



Ecomorphology of six goatfish species (Mullidae) from Toliara Reef, Madagascar

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Abstract Despite the role that goatfishes play in reef ecosystems, knowledge of their ecomorphological diversity remains scarce. Here, we explore the ecomorphology of six species of goatfishes living in sympatry at Toliara Reef (South-West of Madagascar) by using a combination of morphometric and isotopic ($\delta^{13}\text{C}$, $\delta^{15}\text{N}$ and $\delta^{34}\text{S}$) data. The shape of cephalic region was quantified by landmark-based geometric morphometrics and linear measurements, permitting to distinguish each genus and species according to head elongation, cheek size and pectoral fins insertions. Isotopic ratios, used as trophic niche proxy, allowed to highlight clear trophic niche segregation among species built on C and S isotopic ratios, possibly linked to variation in selected prey type and feeding areas. Interspecific variation of these two ratios could also be linked to a cross-reef distribution with species living in the lagoon relying on local

primary production and outer-shelf dwellers depending on oceanic inputs. The absence of variation in $\delta^{15}\text{N}$ values suggests that the six species share similar trophic position. Our comparative analyses revealed a significant relationship between isotopic and morphometric data while taking phylogenetic relationships into account. These results allowed the formulation of hypotheses regarding differences in goatfishes feeding strategies: “long head” species mainly search for preys in anfractuosités or deep in the substrate while “short head” species use their head to dig in superficial soft bottoms. Overall, we highlight ecomorphological partitioning among sympatric goatfishes based on their morphology and feeding habits, and we show that head shape could be used as a trophically relevant trait in Mullidae.

Keywords Goatfishes · Mullidae · Functional morphology · Geometric morphometrics · Stable isotopes · Trophic ecology

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Introduction

Reef-associated teleost fishes constitute highly diverse assemblages of vertebrates, showing an extraordinary panel of body forms and lifestyles. It is now largely recognized that the ecological and morphological diversity is the result of adaptations allowing ecological niche partitioning (Ronco et al. 2021). Hutchinson (1957) proposed that the ecological niche of a species

can be described by its position in an environmental space defined by n axes corresponding to abiotic and biotic factors. This notion illustrates the position that a species occupies in an ecosystem based on the conditions necessary to its development (Polechova and Storch 2008). The Hutchinsonian niche concept expresses the relationship of an individual or a population to all aspects of its environment. Thus, two sympatric species' populations cannot share the same ecological niche, otherwise interspecific competition would be too intense and would lead to the extinction of one of the two species. Populations evolve different strategies to use resources (e.g., food and habitat), reducing niche overlap and ultimately allowing their coexistence. The trophic niche, a subspace of the ecological niche, is a major axis of ecological diversification (Silvertown 2004). From an evolutionary perspective, resource partitioning acts as one of the key factors in the process of diversification by promoting the coexistence of closely related species (Colwell and Fuentes 1975).

Goatfishes (Mullidae) belong to the Syngnathiform order and include 98 species grouped in 6 genera (Eschmeyer 2021): *Mullus* (Linnaeus 1758), *Upeneus* (Cuvier 1829), *Upeneichthys* (Bleeker 1855), *Mulloidichthys* (Whitley 1929), *Pseudupeneus* (Bleeker 1862) and *Parupeneus* (Bleeker 1863). Goatfishes have a worldwide distribution in tropical and subtropical oceans with a few species in temperate areas (Uiblein 2007). They mainly live in coastal ecosystems such as seagrass meadows, muddy substrates, sandbanks, and in rocky or coral reefs (Munro 1976; McCormick 1995). The main morphological trait distinguishing Mullidae from other Syngnathiform families is the presence of a pair of hyoid barbels (Sato 1937; Gosline 1984; McCormick 1993; Longo et al. 2017; Santaquiteria et al. 2021). These articulated barbels are surrounded by soft tissues covered by sensory cells (Sato 1937; Gosline 1984; McCormick 1993). Besides variation in color patterns, goatfishes do not appear morphologically diverse at first glance. It is therefore often assumed that all goatfishes are mainly benthic carnivores, using barbels to extract small prey from the substrate (crustaceans, mollusks, worms) (Nakamura et al. 2003; Kolasinski et al. 2009; El Bakali et al. 2010). However, some species such as *Pseudupeneus maculatus*, *Mulloidichthys martinicus* or *Parupeneus barberinus* occasionally feed on prey (zooplankton or

hyperbenthic invertebrates) in the water column close to the bottom by using their protrusible jaws (Sierra et al. 1994; Lukoschek and McCormick 2001; Krajewski and Bonaldo 2006). Fishes have also been found in the stomach of large adult goatfishes (Labropoulou et al. 1997; Shanti Prabha and Manjulatha 2008). Although some goatfish species live in sympatry, trophic niche partitioning seems limited based on our current knowledge of their ecology.

Ecomorphological studies aim to investigate the correlation between morphological traits of a species and its ecology (e.g., feeding ecology, habitat, environmental conditions) (Leisler et al. 1985; Norton et al. 1995; Wainwright and Richard 1995) and may ultimately allow the identification of relevant morphological traits associated to ecological diversification (Aguilar-Medrano et al. 2011; Santos et al. 2011). To dive into the trophic ecological aspect, two main approaches are commonly used. On the one hand, the analysis of stomach contents provides a snapshot of the most recent meal. On the other hand, the use of stable isotopes provides a time-integrative tool for assessing trophic level through nitrogen stable isotope ($\delta^{15}\text{N}$) (e.g. Blanco-Parra et al. 2012; Tripp-Valdez et al. 2015; Nawrocki et al. 2020), feeding habits (i.e. diet, foraging location) through carbon and sulfur stable isotopes ($\delta^{13}\text{C}$ and $\delta^{34}\text{S}$, respectively) (e.g. Cocheret de la Morinière et al. 2003; Lepoint et al. 2008; Kadye and Booth 2012; Layman et al. 2012) and trophic niche width of species, characterized by the dispersion of individual isotope compositions into an isotopic space (e.g. Cummings et al. 2012; Wang et al. 2018). The coupling of morphological studies with ecological investigations has been successfully applied to explain the coexistence of many sympatric fish species (e.g., Keppeler et al. 2015; Ornelas-García et al. 2018; Delariva and Neves 2020). However, in such ecomorphological studies implying more than one species (i.e., a comparative framework), it is strongly advised to integrate species' phylogenetic relationships to consider the potential covariance due to shared evolutionary history (Westneat 1995).

Studies focusing on the ecological niches of goatfishes can provide supports on fine-scale ecological divergence in sympatric species. For instance, Lombarte et al. (2000) demonstrated habitat partitioning

between two Mediterranean goatfish species: *Mullus surmuletus* and *Mullus barbatus*. The former lives in shallow areas (10–50 m) on rocky substrate while the latter occupies deeper areas (50–200 m) on muddy substrate. Thus, both species can forage for the equivalent type of preys without potential competitive exclusion. Ontogenetic changes in feeding behavior and substrate occupation have also been highlighted in the Red Sea goatfish *Parupeneus forsskali* (Uiblein 1991). Indeed, small individuals forage mainly on soft bottoms using their barbels to detect ophiurids in the upper layer of the substrate. Then, medium sized individuals search for fish eggs in coral crevices with their barbels and finally, larger individuals shift back to soft bottoms but use their heads to dislodge polychaetes buried deeper in the sediment. Consequently, the trophic competition among conspecifics of Red Sea goatfishes is strongly limited along their ontogeny.

The general objective of the present study is to explore the ecomorphological diversity of six goatfish species living in sympatry at Toliara Reef (South-West of Madagascar) and to search for an implication of trophic segregation. Firstly, morphological and ecological diversity will be assessed. The morphological study, based on traditional and landmark-based geometric morphometrics, will focus on the cephalic region.

The ecological aspect will be investigated using stable isotopes analyses of carbon (¹³C), nitrogen (¹⁵ N), and sulfur (³⁴S). The hypothesis of trophic niche partitioning will be tested by combining stable isotopes and morphometric data. Secondly, the relationship between morphological traits and isotopic data will be tested with phylogenetically informed methods in an ecomorphological perspective.

Materials and methods

Sampling

The six studied species, including *Mulloidichthys flavolineatus* (Lacepède 1801); *Mulloidichthys vanicolensis* (Valenciennes 1831); *Parupeneus barberinus* (Lacepède 1801); *Parupeneus indicus* (Shaw 1803); *Parupeneus macronemus* (Lacepède 1801) and *Parupeneus rubescens* (Lacepède 1801), live in the reef system of Toliara (SW Madagascar — 23.36°S, 43.66°E). All specimens (N_{total}=68, Table 1, Table S1) were bought on the fish market of Toliara in May 2016 and 2018. This market is supplied by local fishermen fishing in the lagoon of the Great Reef of Toliara.

The standard length (SL) of each fish was measured to the nearest millimeter and a piece of epaxial musculature (below dorsal fins) was sampled on fresh fish for further stable isotope analysis. After dissection, fish individuals were fixed in a 10% formalin solution for 15 days and then transferred to a 70% ethanol solution.

Morphometry

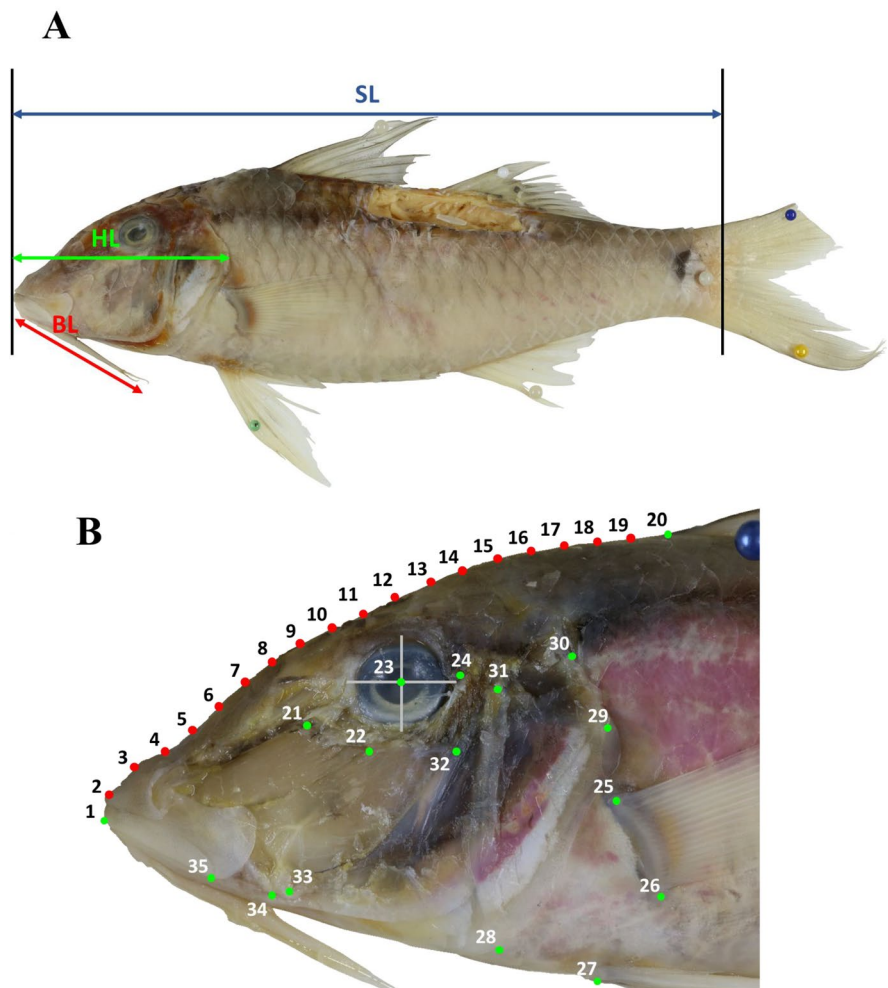
Classic morphometry

Four morphological traits were collected to the nearest millimeter with a caliper to characterize the general body morphology and head characteristics (Fig. 1A). The standard length (SL) was measured from the rostral extremity to the caudal fin insertion. Head length (HL) was measured from the rostral extremity to the posterior extremity of the operculum. The head width (HW) corresponds to the distance between the left and the right cheeks, taken below the eyes. Finally, barbel length (BL) was defined

Table 1 List of studied species with number of specimens used for each type of data sampling. *n* refers to the total number of specimens per species. SL refers to standard length and GM refers to landmark-based geometric morphometrics

Species	SL (mm) Min–Max	Morphometry	GM	Isotopes	<i>n</i>
<i>Mulloidichthys flavolineatus</i>	165.8–225.3	15	15	5	15
<i>Mulloidichthys vanicolensis</i>	162.3–184.2	6	6	4	6
<i>Parupeneus barberinus</i>	130.5–211.2	17	16	11	17
<i>Parupeneus indicus</i>	164–194.3	10	10	8	10
<i>Parupeneus macronemus</i>	136.6–180.7	10	10	10	10
<i>Parupeneus rubescens</i>	125.9–218.8	10	10	9	10
Total		68	67	47	68

Fig. 1 **A** Illustration of morphometric measurements in *Parupeneus barberinus*. SL = standard length, HL = head length, BL = barbel length. **B** head profile of *P. barberinus* illustrating the landmarks (green) and semi-landmarks (red) used for the geometric morphometric analyses (see Table 2 for landmarks description)



from its insertion point on the mandible to its tip. A mean value was calculated for each individual based on three repeated measurements. Potential allometric variation was accounted for by computing three ratios (HL/SL, HW/HL, BL/HL), which were used in subsequent comparative analyses.

Geometric morphometrics

Head shape variation was quantified by using landmark-based geometric morphometric methods (Fig. 1B) (Bookstein 1991; Rohlf and Marcus 1993; Marcus et al. 1996). A thorough quantitative analysis of the cephalic region was chosen because (1) it has been extensively demonstrated that head shape is tightly linked to feeding habits in fishes (e.g., McLellan 1977; Aguilar-Medrano

et al. 2011; Sonnefeld et al. 2014; Abaad et al. 2016; Cooper et al. 2017; Ventura et al. 2017; Carlig et al. 2018), and (2) this region seems to be the most variable structure among goatfish species (Uiblein 2011, 2021).

Specimens were photographed in lateral view with a camera (Canon Eos 6D), and the x- and y-coordinates of 15 homologous landmarks and 20 semi-landmarks (Table 2) capturing head shape (Fig. 1B) were digitized from the left side of each individual using the software TPSDIG, v2.31 (Rohlf 2015). All specimens were superimposed by performing a Generalized Procrustes Analysis (GPA) (Rohlf and Slice 1990). This step allows considering size and shape as two independent components and removing variation due to position, orientation and scale (Rohlf et al. 1996; Rohlf and Slice 1990; Adams et al. 2004). The mean

Table 2 Anatomical description of homologous landmarks

Landmark	Description
1 to 20	Head profile (semi-landmarks)
21 and 22	A1 muscle insertion (adductor mandibulae) on suborbital
23	Orbit centre
24	Adductor arcus palatini
25	Pectoral fin upper insertion
26	Pectoral fin lower insertion
27	Pelvic fin insertion
28	Boundary between subopercle and interoperculum
29	Posterior operculum extremity
30	Upper operculum extremity
31	Upper preoperculum extremity
32	Upper A2 muscle (adductor mandibulae) insertion on preoperculum
33	Mandible articulation
34	Lower ligamentum primordium insertion
35	Upper premaxilla extremity

configuration of all specimens, called consensus, was used as the reference and Procrustes tangent coordinates of each specimen were then used as shape variables for subsequent analyses (Adams et al. 2004).

Stable isotopes

Samples of epaxial muscle tissue were placed in a glass tube and dehydrated in an oven at 50 °C for 48 h before being grounded into a homogenous powder using mortar and pestle. Measurements were performed using an elemental analyzer (Vario Microcube, Elementar, Analysensysteme GMBH, Germany) coupled to an isotope ratio mass spectrometer (Isoprime 100, Isoprime, UK). Isotopic ratios were expressed following the δ notation (‰) (Coplen 2011) based on international standards: Vienna Pee Dee Belemnite for carbon, atmospheric nitrogen for nitrogen and Canon Diablo troilite for sulfur. Substances certified by International Agency for Nuclear Energy (IAEA) were measured for each isotopic ratios: IAEA-C6 for carbon ($\delta^{13}\text{C} = -10.8 \pm 0.5\text{‰}$), IAEA-N1 for nitrogen ($\delta^{15}\text{N} = 0.4 \pm 0.2\text{‰}$), IAEA-S1 for sulfur ($\delta^{34}\text{S} = -0.3\text{‰}$). Sulfanilic acid (Sigma-Aldrich, Overijse, Belgium; $\delta^{13}\text{C} = -25.6 \pm 0.4\text{‰}$; $\delta^{15}\text{N} = -0.1 \pm 0.4\text{‰}$; $\delta^{34}\text{S} = 5.9 \pm 0.5\text{‰}$; means \pm SD) were inter spread every 15 samples in the batch as secondary control. Repeatability precision of the measurements was assessed on 7 repeated goatfish sample and was 0.2‰ for $\delta^{13}\text{C}$, 0.2‰ for $\delta^{15}\text{N}$ and 0.3‰ for $\delta^{34}\text{S}$.

Data treatment and statistics

Morphological diversity

We used ratios from morphometric measurements and head shape data to estimate the degree of morphological variation among species. First, we tested the normality and variance homogeneity of each traditional morphometric traits, i.e., the ratios: HL/SL, HW/HL, BL/HL, using Shapiro–Wilk and Bartlett’s tests, respectively. When data satisfied the parametric requirements (normal distribution and homogenous variance), a one-way analysis of variance (ANOVA) was performed to test for morphological divergence among species. If one or both conditions were not met, a non-parametric Kruskal–Wallis test was conducted.

For geometric morphometric data, a principal component analysis (PCA) was conducted to illustrate shape variation in the cephalic region among species (Bookstein 1991; Rohlf 1993). The PCA, generated in TPSrelw32 (version 1.53), was used (1) to illustrate the general distribution and possible groupings of species in the shape space, and (2) to determine which traits account for most of the variance in the morphological data. In the shape space, convex hulls were added for an illustrative purpose and for highlighting intra-specific variation. Deformation grids generated by the “Thin-plate Spline (TPS)” algorithm

were used to visualize the shape variation along PCA axes (Thompson 1917; Bookstein 1991; Rohlf 1996, 2015). Then, differences among species and between genera based on the two first principal components (PCs) were tested using multivariate analysis of variance (MANOVA) and pairwise comparisons computed with PAST (Version 3.25; Hammer et al. 2001).

Trophic niche partitioning

Isotopic data integrate various facets of fish trophic ecology (Fry 2006; Layman et al. 2012), and we used isotopic raw data as an ecological trait (i.e., proxy for trophic niches). Similarly to morphological data, parametric requirements were checked using Shapiro–Wilk test and Bartlett’s test. Then, ANOVAs or Kruskal–Wallis tests were performed to investigate the hypothesis of isotopic divergence among species in the isotopic space. When tests revealed significant variation among species, post hoc multiple comparison tests were performed (Tukey test for parametric and Dunn test for non-parametric analyses). Convex hulls (i.e., the smallest convex set that contains all data) have been added to the isotopic space for the clarity of data interpretation.

Ecomorphological relationships

The phylogenetic signal may be defined as the statistical nonindependence among species trait values due to their phylogenetic relatedness (Blomberg et al 2003; Revell et al. 2008). Here, we quantified the phylogenetic signal in our traits to test the need to include phylogenetic information in our ecomorphological analyses to interpret them. To do so, the molecular time-calibrated phylogeny of Mullidae from Santaquitera et al. (2021) was pruned to match the species in our dataset to provide an estimate of their evolutionary relationships. All the studied species were present in that phylogeny, except for *P. macronemus*. By using more than 900 Ultraconserved Elements (UCE) markers, Santaquitera et al. (2021) provided strong supports on the monophyly of every mullid genera. Thus, we applied the function *add.species.to.genus* in the package *phytools* (Version 0.7–70; Revell 2012) in R statistical environment (Version 4.0.4; R core team 2021) to randomly place *P. macronemus* among other *Parupeneus* species in the phylogeny. For every further analysis including

phylogenetic information, the inclusion of *P. macronemus* in the Mullidae phylogeny was repeated 500 times in order to include uncertainty due to its random assignment within the monophyletic *Parupeneus* clade. Accordingly, median output values from all subsequent analyses (e.g., lambda statistic, p -value, R^2) were conserved.

We first investigated the phylogenetic signal and ecomorphological patterns in a multivariate context. The phylogenetic signal was estimated using a multidimensional equivalent of Blomberg’s K (Adams 2014) for the four datasets: trophic ecology (stable isotopes), body size, morphological ratios, and head shape (summarized by PC scores) using the function *physignal* from the R-package *geomorph* with 10,000 iterations (Version 4.0.0; Adams and Otárola-Castillo 2013). We investigated the ecomorphological relationship between trophic ecology and morphological characteristics by performing 2-blocks Partial Least Squares (2-blocks PLS) analyses. On the one hand, PLS analyses were conducted without phylogenetic correction if the phylogenetic signal was close to 0 and non-significant (regular PLS using the *two.b.pls* function from *geomorph*). On the other hand, PLS analyses were phylogenetically corrected if a significant phylogenetic signal was detected (phylo-PLS using the *phylo.integration* function from *geomorph*). The relationship was assessed between the full isotopic dataset and the three morphological datasets, i.e., a first one including the three ratios, a second one made of shape data (PC1 and PC2), and a third one including the body size (SL). Regular PLS were run on all observations (i.e., individual data) while phylo-PLS were conducted on mean values per species.

In order to refine our ecomorphological analyses, we investigated ecomorphological trends in a univariate context using linear regression models. We computed phylogenetic generalized least squares (PGLS), in which phylogenetic signal is estimated simultaneously with the regression model (Revell 2010). This approach is particularly useful when the suitability of our data for phylogenetic regression is questioned (Revell 2010). Along this procedure, the error structure of the generalized least squares model is optimized by the simultaneous calculation of the lambda parameter of Pagel (1999) for the studied traits. The value of Pagel’s lambda reflects the phylogenetic signal of each combination of traits (Freckleton et al. 2002; Revell 2010): a lambda value close to 0 means

no phylogenetic signal and the fitted model tends to be an ordinary least squares model; while a lambda value close to 1 reflects a strong phylogenetic signal and the fitted model converges to a phylogenetic regression model where traits followed a Brownian motion model of evolution. Relationships were assessed between each pair of morphological traits (i.e., body size, ratios and shape data summarized by PC scores) and isotopic value. These tests were run with the function *pgls* from the *caper* R-package (Version 1.0.1; Orme 2013). To date, such phylogenetically informed tests do not allow the integration of intraspecific variation. Accordingly, we ran these tests on species mean values.

Results

Morphology

The standard length of all specimens varied from 12.6 to 22.5 cm (Table 1). No significant differences in body size were detected among the six species (ANOVA, $F = 1.395$, $df = 5$, $P = 0.248$).

The six goatfish species significantly differed in their proportional head length (ANOVA, $F = 25.34$, $df = 5$, $P < 0.001$), head width (ANOVA, $F = 17.52$, $df = 5$, $P < 0.001$) and barbel length (ANOVA, $F = 31.13$, $df = 5$, $P < 0.001$). Measurements revealed a proportionally shorter head compared to body length in *Mulloidichthys* species than in *Parupeneus*

species with *P. macronemus* having the proportionally longest head (Fig. 2A). *Mulloidichthys vanicolensis* and *P. indicus* showed a proportionally wider head compared to head length than other species where *P. barberinus* had the narrowest head (Fig. 2B). *Parupeneus macronemus* showed the longest hyoid barbel length while *M. flavolineatus* and *P. rubescens* both had barbels shorter than other species (Fig. 2C).

Regarding head shape data analyses, the two first PCs account for 72.5% of the total shape variation (PC1 = 61.35% and PC2 = 11.25% of the total shape variance, Fig. 3). A visual exploration of the shape space revealed that each species occupies a distinct subspace, except *P. macronemus* and *P. rubescens* which share the same zone. This observation was confirmed by MANOVAs revealing significant shape difference among genera and species (genera: $F = 193.3$, $df = 1$, $P < 0.001$; species: $F = 58.1$, $df = 5$, $P < 0.001$). The *Mulloidichthys* and *Parupeneus* genera are separated along the PC1 axis. Species within each genus are segregated along the PC2 axis. Deformation grids allow the interpretation of shape variation associated with the two first PC axes (Fig. 3). Having positive PC1 values, the two *Mulloidichthys* species have a shorter and more rounded snout, more anteriorly positioned eye, more ventrally inserted adductor mandibulae muscles (small cheek), narrower operculum and more anteriorly inserted pectoral fin than *Parupeneus* species. With positive values along PC2 axis, *P. macronemus* and *P. rubescens* have a more lengthened head

Fig. 2 Boxplots with the three morphometric ratios. **A** HL/SL = head length/standard length, **B** HW/HL = head width/head length, **C** BL/HL = barbel length/head length

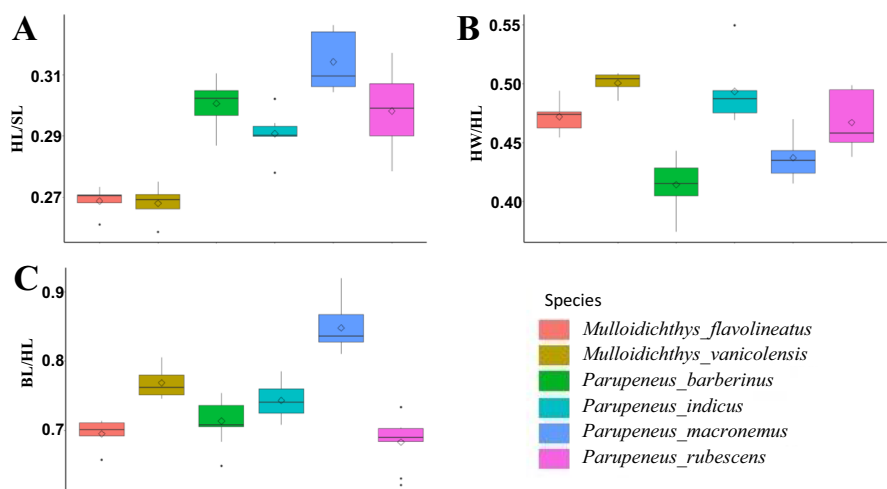
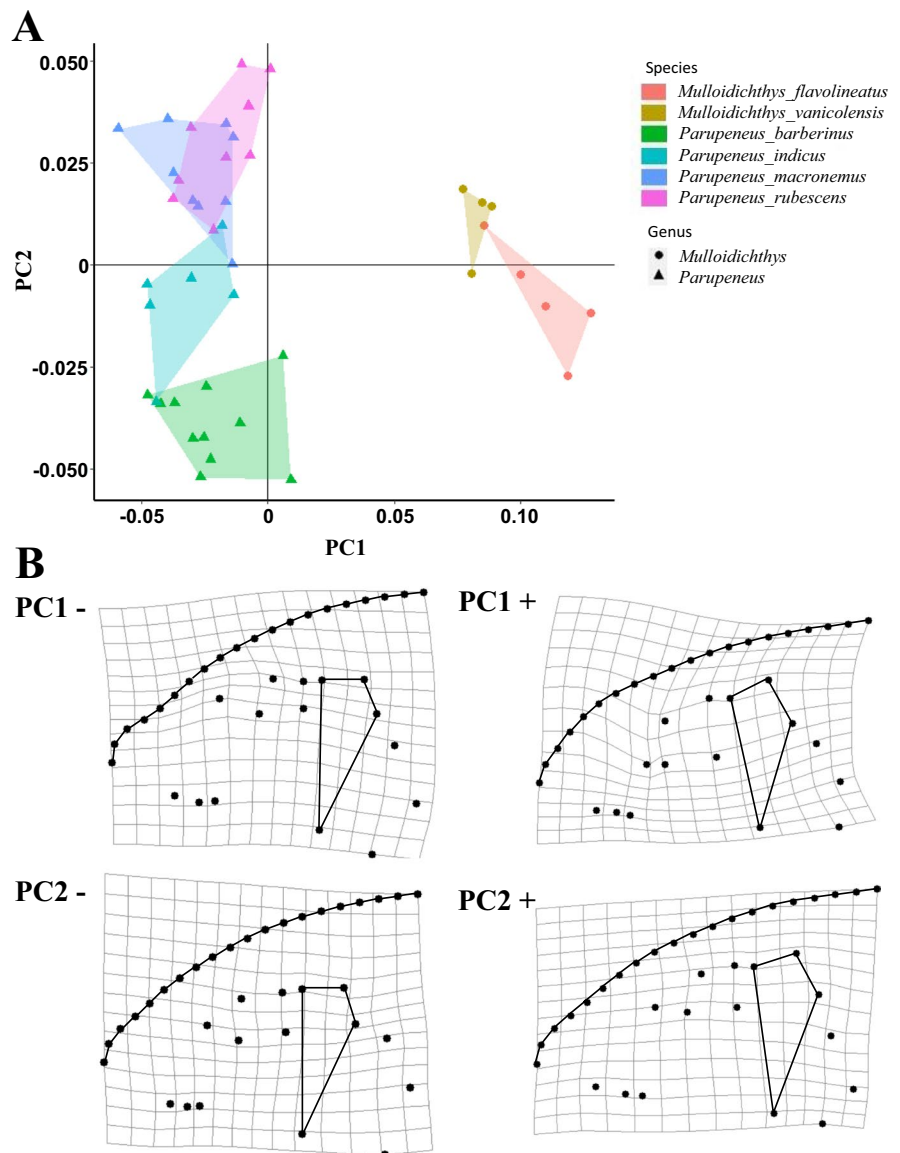


Fig. 3 **A** Morphospace illustrating head shape variation among the six goatfishes. Principal component analysis (PCA) was performed on shape data where PC1 = 61.35% and PC2 = 11.25% of the total shape variation. Convex hulls are added for an illustrative purpose only, showing the intra-specific variation. **B** Deformation grids illustrating shape variation associated with PC1 and PC2 (minimal (- PC) and maximal (+ PC) values)



profile (supraoccipital region is low) with a more caudally located eye, a shorter dentigerous process of the premaxilla and a wider operculum than *P. barberinus*. *Parupeneus indicus* has an intermediate shape between *P. barberinus* and the group formed by *P. macronemus* and *P. rubescens*. The same variation along PC2 is observed between the two species of *Mulloidichthys* with *M. vanicolensis* showing a more elongated snout, more caudally located eye, more dorsally inserted adductor mandibulae muscles and pelvic fins, a wider operculum

and more ventrally inserted pectoral fins than *M. flavolineatus*.

Trophic ecology

Isotopic values from the six studied species ranged between -18.6‰ and -10.2‰ for $\delta^{13}\text{C}$, 7.4‰ and 10.7‰ for $\delta^{15}\text{N}$, 6.8‰ and 19‰ for $\delta^{34}\text{S}$, respectively (Table 3). Significant differences were found among species along the $\delta^{13}\text{C}$ (Kruskal–Wallis, $\chi^2=29.11$, $df=5$, $P<0.001$) and $\delta^{34}\text{S}$ axes (Kruskal–Wallis, $\chi^2=25.84$,

Table 3 Isotopic ratios of the studied mulloid species

Species	$\delta^{13}\text{C}$ (mean \pm SD)	$\delta^{15}\text{N}$ (mean \pm SD)	$\delta^{34}\text{S}$ (mean \pm SD)
<i>Mulloidichthys flavolineatus</i>	-10.65 \pm 0.46	9.08 \pm 0.69	10.15 \pm 3.07
<i>Mulloidichthys vanicolensis</i>	-12.90 \pm 1.01	9.11 \pm 0.27	12.61 \pm 1.31
<i>Parupeneus barberinus</i>	-11.68 \pm 0.64	8.39 \pm 0.61	12.51 \pm 1.79
<i>Parupeneus indicus</i>	-14.56 \pm 2.06	8.61 \pm 0.95	11.60 \pm 3.54
<i>Parupeneus macronemus</i>	-15.24 \pm 1.34	9.44 \pm 0.49	16.86 \pm 0.26
<i>Parupeneus rubescens</i>	-15.11 \pm 2.21	9.17 \pm 1.04	15.53 \pm 1.60

Table 4 Results from Tukey’s and Dunn’s post hoc tests pairwise comparisons for $\delta^{13}\text{C}$ (lower diagonal) and $\delta^{34}\text{S}$ (upper diagonal). Asterisks highlight the significant *p*-values (*p*-value < 0.05)

Species	<i>Mulloidichthys flavolineatus</i>	<i>Mulloidichthys vanicolensis</i>	<i>Parupeneus barberinus</i>	<i>Parupeneus indicus</i>	<i>Parupeneus macronemus</i>	<i>Parupeneus rubescens</i>
<i>M. flavolineatus</i>		1	1	1	0.0021*	0.052
<i>M. vanicolensis</i>	0.41		1	1	0.15	1
<i>P. barberinus</i>	1	1		1	0.01*	0.32
<i>P. indicus</i>	0.025*	1	0.19		0.0057*	0.13
<i>P. macronemus</i>	0.0002*	0.63	0.0013*	1		1
<i>P. rubescens</i>	0.0013*	1	0.0092*	1	1	

df=5, *P*<0.001) but not for $\delta^{15}\text{N}$ values (ANOVA, *F*=2.16, *df*=5, *P*=0.078).

Regarding $\delta^{13}\text{C}$ values, pairwise comparisons using Dunn’s post hoc test allowed the identification of three groups of species (Table 4, Fig. 4): (1) *M. flavolineatus* and *P. barberinus* show the highest $\delta^{13}\text{C}$ values, (2) *P. rubescens* and *P. macronemus* have the lowest ones, and (3) *M. vanicolensis* and *P. indicus* have intermediate $\delta^{13}\text{C}$ values. Pairwise comparisons performed on $\delta^{34}\text{S}$ values (Table 4, Fig. 4B) revealed only two distinct groups where *P. macronemus* shows the highest $\delta^{34}\text{S}$ values, while the 5 remaining species have low but widely distributed values of $\delta^{34}\text{S}$.

Linking morphology to trophic ecology

Two-blocks PLS analyses were conducted to investigate relationships between trophic ecology (isotopes) and three morphological sets (body size, ratios and shape data) in a multivariate context. Phylogenetic signal was lower than 1 and non-significant for all multivariate dataset except head shape (isotopes: $K_{\text{mult}}=0.4$; *P*=0.20; ratios: $K_{\text{mult}}=0.19$; *P*=0.77; head shape $K_{\text{mult}}= 1.29$; *P*=0.02, body size $K_{\text{mult}}= 0.31$; *P*=0.4), indicating that closely related species have more similar head morphologies than expected under

a Brownian motion model. As head shape showed a strong phylogenetic signal (K_{mult} value exceeding 1), the combination of PC1 and PC2 was treated with a phylogenetically corrected PLS (Table 5), while the two other groups of traits, with K_{mult} values close to 0, were treated without phylogenetic correction (regular 2-blocks PLS). The PLS analyses revealed significant relationships between isotopes and both morphometric and head shape datasets (Table 5). *R*² values of 0.47 (ratios) and 0.98 (head shape) support an association between the trophic ecology and the studied morphological traits of the cephalic region.

To explore the ecomorphological relationships in further details, PGLS analyses have been performed on species mean trait values (Table 6). Along these tests, most of the trait combinations showed lambda estimation values close to 0 and thus, analyses could be interpreted as ordinary least squares models (without phylogenetic correction) except for HW/HL and PC2 vs. $\delta^{13}\text{C}$ values where PGLS model has been adjusted with phylogenetic information. These regression analyses highlighted significant negative relationships between SL and $\delta^{34}\text{S}$ (*R*²=0.92), and between PC2 and $\delta^{13}\text{C}$ (*R*²=0.68) while revealing a significant positive relationship between PC2 and $\delta^{15}\text{N}$ (*R*²= 0.70) (Table 6).

Fig. 4 Bivariate plots showing the distribution of goatfishes in the isotopic space: **A** $\delta^{13}\text{C}$ versus $\delta^{15}\text{N}$ and **B** $\delta^{13}\text{C}$ versus $\delta^{34}\text{S}$. Convex hulls are added for an illustrative purpose only, showing the intra-specific variation

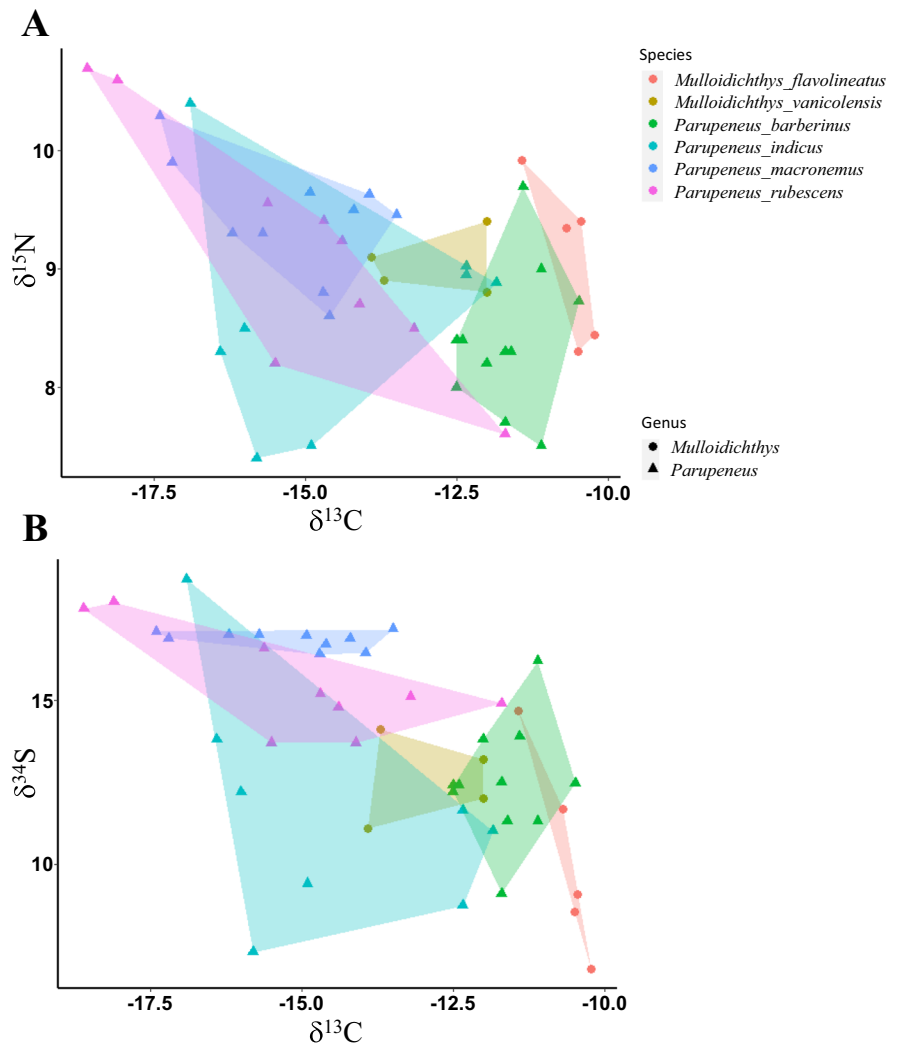


Table 5 Summary statistics of regular and phylogenetic-informed 2-block PLS analyses testing the relationships between (1) standard length (SL), (2) combined morphometric ratios (HL/SL=head length/standard length, BL/HL=barbel length/head length, HW/HL=head width/head length) and (3) head shape (GM: PC1 and PC2) with the three isotopic ratios ($\delta^{13}\text{C}$, $\delta^{15}\text{N}$, $\delta^{34}\text{S}$). Asterisks highlight the significant p -values (p -value < 0.05)

	2-block PLS		Phylo 2-blocks PLS	
	R^2	P -value	R^2	P -value
SL	0.1626	0.5248		
Ratios	0.4698	0.0047*		
GM			0.9799	0.0044*

Discussion

Most studied morphological traits (ratios and head shape) differ significantly among the six mulloid species. In addition, isotopic space based on ratios of carbon ($\delta^{13}\text{C}$) and sulfur ($\delta^{34}\text{S}$) points to some trophic segregation. Our results also reveal that head shape and fish body size are trophically relevant morphological traits in goatfishes as they are significantly related to isotopic variation.

Morphological diversity

Morphological traits, such as number and shape of scales, color patterns, myology or body and head

Table 6 Summary statistics of phylogenetic generalized least squares (PGLS) correlation tests with phylogenetic corrections, testing the relationships between (1) standard length (SL), (2) morphometric ratios (HL/SL= head length/standard length, BL/HL=barbel length/head length, HW/HL= head width/head length) and (3) head shape (GM: PC1 and PC2) with the three isotopic ratios ($\delta^{13}\text{C}$, $\delta^{15}\text{N}$, $\delta^{34}\text{S}$). Lambda is an indicator of phylogenetic signal (0 means no phylogenetic signal and 1 means a strong phylogenetic signal following a perfect Brownian motion). Asterisks highlight the significant p -values (p -value < 0.05)

	Lambda	Slope	PGLS p -value	Adjusted R^2
SL				
$\delta^{13}\text{C}$	0	0.13	0.08	0.48
$\delta^{15}\text{N}$	0	-0.02	0.25	0.14
$\delta^{34}\text{S}$	0	-0.21	0.002*	0.92
Ratios				
$\delta^{13}\text{C}$				
HL/SL	0	-72.57	0.18	0.25
HW/HL	0.98	-30.75	0.09	0.44
BL/HL	0.15	-12.48	0.40	-0.02
$\delta^{15}\text{N}$				
HL/SL	0	1.21	0.92	-0.25
HW/HL	0	2.92	0.63	-0.17
BL/HL	0	2.91	0.35	0.02
$\delta^{34}\text{S}$				
HL/SL	0	116.14	0.07	0.50
HW/HL	0	-21.34	0.59	-0.15
BL/HL	0	20.30	0.31	0.06
GM				
$\delta^{13}\text{C}$				
PC1	0	20.87	0.13	0.34
PC2	0.84	-56.58	0.03*	0.68
$\delta^{15}\text{N}$				
PC1	0	1.39	0.67	-0.19
PC2	0	13.41	0.02*	0.70
$\delta^{34}\text{S}$				
PC1	0	-22.83	0.23	0.16
PC2	0.38	66.27	0.11	0.41

shape are useful tools to assess the phylogenetic relationships among fishes (Strauss and Bond 1990). For more than a decade, Uiblein used a combination of these phenotypic traits to disentangle the Mullidae’s phylogeny (Uiblein 2011, 2021; Uiblein and Gouws 2015). Uiblein routinely used head length to describe species and build taxonomic identification keys at the species level (Uiblein and Heemstra 2010;

Uiblein and McGrouther 2012; Uiblein and Causse 2013; Uiblein and Gouws 2015; Uiblein and White 2015; Uiblein et al. 2017a, b, 2020). According to our results, proportional head length could also be used as an informative character to discriminate some genera. In this study, the two *Mulloidichthys* species show a clear difference in proportional head length in comparison to the *Parupeneus* species, with the former having a very short rostral region and the latter having a longer one. *Parupeneus macronemus* shows the most elongated head and the longest barbels of the studied species (Fig. 2). The pair of hyoid barbels is a synapomorphy shared by Mullidae but their morphology varies among species (Uiblein 2021, present study). Conversely, head width seems less variable among goatfishes and is consequently less relevant from a taxonomic point of view (Fig. 2). Using geometric morphometrics, we also highlighted that head shape is taxonomically relevant. Head shape analyses, which have been used here for the first time on mullids, also allowed a clear distinction between the two genera: *Mulloidichthys* and *Parupeneus* (Fig. 3), making it a promising tool to assess phylogeny of Mullidae and to understand their morphological evolution. The divergence between these two genera is linked to variation in head elongation, cheek size (adductor mandibulae muscle) and insertions of pectoral fins. *Parupeneus* is characterized by longer and more elongated head and snout coupled with more caudally inserted pectoral fins. Conversely, *Mulloidichthys* has a shorter head, a rounder snout, and more anterior pectoral fins insertions.

The cephalic region of all goatfishes is formed by the same osteological structures but varying in size and shape (Kim 2002). The detailed study of Kim (2002) illustrated variation in myological traits and differences in the insertions of adductor mandibulae muscle (4 sections: A1, A2, A3, and Aw) among genera. The size of adductor mandibulae muscle is tightly linked to head length (Vincent et al. 2007). Indeed, adductor mandibulae muscle was greater in the genus *Parupeneus*, including species with the most elongated heads. Characteristics of the adductor mandibulae muscle can inform on the performance of opening/closing jaws (Huby et al. 2019). A large adductor mandibulae muscle allows a fast mouth closing or a strong bite, depending on its insertion point on the jaws (Wainwright 1995; Huby et al. 2019). Goatfishes catch their prey by suction feeding (Gosline 1984), so the size of the adductor mandibulae muscle could

inform on feeding performances correlated to rapid mouth closing movements. We hypothesize that *Parupeneus* species, showing longer head and larger adductor mandibulae muscles than *Mulloidichthys*, have better suction performances and then forage on bigger preys or preys embedded deeper in the substrate. However, our shape data are probably not sufficient to fully validate this statement and additional measurements (e.g., muscles weight) or kinematic data should help to better characterize feeding performances in goatfishes (Wainwright 1995; Olivier et al. 2014, 2016).

Isotopic diversity

At the reef fish community level, most goatfishes are generally assumed to be benthic invertebrate feeders (Wahbeh and Ajia 1985; Lombarte et al. 2000; Lukoschek and McCormick 2001; Nakamura et al. 2003; Mahé et al. 2005; Kolasinski et al. 2009; El Bakali et al. 2010; Esposito et al. 2014). Beyond such a generalization, our isotopic data revealed clear isotopic variation among the six sympatric species: *Mulloidichthys flavolineatus*, *Mulloidichthys vanicolensis*, *Parupeneus barberinus*, *Parupeneus indicus*, *Parupeneus macronemus* and *Parupeneus rubescens*.

The highlighted variation among goatfish species in the isotopic space could certainly be linked to differences in their trophic ecology in its broadest sense (i.e., including difference in diet and/or feeding area). On the one hand, the absence of difference in $\delta^{15}\text{N}$ values among species suggests that the studied goatfishes likely occupy similar trophic position on the vertical axis of the food web (Jennings et al. 2001; Romanuk et al. 2011; Chouvelon et al. 2014). On the other hand, groups of species segregate along the $\delta^{13}\text{C}$ and $\delta^{34}\text{S}$ axes (i.e., horizontal position within the food web) showing divergence in the origin of consumed resources and/or difference in prey species belonging to the same trophic position (Fig. 4) (Cocheret de la Morinière et al. 2003; Lepoint et al. 2008; Kadye and Booth 2012; Layman et al. 2012). Indeed, disparities in $\delta^{13}\text{C}$ and $\delta^{34}\text{S}$ values among goatfishes likely highlight differences in targeted preys, possibly exacerbated by differences in feeding behavior but also differences in feeding areas (e.g., outer reef vs. lagoonal feeding areas). Finally, as sulfur isotopic fractionation in sediments results from the microbial sulfur cycle, divergences along the $\delta^{34}\text{S}$ axis could be correlated

to the sediment depth exploited by goatfishes when searching for prey items (Jørgensen et al. 2019).

Coupling sulfur and carbon isotopes previously allowed to make inferences on fishes' spatial occupation at coastal-scale (Fry 2006). In our case, beyond differences in diet composition, our results could also support the hypothesis of a spatial partitioning in foraging areas among the six goatfishes at Toliara Reef. As Gajdzik et al. (2016) demonstrated in damselfishes, the relationship between $\delta^{13}\text{C}$ and $\delta^{34}\text{S}$ can correspond to variation in the occupation of the reef by fishes. Low values of $\delta^{34}\text{S}$ and high $\delta^{13}\text{C}$ are linked to species living in the lagoon or on the reef structure and conversely, species occupying the outer shelf of the reef show high $\delta^{34}\text{S}$ values and low $\delta^{13}\text{C}$ values. Accordingly, the isotopic values (Fig. 4B, Tables 3 and 4) would suggest that *P. macronemus*, having high $\delta^{34}\text{S}$ values and low $\delta^{13}\text{C}$, live and forage on the outer shelf of the reef relying on oceanic inputs. *Parupeneus barberinus* and *M. flavolineatus*, having lower $\delta^{34}\text{S}$ values and higher values of $\delta^{13}\text{C}$, would mainly feed on preys on the reef itself (including the back reef lagoonal system), an area where basal resources mix up imported oceanic production and local primary production. *Parupeneus rubescens*, *P. indicus*, and *M. vanicolensis* with intermediate values of $\delta^{13}\text{C}$ and $\delta^{34}\text{S}$ probably live on both sides of the reef or switch from one side to the other, helped by the tides which are important in that area (up to 3 m; Chevalier et al. 2015). Such a trophic niche partitioning according to spatial segregation is already known for goatfishes living in other regions. For example, in the Mediterranean Sea, *Mullus barbatus* occupies deeper floors between 50 and 200 m while *Mullus surmuletus* lives in shallower waters under 50 m (Lombarte et al. 2000). Golani (1994) showed that the co-existence of four Mediterranean goatfish species presenting important diet overlap was also allowed by depth range specialization. Along the south-western coast of Australia, *Upeneichthys stotti* occupies preferentially the deep offshore waters whereas *Upeneichthys lineatus* generally lives in the shallow inshore waters; however, they can also be found simultaneously on the inner continental shelf (Platell et al. 1998).

Ecomorphology

The absence of strong phylogenetic signal in morphometric ratios and body size, meaning that

phylogenetically closely related species are not necessarily phenotypically similar and vice versa, suggests an evolutionary lability of these ecomorphological traits in the Mullidae (Kamilar and Cooper 2013). In contrary, head shape evolution, characterized by an important phylogenetic signal, may either follow a neutral evolution (approximated by a Brownian Motion model of trait evolution) or reflect a phylogenetic niche conservatism where closely related species are more similar ecologically than would be expected by simple Brownian motion (Losos 2008; Kamilar and Cooper 2013). Indeed, phylogenetically closely related species share more common evolutionary history than distantly related species, so they tend to have similar phenotypic and niche-related traits (Liu et al 2015).

The combination of morphological and trophic (stable isotopes) data in exploratory multivariate two-blocks PLS analyses, with and without phylogenetic information, revealed a relationship between the head morphology and isotopic ratios. This supports the fact that the morphological variation of the cephalic region in mullids is associated with variation in their trophic ecology (Table 5). Results of linear models provide more detailed information about ecomorphological hypotheses in goatfishes. Species with high $\delta^{13}\text{C}$ values present generally a short head, an anteriorly positioned eye, and have a wide operculum (low PC2 values, Fig. 4) (Table 6). *Mulloidichthys flavolineatus* showing the highest $\delta^{13}\text{C}$ values fully fits to these criteria (Fig. 3). In contrast, *P. macronemus*, which has lower $\delta^{13}\text{C}$ values and high $\delta^{34}\text{S}$ values (Fig. 4), is smaller than the other species and presents a long head (Fig. 3). Gosline (1984) described the behavior of *Parupeneus* specimens searching for preys in anfractuositities, helped by their long snout. This last trait could be involved in deep digging (Lopez-Fernandez 2014) as *Parupeneus barberinus* has already been seen foraging in the sediment using its snout (McCormick 1995). On the other hand, *Mulloidichthys* species, having short snout, may use their head as a shovel to dig in the upper substrate (Gosline 1984; Krajewski et al. 2006). *Mulloidichthys flavolineatus* has been recorded blowing away sand to uncover a prey detected with the barbels or dig into sand with the snout to search for preys (Hobson 1974). As reported and illustrated by Krajewski et al. (2006), *Mulloidichthys martinicus* mainly searches preys by horizontally moving the snout against the

substratum. The combination of these behavioral descriptions with our morphological data strengthens the hypothesis that morphological variations in goatfishes could be linked to differences in feeding strategies and/or prey types and, consequently, trophic niche partitioning.

Moreover, the strong negative correlation between body size and $\delta^{34}\text{S}$ revealed an allometric shift in the goatfishes' diet with larger individuals (lower $\delta^{34}\text{S}$ values) relying more on benthic preys than smaller ones (higher $\delta^{34}\text{S}$ values) (Szpak et al. 2020). We can extrapolate and suggest that resources partitioning (feeding and habitat) is driven by variations in body size. Finally, following the hypothesis of Gajdzik et al. (2016), we suggest that smaller individuals (with higher $\delta^{34}\text{S}$ values) live and feed on the outer shelf of the reef after the pelagic larval phase settlement and then move to the lagoon when they have reached a larger body size (lower $\delta^{34}\text{S}$ values).

Hyoid barbels are anatomical features linked to feeding strategies. Uiblein (1998) has suggested that species with short barbels like *Upeneus sulphureus* show more epibenthic food searching behaviors in opposition to species with long and thick barbels like *U. sundaicus*. This could be supported by the fact that larger barbel surface may carry more taste buds (McCormick 1993; Uiblein 1998) allowing to better detect embedded preys (Sato 1937). Longer barbels could also help to detect prey from anfractuositities or crevices (Hobson 1974; Uiblein 1991; McCormick 1995). Although our analyses did not highlight a relationship between barbel length and trophic data, *P. macronemus* may forage in crevices or deep in the substrate with its long barbels, while *P. rubescens* and *M. flavolineatus*, with the shortest barbels, may forage in the epibenthic area and, sometimes in the pelagic compartment.

To conclude, the six studied mullid species from the Toliara Reef exhibit some ecomorphological diversity most probably related to reef spatial occupation and feeding habits, allowing their sympatry. This segregation is associated with functionally and taxonomically relevant morphological trait variation such as head shape. Applying the same approach for studying assemblages of goatfishes from various geographical areas would allow to compare them and explore the recurrence of ecomorphological diversity of goatfishes. From an evolutionary perspective, head shape appears as a trophically relevant trait for studying the radiation of goatfishes.

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Data availability The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

Declarations

Ethics approval and consent to participate All specimens were bought on Toliara’s local fish markets, already dead. Exporting permits for the samples were obtained via the fishery institution Direction Régionale pour la Pêche et de l’Economie Bleue Atsimo Andrefana (Toliara), 069/22/MeSupRes/U.U/IH.SM. All authors have agreed to participate.

Consent for publication All authors have read and agreed to the published version of the manuscript.

Competing interests The authors declare no competing interests.

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