9	AF398229	JF900249	JF900178
Altfas	*Altolamprologus fasciatus*	Lamprologini			13	11	11	6	EF462255	JF900250	JF900179
Asplep	*Asprotilapia leptura*	Ectodini			11	7	10	9	AY337772	JF900251	JF900180
Astbur	*Astatotilapia burtoni*	Haplochromini			9	11	7	10	JF900319	JF900252	JF900181
Auldew	*Aulonocranus dewindtii*	Ectodini			36	19	16	10	AY337782	JF900253	JF900182
Batgra	*Bathybates graueri*	Bathybatini			10	25	18	2	AY663726	JF900254	JF900183
Batvit	*Bathybates vittatus*	Bathybatini			3	3	1	3	AY663728	JF900255	JF900184
Bentri	*Benthochromis tricoti*	Benthochromini			6	6	7	5	AF317264	JF900256	JF900185
Boumic	*Boulengerochromis microlepis*	Boulengerochromini			18	11	12	5	AF317229	JF900257	JF900186
Calmac	*Callochromis macrops*	Ectodini			16	15	10	10	AY337795	JF900258	JF900187
Chabri	*Chalinochromis brichardi*	Lamprologini			7	9	9	4	EF679241	JF900259	JF900188
Cphgib	*Cyphotilapia frontosa*	Cyphotilapiini			15	12	13	10	EF679242	JF900260	JF900189
Cteben	*Ctenochromis benthicola*	Cyphotilapiini			6	4	4	4	JF900320	JF900261	JF900190
Ctehor	*Ctenochromis horei*	Haplochromini			31	14	17	12	EU753935	JF900262	JF900191
Cyafur	*Cyathopharynx furcifer*	Ectodini			45	34	11	10	AY337781	JF900263	JF900192
Cyplep	*Cyprichromis leptosoma*	Cyprichromini			16	12	11	8	AF398224	JF900264	JF900193
Enamel	*Enantiopus melanogenys*	Ectodini			20	7	10	7	AY337770	JF900265	JF900194
Ectdes	*Ectodus descampsi*	Ectodini			8	4	8	2	AY337790	JF900266	JF900195
Erecya	*Eretmodus cyanostictus*	Eretmodini			16	16	15	9	AF398220	JF900267	JF900196
Gnaper	*Gnathochromis permaxillaris*	Limnochromini			17	11	10	9	JF900321	JF900268	JF900197
Gnapfe	*Gnathochromis pfefferi*	Tropheini			13	10	10	8	U07248	JF900269	JF900198
Gralem	*Grammatotria lemairii*	Ectodini			20	15	13	7	AY337787	JF900270	JF900199
Gwcchr	*Greenwoodochromis christyi*	Limnochromini			9	5	8	3	AY682528	JF900272	JF900201
Hapmic	*Haplotaxodon microlepis*	Perissodini			15	10	10	2	EF437497	JF900273	JF900202
Haptri	*Haplotaxodon trifasciatus*	Perissodini			4	6	4	11	EF437492	JF900274	JF900203
Intloo	*Interochromis loockii*	Tropheini			10	12	10	11	JF900322	JF900304	JF900232
Julorn	*Julidochromis ornatus*	Lamprologini			11	10	10	11	EF462229	JF900275	JF900204
Lamcal	*Lamprologus callipterus*	Lamprologini			21	13	11	10	AF398226	JF900276	JF900205
Lamlem	*Lamprologus lemairii*	Lamprologini			13	12	13	8	EF462271	JF900277	JF900206
Lamorn	*Lamprologus ornatipinnis*	Lamprologini			6	5	6	4	EF462260	JF900278	JF900207
Lchabe	*Limnochromis abeelei*	Limnochromini			8	12	10	3	AY682533	JF900279	JF900208
Lchaur	*Limnochromis auritus*	Limnochromini			5	5	5	3	AF398216	JF900281	JF900210
Lchsta	*Limnochromis staneri*	Limnochromini			7	5	7	5	AY682541	JF900271	JF900200
Lepatt	*Lepidiolamprologus attenuatus*	Lamprologini			26	18	13	11	EF462274	JF900282	JF900211
Lepelo	*Lepidiolamprologus elongatus*	Lamprologini			35	22	19	10	EF462268	JF900283	JF900212

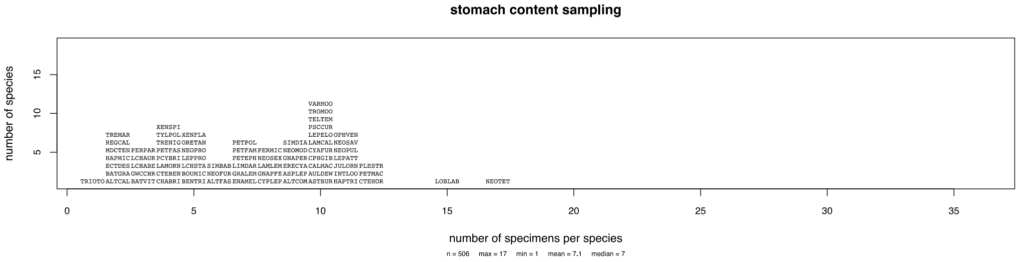
Leppro	*Lepidiolamprologus profundicola*	Lamprologini			7	9	8	5	EF462276	JF900284	JF900213
Limdar	*Limnotilapia dardennii*	Tropheini			29	23	12	7	GQ995724	JF900285	JF900214
Loblab	*Lobochilotes labiatus*	Tropheini			32	14	14	15	U07254	JF900286	JF900215
Mdcten	*Microdontochromis tenuidentatus*	Ectodini			9	6	8	2	AY337784	JF900287	JF900216
Neofur	*Neolamprologus furcifer*	Lamprologini			13	8	9	6	EF679252	JF900288	JF900217
Neomod	*Neolamprologus modestus*	Lamprologini			25	17	12	9	DQ055012	JF900289	JF900218
Neopro	*Neolamprologus prochilus*	Lamprologini			6	4	7	5	EF462248	JF900290	JF900219
Neopul	*Neolamprologus pulcher*	Lamprologini			21	10	10	11	EF462244	JF900291	JF900220
Neosav	*Neolamprologus savoryi*	Lamprologini			22	10	9	11	HM623796	JF900292	JF900221
Neosex	*Neolamprologus sexfasciatus*	Lamprologini			13	11	13	8	HM623828	JF900293	JF900222
Neotet	*Neolamprologus tetracanthus*	Lamprologini			21	23	11	17	EF462220	JF900294	JF900223
Ophven	*Ophthalmotilapia ventralis*	Ectodini			17	14	20	11	AY337774	JF900295	JF900224
Oretan	*Oreochromis tanganicae*	Tilapiini			9	7	2	5	AF317240	JF900296	JF900225
Pcybri	*Paracyprichromis brieni*	Cyprichromini			5	7	10	4	AY740378	JF900297	JF900226
Permic	*Perissodus microlepis*	Perissodini			16	30	11	8	AF398222	JF900298	JF900227
Perpar	*Perissodus paradoxus*	Perissodini			4	4	4	3	EF437500	JF900299	JF900228
Peteph	*Petrochromis ephippium*	Tropheini			12	10	10	7	JF900323	JF900300	JF900229
Petfam	*Petrochromis famula*	Tropheini			10	10	12	7	JF900324	JF900301	JF900230
Petfas	*Petrochromis fasciatus*	Tropheini			8	6	9	4	JF900325	JF900302	JF900231
Petmac	*Petrochromis macrognathus*	Tropheini			18	12	11	12	AY930068	JF900304	JF900233
Petpol	*Petrochromis polyodon*	Tropheini			10	14	10	7	JF900326	JF900305	JF900234
Plestr	*Plecodus straeleni*	Perissodini			11	9	10	12	EF437481	JF900306	JF900235
Psccur	*Pseudosimochromis curvifrons*	Tropheini			13	8	10	10	GQ995777	JF900307	JF900236
Regcal	*Reganochromis calliurus*	Limnochromini			4	3	4	2	AY682544	JF900308	JF900237
Simbab	*Simochromis babaulti*	Tropheini			7	7	11	6	GQ995782	JF900309	JF900238
Simdia	*Simochromis diagramma*	Tropheini			27	13	16	9	AY930087	JF900310	JF900239
Teltem	*Telmatochromis temporalis*	Lamprologini			11	18	11	10	EF462234	JF900311	JF900240
Tremar	*Trematocara marginatus*	Trematocarini			11	9	10	2	JF900327	JF900312	JF900241
Trenig	*Trematocara nigrifrons*	Trematocarini			19	12	10	4	JF900328	JF900313	JF900242
Trioto	*Triglachromis otostigma*	Limnochromini			2	2	2	1	AY337769	JF900280	JF900209
Tromoo	*Tropheus moorii*	Tropheini			28	16	21	10	AY930093	JF900314	JF900243
Tylpol	*Tylochromis polylepis*	Tylochromini			11	10	10	4	U07268	JF900315	JF900244
Varmoo	*Variabilichromis moorii*	Lamprologini			23	21	17	10	DQ055016	JF900316	JF900245
Xenfla	*Xenotilapia flavipinnis*	Ectodini			8	9	9	5	AY337794	JF900317	JF900246
Xenspi	*Xenotilapia spiloptera*	Ectodini			32	21	14	4	AY337788	JF900318	JF900247
total	**71**	**14**			**1049**	**816**	**727**	**506**			

(B) Frequency distribution of the specimens used for body and LPJ shape, and for stable isotope and stomach and gut content analyses.


body shape sampling


lower pharyngeal jaw shape sampling


stable isotope sampling


stomach content sampling

(C) Additional ND2 sequences used in the lineage-through-time (LTT) plots.

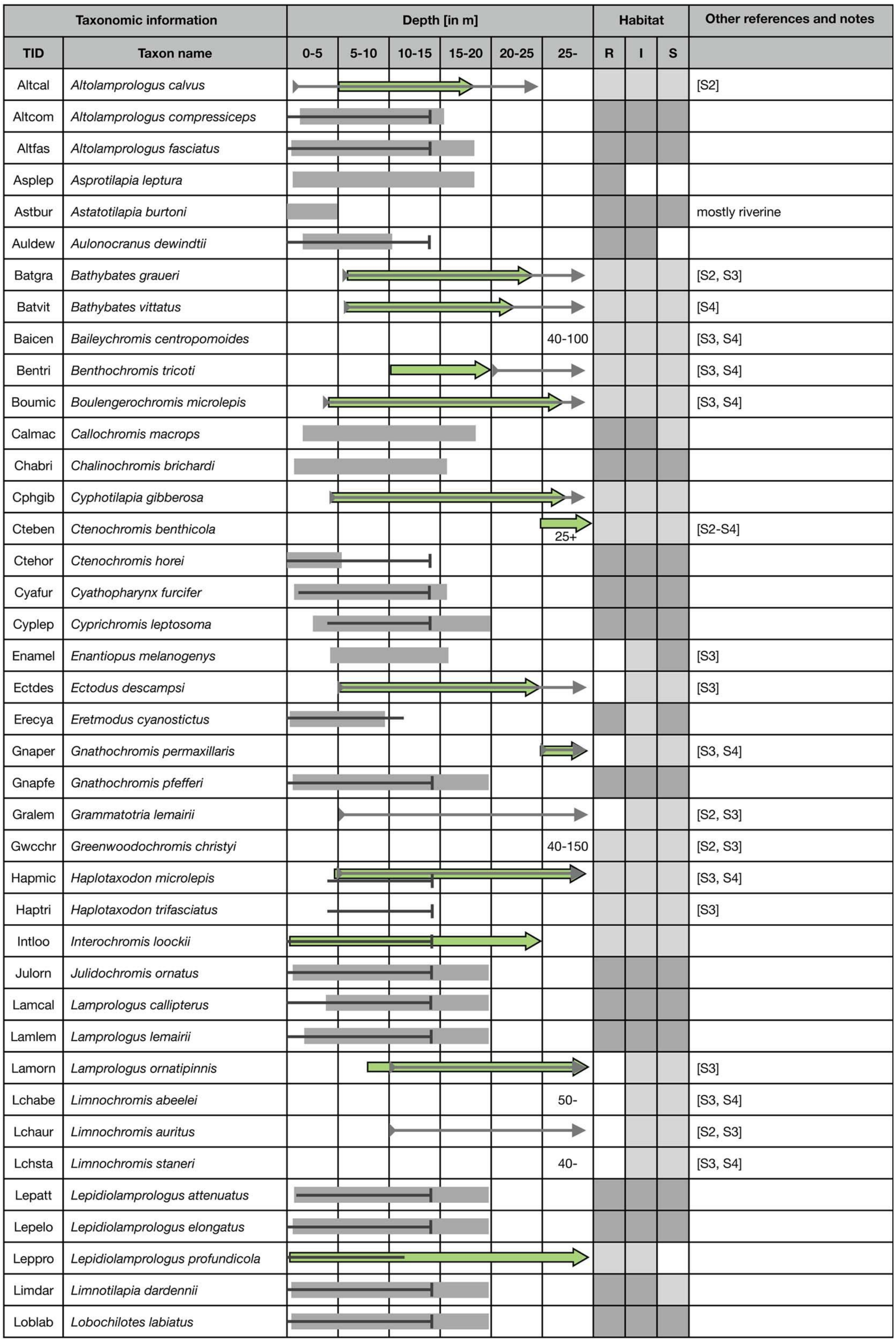
TID	**Taxon name**	**Tribe**	**GenBank accession number ND2**
Altshe	*Altolamprologus sp. 'shell'*	Lamprologini	EF191107
Baicen	*Baileychromis centropomoides*	Limnochromini	AY682509
Batfas	*Bathybates fasciatus*	Bathybatini	AY663732
Batfer	*Bathybates ferox*	Bathybatini	AY663736
Bathor	*Bathybates hornii*	Bathybatini	AY663735
Batleo	*Bathybates leo*	Bathybatini	AY663729
Batmin	*Bathybates minor*	Bathybatini	AY663722
Benmel	*Benthochromis melanoides*	Benthochromini	AY682512
Calple	*Callochromis pleurospilus*	Ectodini	AY337771
Calsta	*Callochromis stappersii*	Ectodini	AY337775
Carsch	*Cardiopharynx schoutedeni*	Ectodini	AY337791
Chapop	*Chalinochromis popeleni*	Lamprologini	U07244
Cunlon	*Cunningtonia longiventralis*	Ectodini	AY337780
Cypmic	*Cyprichromis microlepidotus*	Cyprichromini	AY740346
Cyppav	*Cyprichromis pavo*	Cyprichromini	AY740382
Cypzon	*Cyprichromis zonatus*	Cyprichromini	AY740347
Hemste	*Hemibates stenosoma*	Bathybatini	AY663716
Juldic	*Julidochromis dickfeldi*	Lamprologini	EF462230
Julmar	*Julidochromis marlieri*	Lamprologini	AF398230
Julreg	*Julidochromis regani*	Lamprologini	EF462228
Jultra	*Julidochromis transcriptus*	Lamprologini	EF462231
Lamkun	*Lamprologus kungweensis*	Lamprologini	EF191084
Lamlap	*Lamprologus laparogramma*	Lamprologini	EF462278
Lammel	*Lamprologus meleagris*	Lamprologini	DQ055027
Lamoce	*Lamprologus ocellatus*	Lamprologini	EF462259
Lamsig	*Lamprologus signatus*	Lamprologini	EF191086
Lamspe	*Lamprologus speciosus*	Lamprologini	EF191102
Lamteu	*Lamprologus teugelsi*	Lamprologini	DQ055059
Lepbou	*Lepidiolamprologus boulengeri*	Lamprologini	DQ055040
Lephec	*Lepidiolamprologus hecqui*	Lamprologini	DQ055041
Lepken	*Lepidiolamprologus kendalli*	Lamprologini	EF462269
Lepnka	*Lepidiolamprologus nkambae*	Lamprologini	EF462270
Lesper	*Lestradea perspicax*	Ectodini	AY337765
Lessta	*Lestradea stappersii*	Ectodini	AY337792
Mdcrot	*Microdontochromis rotundiventralis*	Ectodini	AY337793
Neobif	*Neolamprologus bifasciatus*	Lamprologini	HM623809
Neobre	*Neolamprologus brevis*	Lamprologini	EF462264
Neobri	*Neolamprologus brichardi*	Lamprologini	AF398227
Neobue	*Neolamprologus buescheri*	Lamprologini	EF462243
Neocal	*Neolamprologus calliurus*	Lamprologini	DQ093112
Neocau	*Neolamprologus caudopunctatus*	Lamprologini	EF462272
Neochr	*Neolamprologus christyi*	Lamprologini	HM623826
Neocun	*Neolamprologus cunningtoni*	Lamprologini	DQ055054
Neocyl	*Neolamprologus cylindricus*	Lamprologini	EF462224
Neodev	*Neolamprologus devosi*	Lamprologini	EF437476
Neofal	*Neolamprologus falcicula*	Lamprologini	EF462246
Neogra	*Neolamprologus gracilis*	Lamprologini	HM623798
Neohel	*Neolamprologus helianthus*	Lamprologini	DQ055013
Neolel	*Neolamprologus leleupi*	Lamprologini	EF462251
Neolou	*Neolamprologus leloupi*	Lamprologini	EF191103
Neoloc	*Neolamprologus longicaudata*	Lamprologini	EF462250
Neolon	*Neolamprologus longior*	Lamprologini	HM623793

Neomar	*Neolamprologus marunguensis*	Lamprologini	AY740390
Neomee	*Neolamprologus meeli*	Lamprologini	DQ055051
Neomon	*Neolamprologus mondabu*	Lamprologini	EF462242
Neomul	*Neolamprologus multifasciatus*	Lamprologini	EF462266
Neomux	*Neolamprologus mustax*	Lamprologini	EF462223
Neonig	*Neolamprologus niger*	Lamprologini	AY740391
Neogri	*Neolamprologus nigriventris*	Lamprologini	EF462239
Neoobs	*Neolamprologus obscurus*	Lamprologini	HM623824
Neooli	*Neolamprologus olivaceous*	Lamprologini	AY740393
Neopec	*Neolamprologus pectoralis*	Lamprologini	EF462238
Neopet	*Neolamprologus petricola*	Lamprologini	HM623827
Neosim	*Neolamprologus similis*	Lamprologini	EF462261
Neosek	*Neolamprologus sp. 'eseki'*	Lamprologini	HM623794
Neokip	*Neolamprologus sp. 'Kipili'*	Lamprologini	HM623802
Neondo	*Neolamprologus sp. 'ndobnoi'*	Lamprologini	HM623802
Neospl	*Neolamprologus splendens*	Lamprologini	HM623799
Neotoa	*Neolamprologus toae*	Lamprologini	EF462222
Neotre	*Neolamprologus tretocephalus*	Lamprologini	EF462219
Neovar	*Neolamprologus variostigma*	Lamprologini	EF462253
Neoven	*Neolamprologus ventralis*	Lamprologini	EF462233
Neowal	*Neolamprologus walteri*	Lamprologini	HM623808
Neowau	*Neolamprologus wauthioni*	Lamprologini	EF191118
Ophboo	*Ophthalmotilapia boops*	Ectodini	AY337773
Ophhet	*Ophthalmotilapia heterodonta*	Ectodini	EF679254
Ophnas	*Ophthalmotilapia nasuta*	Ectodini	AY337783
Pcynig	*Paracyprichromis nigripinnis*	Cyprichromini	AY740339
Perecc	*Perissodus eccentricus*	Perissodini	EF437511
Petort	*Petrochromis orthognathus*	Tropheini	U07262
Petkat	*Petrochromis sp. 'Katete'*	Tropheini	GQ995748
Petmos	*Petrochromis sp. 'moshi'*	Tropheini	GQ995765
Pettex	*Petrochromis sp. 'Texas'*	Tropheini	GQ995766
Pettre	*Petrochromis trewavasae*	Tropheini	GQ995761
Pleela	*Plecodus elaviae*	Perissodini	EF437504
Plemul	*Plecodus multidentatus*	Perissodini	EF437505
Simmar	*Simochromis marginatus*	Tropheini	AY930088
Simple	*Simochromis pleurospilus*	Tropheini	GQ995783
Spaery	*Spathodus erythrodon*	Eretmodini	DQ055008
Spamar	*Spathodus marlieri*	Eretmodini	HM623786
Tanirs	*Tanganicodus irsacae*	Eretmodini	DQ055007
Telbif	*Telmatochromis bifrenatus*	Lamprologini	AF398228
Telbri	*Telmatochromis brichardi*	Lamprologini	EF462236
Teldho	*Telmatochromis dhonti*	Lamprologini	EF679266
Telvit	*Telmatochromis vittatus*	Lamprologini	EF462237
Ttcmac	*Telotrematocara macrostoma*	Trematocarini	AY663715
Treuni	*Trematocara unimaculatum*	Trematocarini	AF317268
Trioto	*Triglachromis otostigma*	Limnochromini	AF398217
Trobri	*Tropheus brichardi*	Tropheini	AY930086
Trodub	*Tropheus duboisi*	Tropheini	AY930085
Tropol	*Tropheus polli*	Tropheini	AY930084
Xenhec	*Xenochromis hecqui*	Ectodini	EF437513
Xenbat	*Xenotilapia bathyphila*	Ectodini	AY337789
Xenbou	*Xenotilapia boulengeri*	Ectodini	HM135111
Xencau	*Xenotilapia caudafasciata*	Ectodini	AY337777
Xenlon	*Xenotilapia longispinis*	Ectodini	AY337779

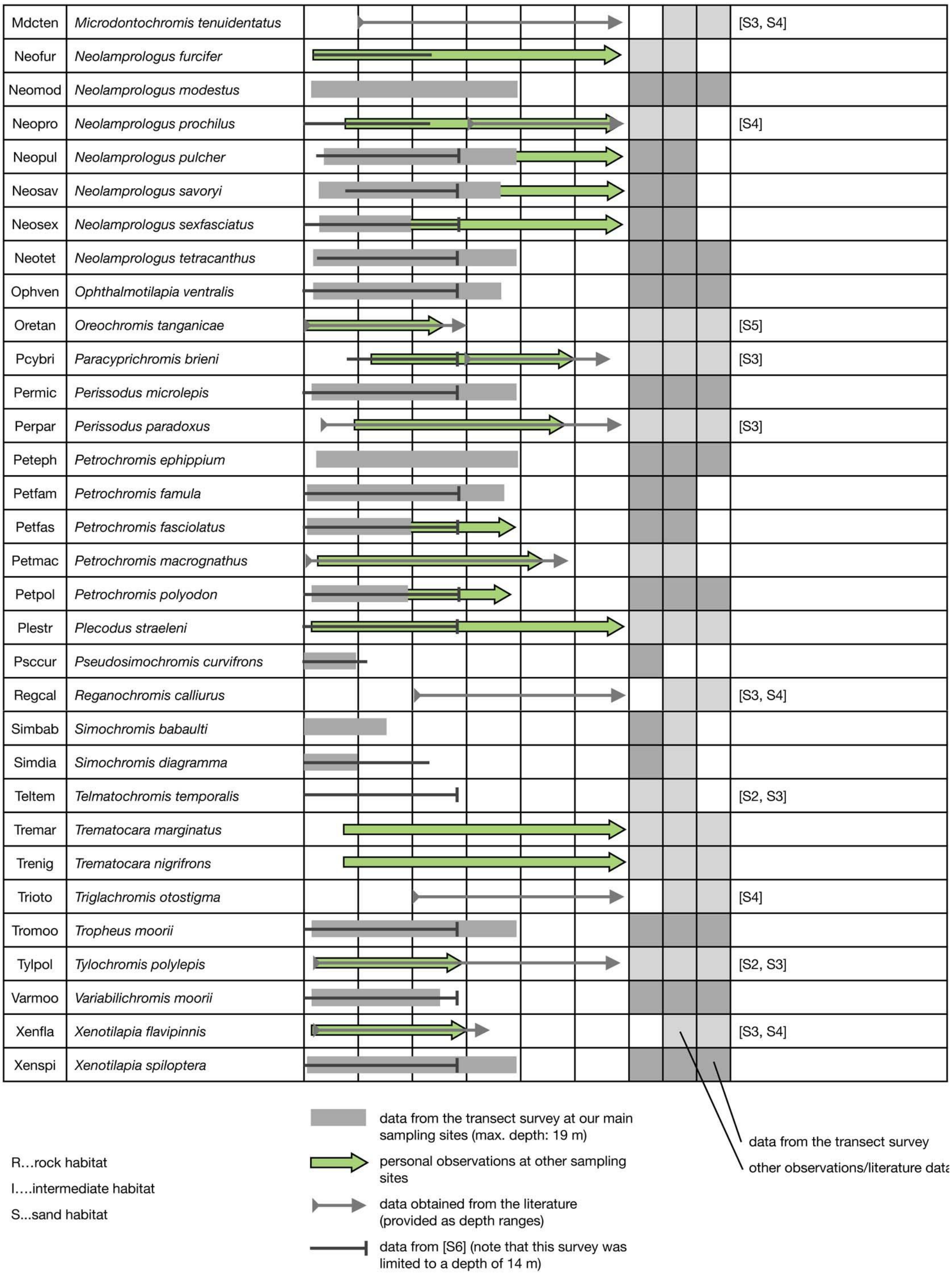
Xenoch	*Xenotilapia ochrogenys*	Ectodini	AY337767
Xensim	*Xenotilapia sima*	Ectodini	AY337785
Xenpap	*Xenotilapia sp. 'papilio sunflower'*	Ectodini	AY337776

TID	Taxon identifier, which is also used in Figures 1 and 2
LPJ	Lower pharyngeal jaw bone
$\mathbf{N_{body}}$	Number of specimens used for morphometric analyses of body shape
$\mathbf{N_{LPJ}}$	Number of specimens used for morphometric analyses of the lower pharyngeal jaw bone
$\mathbf{N_{SIA}}$	Number of specimens used for stable isotope analyses
$\mathbf{N_{SGCA}}$	Number of specimens used for stomach and gut content analyses

Table S2. Depth Distribution of Species Used in This Study



Taxonomic information		Depth [in m]						Habitat			Other references and notes
TID	Taxon name	0-5	5-10	10-15	15-20	20-25	25-	R	I	S	
Altcal	*Altolamprologus calvus*							░	░	░	[S2]
Altcom	*Altolamprologus compressiceps*							█	█	█	
Altfas	*Altolamprologus fasciatus*							█	█	█	
Asplep	*Asprotilapia leptura*							█			
Astbur	*Astatotilapia burtoni*							█	█	█	mostly riverine
Auldew	*Aulonocranus dewindtii*							█	█		
Batgra	*Bathybates graueri*							░	░	░	[S2, S3]
Batvit	*Bathybates vittatus*							░	░	░	[S4]
Baicen	*Baileychromis centropomoides*						40-100	░	░	░	[S3, S4]
Bentri	*Benthochromis tricoti*							░	░	░	[S3, S4]
Boumic	*Boulengerochromis microlepis*							░	░	░	[S3, S4]
Calmac	*Callochromis macrops*							█	█	░	
Chabri	*Chalinochromis brichardi*							█	█	█	
Cphgib	*Cyphotilapia gibberosa*							░	░	░	
Cteben	*Ctenochromis benthicola*						25+	░	░	░	[S2-S4]
Ctehor	*Ctenochromis horei*							█	█	█	
Cyafur	*Cyathopharynx furcifer*							█	█	█	
Cyplep	*Cyprichromis leptosoma*							█	█	█	
Enamel	*Enantiopus melanogenys*								░	█	[S3]
Ectdes	*Ectodus descampsi*								░	░	[S3]
Erecya	*Eretmodus cyanostictus*							█	░	█	
Gnaper	*Gnathochromis permaxillaris*								░	░	[S3, S4]
Gnapfe	*Gnathochromis pfefferi*							█	█	█	
Gralem	*Grammatotria lemairii*								░	░	[S2, S3]
Gwcchr	*Greenwoodochromis christyi*						40-150	░	░	░	[S2, S3]
Hapmic	*Haplotaxodon microlepis*							░	░	░	[S3, S4]
Haptri	*Haplotaxodon trifasciatus*							░	░	░	[S3]
Intloo	*Interochromis loockii*							░	░	░	
Julorn	*Julidochromis ornatus*							█	█	█	
Lamcal	*Lamprologus callipterus*							█	█	█	
Lamlem	*Lamprologus lemairii*							█	█	█	
Lamorn	*Lamprologus ornatipinnis*								░	░	[S3]
Lchabe	*Limnochromis abeelei*						50-		░	░	[S3, S4]
Lchaur	*Limnochromis auritus*								░	░	[S2, S3]
Lchsta	*Limnochromis staneri*						40-		░	░	[S3, S4]
Lepatt	*Lepidiolamprologus attenuatus*							█	█	█	
Lepelo	*Lepidiolamprologus elongatus*							█	█	█	
Leppro	*Lepidiolamprologus profundicola*							░	░		
Limdar	*Limnotilapia dardennii*							█	█	░	
Loblab	*Lobochilotes labiatus*							█	█	█	



		R	I	S	
Mdcten	*Microdontochromis tenuidentatus*		░	░	[S3, S4]
Neofur	*Neolamprologus furcifer*	░	░		
Neomod	*Neolamprologus modestus*	▓	▓	▓	
Neopro	*Neolamprologus prochilus*	░	░		[S4]
Neopul	*Neolamprologus pulcher*	▓	▓		
Neosav	*Neolamprologus savoryi*	▓	▓		
Neosex	*Neolamprologus sexfasciatus*	▓	▓		
Neotet	*Neolamprologus tetracanthus*	▓	▓	▓	
Ophven	*Ophthalmotilapia ventralis*	▓	▓	▓	
Oretan	*Oreochromis tanganicae*	░	░	░	[S5]
Pcybri	*Paracyprichromis brieni*	░	░	░	[S3]
Permic	*Perissodus microlepis*	▓	▓	▓	
Perpar	*Perissodus paradoxus*	░	░	░	[S3]
Peteph	*Petrochromis ephippium*	▓	▓	▓	
Petfam	*Petrochromis famula*	▓	▓		
Petfas	*Petrochromis fasciolatus*	▓	▓		
Petmac	*Petrochromis macrognathus*	░	░		
Petpol	*Petrochromis polyodon*	▓	▓	▓	
Plestr	*Plecodus straeleni*	░	░	░	
Psccur	*Pseudosimochromis curvifrons*	▓			
Regcal	*Reganochromis calliurus*		░	░	[S3, S4]
Simbab	*Simochromis babaulti*	▓	░		
Simdia	*Simochromis diagramma*	▓	░		
Teltem	*Telmatochromis temporalis*	░	░		[S2, S3]
Tremar	*Trematocara marginatus*	░	░	░	
Trenig	*Trematocara nigrifrons*	░	░	░	
Trioto	*Triglachromis otostigma*		░	░	[S4]
Tromoo	*Tropheus moorii*	▓	▓	▓	
Tylpol	*Tylochromis polylepis*	░	░	░	[S2, S3]
Varmoo	*Variabilichromis moorii*	▓	▓	▓	
Xenfla	*Xenotilapia flavipinnis*		░	░	[S3, S4]
Xenspi	*Xenotilapia spiloptera*	▓	▓	▓	

R…rock habitat
I….intermediate habitat
S...sand habitat

data from the transect survey at our main sampling sites (max. depth: 19 m)

personal observations at other sampling sites

data obtained from the literature (provided as depth ranges)

data from [S6] (note that this survey was limited to a depth of 14 m)

▓ data from the transect survey

░ other observations/literature dat

Supplemental Experimental Procedures

Sampling

Sampling at Lake Tanganyika, East Africa, was performed in autumn 2007, 2008, and 2011, and in spring 2010 under the permission and with guidance from the Department of Fisheries, Lake Tanganyika Research Unit, Mpulungu, Republic of Zambia. Cichlid fishes were caught with gill-nets set by snorkeling and scuba diving, by harpooning, by angling, or, in a few cases, obtained from local fishermen. For sample preparation in the field, we followed our standard operating procedure (SOP): Fishes were sized (total and standard length), weighted, sexed (whenever possible) and photographed in a standardized way using either a Nikon Coolpix P5000 or a Nikon D5000 digital camera; then, a fin-clip and a piece of white muscle tissue were taken as tissue sample (for DNA extraction and stable isotope analysis) and preserved in 96% ethanol; finally, we dissected and sun-dried the lower pharyngeal jaw apparatus and preserved the intestines in ethanol for stomach and gut content analyses. Two specimens per species were taken as voucher and preserved in ethanol. In total, we sampled more than 1000 specimens for this study (see Table S1 for details). The core dataset contains 71, thus covering more than a third of all Tanganyikan cichlid species, including all major lineages (‘tribes’), and about 80% of the recognized genera. Note that we use a six letter code for the species, with the first three letters indicating the genus name and the last three letters abbreviating the species name.

Line Transect Survey

In order to obtain depth-distribution and habitat data for the most common species in our core data set, we performed transect surveys using scuba diving at our three main sampling locations in the South of Lake Tanganyika (in August and September 2011; see Table S2). Two independent rounds of fish counts were performed at each of the three locations. The sampling sites were: Toby_right_1 (8° 37' 20.97" S 31° 12' 00.37" E; transect length: 70 m), Toby_right_2 (8° 37' 19.31" S 31° 11' 59.58" E; transect length: 108 m), Toby_left_1 (8° 37' 28.79" S 31° 12' 01.75" E; transect length: 98 m), Toby_left_2 (8° 37' 30.40" S 31° 12' 01.23" E; transect length: 106 m), Mbita_1 (8° 45' 16.57" S 31° 05' 23.74" E; transect length: 60 m), and Mbita_2 (8° 45' 16.75" S 31° 05' 21.92" E; transect length: 50 m).

We used a 120 m rope with markings every 2 m, which was placed in a 90° angle to the shore. The end of the transect was determined by the beginning of sandy flats, where fish densities approximate null. Before starting with the transect dives, we determined the depth of each 2 m marking with a diving computer (Suunto Gekko) and recorded the habitat between two consecutive markings as rocks (R), sand (S) and intermediate between sand and rocks (I). Scuba dives were performed in teams of two or three divers, who recorded a predefined set of species as they were diving along the transect line and in an area of 2 m left and right of the rope. At the end of the rope, the divers rested for a period of 10 min in order to leave enough time for the fish to restore. After that, the divers returned to the shore counting the same set of species a second time (see [7]). Up to five transect dives were performed at each transect; the more shallow areas were partly covered by snorkeling.

Phylogenetic Analyses

DNA Extraction DNA was extracted from ethanol preserved tissue samples (see above) using a Qiagen Biosprint 96 DNA extraction robot and following the manufacturer’s protocol.

Molecular Methods PCR amplification of the entire mitochondrial NADH Dehydrogenase Subunit 2 (ND2) gene followed the strategy described before [20] – this time, however, using Sigma RedTaq DNA polymerase (Sigma Aldrich). For the amplification of the two nuclear gene segments, *ednrb1* and *phpt*, we used the Phusion High-Fidelity DNA polymerase (New England BioLabs) in a total volume of 20µl (10µl Phusion High-Fidelity DNA master mix, 6µl water, 1µl of each primer [10µM], and 2 µl of diluted DNA extract [1:10]). For *ednrb1*, we used published primers [S8, S9]. The primers for *phpt* were 38a_F (5’-AGC AGG GTT GAC CTT CTC AA - 3’) and 38a_R (5’ – TGG CTA AAA TCC CCG ATG TA – 3’). PCR products were purified with the ExoSAP-IT protocol (USB) and used as template for cycle sequencing reactions in both directions with the BigDye Terminator v.3.1 kit (Applied Biosystems) in 10µl reactions. After dye removal with the BigDye XTerminator purification kit (Applied Biosystems), samples were run on an ABI3130*xl* capillary genetic analyzer (Applied Biosystems). All sequences were checked by eye and assembled with CODONCODEALIGNER v.3.5.6 (CodonCode Corporation). ND2 sequences for most of the species were already available from previous studies [20, 21, 34, S10]; all sequences of the nuclear loci have been newly sequenced. GenBank accession numbers of all sequences used in this study are shown in Table S1.

Phylogenetic Inference No additional alignment procedure was necessary for ND2 (all sequences had the identical length of 1'047 bp); the two nuclear gene segments were aligned with MAFFT [S11] resulting in an alignment length of 542 bp for *ednrb1* and 424 bp for *phpt*. We relied on maximum likelihood and Bayesian methods for phylogenetic analysis using PAUP* [S12], MRBAYES [S13] and the BEAST package [S14]. The appropriate model of molecular evolution for the heuristic tree searches in PAUP* was determined with JMODELTEST [S15] and applying the Akaike Information Criterion. MRBAYES was run for 10'000'000 generations with a burn-in of 10% (after monitoring the level of convergence). Data were partitioned in BEAST. Three rounds of analyses were performed, first with the core data set combining the mitochondrial and nuclear DNA sequences in 71 taxa, then with the core data set including *Baileychromis centropomoides,* and third with a mitochondrial data set including the ND2 sequences of 180 taxa (i.e. ca. 90% of all Tanganyika species). The latter analysis was aimed as starting point for the lineage-through-time plots (see below).

Incongruence Testing To statistically test for incongruence between the molecular phylogeny and the grouping of taxa according to their overall and trophic morphology ('cluster analysis'; see below), we applied two classic tests implemented in PAUP*, the Kishino-Hasegawa (KH) and the Shimodaira-Hasegawa (SH) test both under a resampling-estimated log-likelihood (RELL). Note that these tests merely inform that the two topologies built from morphological characters are not supported by our molecular data and cannot *per se* be taken as evidence for convergent evolution. Valid tests for evaluating convergent evolution (pairwise distance-contrast and disparity-through-time plots) and are described below.

Lineage-Through-Time Plots In order to reconstruct diversification rates in the species flock of cichlids from Lake Tanganyika, we performed a LTT analysis with our new extensive data set including about 90% of all species. Such an analysis has been conducted before [21], albeit with a smaller data set. Still, we follow the exact same procedure as described before [21] using BEAST and the APE package [S16] in R. The main difference to the study of Day *et al.* [21] is that we refrain from inferring an absolute time scale for the Lake Tanganyika radiation, due to the lack of fossil calibrations and uncertainties with respect to the onset of the radiation (see discrepancies in previous estimates; [20, 24, 34, S10, S17]). Instead, we use a relative timing, just as with the disparity through time plots (see below), allowing for maximum compatibility between disparity and diversity plots.

Geometric Morphometric and Morphological Analyses

Body Shape We assessed the body shape of 1049 individuals using landmark-based geometric morphometric methods. The exact numbers of specimen per species are given in Table S1. *xy* coordinates of 17 landmarks, distributed across the whole fish body (see Figure. S7A), and the scale of each picture were recorded using TPSDIG [30]. Raw landmark coordinates were procrustes aligned and the resulting procrustes coordinates were used for a pooled-within-species regression of shape against centroid size in MORPHOJ 1.02d [31]. The resulting residuals were averaged for each species and used for principal component analysis (PCA), disparity through time analyses, and for the calculation of pairwise distances between species.

In a second analysis, focusing specifically on the similarity between *Baileychromis centropomoides* and *Lates sp.*, we determinded the landmark configurations of *B. centropomoides* (N=4) and all four endemic *Lates* species (*L. angustifrons*, *L. mariae*, *L. microlepis* and *L. stappersi;* based on drawings from [S18]). We first performed a canonical variates analysis (CVA) in MORPHOJ with the data from *B. centropomoides* and *Lates* and then incorporated *B. centropomoides* and *L. stappersi* (the most similar species) into the core data set and performed another CVA (Figure 3B). We also determined procrustes distances of all cichlid species to *L. stappersi*. *B. centropomoides* shows the by far smallest procrustes distances to *L. stappersi*.

Pharyngeal Jaw Shape For LPJ assessment we recorded *xy* coordinates of 28 evenly distributed landmarks describing the outline of the bone (Figure S7B). We arranged two sets of nine equidistant lines perpendicular to the posterior outline and the anterior-posterior axis respectively. That way, we could treat the intersections of these lines with the outline of the jaw as semi-landmarks. Our initial set was composed of 8 true landmarks and 20 semi-landmarks. We subjected this data set to an iterative sliding-process in TPSRELW (10 iterations) using the minimum bending energy criterion to retain information of outline curve shape and minimize differences in landmark positions along the curve. We then pruned this data set to 14 landmarks, comprised of the 8 true landmarks (red dots in Figure S7B) and 6 slid semi-landmarks (blue dots in Figure S7B). The subsequent analyses were the same as for body shape, with the exception of accounting for the symmetry of the LPJ.

Cluster Analysis We clustered the species for their similarity in body and pharyngeal jaw shape using agglomerative hierarchical clustering in R. We used the agnes() function of the package CLUSTER [S19] and Ward's clustering method on Mahalanobis distance matrices derived from CVA in MORPHOJ.

Stomach and Gut Content Analyses

To assess the trophic specializations of the studied species, we performed stomach and gut content analyses in 506 specimens (note: this number is somewhat smaller than the number of specimens used for the other analyses, as some of the intestinal tracts were empty). For stomach and gut content analyses, the intestinal tracts were opened under a binocular (Leitz) and the entire contents were removed. Stomach and gut contents were separated up into one or more of the following categories: sand, aufwuchs (algae), plant material, mollusks, insects (imagines and larvae), crustaceans, fish (remains), fish eggs, and fish scales. We determined volume (in %) and weight (in µg; using a Kern ALS 120-4 scale) of each category. To prevent bias, roughly the same amount of time was spent on the stomach and gut content of each specimen, and the samples were blinded, i.e. the assayer was unaware of the species ID. The volumetric data, illustrated in Figure 2E, were then used to calculate Schoener's index of proportional diet overlap [S20], and to perform a PCA. We then performed a bootstrap analysis with 10.000 replicates to test whether convergent species pairs show greater similarities in Schoener's index than random pairs of species.

Stable Isotope Analyses

Stomach and gut content analyses as described above have the drawback that they only cover food uptake in the last few hours (in case of tropical fish) or days before the capture of the specimens. This problem can be overcome by determining the chemical signature of food uptake via the analysis of stable isotopes. We here apply a stable isotope analysis (SIA) on the basis of the signature of C and N stable isotopes (^{13}C and ^{15}N). To this end, we used white muscle tissue samples from 727 specimen (see Table S1), which were kept in ethanol and dried at 60°C for 24h in the laboratory. We pulverized the dried tissue using Zirconia beads and a bead-beater, and elutriated the powder in pure ethanol. The suspension was centrifuged and the supernatant decanted. The pellet was then dried at 60°C overnight and amounts of 500 µg were weighed into tin capsules and analyzed on an elemental analyzer (Thermo Finnigan) coupled to a Finnigan Delta V Advantage IRMS (Isotope Ratio Mass Spectrometer), with standard setups for N_2 and CO_2 analysis [S21]. The isotopic composition is expressed in the conventional delta notation as permil (‰) deviation *versus* atmospheric N_2 and Pee Dee Belemnite. Because of sampling at two different times of the year, in three different years and in different localities our sampling captures possible within species variation in trophic ecology.

Correlation between Morphological Clusters and Stable Isotope Signatures

We used distance based redundancy analysis as implemented in the function capscale() in the R package VEGAN [S22] and anova.cca() to test for significance of the association between morphological distances between species and their stable isotope signatures. We also estimated the phylogenetically independent correlations between data sets using phylogenetic canonical correlation analysis. We calculated principle components for each data set and used these to find the axes of largest correlation using phyl.cca() from the PHYTOOLS package [S23]. This revealed a highly significant (p=0.0000007) correlation (cor=0.68) between LPJ shape and stable isotope signatures, corroborating our findings from the disparity through time analyses.

Pairwise Distance-Contrast Plots

To estimate the extent of convergence within the Lake Tanganyika cichlid species flock we compared the phylogenetic distance between each pair of species to its morphological distance. We derived the phylogenetic distance from our molecular phylogeny using the cophenetic() function in R. The morphological distance was calculated as Euclidean distance from the pooled-within-species regressions of shape against centroid size using R's dist() function. In total we had 2485 species comparisons, therefore we used hexagonal binning (x = 10 bins) to overcome problems with overplotting. This also allowed us a direct comparison to our modeled trait evolution scenario. To this end we calculated the variance-covariance matrix from our data considering the phylogeny by using ic.sigma() function in the R-package GEIGER [S24]. We then simulated neutral trait evolution on our phylogeny using sim.char() with Brownian motion. For a comparison to a Ornstein-Uhlenbeck model of trait evolution, we transformed the phylogeny with ouTree() using a wide range of alpha values. The species comparisons that we derived from these simulations were then compared to our actual data by subtracting the binning counts of the simulations from those of the data. This led to negative combined counts in bins with simulated comparisons being in the majority and positive ones in bins with data being in the majority. We tested for statistical significance of the difference of pointwise means between simulations and data (each 1/10th of the x-axis) by bootstrapping (1000 bootstraps). As both simulations, Brownian motion and Ornstein-Uhlenbeck revealed highly congruent results, we only show one of them, Brownian motion, in Fig. 4.

We also estimated the number of convergent species pairs by counting those species comparisons falling below the lower 95% confidence threshold of the neutral evolution simulations. This revealed 122 and 132 species pairs that are convergent in body and LPJ shape, respectively.

Habitat and Depth Overlap

Based on our transect surveys (see above), further observations and catch-records, and available literature [S2-S6, S25], we characterized the depth distribution and the habitat for each species in our core-data set (see Table S2). These data were used to assess habitat and depth overlap between convergent forms.

We also used our transect data on 16 focal species to determine how many species co-occurred at least once within a single 2 m transect. Out of 120 comparisons, only a single species pair was never found together (Neopul-Simbab). This once more highlights the high degree of sympatry of the species in included in this study.

Disparity-through-Time Plots

Following the method of Harmon *et al*. [33], we plotted the trajectory of average subclade disparity against time for shape and stable isotope data. We compared those trajectories to ones generated from Brownian motion simulations of trait evolution using our molecular phylogeny. Positive deviations of the data from the simulations indicate a higher overlap in morphospace among subclades than would be expected under neutral evolution. As disparity measures we used average squared Euclidean distances. We averaged over 100 simulation runs to get a more reliable estimate of Brownian motion trait evolution. The plots are shown up to 80% of the time span only (from root age to present), since this analysis is prone to be affected by tip overdispersion as it approaches present due to missing terminal taxa. This analysis has been performed with the entire core data-set and with a subset of 64 taxa, in which we removed the ancestral lineages Bathybatini, Trematocarini, Tilapiini and *Tylochromis*. Figure 4 depicts the latter analysis.

A potential problem with disparity-through-time analyses is that they might be influenced by varying rates of morphological evolution between sub-clades. This is not the case in cichlids from Lake Tanganyika, as it has previously been shown that the rate of morphological evolution is relatively constant between tribes [23].

Evolutionary PCA

For body shape and LPJ shape, we estimated the ancestral character states at each node in the phylogeny from the regressions against centroid size residuals. This allowed us to calculate the extent and direction of shape change along each branch. These branch-wise estimates were then subjected to principal component analysis to find the axes of greatest evolutionary divergence within the Tanganyikan species flock. All evolutionary principal component analyses were performed in MORPHOJ. We illustrated the shape changes along the heaviest loaded axes by contrasting the reconstructed root state with the derived state along the respective axis and a scale factor of 0.1. The illustration is a warped outline drawing, with interlandmark outlines being estimated and shown for illustration purposes, but for which we have no further information on their accuracy. To counteract the distraction by largely distorted outlines, such as fins, which we never observed in nature and for which we have no direct morphometric information, we manually adjusted those outlines to be more similar in the plots. This did not influence any of our analyses or interpretations.

CT Scanning of the Pharyngeal Jaw Apparatus

To illustrate the arrangement of dentigerous bones in the pharyngeal jaw apparatus of Tanganyikan cichlids we performed a computed tomography (CT) scan. The head of an adult male *Astatotilapia burtoni* was scanned at 18µm voxel size resolution in a SkyScan 1176 in-vivo hi-res microCT scanner. Cross sections were computed from the raw images in NRECON and used to construct a virtual 3D model in OSIRIX. We removed all but the tooth-bearing pharyngeal bones from the virtual model and compiled a movie showing the phranyngeal jaw apparatus in rotation around the dorsal-ventral axis (see Movie S1).

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Chapter 4

Parallel ecological diversification in Antarctic notothenioid fishes as evidence for adaptive radiation

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SR conducted laboratory analyses (sequencing and stable isotope analysis preparations), analysed the data and drafted the manuscript, MMa designed the study, helped with data analysis and helped drafting the manuscript, MD contributed samples and helped drafting the manuscript, MMu conducted the comparative analyses, helped with statistics and contributed in drafting the manuscript, ML helped with stable isotope analysis and data interpretation, RH helped drafting the manuscript, WS designed the study and helped with data interpretation and drafting the manuscript.

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Parallel ecological diversification in Antarctic notothenioid fishes as evidence for adaptive radiation

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Abstract

Antarctic notothenioid fishes represent a rare example of a marine species flock. They evolved special adaptations to the extreme environment of the Southern Ocean including antifreeze glycoproteins. Although lacking a swim bladder, notothenioids have diversified from their benthic ancestor into a wide array of water column niches, such as epibenthic, semipelagic, cryopelagic and pelagic habitats. Applying stable carbon (C) and nitrogen (N) isotope analyses to gain information on feeding ecology and foraging habitats, we tested whether ecological diversification along the benthic–pelagic axis followed a single directional trend in notothenioids, or whether it evolved independently in several lineages. Population samples of 25 different notothenioid species were collected around the Antarctic Peninsula, the South Orkneys and the South Sandwich Islands. The C and N stable isotope signatures span a broad range (mean δ^{13}C and δ^{15}N values between −25.4‰ and −21.9‰ and between 8.5‰ and 13.8‰, respectively), and pairwise niche overlap between four notothenioid families was highly significant. Analysis of isotopic disparity-through-time on the basis of Bayesian inference and maximum-likelihood phylogenies, performed on a concatenated mitochondrial (cyt *b*) and nuclear gene (*myh6*, *Ptr* and *tbr1*) data set (3148 bp), showed that ecological diversification into overlapping feeding niches has occurred multiple times in parallel in different notothenioid families. This convergent diversification in habitat and trophic ecology is a sign of interspecific competition and characteristic for adaptive radiations.

Keywords: disparity-through-time, marine speciation, niche overlap, pelagization, phylogeny, stable nitrogen and carbon isotopes

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Introduction

Adaptive radiation, the evolution of ecological and phenotypic diversity within a rapidly multiplying lineage, is thought to be responsible for a great portion of the diversity of life (Simpson 1953; Schluter 2000). The most famous examples of adaptive radiations are the Darwin's finches on Galápagos, the Caribbean *Anolis* lizards and the East African cichlid fishes. One of the key features of an adaptive radiation is the correlation between the morphologically diverse phenotypes of the 'participating' species and the various habitats that these occupy (Schluter 2000). While it is conceivable how such an 'adaptive disparity' is fulfilled by the paradigmatic Darwin's finches, anoles and cichlids with their characteristic adaptations in beaks, limbs and trophic structures, respectively, the inference of phenotype-environment correlation remains a challenge in other cases of adaptive radiation (Schluter 2000; Gavrilets & Losos 2009).

In fishes, most studies on adaptive radiation focus on freshwater systems, with the cichlid species flocks of

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the East African Great Lakes being the prime examples (Salzburger 2008, 2009). The Antarctic notothenioids represent a marine species flock that evolved under extreme environmental conditions (Eastman & Clarke 1998; Eastman 2000). The perciform suborder Notothenioidei diversified into at least 130 species in eight families, encompassing over 100 Antarctic species (Eastman 2005; Eakin *et al.* 2009). Three ancestral families, Bovichtidae, Pseudaphritidae and Eleginopidae, comprise eleven primarily non-Antarctic species, distributed around southern South America, the Falkland Islands, southern New Zealand and southeastern Australia (Eastman 1993). The remaining families Artedidraconidae, Bathydraconidae, Channichthyidae, Harpagiferidae and Nototheniidae are, with few exceptions, endemic to Antarctic waters and are usually referred to as the 'Antarctic clade' (e.g. Eastman 1993). Notothenioids dominate the Antarctic continental shelf and upper slope, accounting for approximately 46% of the species diversity and over 90% of the fish biomass (Eastman & Clarke 1998; Eastman 2005).

Antarctic waters are constrained by the Antarctic Circumpolar Current (ACC). The Antarctic Polar Front, the northern boundary of the ACC between 50°S and 60°S, acts as major oceanographic barrier, effectively isolating the Southern Ocean faunal assemblages from those of the Indian, Pacific and Atlantic oceans. Through the establishment of a thermally and oceanographically isolated area and the inhibition of faunal admixture, the Antarctic Polar Front is, hence, a likely driver of notothenioid evolution (Coppes Petricorena & Somero 2007). As a means to adapt to Southern Ocean environmental conditions, the Antarctic notothenioids evolved special anatomical and physiological features and, at the same time, lost traits no longer 'needed' in permanently cold waters: (i) The evolution of antifreeze glycoproteins is regarded as an evolutionary key innovation of notothenioids (Eastman 1993; Matschiner *et al.* 2011), facilitating permanent life in subzero temperate waters. (ii) All notothenioids lack a functional swim bladder. Several pelagic species, however, have evolved neutral buoyancy by a combination of skeletal mineralization and the accumulation of lipid deposits (Eastman 1993; Klingenberg & Ekau 1996). (iii) Some notothenioids have lost the classical heat-shock protein response (Place & Hofmann 2005; Clark *et al.* 2008). (iv) The Channichthyidae represent the only known vertebrate group that lacks erythrocytes in the adult state and that is unable to synthesize a functional version of the respiratory oxygen transporter haemoglobin (Ruud 1954; Near *et al.* 2006).

Here, we investigate niche evolution in notothenioids, using a set of 25 representative species (and 365 individuals) that belong to four of the five notothenioid families in the exceptionally species-rich Antarctic clade. Apparently, Antarctic notothenioids diversified along the benthic-pelagic axis in the absence of competition from other fish taxa (Eastman 1993, 2005). From a morphological perspective, this process termed 'pelagization' appears to have occurred independently in several clades (Klingenberg & Ekau 1996; Bargelloni *et al.* 2000).

We used isotopic signatures as indicators for ecological specialization to assess the diversity of lifestyles and feeding strategies/habits of the Antarctic clade, as has been done for adaptively radiating rockfishes (Ingram 2011), and to further test whether these strategies/habits evolved clade-specifically and unidirectionally or independently in several lineages. Stable isotope analysis (SIA) makes use of the fact that the C and N stable isotope signatures ($\delta^{13}C$ and $\delta^{15}N$) of organisms are directly related to their diet. In general, the ratio of the heavier over the lighter stable isotope is greater in consumers than in food material and thus continuously increases with trophic level (TL; e.g. Hobson & Welch 1992; Hobson *et al.* 1994). This is particularly true for nitrogen, where N isotope fractionation leads to trophic shifts of 3–5‰ (DeNiro & Epstein 1978; Minagawa & Wada 1984; Post 2002). The C isotope fractionation is less pronounced during food chain processing, with a typical 1‰ increase per TL (Hobson & Welch 1992). Yet, carbon isotopic values can often be used to assess constraints on the primary carbon source, which can vary strongly between different feeding grounds (e.g. inshore vs. offshore and pelagic vs. benthic). Thus, while N isotope ratios can be used to predict the relative TL of an organism, its C isotopic composition yields valuable information with regard to its habitat (e.g. Hobson *et al.* 1994).

To reconstruct the evolution of ecological specialization in notothenioids, which has not been studied in detail, we established a new phylogeny of the studied species based on mitochondrial and nuclear markers [3148 base pairs (bp) in total]. This phylogeny extends previous work (e.g. Near & Cheng 2008) by the use of multiple nuclear markers and by the longest total sequence length used in notothenioid phylogenetics to date. Phylogeny and time estimation were fully integrated with SIA by the application of a disparity-through-time (DTT) analysis.

According to the results of earlier studies (Klingenberg & Ekau 1996; Eastman & McCune 2000), we expected to find evidence for independent colonization of ecological niches in different lineages. Furthermore, should previous descriptions of the notothenioid diversification as an adaptive radiation be appropriate, the pattern of average subclade disparity throughout the radiation could be expected to resemble those found in other adaptive radiations like *Liolaemus* lizards (Harmon *et al.* 2003) or Tanganyikan cichlid fishes (Gonzalez-Voyer *et al.* 2009) and to be different from patterns observed in putative non-adaptive radiations, such as rats (Rowe *et al.* 2011).

Materials and methods

Sample collection

Sampling took place during three expeditions in the austral summer to the Scotia Sea: The ICEFISH 2004 cruise with RV Nathaniel B. Palmer (Jones *et al.* 2008), cruise ANT-XXIII/8 with RV Polarstern, and the 2008/09 US AMLR Survey with RV Yuzhmorgeologiya (Jones *et al.* 2009) (Fig. 1 and Table 1, Tables S1 and S2, Supporting information). White muscle tissue samples were preserved in 95% ethanol and stored at −20 °C for subsequent investigations. A total of 365 adult individuals of 25 Antarctic notothenioid species were processed for SIA. Molecular analyses were performed with 39 individuals of the same 25 species and three representatives of non-Antarctic notothenioid families serving as outgroups (Table 1).

DNA extraction, amplification, sequencing and alignment

Genomic DNA from approx. 10 mm^3 white muscle tissues was extracted by proteinase K digestion, followed by sodium chloride extraction and ethanol precipitation. Marker selection was based on the genome-wide marker comparison of Li *et al.* (2007). We included a fast-evolving gene (*myh6*), a gene evolving at intermediate rates (*Ptr*) and a slowly evolving gene (*tbr1*). As a representative mitochondrial marker (mtDNA), we used cytochrome *b* (cyt *b*), which had previously been proven suitable for phylogenetic analyses in notothenioids (Chen *et al.* 1998; Matschiner *et al.* 2011). Nuclear markers were amplified with the following primer pairs: myh6_F507/myh6_R1325, Ptr_F458/Ptr_R1248 and tbr1_F86/tbr1_R820 (Li *et al.* 2007); the amplification of cyt *b* was performed using the primers NotCytBf and H15915n (Matschiner *et al.* 2011). Sequences of the three outgroup species and *Pogonophryne scotti*, as well as *Ptr* sequences of *Notothenia coriiceps* and *Trematomus newnesi* were obtained from GenBank (see Data accessibility and Table S4, Supporting information).

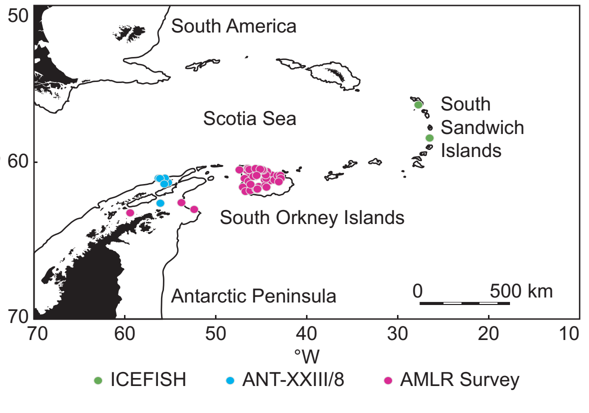

Fig. 1 Sampling sites off the northern Antarctic Peninsula, the South Orkney Islands and the South Sandwich Islands. The solid line indicates the 1000 m depth contour.

The gene fragments were amplified using different polymerase chain reaction (PCR) protocols. Cyt *b*, *myh6* and *Ptr* PCR products were achieved using the Finnzymes' Phusion® High-Fidelity DNA Polymerase (Finnzymes). Individual reaction volumes contained 8.6 μL ddH$_2$0, 10.0 μL 2 × Phusion® Master Mix with HF Buffer [containing 0.04 U/μL Phusion® DNA Polymerase, 2 × Phusion® HF Buffer, 400 μM of each deoxynucleotides (dNTP)], 0.2 μL forward primer, 0.2 μL reverse primer and 1.0 μL DNA template. The PCR profiles included initial denaturation (30 s, 98 °C), followed by 30 (cyt *b*) or 40 cycles (*myh6*, *Ptr*) of denaturation (10 s, 98 °C), annealing (30 s, 56 °C) (53 °C for *Ptr*), extension (30 s, 72 °C) and a final extension phase (10 min, 72 °C). *Tbr1* amplification was achieved using REDTaq® DNA Polymerase (Sigma-Aldrich). The PCR mixes contained 5.5 μL ddH$_2$O, 1.25 μL 10× Taq buffer (Sigma-Aldrich), 1.0 μL MgCl$_2$, 1.25 μL dNTP mix, 1.0 μL forward primer, 1.0 μL reverse primer, 0.5 μL REDTaq® DNA Polymerase (Sigma-Aldrich) and 1.0 μL DNA template. Amplifications of *tbr1* were carried out using the following temperature profile: initial denaturation (2 min, 94 °C) followed by 32 thermocycles of denaturation (30 s, 94 °C), annealing (30 s, 57 °C), extension (1 min, 72 °C) and a final extension phase (7 min, 72 °C). All amplification products were purified using the ExoSAP-IT (USB) standard protocol, adding 0.5 μL ExoSAP-IT and 3.5 μL ddH$_2$O to 2.5 μL PCR templates, incubating (15 min, 37 °C; 15 min, 80 °C) and, in some cases, using the GenElute™ Gel Extraction Kit (Sigma-Aldrich). The purified PCR products were used as templates for cycle sequencing reactions with the BigDye® Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems), following the manufacturer's instructions. The reaction volumes included 0.5 μL primer, 1.0 μL BigDye® Terminator Reaction Mix (Applied Biosystems) and 3.0–6.5 μL purified DNA in a total volume of 8 μL. The nuclear markers were sequenced with one forward and reverse primer each. Sequencing of cyt *b* was additionally performed with two different forward primers: NotCytBf (Matschiner *et al.* 2011) and

Table 1 Sampled species with collection site, sample size for stable isotope analysis (n) and lifestyle of adult individuals. Lifestyle descriptions are often based on trawl depth and may not be definite.

Sample	Location (n)	Lifestyle of adults
Bovichtidae		
Bovichtus diacanthus	Tristan da Cunha	
Pseudaphritidae		
Pseudaphritis urvillii	Victoria, Australia	
Eleginopidae		
Eleginops maclovinus	South America	
Nototheniidae		
Aethotaxis mitopteryx	AP (4), SO (7)	Pelagic[*,†,‡,§], benthopelagic[¶]
Dissostichus mawsoni	AP (2), SO (5)	Pelagic[†,§]
Gobionotothen gibberifrons	AP (10), SO (10)	Benthic[†,‡]
Lepidonotothen larseni	SO (10), SSI (10)	Semipelagic[†]
Lepidonotothen nudifrons	SO (10)	Benthic[†,§]
Lepidonotothen squamifrons	AP (10), SO (10)	Benthic[†]
Notothenia coriiceps	AP (10), SO (11)	Benthic[§]
Notothenia rossii	SO (11)	Semipelagic[†]
Pleuragramma antarcticum	AP (10), SO (10)	Pelagic[*,†,§]
Trematomus eulepidotus	AP (10), SO (10)	Epibenthic[*,†,‡]
Trematomus hansoni	SO (11)	Benthic[†,‡]
Trematomus newnesi	AP (10), SO (10)	Cryopelagic[†]
Trematomus nicolai	SO (6)	Benthic[*,†,‡,**,††], benthopelagic[‡‡]
Trematomus tokarevi	SO (11)	Benthic[††]
Artedidraconidae		
Pogonophryne barsukovi	SO (8)	Benthic[§§]
Pogonophryne scotti	SO (10)	Benthic[†,§§]
Bathydraconidae		
Gymnodraco acuticeps	AP (15)	Benthic[†]
Parachaenichthys charcoti	SO (11)	Benthic[†]
Channichthyidae		
Chaenocephalus aceratus	AP (10), SO (10)	Benthic[†,¶¶]
Chaenodraco wilsoni	AP (10)	Pelagic[***]
Champsocephalus gunnari	AP (11), SO (10)	Pelagic[†,¶¶]
Chionodraco rastrospinosus	AP (10), SO (10)	Benthic[†], benthopelagic[†††]
Cryodraco antarcticus	AP (10), SO (10)	Pelagic[†], benthic[¶¶]
Neopagetopsis ionah	AP (6), SO (6)	Pelagic[¶¶]
Pseudochaenichthys georgianus	SO (10)	Pelagic[†,¶¶], semipelagic[†]

[*] DeWitt *et al.* (1990); [†] Eastman (1993); [‡] Klingenberg & Ekau (1996); [§] Kock (1992); [¶] Kunzmann & Zimmermann (1992); [**] Kuhn *et al.* (2009); [††] La Mesa *et al.* (2004); [‡‡] Brenner *et al.* (2001); [§§] Lombarte *et al.* (2003); [¶¶] Kock (2005); [***] Kock *et al.* (2008); [†††] Hureau (1985b).

AP, Antarctic Peninsula, SO, South Orkney Islands, SSI, South Sandwich Islands.

cyt*b*centralF (5′- CYA CCC TNA CYC GYT TCT TTG C -3′), which was newly designed to bind at a central position of cyt *b* (bases 518–539 in cyt *b* of *Chionodraco rastrospinosus*). The reaction conditions were as follows: initial denaturation (1 min, 94 °C) followed by 25 cycles of denaturation (10 s, 94 °C), annealing (20 s, 52 °C) and elongation phase (4 min, 60 °C). Unincorporated BigDye® terminators were removed with the BigDye® XTerminator™ Purification Kit (Applied Biosystems). To this end, 14.5 μL ddH_2O, 22.5 μL SAM™ solution and 5.0 μL XTerminator™ beads were added to the sequencing products, then shaken (30 min, 2000 rpm), and finally centrifuged (2 min, 211 *g*). All sequences were read with an ABI3130*xl* Capillary Sequencer (Applied Biosystems). Sequence reads were verified by eye, and forward and reverse fragments were assembled using CODONCODE ALIGNER v.3.5.6 (CodonCode Corporation).

All sequences were aligned per locus with the multiple sequence alignment program MAFFT v.6.717b (Katoh & Toh 2008). The alignments were trimmed in MESQUITE v.2.72 (Maddison & Maddison 2009) so that each alignment started and ended with codon triplets, and we also checked for stop codons. Alignments were concatenated and partitioned by molecule type and codon position to account for heterogeneity in evolutionary rates and substitution patterns. Thus, the first and second codon positions of mitochondrial cyt *b* (‘mit12’), the third codon positions of mitochondrial cyt *b* (‘mit3’), the first and second codon positions of nuclear genes ('nuc12') and the third positions of nuclear genes ('nuc3') were used as separate partitions. In a second partitioning scheme, the data set was partitioned with respect to the four genes. The best-fitting models of molecular evolution for each of the eight partitions were estimated with the computer program jMODELTEST v.0.1.1 (Posada 2008), using the Bayesian information criterion (BIC; Schwarz 1978). Selected models were TPM2uf+G (*myh6*), K80+G (*Ptr*), HKY+I (*tbr1*), TrN+G+I (cyt *b*), HKY+I+G (mit12), K80+I (nuc12) and TrN+G (mit3, nuc3).

Phylogenetic analysis

Phylogenetic tree reconstructions were carried out using maximum-likelihood (ML) and Bayesian inference (BI) approaches. Maximum-likelihood phylogenetic inference was performed with both partitioning schemes, applying the respective models of molecular evolution for each partition, in a partition-enabled version of GARLI, GARLI-PART v.0.97 (Zwickl 2006). Heuristic searches were used to find the topology with the best likelihood score. The searches were conducted using automatic termination, after a maximum of 5 million generations, or, alternatively, after 10 000 generations without significant ($P < 0.01$) improvement in scoring topology. Bootstrap (BS) analysis was performed with 100 BS replicates, which were summarized using PAUP* v.4.0a110 (Swofford 2003). The non-Antarctic notothenioid species *Bovichtus diacanthus* was defined as outgroup on the basis of well-supported phylogenetic information (e.g. Near & Cheng 2008; Matschiner *et al.* 2011).

Bayesian phylogenetic analyses were performed with the software BEAST v.1.5.3 (Drummond & Rambaut 2007). For divergence date estimation, the separation of Bovichtidae, Pseudaphritidae and Eleginopidae from the Antarctic lineage (nodes A, B, and C in Fig. 3), as well as the initial diversification of the Antarctic clade (node D) were temporally constrained according to the results of Matschiner *et al.* (2011). Specifically, normal prior distributions were used for each of these splits to approximate highest posterior density (HPD) intervals found by Matschiner *et al.* (2011). Thus, the root of Notothenioidei (node A) was constrained with a mean divergence prior to 71.4 million years ago (Ma; 2.5% quantile: 89.1 Ma, 97.5% quantile: 53.8 Ma), and nodes B-D were constrained at 63.0 (79.5–46.6) Ma, 42.9 (56.5–29.4) Ma and 23.9 (31.3–16.4) Ma, respectively. While these time constraints generally agree with the interpretation of *Proeleginops grandeastmanorum* from the La Meseta Formation on Seymour Island (~40 Ma; Eastman & Grande 1991) as an early representative of the eleginopid lineage (Balushkin 1994), we deliberately avoided using it as a time constraint owing to its debated taxonomical assignment (Near 2004). With the exception of outgroup relationships, which were used for time calibration, no topological constraints were applied. Divergence dates were estimated using the uncorrelated lognormal relaxed molecular clock and the reconstructed birth-death process as a tree prior (Gernhard 2008). Following Shapiro *et al.* (2006), we implemented the codon position-specific model of sequence evolution $HKY_{112} + CP_{112} + \Gamma_{112}$, but we furthermore tested $GTR_{112} + CP_{112} + \Gamma_{112}$ and the model combination selected by BIC for codon-specific partitions. For each of the three combinations, 10 independent analyses were performed with 20 million generations each. Replicates were combined in LOGCOMBINER v.1.5.3 (Drummond & Rambaut 2007) after removing the first 2 million generations of each run as burn-in. Convergence of run replicates was verified by effective sample sizes > 1200 for all parameters and by comparison of traces within and between replicates in TRACER v.1.5 (Rambaut & Drummond 2007). The three settings were compared with Bayes factors (BF), using the harmonic mean approach as implemented in TRACER. While we acknowledge that the harmonic mean estimator may be biased towards more parameter-rich models (Lartillot & Hervé 2006), we chose this approach owing to the lack of suitable alternatives. As the inclusion of multiple individuals per species may violate assumptions of constant diversification implicit in the birth–death tree prior, BI analyses were repeated with a reduced data set containing only one individual of each species.

Stable isotope analysis

In this study, approximately 10 mm^3 of white muscle tissue was used for the SIA. White muscle tissue is less variable with regard to the carbon and nitrogen isotope composition and has a longer retention time than other tissue types (Pinnegar & Polunin 1999; Quevedo *et al.* 2009). Samples were dried (24 h, 60 °C) and then ground in a Zirconia bead mill (30 min, 1800 bpm). Then, the sample powder was rinsed from the beads using 1 mL 99% ethanol, and the supernatant was evaporated (24 h, 60 °C). The ethanol treatment had no effect on subsequent carbon isotope analyses (e.g. Syväranta *et al.* 2008). For C and N isotope measurements, between 0.5 and 0.8 mg sample powder was filled into 5 × 9 mm tin capsules and introduced into an elemental analyser (Thermo Finnigan) coupled to a Finnigan Delta V Advantage Isotope Ratio Mass Spectrometer, with standard setup for N_2 and CO_2 analysis. Measurements were replicated for about 10% of the samples (42 samples). The isotopic composition is expressed in the conventional delta notation as permil (‰) deviation vs. atmospheric N_2 (AIR) and carbonate standards (V-PDB): $\delta = [(R_{\mathrm{sample}}/R_{\mathrm{standard}}) - 1] \times 1000$, with R representing the ratio of the heavy to the light isotope (i.e. $^{13}C/^{12}C$ and $^{15}N/^{14}N$) in the sample and in the standard material, respectively. EDTA ($\delta^{13}C = -30.25$‰, $\delta^{15}N = -1.1$‰) and ammonium oxalate ($\delta^{13}C = -17.02$‰, $\delta^{15}N = 32.7$‰) were used as internal standards, calibrated against international nitrogen (IAEA-N1, IAEA-N2) and carbon (NBS22) standards. The analytical reproducibility based on replicate sample and standard measurements was better than 0.2‰ for both $\delta^{13}C$ and $\delta^{15}N$. Isotope values are presented as mean ± standard deviation (SD). Variable lipid content can have a biasing effect on the interpretation of bulk C and N stable isotope data. In marine fish samples, this effect seems to be minor (Kiljunen *et al.* 2006; Logan *et al.* 2008), and hence, we did not perform a lipid removal step. Nevertheless, we performed a posteriori 'mathematical lipid correction' after the study of Logan *et al.* (2008). The correction, however, did not affect the species distribution pattern, and thus, only the uncorrected values are presented in this study. (The corrected data set is available upon request.)

Statistical analysis

The correlation of $\delta^{13}C$ and $\delta^{15}N$ was tested with a Pearson correlation, whereby we accounted for phylogenetic non-independence using phylogenetic independent contrast ('pic' function in the R package 'ape'; Paradis *et al.* 2004; R Development Core Team 2009). We tested for the effect of geographic sites on isotopic signatures by comparison of pooled $\delta^{13}C$ and $\delta^{15}N$ values between AP and SO (*t*-test). Here, only values from species with similar sample sizes at both locations were considered. Pairwise niche overlap between all families and additional comparisons of the nototheniid *Lepidonotothen–Trematomus* clade with the other families were tested with a multivariate analysis of variance (MANOVA). To assess the group overlap in isotopic signatures, we calculated Wilk's lambda (Wilk's λ) for each comparison.

We analysed the subdivision of ecological niche space throughout the radiation using the BI phylogeny (Fig. 3) and the averaged stable isotope data for each species. Average subclade disparity was calculated at each splitting event and plotted against time. A Brownian motion (BM) model of trait evolution was employed for comparison. Disparity-through-time analyses were conducted in R using the package 'geiger' (Harmon *et al.* 2008). Using 475 trees drawn from the posterior distribution of the BI analysis and 500 permutations of the stable isotope data, we assessed the robustness of the observed pattern against phylogenetic uncertainty and intraspecific variation.

Results

Phylogenetic analysis

The alignments had lengths of 1099 bp (cyt *b*), 705 bp (*myh6*), 702 bp (*Ptr*) and 642 bp (*tbr1*), resulting in a total of 3148 bp with only 0.3% missing data. The *myh6* alignment contained a short insertion (6 bp) in the non-Antarctic outgroup *B. diacanthus*; these 6 bp were excluded from the following phylogenetic analyses. Sequences are available at GenBank under the accession numbers JF264479–JF264629. Bayes factors provided 'very strong' (Kass & Raftery 1995) evidence that the codon position-specific combination of substitution models selected by BIC yielded a better fit than both the $HKY_{112} + CP_{112} + \Gamma_{112}$ (log 10 BF 6.215) and $GTR_{112} + CP_{112} + \Gamma_{112}$ (log 10 BF 19.19) models.

Our ML and BI phylogenetic analyses produced identical topologies and confirmed the monophyly of the Antarctic clade with high support values (BS 100%; Fig. 2, Fig. S1, Supporting information). Yet, BS support and Bayesian posterior probability (BPP) were low at the base of the diversification of the Antarctic clade (but high at species-level relationships). In all cases, clustering of individuals from different populations of the same species was strongly supported (BS ≥ 93% and BPP = 1.00). The three families Artedidraconidae, Bathydraconidae and Channichthyidae were recovered as monophyletic, while the Nototheniidae appeared paraphyletic. An ancestral position was assigned to *Aethotaxis mitopteryx*. The monophyly of a clade containing *Lepidonotothen* and *Trematomus* was highly supported (BS 100% and BPP 1.00), and *Notothenia* appeared as the sister group to the more derived 'high-Antarctic clade', comprising the families Artedidraconidae, Bathydraconidae and Channichthyidae. Both the high-Antarctic clade and the channichthyid family were found monophyletic with BS 100% and BPP 1.00. The two artedidraconids, *P. barsukovi* and *P. scotti*, grouped together in all analyses (with high support values). Monophyly of the two bathydraconid representatives was weakly supported (BS 35% and BPP 0.67). Within the family of Channichthyidae, *Champsocephalus gunnari* was placed as sister species of all other representatives followed by a clade containing *Pseudochaenichthys georgianus* and *Neopagetopsis ionah* and a clade containing the four genera *Chionodraco*, *Chaenodraco*, *Chaenocephalus* and *Cryodraco*. The ML reconstruction with gene-specific partitions resulted in minor topological differences (Fig. S1, Supporting information). Reduction in the data set to one individual per species did not change the tree

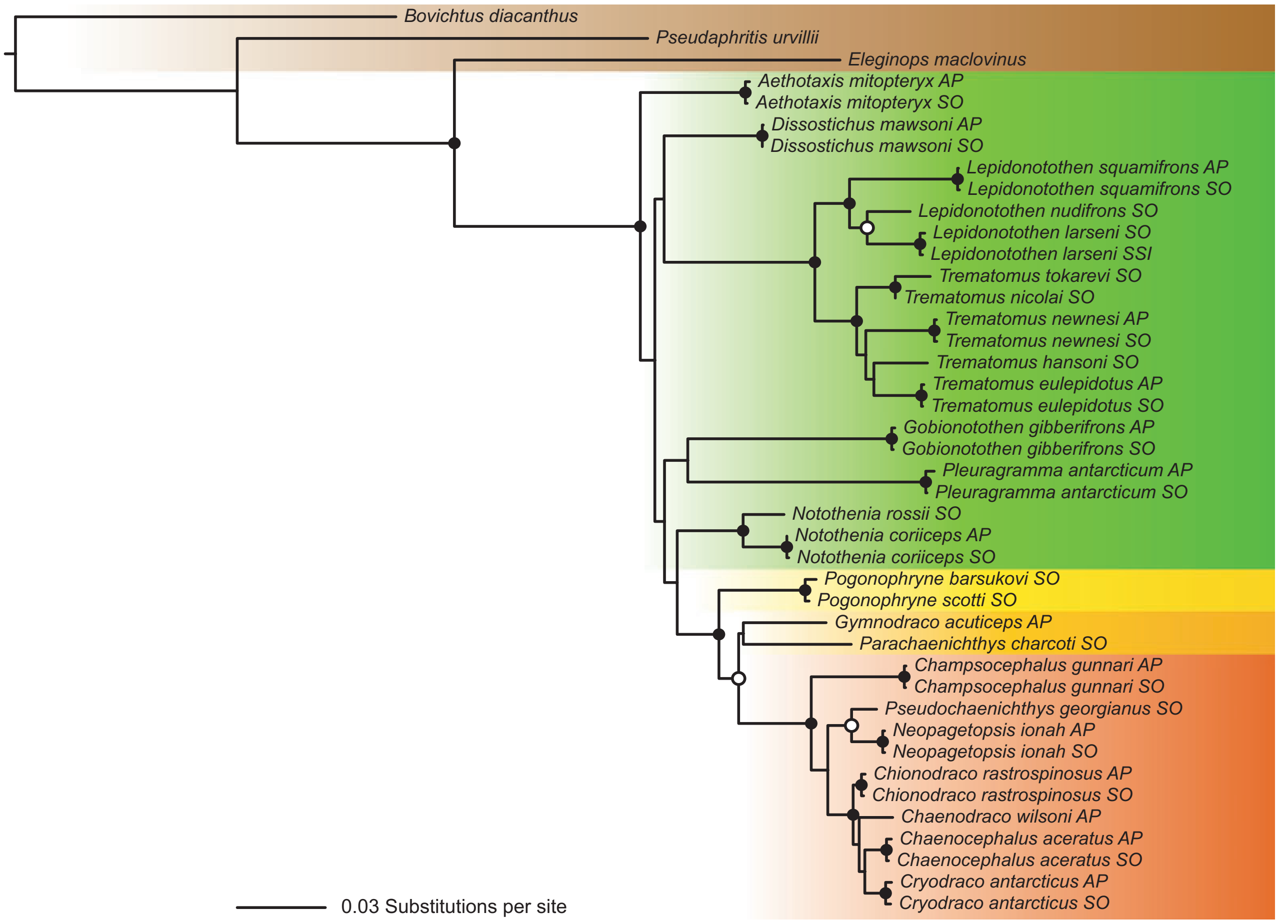

Fig. 2 Maximum-likelihood tree of the notothenioid phylogeny based on the codon position–specific partitioning scheme. Filled circles indicate strongly supported nodes, and moderately supported nodes are marked by open circles Bootstrap (BS ≥ 95 and BS ≥ 70). All species are coloured according to family: brown = non-Antarctic species, green = Nototheniidae, yellow = Artedidraconidae, orange = Bathydraconidae and red = Channichthyidae.

topology with the exception of *Dissostichus mawsoni*, which appeared basal to a group containing the high-Antarctic clade as well as *Nototheniia*, *Pleuragramma* and *Gobionotothen* and the relationships within the *Trematomus* genus (Fig. S1, Supporting information).

According to our time-calibrated phylogeny, diversification of the well-supported nototheniid clade combining *Lepidonotothen* and *Trematomus* began 12.0 Ma (95% HPD 16.4–7.9 Ma; node H) (Fig. 3). The high-Antarctic clade separated from the Nototheniidae around 18.6 Ma (95% HPD 24.0–13.4 Ma; node E). Within the high-Antarctic clade, artedidraconids separated from bathydraconids and channichthyids around 14.6 Ma (95% HPD 15.5–7.0 Ma; node F). The split between Bathydraconidae and Channichthyidae occurred around 2 million years later (12.5 Ma; 95% HPD 16.7–8.5 Ma; node G). The radiation of Channichthyidae, the most derived notothenioid family, began 7.7 Ma (95% HPD 10.6–5.0 Ma; node I).

Stable C and N isotope ratios

The stable carbon and nitrogen isotope composition for the 25 notothenioid species exhibited a comparatively large variability, with values between −27.8‰ and −19.7‰ for $\delta^{13}C$ and between 7.3‰ and 15.6‰ for $\delta^{15}N$ (Fig. 3). Mean values ranged between −25.4‰ and −21.9‰ for $\delta^{13}C$ (SD: 0.3‰ to 1.8‰) and 8.5‰ to 13.8‰ for $\delta^{15}N$ (SD: 0.2‰ to 1.7‰; Fig. 4). Intraspecific ranges of isotopic signatures span from 1.0‰ to 8.1‰ for $\delta^{13}C$ and from 0.4‰ to 5.7‰ for $\delta^{15}N$. Overall, mean intraspecific ranges ($\delta^{13}C$: 2.79‰, $\delta^{15}N$: 2.80‰) were small compared to interspecific ranges of isotopic signatures ($\delta^{13}C$: 8.12‰, $\delta^{15}N$: 8.29‰). The isotopic signatures of $\delta^{13}C$ and $\delta^{15}N$ correlated significantly (0.69; $P < 0.001$), and the correlation remained significant ($P < 0.01$) after correcting for phylogenetic non-independence. No significant difference between values from AP and SO locations was found ($P > 0.16$; *t*-test), even though the

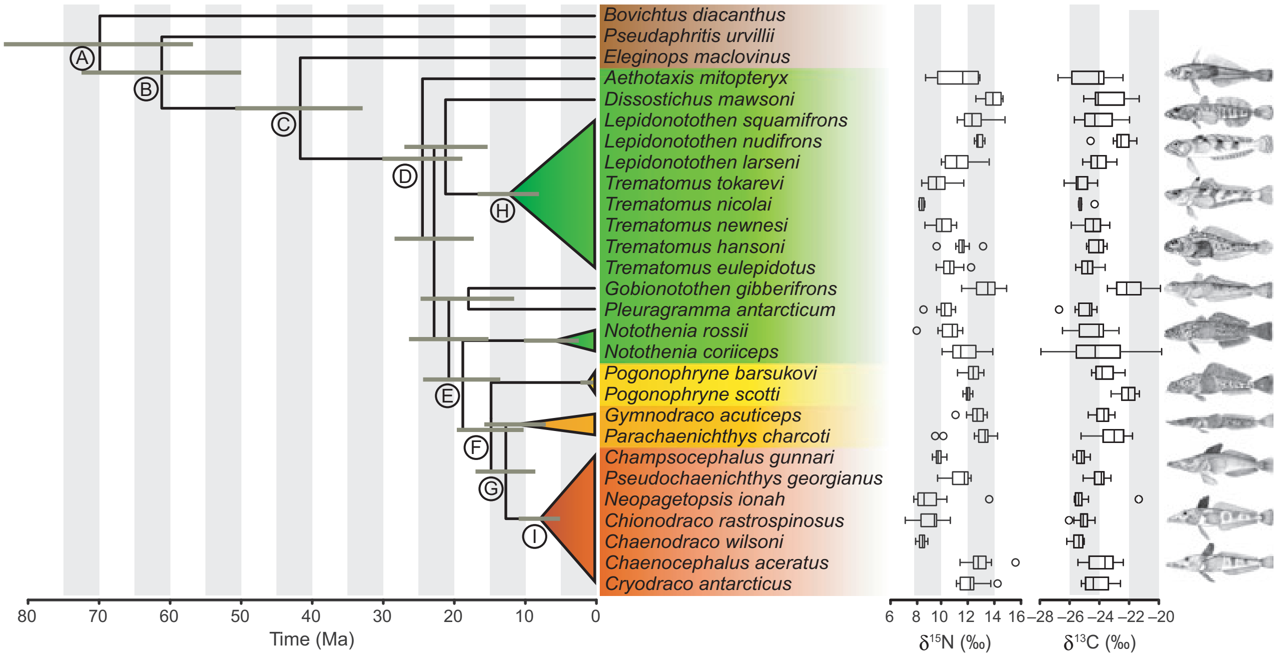

Fig. 3 Left: Time-calibrated phylogeny based on codon-specific partition, inferred with Bayesian inference. Time axis is given in million years ago and nodes labelled A-I are mentioned in the text. Grey node bars indicate upper and lower 95% HPD. All species are coloured according to family: brown = non-Antarctic species, green = Nototheniidae, yellow = Artedidraconidae, orange = Bathydraconidae and red = Channichthyidae. Right: Boxplot of stable isotope values of all included notothenioids. Representative habitus are illustrated at the right, from top to bottom: *Aethotaxis mitopteryx*[d], *Dissostichus mawsoni*[d], *Lepidonotothen nudifrons*[d], *Lepidonotothen larseni*[d], *Trematomus tokarevi*[d], *Gobionotothen gibberifrons*[d], *Notothenia rossii*[b], *Pogonophryne barsukovi*[c], *Gymnodraco acuticeps*[a], *Pseudochaenichthys georgianus*[e], *Chionodraco rastrospinosus*[e] and *Chaenocephalus aceratus*[e]. [a]Boulenger (1902); [b]DeWitt *et al.* (1990); [c]Eakin (1990); [d]Hureau (1985a); [e]Hureau (1985b).

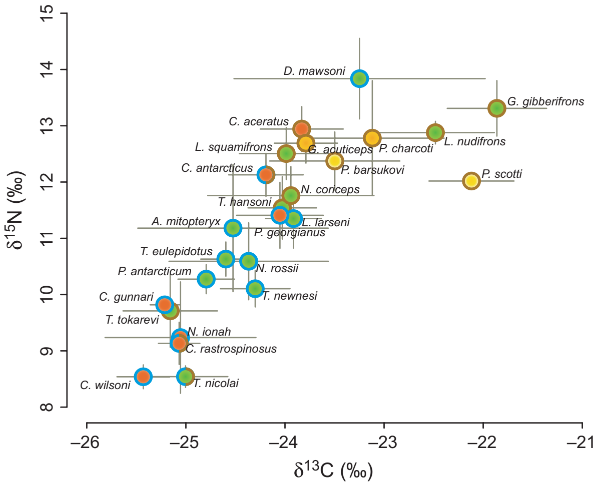

Fig. 4 Scatter plot of carbon and nitrogen isotopic values. Grey bars indicate 95% confidence intervals. All species are coloured according to family (brown: non-Antarctic species, green: Nototheniidae, yellow: Artedidraconidae, orange: Bathydraconidae, red: Channichthyidae), and strokes indicate corresponding lifestyle [blue = pelagic, benthopelagic, semipelagic and epibenthic; brown = benthic; and semicircles when references (Table 1) disagree].

mean values differed slightly (AP δ^{13}C: −24.37‰, SO δ^{13}C: −24.13‰; AP δ^{15}N: 11.30‰, SO δ^{15}N: 10.99‰).

With regard to inferred lifestyle patterns, our SIA data are consistent with previous studies (Hobson *et al.* 1994; Post 2002) in that species that are commonly classified as pelagic clustered around lower δ^{13}C values, while benthic species possessed relatively higher δ^{13}C signatures. However, there are notable exceptions to this: *D. mawsoni*, *C. rastrospinosus*, *Trematomus nicolai* and *T. tokarevi* (Fig. 4, Table 1 and Data S1, Supporting information). Most species had relatively high δ^{15}N signatures, indicating feeding at upper TL. The two well-represented families Nototheniidae and Channichthyidae covered a wide range of isotopic signatures, while bathydraconids and artedidraconids displayed a relatively low variability in both δ^{13}C and δ^{15}N (although the number of individuals was significantly lower). Overlap of the C and N isotope compositions as proxies for niche space was found in all pairwise comparisons (MANOVA) of the four Antarctic notothenioid families (Table 2). Wilk's λ was largest for comparisons of Nototheniidae with all other families ($\lambda > 0.91$; Table 2), and lower values were found for comparisons

Table 2 Pairwise niche overlap comparisons for the four Antarctic notothenioid families, performed with MANOVA (Wilk's λ)

Family 1	Family 2	Wilk's λ
Artedidraconidae	Nototheniidae	0.936
	Lepidonotothen–Trematomus clade	0.791
Bathydraconidae	Nototheniidae	0.913
	Lepidonotothen–Trematomus clade	0.818
Channichthyidae	Nototheniidae	0.930
	Lepidonotothen–Trematomus clade	0.932
Artedidraconidae	Bathydraconidae	0.681
Artedidraconidae	Channichthyidae	0.629
Bathydraconidae	Channichthyidae	0.781

including the lesser-represented families Artedidraconidae and Bathydraconidae ($\lambda > 0.68$). Notably, within-family variation resulted mostly from interspecific variation, instead of intraspecific variation, and closely related species with small intraspecific variation could be found at both ends of the ranges (e.g. *T. nicolai* and *Lepidonotothen nudifrons*; Fig. 3).

Using the DTT method, we assessed how the stable isotope space (as a proxy for ecological niche space) used by the whole clade was subdivided by smaller and smaller subclades as the radiation proceeded. We find positive deviations from the averaged neutral-evolution BM model, indicating larger overlap in niche space between subclades than would be expected if evolution proceeded neutrally (Fig. 5). This result was found to be robust against phylogenetic uncertainty and intraspecific variation by visual inspection of repeated DTT analyses.

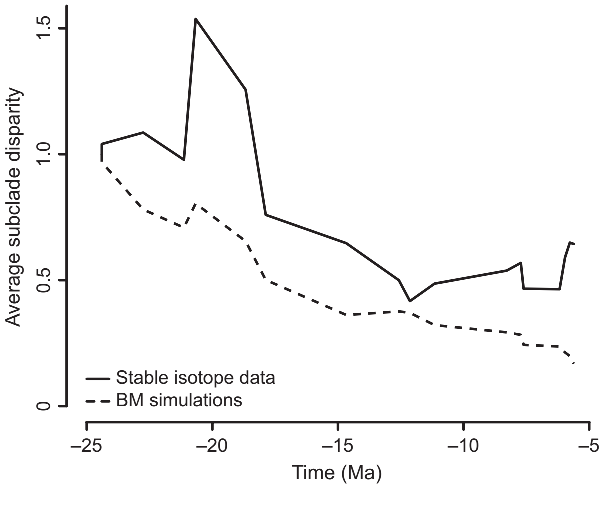

Fig. 5 Disparity-through-time plot for the stable isotopic signatures of Antarctic notothenioid fishes and Brownian motion simulations of character evolution. Time axis is given in million years ago.

Discussion

Phylogenetic relationships

Previous molecular phylogenetic analyses of notothenioids were based on mitochondrial DNA sequences (Bargelloni *et al.* 2000; Stankovic *et al.* 2002; Near 2004; Near *et al.* 2004), on a combination of mtDNA with a single nuclear gene (Near & Cheng 2008) or on morphological characters in addition to molecular data (Derome *et al.* 2002; Sanchez *et al.* 2007). The family-level phylogeny of notothenioids is thus relatively well established. Several questions remain, however, such as the position of the genus *Gobionotothen* (Near *et al.* 2004; Sanchez *et al.* 2007; Near & Cheng 2008) or whether Bathydraconidae are mono- or paraphyletic (e.g. Derome *et al.* 2002; Near & Cheng 2008).

In agreement with most previous studies (e.g. Near 2004; Near & Cheng 2008), our results support paraphyly of the family Nototheniidae. The low support values at the beginning of the Antarctic diversification are characteristic for rapid diversifications. Consequently, the basal position of *D. mawsoni* and the sister species relationships of *G. gibberifrons* and *Pleuragramma antarcticum* remain questionable. As in previous studies (Near 2004; Near & Cheng 2008), the three neutrally buoyant species *A. mitopteryx*, *D. mawsoni* and *P. antarcticum* diverged early within the Antarctic clade but did not cluster together. Phylogenetic relationships of the two genera *Notothenia* and *Lepidonotothen* are consistent with former studies (Bargelloni *et al.* 2000; Near & Cheng 2008). Also, the topology of the nototheniid subfamily Trematominae agrees with previous findings (Sanchez *et al.* 2007; Kuhn & Near 2009), except for *T. tokarevi* and *T. nicolai*, which appeared at basal positions in the phylogeny based on codon position–specific substitution models (Fig. 2, Fig. S1, Supporting information). The early split of the two included bathydraconid species relative to the divergence between Bathydraconidae and Channichthyidae could indicate paraphyly of the former, as was concluded in previous studies (e.g. Derome *et al.* 2002; Near *et al.* 2004; Near & Cheng 2008). Resulting support values within the channichthyids were high, and the recovered topology was in complete agreement with the study of Derome *et al.* (2002). The three genera *Champsocephalus*, *Neopagetopsis* and *Pseudochaenichthys* seem to be well established as the most basal channichthyids (Chen *et al.* 1998; Near *et al.* 2003). In disagreement with former findings, *C. rastrospinosus* and *Chaenodraco wilsoni* did not cluster monophyletically (Chen *et al.* 1998). Near *et al.* (2003) also recovered these two species as paraphyletic but placed *Chaenocephalus aceratus* as the sister taxon to the genera *Cryodraco*, *Chaenodraco* and *Chionodraco,* which disagrees with our findings. Near & Cheng (2008) determined *C. aceratus* as the closest related species of *C. rastrospinosus.*

Inferred split dates (Fig. 3) roughly agree with those found by Near (2004) and Matschiner *et al.* (2011): Divergence estimates for the *Lepidonotothen–Trematomus* clade and the high-Antarctic clade were 12.0 (95% HPD 16.4–7.9) Ma and 18.6 (95% HPD 24.0–13.4) Ma, respectively, while Near (2004) reported them to be 14 ± 0.4 Ma and Matschiner *et al.* (2011) found these splits at 10.3 (95% HPD 15.2–6.1) Ma and 14.7 (95% HPD 20.0–9.9) Ma. According to our estimates, the radiation of the Channichthyidae began 7.7 (95% HPD 10.6–5.0) Ma ago, in good agreement with the estimates of Near (2004) (8.5 ± 0.3 Ma) and Matschiner *et al.* (2011) (6.2 Ma; 95% HPD 9.4–3.4 Ma).

Foraging ecology of notothenioids

So far, it has been shown that some particular feeding strategies are poorly represented or even absent in notothenioids, such as active skeleton-breaking predation (Clarke *et al.* 2004) or planktivory (Eastman & Grande 1989; Eastman 1993). The latter is probably due to restricted phytoplankton production during the austral winter (Clarke *et al.* 2004). The drawback of traditional dietary proxies (stomach content analyses and foraging observations) is that they only captures a snapshot of food uptake. Contrarily, SIA provides time-integrated information on the feeding 'ecology' for a period of weeks to years (McIntyre & Flecker 2006). Isotopic signatures could theoretically be influenced by geographic differences, sampling season and the age of sampled individuals, especially when ontogenic shifts occur in the investigated species. However, our sampling design accounted for these potential problems, as only adult specimens were collected, and all expeditions took place during austral summers. Also, most species were collected at the same two sampling locations, AP and SO, and populations from these two sites did not differ in isotopic signatures. Thus, the observed interspecific differences suggest ecological specialization rather than effects of geographical distribution or life history traits.

Our SIA data confirm that notothenioids occupy a wide variety of ecological niches (Figs 3 and 4). Comparatively high δ^{15}N values suggest that most investigated species reside at a high TL and may be considered tertiary consumers (see also Dunton 2001; Pakhomov *et al.* 2006). The wide range of the carbon stable isotope signatures reflects the notothenioids' variety in habitats along the benthic-pelagic axis (Fig. 4). However, our results are only partly congruent with the lifestyles and feeding reports based on stomach content analyses (Fig. 4, Table 1, Table S3 and Data S1, Supporting information).

At the family level, Nototheniidae are – in terms of habitat and feeding strategies – the most diverse clade among Antarctic notothenioids (La Mesa *et al.* 2004; this study) and include plankton, nekton and benthos feeders, as well as species that combine several feeding modes (Gröhsler 1994). The five included *Trematomus* species were differentiated in both isotopic signatures, thus indicating trophic niche separation (see also Brenner *et al.* 2001). Artedidraconids and bathydraconids represent the most benthic families among notothenioids (Fig. 4; Olaso *et al.* 2000; La Mesa *et al.* 2004). Their δ^{15}N values suggest feeding habits at higher TL (Olaso *et al.* 2000; Jones *et al.* 2009). The well-studied channichthyids clustered into three groups according to their diet (Fig. 4: *C. wilsoni*, *N. ionah*, *C. rastrospinosus* and *C. gunnari* at low TL; *P. georgianus* and *Cryodraco antarcticus* at intermediate TL; and *C. aceratus* at high TL; see also Kock 2005). Carbon signatures indicated a rather pelagic lifestyle for most channichthyid species, with the exception for *C. aceratus*, which we can classify as benthic top predator, in agreement with previous findings (Kock 2005; Reid *et al.* 2007).

The DTT plot (Fig. 5) indicates larger overlap of subclades in niche use than expected from a model of neutral evolution. This is characteristic for adaptive radiations (Harmon *et al.* 2003; Gonzalez-Voyer *et al.* 2009) and differs from patterns of putative nonadaptive radiations, which show a negative deviation from the averaged neutral-evolution BM model (e.g. Rowe *et al.* 2011). Taking into account the considerable variation in stable isotope signatures found in notothenioids as a whole (Fig. 4) – basically ruling out stasis in the evolution of niche use – as well as the robustness of this pattern against intraspecific variation, these results suggest convergent evolution in niche use between species of notothenioid subclades, especially between those clades separating around 20 Ma (Figs 3 and 5). This emphasizes the importance of ecological niche differentiation in the adaptive radiation of notothenioids.

Adaptive radiation and ecological diversification in notothenioids

Our integrative analyses, combining both the phylogenetic relationships and the isotopic signatures of 25 notothenioid species, reveal that ecological diversification into overlapping feeding niches has occurred multiple times in parallel in different notothenioid families (Figs 3 and 5). Using carbon and nitrogen stable isotope ratios as indicators of TL, feeding strategy and macrohabitat, we find great variation within, and substantial overlap between the more basal nototheniids and the derived channichthyids. The representatives of the benthic artedidraconids and bathydraconids also overlap and cluster at high TLs and $\delta^{13}C$ values. Our results further confirm partitioning of habitat and trophic resources within notothenioid fishes, indicating that diversification along the benthic–pelagic axis and to different TLs took place independently in at least two of five notothenioid families of the Antarctic clade (Nototheniidae and Channichthyidae; Fig. 3 and Table 2).

Convergent diversification in habitat and trophic ecology suggests interspecific competition and is a characteristic of adaptive radiations (e.g. Losos 1995; Schluter 2000). For example, *Anolis* lizards of the Caribbean have independently evolved four to six so-called ecomorphs on each of the four large islands of the Greater Antilles, including species specialized to live on grass, twigs, trunks and tree crowns. Variation in limb lengths of anole ecomorphs supports these different lifestyles, so that e.g. the trunk-ground ecomorph possesses relatively long legs adapted to running and jumping on broad surfaces, while the twig ecomorph has short legs and moves slowly on narrow surfaces (Losos 2009). In this context, diversification of notothenioids along the benthic-pelagic axis, as evidenced by their isotopic composition, and the respective adaptations in buoyancy (Eastman 1993) can be considered analogous to the *Anolis* diversification along the ground-tree axis. The notothenioid adaptive radiation shows further analogies to that of Caribbean anoles in terms of species richness (both around 120 species) and age (about 24 and 15–66 Ma, respectively) (Fig. 3; Eastman 2005; Nicholson *et al.* 2005; Losos 2009; Matschiner *et al.* 2011). Not all descendents of the *Anolis* radiation remained within the confined area of the radiation (Nicholson *et al.* 2005), and neither did the notothenioids: *Notothenia angustata*, *N. microlepidota* and the genus *Patagonotothen* secondarily escaped Antarctic waters and occur in New Zealand and South America (Eastman 2005). Moreover, both radiations were probably triggered by key innovations: subdigital toepads support the particular arboreality of *Anolis* lizards, whereas antifreeze glycoproteins in blood and tissues allow notothenioid survival in ice-laden Antarctic waters (Chen *et al.* 1997; Losos 2009; Matschiner *et al.* 2011).

Compared to another well-studied adaptive radiation, that of cichlid fishes in East African lakes, the rate at which lineage formation seems to have occurred is much smaller in Antarctic notothenioids. In the Great Lakes of East Africa, cichlid fishes have diversified into at least 1500 species that differ greatly in naturally and sexually selected traits, including body shape, mouth morphology and colouration (Salzburger 2009). Comparison of cichlid species flocks between East African lakes, as well as mathematical models, have shown that larger habitats effectuate higher diversification rates, as they provide greater habitat heterogeneity and facilitate isolation by distance ('area effect'; Salzburger & Meyer 2004; Gavrilets & Vose 2005; Seehausen 2006). Different adaptive radiations may not be directly comparable as they depend on many ecological, genetic and developmental factors, with an important contribution of historical contingencies (Gavrilets & Losos 2009). Cichlids are known for their philopatry and low dispersal abilities (Danley & Kocher 2001; Salzburger & Meyer 2004), whereas most notothenioids have prolonged pelagic larval stages, enhancing long-range migration (Eastman 1993). Notothenioid populations are characterized by fragmented habitat, historical demographic fluctuations (Patarnello *et al.* 2011) and the absence of genetic structuring over large distances (Matschiner *et al.* 2009; and references therein), whereas many cichlid species posses significant population structuring even on extremely small scales (e.g. Arnegard *et al.* 1999; Rico & Turner 2002). Genetic differentiation over small scales has rarely been found in notothenioids (but see Clement *et al.* 1998). Eastman & McCune (2000) suggested that the smaller species number of notothenioids, compared with cichlid species flocks, could be explained by the absence of certain prime inshore habitats in the Southern Ocean. Alternatively, the notothenioid adaptive radiation may not yet have entered its final stage, namely the diversification with respect to communication. Streelman & Danley (2003) suggested a three-stage model of adaptive radiation (see also Danley & Kocher 2001), in which diversification first occurs with respect to macrohabitats, then with respect to microhabitats and finally with respect to communication (e.g. mating traits such as colouration; see also Gavrilets & Losos 2009). Full species richness would only be achieved through this final step. Streelman & Danley (2003) further suggested that divergence of habitat and trophic morphology is driven by natural selection, whereas diversification along the axis of communication is forced by sexual selection. It is as of yet unclear whether the radiation of notothenioids followed discrete stages. Here, we provide conclusive evidence that the species are separated along the benthic-pelagic axis (i.e. according to macrohabitats; Figs 3 and 4) and probably also as a function of bottom topography and sediment types (Kock & Stransky 2000). Much less is known about microhabitat diversification, although our data suggest that closely related species do differ with respect to foraging strategies (e.g. genera *Lepidonotothen* and *Trematomus*; Figs 3 and 4). Recent evidence further indicates the possibility of divergence along Streelman and Danley's axis of communication, as egg guarding and parental care were observed in all major notothenioid lineages except within the Artedidraconidae (Kock *et al.* 2006; Barrera-Oro & Lagger 2010 and references therein).

On the other hand, because of the paucity of the Antarctic fossil record, it cannot be excluded that the notothenioid radiation has already surpassed its maximum species richness. It is an important characteristic that young adaptive radiations often 'overshoot' in terms of species number and that, generally, niche filling causes declining speciation rates (e.g. Seehausen 2006; Gavrilets & Losos 2009; Meyer *et al.* 2011). That notothenioids already underwent periods of 'overshooting' and niche filling could possibly explain the smaller diversity of Notothenioidei compared to the younger cichlid radiation in the East African Lakes. However, in this case, an early burst of diversification should have left its footprint in a 'bottom-heavy' phylogeny (Gavrilets & Vose 2005). A more extensive study, including many more representatives of the nototheniods, would be necessary to reconstruct the succession of their adaptive radiation.

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S.R. is interested in the diversity of animals, including their evolution and adaptations to the environment. M.Ma. works on the molecular processes underlying adaptive radiation. M.D. is interested in population ecology and evolutionary biology. M.F.L. is specialized on stable isotope biogeochemistry in aquatic environments. R.H. is interested in causes and pathways of adaptation and speciation in the sea. W.S. and M.Mu.'s research focuses on the understanding of the genetic basis of adaptation, evolutionary innovation and animal diversification, using the East Africa' cichlid radiations as main model system. The laboratory's homepage at http://www.evolution.unibas.ch/salzburger provides further details on the group's (research) activities.

Data accessibility

All DNA sequences from this study are available under GenBank accessions: JF264479–JF264516 (cyt *b*); JF264517–JF264554 (*myh6*); JF264555–JF264590 (*Ptr*); and JF264591–JF264629 (*tbr1*). GenBank accession numbers for sequences of other studies are the following: *B. diacanthus* (HM049936; HM050034; HM050153; HM050214); *Eleginops maclovinus* (DQ526429; HM050045; HM050163; HM050225); *N. coriiceps* (HM050183); *P. urvillii* (HM049963; HM050074; HM050195; HM050258); *P. scotti* (HM049962; HM050072; HM050193); and *T. newnesi* (HM050204) (see Table S4, Supporting information). All stable isotope values are given in Table S5, (Supporting information).

Supporting information

Additional supporting information may be found in the online version of this article:

Fig. S1 Maximum-likelihood tree based on the codon position-specific partitioning with numbered nodes (1–19).

Table S1 Antarctic notothenioid samples with corresponding collection id (Table S2) and sample size (*n*) for stable isotope analysis.

Table S2 Collection id for all Antarctic notothenioid samples.

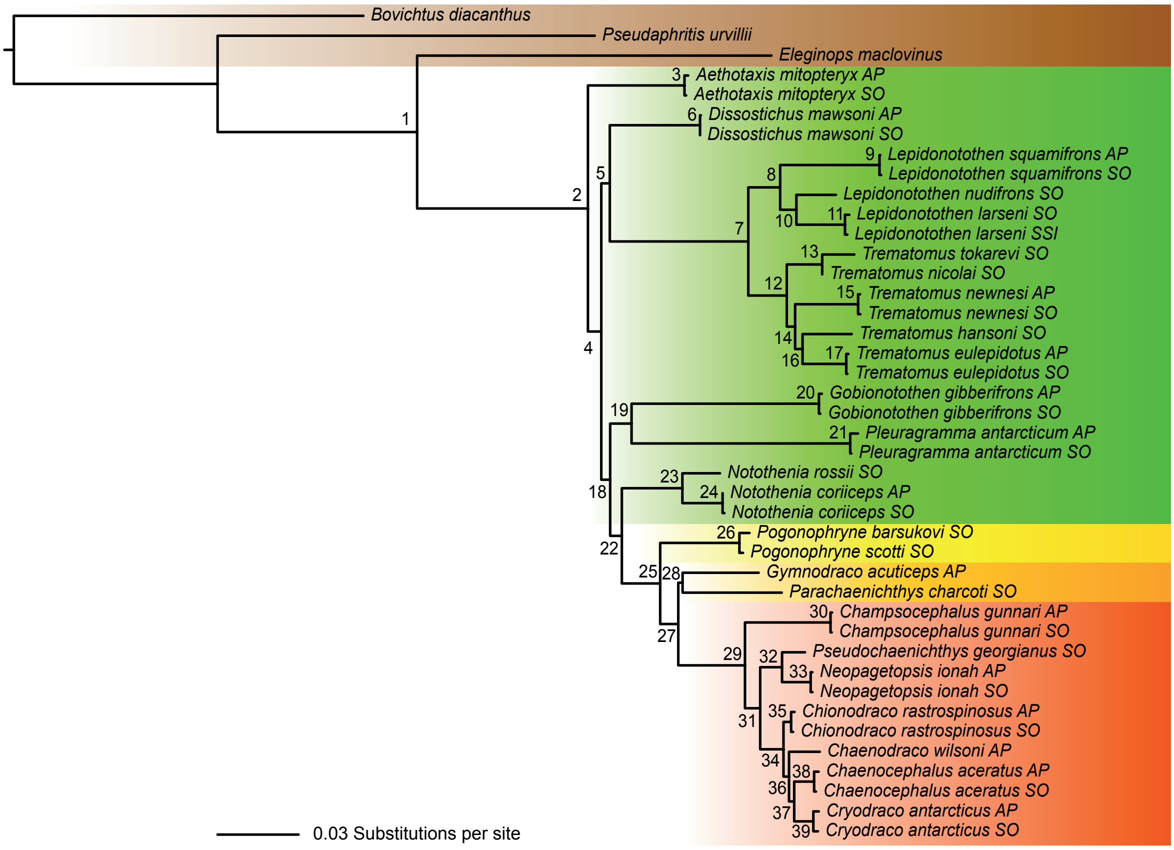
Table S3 Lifestyle and feeding for all included Antarctic notothenioid species.

Table S4 GenBank accession numbers for all used samples.

Table S5 Stable isotope values of all investigated species.

Data S1 Discussion of stable isotope analysis results of individual species.

Supplemental information for chapter 4



Node number	BS partition by codon position 1-2 ind. per species	BPP partition by codon position 1-2 ind. per species	BS partition by gene 1-2 ind. per species	BPP partition by codon position 1 ind. per species
1	100	*	100	*
2	100	*	100	*
3	100	1.00	100	-
4	26	0.38	**	0.39
5	11	0.30	**	**
6	100	1.00	100	-
7	100	1.00	100	1.00
8	96	1.00	95	1.00
9	100	1.00	100	-
10	75	0.99	82	0.98
11	100	1.00	100	-
12	100	1.00	98	1.00
13	100	1.00	100	1.00
14	51	0.70	**	**
15	100	1.00	100	-
16	53	0.80	46	0.61
17	100	1.00	100	-
18	30	0.73	**	0.68
19	40	0.90	**	0.80
20	100	1.00	100	-
21	100	1.00	100	-
22	65	0.99	72	0.99
23	100	1.00	100	1.00
24	100	1.00	100	-
25	100	1.00	100	1.00
26	100	1.00	100	1.00
27	86	1.00	77	1.00
28	35	0.67	34	0.76
29	100	1.00	100	1.00
30	100	1.00	100	-
31	58	1.00	57	1.00
32	78	1.00	79	1.00
33	100	1.00	100	-
34	94	1.00	87	1.00
35	93	1.00	92	-
36	68	0.94	66	0.94
37	66	1.00	78	0.99
38	100	1.00	100	-
39	100	1.00	100	-

* constrained as monophyletic; ** node not present due to topological differences; - node not present due to exclusion of taxa.

Fig. S1 ML tree based on the codon position-specific partitioning with numbered nodes (1-19). BS and BPP values for the corresponding nodes in ML and BI analyses are listed in the table below. All species are coloured according to family (see Fig. 2).

Table S1 Antarctic notothenioid samples with corresponding collection id (Table S2) and sample size (n) for stable isotope analysis

Samples	Collection id (n)
Nototheniidae	
Aethotaxis mitopteryx	56 (4), 11 (4), 47 (2), 49 (1)
Dissostichus mawsoni	56 (2), 11 (2), 17 (3)
Gobionotothen gibberifrons	56 (10), 10 (10)
Lepidonotothen larseni	9 (2), 10 (4), 36 (4), 1 (6), 2 (4)
Lepidonotothen nudifrons	17 (2), 18 (1), 40 (2), 44 (2), 46 (1), 53 (2)
Lepidonotothen squamifrons	54 (10), 16 (4), 17 (6)
Notothenia coriiceps	7 (10), 18 (3), 22 (1), 38 (1), 40 (1), 41 (4), 50 (1)
Notothenia rossii	12 (1), 17 (5), 21 (2), 29 (1), 32 (1), 51 (1)
Pleuragramma antarcticum	56 (10), 42 (1), 49 (9)
Trematomus eulepidotus	54 (5), 55 (2), 56 (3), 20 (1), 22 (8), 28 (1)
Trematomus hansoni	15 (1), 16 (1), 23 (5), 24 (1), 26 (1), 27 (1), 30 (1)
Trematomus newnesi	8 (10), 13 (1), 39 (1), 41 (8)
Trematomus nicolai	11 (2), 32 (3), 37 (1)
Trematomus tokarevi	31 (1), 33 (2), 36 (2), 38 (1), 48 (3), 52 (1), n.a. (1)
Artedidraconidae	
Pogonophryne barsukovi	20 (2), 35 (1), 42 (1), 48 (2), 49 (2)
Pogonophryne scotti	25 (1), 27 (1), 34 (6), 42 (2)
Bathydraconidae	
Gymnodraco acuticeps	54 (1), 56 (14)
Parachaenichthys charcoti	13 (1), 17 (1), 40 (3), 43 (2), 45 (1), 53 (3)
Channichthyidae	
Chaenocephalus aceratus	3 (9), 5 (1), 10 (10)
Chaenodraco wilsoni	56 (10)
Champsocephalus gunnari	4 (5), 6 (6), 27 (8), 51 (2)
Chionodraco rastrospinosus	55 (1), 56 (9), 19 (8), 22 (2)
Cryodraco antarcticus	56 (10), 23 (3), 28 (7)
Neopagetopsis ionah	56 (6), 11 (3), 47 (1), 49 (1), 52 (1)
Pseudochaenichthys georgianus	14 (1), 16 (9)

Table S2 Collection id for all Antarctic notothenioid samples. AP, Antarctic Peninsula, SO, South Orkney Islands, SSI, South Sandwich Islands, with mean values for latitude, longitude and depth

Collection id	Location	Latitude	Longitude	Depth
1	SSI	56°19'18"S	27°27'02"W	330 m
2	SSI	58°27'11"S	26°12'51"W	270 m
3	AP	61°20'44"S	55°15'23"W	350 m
4	AP	61°15'23"S	54°50'10"W	152 m
5	AP	60°58'59"S	55°11'08"W	299 m
6	AP	60°59'19"S	55°53'18"W	203 m
7	AP	61°00'20"S	55°43'40"W	96 m
8	AP	62°33'48"S	55°41'52"W	162 m
9	SO	60°26'15"S	46°17'46"W	142 m
10	SO	60°25'46"S	46°25'07"W	142 m
11	SO	60°30'53"S	46°35'08"W	457 m
12	SO	60°24'06"S	46°30'57"W	220 m
13	SO	60°28'58"S	46°21'53"W	106 m
14	SO	60°26'37"S	45°38'53"W	237 m
15	SO	60°26'32"S	45°16'51"W	497 m
16	SO	60°29'22"S	45°08'06"W	350 m
17	SO	60°31'53"S	44°45'24"W	310 m
18	SO	60°49'16"S	44°29'27"W	172 m
19	SO	60°36'31"S	44°20'33"W	211 m
20	SO	61°03'06"S	42°49'45"W	425 m
21	SO	60°51'29"S	42°52'18"W	359 m
22	SO	60°52'13"S	43°11'46"W	336 m
23	SO	61°17'30"S	43°05'25"W	469 m
24	SO	61°08'57"S	43°31'56"W	455 m
25	SO	61°02'38"S	44°42'50"W	254 m
26	SO	61°07'55"S	44°35'22"W	314 m
27	SO	61°08'01"S	44°13'59"W	337 m
28	SO	61°11'05"S	43°56'44"W	426 m
29	SO	61°33'52"S	45°15'32"W	259 m
30	SO	61°30'49"S	44°32'42"W	380 m
31	SO	61°36'25"S	44°24'23"W	390 m
32	SO	61°13'00"S	45°55'49"W	240 m
33	SO	61°49'12"S	46°11'30"W	453 m
34	SO	61°43'08"S	45°49'03"W	398 m
35	SO	61°14'04"S	46°23'16"W	274 m
36	SO	61°25'44"S	46°09'28"W	352 m
37	SO	60°54'59"S	45°37'17"W	294 m
38	SO	60°55'18"S	45°51'09"W	208 m
39	SO	60°53'57"S	46°03'26"W	187 m
40	SO	60°46'03"S	46°16'10"W	150 m
41	SO	60°37'59"S	46°31'26"W	130 m
42	SO	61°45'22"S	45°26'20"W	375 m
43	SO	60°39'11"S	46°16'52"W	104 m
44	SO	60°45'10"S	44°13'00"W	166 m
45	SO	60°42'49"S	46°00'02"W	96 m
46	SO	60°30'22"S	47°23'22"W	657 m
47	SO	61°03'16"S	46°49'16"W	764 m
48	SO	61°36'19"S	47°00'49"W	629 m
49	SO	61°52'30"S	46°43'21"W	750 m
50	SO	61°16'02"S	44°54'32"W	322 m
51	SO	60°50'07"S	43°48'18"W	221 m
52	SO	60°36'04"S	44°45'52"W	118 m
53	SO	60°48'03"S	45°53'35"W	128 m
54	AP	63°01'05"S	52°21'56"W	623 m
55	AP	62°35'14"S	53°46'22"W	731 m
56	AP	63°14'18"S	59°25'13"W	759 m

Table S3 Lifestyle and feeding for all included Antarctic notothenioid species. The listed feeding ecology was inferred from stomach content analyses (except for reference e, where it is unclear), and may not reflect the full diet

Species	Lifestyle	Feeding
Nototheniidae		
Aethotaxis mitopteryx	pelagic[b,d,g,h], benthopelagic[l]	gammarid, amphipod[l]
Dissostichus mawsoni	pelagic[d,h]	fish, misc. invert.[f]
Gobionotothen gibberifrons	benthic[d,g]	misc. invert., polychaete, salp, ophiuroid, krill, amphipod, isopod[f]
Lepidonotothen larseni	semipelagic[d]	misc. invert., krill, salp, mysid, amphipod[f]
Lepidonotothen nudifrons	benthic[d,h]	misc. invert., amphipod, polychaete, echinoderm, isopod, krill[f]
Lepidonotothen squamifrons	benthic[d]	salp, misc. invert., krill, fish, amphipod, polychaete, isopod[f]
Notothenia coriiceps	benthic[h]	krill, fish, misc. invert., salp[f]
Notothenia rossii	semipelagic[d]	fish , krill, salp, misc. invert., amphipod[f]
Pleuragramma antarcticum	pelagic[b,d,h]	krill, misc. invert.[f]
Trematomus eulepidotus	epibenthic[b,d,g]	krill, misc. invert., salp, fish, mysid, isopod[f]
Trematomus hansoni	benthic[d,g]	fish, misc. invert., krill, salp, octopus, isopod, mysid, amphipod[f]
Trematomus newnesi	cryopelagic[d]	krill, misc. invert., fish[f]
Trematomus nicolai	benthic[b,d,g,k,m], benthopelagic[a]	fish[f]
Trematomus tokarevi	benthic[m]	amphipod[f]
Artedidraconidae		
Pogonophryne barsukovi	benthic[n]	krill[f]
Pogonophryne scotti	benthic[d,n]	krill, fish, misc. invert., isopod[f]
Bathydraconidae		
Gymnodraco acuticeps	benthic[d]	krill[f]
Parachaenichthys charcoti	benthic[d]	fish, krill, misc. invert.[f]
Channichthyidae		
Chaenocephalus aceratus	benthic[d,i]	fish, krill, misc. invert., mysid[f]
Chaenodraco wilsoni	pelagic[j]	krill[e]
Champsocephalus gunnari	pelagic[d,i]	krill, fish[f]
Chionodraco rastrospinosus	benthic[d], benthopelagic[e]	krill, fish, misc. invert.[f]
Cryodraco antarcticus	pelagic[d], benthic[i]	fish, misc. invert., mysid, krill, amphipod[f]
Neopagetopsis ionah	pelagic[i]	fish, krill, misc. invert.[f]
Pseudochaenichthys georgianus	pelagic[d,i], semipelagic[d]	krill, fish, misc. invert., mysid[f]

[a]Brenner *et al.* 2001; [b]DeWitt *et al.* 1990; [c]Eakin 1990; [d]Eastman 1993; [e]Hureau 1985b; [f]Jones *et al.* 2009; [g]Klingenberg & Ekau 1996; [h]Kock 1992; [i]Kock 2005; [j]Kock *et al.* 2008; [k]Kuhn *et al.* 2009; [l]Kunzmann & Zimmermann 1992; [m]La Mesa *et al.* 2004; [n]Lombarte *et al.* 2003.

Table S4 GenBank accession numbers for all used samples. AP, Antarctic Peninsula, SO, South Orkney Islands, SSI, South Sandwich Islands

Species	Location	*cyt b*	*myh6*	*Ptr*	*tbr1*
Aethotaxis mitopteryx	AP	JF264479	JF264517	JF264555	JF264591
Aethotaxis mitopteryx	SO	JF264480	JF264518	JF264556	JF264592
Chaenocephalus aceratus	AP	JF264481	JF264519	JF264557	JF264593
Chaenocephalus aceratus	SO	JF264482	JF264520	JF264558	JF264594
Champsocephalus gunnari	AP	JF264483	JF264521	JF264559	JF264595
Champsocephalus gunnari	SO	JF264484	JF264522	JF264560	JF264596
Chaenodraco wilsoni	AP	JF264485	JF264525	JF264561	JF264597
Chionodraco rastrospinosus	AP	JF264486	JF264523	JF264562	JF264598
Chionodraco rastrospinosus	SO	JF264487	JF264524	JF264563	JF264599
Cryodraco antarcticus	AP	JF264488	JF264526	JF264564	JF264600
Cryodraco antarcticus	SO	JF264489	JF264527	JF264565	JF264601
Dissostichus mawsoni	AP	JF264490	JF264528	JF264566	JF264602
Dissostichus mawsoni	SO	JF264491	JF264529	JF264567	JF264603
Gobionotothen gibberifrons	AP	JF264492	JF264530	JF264568	JF264604
Gobionotothen gibberifrons	SO	JF264493	JF264531	JF264569	JF264605
Gymnodraco acuticeps	AP	JF264494	JF264532	JF264570	JF264606
Lepidonotothen larseni	SO	JF264495	JF264533	JF264571	JF264607
Lepidonotothen larseni	SSI	JF264496	JF264534	JF264572	JF264608
Lepidonotothen nudifrons	SO	JF264497	JF264535	JF264573	JF264609
Lepidonotothen squamifrons	AP	JF264498	JF264536	JF264574	JF264610
Lepidonotothen squamifrons	SO	JF264499	JF264537	JF264575	JF264611
Neopagetopsis ionah	AP	JF264500	JF264538	JF264576	JF264612
Neopagetopsis ionah	SO	JF264501	JF264539	JF264577	JF264613
Notothenia coriiceps	AP	JF264503	JF264540	HM050183	JF264614
Notothenia coriiceps	SO	JF264502	JF264541	JF264578	JF264615
Notothenia rossii	SO	JF264504	JF264542	JF264579	JF264616
Parachaenichthys charcoti	SO	JF264505	JF264543	JF264580	JF264617
Pleuragramma antarcticum	AP	JF264506	JF264544	JF264581	JF264618
Pleuragramma antarcticum	SO	JF264507	JF264545	JF264582	JF264619
Pogonophryne barsukovi	SO	JF264508	JF264546	JF264583	JF264620
Pogonophryne scotti	SO	HM049962	HM050072	HM050193	JF264621
Pseudochaenichthys georgianus	SO	JF264509	JF264547	JF264584	JF264622
Trematomus eulepidotus	AP	JF264510	JF264548	JF264585	JF264623
Trematomus eulepidotus	SO	JF264511	JF264549	JF264586	JF264624
Trematomus hansoni	SO	JF264512	JF264550	JF264587	JF264625
Trematomus newnesi	AP	JF264513	JF264551	HM050204	JF264626
Trematomus newnesi	SO	JF264514	JF264552	JF264588	JF264627
Trematomus nicolai	SO	JF264515	JF264553	JF264589	JF264628
Trematomus tokarevi	SO	JF264516	JF264554	JF264590	JF264629

File S1 Stable isotope values of all investigated species

Sample	Family	Species_Population	δ^{13}C (‰)	δ^{15}N (‰)
IF0929	Nototheniidae	*A.mitopteryx*_AP	-23.63	12.79
IF0930	Nototheniidae	*A.mitopteryx*_AP	-23.21	12.58
IF0932	Nototheniidae	*A.mitopteryx*_AP	-23.62	12.91
IF0933	Nototheniidae	*A.mitopteryx*_AP	-23.91	12.85
IF0665	Nototheniidae	*A.mitopteryx*_SO	-26.09	9.59
IF0779	Nototheniidae	*A.mitopteryx*_SO	-22.28	11.62
IF0780	Nototheniidae	*A.mitopteryx*_SO	-23.53	12.80
IF1011	Nototheniidae	*A.mitopteryx*_SO	-26.65	9.26
IF1012	Nototheniidae	*A.mitopteryx*_SO	-25.95	9.88
IF1013	Nototheniidae	*A.mitopteryx*_SO	-25.42	8.83
IF1010	Nototheniidae	*A.mitopteryx*_SO	-25.48	9.88
CA-604-01	Channichthyidae	*C.aceratus*_AP	-24.72	12.42
CA-604-02	Channichthyidae	*C.aceratus*_AP	-24.50	12.73
CA-604-03	Channichthyidae	*C.aceratus*_AP	-24.65	11.42
CA-604-04	Channichthyidae	*C.aceratus*_AP	-24.79	12.38
CA-604-06	Channichthyidae	*C.aceratus*_AP	-24.83	13.14
CA-604-07	Channichthyidae	*C.aceratus*_AP	-25.44	12.20
CA-604-08	Channichthyidae	*C.aceratus*_AP	-24.80	12.88
CA-604-09	Channichthyidae	*C.aceratus*_AP	-24.08	12.72
CA-604-10	Channichthyidae	*C.aceratus*_AP	-24.59	12.46
CA-610-01	Channichthyidae	*C.aceratus*_AP	-23.73	15.59
IF0002	Channichthyidae	*C.aceratus*_SO	-23.51	12.21
IF0004	Channichthyidae	*C.aceratus*_SO	-23.08	13.59
IF0005	Channichthyidae	*C.aceratus*_SO	-23.30	12.84
IF0007	Channichthyidae	*C.aceratus*_SO	-23.29	13.78
IF0008	Channichthyidae	*C.aceratus*_SO	-23.39	13.20
IF0009	Channichthyidae	*C.aceratus*_SO	-22.67	12.59
IF0010	Channichthyidae	*C.aceratus*_SO	-22.39	13.60
IF0011	Channichthyidae	*C.aceratus*_SO	-23.08	12.44
IF0013	Channichthyidae	*C.aceratus*_SO	-22.78	13.11
IF0014	Channichthyidae	*C.aceratus*_SO	-23.00	13.54
CG-606-02	Channichthyidae	*C.gunnari*_AP	-24.76	9.97
CG-606-03	Channichthyidae	*C.gunnari*_AP	-25.03	9.92
CG-606-04	Channichthyidae	*C.gunnari*_AP	-25.17	9.99
CG-606-06	Channichthyidae	*C.gunnari*_AP	-24.61	10.27
CG-606-09	Channichthyidae	*C.gunnari*_AP	-25.30	10.01
CG-626-02	Channichthyidae	*C.gunnari*_AP	-25.17	9.81
CG-626-03	Channichthyidae	*C.gunnari*_AP	-24.75	9.60
CG-626-04	Channichthyidae	*C.gunnari*_AP	-25.18	9.49
CG-626-05	Channichthyidae	*C.gunnari*_AP	-25.25	10.27
CG-626-06	Channichthyidae	*C.gunnari*_AP	-24.86	10.43
CG-626-01	Channichthyidae	*C.gunnari*_AP	-25.52	9.99
IF0273	Channichthyidae	*C.gunnari*_SO	-25.42	9.76
IF0276	Channichthyidae	*C.gunnari*_SO	-25.52	9.40
IF0444	Channichthyidae	*C.gunnari*_SO	-25.26	9.67
IF0445	Channichthyidae	*C.gunnari*_SO	-25.02	9.64
IF0448	Channichthyidae	*C.gunnari*_SO	-25.20	9.71
IF0449	Channichthyidae	*C.gunnari*_SO	-24.89	9.85
IF0452	Channichthyidae	*C.gunnari*_SO	-25.57	9.56
IF0453	Channichthyidae	*C.gunnari*_SO	-25.54	9.77
IF0454	Channichthyidae	*C.gunnari*_SO	-25.77	9.34
IF0456	Channichthyidae	*C.gunnari*_SO	-25.69	9.73

IF1082	Channichthyidae	*C.wilsoni*_AP	-26.19	8.08
IF1083	Channichthyidae	*C.wilsoni*_AP	-25.71	8.77
IF1084	Channichthyidae	*C.wilsoni*_AP	-25.18	8.66
IF1085	Channichthyidae	*C.wilsoni*_AP	-25.35	8.08
IF1086	Channichthyidae	*C.wilsoni*_AP	-25.02	8.37
IF1088	Channichthyidae	*C.wilsoni*_AP	-25.39	8.55
IF1089	Channichthyidae	*C.wilsoni*_AP	-25.80	8.65
IF1090	Channichthyidae	*C.wilsoni*_AP	-25.10	9.00
IF1123	Channichthyidae	*C.wilsoni*_AP	-25.11	8.48
IF1124	Channichthyidae	*C.wilsoni*_AP	-25.45	8.75
IF0887	Channichthyidae	*C.rastrospinosus*_AP	-25.12	8.68
IF1126	Channichthyidae	*C.rastrospinosus*_AP	-24.84	9.63
IF1127	Channichthyidae	*C.rastrospinosus*_AP	-25.23	8.51
IF1129	Channichthyidae	*C.rastrospinosus*_AP	-25.71	8.46
IF1130	Channichthyidae	*C.rastrospinosus*_AP	-25.47	10.04
IF1132	Channichthyidae	*C.rastrospinosus*_AP	-25.12	9.70
IF1134	Channichthyidae	*C.rastrospinosus*_AP	-25.13	8.02
IF1135	Channichthyidae	*C.rastrospinosus*_AP	-25.28	8.23
IF1142	Channichthyidae	*C.rastrospinosus*_AP	-26.04	7.30
IF1143	Channichthyidae	*C.rastrospinosus*_AP	-25.70	8.46
IF0257	Channichthyidae	*C.rastrospinosus*_SO	-24.46	9.00
IF0258	Channichthyidae	*C.rastrospinosus*_SO	-25.23	9.54
IF0259	Channichthyidae	*C.rastrospinosus*_SO	-24.29	9.45
IF0260	Channichthyidae	*C.rastrospinosus*_SO	-24.76	8.89
IF0261	Channichthyidae	*C.rastrospinosus*_SO	-24.40	10.69
IF0262	Channichthyidae	*C.rastrospinosus*_SO	-24.88	9.71
IF0263	Channichthyidae	*C.rastrospinosus*_SO	-24.67	9.54
IF0264	Channichthyidae	*C.rastrospinosus*_SO	-24.95	9.59
IF0306	Channichthyidae	*C.rastrospinosus*_SO	-25.05	9.54
IF0308	Channichthyidae	*C.rastrospinosus*_SO	-25.03	9.68
IF0927	Channichthyidae	*C.antarcticus*_AP	-23.31	13.73
IF1060	Channichthyidae	*C.antarcticus*_AP	-23.81	14.24
IF1061	Channichthyidae	*C.antarcticus*_AP	-25.06	11.24
IF1062	Channichthyidae	*C.antarcticus*_AP	-24.92	11.49
IF1064	Channichthyidae	*C.antarcticus*_AP	-25.14	11.15
IF1065	Channichthyidae	*C.antarcticus*_AP	-25.01	12.13
IF1066	Channichthyidae	*C.antarcticus*_AP	-25.21	11.63
IF1067	Channichthyidae	*C.antarcticus*_AP	-24.64	11.83
IF1068	Channichthyidae	*C.antarcticus*_AP	-24.93	11.27
IF1070	Channichthyidae	*C.antarcticus*_AP	-24.27	12.67
IF0416	Channichthyidae	*C.antarcticus*_SO	-22.58	12.45
IF0417	Channichthyidae	*C.antarcticus*_SO	-24.19	11.30
IF0420	Channichthyidae	*C.antarcticus*_SO	-24.78	11.66
IF0429	Channichthyidae	*C.antarcticus*_SO	-23.42	12.28
IF0430	Channichthyidae	*C.antarcticus*_SO	-23.68	12.45
IF0432	Channichthyidae	*C.antarcticus*_SO	-23.32	12.31
IF0433	Channichthyidae	*C.antarcticus*_SO	-24.54	11.37
IF0434	Channichthyidae	*C.antarcticus*_SO	-23.36	12.43
IF0435	Channichthyidae	*C.antarcticus*_SO	-24.57	12.25
IF0436	Channichthyidae	*C.antarcticus*_SO	-23.10	12.68
IF0925	Nototheniidae	*D.mawsoni*_AP	-24.08	13.89
IF0926	Nototheniidae	*D.mawsoni*_AP	-24.92	12.61
IF0206	Nototheniidae	*D.mawsoni*_SO	-22.27	14.61
IF0207	Nototheniidae	*D.mawsoni*_SO	-22.14	14.65
IF0208	Nototheniidae	*D.mawsoni*_SO	-21.19	14.40

IF1022	Nototheniidae	*D.mawsoni* _SO	-23.95	13.23
IF1023	Nototheniidae	*D.mawsoni* _SO	-24.18	13.47
IF0877	Nototheniidae	*G.gibberifrons* _AP	-21.34	14.73
IF0878	Nototheniidae	*G.gibberifrons* _AP	-21.86	13.90
IF0879	Nototheniidae	*G.gibberifrons* _AP	-21.89	14.10
IF0967	Nototheniidae	*G.gibberifrons* _AP	-22.18	14.02
IF1025	Nototheniidae	*G.gibberifrons* _AP	-23.03	14.92
IF1027	Nototheniidae	*G.gibberifrons* _AP	-20.06	13.79
IF1028	Nototheniidae	*G.gibberifrons* _AP	-23.33	14.07
IF1029	Nototheniidae	*G.gibberifrons* _AP	-20.83	14.61
IF1030	Nototheniidae	*G.gibberifrons* _AP	-19.76	13.86
IF1031	Nototheniidae	*G.gibberifrons* _AP	-21.32	13.87
IF0001	Nototheniidae	*G.gibberifrons* _SO	-23.11	11.59
IF0021	Nototheniidae	*G.gibberifrons* _SO	-20.79	13.22
IF0022	Nototheniidae	*G.gibberifrons* _SO	-22.77	12.87
IF0052	Nototheniidae	*G.gibberifrons* _SO	-22.97	13.06
IF0053	Nototheniidae	*G.gibberifrons* _SO	-20.25	12.95
IF0054	Nototheniidae	*G.gibberifrons* _SO	-22.17	12.45
IF0055	Nototheniidae	*G.gibberifrons* _SO	-21.90	13.06
IF0056	Nototheniidae	*G.gibberifrons* _SO	-22.64	11.54
IF0060	Nototheniidae	*G.gibberifrons* _SO	-22.51	11.72
IF0076	Nototheniidae	*G.gibberifrons* _SO	-22.52	11.86
IF0883	Bathydraconidae	*G.acuticeps* _AP	-24.56	11.08
IF0899	Bathydraconidae	*G.acuticeps* _AP	-24.46	12.54
IF0900	Bathydraconidae	*G.acuticeps* _AP	-23.84	13.07
IF0910	Bathydraconidae	*G.acuticeps* _AP	-24.75	12.27
IF0911	Bathydraconidae	*G.acuticeps* _AP	-23.43	13.46
IF0912	Bathydraconidae	*G.acuticeps* _AP	-23.76	13.24
IF0913	Bathydraconidae	*G.acuticeps* _AP	-23.69	12.41
IF0914	Bathydraconidae	*G.acuticeps* _AP	-23.86	13.18
IF0915	Bathydraconidae	*G.acuticeps* _AP	-23.34	13.03
IF0917	Bathydraconidae	*G.acuticeps* _AP	-23.44	13.43
IF0918	Bathydraconidae	*G.acuticeps* _AP	-22.95	12.70
IF0919	Bathydraconidae	*G.acuticeps* _AP	-23.08	12.54
IF0920	Bathydraconidae	*G.acuticeps* _AP	-23.33	12.35
IF0921	Bathydraconidae	*G.acuticeps* _AP	-24.68	11.91
IF0923	Bathydraconidae	*G.acuticeps* _AP	-23.66	13.15
IF0035	Nototheniidae	*L.larseni* _SO	-23.66	10.24
IF0037	Nototheniidae	*L.larseni* _SO	-24.54	10.36
IF0039	Nototheniidae	*L.larseni* _SO	-24.99	10.02
IF0043	Nototheniidae	*L.larseni* _SO	-24.07	10.03
IF0077	Nototheniidae	*L.larseni* _SO	-24.51	10.18
IF0078	Nototheniidae	*L.larseni* _SO	-24.25	10.26
IF0610	Nototheniidae	*L.larseni* _SO	-23.16	12.00
IF0611	Nototheniidae	*L.larseni* _SO	-23.23	11.33
IF0612	Nototheniidae	*L.larseni* _SO	-22.69	12.14
IF0613	Nototheniidae	*L.larseni* _SO	-23.61	11.62
LL-49-01	Nototheniidae	*L.larseni_* SSI	-23.81	13.61
LL-49-02	Nototheniidae	*L.larseni* _SSI	-24.53	11.31
LL-49-03	Nototheniidae	*L.larseni* _SSI	-23.96	13.57
LL-49-05	Nototheniidae	*L.larseni* _SSI	-24.27	12.03
LL-49-06	Nototheniidae	*L.larseni* _SSI	-24.36	11.69
LL-49-07	Nototheniidae	*L.larseni* _SSI	-23.18	13.05
LL-51-03	Nototheniidae	*L.larseni* _SSI	-24.49	10.69
LL-51-05	Nototheniidae	*L.larseni* _SSI	-24.05	11.01

LL-51-06	Nototheniidae	*L.larseni* _SSI	-23.20	10.85
LL-51-11	Nototheniidae	*L.larseni* _SSI	-23.73	11.02
IF0229	Nototheniidae	*L.nudifrons* _SO	-22.93	12.68
IF0230	Nototheniidae	*L.nudifrons* _SO	-21.35	12.64
IF0270	Nototheniidae	*L.nudifrons* _SO	-22.68	13.12
IF0271	Nototheniidae	*L.nudifrons* _SO	-21.87	12.71
IF0496	Nototheniidae	*L.nudifrons* _SO	-22.50	13.29
IF0661	Nototheniidae	*L.nudifrons* _SO	-21.88	12.74
IF0664	Nototheniidae	*L.nudifrons* _SO	-22.32	12.50
IF0719	Nototheniidae	*L.nudifrons* _SO	-22.37	13.02
IF0720	Nototheniidae	*L.nudifrons* _SO	-22.46	13.29
IF0909	Nototheniidae	*L.nudifrons* _SO	-24.46	12.79
IF0806	Nototheniidae	*L.squamifrons* _AP	-24.49	11.80
IF0807	Nototheniidae	*L.squamifrons* _AP	-23.98	14.80
IF0808	Nototheniidae	*L.squamifrons* _AP	-24.37	14.31
IF0809	Nototheniidae	*L.squamifrons* _AP	-24.78	12.60
IF0813	Nototheniidae	*L.squamifrons* _AP	-24.44	11.58
IF0814	Nototheniidae	*L.squamifrons* _AP	-24.91	13.65
IF0835	Nototheniidae	*L.squamifrons* _AP	-25.55	13.77
IF0836	Nototheniidae	*L.squamifrons* _AP	-24.88	12.39
IF0837	Nototheniidae	*L.squamifrons* _AP	-24.85	13.01
IF0838	Nototheniidae	*L.squamifrons* _AP	-25.24	13.00
IF0117	Nototheniidae	*L.squamifrons* _SO	-23.82	12.45
IF0119	Nototheniidae	*L.squamifrons* _SO	-22.96	12.53
IF0120	Nototheniidae	*L.squamifrons* _SO	-22.73	11.71
IF0121	Nototheniidae	*L.squamifrons* _SO	-23.79	11.41
IF0215	Nototheniidae	*L.squamifrons* _SO	-22.74	11.82
IF0216	Nototheniidae	*L.squamifrons* _SO	-22.85	12.09
IF0220	Nototheniidae	*L.squamifrons* _SO	-21.84	12.25
IF0221	Nototheniidae	*L.squamifrons* _SO	-23.63	12.18
IF0222	Nototheniidae	*L.squamifrons* _SO	-23.06	11.56
IF0223	Nototheniidae	*L.squamifrons* _SO	-24.83	11.21
IF0938	Channichthyidae	*N.ionah* _AP	-25.59	10.43
IF0939	Channichthyidae	*N.ionah* _AP	-25.68	8.32
IF0940	Channichthyidae	*N.ionah* _AP	-25.18	8.78
IF0942	Channichthyidae	*N.ionah* _AP	-25.44	7.94
IF0943	Channichthyidae	*N.ionah* _AP	-25.61	9.68
IF0944	Channichthyidae	*N.ionah* _AP	-25.60	9.60
IF0245	Channichthyidae	*N.ionah* _SO	-25.30	8.71
IF0670	Channichthyidae	*N.ionah* _SO	-25.66	8.72
IF0776	Channichthyidae	*N.ionah* _SO	-21.35	13.61
IF1016	Channichthyidae	*N.ionah* _SO	-25.35	8.68
IF1017	Channichthyidae	*N.ionah* _SO	-24.73	8.17
IF1018	Channichthyidae	*N.ionah* _SO	-25.16	8.19
NC-627-01	Nototheniidae	*N.coriiceps* _AP	-22.18	13.05
NC-627-02	Nototheniidae	*N.coriiceps* _AP	-25.60	11.47
NC-627-03	Nototheniidae	*N.coriiceps* _AP	-25.16	10.85
NC-627-04	Nototheniidae	*N.coriiceps* _AP	-25.13	10.39
NC-627-05	Nototheniidae	*N.coriiceps* _AP	-25.43	10.20
NC-627-06	Nototheniidae	*N.coriiceps* _AP	-23.44	11.72
NC-627-07	Nototheniidae	*N.coriiceps* _AP	-23.39	12.25
NC-627-08	Nototheniidae	*N.coriiceps* _AP	-25.45	10.88
NC-627-09	Nototheniidae	*N.coriiceps* _AP	-24.15	10.07
NC-627-10	Nototheniidae	*N.coriiceps* _AP	-23.62	11.77
IF0292	Nototheniidae	*N.coriiceps* _SO	-24.98	10.95

IF0491	Nototheniidae	*N.coriiceps*_SO	-21.96	13.38
IF0492	Nototheniidae	*N.coriiceps*_SO	-19.68	12.59
IF0493	Nototheniidae	*N.coriiceps*_SO	-22.18	13.88
IF0520	Nototheniidae	*N.coriiceps*_SO	-22.47	12.61
IF0724	Nototheniidae	*N.coriiceps*_SO	-24.26	11.45
IF0746	Nototheniidae	*N.coriiceps*_SO	-25.42	10.84
IF0748	Nototheniidae	*N.coriiceps*_SO	-25.46	11.40
IF0749	Nototheniidae	*N.coriiceps*_SO	-27.80	10.90
IF0751	Nototheniidae	*N.coriiceps*_SO	-21.54	12.90
IF0752	Nototheniidae	*N.coriiceps*_SO	-23.45	13.39
IF0224	Nototheniidae	*N.rossii*_ SO	-24.19	9.99
IF0224	Nototheniidae	*N.rossii*_SO	-25.22	8.16
IF0225	Nototheniidae	*N.rossii*_SO	-22.55	11.59
IF0226	Nototheniidae	*N.rossii*_SO	-23.58	11.13
IF0227	Nototheniidae	*N.rossii*_SO	-23.62	10.20
IF0327	Nototheniidae	*N.rossii*_SO	-23.54	11.32
IF0328	Nototheniidae	*N.rossii*_SO	-23.89	11.12
IF0554	Nototheniidae	*N.rossii*_SO	-25.21	10.84
IF0636	Nototheniidae	*N.rossii*_SO	-23.74	11.62
IF1005	Nototheniidae	*N.rossii*_SO	-26.14	10.78
IF0272	Nototheniidae	*N.rossii*_SO	-26.35	9.76
IF0231	Bathydraconidae	*P.charcoti*_SO	-21.77	13.50
IF0660	Bathydraconidae	*P.charcoti*_SO	-24.18	12.50
IF0695	Bathydraconidae	*P.charcoti*_SO	-21.90	14.24
IF0696	Bathydraconidae	*P.charcoti*_SO	-22.63	13.39
IF0699	Bathydraconidae	*P.charcoti*_SO	-23.28	13.23
IF0700	Bathydraconidae	*P.charcoti*_SO	-22.39	14.04
IF0728	Bathydraconidae	*P.charcoti*_SO	-23.31	13.30
IF0729	Bathydraconidae	*P.charcoti*_SO	-25.23	9.55
IF0781	Bathydraconidae	*P.charcoti*_SO	-23.00	13.14
IF0785	Bathydraconidae	*P.charcoti*_SO	-22.35	13.53
IF0902	Bathydraconidae	*P.charcoti*_SO	-24.27	10.16
IF1155	Nototheniidae	*P.antarcticum*_AP	-24.34	10.13
IF1156	Nototheniidae	*P.antarcticum*_AP	-26.58	10.05
IF1157	Nototheniidae	*P.antarcticum*_AP	-24.52	10.75
IF1158	Nototheniidae	*P.antarcticum*_AP	-24.73	11.08
IF1159	Nototheniidae	*P.antarcticum*_AP	-24.03	10.47
IF1160	Nototheniidae	*P.antarcticum*_AP	-24.26	10.89
IF1161	Nototheniidae	*P.antarcticum*_AP	-25.33	9.67
IF1164	Nototheniidae	*P.antarcticum*_AP	-24.16	10.12
IF1165	Nototheniidae	*P.antarcticum*_AP	-24.43	10.38
IF1167	Nototheniidae	*P.antarcticum*_AP	-24.30	9.95
IF0560	Nototheniidae	*P.antarcticum*_SO	-25.31	9.84
IF0563	Nototheniidae	*P.antarcticum*_SO	-24.51	10.24
IF0598	Nototheniidae	*P.antarcticum*_SO	-24.49	10.12
IF0599	Nototheniidae	*P.antarcticum*_SO	-25.20	10.81
IF0601	Nototheniidae	*P.antarcticum*_SO	-24.54	10.30
IF0602	Nototheniidae	*P.antarcticum*_SO	-24.81	11.00
IF0603	Nototheniidae	*P.antarcticum*_SO	-25.50	10.76
IF0604	Nototheniidae	*P.antarcticum*_SO	-25.06	10.31
IF0605	Nototheniidae	*P.antarcticum*_SO	-25.34	8.67
IF0607	Nototheniidae	*P.antarcticum*_SO	-24.46	9.95
IF0397	Artedidraconidae	*P.barsukovi*_SO	-24.23	11.93
IF0398	Artedidraconidae	*P.barsukovi*_SO	-23.42	12.54
IF0558	Artedidraconidae	*P.barsukovi*_SO	-22.14	11.23

IF0667	Artedidraconidae	*P.barsukovi*_SO	-23.95	13.21
IF0668	Artedidraconidae	*P.barsukovi*_SO	-22.71	12.28
IF0669	Artedidraconidae	*P.barsukovi*_SO	-24.39	12.68
IF0677	Artedidraconidae	*P.barsukovi*_SO	-23.96	12.93
IF0686	Artedidraconidae	*P.barsukovi*_SO	-23.18	12.19
IF0424	Artedidraconidae	*P.scotti*_SO	-23.08	11.64
IF0425	Artedidraconidae	*P.scotti*_SO	-22.24	12.20
IF0559	Artedidraconidae	*P.scotti*_SO	-22.49	12.09
IF0562	Artedidraconidae	*P.scotti*_SO	-23.03	11.95
IF0575	Artedidraconidae	*P.scotti*_SO	-21.36	12.04
IF0576	Artedidraconidae	*P.scotti*_SO	-21.74	11.89
IF0577	Artedidraconidae	*P.scotti*_SO	-21.53	11.95
IF0578	Artedidraconidae	*P.scotti*_SO	-22.03	12.15
IF0579	Artedidraconidae	*P.scotti*_SO	-21.61	12.38
IF0581	Artedidraconidae	*P.scotti*_SO	-22.07	11.89
IF0090	Channichthyidae	*P.georgianus*_SO	-25.07	9.73
IF0122	Channichthyidae	*P.georgianus*_SO	-23.72	11.79
IF0123	Channichthyidae	*P.georgianus*_SO	-24.00	10.51
IF0125	Channichthyidae	*P.georgianus*_SO	-25.09	10.86
IF0126	Channichthyidae	*P.georgianus*_SO	-23.99	12.25
IF0130	Channichthyidae	*P.georgianus*_SO	-23.74	11.18
IF0132	Channichthyidae	*P.georgianus*_SO	-23.22	12.07
IF0137	Channichthyidae	*P.georgianus*_SO	-24.32	12.02
IF0141	Channichthyidae	*P.georgianus*_SO	-23.69	11.70
IF0149	Channichthyidae	*P.georgianus*_SO	-23.67	11.98
IF0816	Nototheniidae	*T.eulepidotus*_AP	-25.16	9.63
IF0820	Nototheniidae	*T.eulepidotus*_AP	-23.94	11.19
IF0821	Nototheniidae	*T.eulepidotus*_AP	-24.64	10.83
IF0825	Nototheniidae	*T.eulepidotus*_AP	-23.51	12.25
IF0834	Nototheniidae	*T.eulepidotus*_AP	-23.48	11.06
IF0841	Nototheniidae	*T.eulepidotus*_AP	-24.35	10.81
IF0854	Nototheniidae	*T.eulepidotus*_AP	-24.63	10.64
IF1045	Nototheniidae	*T.eulepidotus*_AP	-24.74	10.35
IF1048	Nototheniidae	*T.eulepidotus*_AP	-24.88	10.16
IF1050	Nototheniidae	*T.eulepidotus*_AP	-24.05	10.50
IF0296	Nototheniidae	*T.eulepidotus*_SO	-24.30	11.70
IF0297	Nototheniidae	*T.eulepidotus*_SO	-25.04	9.73
IF0299	Nototheniidae	*T.eulepidotus*_SO	-25.23	10.23
IF0300	Nototheniidae	*T.eulepidotus*_SO	-24.46	10.71
IF0301	Nototheniidae	*T.eulepidotus*_SO	-24.44	11.04
IF0302	Nototheniidae	*T.eulepidotus*_SO	-25.46	9.69
IF0303	Nototheniidae	*T.eulepidotus*_SO	-24.86	10.45
IF0304	Nototheniidae	*T.eulepidotus*_SO	-24.67	10.67
IF0337	Nototheniidae	*T.eulepidotus*_SO	-25.05	10.87
IF0427	Nototheniidae	*T.eulepidotus*_SO	-25.04	10.11
IF0151	Nototheniidae	*T.hansoni*_SO	-23.49	12.11
IF0155	Nototheniidae	*T.hansoni*_SO	-23.91	11.67
IF0399	Nototheniidae	*T.hansoni*_SO	-24.59	9.66
IF0401	Nototheniidae	*T.hansoni*_SO	-24.60	11.57
IF0402	Nototheniidae	*T.hansoni*_SO	-24.56	11.10
IF0403	Nototheniidae	*T.hansoni*_SO	-23.87	11.29
IF0404	Nototheniidae	*T.hansoni*_SO	-23.45	13.15
IF0423	Nototheniidae	*T.hansoni*_SO	-24.05	11.58
IF0458	Nototheniidae	*T.hansoni*_SO	-23.70	11.67
IF0484	Nototheniidae	*T.hansoni*_SO	-23.35	11.38

IF0542	Nototheniidae	*T.hansoni* _SO	-24.73	11.77
TN-685-04	Nototheniidae	*T.newnesi* _AP	-25.20	9.61
TN-685-05	Nototheniidae	*T.newnesi* _AP	-24.45	10.64
TN-685-06	Nototheniidae	*T.newnesi* _AP	-24.16	10.83
TN-685-07	Nototheniidae	*T.newnesi* _AP	-23.86	11.16
TN-685-08	Nototheniidae	*T.newnesi* _AP	-23.25	10.81
TN-685-09	Nototheniidae	*T.newnesi* _AP	-23.60	10.97
TN-685-10	Nototheniidae	*T.newnesi* _AP	-23.25	10.39
TN-685-16	Nototheniidae	*T.newnesi* _AP	-24.26	9.79
TN-685-17	Nototheniidae	*T.newnesi* _AP	-23.74	10.69
TN-685-19	Nototheniidae	*T.newnesi* _AP	-23.17	10.95
IF0733	Nototheniidae	*T.newnesi* _SO	-24.29	9.41
IF0735	Nototheniidae	*T.newnesi* _SO	-24.87	9.68
IF0737	Nototheniidae	*T.newnesi* _SO	-25.47	9.14
IF0739	Nototheniidae	*T.newnesi* _SO	-25.76	8.78
IF0740	Nototheniidae	*T.newnesi* _SO	-24.45	10.22
IF0742	Nototheniidae	*T.newnesi* _SO	-25.31	9.25
IF0743	Nototheniidae	*T.newnesi* _SO	-24.37	9.85
IF0745	Nototheniidae	*T.newnesi* _SO	-23.85	9.89
IF0764	Nototheniidae	*T.newnesi* _SO	-23.95	10.34
IF1006	Nototheniidae	*T.newnesi* _SO	-24.75	9.68
IF0688	Nototheniidae	*T.nicolai* _SO	-25.23	8.70
IF0689	Nototheniidae	*T.nicolai* _SO	-25.27	8.36
IF0690	Nototheniidae	*T.nicolai* _SO	-25.19	8.32
IF0788	Nototheniidae	*T.nicolai* _SO	-25.07	8.49
IF1019	Nototheniidae	*T.nicolai* _SO	-24.19	8.77
IF1020	Nototheniidae	*T.nicolai* _SO	-25.07	8.61
IF0246	Nototheniidae	*T.tokarevi* _SO	-26.24	10.68
IF0502	Nototheniidae	*T.tokarevi* _SO	-26.24	10.41
IF0588	Nototheniidae	*T.tokarevi* _SO	-25.31	9.64
IF0589	Nototheniidae	*T.tokarevi* _SO	-24.43	9.66
IF0673	Nototheniidae	*T.tokarevi* _SO	-23.99	11.70
IF0674	Nototheniidae	*T.tokarevi* _SO	-25.31	9.36
IF0675	Nototheniidae	*T.tokarevi* _SO	-25.43	10.16
IF0682	Nototheniidae	*T.tokarevi* _SO	-24.90	8.54
IF0683	Nototheniidae	*T.tokarevi* _SO	-24.42	8.58
IF0791	Nototheniidae	*T.tokarevi* _SO	-25.40	9.04
IF0796	Nototheniidae	*T.tokarevi* _SO	-25.07	9.04

Text S1 Discussion of SIA results of individual species

Our results are only partly congruent with the lifestyles and feeding reports based on stomach content analyses (Fig. 4 and Tables 1, S3, Supporting information). *Chionodraco rastrospinosus*, for example, has been described as a benthic (Eastman 1993) or benthopelagic (Hureau 1985b) species but shows one of the lowest δ^{13}C values, suggesting a pelagic lifestyle. Our SIA results are, however, consistent with buoyancy assessments by Eastman & Sidell (2002), who reported low weight in seawater for *C. rastrospinosus,* which is indicative of a pelagic lifestyle. We also obtain conflicting results for *T. nicolai and T. tokarevi*, which are considered as benthic or benthopelagic species and as deep-water species, respectively (see Table 1 and references therein; Andriashev 1978). Our data suggest that both are pelagic species residing at low TLs (Fig. 4). Carbon isotopic signatures of *A. mitopteryx* indicate feeding on higher TL in disagreement with previous reports (Table S3, Supporting information). Finally, *D. mawsoni*, displays the greatest variation in δ^{13}C signatures and the highest mean δ^{15}N value, indicating a broad range of habitats along the benthic-pelagic axis and piscivorous feeding. This agrees with its characterization as one of the largest notothenioid species (up to 1.75 m in length) and a top predator (DeWitt *et al*. 1990). It has been suggested that individual specialization to different habitats is more common in predators due to higher intraspecific competition (Quevedo *et al.* 2009).

References

Andriashev AP (1978) *Trematomus tokarevi*, a new species of the family Nototheniidae (Pisces) from the abyssal waters near Antarctica. *Journal of Ichthyology*, **18**, 521-526.

DeWitt HH, Heemstra PC, Gon O (1990) Nototheniidae. In: *Fishes of the Southern Ocean* (eds Gon O, Heemstra PC), pp. 279-331. J.L.B. Smith Institute of Ichthyology, Grahamstown, South Africa.

Eastman JT (1993) *Antarctic Fish Biology: Evolution in a Unique Environment*. Academic Press, San Diego, California.

Eastman JT, Sidell BD (2002) Measurements of buoyancy for some Antarctic notothenioid fishes from the South Shetland Islands. *Polar Biology*, **25**, 753-760.

Hureau JC (1985b) Family Nototheniidae – Antarctic rock cods. In: *FAO Species Identification Sheets for Fishery Purposes. Southern Ocean. CCAMLR Convention Area. Fishing Areas 48, 58 and 88* (eds Fischer W, Hureau JC), 2, pp. 323-385. Food and Agriculture Organization of the United Nations, Rome, Italy.

Quevedo M, Svanbäck R, Eklöv P (2009) Intrapopulation niche partitioning in a generalist predator limits food web connectivity. *Ecology*, **90**, 2263-2274.

Curriculum vitae

MORITZ MUSCHICK

Ph.D. | M.Sc.

* 23. October 1980 in Wolfenbüttel, Germany
contact: moritzmuschick@me.com

EDUCATION

UNIVERSITY STUDIES & COURSES

2001 – 2007	Studying biology at the University of Constance, Germany. Received the Vordiplom (~ B.Sc.) in 2004 and the Diplom (~ M.Sc.) in biology in 2007. The master thesis was prepared in the group of Axel Meyer, entitled: „Evolution of the Midas Cichlid Species Complex in Crater Lake Apoyo, Nicaragua“.
2007 – 2011	Ph.D. studies in the group of Walter Salzburger at the University of Basel, Switzerland, on the topic of convergence and plasticity in the evolution of cichlid fishes. Received the Ph.D. in Zoology on 25th November 2011; grade: *summa cum laude*; examiners: W. Salzburger and P. Nosil
2008 5th/6th Nov	Foundations of the Theory of Speciation Course at the University of Lausanne, Switzerland by Sergey Gavrilets
2008 Nov/Dec	Analysis of Organismal Form Online course with the University of Manchester by Christian Klingenberg
2009 January	Workshop on Molecular Evolution Cesky Krumlov, Czech Republic by Michael Cummings and Scott Handley (Organisers)
2009 June	Evolutionary Biology in Guarda Guarda, Switzerland One-week course on proposal writing by Sebastian Bonhoeffer and Dieter Ebert (Organisers)

SCIENTIFIC EXPERIENCE

2002 - 2007	Working as a ‘helping scientist’ at the Chair for Zoology and Evolutionary Biology held by Axel Meyer at the University of Constance, Germany.
2007 – 2011	Working as a scientific assistant in the group of Walter Salzburger at the University of Basel, Switzerland.
2011	Collaborating with Patrik Nosil on the topic of niche-dimensionality hypothesis in Lake Tanganyikan cichlids as a visiting student in his lab at the University of Colorado, Boulder.
since 2012	investigating the genomic basis of adaptive radiation in *Timema* stick-insects as a postdoc in Patrik Nosil’s lab in Sheffield, UK.

TEACHING EXPERIENCE

2002 – 2007	Teaching students in the Animal Identification Course held annually by Gregor Schmitz at the University of Constance, Germany.
2008 - 2010 March	Teaching students in the practical course Zoology & Evolution organised by Walter Salzburger at the University of Basel, Switzerland.
2010 - 2011	Supervising Marco Colombo and Robin Kovac, master students in the Salzburger group.
2011	Co-supervising a three-week students excursion from the University of Basel to Lake Tanganyika
2012 – 2013	Co-supervising Michaela Maurer and Rachel Spinks, students in the Salzburger group

FIELD WORK EXPERIENCE

1997 July – Oct	Working as field assistant with Birgit Dörges and Jürgen Heucke, University of Braunschweig, Germany, in Northern Territory, Australia on adaptations in physiology and group structure to changing environmental conditions in the dromedary *Camelus dromedarius*.
1999 July/Aug	Working as field assistant with Kathrin Lampert, University of Würzburg, Germany, in the Comoé National Park, Côte d'Ivoire, on alternative life cycle strategies in the West African reed frog *Hyperolius nitidulus*.
2000 – 2001	Attending to civilian service in the 'NABU Naturschutzstation' in Kranenburg, Germany. Included bird observations and diversity assessments of freshwater molluscs.
2009 September	Supervising a students excursion to the volcanic crater-lakes of Nicaragua. Teaching field-work techniques and cichlid fish evolution.
2007, 2008, 2010, 2011	Collecting cichlid fish samples in Lake Tanganyika, Zambia, to study the convergent evolution of trophic adaptations.
2011	Expedition to Lake Tanganyika's remote areas, funded by the National Geographic Society
2012	Sampling expedition throughout California together with Patrik Nosil in search of *Timema* stick-insect species

PUBLICATIONS

Citation statistics can be viewed at: http://scholar.google.com/citations?hl=en&user=KVhG148AAAAJ

PUBLISHED

Muschick M, Indermaur A & Salzburger W
Convergence within an adaptive radiation of cichlid fishes.
Current Biology 22:2362–2368 **(2012)**

Colombo M, Diepeveen E, Muschick M, Santos E, Indermaur A, Boileau N, Barluenga M & Salzburger W
The ecological and genetic basis of convergent thick-lipped phenotypes in cichlid fishes
Molecular Ecology early view DOI: 10.1111/mec.12029 **(2012)**

Dittmann M, Roesti M, Indermaur A, Colombo M, Gschwind M, Keller I, Kovac R, Muschick M & Salzburger W
Depth-dependent abundance of Midas cichlid fish (*Amphilophus spp*.) in two Nicaraguan crater lakes.
Hydrobiologia 686:277-285 **(2012)**

Rutschmann S, Matschiner M, Damerau M, Muschick M, Lehmann MF, Hanel R & Salzburger W
Parallel ecological diversification in Antarctic notothenioid fishes as evidence for adaptive radiation.
Molecular Ecology 20:4707-4721 **(2011)**

Muschick M, Barluenga M, Salzburger W & Meyer A
Adaptive phenotypic plasticity in the Midas cichlid fish pharyngeal jaw and its relevance in adaptive radiation.
BMC Evolutionary Biology 11:116 **(2011)**

Barluenga M, Stölting KN, Salzburger W, Muschick M & Meyer A
Evolutionary biology - Evidence for sympatric speciation? Reply.
Nature 444:E13-E13 **(2006)**

Barluenga M, Stölting KN, Salzburger W, Muschick M & Meyer A
Sympatric speciation in a Nicaraguan crater lake cichlid fish.
Nature 439:719-723 **(2006)**

SUBMITTED

Muschick M & Salzburger W
Pharyngeal jaws and their evolutionary, ecological and behavioural significance.
Invited review submitted to *Journal of Fish Biology*

CONFERENCES & SEMINARS

ORAL PRESENTATIONS

SEEDS 2008 meeting, University of Lausanne, Switzerland, 23rd October 2008
Parallelism in pharyngeal jaws of cichlid fishes from Lake Tanganyika, East Africa

Internal seminar, Zoological Institute, University of Graz, Austria, 27th July 2009
Cichlid pharyngeal jaws – Their role in speciation and adaptive radiation

Willi Hennig Symposium on Phylogenetics and Evolution, University of Hohenheim, Germany, 29th September to 2nd October 2009
Evolution of pharyngeal jaw morphology in Tanganyikan cichlid fishes

Zürich Interaction Seminar, ETH Zürich, Switzerland, 5th October 2009
Evolution of pharyngeal jaw morphology in Tanganyikan cichlid fishes

Seminar at the Eawag (Swiss federal institute for aquatic research), Kastanienbaum, Switzerland, 9th December 2009
Evolution of body and pharyngeal jaw morphology in Tanganyikan cichlid fishes

Seminar at the Zurich University of Arts, Switzerland, 13th October 2010
Scientific Visualization

Evolution meeting, Norman, Oklahoma, 17th -23rd June 2011
Convergent evolution in the adaptive radiation of cichlid fishes in Lake Tanganyika, East Africa

Seminar at the Museum for Comparative Zoology, Harvard University, Cambridge, MA, 17th November 2011
Convergent evolution in the adaptive radiation of cichlid fishes in Lake Tanganyika, East Africa

Joint meeting Evolution & ESEB, Ottawa, Canada, 6th – 10th July 2012
Dimensionality and convergence in an adaptive radiation of cichlid fishes

ICDP workshop 'DeepCHALLA', Nairobi, Kenya, 10th – 13th September 2012
Cichlid evolution, crater lakes, and the promises of DeepCHALLA

Cichlid Science 2012, Leuven, Belgium, 16th – 19th September 2012
Convergence within the adaptive radiation of cichlid fishes in Lake Tanganyika

POSTER PRESENTATIONS

SMBE 2008 conference, Universitat de Barcelona, Spain, 5th to 8th June 2008
Parallelism in pharyngeal jaws of cichlid fishes from Lake Tanganyika, East Africa
Moritz Muschick and Walter Salzburger

ESEB 12th congress, Turin, Italy, 24th to 29th August 2009
Comparing adaptive radiations of Lake Tanganyika cichlid fishes within and across tribes
Moritz Muschick and Walter Salzburger

ESEB 13th congress, Tuebingen, Germany, 20th to 25th August 2011
Convergence is the natural outcome of processes driving adaptive radiations
Moritz Muschick and Walter Salzburger

North of England Young Evolutionary Ecologist Symposium, Liverpool, UK, 29th/30th March 2012
Convergence is the natural outcome of processes driving adaptive radiations
Moritz Muschick and Walter Salzburger

OTHER MEETINGS AND CONFERENCES ATTENDED

2nd Congress of Conservation of Biological and Cultural Diversity in the Andes and the Amazon Basin / 4th Ecuadorian Botanical Congress, Universidad Technica Particular, Loja, Ecuador, 25th to 30th August 2003

Phylogenetisches Symposium, Naturhistorisches Museum Braunschweig, Germany, 21st and 22nd November 2009

Latsis Symposium, ETH Zürich, Switzerland, 23rd and 24th November 2009

Continental Drilling in the East African Rift Lakes Workshop, Providence, RI, 14th to 16th November 2011

CONFERENCES & SYMPOSIA ORGANISED

Cichlid Science 2010 meeting in Basel, Switzerland, 26th to 29th August 2010. Initiation and organisation together with Britta Meyer, Adrian Indermaur and Yuri Klaefiger.

"Parallel Evolution" symposium at ESEB meeting in Tübingen, Germany, 20th to 25th August 2011. Organisation together with Walter Salzburger.

GRANTS

Travel grants by the Dr.-Oskar-Sommer Foundation for fieldwork in Australia (1997) and Ivory Coast (1999).

Travel grants by the University of Basel for participation in conferences and workshops 2008-2011.

Travel grant by the European Science Foundation's 'Frontiers in Speciation Research' program 2011.

Support grant for completing dissertation research by Freiwillige Akademische Gesellschaft, Basel, 2011.

Research fellowship for prospective researchers by the Swiss National Science Foundation, 2012-13.

REVIEWING SERVICES

BMC Evolutionary Biology
International Journal of Evolutionary Biology
Hydrobiologia
PLoS ONE
Evolution

OUTREACH

Was macht ein Evolutionsbiologe? Und wie wird man einer? (What does an evolutionary biologist do? And how to become one?)

Presentation and discussion with final-year highschool students of the Christophorusschule, Braunschweig, Germany 5th July 2012

SOCIETY MEMBERSHIPS

Freiwillige Akademische Gesellschaft (Basel, Switzerland) - *since 2011*
European Society for Evolutionary Biology (ESEB) - *since 2011*
Society for the Study of Evolution (SSE) - *since 2011*
Gesellschaft für Naturkunde (Braunschweig, Germany) - *since 2000*
Deutsche Zoologische Gesellschaft (München, Germany) - *since 2012*

PROFESSIONAL SKILLS

geometric morphometric shape analysis
R statistical programming language: experienced in applying and developing methods for comparative analysis
phylogenetic reconstruction
microCT scanning and 3D reconstruction
SCUBA Diving: PADI Advanced Open Water Diver
languages: German (native), English (fluent)

PHOTOGRAPHY

Museumsnacht Basel 2012 photo competition, 1st Prize

Research photo competition, University of Sheffield, 2012, 3rd Prize

Cover image for the Genomics of Adaptation special feature 2012 Proceedings of the Royal Society B
Image caption: *Timema cristinae* stick-insect resting on its host plant *Ceanothus spinosus*