

Covariation of trophic and habitat-related traits in chondrostoms (Cyprinidae): implications for repeated and diversifying evolutionary processes

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Keywords

fish ecomorphology; repeated evolution; allometric repatterning; Procrustes analysis; adaptation.

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Abstract

The tenet that ecological adaptation can lead to recurrent ecomorphological trends resulting from repetitive processes has long been a primary topic of investigation in evolutionary ecology. To explore this aspect further, this study provides an analysis of the morphological diversity in chondrostoms (Cyprinidae). This freshwater fish group shows a tendency towards bottom-feeding specialization, which has led to evolutionary innovations in body and mouth shape traits, which are currently used for the classification of genera. Body, lower lip (LL) and corner ray shape were analysed for nine species in total. Allometric relationships among the three morphometric traits were considered to be responsible for LL shape variability and there was significant covariation between LL and body shape, which reflected habitat use. Smaller and opportunistic-feeding species inhabiting stream or small-sized rivers were characterized by a deeper body (increased feeding maneuverability), an arched LL and a terminal mouth position. Conversely, larger and diet-specialized species were characterized by a fusiform body (increased swimming performance), a straight LL and an inframouth position on an elongated snout (optimized bottom feeding). The results suggest that interspecific mouth shape variability may have originated from two types of 'deformation' processes, both leading to a straight mouth shape and acting either jointly or independently. Also, given the plesiomorphic state of several of the species under study, the specialization towards a benthic lifestyle in chondrostoms from different phylogenetic lineages is thought to have occurred repeatedly to overcome a number of functional constraints, including foraging efficiency and swimming performance.

Introduction

In the study of ecomorphology, the question of what processes are responsible for molding morphological variability at the lower taxonomic levels (i.e. family, genus) becomes especially relevant in the light of recurring evolutionary trends. These indicate that similar adaptations can result from repetitive evolutionary processes each with a different likelihood of occurrence (Gompel & Prud'homme, 2009). At the same time, repeated evolution can be facilitated by similar genetic (Elmer & Meyer, 2011) and/or developmental pathways (Nijhout, 1991), hence as a result of interactions between genotype and developing phenotype (Vilizzi & Kováč, 2014).

Freshwater fish represent an ideal taxonomic group for investigating ecomorphological trends and relationships, as proven by the extensive literature in support (Table S1). Thus, several cases of repeated evolution have been documented at the genus level, suggesting that similar morphological diversification events may occur through similar evolutionary processes in comparable environments (e.g. as in cavefishes, sticklebacks, whitefishes and cichlids: Elmer & Meyer, 2011). Likewise, at the family level, the same morphological structures can be recurrently targeted by selection processes, as observed in Centrarchidae (e.g. pumpkinseed *Lepomis gibbosus*: Brinsmead & Fox, 2002) and Cyprinidae (e.g. roach *Rutilus rutilus*: Haas, Blum & Heins, 2010), which are known

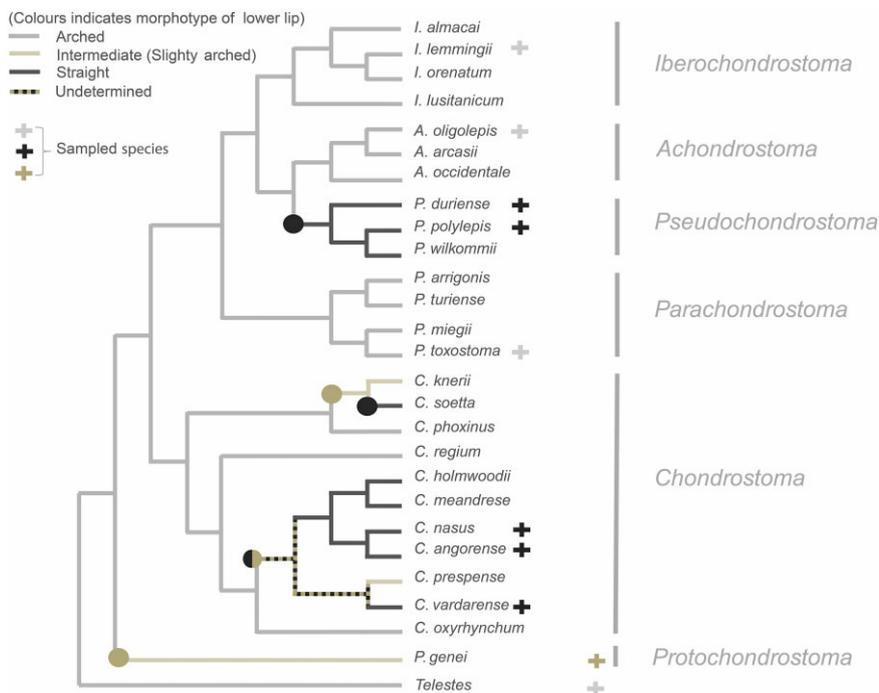


Figure 1 Character mapping based on molecular phylogeny of the lower lip (LL) morphology in the nine chondrostom species under study and relative to the (control) *Telestes* group. The original topology of the tree (from Robalo *et al.*, 2007) was completed with the inclusion of *Chondrostoma vardarensis* (based on Perea *et al.*, 2010). Character mapping of the LL based on Robalo *et al.* (2007). Note that genus names from Robalo *et al.* (2007) have changed in accordance with more recent taxonomy.

to undergo similar changes in body depth between lotic and lentic populations.

Chondrostoms are a Eurasian freshwater fish group that has diversified since the Miocene (Durand *et al.*, 2003; Robalo *et al.*, 2007; Perea *et al.*, 2010). Although currently living species occur predominantly in stream (lotic) habitats, some populations are also encountered in lentic systems. Within this group, there has been an evolutionary tendency towards trophic specialization, as reflected by changes in mouth position and lower lip (LL) shape. Phylogenetically, the most parsimonious hypothesis in regard purports that chondrostoms comprise six different lineages, which have been recently classified into corresponding genera: *Achondrostoma*, *Chondrostoma*, *Iberochoondrostoma*, *Parachondrostoma*, *Protochoondrostoma* and *Pseudochoondrostoma* (Robalo *et al.*, 2007).

Based on the above-mentioned classification, the last common chondrostom ancestor had a terminal mouth, a short snout and a hornless arched LL shape (Durand *et al.*, 2003; Doadrio & Carmona, 2004), reflecting the ‘plesiomorphic’ state that characterizes most of the currently living species (e.g. *Achondrostoma oligolepis* and *Iberochoondrostoma lemmingii*). Whereas, in other species the mouth migrated to a more inferior position (e.g. *Parachondrostoma toxostoma*) and in some cases this shift was accompanied by corresponding changes in LL shape, which have been described as either straight (e.g. *Chondrostoma nasus*) or intermediate (slightly arched: e.g. *Protochoondrostoma genei*) (Fig. 1). Species with intermediate or straight shapes are found in four lineages and branched off from sister lineages with arched shape species. These four lineages include: (1) *Protochoondrostoma* (with the only species *Protochoondrostoma genei*), which branched away from all other lineages; (2) *Pseudochoondrostoma* (including

Pseudochoondrostoma duriense, *Pseudochoondrostoma polylepis* and *Pseudochoondrostoma wilkommii*), which branched together with the *Achondrostoma* arched-shape species; (3) a first *Chondrostoma* subgroup (including *C. knerii* and *C. soetta*), which branched together with arched-shape *C. phoxinus*; and (4) a second *Chondrostoma* subgroup (including *C. holmwoodii*, *C. meandrese*, *C. nasus*, *C. angorensis*, *C. prespense* and *C. vardarensis*), which branched together with arched-shape *C. oxyrhynchum*.

Despite a degree of uncertainty surrounding morphological evolution in chondrostoms, it has been suggested that independent evolution of the LL shape trait may have occurred at least three times, namely twice in *Chondrostoma* and once in *Pseudochoondrostoma*. It has also been hypothesized that the LL shape trait may be correlated with foraging specialization (Nelva-Pasqual, 1985). This is because a straight LL morphotype is thought to enhance grazing performance compared with an arched LL morphotype, which in turn would guarantee a generalist feeding capacity reflecting a more generalist diet. This hypothesis has been confirmed in the French chondrostom species complex, for which dietary analyses have revealed that the arched LL morphospecies *Parachondrostoma toxostoma* can be regarded as a generalist feeding on both invertebrates and diatoms, in contrast to the straight LL morphospecies *C. nasus*, which is considered to be a diatom specialist (Nelva-Pasqual, 1985; Corse *et al.*, 2010).

Because trophic specialization in fish is closely related to habitat use, multiple functional traits need to be accounted for whenever attempting ecomorphological predictions. This is especially true for body shape, which is known to reflect closely life-history traits in fishes (e.g. Záhorská *et al.*, 2009; Novomeská *et al.*, 2013). In this respect, current ecological

knowledge of chondrostoms points to interspecific differences in habitat use (Table 1) and in this regard the independent evolution of a straight LL shape within the group suggests a repeatedly adaptive process towards a new ecological niche.

The aim of this study was to investigate what molds ecomorphological diversity in chondrostoms and what are the selective forces at play. To this end, an extensive morphological analysis of LL shape and body shape traits was conducted on nine chondrostom species representative of each of the six currently recognized genera. An analysis of covariation between LL shape and body shape also was performed to test whether changes in the former traits are correlated with other morphological evolutionary characters. Finally, a correlation analysis between morphological data and species-specific ecological knowledge was performed to test whether LL and body shape covariation in chondrostoms is correlated with habitat changes.

Material and methods

Specimens from nine representative chondrostom species and one 'control', non-chondrostom cyprinid (i.e. *Telestes souffia*; Durand *et al.*, 2003) were sampled by electrofishing in 2012 from eight rivers across southern Europe, which were chosen to encompass the current distributional range of the group (Table 2; also Table S2). Upon capture, fish were anaesthetized in Eugenol (Clarben SA, Madrid, Spain), placed on a polystyrene board equipped with a ruler and measured for fork length (FL; nearest 0.01 cm). Digital pictures were taken of the ventral and lateral side of the body, after which each specimen was returned immediately to the water. Prior to analysis, pictures of fish with incorrect LL posture (hence, affecting head expansion, which should be kept minimal) and body posture were removed from the dataset. Using digitizing software (tpsDIG2; Rohlf, 2004), 20 landmarks for body shape (Fig. 2a) and 37 for LL shape (Fig. 2b) were identified (after Corse *et al.*, 2009) and two morphometric traits also were measured: (1) LL width (LLW), taken as distance between landmark 1 and 37, and (2) LL height (LLH), taken as distance between landmark 19, and the middle of LLW (Fig. 2c). From these two measures, the ratio $R_{HW} = LLH/LLW$ was then calculated to determine whether species-specific LL shape differences are due to allometrical relationships (Corse *et al.*, 2012).

Landmarks for LL shape were analysed by generalized Procrustes analysis (GPA; Gower, 1975), with regression on FL performed to adjust for ontogenetic variation (MorphoJ; Klingenberg, 2011). To quantify shape differences between specimens, residuals from GPA were analysed by principal component analysis (PCA) and coupled with a wireframe graph to characterize shape deformation between specimens on both sides of the first two PCA axes of variation (i.e. PCA1 and PCA2). To assess whether LL variability resulted from a different allometrical relationship between LLW and LLH, a preliminary correlation analysis between R_{HW} and both PCA1 and PCA2 was performed, with statistical significance permutationally tested. However, because of the limited performance of the R_{HW} , a different ratio was

Table 1 Ecological knowledge of the nine chondrostom species (plus the non-chondrostom 'control' cyprinid *Telestes souffia*) under study

Species	Horny layer	Habitat	Current	Water course	Spawning migration	Feeding behaviour
<i>Achondrostoma oligolepis</i>	No	Lower stretches of rivers, coastal rivers and terminal segments of main rivers	Slow to moderate	Stream	No	–
<i>Chondrostoma angorense</i>	Thick	–	Moderate to fast	Rivers	–	Diatoms and cyanobacteria from hard substrates
<i>Chondrostoma nasus</i>	Thick	Large- to medium-sized rivers with rocks or gravel substrates	Moderate to fast	Large rivers	Yes	Benthic diatoms and detritus from hard substrates
<i>Chondrostoma vardarensis</i>	Thick	Lowland water courses from rivers to brooks, over stony to rocky substrates	Moderate to fast	Rivers	Yes	–
<i>Iberochondrostoma lemmingii</i>	No	Middle reaches of streams and lower reaches of rivers with aquatic vegetation	Slow to moderate	Rivers	No	Algae and zooplankton
<i>Parachondrostoma toxostoma</i>	No	Middle reaches of streams, clear water, cobble substrates	Slow to moderate	Rivers	No	Invertebrates and algae
<i>Protochondrostoma genei</i>	Thin	Middle reaches of rivers and their main tributaries	Moderate to fast	Rivers	Yes	Mainly aquatic insects and epilithic algae
<i>Pseudochondrostoma duriense</i>	Thick	Middle reaches of rivers	Moderate to fast	Large rivers	Yes	Vegetal matter supplemented by small invertebrates and detritus
<i>Pseudochondrostoma polyplepis</i>	Thick	Middle reaches of rivers	Moderate to fast	Large rivers	Yes	Vegetal matter supplemented by small invertebrates and detritus
<i>Telestes souffia</i>	No	Middle reaches of streams and rivers with clear water and gravel substrates	Slow to moderate	Rivers	No	Invertebrates and algae

Horny layer (whenever present) thickness after Robalo *et al.* (2007) and complemented with information from FishBase (Froese & Pauly, 2014). Habitat information refers to the species' distribution along an upstream–downstream gradient from Kottelat & Freyhof (2007). Current indicates the tolerance of species to current. Water course indicates the main species' position along the gradient. These last two variables were based on empirical knowledge (E. Corse, pers. comm.). Feeding behaviour information from Kottelat & Freyhof (2007). A dash (–) indicates unavailable information.

Table 2 Chondrostom species plus a non-chondrostom ‘control’ cyprinid analysed in this study with indication of lower lip (LL) shape characteristics (after Robalo *et al.*, 2007), sampling area (within-basin locations in Table S2), number (*n*) of individuals, body size (measured as fork length, FL) and habitat use variables

Species	LL shape	Basin	Country	<i>n</i>	FL (mean ± SE)	Habitat use		
						<i>L</i> _{max}	course	Current
<i>Achondrostoma oligolepis</i> (Steindachner, 1866)	Arched	Mondego; Vouga	Portugal	40	8.12 ± 0.24	25	1	1
<i>Chondrostoma angorense</i> (Elvira, 1987)	Straight	Sankarya	Turkey	16	12.58 ± 0.45	24	2	2
<i>Chondrostoma nasus</i> (Linnaeus, 1758)	Straight	Loire; Seine	France	40	17.95 ± 1.01	50	3	2
<i>Chondrostoma vardarense</i> (Karaman, 1828)	Straight	Aliakmon	Greece	40	24.03 ± 0.32	35	2	2
<i>Iberochondrostoma lemmingii</i> (Steindachner, 1866)	Arched	Guadiana; Quarteira	Portugal	40	9.63 ± 0.22	25	2	1
<i>Parachondrostoma toxostoma</i> (Vallot, 1837)	Arched	Orbieu; Rhône	France	41	17.21 ± 0.50	30	2	1
<i>Protochondrostoma genei</i> (Bonaparte, 1839)	Slightly arched	Tiber	Italia	40	12.61 ± 0.39	30	2	2
<i>Pseudochondrostoma duriense</i> (Coelho, 1985)	Straight	Douro	Portugal	36	8.72 ± 0.33	40	3	2
<i>Pseudochondrostoma polylepis</i> (Steindachner, 1864)	Straight	Mondego	Portugal	35	14.09 ± 0.44	40	3	2
Control								
<i>Telestes souffia</i> (Risso, 1827)	Arched	Rhône	France	25	12.55 ± 0.65	25	2	1

*L*_{max} = total length of the fish (as measured from the tip of the snout to the tip of the longer lobe of the caudal fin: after Froese & Pauly, 2014) from Fishbase (Muus & Dahlström, 1968); Water course: 1 (stream), 2 (river), 3 (large river); (Tolerance to) Current: 1 (slow to moderate) and 2 (moderate to fast).

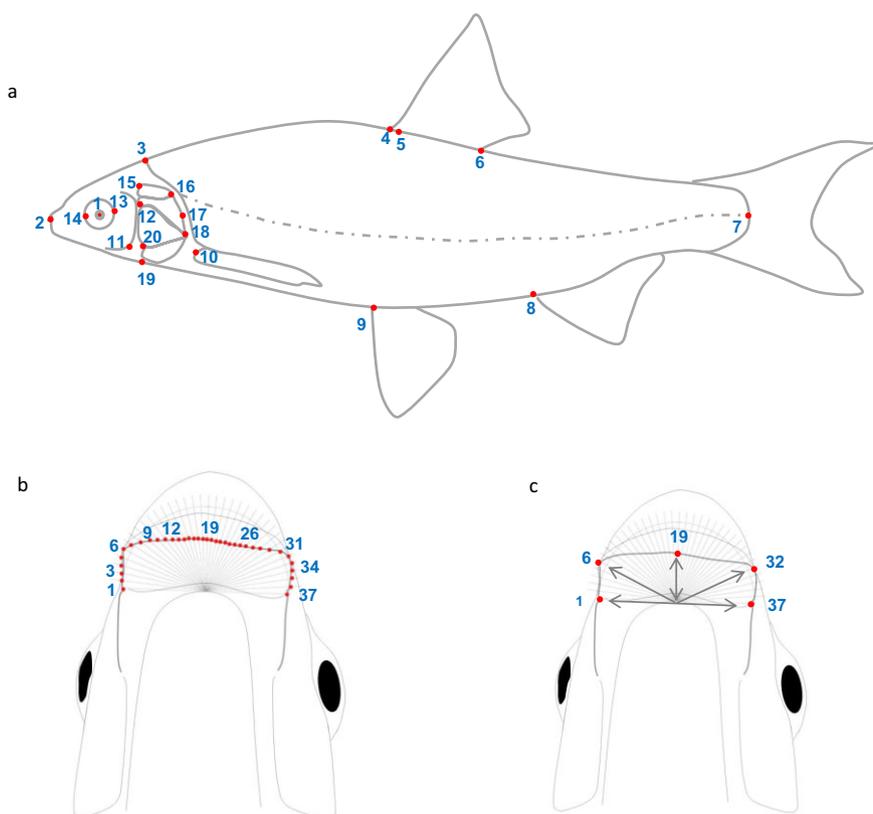


Figure 2 (a) Landmarks used for body shape analysis (20 in total). (b) Landmarks used during lower lip (LL) shape analysis (37 in total, with indication of a subset for clarity). (c) Width, height and corner ray of the LL as indicated by grey arrows. For the LL corner ray, the length taken for analysis corresponds to the mean between the left and right corner rays.

employed. This was based on the ratio (from all possible ones given the rays constituting the radian: Fig. 2b) that maximized correlation with PCA2. This involved identification of a third morphometric trait called LL corner ray (LLCR), corresponding to the length between the radian

center and the corner of the LL. The LLCR was calculated for both sides (i.e. left and right) of the LL, with the mean value of the two sides computed for each specimen, and the ratio $R_{CC} = LLCR/LLW$ (CC = center to corner) was obtained.

Landmarks for body shape were analysed similar to those for LL shape and a correlation analysis between the two resulting landmark datasets was performed by a partial least squares (PLS) function (two separate blocks option: MorphoJ), which was measured with a global RV coefficient evaluated for both PLS axes (Klingenberg & Ekau, 1996). Statistical significance was assessed permutationally ($n = 10\,000$ iterations), under the assumption of complete independence between the two datasets. Linear modelling also was conducted as an ancillary to PLS analysis under R (Wilkinson & Rogers, 1973; Chambers, 1992).

For ecological analysis, habitat use for each species was characterized based on available ecological knowledge (Table 1) and summarized into: (1) L_{\max} , defined as the maximum recorded body size for the species (after FishBase: Froese & Pauly, 2014); (2) Water course, categorized into 1 (stream), 2 (river) and 3 (large river); and (3) tolerance to current, categorized into 1 (slow to moderate) and 2 (moderate to fast) (Table 2). The resulting habitat use data were analysed by PCA to determine the ecological distances between species using library 'ade4' (Chessel *et al.*, 2004) in R (R Development Core Team, 2010).

Results

In total, 327 chondrostom specimens in the nine species under study were sampled. These included 81 individuals of *C. nasus* and *Parachondrostoma toxostoma*, which were available from Corse *et al.* (2012). Also, 25 specimens of *T. souffia* were sampled and added to the dataset as the non-chondrostom (control) cyprinid species. Notably, as lateral body pictures of *Protochondrostoma genei* could not be obtained, morphological data were not available for this species.

LL shape

The first two PCA axes contributed 89.2% of the total inertia in the LL landmarks dataset and both indicated a strong species effect (Kruskal test: $K_9 = 230.82$, $P < 0.001$ for PCA1; $K_9 = 167.38$, $P < 0.001$ for PCA2) (Fig. 3). Accordingly, while the arched morphotype (plesiomorphic) species *A. oligolepis*, *C. vardarensis*, *I. lemmingii* and *Parachondrostoma toxostoma* clustered more closely with control *T. souffia*, the other species were distributed in succession along PCA1, with *Pseudochondrostoma duriense* (straight LL) located at the extreme end of the gradient. Whereas, PCA2 segregated mainly *C. angorensis* and *Pseudochondrostoma polylepis*. Morphologically, the two PCA axes represented two different types of deformation ranging from an arched to a straight morphotype, with PCA1 indicating a progressive downward shift of the standing arch of the LL, and PCA2 a progressively upward shift of its sides (Fig. 3).

As previously indicated, R_{HW} was strongly correlated with PCA1 ($r = -0.79$, prediction ratio = 77.70%, $P < 0.001$) but not with PCA2 (-0.01 , 0.13%, $P = 0.495$), which was instead strongly correlated with R_{CC} (-0.57 , 67%, $P < 0.001$). Values for both R_{HW} and R_{CC} ranged from 0 to 1 across all species (Fig. 4), indicating larger LLW values relative to those for

LLH and LLCR. This revealed an allometric relationship in all species between LLW and the other two morphometric traits. Specifically, PCA1 deformation was due to an allometric relationship between LLH and LLW (hereafter, 'allometric deformation'), whereas PCA2 deformation was related to the relative length of the LLCR relative to LLW (hereafter, 'corner ray deformation').

LL and body shape covariation

Comparisons between LL shape and body shape were based on a total of 279 specimens because of the 33 lateral side pictures not fulfilling quality requirements and therefore being excluded from analysis. There was a significant correlation between the two datasets, as indicated by PLS (RV = 0.16, $P < 0.001$). Shape changes along the first PLS axis, which explained $\approx 95\%$ of the total covariation, showed that a straight LL deformation was correlated with a more fusiform body shape, an elongated snout and a straighter gill cover shape (Fig. 5a,b). *Pseudochondrostoma duriense* and *T. souffia* were the species located at the two opposite ends of the morphological gradient (Fig. 5c). Along this, an elongated snout was characterized by an increase in the distance between landmarks 1 and 2 and a fusiform shape by a shallow body depth because of a relatively short distance between the dorsal fin (landmarks 4, 5 and 6) and the anal and pelvic fins (landmarks 8 and 9, respectively). Gill cover shapes were mainly related to distance changes between landmarks 18 and 19/20 (basal part of the operculum) and were dorsoventral and anteroposterior dilated in straight LL morph specimens. Finally, LL covariation of the first PLS axes described a deformation from an arched to a straight morphotype.

Following PLS analysis, a new coordinate was calculated for each specimen to summarize the relative distance along the LL–body bivariate gradient in one dimension (Fig. 5c), which was described by a linear model ($r = 0.17$, prediction ratio = 30%, $P < 0.001$). For each specimen, a new coordinate corresponded to the mean value of the x and y projections on that model, which was then used in subsequent analyses.

Correlation between LL–body covariation and habitat use

The first two components of a PCA conducted on the habitat data explained 93.0% of the total inertia (Fig. 6). The contribution of the three habitat variables to the first axis of variation was similar ($L_{\max} = 35.6\%$; Water course = 35.7%; Current = 28.7%) and positively correlated, with current representing the main contribution to the second axis (71.2%). Small-sized species with preference for stream and low-current environments such as *A. oligolepis* were located opposite to the large-sized species occurring mainly in large rivers with strong currents (i.e. *C. nasus*, *Pseudochondrostoma polylepis* and *Pseudochondrostoma duriense*).

Based on a plot between the one-dimension coordinates of the LL–body shape covariation and the first PCA habitat components, an ecomorphological gradient was identified

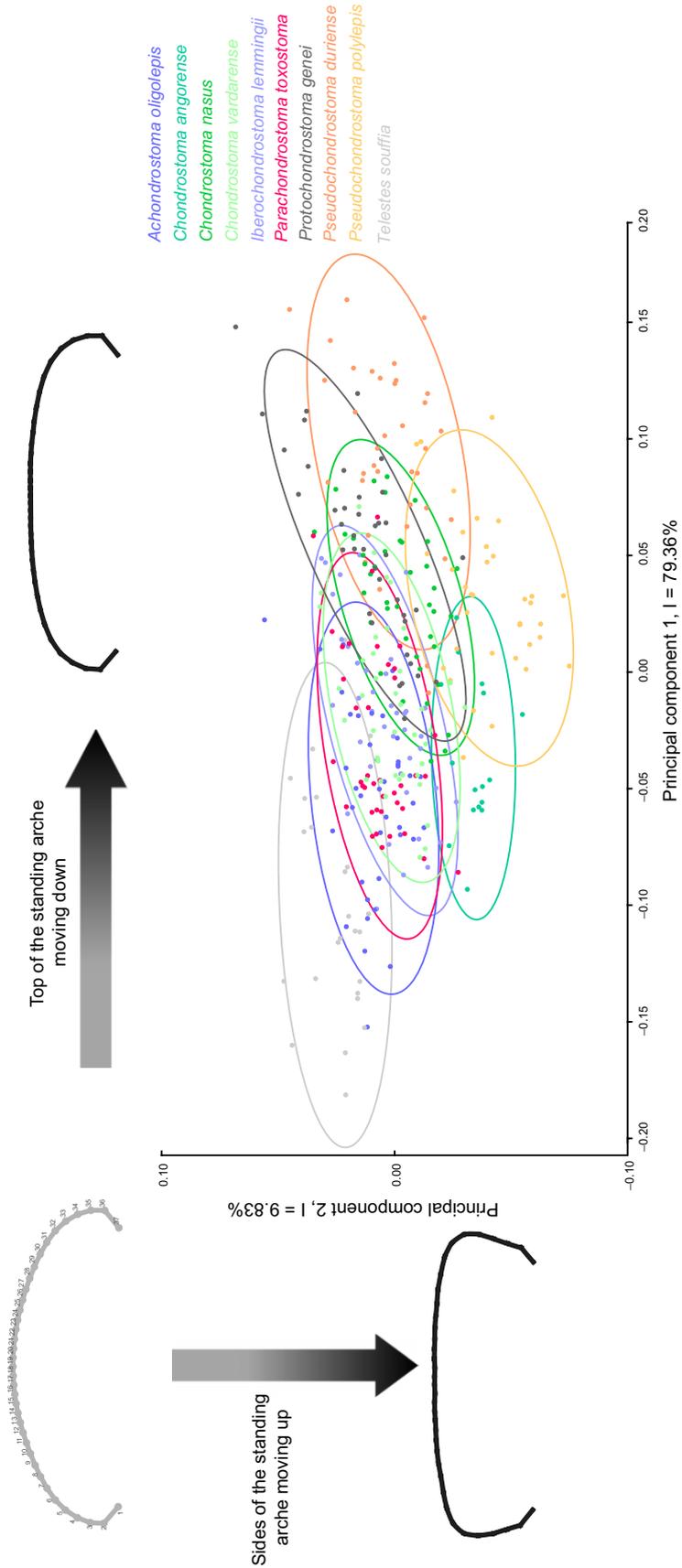


Figure 3 Principal component analysis based on the 37 landmarks for lower lip shape following Procrustes superimposition.

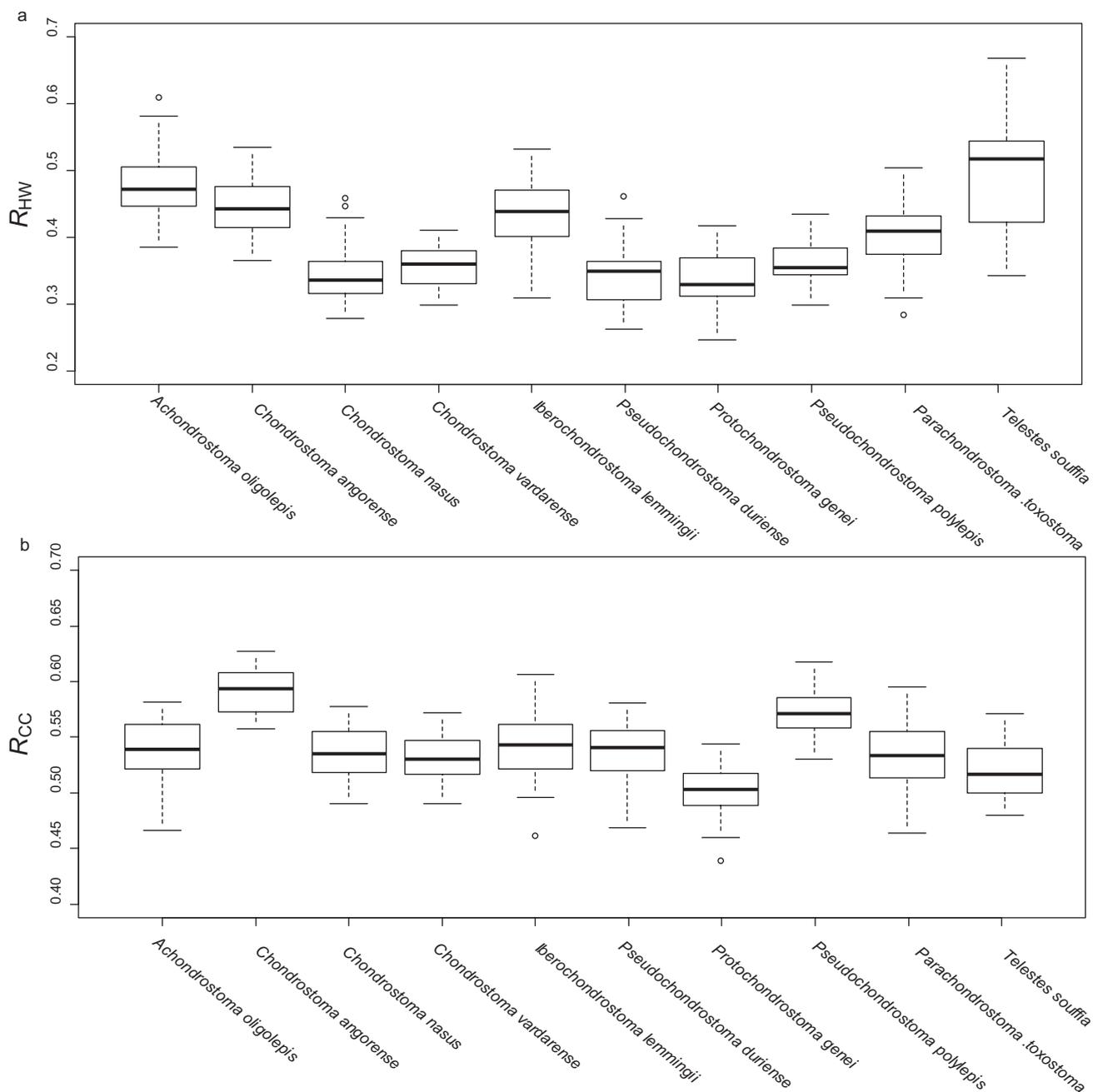


Figure 4 Distribution of the two morphometrical ratios according to species. (a) R_{HW} values (ratio of height and the width of lower lip, LL). (b) R_{CC} values (ratio of center to the corner ray and LL width).

($r = 0.037$, prediction ratio = 77%, $P = 0.003$) (Fig. 7). Along this gradient, *A. oligolepis* and *Pseudochondrostoma polylepis* showed that deep-bodied species with an arched LL are smaller and mainly encountered in streams or small-sized rivers, whereas species displaying a more fusiform body shape and straighter LLs are larger and associated with stronger currents.

Discussion

This study has shown that evolution towards a straight LL shape in chondrostoms originated from two deformations, which have acted either independently or concurrently depending on the species. Thus, a straight LL morphotype can result from an allometric deformation (as in

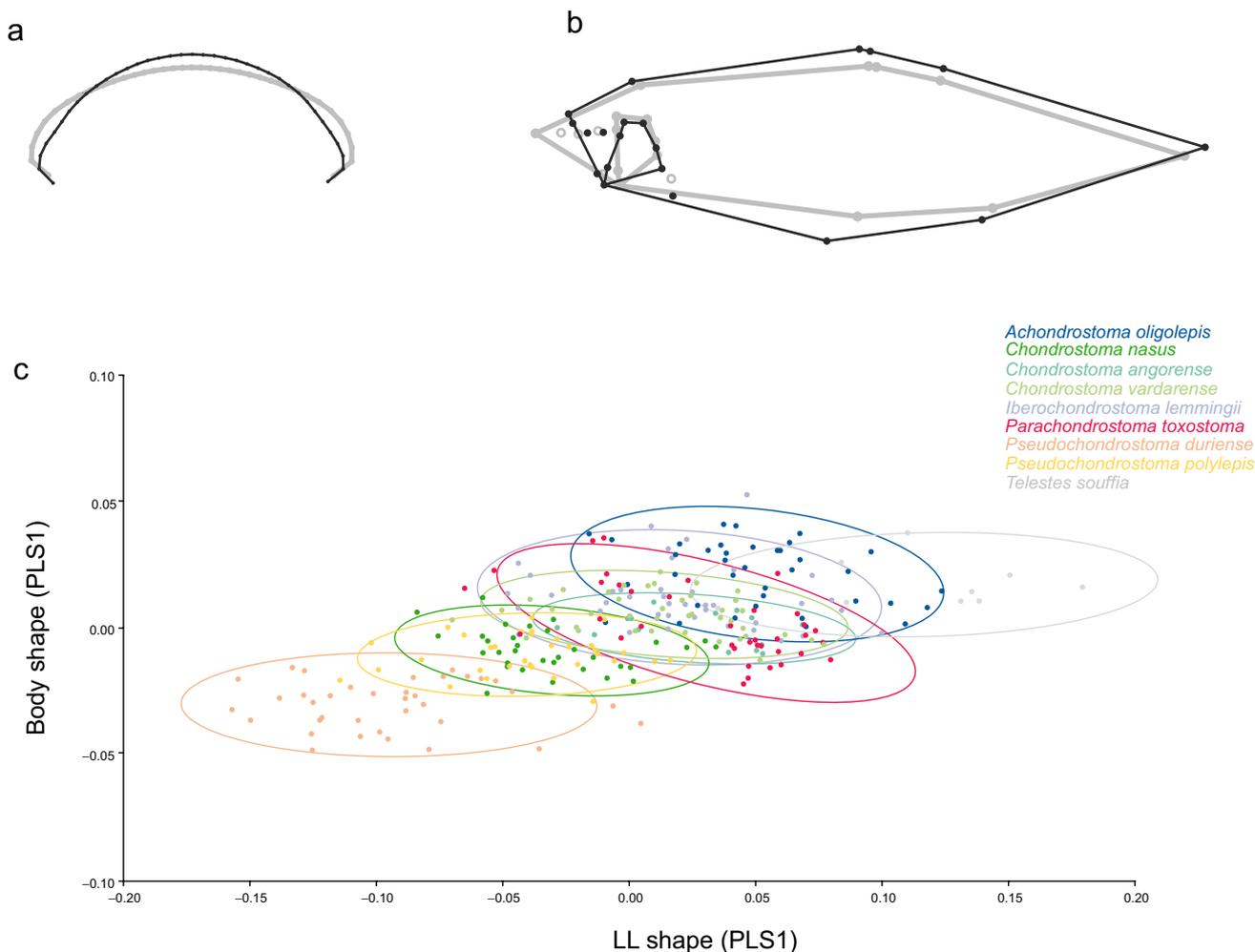


Figure 5 Covariation between lower lip (LL) shape and body shape using partial least square analysis (PLS) of the corresponding two datasets. (a) LL deformation. (b) Correlated body deformation. (c) Distribution of individuals in each of the nine chondrostom species (plus control *Telestes souffia*) along the two PLS axes of variation.

Pseudochondrostoma duriense), a corner ray deformation (as in *C. angorense*) or from both deformations (as in *Pseudochondrostoma polylepis*). In addition, these two different types of deformation do not appear to have a phylogenetic basis. This is because not only were they both found in two independent lineages (i.e. *Chondrostoma* and *Pseudochondrostoma*), but they were also inconsistent within such lineages (i.e. *C. nasus* vs. *C. angorense* and *Pseudochondrostoma polylepis* vs. *Pseudochondrostoma duriense*); thereby implying that a straight LL shape may have shifted during evolution.

With regard to the question whether these two deformation types are correlated with any differential feeding capacity, no evidence in support has so far been provided among the straight LL morphospecies. These are considered to be perolithon grazers based on empirical evidence (i.e. feeding traces onto stones; E. Corse, pers. obs.) because of a dearth of published dietary studies. Clearly, a more thorough knowl-

edge of chondrostom dietary habits is crucial before any claims can be made as to whether differences in straight LL morphologies have resulted from adaptive processes. In this respect, the *Pseudochondrostoma* genus would make for an interesting case study for comparing the dietary niches of the different species-specific LL morphotypes characterizing the species *Pseudochondrostoma polylepis* and *Pseudochondrostoma duriense*, which are known to co-occur in the same watersheds – a characteristic that would exclude the influence of environmental effects.

In the present context, an alternative hypothesis based on neutral evolution would appear more likely. Specifically, the two deformations may not have represented a differential selective advantage but rather may have resulted from developmental drift changes (True & Haag, 2001). Based on this hypothesis, the target of selection would be the adult phenotype for the LL shape rather than the corresponding developmental process and this would happen because ‘multiple

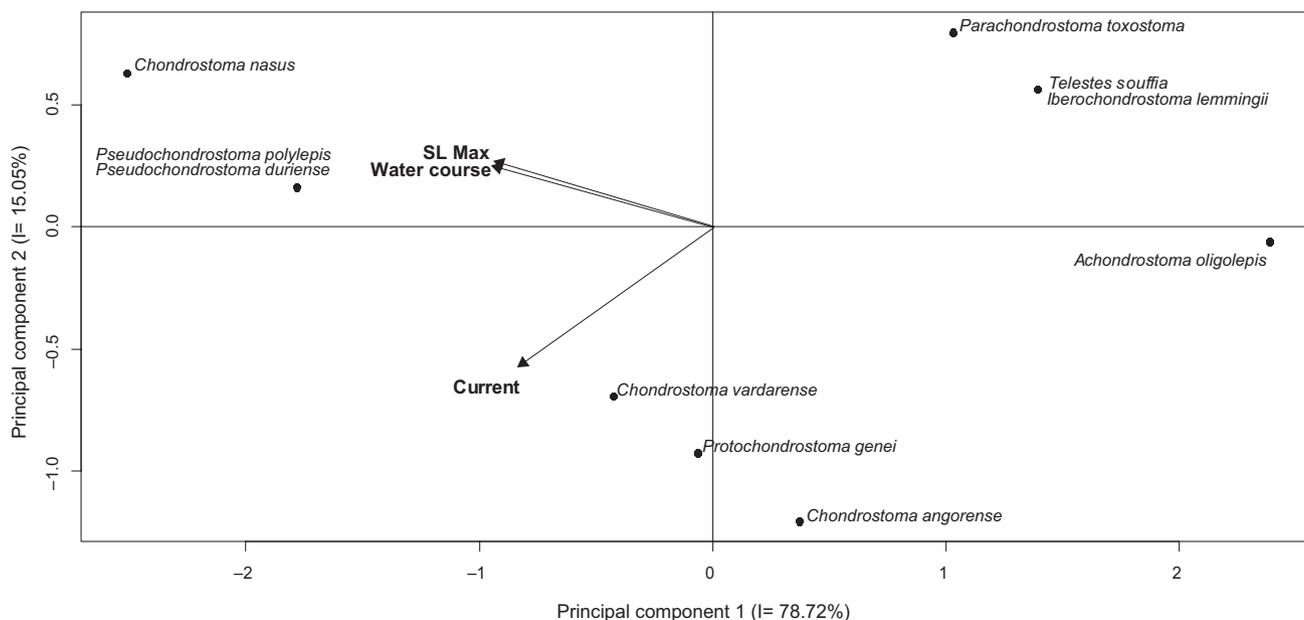


Figure 6 Principal component analysis relating habitat use (Table 2) made on L_{\max} , water course and current variables by species.

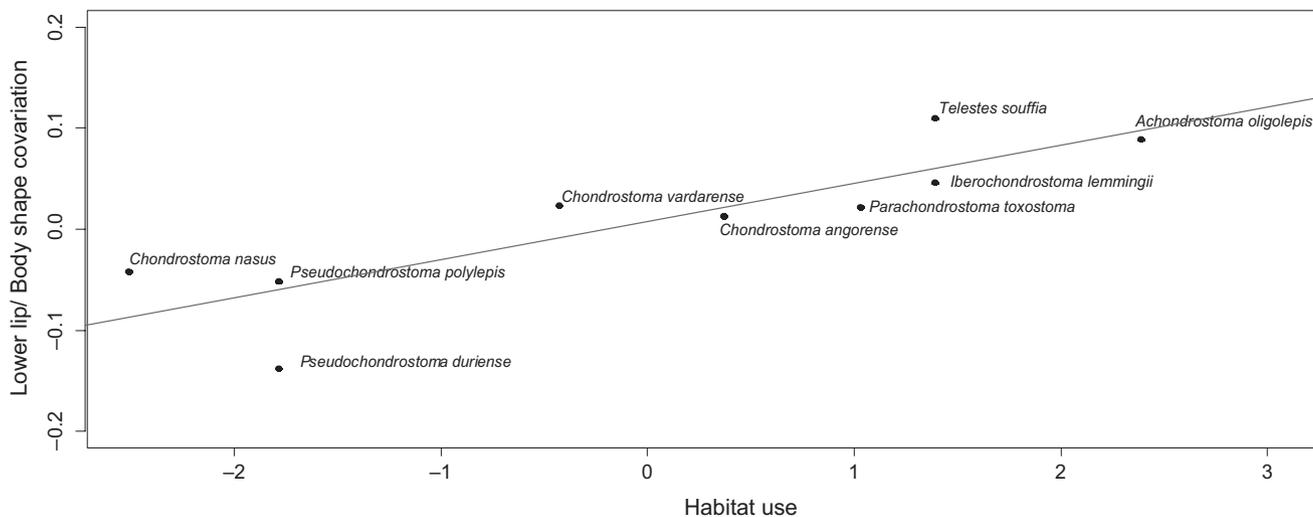


Figure 7 Morphological covariation of lower lip and body shape with habitat use for the nine chondrostom species under study (plus control *Telestes souffia*).

developmental solutions exist to the same evolutionary challenge' (Adams & Nistri, 2010, p. 8). In the case of chondrostoms, this hypothesis is supported by the fact that the two deformations of the LL shape did not have any phylogenetic basis, implying that corresponding developmental trajectories were not static in these lineages, but rather appeared to be highly flexible. Moreover, the present study has suggested that ontogenetic changes have likely resulted from an allometric repatterning of the three 'labile' morphometric traits under study (i.e. LLW, LLH and LLCR).

As a result, the ontogenetic investigation of these morphometric traits appears particularly relevant for understanding developmental differences in LL shape, as well as how convergent phenotypes (i.e. a straight LL morphology) may have arisen through non-convergent developmental changes.

PLS analysis between LL shape and body shape showed that the first axis of deformation was correlated with three functional body traits, namely snout, operculum and body depth. Thus, with the straightening of the LL, the body

becomes more fusiform, the snout elongated and the operculum more dorsoventral and anteroposteriorly dilated. In freshwater fishes, the mouth is the most remarkable trophic trait showing variation in position (i.e. superior, terminal, inferior), shape, relative size and number of taste buds. In this regard, it has been shown that cyprinid species foraging in deeper habitats with lower visibility have inferior mouths with a higher number of taste buds, in contrast to species foraging near the surface, hence in higher visibility environments (Yamamoto *et al.*, 2009; see also Table S1: reference number 22). Also, Elmer *et al.* (2010) showed how the lip morphology is correlated with trophic ecology in Midas cichlids. Snout morphology may also influence trophic performance in fish (Table S1: reference numbers 16–18, 20, 21 and 23), as in the case of the mountain whitefish *Prosopium williamsoni*. In this respect, Whiteley (2007) showed that specimens with an elongated snout (so-called ‘Pinocchio’ morphotype) forage mainly on benthic prey, contrary to the ‘normal’ morphotype that feeds mostly on water column prey.

Overall, it has been proposed that an elongated snout will enhance foraging performance during bottom feeding and allow the removal of substratum particles (Whiteley, 2007). In the case of chondrostoms, species more associated with bottom-feeding behaviour also exhibited elongated snouts in addition to a straight LL. Correlation between these two traits could therefore result from diet specialization in these species and this assumption is congruent with the idea that a straight LL and an elongated snout would enhance substratum scraping behaviour. However, because the latter has never been described within the group, an alternative hypothesis could explain a similar evolution as a result of bone progression (i.e. kinethmoid) to improve protrusion performance, as observed in the Atherinidae (Humphries, 1993). Once again, further studies on feeding behaviour in chondrostoms are required to clarify the role of elongated snouts in bottom-foraging abilities.

On the other hand, the possible causes for the covariation observed between operculum and LL shape remain unclear. This is mainly because the operculum, in virtue of its position, can be associated with different structures such as branchial arches or head bones, which in turn are related to different metabolic functions including respiration (i.e. dilation of the operculum chamber: Steen & Krusse, 1964), osmoregulation (Evans, 2005) and mastication (Sibbing, 1982). In addition, the operculum is known to be associated with foraging performance because of its anatomic connection with the lower jaw through a series of ligaments and bones (Lauder & Liem, 1983). Moreover, the height of the lower jaw is considered to be correlated with operculum depth (as in Leuciscinae: Kottelat & Freyhof, 2007). However, operculum shape changes resulting from ecological causes have been demonstrated in sticklebacks (Kimmel *et al.*, 2008) and specifically as an adaptation to lake environments leading to lateral size dilatation and dorsoventral diminution.

Body depth appears to be an important functional trait related to trophic adaptation in fish (Table S1: reference numbers 3, 5, 6, 8, 10 and 25) and it is often implied in cost-benefit ratio improvements during morphological adapta-

tion to prey foraging (Hart, 1993). For example, sticklebacks with a benthic lifestyle are characterized by a deeper body that increases maneuverability, whereas limnetic morphotypes foraging on plankton display more fusiform body shapes (Walker, 1997). Therefore, it can be hypothesized that in chondrostoms, a deeper body shape may enhance foraging performance on invertebrates by enhancing maneuverability. This assumption is supported by the fact that invertebrates constitute a more important part in the diet of the arched morph species compared with that of the straight morph species foraging mainly on periphyton. However, in the present study, the fusiform body shape deformation (i.e. low body depth) that was observed in species with a straight LL shape did not appear to be directly linked with diet specialization.

Studies on freshwater fish have indicated that a fusiform body deformation may result from adaptation to current conditions (Table S1: reference numbers 4, 7, 9, 11 and 12). In the present study, correlation analysis between morphological and ecological data showed that fusiform-shaped chondrostoms inhabit mostly large rivers. Furthermore, the chondrostom species that are mainly found in large rivers with higher water velocities displayed larger maximal body size (e.g. *C. nasus*, *C. vardarensis*, *Pseudochondrostoma duriense* and *Pseudochondrostoma polylepis*), whereas the smaller-sized (25 cm max FL: *A. oligolepis* and *I. lemmingii*) or intermediate-sized species (30 cm max FL: *Parachondrostoma toxostoma* and *Protochondrostoma genei*) are mainly found in streams or smaller rivers. Although several factors (including predation and temperature) are known to affect body size in freshwater fish, the latter is also considered to be positively related to flow rates (Wikramanayake, 1990; Nikolski, 1933 also see Table S1: reference number 2). It is therefore suggested that a large body size in an elongated form may have resulted from an adaptive response to habitat use characterized by deeper waters with stronger current exposure.

Conclusions

This study has demonstrated that morphological differences in chondrostoms can result from trophic (i.e. straight LL, elongated snout) and habitat use (i.e. fusiform body, larger body size) adaptations, in line with the ‘multivariate traits specialization hypothesis’ (Table S1: reference numbers 23–26). Accordingly, in the present study, the chondrostom species analysed displayed a continuous ecomorphological gradient ranging from diet generalists to diet specialists. Thus, generalist species such as *A. oligolepis* and *Parachondrostoma toxostoma*, which are mainly found in small rivers, tend to have a higher body depth (hence, better maneuverability to forage on drift prey), a terminal mouth and an arched LL, and are opportunistic feeders. All of these characteristics make them more closely related to the hypothetical last common ancestor (i.e. a *Telestes*-like species). Conversely, specialized species such as *C. nasus* and *Pseudochondrostoma polylepis* are characterized by a fusiform body shape, a larger maximum size (hence, better performance to current exposure in deeper environments), an inferior mouth, an elongated snout and a straight LL (optimized for bottom feeding). Finally,

intermediate-diet species such as *Parachondrostoma toxostoma* and *Pseudochondrostoma genei* occupy intermediate niches and present intermediate traits in LL shape together with maximal body size and shape.

Overall, the ecomorphological correlation documented in this study has provided for strong evidence of adaptive evolution in chondrostoms. At the same time, the ecomorphological similarity between species from distant lineages (i.e. *Chondrostoma* and *Pseudochondrostoma*) suggests recurrent and independent evolutionary processes, with selection acting on both feeding and swimming performance. This morphological evolutionary trend appears to be correlated with adaptation to larger river habitats and periphyton grazing. However, the reason why colonization of large rivers has been repeatedly correlated with trophic specialization remains a topic of investigation.

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References

- Adams, D.C. & Nistri, A. (2010). Ontogenetic convergence and evolution of foot morphology in European cave salamanders (Family: Plethodontidae). *BMC Evol. Biol.* **10**, 216.
- Brinsmead, J. & Fox, M.G. (2002). Morphological variation between lake- and stream-dwelling rock bass and pumpkinseed populations. *J. Fish Biol.* **61**, 1619–1638.
- Chambers, J.M. (1992). Linear models. In *Statistical models*: 95–144. Chambers, J.M. & Hastie, T.J. (Eds). Pacific Grove: Wadsworth & Brooks/Cole.
- Chessel, D., Dufour, A.B. & Thioulouse, J. (2004). The ade4 package-I- One-table methods. *R News* **4**, 5–10.
- Corse, E., Costedoat, C., Pech, N., Chappaz, R., Grey, J. & Gilles, A. (2009). Trade-off between morphological convergence and opportunistic diet behavior in fish hybrid zone. *Front. Zool.* **6**, 26.
- Corse, E., Costedoat, C., Chappaz, R., Pech, N., Martin, J.F. & Gilles, A. (2010). A PCR-based method for diet analysis in freshwater organisms using 18S rDNA barcoding on faeces. *Mol. Ecol. Resour.* **10**, 96–108.
- Corse, E., Neve, G., Sinama, M., Pech, N., Costedoat, C., Chappaz, R. & Gilles, A. (2012). Plasticity of ontogenetic trajectories in cyprinids: a source of evolutionary novelties. *Biol. J. Linn. Soc. Lond.* **106**, 342–355.
- Doadrio, I. & Carmona, J.A. (2004). Phylogenetic relationships and biogeography of the genus *Chondrostoma* inferred from mitochondrial DNA sequences. *Mol. Phylogenet. Evol.* **33**, 802–815.
- Durand, J.-D., Bianco, P.G., Laroche, J. & Gilles, A. (2003). Insight into the origin of endemic Mediterranean ichthyofauna: phylogeography of *Chondrostoma* genus (Teleostei, Cyprinidae). *J. Hered.* **94**, 315–328.
- Elmer, K.R. & Meyer, A. (2011). Adaptation in the age of ecological genomics: insights from parallelism and convergence. *Trends Ecol. Evol.* **26**, 298–306.
- Elmer, K.R., Lehtonen, T.K., Kaut, A.F., Harrod, C. & Meyer, A. (2010). Rapid sympatric ecological differentiation of crater lake cichlid fishes within historic times. *BMC Biol.* **8**, 60.
- Evans, D.H. (2005). The multifunctional fish gill: dominant site of gas exchange, osmoregulation, acid-base Regulation, and excretion of nitrogenous waste. *Physiol. Rev.* **85**, 97–177.
- Froese, R. & Pauly, D. (eds) (2014). FishBase. World Wide Web electronic publication. Available at: www.fishbase.org. (accessed 20 June 2013).
- Gompel, M. & Prud'homme, B. (2009). The causes of repeated genetic evolution. *Dev. Biol.* **332**, 36–34.
- Gower, J.C. (1975). Generalized Procrustes analysis. *Psychometrika* **40**, 33–51.
- Haas, T.C., Blum, M.J. & Heins, D.C. (2010). Morphological responses of a stream fish to water impoundment. *Biol. Lett.* **6**, 803–806.
- Hart, P.J. (1993). Teleost foraging: facts and theories. In *Behaviour of teleost fishes*: 2253–2284. Pitcher, T.J. (Ed.). London: Chapman & Hall.
- Humphries, P. (1993). A comparison of the mouth morphology of three co-occurring species of atherinid. *J. Fish Biol.* **42**, 585–593.
- Kimmel, C.B., Aguirre, W.E., Ullmann, B., Currey, M. & Cresko, W.A. (2008). Allometric change accompanies opercular shape evolution in Alaskan threespine sticklebacks. *Behaviour* **145**, 669–691.
- Klingenberg, C.P. (2011). MorphoJ: an integrated software package for geometric morphometrics. *Mol. Ecol. Resour.* **11**, 353–357.
- Klingenberg, C.P. & Ekau, W. (1996). A combined morphometric and phylogenetic analysis of an ecomorphological trend: pelagization in Antarctic fishes (Perciformes: Nototheniidae). *Biol. J. Linn. Soc. Lond.* **59**, 143–177.
- Kottelat, M. & Freyhof, J. (2007). *Handbook of European freshwater fishes*. Berlin: Kottelat, Cornol & Freyhof.
- Lauder, G.V. & Liem, K.F. (1983). The evolution and interrelationships of the actinopterygian fishes. *Bull. Mus. Comp. Zool.* **150**, 95–197.
- Muus, B.J. & Dahlström, P. (1968). *Süßwasserfische*. München: BLV Verlagsgesellschaft.
- Nelva-Pasqual, A. (1985). Biogéographie, démographie et écologie de *Chondrostoma nasus nasus* (L. 1758). *Hotu*

- (Poisson, téléostéen, Cyprinidé). PhD thesis, Université Claude Bernard-Lyon I.
- Nijhout, H.F. (1991). *The development and evolution of butterfly wing patterns*. Washington & London: Smithsonian Institution Press.
- Nikolski, G.V. (1933). On the influence of the rate of flow on the fish fauna of the rivers of central Asia. *J. Anim. Ecol.* **2**, 266–281.
- Novomeská, A., Katina, S., Copp, G.H., Pedicillo, G., Lorenzoni, M., Pompei, L., Cucherousset, J. & Kováč, V. (2013). Morphological variability of black bullhead *Ameiurus melas* in four non-native European populations. *J. Fish Biol.* **82**, 1103–1118.
- Perea, S., Böhme, M., Zupančič, P., Freyhof, J., Šanda, R., Özulug, M., Abdoli, A. & Doadrio, I. (2010). Phylogenetic relationships and biogeographical patterns in Circum-Mediterranean subfamily Leuciscinae (Teleostei, Cyprinidae) inferred from both mitochondrial and nuclear data. *BMC Evol. Biol.* **10**, 265.
- R Development Core Team (2010). *R: a language and environment for statistical computing*: Vienna: R Foundation for Statistical Computing. Available at: <http://www.R-project.org> (accessed October 31, 2014).
- Robalo, J.I., Almada, V.C., Levy, A. & Doadrio, I. (2007). Re-examination and phylogeny of the genus *Chondrostoma* based on mitochondrial and nuclear data and the definition of 5 new genera. *Mol. Phylogenet. Evol.* **42**, 362–372.
- Rohlf, F.J. (2004). TPS program series. Available at: <http://life.bio.sunysb.edu/morph/> (accessed January 17, 2014).
- Sibbing, F.A. (1982). Pharyngeal mastication and food transport in the carp (*Cyprinus carpio* L.): a cineradiographic and electromyographic study. *J. Morphol.* **172**, 223–258.
- Steen, J.B. & Kruyssen, A. (1964). The respiratory function of teleostean gills. *Comp. Biochem. Physiol.* **12**, 127–142.
- True, J.R. & Haag, E.S. (2001). Developmental system drift and flexibility in evolutionary trajectories. *Evol. Dev.* **3**, 109–119.
- Vilizzi, L. & Kováč, V. (2014). Alternative ontogenies and developmental plasticity: implications for ecological and evolutionary studies on species complexes. *Fish Fish.* **15**, 523–531.
- Walker, J.A. (1997). Ecological morphology of lacustrine threespine stickleback *Gasterosteus aculeatus* L. (Gasterosteidae) body shape. *Biol. J. Linn. Soc. Lond.* **61**, 3–50.
- Whiteley, A.R. (2007). Trophic polymorphism in a riverine fish: morphological, dietary, and genetic analysis of mountain whitefish. *Biol. J. Linn. Soc. Lond.* **92**, 253–267.
- Wikramanayake, E.D. (1990). Ecomorphology and Biogeography of a Tropical Stream Fish Assemblage: Evolution of Assemblage Structure. *Ecology* **71**, 1756–1764.
- Wilkinson, G.N. & Rogers, C.E. (1973). Symbolic descriptions of factorial models for analysis of variance. *Appl. Stat.* **22**, 392–399.
- Yamamoto, Y., Byerly, M.S., Jackman, W.R. & Jeffery, W.R. (2009). Pleiotropic functions of embryonic sonic hedgehog expression link jaw and taste bud amplification with eye loss during cavefish evolution. *Dev. Biol.* **330**, 200–211.
- Záhorská, E., Kováč, V., Falka, I., Beyer, K., Katina, S., Copp, G.H. & Gozlan, R.E. (2009). Morphological variability of the Asiatic cyprinid, topmouth gudgeon *Pseudorasbora parva*, in its introduced European range. *J. Fish Biol.* **74**, 167–185.

Supporting information

Additional Supporting Information may be found in the online version of this article at the publisher's web-site:

Table S1. Summary information from representative studies on fish ecomorphology.

Table S2. Chondrostom species plus control non-chondrostom cyprinid analysed in this study with indication of sampling location.