

Kinematics of benthic suction feeding in Callichthyidae and Mochokidae, with functional implications for the evolution of food scraping in catfishes

Sam Van Wassenbergh^{1,*}, Tim Lieben¹, Anthony Herrel², Frank Huysentruyt³, Tom Geerinckx³,
 Dominique Adriaens³ and Peter Aerts^{1,4}

¹Department of Biology, Universiteit Antwerpen, Universiteitsplein 1, B-2610 Antwerpen, Belgium, ²Department of Organismic and Evolutionary Biology, Harvard University, 26 Oxford Street, Cambridge, MA 02138, USA ³Evolutionary Morphology of Vertebrates, Ghent University, K.L. Ledeganckstraat 35, B-9000 Gent, Belgium and ⁴Department of Movement and Sports Sciences, Ghent University, Watersportlaan 2, B-9000 Gent, Belgium

*Author for correspondence (e-mail: sam.vanwassenbergh@ua.ac.be)

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SUMMARY

Food scraping has independently evolved twice from suction feeding in the evolution of catfishes: within neotropical Loricarioidea and paleotropical Mochokidae. To gain insight in the evolutionary transitions associated with the evolution towards scraping, we analyzed prey capture kinematics in two species of benthic suction feeders which belong to taxa that are closely related to the scraper lineages (respectively, *Corydoras splendens* and *Synodontis multipunctatus*), and compared it to prey capture in a more distantly related, generalist suction feeder (*Clarias gariepinus*). Simultaneous ventral and lateral view high-speed videos were recorded to quantify the movements of the lower jaw, hyoid, pectoral girdle and neurocranium. Additionally, ellipse modeling was applied to relate head shape differences to buccal expansion kinematics. Similarly to what has been observed in scrapers, rotations of the neurocranium are minimal in the benthic suction feeders, and may consequently have facilitated the evolution of a scraping feeding mechanism. The hypothesis that fish with a more laterally compressed head rely more heavily on lateral expansion of the buccal cavity to generate suction, was confirmed in our sample of catfish species. Since an important contribution of lateral expansion of the head to suction may avoid the need for a strong, ventral depression of the mouth floor during feeding, we hypothesized that this may have allowed a closer association with the substrate in the ancestors of scrapers. However, our hypothesis was not supported by an ancestral state reconstruction, which suggests that scraping probably evolved from sub-terminal mouthed ancestors with dorsoventrally flattened heads.

Key words: catfish, feeding, prey capture, buccal expansion, suction, hyoid.

INTRODUCTION

Understanding the mechanical consequences of morphological variation is a major component of research in functional morphology and evolutionary biology. However, the relationship between form and function in an evolutionary context is not always easy to deduce. There can be several reasons for this: functional systems may be biomechanically complex (e.g. Van Hasselt, 1979; Aerts et al., 2003), and historical aspects need to be considered when explaining biological design (e.g. Bock, 1999; Herrel et al., 2004; Alfaro et al., 2004; Albertson et al., 2005).

The evolution of scraping systems in suckermouth catfishes is a compelling example of a system that is biomechanically complex (see Geerinckx and Adriaens, 2008; Geerinckx et al., 2007), and for which the phylogenetic history probably influenced the morphology of the present scraping species (Schaefer and Lauder, 1986; Schaefer and Lauder, 1996) (Geerinckx et al., 2009). Interestingly, suckermouth scraping of food such as algae, sessile invertebrates or wood has evolved twice independently during the evolution of catfishes: once within the South-American superfamily Loricarioidea, and once within the African catfish family of the Mochokidae (Fig. 1). Scraping in mochokids has emerged at a lower taxonomic level compared with the loricarioid lineage (Fig. 1), as shown in a recent molecular phylogeny (Sullivan et al., 2006). For both lineages, and for catfish in general, the ancestral prey capture mode is probably suction feeding (Bruton, 1996): generating a flow

of water to draw prey into the mouth by performing a rapid expansion of the mouth cavity. The omnivorous, benthic suction feeders of the Callichthyidae, and the mochokid genera like *Synodontis* and *Microsynodontis*, which show a similar trophic ecology (i.e. benthic suction feeding), are among the closest relatives of loricarioid and mochokid suckermouth species (Sullivan et al., 2006; Vigliotta, 2008).

If we want to ultimately understand the evolutionary transition towards scraping, it is essential to know how the feeding system functions in groups ancestral to scrapers (Schaefer and Lauder, 1986). Interestingly, it appears that the closest relatives of loricarioid and mochokid scrapers have retained the ancestral feeding mode (suction feeding) (Bruton, 1996) and are therefore the ideal groups to explore the ancestral characteristics of the feeding system relative to scrapers. In turn, since suction feeding biomechanics have been described in detail for Clariidae (Bruton, 1979; Van Wassenbergh et al., 2004; Van Wassenbergh et al., 2005; Van Wassenbergh et al., 2006a; Van Wassenbergh et al., 2006b; Van Wassenbergh et al., 2007), a siluroid family of generalist suction feeders that are more distantly related to scrapers, a comparative approach could shed light on which characteristics are essential to allow the evolution of scraping.

When reconstructing the evolutionary transitions in morphology, we tend to search for similarities between the derived (here: suckermouth scraping apparatus) and ancestral states (here: benthic

suction-feeding apparatus) along the phylogenetic tree. For example, the mobility of the premaxilla is already present in the suction feeding Callichthyidae, a group that is closely related to the scraping loricarioids (Schaefer and Lauder, 1986; Schaefer and Lauder, 1996). However, in this respect, an unusual feature seems to occur in Loricarioidei when looking at the general shape of the head (Fig. 2A): whereas loricariid scrapers and astroblepid suckermouths have a dorsoventrally flattened head, the most closely related suction feeders of the Callichthyidae have heads that are relatively narrow and high (laterally flattened), even when compared to other suction feeding catfish (Fig. 2A). Although less pronounced, a similar trend seems to occur within Mochokidae, where scraping species with predominantly dorsoventrally-flattened heads (*Atopochilus*, *Atopodontus*, *Chiloglanis*, *Euchilichthys*) tend to separate from the closely related, more laterally compressed heads of non-scrapers in a head width *versus* head height morphospace (Fig. 2B). Consequently, it appears as if the evolution towards scraping has passed through an intermediate stage where the head became laterally compressed, although no direct evidence is currently available to support this idea.

However, before we can speculate about potential evolutionary scenarios and pathways, the functional consequences of the differences in cranial morphology between suction feeding catfish with different head shapes need to be analyzed. Alexander (Alexander, 1970) predicted that fish with laterally compressed heads rely more on lateral expansion of the buccal cavity (i.e. abduction of the suspensorium and operculum) to generate suction, whereas fish with dorsoventrally flattened heads will be mechanically constrained to generate most of their buccal expansion by depressing the ventral side of the head (i.e. depression of lower jaw, hyoid and pectoral girdle) rather than by lateral expansion. Since ventral depression of the head during feeding interferes with remaining close to the substrate (Adriaens and Verraes 1994; Van Wassenbergh et al., 2006c), understanding how suction feeding ancestors of ventral-mouthed scrapers performed buccal expansion can be a critical aspect in elucidating the functional transition from benthic suction feeding to scraping.

The aims of the present study were to compare kinematics of buccal expansion between a typical, generalist suction feeding catfish, *Clarias gariepinus* (Van Wassenbergh et al., 2005), that is distantly related to the scraping taxa, and benthic suction feeding in representatives from the sister taxa of the South-American scrapers (Callichthyidae: *Corydoras splendens*) and African scrapers (Mochokidae: *Synodontis multipunctatus*). The results will be discussed in the context of the evolution towards scraping in catfish.

MATERIALS AND METHODS

Morphometrics

The diversity in cranial shape of catfishes was quantified by measuring the length (upper jaw tip to opercular slit in lateral view pictures), the height and width (both at the level of the opercular slits) of the head (Fig. 2). Data on 14 species of Callichthyidae and 49 species of other, non-mochokid suction feeders were obtained from a previous study (Van Wassenbergh et al., 2006a), where a list can be found of the species measured. For the present study, 22 species of Loricariidae (*Ancistrus megalostomus*, *A. occlioi*, *Chaetostoma lineopunctata*, *Chaetostomus leucomelas*, *C. mollinasus*, *Farlowella curtirostra*, *F. colombiensis*, *Harttia filamentissima*, *H. microps*, *Hemiancistrus arenarius*, *H. landoni*, *Loricaria beni*, *L. gymnogaster*, *L. pujanensis*, *Otothyris canaliferus*, *Plecostomus bolivianus*, *P. popoi*, *P. pularum*, *Pterygoplichthys juvenis*, *Rhinelepis levis*, *Sturisoma festivum*), 17

species of suction feeding Mochokidae (*Synodontis afrofischeri*, *S. dhonti*, *S. fuelleborni*, *S. granulatus*, *S. matthesi*, *S. multipunctatus*, *S. nigromaculatus*, *S. petricola*, *S. polli*, *S. punctulatus*, *S. ruandae*, *S. rufigiensis*, *S. rukwaensis*, *S. tanganyicae*, *S. victoriae*, *S. zambezensis*, *S. zanzibaricus*) and six species of scraping Mochokidae (*Chiloglanis harbinger*, *C. niger*, *C. polypogon*, *C. reticulatus*, *C. sanagaensis*, *Atopochilus vogti*) were added. Except for the *Synodontis* species and *Atopochilus vogti*, which were measured from lateral and dorsoventral drawings from Eccles (Eccles, 1992), all pictures were provided by the California Academy of Sciences, San Francisco (<http://www.calacademy.org/research/ichthyology>).

Study animals

Four *Corydoras splendens* Castelnau 1855 (cranial length=33.4±2.84mm; mean ± s.d.) and two *Synodontis multipunctatus* Boulenger 1898 (cranial length=26.1±0.6mm) specimens were obtained through the commercial trade. All animals were kept in separate, Plexiglas aquaria (35×18×30cm) during the course of the training and recording period. The aquaria were equipped with a mirror placed underneath the transparent floor at an angle of 45°. This mirror allowed simultaneous video recording of the feeding animal from a lateral and a ventral view, with a single camera. A constant water temperature of 24°C was maintained under a 12h:12h light:dark photoperiod. Before the recording sessions, fish were fed a variety of benthic prey, ranging from food pellets to fly larvae. Data on feeding kinematics of *Clarias gariepinus* were obtained from previous work (Van Wassenbergh et al., 2005; Van Wassenbergh et al., 2007).

High-speed video recording

High-speed videos of prey capture were recorded using a Redlake MotionPro camera, at 250Hz for *Corydoras splendens* or 500Hz for the slightly smaller *Synodontis multipunctatus*. Head size differences between the species forced us to use different prey for each species in order to mimic feeding on relatively large, benthic prey. Large pieces of earth worm (*Lumbricus terrestris*) with a diameter of approximately 80% of maximal gape size of the fish were used for *C. splendens*. *S. multipunctatus* was fed bloodworms (*Glycera* sp.) of approximately 10mm in length. Both prey were presented on the bottom of the aquarium. Three arrays of eight ultrabright, red LEDs provided the necessary illumination.

Only the prey capture sequences in which the fish approached the prey along a plane approximately perpendicular to the axis of the camera lens, and in which minimal or no roll or yaw could be discerned during prey capture, were used for further analysis. Note that yaw was observed in more than 80% of the recordings of *Synodontis multipunctatus* because these catfish typically re-oriented their head during suction after the prey has touched one of their long, maxillary barbels which extend towards the side of their head (see also Van Wassenbergh et al., 2007). Despite these practical limitations, we managed to analyze 13 sequences for each species (*C. splendens*: 6+3+3+2; *S. multipunctatus*: 6+7). From these thirteen videos used for the kinematical analysis, the six videos with the best sharpness and contrast for both lateral and ventral views were selected for volume modeling (see below).

Kinematical analysis

In order to study the movement of the most important elements of the feeding apparatus during prey capture in *Corydoras splendens* and *Synodontis multipunctatus*, and to enable comparison with previously published data on suction feeding kinematics in other

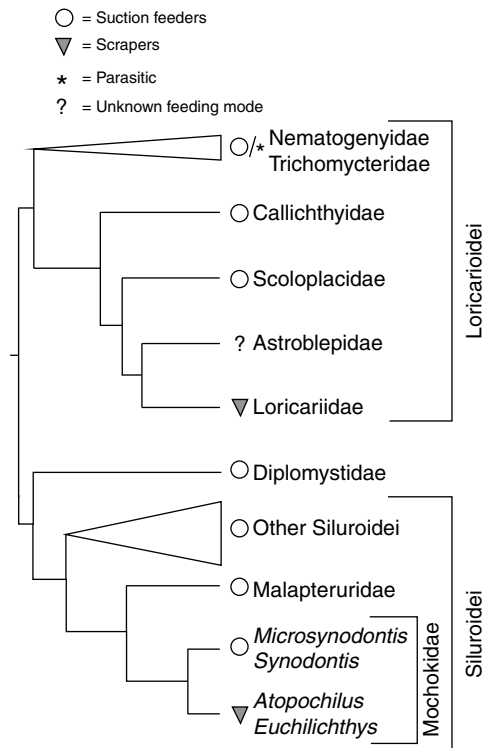


Fig. 1. Simplified phylogeny of catfishes (Siluriformes) based on molecular data (Sullivan et al., 2006), indicating the two lineages where scraping has evolved independently: Loricarioidei and Mochokidae. Triangles in the tree indicate a taxonomically large group.

catfish, a standard kinematical analysis was performed. To do so, five anatomical landmarks were digitized frame-per-frame on the lateral-view, high-speed video images using Didge software (Alistair Collum, Creighton University, Omaha, NE, USA), following the position of the eye, the rostral base of the dorsal fin, the lower jaw tip, the hyoid tip and the tip of the cleithrum (Fig. 3).

The x and y coordinates of all landmarks were recalculated to a frame of reference moving with the neurocranium. In this frame, the eye was taken as origin and the x -axis was approximately parallel with the roof of the buccal cavity (Fig. 3). To calculate this, the instantaneous inclination of the roof of the buccal cavity was assumed to correspond to the line between the landmark on the eye and the landmark on the dorsal fin base (which lies anterior of the articulation between neurocranium, including swimbladder capsule and Weberian apparatus, and vertebral column) after rotating it by 30° in the clockwise direction for fish facing towards the left (see Fig. 3). This enabled us to calculate the following kinematical variables: lower jaw depression ($-\Delta y$ lower jaw tip), hyoid depression ($-\Delta y$ hyoid tip), cleithrum depression ($-\Delta y$ cleithrum tip) and neurocranium pitch (increase of the x -axis angle with respect to the horizontal; downward facing pitch angles are defined as positive, upward facing is represented by negative angles). Digitization noise was reduced by applying a low-pass fourth-order zero phase-shift Butterworth filter (cut-off frequency of 25 Hz) to the raw data. The start of lower jaw depression was set as time=0.

Buccal expansion modeling

The increase in the volume of the buccal cavity is responsible for the flow of water (and prey) into the mouth. In order to evaluate potential interspecific differences in buccal expansion, the buccal

volume increase during suction was modeled using the ellipse method of Drost and Van den Boogaart (Drost and Van den Boogaart, 1986). The expanding buccal volume was approximated by a series of elliptical cylinders, in which the major and minor axis of each ellipse corresponds to the width and height of the buccal cavity at a certain position along the head's mediosagittal axis. The following data were needed for this: (1) a measurement of the dimensions of the buccal cavity (i.e. the width and height at specific points along the mediosagittal axis), for example from the head in compressed state, and (2) measurements of the changes of these ellipse axes in time during suction feeding (e.g. Van Wassenbergh et al., 2007).

The first type of data was obtained from lateral and ventral view radiographs of an unexpanded buccal cavity of a specimen overdosed with MS222 from each of the species in which the bucco-pharyngeal cavity was filled with a radio-opaque (barium) fluid (Fig. 4). The line connecting the upper jaw tip to a point equidistant between the base of the right and left pectoral fin served as mediosagittal axis. Height and width of the buccal cavity were measured at 21 points equally distributed along this line. It was assumed that this situation (i.e. the buccal volume distribution for the preserved specimen at rest) reflects the moment prior to the start of the suction event.

The second type of data was obtained from the high-speed videos. The upper and lower contours of the catfish head were digitized frame-by-frame (50 points each) in lateral and ventral view. At the same time, the coordinates of the mediosagittal axis described above were also digitized. The contour coordinates were recalculated to a new frame of reference moving with the fish, with the upper jaw tip as origin and the mediosagittal axis as the x -axis. Next, the distance between the left and right contours were extracted at 21 equally spaced intervals along the mediosagittal line between upper jaw tip and pectoral fin base. Similar to the procedure outlined for the standard kinematical analysis, digitization noise was reduced using a Butterworth filter algorithm applied to the profiles of length and width *versus* time. Finally, buccal volumes were calculated for each video frame by assuming that the thickness of the tissue layer between the internal (buccal cavity) and the external (head contours) boundaries of the head remains constant. To allow comparison between individuals of different size, all models were isometrically scaled to a head length of 25 mm.

Finally, the contribution of ventral expansion and lateral expansion to the total volume increase was calculated. The buccal volume increase due to ventral expansion equals the expansion of the model in which the widths of the ellipse cross-sections are held constant in time. This means that the otherwise time-varying ellipse widths are now set equal to the ones measured at time=0 ms for all time samples, and only the ellipse heights change as a function of time. Similarly, the buccal volume increase due to lateral expansion equals the expansion of the model in which the height of the model is held constant in time. This means that the otherwise time-varying ellipse heights are now set equal to the ones measured at time=0 ms for all time samples, and only the ellipse widths change as a function of time. Note that this implies that the sum of ventral expansion *only* and lateral expansion *only* will probably be less than the calculated total volume increase (because of the additive effect of simultaneous expansion in both directions).

Statistics

In order to test whether species differ in the measured kinematical variables, two-way analyses of variance (ANOVAs) were performed. The independent variables in these analyses are species (fixed) and individual (nested within species; random). In the case

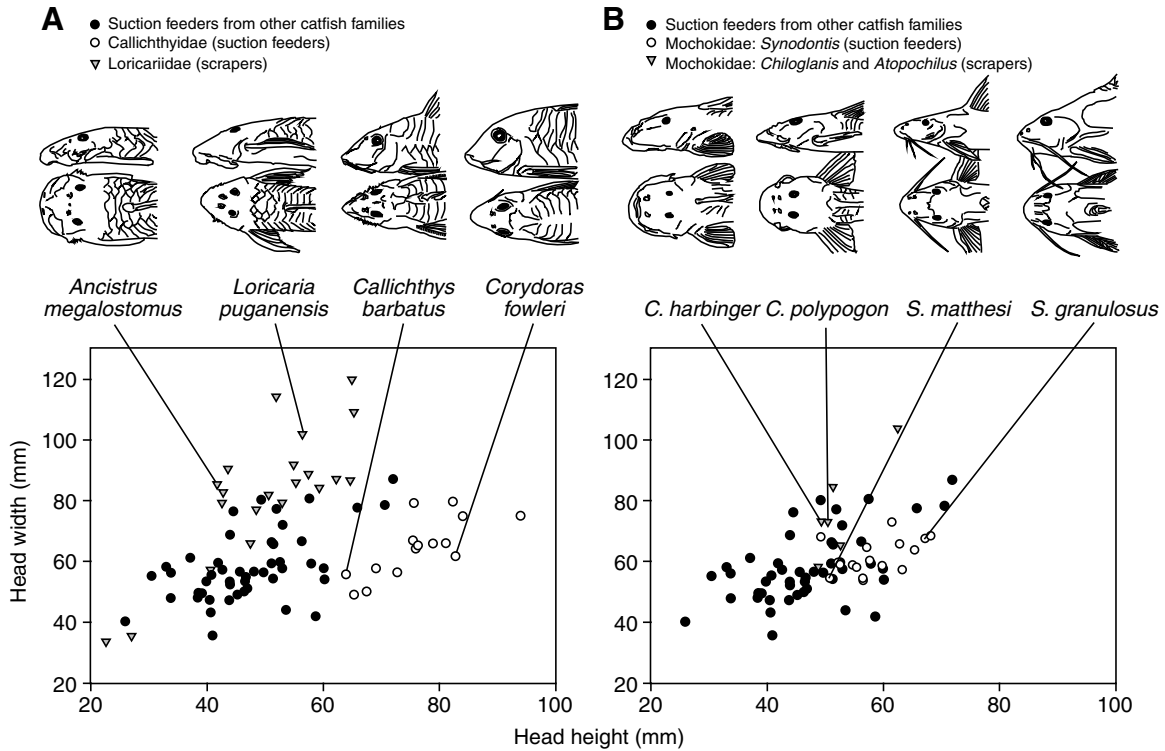


Fig. 2. Morphospace of cranial width *versus* cranial height at the level of the opercular slits, with each point representing a single species. All heads were isometrically scaled to a length of 72 mm. Non-loricarioid, non-mochokid suction feeders are shown [data from Van Wassenbergh et al. (Van Wassenbergh et al., 2006a)] in contrast to (A) scraping and non-scraping Loricarioidei or (B) Mochokidae. Note that closest relatives of the relatively broad-headed scraping Loricarioidei have a relatively high and narrow head with respect to other suction feeding catfish (A). The same trend, although less pronounced, can be observed within the Mochokidae (B).

that significant differences were found ($P < 0.05$), a relatively conservative *post-hoc* test (Tukey's honest significant difference test) was performed to test which species were different from one another.

Since the number of sequences analyzed per individual is (for practical reasons) limited for the volume modeling data ($N = 1, 2$ or 3), we were forced to treat within-species variation in these data as a combined effect of strike-to-strike and between-individual variation. First, overall differences between species in the characteristics of buccal expansion were tested using one-way ANOVA. Again, in the case that significant differences were found, a Tukey's *post-hoc* test was performed to compare the species separately. Second, least-squares linear regression analyses were performed to test Alexander's (Alexander, 1970) hypothesis regarding the contribution of expansion in the lateral or ventral direction in relation to the aspect ratio of the initial buccal volume. This aspect ratio was calculated as the mean aspect ratio (height/width) of the ellipse cross-sections of the modeled buccal cavity of each species (Fig. 4). The least-squares method was chosen because variation in the initial buccal aspect ratio within species is negligibly low compared with the exhibited strike-to-strike variation in buccal expansion (Sokal and Rohlf, 1995). All statistical analyses were performed with SPSS 15.0 (SPSS, Chicago, USA).

Ancestral state reconstruction

To evaluate evolutionary changes in head shape and mouth position, we reconstructed ancestral states of these traits using squared-change parsimony in Mesquite v2.01 (Maddison and Maddison, 2007). The relationships between terminal taxa are based on the Bayesian

phylogeny of *rag1* and *rag2* sequences in Sullivan et al. (Sullivan et al., 2006) which is largely supported by a more recent analysis of catfish interrelationships (Lundberg et al., 2007). In addition to some of the species included in Fig. 2 (see above), head shape (laterally or dorsoventrally flattened) and mouth position (terminal, sub-terminal or ventral) was scored based on pictures of the following species: Amphiliidae: *Amphilius uranoscopus* (Skelton, 1986), *Doumea typica* (pictures D.A.); Callichthyidae: *Aspidoras depinnae* (Britto, 2000), *Callichthys serralabium* (Lehmann and Reis, 2004); Mochokidae: *Microsynodontis batesii* (Ng, 2004),

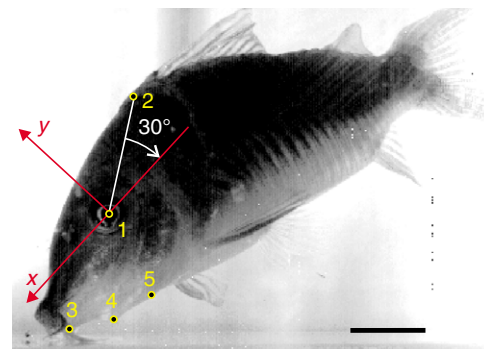


Fig. 3. Illustration of the frame of reference moving with the neurocranium, and the landmarks digitized for the kinematical analysis. These landmarks are: (1) the center of the eye, (2) the rostral base of the dorsal fin, (3) the tip of the lower jaw, (4) the tip of the hyoid and (5) the tip of the cleithrum. Scale bar, 10 mm.

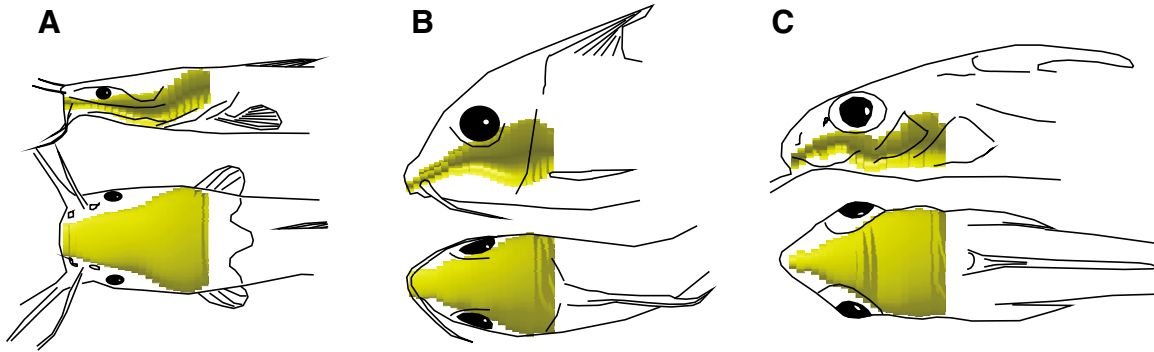


Fig. 4. Reconstruction of the starting volumes of the buccal cavities of *Clarias* (A), *Corydoras* (B) and *Synodontis* (C) from lateral view (top drawings) and dorsal view (bottom drawings) based on the ellipse method (Drost and Van den Boogaart, 1986) applied to X-ray images. Although the degree of lateral flattening is less pronounced at the level of the buccal cavity compared with the external head shape (because of the relatively high neurocranium in *Corydoras* and *Synodontis* compared with *Clarias*), a strong correlation ($R^2=0.983$) exists between external shape and buccal cavity shape among these three species.

Synodontis macrophthalmus, *Synodontis congicus* (Poll, 1971), *Mochocus brevis* (Boulenger, 1911); Malapteruridae: *Malapterurus electricus* (Norris, 2002); Scoloplacidae: *Scoloplax dicra* (Schaefer et al., 1989), Schilbidae: *Schilbe angolensis*, *Schilbe djeremi*, *Siluranodon auritus* (De Vos, 1995); Clariinae: *Chrysichthys brachynema* (Hardman, 2008).

RESULTS

Kinematics of lower jaw, hyoid, cleithrum and neurocranium

All species showed a typical, rostrocaudal sequence of ventral excursion of the lower jaw, hyoid and cleithrum (Figs 5 and 6). For example, in *Corydoras splendens* and *Synodontis multipunctatus*, maximum lower jaw depression (after 28.2 and 31.2 ms, respectively) precedes maximum hyoid depression (after 43.2 and 36.6 ms, respectively), which in turn precedes maximum cleithrum depression (after 91.0 and 50.4 ms, respectively). Suction feeding in both species nearly always followed contact of the prey with one of the barbels. All individuals of these species were capable of performing consecutive suction acts (mostly two, sometimes even three) to capture and transport the prey into the buccal cavity. Jaw prehension to pick up prey from the substrate was never observed.

Although the average kinematical profiles tended to show larger excursions of the lower jaw, hyoid and cleithrum in *Clarias gariepinus* compared with *Corydoras splendens* and *Synodontis multipunctatus* (Fig. 6), owing to considerable strike-to-strike variation and variation between individuals of a single species (see large standard error in Fig. 6), no significant differences were observed between these species in the magnitude of depression of the lower jaw tip (ANOVA, Wald $\chi^2=0.037$, d.f.=2, $P=0.981$), the hyoid tip (ANOVA, Wald $\chi^2=0.095$, d.f.=2, $P=0.954$), and the cleithrum tip (ANOVA, Wald $\chi^2=0.042$, d.f.=2, $P=0.979$).

However, the position and kinematics of the neurocranium during prey capture did differ significantly between the species. Firstly, the neurocranium pitch angle (i.e. the angle between the roof of the buccal cavity and the horizontal at the onset of feeding) differed considerably between the species (ANOVA, Wald $\chi^2=11275$, d.f.=2, $P<0.0001$). The mean pitch angle was the lowest in *Clarias gariepinus* (-5.0 ± 2.6 deg; mean \pm s.e.m.), the highest in *Corydoras splendens* (38.5 ± 1.8 deg), and intermediate in *Synodontis multipunctatus* (6.7 ± 1.4 deg; Fig. 6). All species differed significantly from each other in their mean pitch angle (Tukey's *post-hoc* test, P always <0.0001).

Secondly, the total decrease in neurocranium pitch angle (caused by neurocranial elevation) during suction feeding also differed significantly between the species (ANOVA, Wald $\chi^2=42.8$, d.f.=2, $P<0.0001$). *Clarias gariepinus* showed the largest pitch angle decrease (5.2 ± 0.9 deg), whereas *Corydoras splendens* had the least time-varying pitch angle (2.2 ± 0.7 deg). These two species differed significantly from each other in this kinematical variable (Tukey's *post-hoc* test, $P=0.027$). Again, *Synodontis multipunctatus* was intermediate between the other two other species (3.2 ± 0.9 deg), but did not differ significantly from *C. gariepinus* (Tukey's *post-hoc* test, $P=0.66$) or from *C. splendens* (Tukey's *post-hoc* test, $P=0.17$).

Buccal volume expansion

All species studied relied on ventral expansion of the buccal cavity as well as on lateral expansion (Fig. 7). The total volume increase (scaled to equal head lengths of 25 mm) calculated by the model significantly differed between the species (ANOVA, $F_{2,15}=4.02$, $P=0.04$). On average, the highest volume increase, as observed in *Synodontis multipunctatus* (832 ± 117 mm³), was significantly higher than in *Corydoras splendens* (463 ± 59 mm³; Tukey's *post-hoc* test, $P=0.043$). *Clarias gariepinus* was intermediate between these two bottom-feeding specialists (756 ± 105 mm³), and did not differ statistically from these species (Tukey's *post-hoc* test, $P>0.84$) when scaled to the same head size.

Although *Corydoras splendens* appeared to have a lower volume increase from ventral expansion (285 ± 44 mm³) compared with *Clarias gariepinus* (496 ± 39 mm³) and *Synodontis multipunctatus* (486 ± 93 mm³), the species did not differ significantly in this variable (ANOVA, $F_{2,15}=3.50$, $P=0.057$). Also the volume increase due to lateral expansion did not differ significantly between the species (ANOVA, $F_{2,15}=2.66$, $P=0.102$). Here, the average value was lowest in *Clarias gariepinus* (158 ± 39 mm³), followed by *Corydoras splendens* (205 ± 9 mm³) and *Synodontis multipunctatus* (284 ± 54 mm³).

If, however, the volume increase due to lateral expansion is expressed as a percentage of the total volume increase (resulting in a metric of how important lateral expansion is for generating suction), our species did differ significantly (ANOVA, $F_{2,15}=4.32$, $P=0.033$). The smallest contribution of lateral expansion to the total volume increase was calculated for *Clarias gariepinus* ($19.1\pm 2.6\%$), whereas *Corydoras splendens* showed the highest value ($51\pm 11\%$); these were significantly different (Tukey's *post-hoc* test, $P=0.026$).

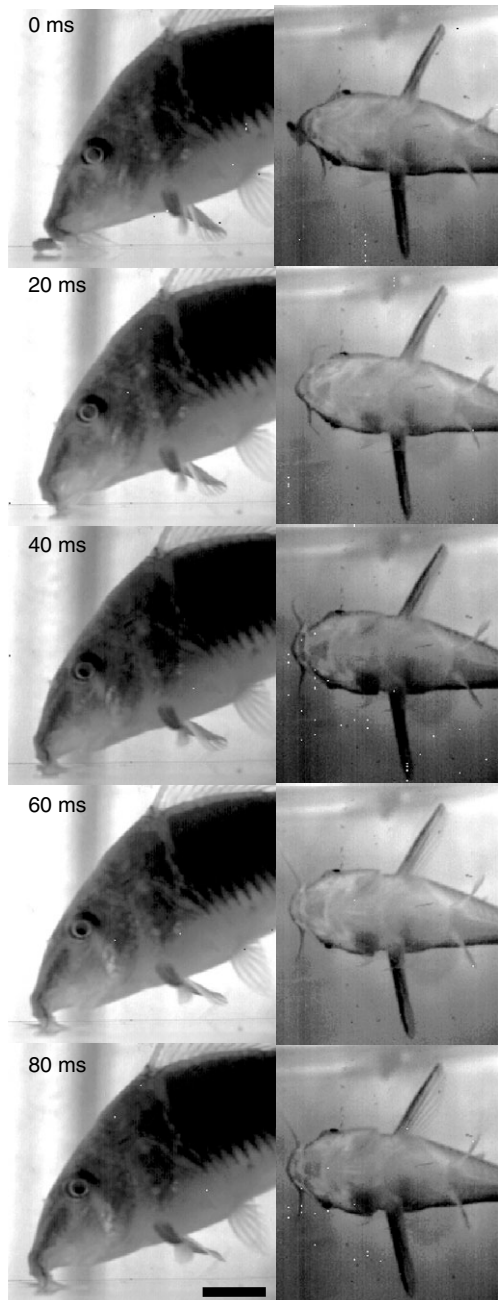


Fig. 5. Example of a prey capture sequence in *Corydoras splendens* in lateral view (left column) and ventral view (right column). Scale bar, 10 mm.

Synodontis multipunctatus took an intermediate position in lateral expansion contribution to suction ($36 \pm 7\%$), and did not differ significantly from both other species (Tukey's *post-hoc* test, $P > 0.29$). The relative contribution of ventral expansion to the total volume increase did not differ significantly between the species studied (ANOVA, $F_{2,15} = 0.58$, $P = 0.56$).

The regression analysis showed that the amount of ventral expansion decreased significantly when the initial buccal volumes become more laterally flattened in the species studied ($R^2 = 0.22$; $P = 0.047$; Fig. 8A). Furthermore, the relative contribution of lateral expansion to the total volume increase of the buccal cavity increased significantly with an increasingly laterally flattened head shape ($R^2 = 0.22$; $P = 0.008$; Fig. 8B). All other buccal expansion variables

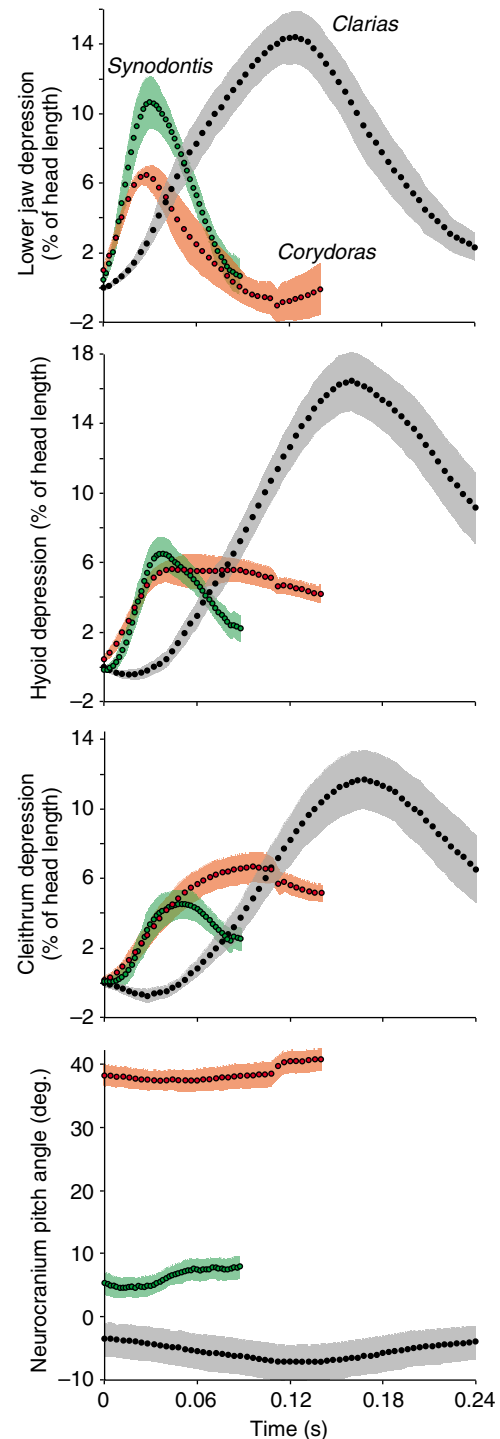


Fig. 6. Mean kinematic profiles of lower jaw depression, hyoid depression, cleithrum depression and neurocranium pitch angle in *Clarias gariepinus* [black; data from Van Wassenbergh et al. (Van Wassenbergh et al., 2005)], *Corydoras splendens* (red) and *Synodontis multipunctatus* (green). Shaded areas indicate standard errors ($N = 13$ sequences per species; two individuals of *C. gariepinus* and *S. multipunctatus*, four individuals of *C. splendens*). Note that the difference in speed (longer prey-capture time for *Clarias gariepinus*) is due to body size differences (head length *Clarias* = 80 ± 13 mm).

that were quantified were not significantly correlated with the average height to width ratio of the buccal cavity in the three species studied (total volume increase: $R^2 = 0.17$, $P = 0.09$; volume increase

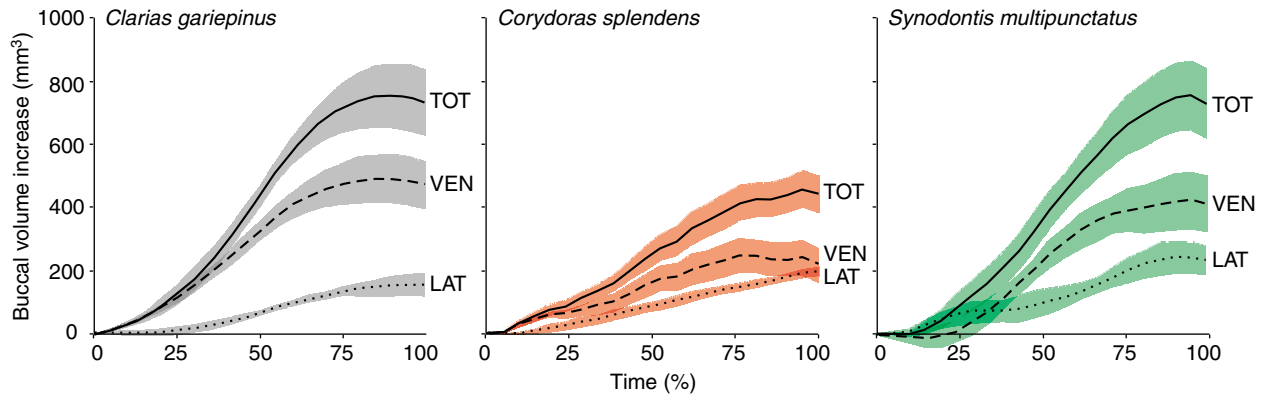


Fig. 7. Increase in the volume of the bucco-pharyngeal cavity during suction feeding calculated using ellipse models (see also Fig. 4 for start volumes). The 100% relative time (x-axis) corresponds to one frame after maximal volume. All models were scaled to a head length of 25 mm. Total volume increase (ventral and lateral expansion; solid line); VEN, the volume increase due to only ventral expansion (dashed line); and LAT, the volume increase due to only lateral expansion (dotted line). Shaded areas indicate standard errors.

due to lateral expansion: $R^2=0.05$, $P=0.36$; relative contribution of ventral expansion to the total volume increase: $R^2=0.05$, $P=0.40$).

DISCUSSION

Benthic suction feeding kinematics

Several constraints can be recognized for suction feeding close to the substrate. First, ground-impact on the elements of the feeding system that expand the buccal cavity in the ventral direction needs to be avoided (Adriaens and Verraes, 1994; Van Wassenbergh et al., 2006c). The present kinematical analysis of two specialist benthic suction feeding catfish species (*Corydoras splendens* and *Synodontis multipunctatus*) showed an increased pitch angle of the head with respect to the substrate during feeding compared to a generalist suction feeding catfish (*Clarias gariepinus*; Fig. 6). This behavior allows these species to rotate the lower jaw, hyoid and pectoral girdle ventrally without being hindered by the substrate. This feeding behavior is also observed in benthic feeding cichlid fish (Liem, 1980).

A second kinematical difference between the specialist benthic suction feeders and the generalist species was observed in the magnitude of rotation of the neurocranium with respect to the substrate. The benthic feeders tend to hold a more stable inclination of the neurocranium during suction feeding compared with the generalist *Clarias gariepinus*. Yet, it should be noted that the latter species can fine-tune the position of the upper jaw during suction by varying the amount of neurocranial elevation (Van Wassenbergh et al., 2006b). Therefore, the capacity to suction feed without considerable neurocranial elevation is probably a more general trait among catfishes. Little or no cranial elevation during feeding has also been reported for benthic suction feeding sharks (Motta et al., 2002) and may be advantageous for benthic species feeding between narrow rocky cavities to minimize neurocranial motion. Alternatively, or additionally, holding the mouth at a fixed position close to the substrate (which may not always be possible if the upper jaws are elevated together with the neurocranium) may beneficially influence the hydrodynamics of suction feeding (see Nauwelaerts et al., 2007).

Alexander's hypothesis

Alexander (Alexander, 1970) predicted that fish with laterally compressed heads rely more on lateral expansion of the buccal cavity (i.e. abduction of the suspensorium and operculum) to generate

suction, whereas fish with dorsoventrally flattened heads will be mechanically constrained to generate their buccal expansion by depressing the ventral side of the head (i.e. depression of lower jaw, hyoid and pectoral girdle) rather than by lateral expansion. The three

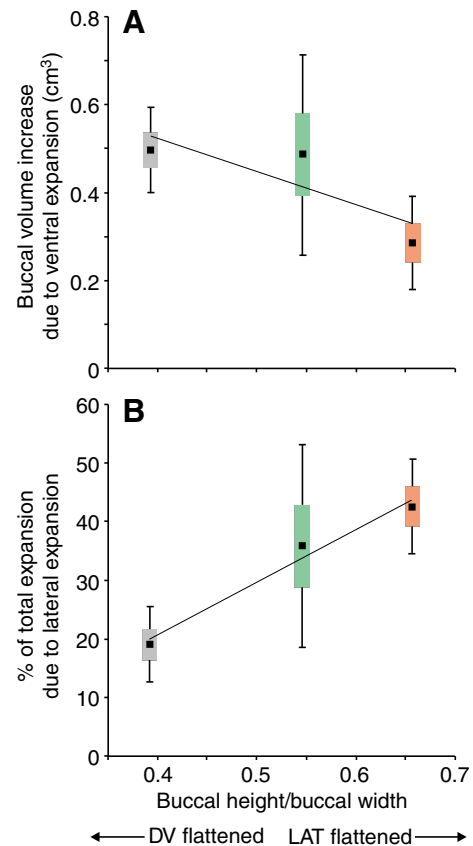


Fig. 8. Correlations between the average aspect ratio of the cross-sections of the buccal cavity (height/width) and (A) the volume increase due to ventral expansion, and (B) the relative contribution of lateral expansion to the total volume increase during suction feeding in the three species studied [ordered with increasing buccal height to width ratio, or from dorsoventrally (DV) flattened to more laterally (LAT) flattened: *Clarias gariepinus* (gray), *Synodontis multipunctatus* (green) and *Corydoras splendens* (red)]. Boxes represent s.e.m., whiskers represent s.d.

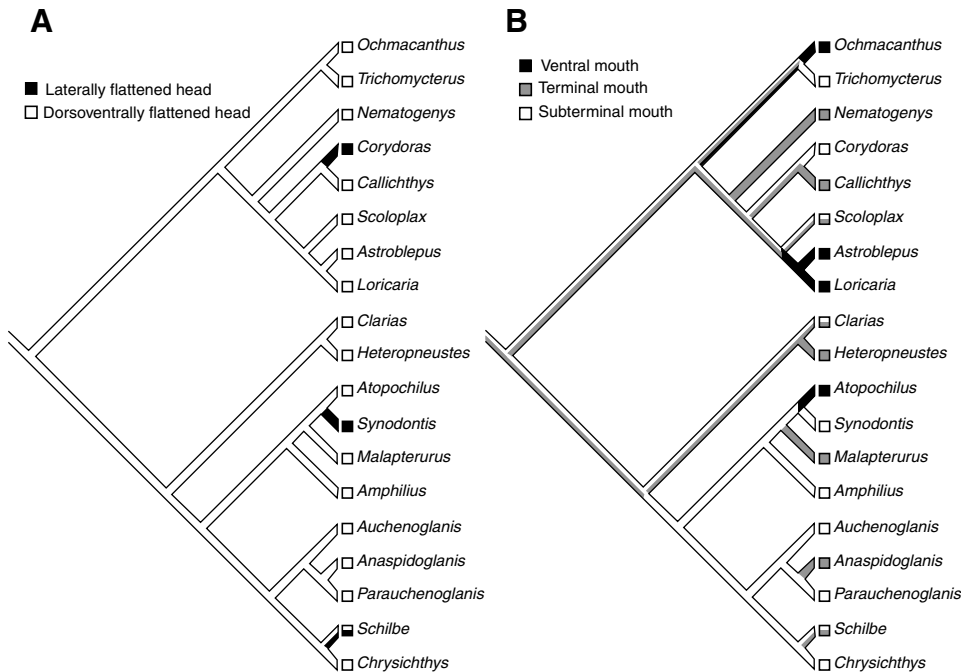


Fig. 9. Ancestral state reconstruction of head shape (A) and mouth position (B) in catfish assuming squared-change parsimony based on the Bayesian phylogeny of Sullivan et al. and Lundberg et al. (Sullivan et al., 2006; Lundberg et al., 2007). This reconstruction suggests that the ancestral state is a dorsoventrally flattened head, with evolution of a lateral flattened head only occurring within the *Corydoras*, *Synodontis* and *Schilbe* lineages (A). Mouth position is a phylogenetically highly variable trait, for which the ancestral state for catfishes can be either a terminal or subterminal mouth (B). These data suggest that a ventral mouth evolved in the sucker-mouth lineages (*Astroblepus*, *Loricaria* and *Atopochilus*) and Trichomycteridae (*Ochmacanthus*).

species included in this study have heads that are unequally dorsoventrally compressed (Fig. 2) (Van Wassenbergh et al., 2006a). *Clarias gariepinus* shows the most pronounced dorsoventral flattening of the buccal cavity (mean aspect ratio=0.39), followed by *Synodontis multipunctatus* (0.55) and *Corydoras multipunctatus* (0.66). Our study thus provided an ideal opportunity to test Alexander's hypothesis.

The presented data confirmed Alexander's hypothesis in that the contribution of lateral expansion to suction increased as the buccal cavity becomes more laterally flattened (Fig. 8B). Logically, the relative contribution of ventral expansion showed the opposite trend, although, owing to a higher interspecific variability for this variable, no significant correlation with buccal aspect ratio could be demonstrated. Yet, the total volume increase due to ventral expansion (expressed in absolute volumes) did show a negative correlation with increasing lateral compression of buccal cavity shape (Fig. 8A). Both correlations (Fig. 8) thus support Alexander's hypothesis.

Evolution of food scraping in catfish

The phylogenetic relationships among catfishes (Fig. 1) indicate that the behavior of acquiring food from the substrate by suction probably lies at the basis of the evolution of a scraping feeding mode. In two independent evolutionary lineages (Loricarioidei and Mochokidae), close relatives of scraping groups exclusively include specialist benthic suction feeding species. In the present study, we analyzed suction feeding kinematics in representatives of each of these lineages, and compared it to an outgroup species (generalist suction feeder *Clarias gariepinus*) in order to gain insights into the evolution of the highly specialized feeding strategy of scraping. Although we realize that phylogenetic statistical models are needed to provide evidence for pre-adaptations or exaptation, our analysis enables us to recognize characteristics that may have facilitated the evolution of food scraping.

An evolutionary scenario for the transition in jaw morphology and jaw kinematics in Loricarioidei has been discussed elsewhere

(Schaefer and Lauder, 1986). This and other studies (Geerinckx et al., 2007; Adriaens et al., 2009) suggest that scraping catfish are characterized by ventrally oriented upper and lower jaws, which possess a high range of mobility, kinematical independence of upper and lower jaws, and a left-right kinematical asymmetry of the lower jaws during feeding. This kinematical versatility of the jaws has probably resulted from decoupling events (e.g. upper jaws from neurocranium, loss of the lower jaw symphysis) during the evolutionary history of Loricarioidei (Schaefer and Lauder, 1986).

However, scrapers do not only differ in jaw movement from species that have retained the ancestral mode of feeding (i.e. suction feeding) (Bruton, 1996). Although currently quantitative kinematical data are only available for the jaws (Adriaens et al., 2009), feeding in sucker-mouth armored catfish (Loricarioidei) showed a roughly constant neurocranial pitch angle, a relatively limited amount of hyoid depression (approximately less than 5% of cranial length; cf. Fig. 6), no cleithrum depression (the cleithrum is anatomically locked to the pectoral girdle) and some abduction of the suspensoria (personal observations of *Pterygoplichthys disjunctivus*, *Ancistrus cf. triradiatus*, *Hemiloricaria parva* and *Farlowella acus*). The ability to feed without considerable neurocranial elevation could therefore have facilitated the evolutionary transition to scraping. As mentioned above, this capacity is already present in the more distantly related group of Clariidae (Van Wassenbergh et al., 2006b). However, the representative species from the sister taxa of scraping catfish showed even less movement of the neurocranium with respect to the substrate when compared to the clariid *Clarias gariepinus*.

Feeding without relying on an extensive lowering of the ventral floor of the buccal cavity can be regarded as a second feature that may facilitate the evolutionary shift from suction feeding to scraping. Whether this is observed in our data, is not entirely clear. On the one hand, there is no evidence that the ventral excursion of the hyoid and cleithrum is limited in the benthic suction feeders with respect to the generalist catfish *Clarias gariepinus*. On the other hand, we

did find a significantly lower volume increase of the buccal cavity due to ventral expansion in the loricarioid *Corydoras splendens* than in *Clarias gariepinus*, but not in the mochokid *Synodontis multipunctatus*.

Furthermore, as mentioned earlier, a relationship exists between the amount of volume increase due to ventral expansion and the shape of the mouth cavity (Fig. 8A). The more laterally compressed the buccal cavity is, the less suction is generated by ventral expansion (Fig. 8A), and the more important lateral expansion becomes (Fig. 8B). Could this relationship, together with the requirements for scraping, explain why we observed the (at first sight counter-intuitive) difference between head shape in scrapers (strongly dorsoventrally flattened; Fig. 2) and their suction feeding close relatives (tending to be more laterally compressed relative to other suction feeding catfish; Fig. 2)? In other words, did ancient catfish first become laterally compressed, enabling them to feed successfully without the need of important ventral expansion of the head, and subsequently evolve into scrapers during the evolutionary history of the mochokid and loricarioid lineages?

To answer this question, we performed an ancestral state reconstruction of head shape (dorsoventrally compressed vs laterally compressed) along the phylogenetic tree of catfish (Fig. 9A). The results of this analysis suggest that evolution of food scraping occurred without an evolutionary precursor characterized by a laterally compressed head. The latter head shape only seems to have evolved from a dorsoventrally flattened head at the level of specialized benthic suction feeding taxa (*Corydoras*, *Synodontis*). Consequently, the most plausible evolutionary scenario of scraping in catfish is that a shift in mouth position from sub-terminal to ventral (Fig. 9B) occurred in a dorsoventrally flattened ancestor. In this scenario, evolution towards a ventrally (and caudally) protruding lower lip as part of the sucker disk, as can be observed in extant species from scraping taxa, may have prevented direct contact between the hyoid and the substrate and could thus have allowed a certain, though relatively limited, amount of hyoid depression without substrate hindrance.

Conclusions

Both benthic suction feeding species studied (each closely related to a lineage in which an independent evolution of scraping occurred) showed a relatively high neurocranial pitch angle when feeding, with the neurocranium being very little rotated with respect to the substrate during feeding. This nearly immobile neurocranium may have facilitated the evolution of a scraping mode of feeding. The relationship between head shape and the direction of expansion during suction feeding, as proposed by Alexander (Alexander, 1970), was confirmed in our sample of catfish species. The reduced ventral expansion in species with more laterally compressed heads may explain the striking difference in head shape of suction feeders that are closely related to scrapers, and more distantly related, non-specialized benthic suction feeding catfish. Although the capacity to feed successfully by suction despite a reduced ventral expansion of the head probably facilitates close contact with the substrate, the current catfish phylogeny suggests that scraping evolved directly from ancestors with dorsoventrally compressed heads in the mochokid and loricarioid lineages.

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