

Research article

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Repeated morphological diversification in endemic Antarctic fishes of the genus *Trematomus*

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Abstract. The iterative nature of ecomorphological diversification is observed in various groups of animals. However, studies explicitly testing the consistency of morphological variation across and within species are scarce. Antarctic notothenioids represent a textbook example of adaptive radiation in marine fishes. Within Nototheniidae, the endemic Antarctic genus *Trematomus* consists of 15 extant species, some with documented large intraspecific variability. Here, we quantify head shape disparity in 11 species of *Trematomus* by landmark-based geometric morphometrics, and we illustrate repeated events of divergence and convergence of their head morphology. Taking advantage of the polymorphism observed in some species of *Trematomus*, we also show that two closely related species or clades (e.g., *Trematomus bernacchii* and *T. hansonii*) are characterised by the same level of morphological disparity as observed at the level of the entire genus. Interestingly, the same main axes of shape variation are shared between and within species, indicating repeated morphological diversification. Overall, we illustrate a similarity of intra- and interspecific patterns of phenotypic diversity providing new insights into the mechanisms that underlie the diversification of Antarctic fishes.

Keywords. Convergence, ecomorphology, geometric morphometrics, head shape, macroevolution, microevolution, phylomorphospace.

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Introduction

Many factors may influence the processes of species and phenotypic diversification. During the last two decades, macroevolutionary studies have taken advantage of time-calibrated phylogenies to test the predictions of adaptive radiation where lineages are multiplying rapidly as a consequence of ecological opportunity (e.g., MATSCHINER *et al.* 2011; LITSIOS *et al.* 2012). Beyond this classic evolutionary theory, many studies also illustrate how intrinsic lineage characteristics such as genome duplication (e.g., TANK *et al.* 2015), anti-predatory defenses (e.g., LIU *et al.* 2018), trophic specialization (e.g., GAJDIK *et al.* 2019), or locomotor strategies (e.g., DORNBURG *et al.* 2011) may constitute novelties that shape clade diversification. Additionally, it was shown that extrinsic factors such as the invasion of a new habitat may provide opportunities for lineages to diversify and lead to variation in the tempo of diversification and morphological evolution (e.g., ALFARO *et al.* 2007; FRÉDÉRICH *et al.* 2016b).

While accumulating evidence of forces acting on the rates of lineage diversification and morphological evolution, studies have also questioned the consistency of diversification (RUBER & ADAMS 2001; LANGERHANS *et al.* 2004; MUSCHICK *et al.* 2012). For example, BURBRINK *et al.* (2012) revealed determinism in the rate of species diversification occurring during the adaptive radiation of four groups of squamates with early pulses of speciation. They showed that the processes of morphological evolution are not so easily predictable. In contrast, it has been found that *Anolis* lizards diversify on similar adaptive landscapes, which leads to exceptional morphological convergence of the entire fauna on four Caribbean islands (LOSOS *et al.* 1998; MAHLER *et al.* 2013). In the marine realm, coral reef damselfishes also experienced iterative ecological radiations in their evolutionary history (FRÉDÉRICH *et al.* 2013, 2016a). Such repeated ecological radiation produced subclades with similar levels of morphological disparity (FRÉDÉRICH *et al.* 2013) and regional assemblages being similar in their eco-functional diversity (GAJDIK *et al.* 2018). These contrasted findings from various taxonomic groups certainly ask for additional research, especially studies focusing on the recurrence of phenotypic diversification at multiple evolutionary levels.

The Nototheniidae (sensu DUHAMEL *et al.* 2014) represent an ideal system to investigate the iterative nature of phenotypic diversification. Commonly known as cod icefishes, this family includes at least 115 species (DUHAMEL *et al.* 2014) and represents the bulk of notothenioids predominating the teleost fauna of the Antarctic continental shelf by abundance and biomass as well as by diversity (EASTMAN 2005). Within this speciose family, *Trematomus* forms a clade of 15 recognized species, including *Pagothenia* and *Cryothernia* (DUHAMEL *et al.* 2014; EASTMAN & EAKIN 2021). Based on time-calibrated molecular phylogenies, the species of *Trematomus* are hypothesized to have experienced an early pulse of lineage diversification (NEAR *et al.* 2012). Documented ecological variation among species of *Trematomus* provides additional support for a scenario of adaptive radiation during their evolutionary history. *Trematomus* exhibit a primarily benthic lifestyle but there are a few members with an epibenthic (*T. loennbergii*, *T. lepidorhinus*, and *T. eulepidotus*) or even a (cryo-)pelagic lifestyle (*T. borchgrevinki* and *T. newnesi*) (LANNOO & EASTMAN 2000; DUHAMEL *et al.* 2014). In addition to differences in feeding ecology (BRENNER *et al.* 2001; RUTSCHMANN *et al.* 2011), they have also diversified according to depth, with inshore (*T. newnesi*, *T. hansonii*, *T. bernacchii*) and deep-water taxa (*T. lepidorhinus* and *T. loennbergii*) (CAUSSE *et al.* 2011; DUHAMEL *et al.* 2014).

Along with this ecological variation among species of *Trematomus*, intraspecific variability in the form of genetic variation and/or phenotypic plasticity has also been described. For example, plasticity is well documented in *T. newnesi*, a species with at least two ecomorphs characterized by differences in mouth size (EASTMAN & DEVRIES 1997; PIACENTINO & BARRERA-ORO 2009). Trophic and genetic data support the hypothesis that populations of *T. bernacchii* are also ecologically heterogeneous. This species is considered to be a benthic feeder and shows opportunism in its feeding strategy (LA MESA *et al.* 2004). Furthermore, it exhibits variation in morphology (BERNARDI & GOSWAMI 1997;

LAUTREDOU *et al.* 2010) and genetic population structure (VAN DE PUTTE *et al.* 2012). This intraspecific diversity provides an opportunity to compare the pattern of morphological variation between and within species throughout an adaptive radiation, thereby providing a unique temporal perspective.

In the present study, we first aimed at describing the head shape disparity in species of *Trematomus* using geometric morphometric methods. The adaptive significance of head shape variation has been linked to trophic resource use in numerous fish taxa (e.g., AGUILAR-MEDRANO *et al.* 2011; SANTOS-SANTOS *et al.* 2015) including notothenioid fishes (HU *et al.* 2016). Second, we compared the variation of this morphological trait, which is directly related to the trophic ecology of fish, to better understand the current processes of morphological diversification in species of *Trematomus*. Finally, we tested whether the level and the axes of shape variation at the interspecific level differ from the ones at the intraspecific level. Overall, our results provide strong support for repeatability in the pattern of morphological evolution in *Trematomus* fishes.

Material and methods

Morphological data and phylogenetic information

We analysed 193 museum specimens from 11 species of *Trematomus* (i.e., 73 % of the extant diversity; Table 1) from the Muséum national d'Histoire naturelle (MNHN, Paris, France) and the Natural History Museum (NHMUK, London, England). Here, as suggested by DUHAMEL *et al.* (2014) and NEAR *et al.* (2018), we consider that *Pagothenia borchgrevinki* belongs to the genus *Trematomus*. The identification of every specimen is listed in Table S1, and sample sizes within species ranged between one and 59 specimens (median = 19 individuals, Table 1).

Specimens were photographed in lateral view with a camera (Canon Eos 6D & Nikon D7000), and the x- and y-coordinates of 16 homologous landmarks capturing head shape (Fig. 1) were digitized from the left side of each individual using the morphometric software TPSDIG, ver. 2.26 (ROHLF 2004). In order to test the hypotheses about the consistency of morphological diversification patterns among and within species of *Trematomus*, we focused on head shape, an ecologically relevant morphological trait. A Generalized Procrustes Analysis was performed to align specimens to a common coordinate system and to remove variation in their position, orientation, and size (ROHLF & SLICE 1990). Procrustes tangent coordinates were then used as shape variables for all specimens (ADAMS *et al.* 2013). Head size was calculated from landmark configurations as centroid size (CS), the square root of summed squared distances of landmarks from the centroid (BOOKSTEIN 1991).

Allometry, referring to the proportion of shape variation explained by size variation (KLINGENBERG 1998), has been extensively documented in fishes (e.g., MEYER 1990; FRÉDÉRICH & SHEETS 2010; SANTOS-SANTOS *et al.* 2015) and our sampling includes specimens with a significant variation in body size (range of body size across all species: 6–31 cm SL; Table 1; Table S1). We therefore used regression to correct for the size effect. Size correction of shape data was performed by a linear regression of all shape variables on log-transformed CS using the function *procD.lm* from the R-library GEOMORPH ver. 3.3.2 (ADAMS & OTÁROLA-CASTILLO 2013). The residuals obtained from a single regression model across all specimens were then used as a new dataset in comparative analyses. For simplicity, this new dataset will be named 'size-corrected shape variables' in the subsequent analyses.

The molecular, time-calibrated, and multigene phylogeny of *Trematomus* prepared by PARKER and colleagues (2022) was pruned to match the species in our morphological dataset and to provide an estimate of the evolutionary relationships among taxa (Fig. 2). This time-tree was built on the DNA alignments from NEAR *et al.* (2018), who used restriction site-associated DNA sequencing (RADseq), and thus is consistent with their phylogenetic hypotheses.

TABLE 1

List of the studied species. N, number of specimens. The range of standard length (SL) for studied specimens is provided.

Species	N	Size range (SL, cm)
<i>Trematomus bernacchii</i>	59	9.8–27
<i>Trematomus borchgrevinki</i>	4	18.7–23
<i>Trematomus eulepidotus</i>	22	11.7–23.6
<i>Trematomus hansonii</i>	30	13.3–30.8
<i>Trematomus lepidorhinus</i>	22	11.5–24.2
<i>Trematomus loennbergii</i>	6	6.0–15.5
<i>Trematomus newnesi</i>	19	6.5–18.2
<i>Trematomus nicolai</i>	1	6.6
<i>Trematomus pennellii</i>	5	12.5–22.1
<i>Trematomus scotti</i>	24	6.0–14.1
<i>Trematomus tokarevi</i>	1	16

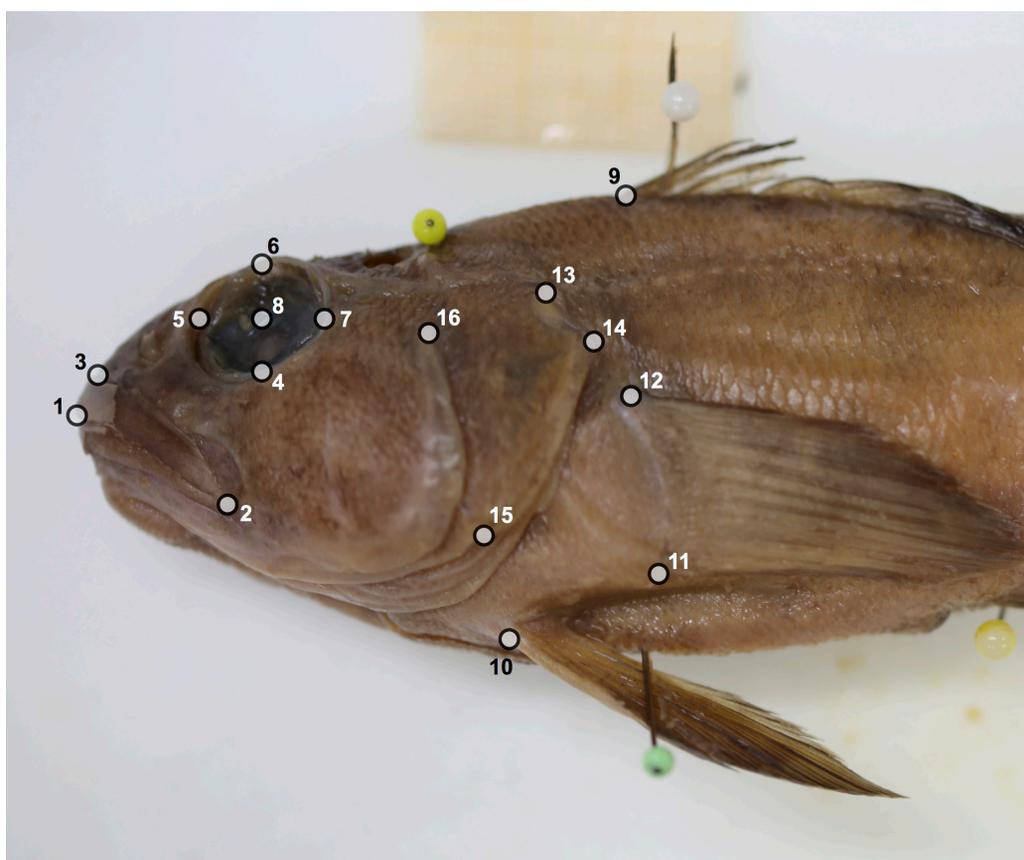


Figure 1 – Morphologically homologous landmarks (LMs) used in the analysis of the *Trematomus* head shape diversity. LMs are here illustrated for *Trematomus bernacchii*: (1) mouth tip; (2) posterior tip of the dentigerous process of the premaxilla; (3) upper bound of the upper lips; (4–7) inferior, anterior, superior, and posterior margin of the eye; (8) centre of the eye; (9) base of the first spiny dorsal fin ray; (10) base of pelvic fin; (11) ventral base of the pectoral fin; (12) dorsal base of the pectoral fin; (13) superior tip of the operculum; (14) posterior tip of the operculum; (15) ventral tip of the subopercle; (16) superior tip of the preoperculum.

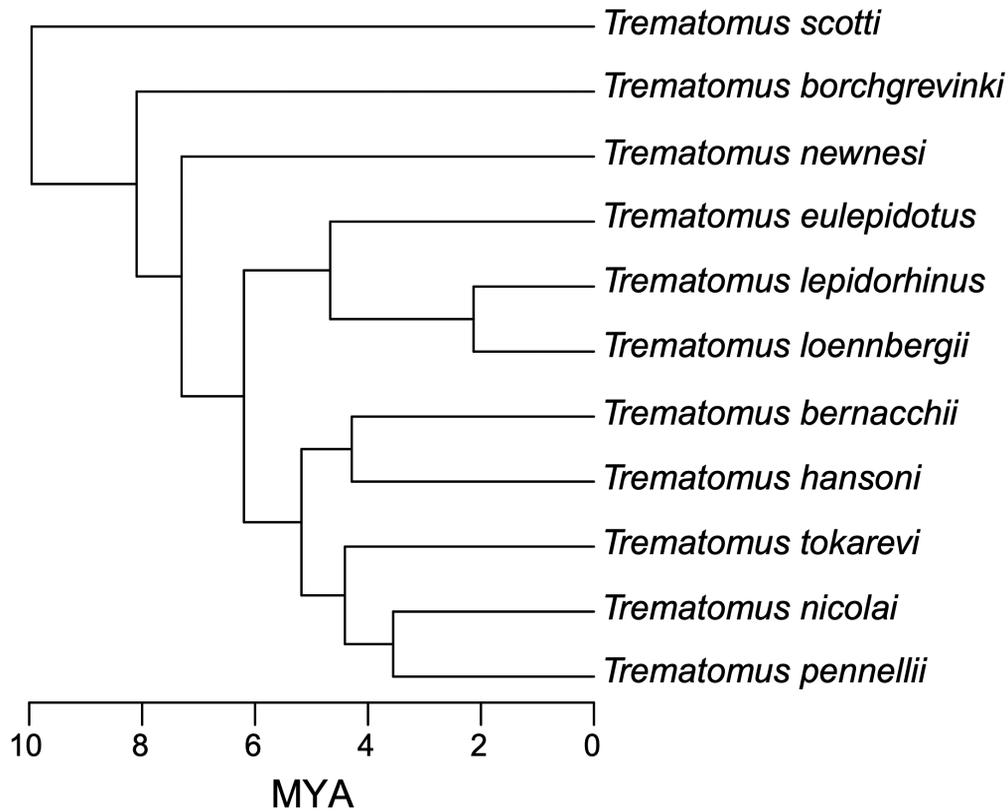


Figure 2 – Consensus time-calibrated tree displaying the estimated phylogenetic relationships among the studied species of *Trematomus*.

Phylomorphospace and head shape variation among *Trematomus*

We first used a phylomorphospace approach (SIDLAUSKAS 2008) to illustrate the main pattern of head shape evolution in species of *Trematomus*. Size-corrected shape data were implemented in a regular principal component analysis (PCA) and the phylogeny projected into this ordination plot. In parallel, the mean head shape for all 11 species was calculated and the estimates of head shape at the internal nodes of the phylogeny were inferred by using FELSENSTEIN's (1985) contrasts algorithm.

We then performed non-parametric MANOVA (COLLYER *et al.* 2015) and canonical variate analyses (CVA) on size-corrected shape variables to test for shape differences among species. Permutation tests ($N=10\,000$) on pairwise comparisons using Procrustes distances were performed to determine which species differed from one another in the shape space. Thin-plate spline (TPS) deformation grids were used to illustrate extreme positive and negative deviations along principal component axes. TPS deformation grids, PCA and CVA were produced with the program MorphoJ (KLINGENBERG 2011). Non-parametric MANOVA (also referred to as Procrustes ANOVA) was computed using the function *procD.lm* from the R-library GEOMORPH. Only species represented by more than one specimen were included in MANOVA and CVA, i.e., excluding *T. nicolai* and *T. tokarevi* (Table 1). Phylomorphospaces were generated by using the function *phylomorphospace* from the R-library PHYTOOLS (REVELL 2012).

Comparing morphological disparity at different evolutionary levels

In order to compare shape variation at different evolutionary scales, we designed a comparative framework where the disparity present in a single species or a group of species would be compared

to the one observed in the rest of the clade. Populations of *T. bernacchii* are trophically (LA MESA *et al.* 2004) and genetically (VAN DE PUTTE *et al.* 2012) heterogeneous, and the feeding ecology of *T. hansonii* is considered extremely diverse (PAKHOMOV 1998). Thus, we first compared the level and the pattern of morphological disparity between the two sister-species *T. bernacchii* and *T. hansonii* to the one observed for the rest of the clade. Similarly, we chose to compare the subclade formed by *T. eulepidotus*, *T. loennbergii* and *T. lepidorhinus* with the rest of the clade. If morphological disparity is proportional to taxonomic diversity, we hypothesised that the clade formed by all species of *Trematomus* shows a higher level of disparity than *T. bernacchii*, *T. hansonii*, the clade formed by *T. bernacchii* and *T. hansonii*, and the clade with *T. eulepidotus*, *T. loennbergii*, and *T. lepidorhinus*. Conversely, a similar pattern of morphological divergence at these different evolutionary scales would sustain the hypothesis of repeated convergences and/or provide some evidence for cryptic diversity in *T. bernacchii* and *T. hansonii* populations.

We calculated the level of shape disparity based on Procrustes variance (ZELDITCH *et al.* 2004) and performed the comparison between groups using the function *morphol.disparity* with permutation test (9999 iterations) in the R package GEOMORPH. We then compared the pattern of shape variation, i.e., the distribution of fishes in the shape space and the main dimensions along which shapes are most disparate, by using the program SpaceAngle8 (IMP-software). Based on PCA of shape variables, this method allows to test if two groups occupy the same subspaces of the morphospace. Detailed explanations on this method and examples can be found elsewhere (e.g., ZELDITCH *et al.* 2004; FRÉDÉRICH & VANDEWALLE 2011). In brief, the angle between two subspaces embedded in the shape space can be defined as the angle through which one subspace must be rotated to match the other. If the angle between the subspaces occupied by two groups does not exceed the bootstrapped within-group variance, we may not reject the null hypothesis stating that the two groups share the same major axes of head shape variation. The range of angles within group is calculated using a bootstrapping procedure (N=2500) and subsequently, this range was compared to the angle between groups. PCA of shape data was performed using PCAGen (IMP-software), and the angles between subspaces defined by the first six PCs (80% of the total shape variance) were calculated by SpaceAngle8 (IMP-software). The package of IMP software, including PCAGen & SpaceAngle8, was created by H. David Sheets and is freely available at <https://www.animal-behaviour.de/imp/>.

In addition to the analyses with SpaceAngle8, we compared the structure of covariance matrices between taxonomic groups by using different approaches including Random Skewers (RS) and the Krzanowski correlation (Kzr). All details about these approaches, which are designed to estimate the similarity or dissimilarity between matrices, are provided in MELO *et al.* (2016). Briefly, RS values vary between -1 (the two matrices have opposite structures) and 1 (the two matrices share the same structure). Zero means that the matrices have distinct structures. Similar to SpaceAngle, Kzr measures the degree to which the first principal components span the same subspace. The Kzr correlation values range between 0 (two subspaces are dissimilar) and 1 (two subspaces are identical). We performed these tests by using the function *MatrixCompare* from the R package EVOLQG (MELO *et al.* 2016). For helping the repetition of statistical analyses. We provided the TPS file with landmark data, grouping factors as well as our R codes as supplementary material ([Supp. file S1](#), [Supp. file S2](#), [Supp. file S3](#)).

Results

Head shape disparity in *Trematomus*

Head shape differed significantly across species (non-parametric MANOVA: $R^2=0.36$, $Z=13.75$, $P=0.0001$) and pairwise comparisons based on Procrustes distances showed significant differences between species ($P < 0.05$, Table 2). Discrimination among species can be interpreted by examining the

ordination of specimens in the shape space defined by CV axes (Fig. 3). This ordination corroborates with findings of significant shape differences among species although some overlap is present in the scatter plots (Fig. 3A). The three first CV axes together explained 84% of the total variation in the dataset. Three groups are distinguished along CV1 axis: a first group including only *T. newnesi*; a second group including *T. bernacchii*, *T. borchgrevinki*, *T. hansonii*, *T. loennbergii*, and *T. pennellii*; and a third group including *T. scotti*, *T. lepidorhinus*, and *T. eulepidotus*. The three species from this last group have

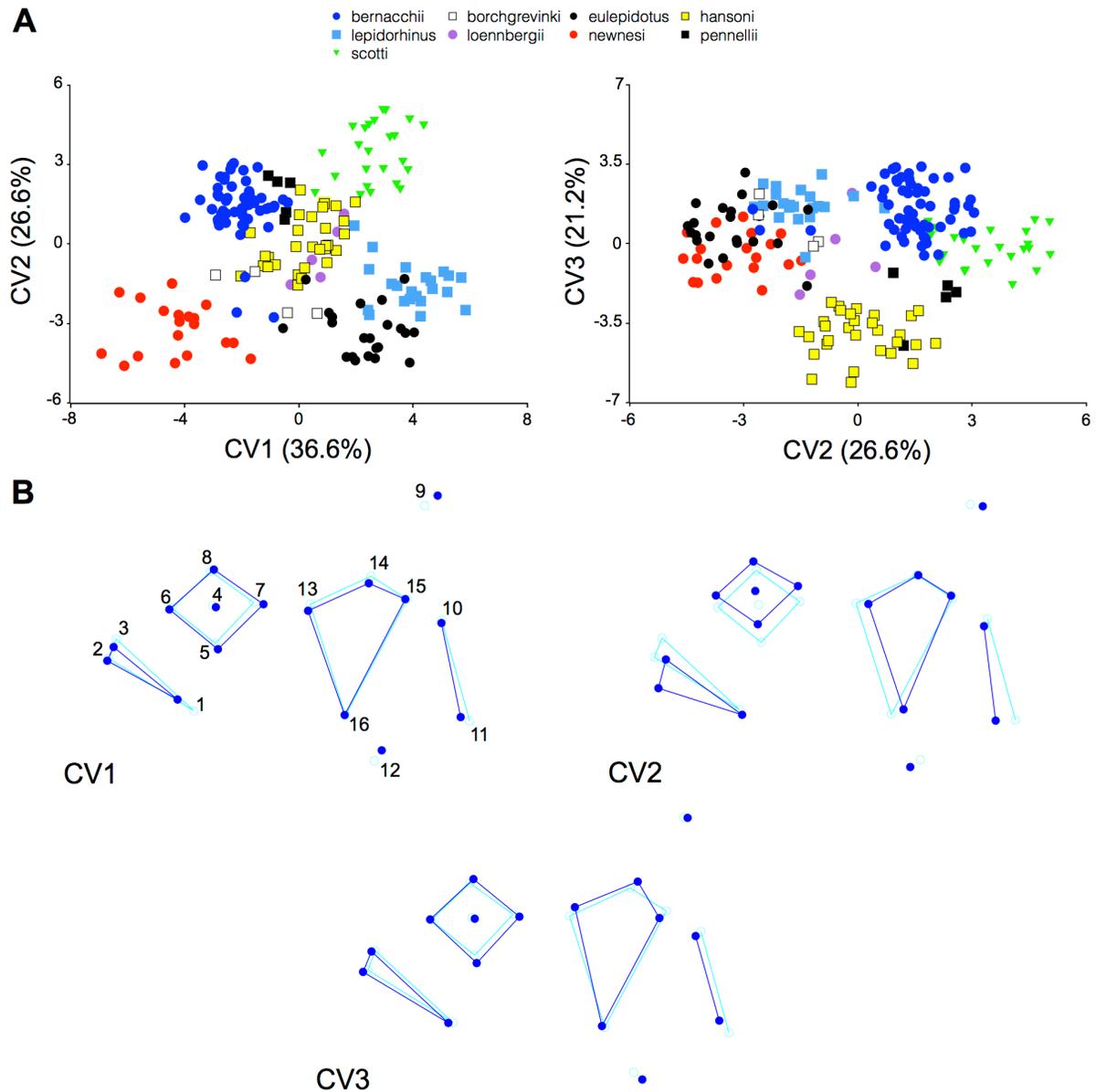


Figure 3 – (A) Ordination of the nine species of *Trematomus* in the shape space defined by the first three canonical axes (CV1, CV2, and CV3) based on the matrix of size corrected shape data. (B) Wireframe graphs depicting head shape variation along the canonical variate axes. Species are indicated by different symbols and colours. Percentage of shape variance summarised by each CV axis is given in parentheses. Wireframe graphs illustrate shape variation from the lowest (light blue) to the highest values (dark blue). The shape variation along CV axes has been exaggerated for better visualization ($\times 5$). The description of landmarks is provided on Figure 1.

TABLE 2

Pairwise comparisons among nine species of *Trematomus* using size-corrected shape data. Procrustes distances are shown below the diagonal, and *P*-values are shown above the diagonal. Results are obtained by permutation tests (N = 10 000). Significant results are italicized.

	<i>bernacchii</i>	<i>borchgrevinki</i>	<i>eulepidotus</i>	<i>hansoni</i>	<i>lepidorhinus</i>	<i>loennbergii</i>	<i>newnesi</i>	<i>pennellii</i>	<i>scotti</i>
<i>T. bernacchii</i>	–	<0.001	<0.001	<0.001	<0.001	0.033	<0.001	0.0469	<0.001
<i>T. borchgrevinki</i>	0.1289	–	0.002	<0.001	0.001	0.001	0.0054	0.0064	<0.001
<i>T. eulepidotus</i>	0.1082	0.0913	–	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
<i>T. hansoni</i>	0.0619	0.1081	0.0923	–	<0.001	0.0122	<0.001	0.0073	<0.001
<i>T. lepidorhinus</i>	0.0901	0.0974	0.0744	0.0853	–	<0.001	<0.001	0.001	<0.001
<i>T. loennbergii</i>	0.0553	0.1236	0.1064	0.0630	0.0776	–	<0.001	0.0012	<0.001
<i>T. newnesi</i>	0.0992	0.0842	0.0813	0.0826	0.1093	0.1051	–	<0.001	<0.001
<i>T. pennellii</i>	0.0579	0.1548	0.1311	0.0731	0.1149	0.0777	0.1289	–	<0.001
<i>T. scotti</i>	0.0688	0.1491	0.1290	0.0855	0.0855	0.0822	0.1474	0.0736	–

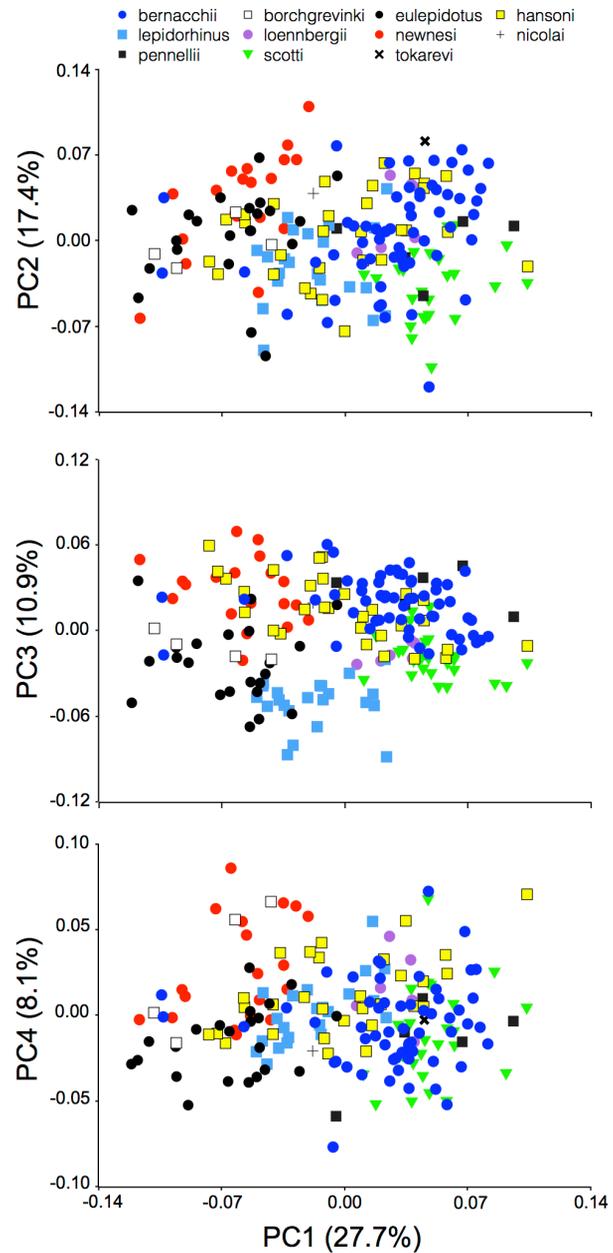


Figure 4 – Ordination of the eleven species of *Trematomus* in the shape space defined by the first four principal component axes (PC1–PC4) based on the matrix of size-corrected shape data. Species are indicated by different symbols and colours. Percentage of shape variance summarised by each PC is given in parentheses.

shorter mouths (LMs 1-3) and larger eyes (LMs 4-8) than the other species (high values along CV1, Fig. 3A). CV2 axis discriminates *T. bernacchii*, *T. pennellii*, *T. scotti* and to some extent *T. hansonii* and *T. loennbergii* from the others due to a more ventrally oriented mouth, more dorsally positioned eyes, shorter operculum and more vertically inserted pectoral fins (Fig. 3B). *Trematomus hansonii* differs from all the other species along CV3. Proportionally, it shows the largest insertion of the pectoral fin among the studied species (LMs 10 & 11; Fig. 3B).

The main shape variation across species can also be examined by a distribution of specimens in the shape space defined by a PCA performed on size-corrected shape variables (Fig. 4). Even if the species are similarly aggregated as in the ordinations produced by CVA scatterplots (Fig. 3A), PCA highlights the extensive intra-specific shape variability. Across all studied species, *T. hansonii*, *T. bernacchii*, and *T. newnesi* show the largest deviation along PC axes (Fig. 4). Generally speaking, the patterns of shape variation highlighted by PCA are similar to those observed for CVA (Fig. 4). The main axis of shape variation captured by PC1 (27.7 % of the total shape variation) concerned the relative size of the operculum and the eye as well as the orientation of the mouth and the pectoral fin attachment (Fig. 5).

The variation in head shape evolution was highlighted in a phylomorphospace (Fig. 6). Some sister lineages are highly divergent in the shape space (e.g. *T. lepidorhinus* vs *T. loennbergii*; *T. eulepidotus* vs *T. lepidorhinus* and *T. loennbergii*; *T. nicolai* vs *T. pennellii*) while some pairs of more distantly-related species are highly similar in their head morphology (*T. eulepidotus* and *T. borchgrevinkii*, *T. bernacchii* and *T. pennellii*; Fig. 6).

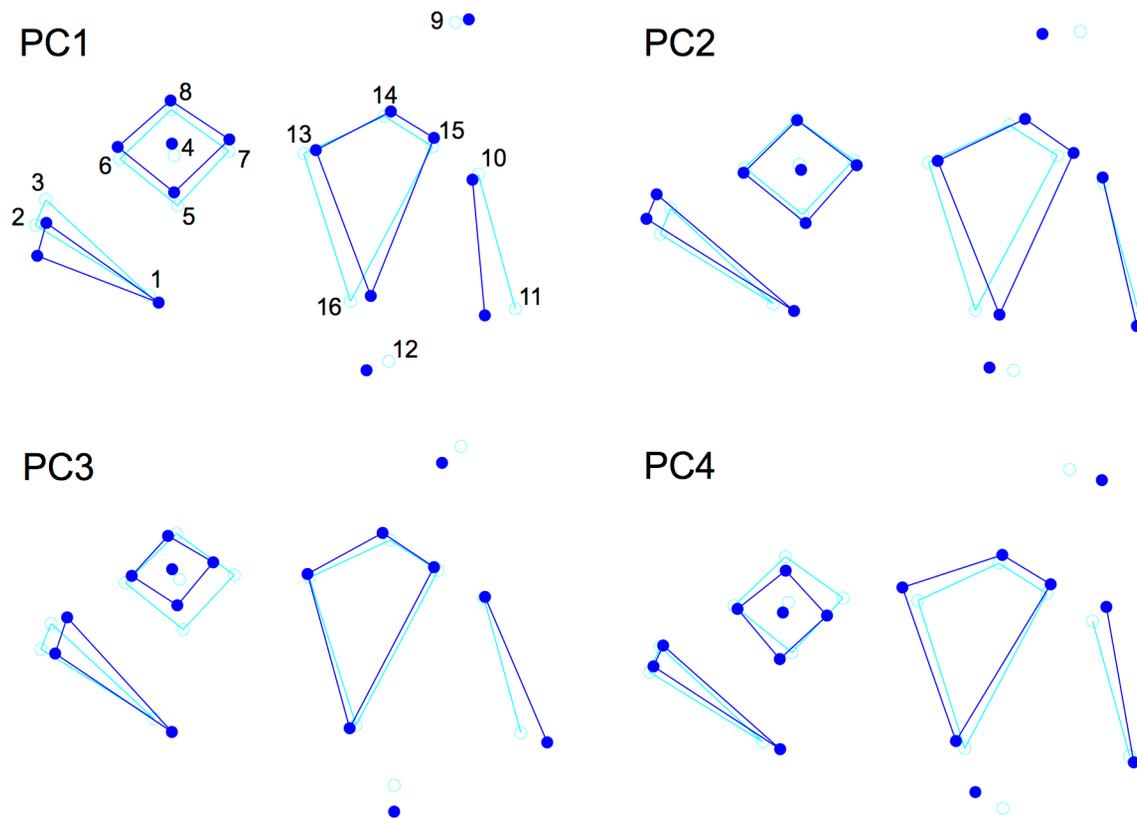


Figure 5 – Wireframe graphs illustrating head shape variation from the lowest (light blue) to the highest values (dark blue) along PC axes. The shape variation along PC axes has been exaggerated for better visualization ($\times 5$). The description of landmarks is provided on Figure 1.

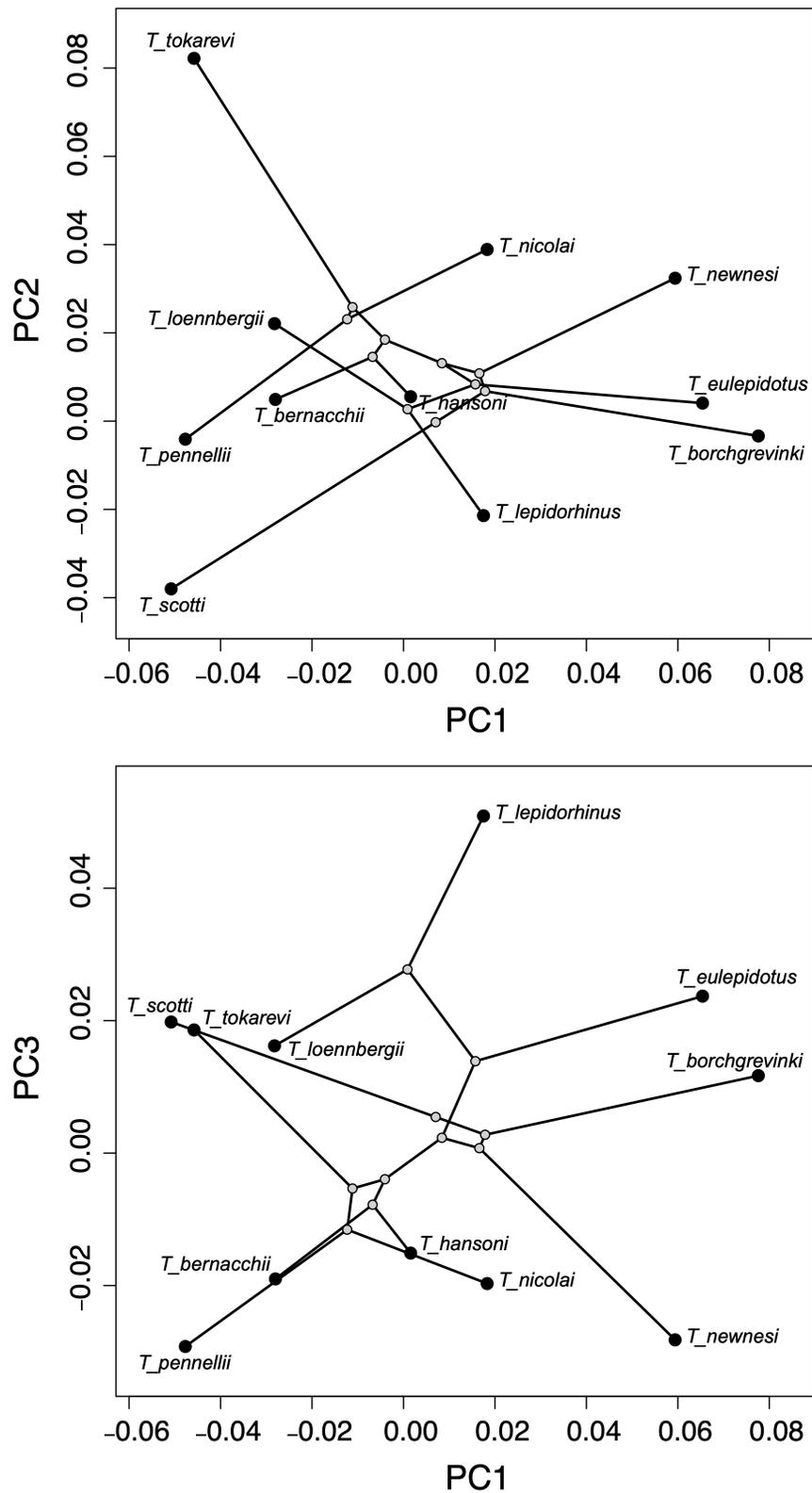


Figure 6 – Phylomorphospace of the head of *Trematomus*. Morphospace viewed as the first three principal component (PC) axes of tangent space where the consensus phylogenetic tree has been projected to visualise the relationships among species (black dots) and the estimated shapes at each internal nodes are highlighted by grey points.

TABLE 3

Comparisons of the level of shape disparity between various taxonomic groups. Procrustes variance for each group and *P*-values are provided. Results are obtained by permutation tests ($N = 10\,000$). Significant results are italicized.

Group 1	Group 2	Variance group 1	Variance group 2	P-value
<i>T. bernacchii</i>	others	0.0087	0.0102	0.055
<i>T. hansonii</i>	others	0.0085	0.0099	0.129
<i>T. bernacchii/T. hansonii</i>	others	<i>0.0087</i>	<i>0.0107</i>	<i>0.003</i>
<i>T. bernacchii</i>	<i>T. hansonii</i>	0.0087	0.0085	0.850
<i>T. eulepidotus/T. loennbergii/T. lepidorhinus</i>	others	0.0096	0.0098	0.841
<i>T. eulepidotus/T. loennbergii/T. lepidorhinus</i>	<i>T. bernacchii/T. hansonii</i>	<i>0.0096</i>	<i>0.0087</i>	<i>0.011</i>

TABLE 4

Comparisons of the patterns of shape disparity between taxonomic groups. Results from SpaceAngle8, Random Skewers (RS) and Krzanowski correlation (Kzr) are provided. In SpaceAngle8, results were obtained by bootstrapping procedures ($N = 2500$). Angles between the subspaces defined by the first six principal components on shape variables are in decimal degrees. The angle between subspaces is considered significant if it exceeds the bootstrapped within-group variance at 95% confidence (CI).

Group 1	Group 2	SpaceAngle		RS	Kzr
		Between groups (95% CI)	Within group 1 / Within group 2		
<i>T. bernacchii</i>	others	93.9 (88–115)	90.8 / 89.6	0.67	0.77
<i>T. hansonii</i>	others	100.3 (88.8–121)	105 / 93.4	0.70	0.78
<i>T. bernacchii/T. hansonii</i>	others	93.9 (85.3–116.4)	89.4 / 75.2	0.70	0.81
<i>T. bernacchii</i>	<i>T. hansonii</i>	94.6 (86.1–120.4)	88.9 / 105	0.72	0.76
<i>T. eulepidotus/T. loennbergii/T. lepidorhinus</i>	others	86.5 (79.1–112.7)	96.5 / 92.3	0.68	0.78
<i>T. eulepidotus/T. loennbergii/T. lepidorhinus</i>	<i>T. bernacchii/T. hansonii</i>	100.5 (94.2–122.9)	89.4 / 96.1	0.68	0.78

Comparison of the pattern of shape disparity between taxonomic groups

A visual exploration of the distribution of fish specimens in the PCA shape space highlighted overlaps between species and illustrated extensive shape variation within species (Fig. 4). The level of morphological variation within *T. hansonii*, *T. newnesi*, and *T. bernacchii* is high and appears to account for a large proportion of the *Trematomus* head shape disparity. This was confirmed by the calculation of shape disparity levels based on Procrustes variance. Most pairwise comparisons revealed that the level of shape disparity did not differ significantly between taxonomic groups, i.e. groups made of one species or a complex of species (Table 3). Only the group made of *T. bernacchii* and *T. hansonii* looked less disparate than the other *Trematomus* species or the subclade “*eulepidotus/loennbergii/lepidorhinus*” ($P < 0.02$; Table 3).

In addition to the similarity in the extent of shape disparity, the major axes of shape variation are shared at all studied temporal scales, both across and within species. For all pairwise comparisons, results from SpaceAngle8 suggested that we cannot reject the null hypothesis that one group occupies the same subspace of shape space as the other group. Indeed, the 95% confidence interval of the angle between the subspaces of two groups was always lower than the ranges of the within-subspace angles (Table 4). As an example, the angle between the subspaces of *T. hansonii* and the other *Trematomus* was 100° ,

and thus lower than the ranges of within-hyperplane angles (105° for *T. hansonii* and 93° for all other *Trematomus*). Values from Random Skewers (RS) and Kzr correlation are close to 1 (Table 4) and thus reinforce results from SpaceAngle. RS and Kzr values highly suggested similarities in the pattern of head shape variation between taxonomic groups.

Discussion

The morphological diversification of lineages of *Trematomus* is characterised by repeated events of head shape divergence and convergence. Interestingly, we also illustrate that one species harbors the same level of morphological disparity as observed at the genus level. Beyond this large amount of head shape variation observed at the species level, the pattern of head shape variation in *Trematomus* is highly conserved across the evolutionary history of the clade.

Previous studies highlighted ecological variation among *Trematomus* lineages along the benthic-pelagic axis (EKAU 1991; KLINGENBERG & EKAU 1996; LANNOO & EASTMAN 2000; RUTSCHMANN *et al.* 2011; WILSON *et al.* 2013) and our morphological study agrees with such a partitioning in their foraging ecology. The pattern of head shape variation in *Trematomus* is relatively similar to the one observed in marine and freshwater fish families characterised by benthic-pelagic transitions along their evolutionary history (COOPER *et al.* 2010; AGUILAR-MEDRANO *et al.* 2011; FRÉDÉRICH *et al.* 2016a 2017; TAVERA *et al.* 2018). Benthic *Trematomus* species (*T. bernacchii*, *T. pennellii* and *T. scotti*) have a ventrally oriented mouth and dorsally positioned eyes (Figs 3–5), optimizing the capture of benthic prey and increasing the ability to detect predators when resting on the bottom. Conversely, the epibenthic (*T. lepidorhinus* and *T. eulepidotus*) and cryopelagic species (*T. newnesi* and *T. borchgrevinki*) have horizontally oriented mouths, which is an adaptation for targeting prey in the water column. *Trematomus hansonii* and *T. loennbergii* show a somewhat intermediate head shape pattern, with tendencies of being more similar to the benthic species.

The relative size of the mouth also reflects the foraging strategy (WAINWRIGHT & RICHARD 1995). The rather small cryopelagic *T. newnesi* (max. TL=25 cm) has a relatively small mouth, whereas the likewise small (max. TL=20 cm) but benthic *T. scotti* has a relatively larger mouth (Fig. 3) (EASTMAN 2019). Despite its relatively small size, *T. scotti* relies on capturing larger benthic prey (e.g., polychaetes, gastropods, benthic amphipods), while *T. newnesi* feeds on smaller planktonic organisms, for example, copepods, euphausiids, and pelagic amphipods (CASAUX & BARRERA-ORO 2013; MOREIRA *et al.* 2014). Other benthic species, such as *T. bernacchii*, *T. hansonii*, and *T. loennbergii* may be able to capture benthic prey in similar size ranges as *T. scotti* due to their larger absolute size.

Concerning locomotion, species of *Trematomus* use a combination of both pectoral and body-caudal fins for swimming (EASTMAN & DEVRIES 1985). It is well known that the performance of pectoral fin locomotion is highly influenced by fin shape and orientation (WAINWRIGHT *et al.* 2002). Interestingly, we revealed some variation in the angle of pectoral fin attachment among *Trematomus* (Figs 3–5), which is directly related to their lifestyle. The pectoral fins of the epibenthic *T. lepidorhinus* and *T. eulepidotus*, and the cryopelagic *T. borchgrevinki* and *T. newnesi* are more horizontally attached than in benthic species. Such a morphology decreases water resistance and optimises free swimming (FULTON 2007; AGUILAR-MEDRANO *et al.* 2013). The large pectoral fin attachment observed in *T. hansonii* (Fig. 3) could be associated with powerful swimming, and it may agree with the hypothesis that this species is not strictly benthic (EKAU 1991; KLINGENBERG & EKAU 1996; KOCK *et al.* 2012). Overall, the tight links between the morphology of the head region in *Trematomus* species and their lifestyle provide strong evidence for the adaptive significance of their diversification. Divergent selection thus played a key role along their evolutionary history.

The visual exploration of phylomorphospace suggested that the diversification of *Trematomus* probably consists of ecomorphological divergence and convergence (Fig. 6). Imperfect morphological convergence and incomplete ecological niche partitioning among *Trematomus* species likely sustain a constant increase of morphological disparity over evolutionary time (STAYTON 2006; COLLAR *et al.* 2014). DORNBURG *et al.* (2017) argued that high-latitude Antarctic nearshore habitats, where many species of *Trematomus* occur, act as evolutionary sinks and *Trematomus* instead originated in the Scotia Arc and Antarctic Peninsula region. Such a pattern of repeated habitat colonization could also explain the presence of morphological convergence.

The present study illustrates that the level of morphological disparity and the main axes of head shape variation are similar at intraspecific and interspecific levels in *Trematomus*. These data provide new insights into the morphological evolution and the diversity in this genus. Our results from SpaceAngle8, Random Skewers as well as Krzanowski correlation suggest that similar selective pressures might be active within and between species. Accordingly, this hypothesis would support that there is a continuum of divergence from the population to the species levels (ROLLAND *et al.* 2018). Competition is certainly one of the main external factors driving the ecological and phenotypic diversity of *Trematomus*. It is likely that competition leading to niche partitioning among species operates in the same way at the population level (DE MEYER *et al.* 2016). The two morphs of *T. newnesi*, for instance, seem to reflect niche partitioning, with large mouth morphs preying more on fish and less on krill than typical mouth morphs (EASTMAN & DEVRIES 1997); although, for instance, their buoyancy is not heterogeneous (EASTMAN & BARRERA-ORO 2010). Concerning internal factors, a high level of phenotypic integration, i.e., covariation of multiple traits (KLINGENBERG 2008), could also contribute to the recurrent pattern of head shape variation in *Trematomus*. Indeed, HU *et al.* (2016) have demonstrated that notothenioid skulls are highly integrated. Thus, the different traits of a head of *Trematomus* are highly linked to one another and the specific changes of traits occur together. Second, the high level of head shape disparity in *T. bernacchii* and *T. hansonii* strengthens previous observations on ecological and phenotypic plasticity in some species of *Trematomus* (EASTMAN & DEVRIES 1997; LA MESA *et al.* 2004; PIACENTINO & BARRERA-ORO 2009). The observed phenotypic variance within *T. bernacchii* may be linked to geographic and genetic variation. Spatial genetic population structure was documented in *T. bernacchii*, but it is unclear how this relates to morphological variation (VAN DE PUTTE *et al.* 2012). Here, we hypothesize that the high level of morphological disparity within one species (e.g., populations of *T. bernacchii*, *T. hansonii*, *T. newnesi*) suggests cryptic diversity or early stages of speciation, an assumption which is also corroborated by molecular data (LAUTREDOU *et al.* 2010). Future work is thus needed to investigate the degree to which the high phenotypic variance within *Trematomus* can be explained by plasticity, adaptive divergence, or spatial patterns of genetic diversity.

Conclusions

Our study of head shape disparity illustrates that polyphenism, i.e., the presence of alternative phenotypes within the same species, could be a relatively common trait in *Trematomus* species and suggests that there is a link between the main axis of morphological variation within species and the nature of phenotypic diversity in the adaptive radiation of *Trematomus*. EASTMAN & DEVRIES (1997) suggested that polyphenism observed in *Trematomus* could be explained by the low level of competition with other teleosts in the shallow waters at the highest latitudes of the Southern Ocean. Indeed, the high disparity level of *T. bernacchii* or *T. hansonii* might be related to an underutilization of resources allowing niche variation within one species. Currently, we have accumulated evidence that polyphenism has a role in promoting speciation and adaptive radiation (PFENNIG & MCGEE 2010; PFENNIG *et al.* 2010). Polyphenism is a hallmark of adaptive radiation, i.e., a rapid evolution, by generating new phenotypes for selection in short periods of time (PFENNIG *et al.* 2010). Acknowledging the link between plasticity/polyphenism and adaptive radiation, the high level of phenotypic variation observed within

some species of *Trematomus* provides further arguments for the adaptive nature of their diversification. Intraspecific variation may form the basis for interspecific diversification in *Trematomus* and this parallel distribution of traits at both population and species levels argues for a deterministic outcome of their morphological diversification. These findings may certainly have important implications for the prediction of notothenioid adaptations when facing global change.

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Supplementary files

Table S1. List of the studied specimens with #ID of museum collections (MNHN, Paris, France and NHM, London, England). The standard length (SL) of every fish specimen is also provided.

File S1. Landmark data. TPS file with landmark data for all studied specimens.

File S2. Identification of studied specimens and grouping factors for disparity analyses.

File S3. Coding for statistics in R. R-codes to perform disparity analysis as well as the comparison of covariance matrices.