Biological Journal of the Linnean Society (1996), *59:* 143–177. With 11 figures

A combined morphometric and phylogenetic analysis of an ecomorphological trend: pelagization in Antarctic fishes (Perciformes: Nototheniidae)

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Received 20 December 1994, accepted for publication 6 December 1995

The Antarctic fish family Nototheniidae (Perciformes) presumably originated from a benthic ancestor, and several lineages have evolved to live or at least feed in the water column, a trend called pelagization. Here, we use information on phylogeny, allometric growth, and diet composition for an integrated analysis of morphological and ecological diversification in this group, mainly focusing on the subfamilies Trematominae and Pleuragramminae. A phylogenetic analysis of data published in earlier systematic studies produced eight equally parsimonious trees, all indicating that several previously recognized taxa are paraphyletic. These phylogenetic trees all suggest multiple origins of pelagic life styles. Multivariate morphometric analyses including nine species showed that juveniles and adults grow according to a common pattern of ontogenetic allometry. The morphometric differences among species are mostly the result of lateral transpositions of the growth trajectories, indicating that embryonic and larval development is more important as a determinant of morphological variation than allometric growth as juveniles and adults. We studied patterns of interspecific variation with principal components and the covariation between morphometric variables and food composition with a partial least-squares analysis. Both analyses revealed a gradient from benthic to pelagic foragers. Measurements of structures involved in swimming have a prominent role in these analyses, suggesting adaptive evolution of these traits. Tracing morphometric traits on the phylogenetic trees revealed a considerable amount of evolutionary plasticity, showing that species related phylogenetically need not be morphologically similar, but can diverge considerably, perhaps as a response to natural selection and adaptation to different habitats and foraging modes. In accordance, a test of phylogenetically independent contrasts showed that bursts of increased morphological change accompanied habitat shifts.

ADDITIONAL KEY WORDS: — Adaptation – allometric growth – common principal components – constraints – niche differentiation – ontogeny – partial least squares – size correction.

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0024–4066/96/010143 + 35 $25.00/0

INTRODUCTION

Morphological and ecological features of organisms are mutually related to one another. On the one hand, morphology at least partly determines many ecological characteristics, such as feeding or locomotor performance (Webb, 1977, 1984; Wainwright, 1988, 1994; Losos, 1990; Norton, 1991; Schluter, 1993; Smith & Van Buskirk, 1995), while on the other hand, ecological factors can induce phenotypic changes (Meyer, 1987; Brönmark & Miner, 1992; Travis, 1994; Smith & Van Buskirk, 1995) and ecological diversification within a clade can drive the adaptive evolution of morphological traits (Baumgartner, Bell & Weinberg, 1988; Losos, 1990; Schluter & McPhail, 1992; Robinson & Wilson, 1994; Snorrason *et al*., 1994; Westneat, 1995). These interactions are the subject of ecomorphology (Motta & Kotrschal, 1992; Ricklefs & Miles, 1994).

The central tenet of ecomorphology is that present covariation between morphological and environmental features results from past natural selection and adaptive evolution. Therefore, to understand ecological and morphological diversity in a study group, an explicitly historical approach is necessary. There are, however, two different views of history as it relates to evolutionary biology. One of these views is that hypotheses about unique historical events are untestable, hence are outside the realm of science, and that historical biology therefore should study more general features instead and search for recurrent patterns (Lauder, 1981, 1982; Eldredge, 1993). This perspective underlies a variety of comparative methods, which estimate a (constant) evolutionary correlation from the changes in the values of two traits along the branches of a phylogeny (e.g. Pagel & Harvey, 1988; Harvey & Pagel, 1991; Losos & Miles, 1994). The other view recognizes that unique events and contingency are crucial for understanding evolutionary history (e.g. Gould, 1989). Several authors recently have advocated phylogenetic methods to study evolution by mapping morphological, ecological, or behavioural traits onto an independently estimated phylogeny (e.g. Coddington, 1988; Donoghue, 1989; Baum & Larson, 1991; Brooks & McLennan, 1991; Winterbottom & McLennan, 1993; Losos & Miles, 1994). Whereas these authors mostly have focused on discrete characters, the approach is also feasible for continuous ones (Brooks & McLennan, 1991; 364–366; Andersen, 1994), and here we apply it in the context of multivariate morphometrics. By estimating the ‘chronicle’ of trait changes (O’Hara, 1988), this procedure provides an understanding of evolutionary associations between ecological and morphological traits in particular clades. Process-oriented tests can then be derived from

generalizations of such phylogenetic patterns (e.g. McPeek, 1995). Ecomorphology, in this sense, encompasses much of Liem's vision of a new synthesis in morphology, whose "mission is to explain diversity and adaptation in both historical and functional terms and to discover *the reasons for the richness of unique phenomena that characterizes biology*" (Liem, 1991: 764; emphasis original).

The fishes of the perciform suborder Notothenioidei provide an excellent opportunity for ecomorphological studies. This group is the most abundant and taxonomically diverse component of the Antarctic fish fauna (Ekau, 1990; Hubold, 1992; Kock, 1992; Eastman, 1993; Miller, 1993). Although the sister group of the notothenioids is not known unambiguously, they are generally thought to have originated from a benthic ancestor (e.g. Andersen, 1984; Andriashev, 1987; Balushkin, 1992; Eastman, 1993) In the Antarctic seas, these fishes utilize such a broad variety of niches that they are the ecological equivalents to fishes from several orders in other regions. This especially applies to the family Nototheniidae. Besides a number of benthic and epibenthic species, this family contains mesopelagic species feeding on plankton or as predators of fish, and some even have colonized the unique habitat under floating ice (Andriashev, 1987; Eastman, 1993). Several authors have discussed this ecological diversification as an evolutionary trend toward pelagic life styles, which occurred in several notothenioid lineages (Voskoboynikova, 1982; Andersen, 1984; Andriashev, 1987; Ekau, 1988, 1991; Hubold, 1992; Eastman, 1993). Because all species lack a swim bladder, the transition to pelagic life was accompanied by extensive morphological and physiological changes to achieve neutral buoyancy and effective swimming performance (reviewed by Eastman, 1993). Previous ecomorphological studies arranged species in a graded series from benthic to pelagic life styles, using morphological criteria to infer their ecological niche (Ekau, 1988, 1991). These results were consistent with data on diet composition (Schwarzbach, 1988) and behavioural observations (Ekau & Gutt, 1991; Eastman, 1993).

Previous studies have used only morphological and ecological information to examine the evolution of pelagic modes of life, and therefore were unable, for instance, to investigate whether pelagic life styles evolved as a single directional trend or in several lineages independently. In contrast, here we perform a phylogenetic analysis of a part of the Nototheniidae using information published in earlier systematic studies. We reanalyse morphometric data for ten species of nototheniid fishes from the Weddell Sea (Ekau, 1988, 1991) to assess interspecific variation and to identify patterns of covariation between morphological and ecological features. In organisms with indeterminate growth such as fishes, comparisons among species are complicated by the effects of size. We take allometric variation into account explicitly by using a multivariate technique correcting for growth effects (Burnaby, 1966; Klingenberg, 1996). Data on diet composition allow us to quantify the ecological niches of the species, and to examine the patterns of covariance between diet and morphometric traits; hence our study is entirely based on evidence that is independent from earlier classifications of 'ecological types' or the results of studies in other species. Finally, we combine this 'equilibrium' approach (Lauder, 1981, 1982) with phylogenetic information to reconstruct the evolutionary history of our study group (O'Hara, 1988; Brooks & McLennan, 1991). Altogether, these analyses allow us to address the morphological and ecological diversification of these fishes in a broad historical view.

MATERIAL AND METHODS

Phylogenetic analysis

Existing hypotheses about the phylogeny of the Nototheniidae (Andersen, 1984; Balushkin, 1989; Eastman, 1993; Voskoboynikova, 1993) agree that the subfamilies Trematominae and Pleuragramminae form a monophyletic group, and these were therefore used as the ingroup for our analysis. Analyses of mitochondrial DNA sequences strongly support the monophyly of the Trematominae, but have not included any member of the Pleuragramminae (Bargelloni *et al*., 1994; Ritchie *et al*., 1995). Therefore, our ingroup comprised the following species (classification and nomenclature following DeWitt, Heemstra & Gon, 1990): *Pagothenia borchgrevinki* (Boulenger, 1902), *P. brachysoma* (Pappenheim, 1912), *Trematomus bernacchii* Boulenger, 1902, *T. eulepidotus* Regan, 1914, *T. hansoni* Boulenger, 1902, *T. lepidorhinus* (Pappenheim, 1911), *T. loennbergii* Regan, 1913, *T. newnesi* Boulenger, 1902, *T. nicolai* (Boulenger, 1902), *T. pennellii* Regan, 1914, *T. scotti* (Boulenger, 1907), *T. tokarevi* Andriashev, 1978, *Aethotaxis mitopteryx* DeWitt, 1962, *Cryothenia peninsulae* Daniels, 1981, *Gvozdarus svetovidovi* Balushkin, 1989, and *Pleuragramma antarcticum* Boulenger, 1902. One species of the ingroup glade, *Trematomus vicarius* Lönnberg, 1905, was omitted from the analysis because of the lack of data for many characters (nevertheless, the available information is included in Table 1).

TABLE 1. Data matrix for the phylogenetic analysis. The letter 'X' denotes polymorphism, i.e. both character states 0 and 1 present. Generic names are given if only one species of that genus is included in the analysis (see text for full names)

	Character								
Species	1 5	10	15	20	25	30	35	40	
Eleginops	00000	00100	00000	00000	11000	0010?	00111	00101	0000
Dissostichus	00000	00121	00011	00000	00000	0000?	00111	00000	0000
Gobionotothen	01000	00000	00001	00101	01110	10000	00111	00101	0010
L. kempi	01000	00010	00000	00101	01110	00000	00000	00000	0000
L. nudifrons	01000	00010	00001	00111	01110	00101	11111	00001	X000
L. larseni	01000	00010	00011	00100	01100	00200	0000X	00001	0000
Notothenia	01000	00011	00000	00101	11110	00001	11111	11100	0010
Paranotothenia	01000	00011	???00	00??1	11100	10001	11111	11???	????
Patagonotothen	01000	00010	00001	00110	01100	00001	00111	00001	1000
T. bernacchii	11100	10010	00?00	?010?	00110	10100	00111	00000	0000
T. eulepidotus	01000	00021	11011	10000	00110	1000?	00000	00101	1111
T. hansoni	01X00	10010	00000	00101	00110	10101	00X11	00011	0000
T. lepidorhinus	01000	00021	11?11	1010?	00110	10000	00000	00001	1010
T. loennbergii	01000	00021	00?01	0011?	00110	10000	00111	00010	1111
T. newnesi	01000	00021	10000	10101	00110	00001	11011	00101	1010
T. nicolai	01000	00010	00001	00010	00110	10001	11011	00101	0110
T. pennellii	01000	00000	00001	00111	00110	10100	00111	00101	1010
T. scotti	01000	00000	00001	00111	00110	10100	00001	00101	1111
T. tokarevi	01000	10010	00?01	0011?	00110	10101	11111	00???	????
*T. vicarius**	X1000	X00??	?????	?????	?0???	?0101	00111	00???	????
P. borchgrevinki	11111	1101?	???1?	?0???	00110	01101	11111	11111	0110
P. brachysoma	11111	110??	01011	10011	00110	01101	11111	11101	1110
Cryothenia	11X02	220??	?????	?????	?0111	?1101	01111	10???	????
Gvozdarus	11101	220??	?????	???1?	?0100	?0111	11111	11???	????
Aethotaxis	11001	11021	01111	01010	00011	10001	11111	01101	0110
Pleuragramma	11102	22021	01111	01010	00111	11111	11111	00101	0010

**T. vicarius* was not included in the phylogenetic analysis.

The outgroups included two species that branched off from the remaining nototheniids early in the phylogeny of the family (according to morphological analyses; but see Bargelloni *et al*., 1994), *Dissostichus eleginoides* Smitt, 1898, and *Eleginops maclovinus* (Cuvier, 1830) (the latter was placed in its own family by Balushkin, 1992). Seven additional outgroup taxa were from the subfamily Nototheniinae, which is commonly thought to be the sister group of the ingroup clade. The species of Nototheniinae were chosen to represent seven of the eight genera recognized by Balushkin (1989): *Gobionotothen gibberifrons* (Lönnberg, 1905), *Lepidonotothen* (Lepidonotothen) *kempi* (Norman, 1937), *Lepidonotothen (Lindbergichthys) nudifrons* (Lönnberg, 1905), *Lepidonotothen (Nototheniops) larseni* (Lönnberg, 1905), *Notothenia (Notothenia) rossii* Richardson, 1844, *Paranotothenia magellanica* (Forster, 1801), and *Patagonotothen guntheri* (Norman, 1937).

The data matrix (Table 1) used for phylogenetic analysis was compiled from the literature; definitions of the characters are given in the Appendix. Of the 44 characters, eight are features of the cephalic lateral line system, 13 characters deal with the visceral skeleton, one with the pectoral girdle, and four with the caudal fin skeleton. Squamation on the lateral lines and on the head is described by 11 characters. Pigmentation of larvae and juveniles (characters 38–44) is the only class of characters not previously used in studies of notothenid relationships. If two alternative character states were mentioned for a species, it was coded as polymorphic unless one of them was described as rare. We could not code characters from descriptions of otolith morphology (Hecht, 1987), whereas other data, e.g. from protein or mitochondrial DNA sequences (di Prisco *et al*., 1991; Bargelloni *et al*., 1994), enzyme electrophoresis (McDonald *et al*., 1992), and karyotypes (Morescalchi *et al*., 1992; Eastman, 1993) were available only for a few species or in some instances are inconsistent among studies. Only after completing this analysis did we become aware of a phylogenetic study of the Trematominae using mitochondrial DNA sequences (Ritchie *et al*., 1995).

We performed heuristic searches using PAUP, version 3.1 (Swofford, 1993), because the number of taxa in the full analysis precluded branch-and-bound or exhaustive searches. Two hundred random addition sequences were used in each analysis, and branch swapping was done with the TBR (tree bisection–reconnection) algorithm (Swofford, 1993). For the analysis of the ingroup alone, we used the branch-and-bound method.

Because most of the characters have previously been used in systematic studies, whose authors presumably chose these characters because they seemed particularly informative, they clearly are not a random sample of possible characters. Therefore, we did not attempt to test the phylogenetic hypotheses statistically, for example by bootstrapping.

Morphometric data

The material used in this study was collected in the Weddell Sea during two cruises of the German research vessel *Polarstern* (January–February 1985 and October–November 1986). Fish were caught in depths between 200 and 1200 m using bottom trawls or Agassiz trawls. Detailed information about stations and species compositions was given by Ekau (1988, 1990). Measurements were taken on

TABLE 2. Sample sizes by species and sex. Only specimens with complete data for the ten morphometric variables are included. 'Juveniles' are specimens whose gonads did not allow identification of sex. Some specimens had missing data for sex; therefore, the total number of specimens is greater than the sum of the sample sizes in the three categories for some species

Species	Juveniles	Females	Males	Total
Aethotaxis mitopteryx	2	11	8	21
Pagothenia borchgrevinki	0	2	1	3
Trematomus bernacchii	0	10	1	12
Trematomus eulepidotus	12	71	52	137
Trematomus hansoni	0	7	8	16
Trematomus lepidorhinus	30	127	36	196
Trematomus loennbergii	1	57	17	79
Trematomus nicolai	0	8	11	19
Trematomus pennellii	1	21	25	47
Trematomus scotti	17	51	44	113

693 specimens from ten species (Ekau, 1988, 1991); of these, 643 specimens had complete data for the morphometric variables considered here (Table 2).

We chose ten measurements for this study (Fig. 1) from a larger set of variables considered by Ekau (1988, 1991). Because this study focuses on evolutionary changes of morphological traits associated with the transition to pelagic life, structures associated with foraging and locomotion are of special interest. The head of fishes bears the feeding and main sensory structures. We included head length (HL) and width (HW), the size of the mouth (ML, the length of the premaxillary and maxillary), and orbital length (OL). For sustained slow swimming, nototheniids predominantly use the labriform swimming mode, powered by movements of the pectoral fins, but for fast bursts, they swim in the subcarangiform mode, using the posterior trunk and tail fin as propulsors (Montgomery & Macdonald, 1984; Archer & Johnston, 1989; Kunzmann & Zimmermann, 1992; Eastman, 1993). The corresponding morphometric variables are pectoral fin length (PFL), the lengths of the second dorsal (DFL) and anal fins (AFL), body depth at the first ray of the anal fin (BDA), and the depth of the caudal peduncle (CPD). In addition, standard length

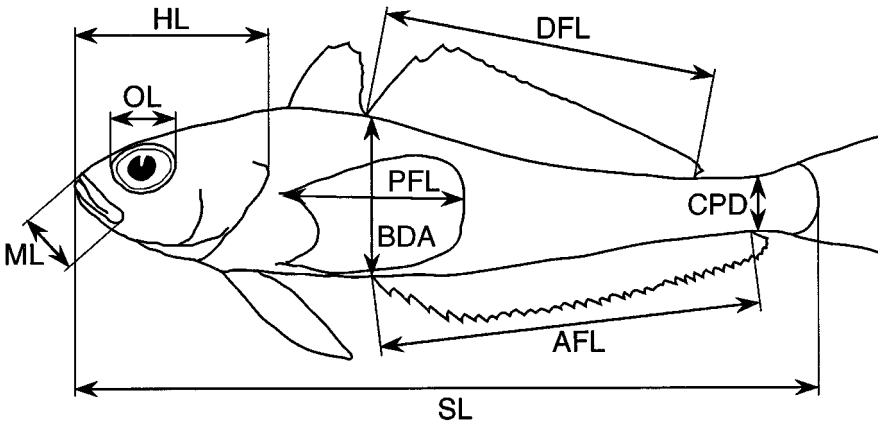

Figure 1. Measurements used for morphometric analyses. One variable, head width (HW) measured at the opercles, is not shown in the drawing.

(SL) was included to facilitate comparisons with other studies, in which it is often used as a measure of overall size.

In addition to these measurements, the age of most specimens was determined from annual growth increments of otoliths. Smaller otoliths were examined under a dissecting microscope without preparation, whereas larger ones needed to be sectioned (for details, see Ekau, 1988). Moderate inaccuracies in the age estimates based on otoliths, as some studies have found mostly for adult fish (White, 1991; Kock, 1992), do not invalidate our analysis because we use age only to detect general patterns of ontogenetic change, rather than for quantitative estimation of growth parameters. Moreover, differences between observers can be ruled out as a source of errors (Kock, 1992), because the ages for all specimens were determined by the same person (W.E.).

Statistical analysis

Multivariate allometry

Our study is mostly based on the multivariate generalization of allometry using principal component analysis (Jolicoeur, 1963) and newer techniques derived from it (reviewed by Klingenberg, 1996). Because we could not assume multivariate normality in most cases and due to the small sample sizes for some species, statistical tests were done using the bootstrap method (Efron & Tibshirani, 1993). Compared with parametric tests, this computer-based approach also permits a greater flexibility to choose test statistics. All measurements were transformed to natural logarithms before the analyses.

For the analysis of allometric growth, we identified the patterns of ontogenetic allometry, which represent the directions of growth trajectories in the space of log-transformed measurements (Klingenberg & Zimmermann, 1992; Klingenberg, 1996). These patterns of multivariate allometry were computed as the coefficients of the first principal components (PC1s) of the covariance matrix for each species (except for *Pagothenia borchgrevinki*, which had to be excluded because of the small sample size). We used the bootstrap approach to compute standard errors for PC1 coefficients and to compare them to the value expected for isometry, with 1000 replicates for each species (Efron & Tibshirani, 1993). Directions of growth trajectories were compared between species by calculating the angles between their PC1 axes. The angle α between two PC1 vectors $\mathbf{b}$ and $\mathbf{c}$ (normalized so that $\mathbf{b'b} = 1$ and $\mathbf{c'c} = 1$) was computed as $\alpha = \arccos(\mathbf{b'c})$ (e.g. Klingenberg, 1996).

Morphometric comparisons between species have to take allometric growth into account explicitly; otherwise, differences between species due to lateral transpositions of the growth trajectories are confounded with differences in the size composition of the samples. The methods used to correct for these size effects all assume that the species share a common allometric pattern, i.e. that their growth trajectories are parallel (Rohlf & Bookstein, 1987; Klingenberg, 1996). We tested this assumption with a bootstrap test, which does not rely on multivariate normality (Efron & Tibshirani, 1993). As the test statistic, we used the largest angle between the PC1 axes of any pair of species. The distribution of this test statistic under the null hypothesis of parallel growth trajectories was simulated by using principal component (PC) scores of each species rather than the original variables (Klingenberg, 1996). Because this manoeuvre only involves a rotation of the

coordinate system, it does not change the relative positions of the data points within each species, but it renders parallel the growth trajectories of all species. From this modified data set, bootstrap samples were randomly drawn for each species, with replacement, the PCs of each species were extracted, and the maximal angle between the PC1s of any pair of species was computed. We repeated this step 10 000 times. To establish the significance level achieved for the test, we counted how often the maximal angle equaled or exceeded the one obtained for the original data, and divided this count by 10 000.

An allometric pattern shared among species was estimated with common principal component analysis (Airoldi & Flury, 1988; Flury, 1988; Klingenberg & Zimmermann, 1992; Klingenberg, 1996). The assumption underlying the common principal component (CPC) model is that several groups share the same PCs, but that they may differ in the amounts of variation associated with each PC axis. In the context of allometry, this implies that the growth trajectories of all species are parallel, but that the amount of growth may differ among species. To compute CPCs, we used an implementation of the FG algorithm (Flury, 1988) written in the SAS/IML language (available from C.P.K. on request; the CPC technique is also available in the NTSYS-pc software and the IMSL/STAT library as routine KPRIN).

We used Burnaby's procedure to separate variation in 'size' due to growth from variation in directions perpendicular to the growth trajectories which can be considered as 'growth-invariant' morphological variability (Burnaby, 1966; Rohlf & Bookstein, 1987; Klingenberg, 1996). This technique can be used to display differences between species due to lateral transpositions of growth trajectories (Klingenberg & Spence, 1993). It is important to note that Burnaby's procedure does not separate 'size' from 'shape' in a geometric sense, because 'shape' variation associated with size through allometric growth is included in the 'size' component. In this study, we used the first common principal component (CPC1) as the growth axis. Instead of removing the variation along the CPC1 from the original variables as proposed by Burnaby (1966), it is mathematically equivalent but more convenient to simply omit the CPC1 and carry out analyses using the scores of the remaining components (CPC2–CPC10) as variables (Klingenberg, 1996), similar to a procedure proposed by Thorpe (1983). In the multidimensional space of morphometric variables, this results in a projection of the data points onto the subspace perpendicular to the CPC1, or growth trajectory. We retained all dimensions of this subspace (CPC2–CPC10), because our goal at this stage of the analysis was the elimination of growth effects from the interspecific comparison, rather than data reduction; moreover, Ricklefs & Miles (1994: 26) noted that components with little morphological variation (small eigenvalues) can contain ecologically relevant information.

To display growth-invariant differences among species, we used CPC2–CPC10 as variables in a multivariate analysis of variance (MANOVA) with species as the classification criterion (see also Klingenberg & Spence, 1993). The first two principal components of the between-species matrix of sums of squares and cross-products accounted for most between-species variation. We used these two growth-free axes for ordinations, but we did not carry out formal statistical tests.

Associations between morphology and diet composition

The mode of feeding is a major determinant of the ecological niche of a fish species (e.g. Schluter & McPhail, 1992; Robinson & Wilson, 1994; Snorrason *et al*.,

1994), and there is a diversity of feeding modes in nototheniids (Daniels, 1982; Eastman, 1985, 1993; Schwarzbach, 1988). Therefore, we used food composition to quantify niche differentiation for comparison with morphometric measurements.

Quantitative data on the abundance of food items from stomach analyses were available in the literature (Hureau, 1970; Foster, Cargill & Montgomery, 1987; Schwarzbach, 1988; Kiest, 1993; Montgomery *et al*., 1993) for all species in our morphometric analysis except *Aethotaxis mitopteryx*; the only study of stomach contents in that species yielded only two identifiable items (Kunzmann & Zimmermann, 1992). For species represented by large samples in more than one study, we combined the data by adding absolute numbers of food items (computed from percentages if necessary), thereby giving greater weight to studies with large sample sizes. Data consisted of the percentages of major food categories among identified items in the diets (Table 3). Because the differences in food utilization between species are more important for this study than the actual diet composition, we standardized these data before the analyses to have zero means and unit variance for each category, thus correcting for differences in the sizes of food organisms. The size ranges of fish analysed in the diet studies were comparable to those in our morphometric data set. Using the average diet compositions and growth-corrected morphometric variables therefore prevents artifacts due to ontogenetic niche shifts; if these occur, they would tend to blur the distinctions among species rather than generate spurious ecomorphological associations, thereby making the analysis more conservative.

To study the associations between food compositions and the morphometric variables discussed above, we used the method of partial least squares (PLS; Bookstein *et al*., 1990; Streissguth *et al*., 1993), which is based on a singular value decomposition of the matrix of cross-covariances between the two sets of variables. This method, originally introduced to psychometry as "inter-battery factor analysis" by Tucker (1958), has been used in a variety of contexts, such as "dose-response" studies (Bookstein *et al*., 1990; Streissguth *et al*., 1993) and in ecology to relate measurements of environmental variables to species abundances (Chessel & Mercier, 1993).

The PLS technique finds pairs of linear combinations of the original variables, each pair consisting of a food axis and a morphometric axis, which have maximal covariances between the two sets. Each axis in one set of variables is only correlated with the corresponding axis in the other set, but uncorrelated with the remaining axes in that set. The axes of each set are orthogonal to one another, and the squared coefficients of each axis sum up to unity; therefore, this technique can be interpreted as a rigid rotation of the two coordinate systems, and is analogous to principal component analysis (PCA) to some extent (see also Streissguth *et al*., 1995: 65–66). Whereas the rotation in PCA is designed to find linear combinations successively accounting for the maximal amount of variance while being uncorrelated to all previous ones within a single set of variables, in PLS the coordinate systems of two sets of variables are rotated to find successive pairs of axes that maximize the covariance between sets, and where each axis is uncorrelated with the previous axes in the other variable set. The importance of each pair of axes can be assessed by the squared covariance between the two linear combinations, expressed as a percentage of the sum of squared covariances between food composition and morphometric variables; this is analogous to the percentage of 'explained' total variance in PCA.

Canonical correlation analysis is another technique often used for similar

TABLE 3. Food composition. Tabled values are the percentages (by number) of the major food categories in the diets. Values may not add up to 100 because some less abundant food items, such as chaetognaths or eggs, were not included in the table, and due to rounding errors

Species	Euphausiacea	Copepoda*	Amphipoda†	Mysidacea	Other crustaceans‡	Polychaeta†	Other benthic invertebrates§	Pteropoda	Fish
P. borchgrevinki	3	4	8	0	0	0	0	83	1
T. bernacchii	1	0	25	0	18	33	20	2	1
T. eulepideotus	56	15	6	1	6	2	1	11	1
T. hansoni	22	24	14	0	7	16	11	0	4
T. lepidorhinus	1	18	15	15	11	26	2	0	8
T. loennbergii	0	10	16	3	26	20	15	0	7
T. nicolai	0	0	78	0	0	0	0	7	16
T. pennellii	0	1	49	1	20	21	6	1	2
T. scotti	0	3	35	1	22	26	12	0	0

*Pelagic copepods are more abundant than benthic ones in the diets, where information is available.
†Benthic amphipods and polychaetes are more abundant than pelagic ones in the diets, where information is available.
‡Isopoda, Tanaidacea, Decapoda, Cumacea, etc.
§Gastropda, Echiurida, Priapulida, Sipunculida, Holothuroidea, etc.

problems (e.g. Miles & Ricklefs, 1984; Miles, Ricklefs & Travis, 1987; Losos, 1990). It is designed to find pairs of linear combinations that are maximally correlated between two sets of variables, regardless of the absolute amount of variation and covariation for which they account. Therefore, it can produce linear combinations that are almost perfectly correlated between variable sets, but only account for minute amounts of variation in either set. This problem is especially serious when the number of cases (species) is similar to the number of variables in the two sets, i.e. if the covariance matrices within sets are approaching singularity, as in our study. Therefore, we did not use canonical correlation analysis.

Because our analysis addresses patterns of interspecific covariation between morphological and ecological variables, we used species averages of the CPC2–CPC10 scores as the morphometric data, omitting the CPC1 or within-species 'size' axis. Using averages gives equal weight to every species irrespective of sample size. Because the morphometric variation is scaled in a biologically interpretable way (log-transformed measurements), there was no need to standardize the CPC scores. Cross-covariances between the CPCs and the standardized variables for food composition (see above) thus reflect the magnitude of associations between niche differentiation and morphological variation among species. To avoid singular matrices, we used a principal component transformation before the analysis. For the presentation of results, however, we subsequently rotated the axes back to the original coordinate system. Because our set of species is not a random sample of some underlying distribution, and because there is no possibility to assess the sampling variation of the stomach content data, we used the results of the PLS analysis as a exploratory tool to characterize the patterns of covariance, but not for statistical testing.

Phylogenetic reconstruction of ecological and morphometric change

We mapped the life styles of the species in our morphometric study onto the phylogeny, using MacClade, version 3.01 (Maddison & Maddison, 1992). Following Eastman (1993), we distinguished benthic, epibenthic, pelagic, and cryopelagic life styles among these species. Benthic and epibenthic species differ in their activity patterns, as the latter more often swim above the substrate, rather than resting on it (Ekau & Gutt, 1991), and show more spontaneous activity in aquaria (Eastman, 1993). Cryopelagic species differ from pelagic ones in their close association with the sea ice (Andriashev, 1970; Eastman & DeVries, 1985; Eastman, 1993). Although *Aethotaxis mitopteryx* has been described as benthopelagic because the majority of specimens were caught within a few metres of the sea floor (Kunzmann & Zimmermann, 1992), skeletal reduction, including partial retention of the notochord, and the extremely high lipid content suggest it has a pelagic life style (Eastman, 1993; Friedrich & Hagen, 1994).

To study the evolution of morphometric variation among species, we mapped the scores from the preceding analyses onto the phylogenetic trees, using squared-change parsimony to reconstruct the scores for internal nodes (e.g. Huey & Bennett, 1987; Maddison, 1991). In a multivariate context, squared-change parsimony finds the tree reconstruction that minimizes the Euclidean distance along its branches (rather than Manhattan distance, as in linear parsimony), due to the Pythagorean theorem. Because our phylogenetic analysis did not provide reliable estimates of branch lengths, we used the unweighted version of this method (Maddison, 1991). Unlike other studies that used this method primarily to compute evolutionary correlations

among traits (e.g. Losos, 1990; Martins & Garland, 1991), we combine it with ordination and explicitly focus on reconstruction of ancestral states as a tool to study the history of morphological diversification within a clade.

For both the analysis of growth-invariant variation among species and for the partial least squares analysis, we separately reconstructed the scores of the first and second axes for all internal nodes, including the ingroup node (the root node for the species included here, bold lines in Fig. 1). Ancestral states were reconstructed from the average scores of all species in our data set using the MacClade software, version 3.01 (Maddison & Maddison, 1992). Although *Aethotaxis mitopteryx* could not be used in the PLS analysis, we computed its average scores for the morphometric axes and included them in this step.

To assess these patterns of evolution in a statistically more rigorous way, we conducted two analyses of phylogenetically independent contrasts (Felsenstein, 1985; Martins & Garland, 1991; Garland, Harvey & Ives, 1992; McPeek, 1995). Because this study deals with multiple variables, we did not analyse contrasts for the variables separately, but instead we considered the amount of morphological change, measured as Euclidean distance in the multidimensional space spanned by these variables. For this purpose, we added all the squared values for contrasts for one pair of branches or a single branch (see below), and then took the square root to compute Euclidean distance.

The first analysis of contrasts examined if there is an overall correlation between the amount of evolutionary change in morphology and in diet. Here, we used the nine species for which data on both food and morphometric variation were available. We computed independent contrasts for CPC2–CPC10 and for all food variables, which were standardized as for the PLS analysis. For this analysis, we used the algorithm described by Felsenstein (1985), and set all initial branch lengths to unity, as no reliable estimates of branch lengths (in units of expected variance of evolutionary change) were available. This is different from using unstandardized contrasts (Martins & Garland, 1991), as variance from estimating values for internal nodes is considered in contrasts deeper in the phylogeny: standardizing the contrasts discounts for the uncertainty in ancestral trait values. Then, standardized contrasts for both variables were transformed to Euclidean distance, yielding a vector of distances for each set of variables. Finally, we computed correlations between corresponding elements for these vectors; note that ordinary product-moment correlations are appropriate here, rather than the correlation coefficient for regression through the origin (Martins & Garland, 1991), because the Euclidean distances are bound to be positive. We tested these correlations with a permutation test, using 10 000 random permutations (Pitman, 1937; Good, 1994). As the expectation was a positive association between the amounts of change in morphology and diet, we used a one-tailed test.

The second analysis focused on a more specific aspect: was there more morphological change on those branches of the phylogenetic tree where a habitat shift took place than on other branches? For this analysis, we used the technique proposed by McPeek (1995) to estimate contrasts on single branches, and all 10 species for which we had morphometric data. Among these species, the pelagic and cryopelagic life styles originate either in the terminal branches leading to *Aethotaxis mitopteryx* and *Pagothenia borchgrevinki*, respectively, or in their common ancestor (see Results). Despite this uncertainty, the contrast between these two species does contain at least one habitat transition. Conversely, it is unclear if there is a habitat

change on the branch linking the common ancestor of *Aethotaxis* and *Pagothenia* to the rest of the tree; therefore we excluded this branch from the analysis. In one tree reconstruction (tree C), the other unambiguous habitat shift (in the common ancestor of *Trematomus eulepidotus*, *T. lepidorhinus* and *T. loennbergii*) is on a branch originating from the root node for these species; therefore, the contrast for this branch cannot be estimated so that it is statistically independent of the others (McPeek, 1995), and instead we used the contrast of the pair of branches originating at the root node. This contrast contains one branch with no habitat shift, and is therefore likely to make the analysis conservative. For all three ingroup tree reconstructions, these analyses resulted in two contrasts with and six contrasts without habitat shifts. Standardized contrasts from single branches can be compared directly to those from pairs of branches, because they estimate rates of change (under a Brownian motion model, see McPeek, 1995). We compared the mean Euclidean distances, computed from the standardized contrasts as above, between these groups with a bootstrap test (Efron & Tibshirani, 1993; 10 000 bootstrap iterations per test).

RESULTS

Phylogeny

A preliminary analysis of the entire data set (excluding *Trematomus vicarius*) produced 386 most parsimonious trees with a length of 139 steps (consistency index 0.39, retention index 0.61). In all of these trees, the subfamily Pleuragramminae was nested within the Trematominae (as a clade together with the genus *Pagothenia*). This supports the hypothesis of ingroup monophyly together with the phylogenies derived from molecular data (Bargelloni *et al.*, 1994), unfortunately omitting or uninformative for the Pleuragramminae, which strongly suggest that the Trematominae form a clade distinct from the other Nototheniidae (Eleginopinae and Nototheniinae).

An analysis constraining the ingroup to be monophyletic, but without further assumptions about outgroup structure, yielded 14 most parsimonious trees with a length of 140 steps (consistency index 0.39, retention index 0.61). Eight of these trees were consistent with the two most parsimonious unrooted trees for the ingroup alone (tree length 90 steps, consistency index 0.53, retention index 0.67), and only these will be considered further.

The eight preferred trees share a common pattern, and the differences among them result from variations in only three features. (1) The trees for the ingroup can be rooted in three different ways, designed A (Fig. 2a, b), B (Fig. 2c), and C (Fig. 2d), which result in different hypotheses of relationships among *Trematomus* species. (2) For each of these arrangements, there are two equally parsimonious placements for *Gvozdarus*, either as the sister taxon of the clade containing *Pleuragramma* and *Cryothenia* (Fig. 2a, c, d) in trees A1, A3, B1, C1, or as the sister taxon of the genus *Pagothenia* (Fig. 2b) in trees A2, A4, B2, C2. (3) Finally, there are two alternative hypotheses of relationships among outgroups: one, with *Gobionotothen* as the sister taxon for the ingroup (Fig. 1a, c, d), occurs with all three rooting arrangements (trees A1, A2, B1, B2, C1, C2), whereas the other is only valid with arrangement A (trees A3, A4), and has the clade (*Gobionotothen*, (*Notothenia*, *Paranotothenia*)) as the sister taxon of the ingroup (Fig. 2b). Altogether, these possibilities result in only three different

topologies for the species included in the morphometric study (bold lines in Fig. 2), corresponding to arrangements A, B, and C.

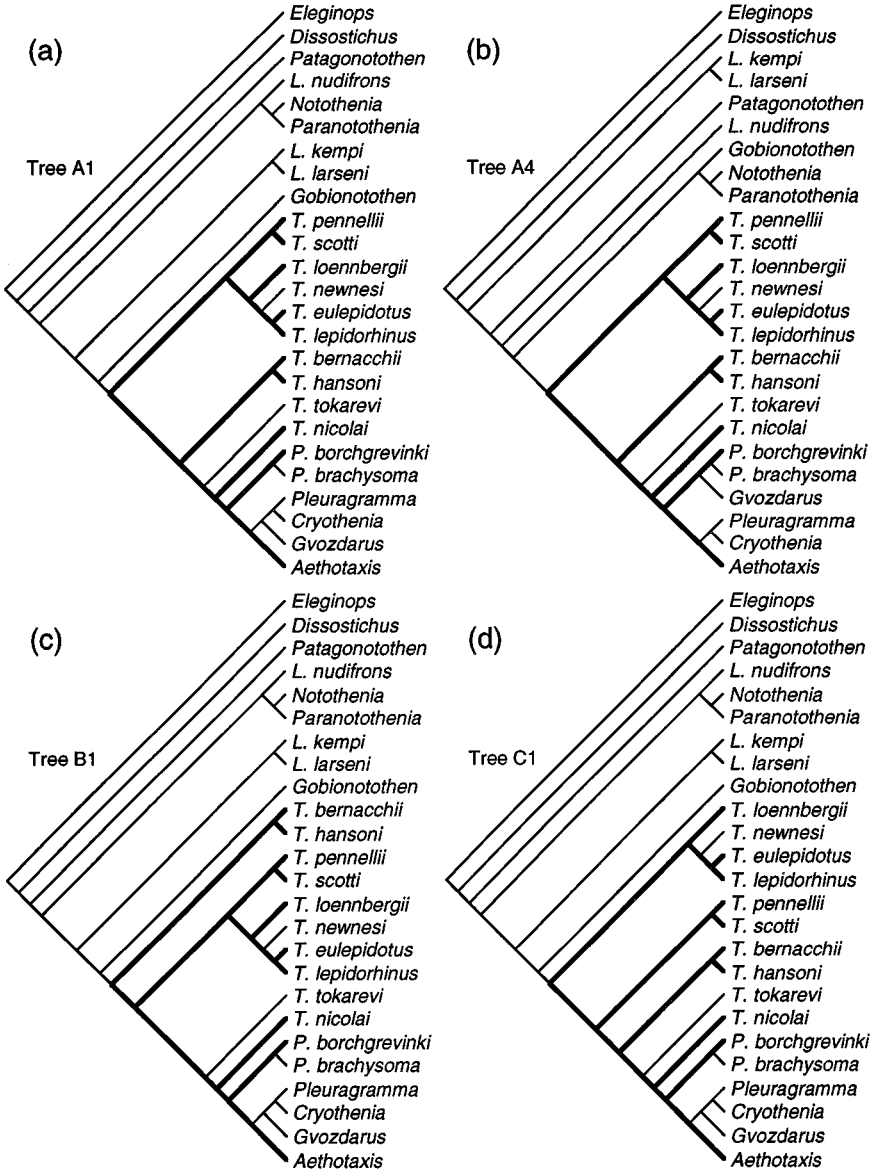

Figure 2. Phylogenetic relationships within the Nototheniidae. Only four of the eight trees are shown, because the trees result from combining alternatives for ingroup rooting (a, c, d), the placement of *Gvozdarus* (a, b), and outgroup relationships (a, b; see text for details). The ingroup included all species of the Trematominae (except for *Trematomus vicarius*) and Pleuragramminae, and nine outgroup taxa were chosen to represent the remaining subgroups within the Nototheniidae (see Material and methods for complete names). The ingroup was constrained to be monophyletic. Bold lines indicate the relationships among the species included in the morphometric study.

Multivariate allometry

The first principal component (PC1) accounted for 95–98% of the total variance in each species (Table 4), indicating that the multivariate model of simple allometry fits well to the growth of the nine species examined (*Pagothenia borchgrevinki* had to be excluded due to insufficient sample size). Therefore, growth trajectories of all species can be approximated by straight lines in the space of log-transformed measurements. The estimates of PC1 coefficients are fairly stable, as indicated by the small standard errors. Many of the coefficients are relatively close to the value 0.316 (= $1/\sqrt{10}$) for isometry. There are, however, some consistent deviations from isometry. Orbital length (OL) invariably shows negative allometry, whereas head width (HW) and for most species body depth (BDA) show positive allometry.

The allometric patterns are fairly similar among species (Table 4). The angles between the growth trajectories (PC1 axes) of different species vary from 2.5° (between *Trematomus eulepidotus* and *T. scottii*) to 8.7° (between *Aethotaxis mitopteryx* and *T. bernacchii*). The bootstrap test of the maximal angle between the PC1 axes of all species did not reveal a statistically significant difference from a zero angle ($P = 0.14$). Therefore, a simplified model can be used, in which all species share a common allometric pattern. We estimated such a shared allometric pattern as the first common principal component (CPC1) of the log-transformed data. The CPC1 coefficients (Table 5) are a 'compromise' between the PC1s of the individual species. The common allometric pattern accounts for 95.3% (*T. nicolai*) to 98.3% (*T. lepidorhinus*) of the total variance; this is only a slight decrease from the amount of variation taken up by the PC1s of each species.

We used the CPC1 as the common allometric pattern for separating variation in 'size' from lateral transposition of growth trajectories (Fig. 3). *T. scotti* has the smallest average CPC1 score, for the most part, because it has a substantially smaller asymptotic size than the other species (Ekau, 1988). Of the other species, complete ontogenetic series including juveniles as small as those of *T. scotti* were only available for *T. eulepidotus* and *T. lepidorhinus*. For interpreting the interspecific variation in CPC1 scores, it is important to keep in mind that these fish have indeterminate growth, and that the size composition of the specimens caught may be influenced by the fishing gear. Average CPC1 scores thus reflect both differences among species and sample composition, emphasizing the need for size correction in interspecific comparisons.

The first two growth-invariant axes take up 44% and 27% of the morphometric variation among species in directions orthogonal to that of the CPC1 axis. These two growth-invariant axes produce an ordination of the species that is consistent with previous classifications into ecological types (Fig. 4; Ekau, 1988, 1991; Eastman, 1993). The first axis displays a gradient from pelagic and epibenthic (low scores) to benthic species (high scores). Both axes together clearly separate *Aethotaxis*, which has a pelagic life style, from the remaining species. The epibenthic *T. eulepidotus*, *T. lepidorhinus*, and *T. loennbergii* form a cluster together with the cryopelagic *P. borchgrevinki*, all with relatively low scores on the first axis. Among the species characterized as benthic, *T. bernacchii*, *T. hansoni*, *T. nicolai*, and *T. pennellii* have high scores for both axes, whereas *T. scotti* is distinct from them as it has a lower score for the second axis and a slightly lower score for the first axis. These patterns of morphological differences among species can be related to the original variables (Fig. 5). The first axis mainly is a contrast between head width (HW) and the lengths of

TABLE 4. Multivariate patterns of ontogenetic allometry. Tabled values are the PC1 coefficients for each species, and their bootstrap standard errors (in parentheses). Positive and negative allometry are indicated by '+' and '–' respectively, for those coefficients whose bootstrapped 95% confidence intervals did not include 0.316, the value for isometry. '% Variance' is the percentage of the total variance taken up by the PC1. *Pagothenia borchgrevinki* could not be included, because sample size was too small

Variable	*Aethotaxis*	*T. bernacchii*	*T. eulepidotus*	*T. hansoni*	*T. lepidorhinus*	*T. loennbergii*	*T. nicolai*	*T. pennellii*	*T. scotti*
SL	0.304–	0.291–	0.298–	0.299–	0.307–	0.297–	0.291–	0.303–	0.300–
	(0.005)	(0.018)	(0.002)	(0.006)	(0.002)	(0.003)	(0.008)	(0.006)	(0.002)
HL	0.343+	0.292	0.291–	0.309	0.271–	0.286–	0.281	0.288–	0.300–
	(0.006)	(0.021)	(0.003)	(0.008)	(0.002)	(0.003)	(0.013)	(0.005)	(0.002)
AFL	0.283–	0.231–	0.289–	0.304–	0.337+	0.291–	0.288	0.288–	0.288–
	(0.011)	(0.051)	(0.006)	(0.008)	(0.003)	(0.004)	(0.033)	(0.006)	(0.004)
DFL	0.288–	0.286–	0.303–	0.305	0.309–	0.303–	0.315	0.299–	0.307–
	(0.012)	(0.027)	(0.003)	(0.011)	(0.002)	(0.004)	(0.019)	(0.005)	(0.004)
BDA	0.316	0.416+	0.372+	0.327	0.382+	0.395+	0.401+	0.351+	0.340+
	(0.016)	(0.052)	(0.004)	(0.022)	(0.003)	(0.006)	(0.026)	(0.005)	(0.005)
CPD	0.320	0.300	0.306	0.298	0.305–	0.344+	0.286	0.321	0.320
	(0.009)	(0.038)	(0.008)	(0.020)	(0.004)	(0.006)	(0.019)	(0.007)	(0.006)
HW	0.341+	0.391	0.380+	0.393+	0.335+	0.379+	0.389	0.416+	0.366+
	(0.006)	(0.051)	(0.017)	(0.021)	(0.005)	(0.006)	(0.043)	(0.012)	(0.007)
OL	0.302	0.234	0.281–	0.273–	0.249–	0.235–	0.240	0.254–	0.283–
	(0.024)	(0.032)	(0.006)	(0.019)	(0.005)	(0.006)	(0.031)	(0.012)	(0.007)
ML	0.349+	0.352	0.135	0.360+	0.315	0.338+	0.353+	0.318	0.334+
	(0.011)	(0.032)	(0.004)	(0.012)	(0.003)	(0.005)	(0.017)	(0.007)	(0.006)
PFL	0.312	0.317	0.310	0.275–	0.333+	0.258–	0.280	0.296	0.314
	(0.012)	(0.037)	(0.010)	(0.019)	(0.004)	(0.006)	(0.016)	(0.012)	(0.006)
% Variance	97.5	95.3	97.6	98.0	98.7	98.0	95.7	97.3	97.7
	(0.8)	(6.8)	(0.6)	(2.9)	(0.1)	(0.3)	(4.3)	(0.5)	(0.2)

TABLE 5. Coefficients of the first common principal component (CPC1) and their bootstrap standard errors

Variable	CPC1 coefficient	Standard error
SL	0.302	0.001
HL	0.284	0.002
AFL	0.307	0.003
DFL	0.306	0.002
BDA	0.374	0.003
CPD	0.313	0.003
HW	0.371	0.006
OL	0.259	0.004
ML	0.322	0.003
PFL	0.308	0.004

the anal and pectoral fins (AFL and PFL), whereas the second axis contrasts the size of the mouth (ML) with body depth (BDA and to a lesser extent CPD).

Associations between morphology and diet composition

The first two pairs of axes produced by the partial least squares analysis account for 58% and 24% of the sum of squared cross-covariances, and therefore give a fairly complete summary of the associations between morphometric variables and diet composition. The correlations between the morphometric and food axes are 0.77 for

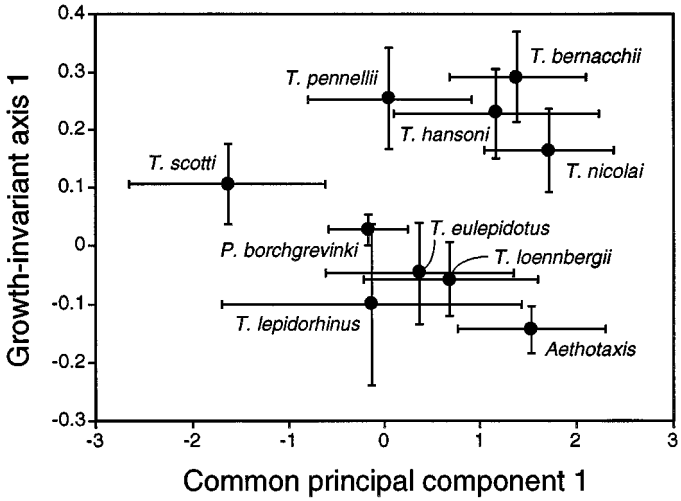

Figure 3. Morphometric variation within and between species. The CPC1 can be interpreted as within-species 'size' axis reflecting growth allometry, and accounts for the largest proportion of variation within species. The growth-invariant axis 1 is the direction of maximal variation among species orthogonal to the CPC1; it thus displays differences between species corrected for the effects of 'size' (Burnaby's technique). Dots represent species averages, and the bars indicate the standard deviations of individual scores. Note the differences in scaling of the axes.

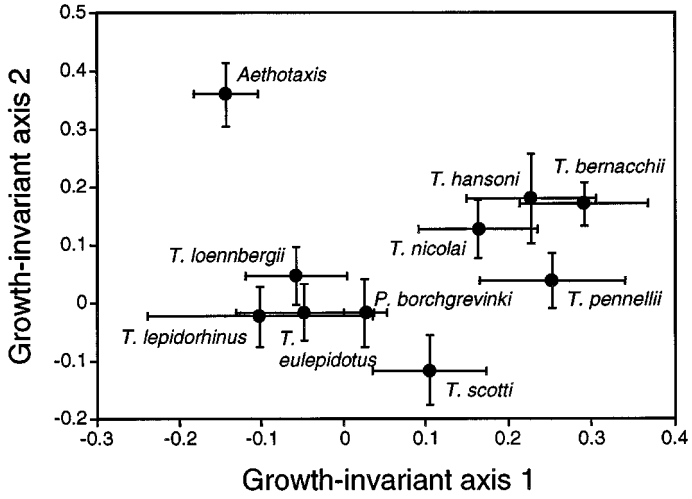

Figure 4. Morphometric variation among species. The first two growth-invariant axes summarize 71% of the variation among species in directions orthogonal to the common direction of growth trajectories, as estimated by the CPC1. Differences between species can therefore be interpreted as lateral transpositions of growth trajectories. Dots represent species averages, and the bars indicate the standard deviations of individual scores.

the first pair and 0.78 for the second pair (note that these correlations are *not* the quantities maximized by the procedure).

The first food axis is clearly dominated by a contrast of pteropods, copepods, mysids and euphausiaceans, which mainly live in the water column, against the predominantly benthic amphipods, polychaetes, and other invertebrates (Fig. 6a).

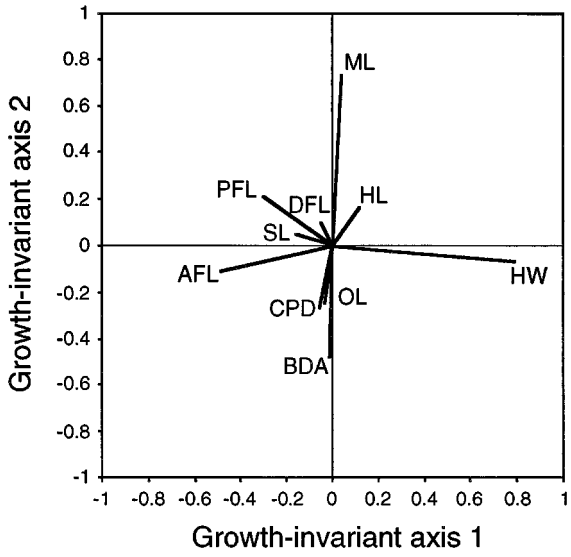

Figure 5. Relative importance of morphometric variables for size-adjusted variation among species. The lines indicate the coefficients of the two growth-invariant axes for the original coordinate system, as in a biplot (e.g. Marcus, 1993), and can be interpreted as the projections of a unit vector for each variable onto the plant of the two growth-invariant axes.

The corresponding morphometric axis opposes measurements of the head (HW, ML, and OL) to body depth (CPD and BDA) and pectoral fin length (Fig. 6b). This means that pelagic feeders tend to have longer pectoral fins and deeper bodies, but smaller heads than benthic feeders. The coefficients for the second pair of axes are more difficult to interpret. The second food axis is mainly a contrast between pteropods and most other food classes, especially mysids. The corresponding morphometric axis sets head width and caudal peduncle depth against anal fin length and orbital length.

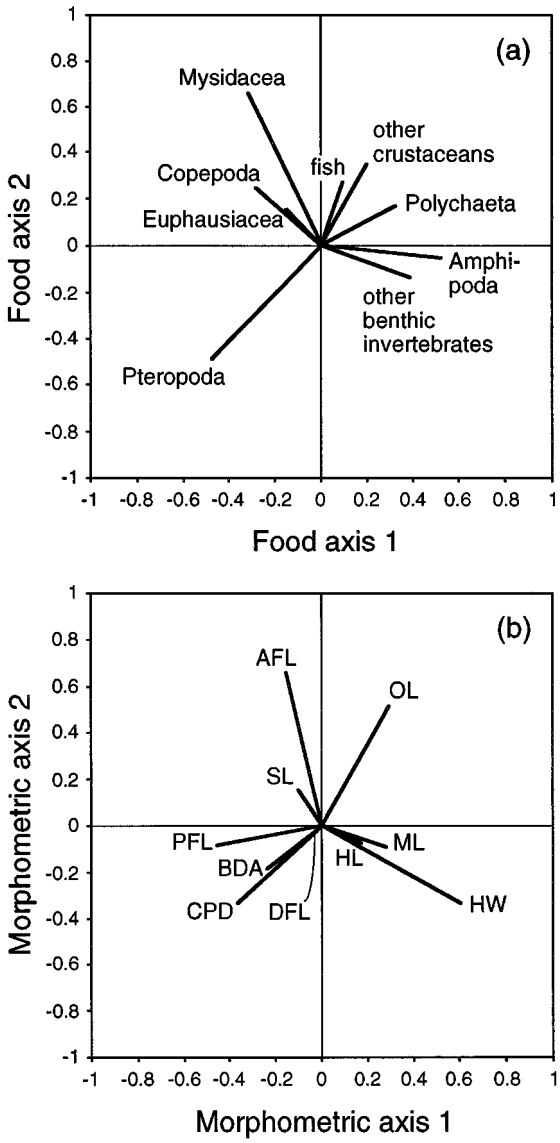

Figure 6. Plot of coefficients for the first two pairs of axes from the partial least squares analysis. Each food axis (a) has maximal covariance with the corresponding morphometric axis (b). The arc in (b) indicates the tip of the line for the variable DFL, as it is hidden by the lines for BDA and CPD.

The first two pairs of axes from the PLC analysis define two 'parallel' ordinations of the food and morphometric data (Fig. 7), which are concordant in their general features, but not in all details. The cryopelagic *P. borchgrevinki* is separated in the lower left part of the graphs from the other species, which live on or near the bottom; its diet with a large proportion of pteropods is unique among these species (Table 3). Among the other species there appears to be a gradient, more clearly for the morphometric than the diet variables, from the more pelagic species in the upper left corner to the benthic ones at the lower right. The three epibenthic species *T. lepidorhinus*, *T. loennbergii*, and *T. eulepidotus* form a fairly tight cluster in the space of morphological variables, but their diets differ considerably: *T. eulepidotus* has the most pelagic food composition (with considerable fractions of euphausiaceans, copepods and pteropods), whereas that of *T. loennbergii* is similar to those of the benthic species (with substantial proportions of benthic 'other crustaceans', polychaetes, amphipods, and invertebrates). Among the species classified as benthic, *T. scotti* is relatively similar to the epibenthic species in its morphology, but its diet resembles closely to that of *T. bernacchii*, *T. pennelli*, and *T. nicolai*. Conversely, *T. hansoni*, which resembles the latter three species in its morphology, has an intermediate position between them and the more pelagic species in the space of food variables.

Scores for the morphometric axes vary considerably within species, especially for *T. lepidorhinus* and *T. eulepidotus*. Some of this variation is associated with age, but the patterns of ontogenetic change differ among species (Fig. 8). *T. eulepidotus* shows a consistent increase in the scores for the first morphometric axis after age class 2 (Fig. 8a), whereas the scores for *T. lepidorhinus* do not change noticeably after a dramatic

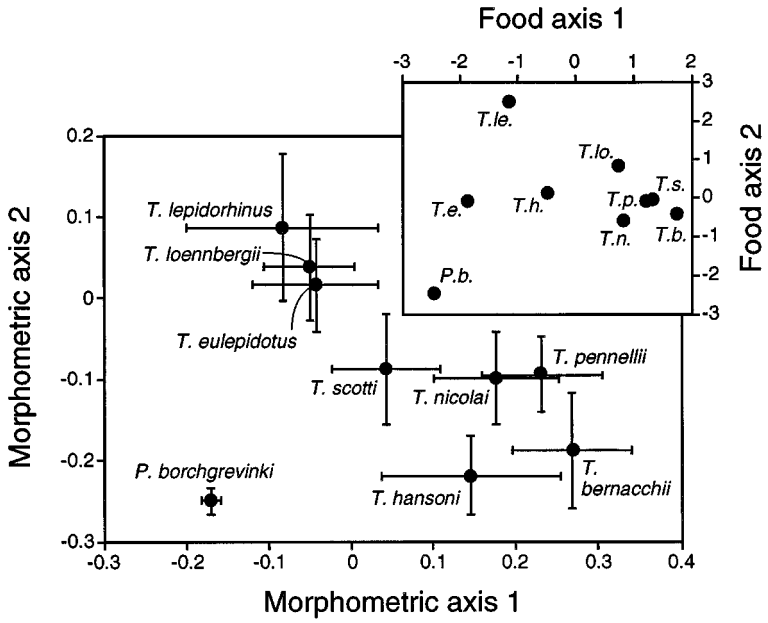

Figure 7. Results of the partial least squares analysis of covariances between the diet and morphometric variation. The first two pairs of axes account for 82% of the squared cross-covariances between diet and morphometric variation. The main graph is a plot of the first two morphometric axes; dots represent average scores of each species and bars the standard deviations of individual scores. The inset shows the first two food axes; because the data were average diet compositions, no measures of variability within species are available.

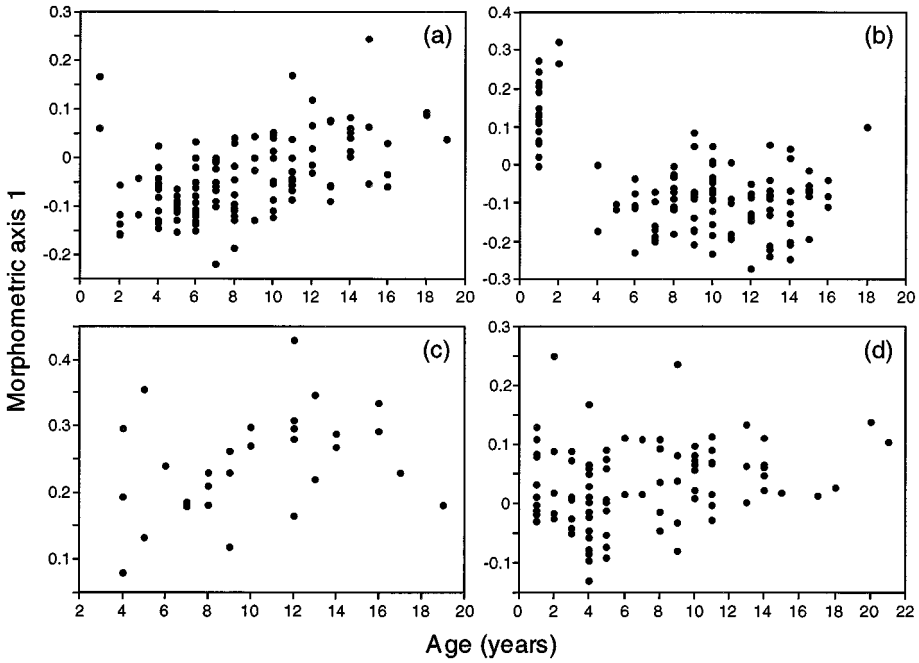

Figure 8. Ontogenetic changes in the scores for the first morphometric axis from the partial least squares analysis. (a) *Trematomus eulepidotus*, (b) *T. lepidorhinus*, (c) *T. pennellii*, (d) *T. scotti*.

drop between age classes 2 and 4 (Fig. 8b). A similar ontogenetic change may also explain the high scores for the two specimens of age class 1 in *T. eulepidotus*. Other species show no such clear-cut patterns (e.g. Fig. 8c, d).

Phylogenetic reconstruction of habitat shifts and morphometric change

Phylogenetic reconstructions of habitat shifts, according to the classes distinguished by Eastman (1993), give similar results for the three alternative phylogenetic trees of the species considered here (Fig. 9). The benthic habitat is ancestral; this life style is assigned to the root node whether *Gobionotothen* (all three trees) or the clade of *Gobionotothen, Notothenia*, and *Paranotothenia* (tree A only) are considered as sister group of the ingroup (see Fig. 2). The epibenthic life style has a single origin in the common ancestor of *T. eulepidotus, T. lepidorhinus*, and *T. loennbergii* in all three phylogenetic trees. Reconstructions for the pelagic and cryopelagic life styles are more ambiguous, and depend critically on the species not included in our morphometric study. If *Gvozdarus* is considered as the sister taxon of *Pagothenia* (Fig. 2b), the most parsimonious scenario is that the common ancestor of these species and the remaining Pleuragramminae was pelagic, and that *Pagothenia* switched from a pelagic to the cryopelagic habitat. Conversely, if *Pagothenia* is taken as the sister group of the Pleuragramminae (Fig. 2a, c, d), there are three equally parsimonious reconstructions, with a hypothetical ancestor that could either be benthic, pelagic or cryopelagic.

The reconstructions of the phylogenetic trajectories of the scores for growth-

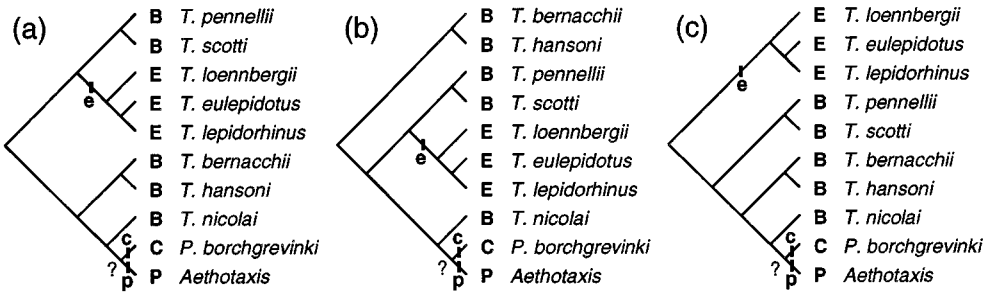

Figure 9. Phylogenetic reconstruction of habitat shifts in the study group. (a) Tree A, (b) tree B, (c) tree C. Abbreviations for life styles are B for benthic, E for epibenthic, C for cryopelagic, and P for pelagic. The reconstructed life style for the hypothetical ancestor of all these species (ingroup node) is benthic. The question marks indicate ambiguous reconstructions for the origins of cryopelagic and pelagic life styles (see text for details); the possibility shown here is that of independent origins from a benthic ancestor of these species.

corrected variation among species reveal a considerable amount of divergent evolution (Fig. 10). Only the pair of sister species *T. bernacchii* and *T. hansoni* and the clade consisting of *T. eulepidotus*, *T. lepidorhinus*, and *T. loennbergii* appear as compact groups, i.e. with short connecting branches. For the remaining species, however, morphometric similarity does not match phylogenetic relationships. This also leads to the absence of consistent evolutionary trends under any of the scenarios suggested by the three phylogenetic trees. Whereas diversification occurred extensively in both main lineages of tree A (Fig. 10a), the other two scenarios imply it was concentrated mainly in only one lineage (Fig. 10b, c).

The morphometric axes from the partial least squares analysis shows a different evolutionary pattern, as several branches are directed from the upper left to the lower right of the plots or vice versa (Fig. 11). Depending on which of the alternative trees is chosen, one can infer that evolutionary trajectories went in both (Fig. 11a, c) or mainly in one direction (Fig. 11b). The only strong deviation from this pattern occurred in the lineage leading to *P. borchgrevinki*; weaker ones can be seen for *T. nicolai* and for the pairs of sister species *T. pennellii–T. scotti* and *T. hansoni–T. bernacchii*.

To examine the phylogenetic patterns of morphological evolution statistically, we performed a test of the correlation between phylogenetically independent contrasts of the morphometric and diet data. As we restricted this test to the overall amounts of evolution in the two data sets, we used the standardized Euclidean distance between the tips of each pair of branches (for details, see Material and methods). The correlations for the eight contrasts were positive and weak to moderate for all three trees, but did not differ significantly from zero in a permutation test (tree A, $r = 0.32$, $P = 0.24$; tree B, $r = 0.23$, $P = 0.30$; tree C, $r = 0.32$, $P = 0.24$).

Whereas the preceding test estimated a single correlation for all branches of a phylogenetic tree simultaneously, our second test examined the hypothesis that more morphological change is associated with those branches on which a habitat shift occurred. The average standardized contrast with a habitat change was more than twice the average of contrasts without habitat shifts for all three trees, and this difference was statistically significant even for tree C, where we had to use a slightly more conservative procedure (tree A, average with/without habitat shift

0.279/0.126, $P < 0.001$; tree B, 0.278/0.127, $P < 0.001$; tree C, 0.269/0.132, $P = 0.04$).

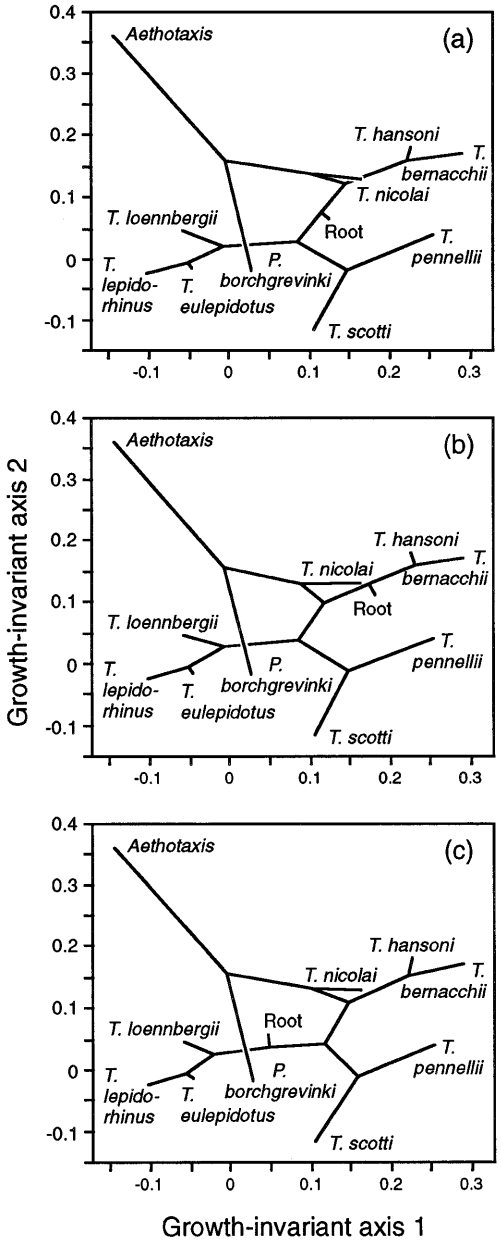

Figure 10. Phylogenetic trajectories in the space of growth-invariant morphometric variation among species. The diagrams show a scatterplot (as in Fig. 2) of the species averages and scores of internal nodes reconstructed by squared-change parsimony. These points are linked according to the topology of the respective cladogram, so that the lines trace the hypothetical evolutionary pathways through the space defined by the morphometric variation among species; in other words, the cladogram has been 'bent' and 'stretched' or 'shrunk' to fit the scatter plot. The branch labelled 'Root' indicates the common ancestor of the species considered here, the ingroup node (the length and direction of that branch are arbitrary). The three scenarios correspond to tree A (a), tree B (b), and tree C (c).

DISCUSSION

Ecomorphology is based on the premise that morphological traits reflect the ecological characteristics of an organism because of its history of adaptive evolution.

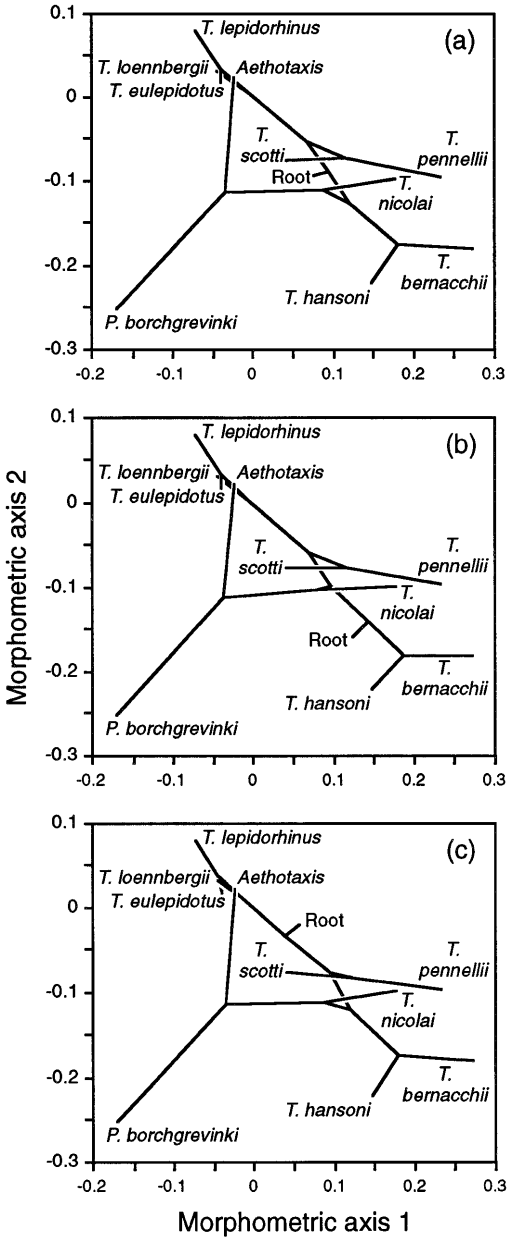

Figure 11. Phylogenetic trajectories of morphometric variation associated with diet composition. Branch tips indicate the average scores for the first two morphometric axes of the partial least squares analysis, and internal nodes have been reconstructed from these with squared-change parsimony. The three scenarios correspond to tree A (a), tree B (b), and tree C (c); see the legend of Fig. 9 for further explanations.

Yet morphological and ecological characteristics are not only the products of the phylogeny of a taxon, but in turn they can influence its evolutionary fate by affecting probabilities of extinction and speciation. Morphometric analyses of these interactions are further complicated by the effects of allometric growth. The nototheniids provide a remarkable example of an adaptive radiation and therefore are especially interesting for such studies. Using all the information available to us, we have carried out an analysis of phylogeny, examined morphometric variation and its basis in allometric growth, and studied the covariation between morphology and food composition. Here, we attempt to integrate the results to obtain a unified view of the evolutionary history of our study group and its ecological diversification.

Phylogeny

Although they are mainly based on the characters used in previous systematic studies, our results differ considerably from published phylogenetic hypotheses (Andersen, 1984; Balushkin, 1989; Eastman, 1993; Voskoboynikova, 1993) or classifications (Balushkin, 1989, 1992; DeWitt *et al*., 1990; Eastman, 1993). Most subfamilies in these classifications turned out to be paraphyletic: the subfamily Pleuragramminae (which may or may not be monophyletic, depending on the placement of *Gvozdarus*) is nested within the Trematominae, which itself is contained entirely within the Nototheniinae (but this latter point is not strongly supported). Moreover, in all our phylogenetic trees, the genus *Trematomus* is also paraphyletic.

These results are hardly surprising, considering that no synapomorphies were found for the genus *Trematomus* in an earlier study mostly based on the same characters (Andersen, 1984), and some published synapomorphies for other taxa seem to be questionable (discussed by DeWitt *et al*., 1990). The analyses support the classification of *Trematomus* species by DeWitt *et al*. (1990): *T. bernacchii*, *T. hansoni*, and *T. tokarevi* are not closely related to *Pagothenia* and therefore should not be placed in that genus (*contra* Andersen, 1984), and the separation of *T. newnesi* from the remaining species by recognizing the genus *Pseudotrematomus* (e.g. Balushkin, 1989, 1992; Miller, 1993) seems unjustified.

Molecular data are mostly consistent with this picture. In the study of mitochondrial DNA sequences by Bargelloni *et al*. (1994), the family Nototheniidae was found to be paraphyletic, with the subfamily Trematominae as the sister group of other Nototheniidae as well as the Artedidraconidae, Bathydraconidae and Channichthyidae. Furthermore, a molecular phylogeny of *Trematomus* showed the genus to be paraphyletic, with *Pagothenia borchgrevinki* nested within it (Ritchie *et al*., 1995). The same study supported a clade with *T. bernacchii* and *T. hansoni* and another one consisting of *T. eulepidotus, T. lepidorhinus* and *T. loennbergii*. Beside these similarities to our study, however, there are also a number of differences (e.g. the placement of *T. newnesi, T. pennellii* and *T. scotti*). Nevertheless, even molecular data did not resolve the relationships among *Trematomus* species unambiguously, as the topologies of the phylogenetic trees differ between the three methods used (parsimony, maximum likelihood, neighbour joining). These studies and ours are partly complementary; for instance, Bargelloni *et al*. (1994) show that the Trematominae are a clade clearly separate from the Nototheniinae, whereas our data do not resolve this point well. Conversely, our data suggest a close relationship between the Pleuragramminae and

Trematominae, whereas no members of the Pleuragramminae have been included in studies of DNA sequences.

We emphasize, however, that analyses based on a broader set of characters will be necessary for a thorough study of congruence between morphological and molecular phylogenies and for a revision of the phylogeny and classification of the Nototheniidae. Here, we use these phylogenetic hypotheses as the best evidence currently available for comparative analyses.

Some of the characters we used may be subject to natural selection associated with the transition to pelagic life styles; for example, numerous osteological characters may have been affected by skeletal reduction to attain neutral buoyancy (Eastman, 1993). The convergent evolution toward a more pelagic life style in several lineages thus might have contributed to the substantial amount of homoplasy in the data set (Wake, 1991). This may result in an erroneous reconstruction of the phylogeny. Because many morphological characters are potentially related to the ecological niches of the species, we cannot rule out this possibility definitely without additional information from independent characters (e.g. Brooks & McLennan, 1991). The parsimony procedures used to estimate phylogenetic trees tend to underestimate parallelisms and convergences (Maddison & Maddison, 1992; Miles & Dunham, 1993), and such errors should thus tend to obscure real ecomorphological associations, rather than to produce spurious ones. Therefore, our analysis should be considered conservative.

Ontogenetic basis of morphological variation

Because these fishes have indeterminate growth, accounting for allometric variation is indispensable for interspecific comparisons (Thorpe, 1983; Klingenberg, 1996). Moreover, examining the ontogenetic sources of morphometric variation can shed light on evolutionary processes, for instance, by pinpointing the age at which interspecific differences first appear.

Within each species, the model of simple multivariate allometry fits the data well and the bootstrap test for the directions of the growth trajectories did not reject the hypothesis of a common pattern of ontogenetic allometry. Therefore, our morphometric data indicate that differences among species evolved mostly through lateral transposition of growth trajectories, which are visible as clear differences among species in growth-corrected morphometric variables (Figs 3, 4, 7). In the more familiar language of bivariate allometry, this means that there are differences in the *y*-intercepts, whereas the allometric slopes are the same for all species (see also Klingenberg & Spence, 1993; Klingenberg, 1996).

Therefore, interspecific differences mainly develop by divergent growth in embryonic and larval stages not included in our samples, before individuals of all species switch to the common mode of allometric growth in juveniles and adults. Similarly, the differences in head and jaw morphology between four trophic morphs of arctic charr originate within a few months of the onset of feeding (Skúlason, Noakes & Snorrason, 1989). There are no morphometric studies for larvae of nototheniids, but results from other fishes indicate ample variability of allometric patterns even within families (Strauss & Fuiman, 1985; Klingenberg & Froese, 1991). Yet there is some direct evidence for the transition between the two growth modes

in *Trematomus lepidorhinus*, where morphometric scores change sharply from one level in the youngest two year classes to a different one in older fish (Fig. 8b).

Growth after metamorphosis seems to account only for a minor part of the variation among species. The only clear case is *T. scotti*, which attains a much smaller final size than the other species (Ekau, 1988), although it reaches a similar age (Fig. 8d); growth curves given by Ekau (1988) suggest that this species has particularly low growth rates, resulting in the lowest growth performances of all species reviewed by Kock (1992: fig. 41). If these data are interpreted in terms of heterochrony, *T. scotti* can be considered to be neotenous for size.

Additional support for heterochrony as a factor in the evolution of this group comes from the observation that some of the osteological variation among adults of nototheniid species corresponds to ontogenetic variation within a species (for example the fusion of hypuralia; Andersen, 1984; Balushkin, 1989; Voskoboynikova & Tereshchuk, 1991). Voskoboynikova (1982) and Balushkin (1989) mentioned several examples of paedomorphosis related to skeletal reduction in notothenioid fishes, but the most spectacular example is the reduction of vertebrae and retention of the larval notochord in adult *Pleuragramma antarcticum* (Eastman, 1993).

Covariation of ecological and morphological traits

Morphometric analyses of growth-adjusted variation among species produced results generally consistent with previous classifications of ecological types. Gradients between species living on the bottom and in the water column were apparent in all our exploratory analyses, irrespective of whether they considered only morphometric variation (Figs 3, 4, 10) or also included ecological variables (Figs 7, 10), and formal statistical tests demonstrated that phylogenetic contrasts involving a habitat shift are associated with larger amounts of morphometric change than other contrasts. An earlier morphometric study using another technique and different variables found similar patterns, but did not take allometric growth into account formally (Ekau, 1991). This correspondence of the results from several analyses suggests that ecological and morphological variation are related in these fishes.

While ordinations consistently distinguish benthic from more pelagic species in different analyses, the coefficients of the morphometric variables emphasize the relative importance of structures involved in swimming. In the analyses of between-species variation (Figs 4, 5) and of covariation between morphology and food composition (Figs 6b, 7) the main pattern is a contrast between benthic, wide-headed fish with short pectoral fins and posterior bodies (as indicated by the lengths of anal fins) and more elongated, slimmer ones living in the water column. The pectoral fins are especially important for species feeding in the water column, as most nototheniids use the labriform swimming mode for sustained locomotion (Montgomery & Macdonald, 1984; Archer & Johnston, 1989; Kunzmann & Zimmermann, 1992; Eastman, 1993). An elongated posterior part of the body might assist acceleration for quick dashes in the subcarangiform swimming mode (Montgomery & Macdonald, 1984; Webb, 1984). The larger head width and mouth length in benthic species may reflect that benthic prey items tend to be larger than planktonic ones (Foster & Montgomery, 1993; see also Keast & Webb, 1966). Nevertheless, not all coefficients of morphometric variables have such straightforward functional interpretations, and

some caution is needed because it was not possible to test these patterns statistically.

The evidence for associations between morphology and food composition is purely correlative, although they can be interpreted functionally and by comparison with information from other sources. The general patterns are consistent with observations made in aquaria (Hubold, 1992; Eastman, 1993) and in the natural habitats (Moreno, 1980; Ekau & Gutt, 1991), but other factors also should be taken into account. For example, Hubold (1992) noted that the species composition of fish caught by trawling differs from catches in baited traps, indicating that species vary in their activity patterns, and therefore in their susceptibility to be caught in different kinds of fishing gear. Choice of habitat (Ekau & Gutt, 1991) and other components of foraging behaviour may account for the differences in the diets among the morphometrically similar *T. eulepidotus*, *T. lepidorhinus*, and *T. loennbergii*. The importance of behaviour is further underscored by field observations of two benthic species, *Trematomus bernacchii* and *T. scotti*, resting on upright sponges, which may allow them to feed on plankton without actually leaving the substrate (Moreno, 1980, Ekau & Gutt, 1991). Such behavioural specializations may be important for niche differentiation without being reflected in morphological traits.

The analysis of phylogenetically independent contrasts revealed weak to moderate positive overall correlations between the magnitude of morphological and diet changes. Yet these correlations were not significantly different from zero, reflecting the low power of the test resulting from the small number of species in this data set and the variation among the branches of the phylogenetic trees. A substantially larger number of species will be necessary to provide a firm assessment of covariation between diet and morphology. Conversely, our second test of phylogenetically independent contrasts showed that much more morphological change occurred in conjunction with habitat shifts than on the other branches of the cladograms.

Because our exploratory analyses suggest functional interpretations for the major patterns of variation in morphometric traits, it seems likely that much of the interspecific variation is due to adaptations for swimming and foraging. Although it is not possible to test the hypothesis of adaptation rigorously for our example, there is some support from similar evolutionary trends in other groups of fishes (see also Robinson & Wilson, 1994). In sticklebacks and Arctic charr, some differences between benthic and limnetic (open-water) forms are similar to the morphological variation we found in nototheniids (Baumgartner *et al.*, 1988; Schluter, 1993; Snorrason *et al.*, 1994). The species flock of sculpins in Lake Baikal provides a spectacular example of ecological diversification similar to that in nototheniids, including the origin of pelagic species from benthic ancestors (Smith & Todd, 1984).

Evolutionary history of diversification

The phylogenetic analysis clearly demonstrates that the shift from benthic to pelagic life did not occur as a single, directional trend. Although there are some differences in topology, the phylogenies based either on morphological (Fig. 2) or molecular characters (Ritchie *et al.*, 1995) support this conclusion. Irrespective of which phylogenetic tree is used, pelagic (including epibenthic and cryopelagic) life

styles independently evolved at least twice among the species included in our study (Fig. 9).

One of the lineages that evolved pelagic life styles is the clade consisting of *Trematomus loennbergii*, *T. eulepidotus*, *T. lepidorhinus* and *T. newnesi*. On all our phylogenetic trees (Figs 2, 9), parsimony methods (e.g. Maddison & Maddison, 1992) unequivocally map the origin of pelagic life onto the branch giving rise to this clade, because all surrounding nodes are assigned a benthic life style. The phylogenies of Ritchie *et al*. (1995) suggest that *T. newnesi* evolved independently from the other three species; its pelagic life style thus may have originated separately.

The second origin of pelagic life styles is in the common ancestor of the clade containing the genera *Pagothenia*, *Aethotaxis*, *Gvozdarus*, *Cryothenia* and *Pleuragramma*. Within this clade, however, there is extensive variation in both ecological niches (Eastman, 1993) and in morphological traits, as reflected by the recognition of four monotypic genera and by the large morphometric differences we found between *Aethotaxis* and *P. borchgrevinki*. Because of the close associations with the sea ice (Andriashev, 1970; Eastman, 1993), we consider the cryopelagic life style to be sufficiently different from the mesopelagic one to treat them separately. Depending on the placement of *Gvozdarus* (see Results; Fig. 2), mapping life styles onto the phylogenetic trees suggests either that the cryopelagic genus *Pagothenia* evolved independently, presumably from a benthic ancestor, or alternatively, that it originated from a pelagic ancestor.

A substantial amount of evolutionary plasticity is demonstrated by superimposing the reconstructed phylogenies on ordination plots of morphometric variation (Figs 10, 11). Clearly, the species most closely related phylogenetically are not always close in the space of morphometric variables. At least if one suspects relationships between ecological and morphometric variation, as we have shown them here, morphometric characters seem to be of limited use for estimating phylogenies.

Such evolutionary flexibility of morphometric variables implies a considerable potential for adaptive evolution, but also increases the historical 'noise' in the analysis due to other causes of evolutionary change. Comparative analyses often seek to identify patterns, e.g. correlations between variables, across all branches of a phylogeny (Pagel & Harvey, 1988; Harvey & Pagel, 1991; Martins & Garland, 1991; Garland *et al*., 1992; Losos & Miles, 1994); such patterns are then understood as rules underlying the evolutionary process (see Lauder, 1981, 1982; Eldredge, 1993). The evolutionary 'noise' obscures such patterns, and such studies therefore need to consider large study groups, and describe the patterns in general terms, to achieve sufficient power to confirm them statistically. The number of species in our study was not sufficient to support an evolutionary relationship between diet and morphology, even after simplifying the problem by considering the overall amounts of change in the two sets of variables.

An alternative approach is specifically historical, and therefore emphasizes the need for different explanations that take into account sequences of unique evolutionary events and the role of contingency (O'Hara, 1988; Gould, 1989; Brooks & McLennan, 1991; Losos & Miles, 1994). Studies start with a hypothesized reconstruction of evolutionary events that provides the basis for subsequent inference about the processes involved, a two-step procedure analogous to writing chronicles and narratives in the study of human history (O'Hara, 1988). This approach is compatible with the one emphasizing 'rules' and processes, because testable generalizations are possible if evolutionary events are taken as instances of a more

general class of events (Eldredge, 1993), here for instance, by interpreting the origin of the cryopelagic life style in *Pagothenia* as a habitat shift. Such distinctions among branches in the phylogeny according to the occurrence of evolutionary changes provide the opportunity to better resolve historical detail. Together with a new method of computing independent contrasts for single branches of the phylogeny (McPeek, 1995), this approach enabled us to corroborate the hypothesis that habitat shifts are associated with increased amounts of morphological change.

These patterns, while testable in a rigorous manner, are far from the resolution of the exploratory analyses. Many more species within a phylogenetically well-known group, all with morphometric data and information on their diet, would be necessary to test statistically the relations between morphological and ecological traits found in our PLS analysis. One alternative is to consider a selection of well-defined traits of the organisms' environment, morphology, and performance that is based on a priori functional hypotheses about a specific aspect of the biology of the study organisms. Examples are studies of jaw morphology, biomechanics, and the proportion of large, evasive or hard-shelled prey in fish (Wainwright, 1988; Wainwright & Lauder, 1992; Westneat, 1995), or of the perch diameter, leg measurements, and jumping distance in lizards (Losos, 1990). Quite frequently, however, evolutionary biologists are interested in multiple aspects of the biology of a study group, or in groups where such detailed information is not available. Then, a comparative approach is most promising if it includes both exploratory analyses and rigorous statistical tests of specific aspects.

In this way, our analysis of morphometric and ecological diversification in the Nototheniidae revealed a considerable amount of evolutionary flexibility, as pelagic life styles independently originated on several branches of the phylogeny, and even closely related species can differ dramatically in morphometric traits. The analysis of phylogenetically independent contrasts showed that habitat shifts were accompanied by bursts of increased morphometric change. Morphological differences among species, mainly in traits related to locomotion, are associated with food composition, and presumably evolved as responses to changing functional demands during ecological niche shifts. Conversely, the patterns of allometric growth are mostly conserved; morphological evolution occurred mainly through lateral transposition of growth trajectories rather than through changes of their directions.

ACKNOWLEDGEMENTS

We thank H. H. DeWitt, J. T. Eastman, B. D. Flury, P. A. Hastings, R. G. Miller, J. S. Nelson, P. A. Ritchie, and N. G. Yoccoz for providing advice and informations in various stages of this study. P. A. Ritchie provided a copy of an unpublished manuscript, and R. J. Behnke arranged for the translation into English and publication of the articles by Voskoboynikova (1993) and Balushkin (1994). This manuscript has been improved substantially thanks to comments on earlier versions by J. T. Eastman, J. S. Nelson, and two anonymous reviewers. We are grateful to them all. Financial support was provided by an Izaak Walton Killam Memorial Scholarship to C. P. K. and grants from the Natural Sciences and Engineering Research Council of Canada to J. R. Spence.

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APPENDIX

The following list describes the characters used in the phylogenetic analysis, their states, and character type (only for multistate characters).

I. CEPHALIC LATERAL LINE SYSTEM (Jakubowski, 1970, 1971; Andersen, 1984; Balushkin, 1989; DeWitt *et al*., 1990).

1. Preopercular-mandibular canal: (0) continuous; (1) separate preopercular and mandibular canals.
2. Preopercular and temporal canals: (0) joined; (1) separate.
3. Infraorbital canal below eye (behind fourth pore): (0) continuous; (1) interrup
4. Infraorbital canal behind eye (at first pore below junction with supraorbital and temporal canals): (0) continuous; (1) interrupted.
5. Coronal commissure: (0) continuous; (1) incomplete; (2) absent. Unordered.
6. Lateral part of supratemporal canal (adjacent to first pore from junction with temporal canal): (0) continuous; (1) interrupted (i.e., a gap between lateral-most pore and the medial segments of the canal); (2) absent (i.e., no medial canal segment). Unordered.
7. Medial part of supratemporal canal: (0) continuous; (1) interrupted; (2) absent. Unordered.
8. Canaliculi leading from main canals to pores: (0) absent; (1) present.

II. VISCERAL SKELETON (Balushkin & Voskoboynikova, 1980; Voskoboynikova, 1980, 1982, 1993; Iwami, 1985; Balushkin, 1989).

9. Ascending process of premaxilla: (0) large (comparable to the length of the premaxilla); (1) moderate; (2) small. Ordered. States are assigned according to descriptions and figures in Voskoboynikova (1980, 1993), and figures in Balushkin (1989; some Nototheniinae) and Iwami (1985, fig. 115; *Pagothenia borchgevinki*).
10. Longitudinal ridge on maxilla: (0) present; (1) reduced or absent.
11. Inclination of the palatine: (0) strongly inclined forwards; (1) steep.
12. Shape of the palatine: (0) robust; (1) slender.
13. Ectopterygoid: (0) small; (1) subequal to mesopterygoid.
14. Mesopterygoid: (0) robust; (1) smaller and elongate.
15. Posterior process of the quadrate: (0) short and broad; (1) elongate.
16. Hyomandibular, posterior process for articulation of the operculum: (0) positioned low on hyomandibular (approximately in line with the base of the anterior articular head of the hyomandibular); (1) higher. This character is briefly mentioned by Voskoboynikova (1980: 86), but the present description has been expanded according to figures in Voskoboynikova (1980, 1993), Iwami (1985), and Balushkin (1989).
17. Hyomandibular, canals of the facial nerve: (0) openings free on the outer surface; (1) openings at the base of the anterior articular head.
18. Preopercle and symplectic: (0) separate; (1) articulation by outgrowth of preopercle.
19. Opercle, posterior end of dorsal margin: (0) not enlarged; (1) enlarged lobe or spine.
20. Opercle, dorsal process: (0) small and triangular; (1) drawn out, spine-like.
21. Junction between ceratohyal and epihyal: (0) cartilage; (1) sutured.

III. PECTORAL GIRDLE (Andersen, 1984; DeWitt *et al*., 1990).

22. Scapular foramen. (0) within scapula only; (1) extending into coracoid.

IV. CAUDAL FIN SKELETON (Andersen, 1984; Balushkin, 1989, 1994; DeWitt *et al*., 1990).

23. Hypurals H1 and H2: (0) separate; (1) fused.
24. Hypurals H3 and H4: (0) separate; (1) fused.
25. Hypurals H4 and H5: (0) separate; (1) fused.
26. Hypurals H3 and H4: (0) free; (1) fused or sutured to vertebra.

V. SQUAMATION (Hureau, 1985; DeWitt *et al*., 1990; Shandikov & Kratkiy, 1990; Miller, 1993).

27. Upper lateral line: (0) tubed scales; (1) pored scales (at most a few tubed scales).
28. Middle lateral line: (0) tubed scales; (1) pored scales (at most a few tubed scales).
29. Lower lateral line: (0) absent; (1) present, with pored scales.
30. Scales on head: (0) mostly or entirely ctenoid (in some species non-ctenoid scales, e.g., on cheeks or lower jaws); (1) completely non-ctenoid.
31. Occipital region: (0) scaly; (1) naked.
32. Interorbital region: (0) scaly; (1) naked.
33. Snout: (0) scaly; (1) naked.
34. Preorbitals: (0) scaly; (1) naked.
35. Lower jaw: (0) scaly; (1) naked.
36. Cheeks: (0) entirely scaly; (1) at least partly naked.
37. Opercles: (0) entirely scaly; (1) at least partly naked.

VI. LARVAL PIGMENT PATTERNS (de Ciechomski & Weiss, 1976; Efremenko, 1979, 1984; Moreno, 1980; Slosarczyk, 1983; Gon, 1988; Kellermann, 1990; North & Kellermann, 1990).

38. Dorsal pigment band: (0) reduced or absent, (1) present over most of the postanal section.
39. Lateral pigment: (0) present, (1) absent.
40. Ventral pigment band: (0) reduced or absent, (1) present over most of the postanal section.
41. Ventral abdominal melanophores: (0) absent, (1) present.
42. Pectoral fin base: (0) unpigmented, (2) with pigment cells.
43. Anterior dorsal neck region: (0) unpigmented, (1) pigment cells present.
44. Abdominal region of juveniles: (0) at most partly pigmented, (1) completely covered by melanophores.