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First Application of Morphometrics in a Study of Variations in Uncinial Shape Present within the Terebellidae (Polychaeta)

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André Rinaldo Senna Garraffoni and Maurício de Garcia Camargo (2006) First application of morphometrics in a study of variations in uncinial shape present within the Terebellidae (Polychaeta). *Zoological Studies* **45**(1): 75-80. In this study, morphometric analyses of the uncinial shape were used to differentiate morphological groups within 4 Terebellidae subfamilies. Thirty species were examined, and 17 distances were measured and analyzed using non-metric multidimensional scaling (n-MDS) and cluster analysis. The results derived from both techniques were quite similar and clearly demonstrated that there are 2 different character states for the shape of the manubrium (one for the Trichobranchinae and another for the other subfamilies), and that 3 different character states define the overall uncini shape (one for Terebellinae and Thelepodinae, another for Polycirrinae, and yet another for Trichobranchinae). http://zoolstud.sinica.edu.tw/Journals/45.1/75.pdf

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The Terebelliformia (sensu Rouse and Pleijel 2001) is a group of sedentary polychaetes comprising the families Terebellidae, Ampharetidae, Alvinellidae, and Pectiinaridae. Within the taxon Terebelliformia, the family Terebellidae contains more than 400 species grouped in approximately 60 genera within 4 subfamilies: Polycirrinae, Thelepodinae, Trichobranchinae (Rouse and Pleijel 2001, Garraffoni and Lana 2004), and Terebellinae (Holthe 1986, Hutchings 2000, Rouse and Pleijel 2001).

The Terebellidae is characterized by anterior grooved buccal tentacles, which are used to transport fine surface particles to the mouth (Fauchald and Jumars 1979, Hutchings 2000, Rouse and Pleijel 2001), and neurochaetae modified as uncini which have an anchor-like function (Hutchings 2000, Rouse and Pleijel 2001), allowing them to anchor their soft body within tubes in order to avoid the risk of predation (Holthe 1986, Woodin and Merz 1987). Exceptions are found in the genera *Hauchiella* Hartman, 1959; *Enoplobranchus* Webster, 1879; and *Lysilla* Malmgren, 1866, which lack neurochaetae, and the genus *Amaeana* Levinsen, 1893, which has neurochaetal spines rather than uncini. These 4 genera are included within the terebellid subfamily Polycirrinae.

Analyses of neuropodial uncini in the families Terebellidae, Ampharetidae, Alvinellidae, and Pectiinaridae have shown that most morphological variation occurs in suprageneric taxa (Holthe 1986). In spite of these differences observed in each family, however, it is possible to find some common patterns. All uncini of the Terebelliformia have a main fang, an upper part or capitium (with numerous secondary teeth arranged in different positions), and a lower part, where a subrostral process can be present or absent (and a base called a manubrium) (Holthe 1986, Bartolomaeus 1995, Garraffoni and Lana 2004).

Morphometrics allow rigorous quantitative analysis of variations in organism size from shape

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*To whom correspondence and reprint requests should be addressed. E-mail: garraffoni@ufpr.br effects using multivariate statistical methods (Kligenberg 2002). This method is able to distinguish the effect on shape variations of different sizes of the organism or structure of an organism being studied. The most-common approach of morphometrics is to consider the landmark configuration of morphological features (Kligenberg 2002). The landmarks can be interpreted as a way to reduce the shape of an organism to a set of points that characterize their general traits, which are assumed to be homologous across individuals and populations (Rolf and Marcus 1993, David and Laurin 1998, Costa et al. 2004).

Morphometric studies using morphological landmarks to define species or populations have not been widely used in polychaetes. Some studies (Ben-Eliahu 1987, Fauchald 1991) used morphometric studies in the broad sense of the term, but in recent years, new studies on polychaetes using morphometrics which consider landmark configurations have been published (Maltagliati et al. 2001, Costa and Paiva submitted).

The main goal of this paper was to use a morphometric approach to assess the different morphological classes of uncini observed within the Terebellidae. This morphometric study represents the 1st comprehensive study of the topic for Terebelliformia. As pointed out by Guerrero et al. (2003), the results of statistical tests of character variations allow a consistent judgment of the similarity among variants in order to establish character state identity.

MATERIALS AND METHODS

Operational taxonomic units (OTUs)

Thirty species were selected within Terebellidae: 3 species of Trichobranchinae, 4 species of Polycirrinae, 5 species of Thelepodinae, and 18 species of Terebellinae (Table 1).

Measurements

In the present analysis, 17 distances were measured using 9 landmarks distributed in order to show differences observed in the morphology of the thoracic uncini in the Terebellidae (Fig. 1). We also used 4 points (1, 5, 7, and 13) that are not real landmarks because they do not fall on the structure being analyzed. However, these points are directly related to uncinial parts as they are taken from projections of real landmarks. These additional points therefore contributed to a better representation of the morphological variability of the uncini, and no correlations were found between these projections and the real landmarks they were based upon. Bookstein (1991) suggested that 3 main kinds of landmarks may be used: juxtaposition, maximal curvature, and externally constructed points, and also intermediary cases between types 2 and 3. We used an intermediary case between types 2 and 3, because of the nature of our data.

The distances were as follows: distance 1 was the distance between points 2 and 6; distance 2, points 3 and 9; distance 3, points 12 and 11; distance 4, points 11 and 10; distance 5, points 12 and 2; distance 6, points 12 and 3; distance 7, points 12 and 6; distance 8, points 12 and 8; distance 9, points 2 and 1; distance 10, points 6 and 5; distance 11, points 8 and 4; distance 12, points 8 and 7; distance 13, points 8 and 13; distance 14, points 2 and 3; distance 15, points 3 and 6; distance 16, points 6 and 9; and distance 17, points 9 and 2. The uncini distances were measured directly from the original illustrations of each species and were corrected for the original scale. Only *Polycirrus abrolhensis*, *Nicolea* sp. 1, *Terebellides sepultura*, and *T. totae* were measured directly from drawings done by the authors. We also choose to include figures of illustrated anterior thoracic uncini. However, species for which the original description did not mention from which segment the uncinus was dissected were

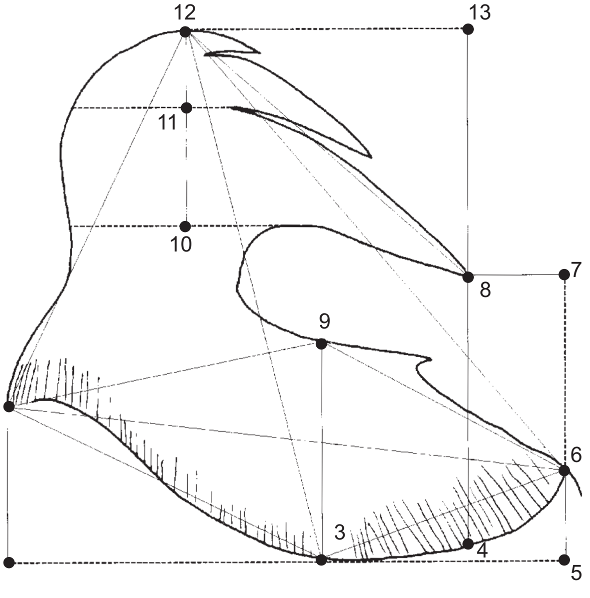

Fig. 1. Morphometric measurements recorded for Terebellidae uncini (modified from Hutchings and Glasby, 1988).

not included in the analysis. The data matrix is available at the web site (http://www.cem.ufpr.br/garraffoni/planilhauncini.xls).

These distances were chosen to avoid some of the biases and weakness pointed out by Strauss and Bookstein (1982) in traditional character sets, such as: 1) most characters tend to be aligned with one of very few axes; 2) coverage of the form is highly uneven by region as well as by orientation, being dense in some areas of the body and sparse in other, and 3) many measurements extend over much of the body, so short distances contain more-localized information than long ones.

In order to describe the different regions of the uncini, we used the terminology proposed by Holthe (1986: 31, fig. 6).

Analysis

Cluster analysis and non-metric multidimensional scaling ordination (n-MDS) were performed from a log-transformed matrix (OTUs x measurements) using the software, Primer (Primer-E, Plymouth Marine Laboratory UK). Characters used by both analyses were standardized, and then Euclidian distance was applied among the OTUs. Euclidian distance was used because it can measure natural distances between any 2 points in space (Clarke and Warwick 1994). n-MDS gives a general overview of the data and can be used for illuminating relationships among species. Using n-MDS, a map or configuration of the objects can be constructed (Clarke and Warwick 1994). Cluster analysis, on the other hand, aims to find "natural groups" of samples such that those within a group are more similar to each other than to those outside of the group. Clarke and Warwick (1994) also pointed out that sometimes it might be of interest to use Euclidean distances in the species space as input to a cluster analysis. ANOSIM (analysis of similarities) is a permutation test analog of the standard ANOVA

Table 1. List of the Terebellidae species used in the present study

Species	Subfamily	Abbreviation
Polycirrus boholensis Grube, 1878	Polycirrinae	Po_bo
Polycirrus disjunctus Hutchings and Glasby, 1986	Polycirrinae	Po_di
Polycirrus medius Hessle, 1917	Polycirrinae	Po_me
Polycirrus abrolhenis Garraffoni and Costa, 2003	Polycirrinae	Po_abro
Amphitrite pachyderma Hutchings and Glasby, 1986	Terebellinae	Am_pa
Arranooba booromia Hutchings and Glasby, 1986	Terebellinae	Arr_bo
Baffinia biseriata Hutchings and Glasby, 1988	Terebellinae	Ba_bi
Lanassa ocellata Hutchings and Glasby, 1988	Terebellinae	Lan_oc
Lanice sinata Hutchings and Glasby, 1990	Terebellinae	Lani_si
Lanicides fascia Hutchings and Glasby, 1988	Terebellinae	La_fa
Lanicides tribranchiata Hutchings and Glasby, 1988	Terebellinae	La_tr
Loimia triloba Hutchings and Glasby, 1988	Terebellinae	Lo_tr
Longicarpus nodus Hutchings, 1990	Terebellinae	Lon_no
Neolepra macrocercus Hutchings and Glasby, 1986	Terebellinae	Ne_ma
Paraeupolymnia uspiana Nogueira, 2003	Terebellinae	Ni_sp1
Phisidia echuca Hutchings and Glasby, 1986	Terebellinae	Ph_ec
Pista sinusa Hutchings and Glasby, 1986	Terebellinae	Pi_si
Pseudoproclea australis Hutchings and Glasby, 1990	Terebellinae	Ps_au
Reteterebella aloba Hutchings and Glasby, 1986	Terebellinae	Re_al
Hutchingsiella cowarrie (Hutchings, 1997)	Terebellinae	Sp_co
Terebella muliarrus Hutchings, 1993	Terebellinae	Te_mu
Tyra owensi Hutchings, 1997	Terebellinae	Ty_ow
Euthelepus setubalensis McIntosh, 1885	Thelepodinae	Eu_st
Streblosoma comatus (Grube, 1859)	Thelepodinae	St_co
Thelepus ambitus Glasby and Hutchings, 1987	Thelepodinae	Th_am
Pseudothelepus binara Hutchings, 1997	Thelepodinae	Pst_bi
Pseudostreblosoma serratum Hutchings and Murray, 1984	Thelepodinae	Pss_se
Octobranchus antarcticus Monro, 1936	Trichobranchinae	Oc_an
Terebellides sepultura Garraffoni and Lana, 2003	Trichobranchinae	Tere_sep
Terebellides totae Elias and Bremec, 1999	Trichobranchinae	Tere_to

(analysis of variance), used to detect differences among groups established a priori in n-MDS. ANOSIM was used here to test possible groups of species belonging to the same subfamily according to the ordination.

The Primer framework (n-MDS + cluster analysis + ANOSIM) is commonly used in ecological studies, but less often used in morphometric studies. Actually, n-MDS and cluster analysis are not new techniques, and they have been applied to morphometric analyses elsewhere (Maltagliati et al. 2001, Chui et al. 2002, Noireau et al. 2002, Costa et al. 2004). ANOSIM, on the other hand, was introduced by the Primer framework (Clarke and Warwick 1994) and should be interpreted in conjunction with the n-MDS. ANOSIM simply calculates the probability of the random occurrence of the observed groups.

RESULTS

The result of the n-MDS of the 30 Terebellidae species (Fig. 2) shows a very low stress value (minimum stress, 0.04) proving high resolution (Clarke and Warwick 1994, Chui et al. 2002). The n-MDS analysis using 17 characters clearly separated the species into 3 clusters according to different quadrants (Fig. 2). The Trichobranchinae was clustered in the 1st quadrant, Polycirrinae in the 2nd quadrant, and Thelepodinae and Terebellinae in the 3rd quadrant. The ANOSIM permutation test (Table 2) confirmed this tendency, as every pair of groups was significantly separated at the level of 5%, except for Terebellidae against Thelepodinae. Very similar classes as defined in the n-MDS were also obtained by cutting the dendrogram at specific height points, but some species from the same subfamily were not necessarily grouped together (Fig. 3).

The n-MDS and cluster analysis revealed 2 distinct morphotypes within the 4 subfamilies. These 2 distinct morphotypes (or 2 distinct clusters in the dendrogram) were obtained by the morphological differences in the size of the manubrium. Trichobranchinae has uncini with a long-shafted manubrium, while Polycirrinae, Thelepodinae, and Terebellinae have uncini with a short manubrium.

The dendrogram (a diagram in which the similarities of 2 samples or groups are considered to have fused), in figure 3, also shows that some species of the Terebellinae have an uncinial shape more similar to species of Thelepodinae than to other Terebellinae species (*Lanicides fascia*, *Pista sinusa*, and *L. tribranchiata*). This is because *L. fascia*, *P. sinusa*, and *L. tribranchiata* have a developed chitinized shaft in the posterior part of the uncini, called the posterior process. The other species of the Terebellinae and Thelepodinae do not have this posterior process.

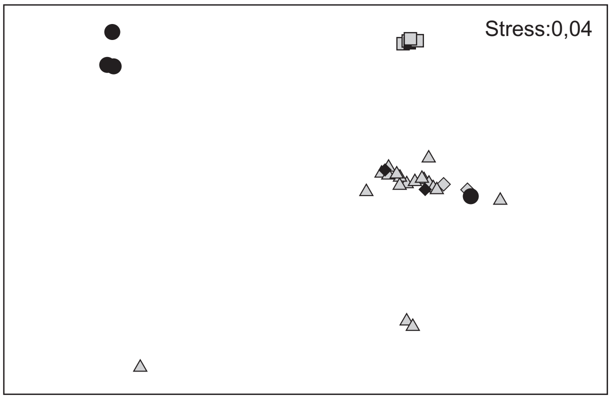

Fig. 2. Two-dimensional plot of uncini character sets analyzed by non-metric multidimensional scaling. Dark gray circles represent Trichobranchinae species, light gray squares represent Polycirrinae species, light gray triangles represent Terebellinae species, and dark gray rhombuses represent Thelepodinae species.

Table 2. Results of the ANOSIM (global R = 0.51, global significance = 0.2) using the Terebellidae subfamilies. Abbreviations: Tere, Terebellinae; Tricho, Trichobranchinae; The, Thelepodinae; Poly, Polycirrinae

Groups	R statistic	Significance level (p)	Possible permutations
Tere, Trico	0.9	0.2	1540
Tere, Poly	0.57	0.5	8855
Tere, The	-0.038	54.5	8855
Trico, Poly	1.0	2.9	35
Trico, The	1.0	2.9	35
Poly, The	1.0	2.9	35

DISCUSSION

Shape is a multidimensional component of variation in the morphological form which is expected to have a high information content regarding the evolutionary process responsible for the observed diversity (Atchley and Hall 1991, Costa et al. 2004). One of the main purposes of morphometric analysis is to establish and delimit the nature of morphological variations as a 1st step to providing an assessment of homology among different features, which can be used in subsequent studies to improve one's understanding of the phylogenetic relationships among the taxa (Reis 1988). Guerrero et al. (2003) also pointed out that the similarity test of character states identified by morphometric analyses can help the systematist judge whether some character variants are sufficiently different to recognize them as a different character or character state.

Observing the morphological groups established by n-MDS and the dendrogram, we can clearly evaluate the differences between the posterior process and the long-shafted manubrium. Unfortunately, there is no information on the ontogeny of the development of this posterior process and the long-shafted manubrium in the literature to help us evaluate our results.

The 2 different morphotypes identified (in n-MDS quadrant 1 vs. quadrants 2 and 3; and in the dendrogram, the 1st dichotomy close to the base), separating the Trichobranchinae from the Polycirrinae, Terebellinae, and Thelepodinae can be defined by the presence of a long-shafted manubrium in the Trichobranchinae. Thus, these 2 different morphotypes, short- and long-shafted manubrium, can be assumed to represent 2 character states linked to the size of the manubrium.

The presence of a posterior process in some Terebellinae species, namely *Lanicides fascia*, *Pista sinusa*, and *L. tribranchiata*, is also considered interesting as it potentially indicates homology with the long-shafted manubrium. However, our results showed that these structures are not homologous, and they are thus treated as independent characters. We suggest that the posterior process underwent different ontogenetic development than that of the manubrium, and the former is only an extensive development of the posterior part while the latter is a development of the entire uncinial base.

Another important result from both analyses is the identification of 3 different shape components showing significant differences among the subfamilies. These 3 different shapes can also be assumed to be 3 character states for the character

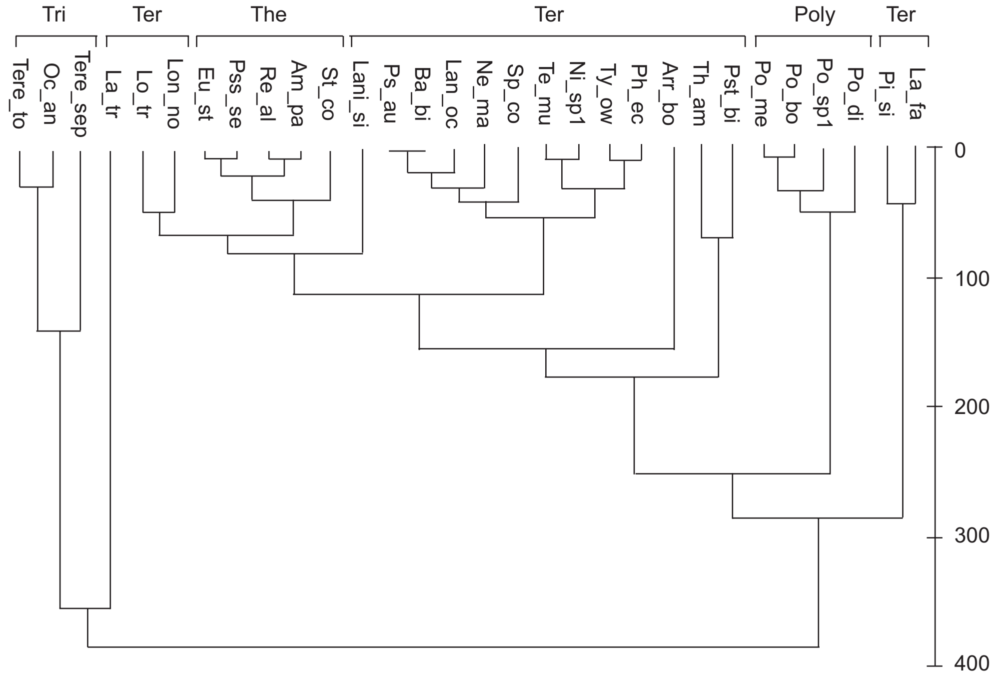

Fig. 3. Dendrogram of the 30 Terebellidae species obtained using Euclidean distances. For species abbreviations see table 1. Ter, Terebellinae; The, Thelepodinae; Poly, Polycirrinar; Tri, Trichobranchinae.

of the overall uncinial shape. In spite of each group having its own overall shape (Figs. 2, 3), morphotypes of the Terebellinae and Thelepodinae are very similar, and it is difficult to divide them into different states.

In summary, morphometry is a powerful tool which allows an objective coding of morphologically quantitative traits into character states, or different morphological patterns into specific levels to understand microevolution and identify morphotypes. Our research provides a robust basis to examine the overall performance of morphological variation in the Terebellidae uncini and can help elucidate the major contribution of these characters to delineating this family. Terebellidae uncini is a rich source of taxonomic information that can be used to help assess evolutionary relationships within the family.

Note added during proofing: After the time that this paper was submitted, we began a new study using morphometrics on Terebellidae uncini in order to complement this first approach. In this new study, we dissected segments 7 and 16 of different Terebellidae species, and took pictures of 3 different uncini on each segment. Using measurement software, we obtained all distances previously used in this paper and additional measurements such as area and perimeter. Currently, we are still analyzing the information using the same procedures that were applied here. In addition, an attempt is being made to establish a mathematical model to predict different taxa (output) from uncini measurements (input) using an artificial neural network (ANN) based on multilayer perceptions.

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