Morphometric analysis of two sympatric species of *Perinereis* (Annelida: Nereididae) from the Brazilian coast

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Species definition is not an easy task, when considering the more than 27 known species concepts. Among them, the morphospecies concept has been one of the most applied since it is based on the use of observable morphological features. Morphometry has been used to delimitate morphospecies or similar taxa complementing the morphological observations and contributing to clarify taxonomic problems. Specimens from the sympatric species Perinereis anderssoni *and* Perinereis ponteni, *collected from the north-eastern to southern coast of Brazil and considered synonymous by some authors, were compared through morphometric analyses for the evaluation of their taxonomic status. Morphometric analysis indicates that notopodial cirri lengths in the median and posterior regions on the body clearly allowed differentiation between the two species. Our results indicate that the number and arrangement of paragnaths demonstrate a pattern of variation that effectively differs and could be used to discriminate these two* Perinereis *species. This distinction was confirmed by the restricted among-population variability within each species, even when populations that are geographically very distant from each other were considered.*

INTRODUCTION

Species definition is not an easy task, when considering the more than 27 known species concepts (Wilkins, 2011). Among them, the morphospecies concept has been one of the most applied since it is based on the use of observable morphological features. It has been used for centuries as the main diagnostic procedure for delimitation of species in taxonomic studies (Cook *et al.*, 2010). This approach is based on looking for phenotype or shape differences among groups of individuals in order to delimitate and discriminate species. Morphological differences can be based on both meristic (countable) and morphometric (measured) traits in what are defined as shape differences. Shape can be assessed by the taxonomic point of view, in a more subjective way, or can be mathematically expressed by combination of measurements in what has been known as morphometric analysis (Zelditch *et al.*, 2004).

Morphometry has been used to delimitate morphospecies or similar taxa complementing the morphological observations and contributing to clarify taxonomic problems (Martin *et al.*, 2003; Lattig *et al.*, 2007). In polychaetes, despite being soft-bodied animals and thus more suitable to measurement errors (Costa-Paiva *et al.*, 2007), this approach

was also successfully applied on delimitation and diagnosis of some species leading to a more accurate taxonomic assessment (Ford & Hutchings, 2005; Garraffoni & Camargo, 2006; Glasby & Hsieh, 2006; Costa-Paiva & Paiva, 2007; Occhioni *et al.*, 2009).

The species *Perinereis ponteni* Kinberg (1866) and *P. anderssoni* Kinberg (1866) were considered synonymous by Hartman (1948, 1959) because they occur in sympatry along the Western Atlantic (Lana, 1984; de León-González & Solis-Weiss, 1998; de León-González, 1999; Amaral *et al.*, 2013; de León-González & Gothel, 2013). Although they are considered as distinct species by some authors (Santos, 1996; Santos & Lana, 2000; Steiner, 2000; Santos & Steiner, 2006; Ipucha *et al.*, 2007; de Léon-Gonzalez & Gothel, 2013), these two species have been treated as synonymous even in recent reports and databases (Espinosa *et al.*, 2007; WORMS, 2014).

These species co-occur on the same rocky shores and intertidal zone environments (Lana, 1984; Santos & Steiner, 2006) and probably exploit the same food resources. Furthermore, these species are very abundant on rocky shores, a highly vulnerable environment. Thus, clarification of the taxonomy of the group members is not only desirable from a systematic point of view, but it will also facilitate reconciling species names with the considerable biological literature available for the family Nereididae.

The criteria used by Santos & Steiner (2006) to distinguish both species and that were used for a prior identification of these species at the outset of the study were: *P. anderssoni*

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possesses fewer number of paragnaths on area I in the proboscis than *P. ponteni*; *P. ponteni* possesses one paragnath on area V while *P. anderssoni* possesses three; *P. anderssoni* presents notopodial ligule longer and wider in median and posterior body chaetigers than *P. ponteni*; in *P. anderssoni* notopodial cirri extends beyond the apex of the notopodial ligules in the anterior region of the body, while in *P. ponteni* it is shorter.

Despite the widespread occurrence of these species along the Atlantic coast, a quantitative assessment of the morphological variability of their populations is lacking and would allow the validity of *P. anderssoni* and *P. ponteni* as different species to be tested. Thus, the present study compared *P. anderssoni* and *P. ponteni* populations from different regions in the Brazilian coast through morphometric analyses to evaluate their taxonomic status.

MATERIALS AND METHODS

Sampling

A total of 498 specimens from 14 populations, distributed from the north-eastern to the southern Brazilian coast, were analysed (Figure 1).

The collection sites were Ilha do Mel-Paraná (PR), Martim de Sá and Picinguaba-São Paulo (SP), Itaipu-Rio de Janeiro (RJ), São Francisco do Conde-Bahia (BA), Pina-Pernambuco (PE), Baía da Traição and Tambaba-Paraíba (PB), Pacheco-Ceará (CE) (Table 1).

All specimens were collected in the intertidal region through scraping the existing coverage on hard substrates. Specimens were anaesthetized with menthol, subsequently fixed in formalin, and transferred to 70% alcohol after 72 h,

Fig. 1. Location of collection sites for the studied populations of *P. anderssoni* and *P. ponteni* along the Brazilian coast (modified from Clímaco 2013).

with the exception of specimens from the São Francisco do Conde, which were fixed in 95% ethanol.

Morphometric analyses

The following variables were evaluated: total length (TL); total number of chaetigers (NC); postero-dorsal tentacular cirri length (PDTC); prostomium width (PW); prostomium length (PL); peristomium width (PEW); width of the 10th chaetiger with and without parapodia (WP10 and W10); width of the 15th chaetiger with and without parapodia (WP15 and W15); jaw length (JL); number of maxillary teeth (MT); notopodial and neuropodial cirri lengths (DC and VC), notopodial and neuropodial ligules length (DLI and VLI), notopodial and neuropodial lobes length (DLO and VLO) from the 10th, 30th and 45th chaetigers; and the number of paragnaths in Areas I to VII–VIII of the proboscis (AI, AII, AIII, AIV, AV, AVI, AVII–VIII).

Morphometric measurements were initially converted to millimetres, and outlier individuals were excluded. Normality and homoscedasticity were tested in the data using the Shapiro–Wilks and Levene tests, respectively. Individuals were observed, described and measured with the aid of compound (Olympus CX31) and stereoscopic (Olympus SZ51) microscopes, coupled to a digital camera (Sony 13MP).

The morphometric variables were statistically evaluated using linear regression and CVA (Canonical Variable Analysis). The meristic variables were statistically evaluated using ANOVA applied to number of paragnaths of the proboscis per Area, SIMPER and PERMANOVA applied for the number of paragnaths in all Areas of proboscis. Statistical analyses were performed with the aid of the statistical programs R environment (R Core Team, 2012), PRIMER 6.0 (Clarke & Gorley, 2006) and Statistica 7.0 (2004).
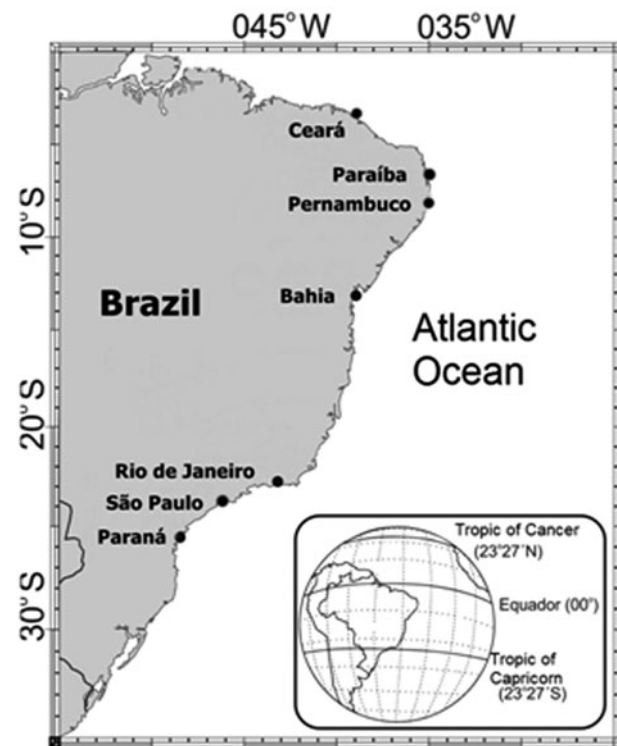
RESULTS

The linear regression analysis, applied on each of the 14 populations (seven of each species) and on each species, indicated that the width of the 10th chaetiger without parapodia (Linear regression, $r^2 = 0.76$, $Tstatistic = 25.79$, $P < 0.0001$) was the best character to estimate total body length in *P. anderssoni*; the width of the 15th chaetiger with parapodia (Linear regression, $r^2 = 0.65$, $Tstatistic = 17.98$, $P < 0.0001$) was the best character to estimate total body length in *P. ponteni*.

According to the CVA analysis, *P. anderssoni* and *P. ponteni* differed in shape significantly. In a first analysis, grouping 14 populations of both species (seven localities of each species), the canonical variable 1 (CV1) explained 45% of the variation found between species. Out of all measured variables, CV1 closely correlated with the notopodial cirri length from chaetigers 30 ($r = -0.56$) and 45 ($r = -0.56$). The notopodial ligule length, in the 45th chaetiger, was the only variable that positively correlated with CV1 (Figure 2 and Table 2).

Among-population variation was also observed both for *P. ponteni* and *P. anderssoni*. In *P. ponteni*, CV1 explained 44% of the among-populations variation, CV2 explained 25% and CV3 explained 14%. CV1 closely correlated with the notopodial ligule length from chaetigers 30 and 10 ($r = 0.77$) and 45 ($r = 0.79$). CV2 closely correlated with total body length

Table 1. Data of collection sites for the studied populations of *P. anderssoni* and *P. ponteni* along the Brazilian coast.

Locality	Collection sites coordinates	Specie	Number of specimens
Ilha do Mel (IM)- PR	25°33′44.11″S 48°19′8.53″W	*P. anderssoni*	50
Ilha do Mel (IM)- PR	25°33′44.11″S 48°19′8.53″w	*P. ponteni*	9
Martim de Sá (MS12)- SP	23°37′32.59″S 45°22′31.60″W	*P. anderssoni*	50
Martim de Sá (MS01)- SP	23°37′32.59″S 45°22′31.60″W	*P. anderssoni*	45
Martim de Sá (MS)- SP	23°37′32.59″S 45°22′31.60″W	*P. ponteni*	50
Picinguaba (PI)-SP	23°21′26.29″S 44°51′56.22″W	*P. ponteni*	50
Itaipu (I)- RJ	22°58′26.71″S 43°2′48.99″W	*P. anderssoni*	50
Itaipu (I)- RJ	22°58′26.71″S 43°2′48.99″W	*P. ponteni*	36
São Francisco do Conde (SFC)- BA	12°40′47.89″S 38°42′29.65″W	*P. ponteni*	24
Pina (PN)- PE	8° 5′23.30″S 34°52′46.41″w	*P. ponteni*	30
Baía da Traição (BT)- PB	6°41′18.90″S 34°55′49.83″O	*P. anderssoni*	18
Baía da Traição (BT)- PB	6°41′18.90″S 34°55′49.83″W	*P. ponteni*	31
Tambaba (TB)- PB	7°22′00.29″S 34°47′50.90″W	*P. anderssoni*	20
Pacheco (P)- CE	3°41′12.42″S 38°38′32.15″W	*P. anderssoni*	35

CE, Ceará; PB, Paraíba; PE, Pernambuco; BA, Bahia; RJ, Rio de Janeiro; SP, São Paulo; PR, Paraná.

($r = -0.59$) and number of chaetigers ($r = -0.36$). All variables correlated positively with CV1 (Figure 3).

For *P. anderssoni*, CV1 explained 37% of the among-populations variation, CV2 explained 27%, and CV3 explained 19%. Out of all measured variables, CV1 closely correlated with the notopodial cirri length from chaetigers 30 ($r = 0.77$) and 10 ($r = 0.75$). CV2 closely correlated with the neuropodial lobe length in the 10th chaetiger ($r = 0.35$) and width of the 15th chaetiger without parapodia ($r = 0.29$).
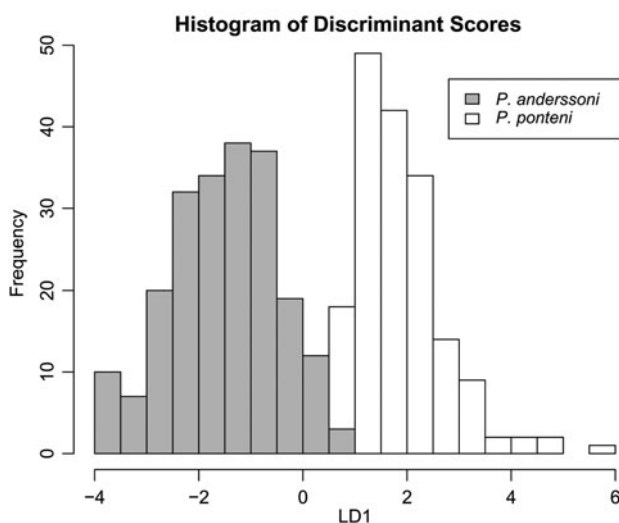

Fig. 2. Histogram of discriminant function on the studied species of *P. ponteni* and *P. anderssoni*.

All variables, except number of chaetigers and postero dorsal tentacular cirri length, were positively correlated with CV1 (Figure 3).

When analysing the species *P. anderssoni* and *P. ponteni* together, the number of paragnaths of all eight proboscis Areas revealed significant interspecific differences, with, as expected, the number of paragnaths of Area V, one of the criteria used in prior species classification, being the most significant to discriminate the species (ANOVA, $F_{(13)} = 1340.47$, $P < 0.0001$) (Figure 4). *Perinereis ponteni* possessed higher number of paragnaths in Areas I, II and VI of the proboscis, and maxillary ring; *P. anderssoni* possessed more paragnaths in Areas III, IV, V and VII–VIII of the proboscis. Conversely, populations of *P. ponteni* showed significant differences in number of paragnaths of all Areas, except in Area VI, in the proboscis (ANOVA, $F_{(6)} = 1.54$, $P = 0.17$); the highest number of paragnaths, considering the mean of all Areas, were observed in Ilha do Mel, and

Table 2. Main discriminant variables of shape for *P. anderssoni* and *P. ponteni*.

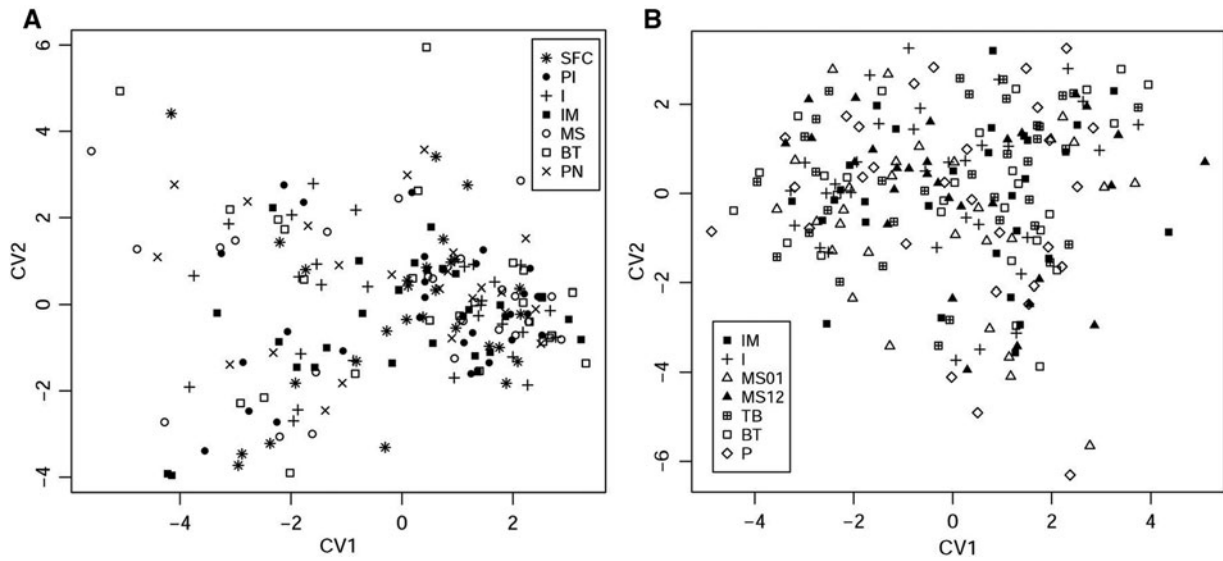| Species		DC30	DC45	AV	AI
P. anderssoni	Mean	0.37	0.32	3.01	4.12
$n = 267$	SD	0.13	0.11	0.16	1.05
	Variance	0.10–1.05	0.09–0.75	2–5	2–8
P. ponteni	Mean	0.23	0.19	1.03	11.03
$n = 230$	SD	0.1	0.08	0.17	3.12
	Variance	0.02–0.95	0.07–0.41	1–2	0–21

Fig. 3. Canonical variables analysis (CVA) on the studied populations: (A) of *Perinereis ponteni*; (B) of *Perinereis anderssoni*. IM, Ilha do Mel; I, Itaipu; MS, Martim de Sá; TB, Tambaba; BT, Baía da Traição; P, Pacheco; SFC, São Francisco do Conde; PI, Picinguaba; PN, Pina.

Fig. 4. Analysis of variance (ANOVA) of paragnaths numbers in Area V, in the proboscis, in *P. anderssoni* (Pa) and *P. ponteni* (Pp) populations. IM, Ilha do Mel; I, Itaipu; MS, Martim de Sá; TB, Tambaba; BT, Baía da Traição; P, Pacheco; SFC, São Francisco do Conde; PI, Picinguaba; PN, Pina.
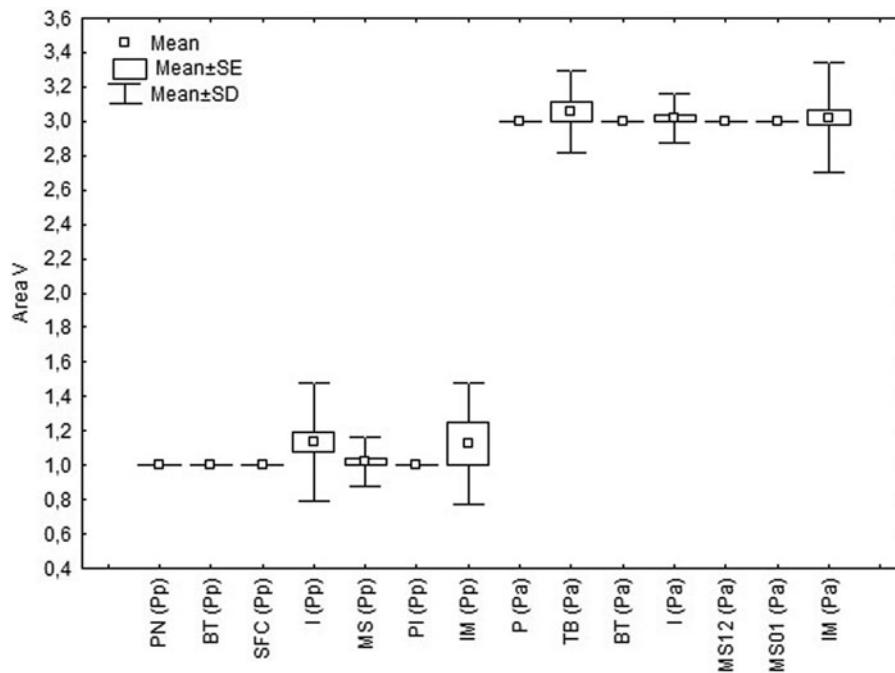
the lowest in Picinguaba. The number of paragnaths of all Areas were statistically different among *P. anderssoni* populations, except Area V of the proboscis (ANOVA, $F_{(6)} = 0.35$; $P = 0.91$); the highest values of numbers of paragnaths, considering the mean of all Areas of the proboscis, were observed in Ilha do Mel and Pacheco, and the lowest values in Martim de Sá.

In qualitative terms, and for both species, the Area VI has bar-shaped paragnaths and in the other Areas paragnaths are conical. Area I has triangular arrangement, Area VI has two bars and Area VII–VIII paragnaths in rows in both species.

In *P. anderssoni* specimens, paragnaths have the following arrangements: triangular in Area II, circular in Area III, rectangular in Area IV, T form in Area V. In *P. ponteni* specimens, paragnaths have the following arrangements: trapezium in Area II, arc in Area III, triangular in Area IV, one conical paragnath in Area V.

The PERMANOVA analysis indicated significant differences between the two species (PERMANOVA, $F_{(1)} = 199.02$; $P < 0.0001$) and also among geographic regions (*P. anderssoni*: PERMANOVA, $F_{(6)} = 10.22$, $P < 0.0001$; *P. ponteni*: PERMANOVA, $F_{(6)} = 6.82$, $P < 0.0001$).

The SIMPER analysis demonstrated that the number of paragnaths of Area IV contributed the most to the similarity between populations, both in *P. anderssoni* and *P. ponteni*; the number of paragnaths of Area I contributed the most to the dissimilarity (24.69%) between two species.

DISCUSSION

Although *Perinereis ponteni* and *P. anderssoni* have been considered synonymous for some authors (Hartman, 1948, 1959; Espinosa *et al.*, 2007; WORMS, 2014), the morphometric analyses performed in this study clearly differentiated the two species, as suggested earlier, from morphological analysis (Santos & Steiner, 2006) and cytogenetics (Ipucha *et al.*, 2007). Little is known about the reproductive biology and population dynamics of these species. Peixoto (2013) suggests that *P. anderssoni* larvae are lecithotrophic, but nothing has been described for *P. ponteni*. Ben-Eliahu (1987) suggests that the proportions between parapodial structures, complemented by meristic data and measurements, allow for a better understanding of morphological variability in nereidid populations. Similarly, Costa-Paiva & Paiva (2007) were able to discriminate closely related species in the genus *Eunice* based on the lengths of antennae and anterior body region. Glasby & Hsieh (2006) also discriminated species in the *P. nuntia* Savigny, 1818 species group (*P. mictodonta, P. nuntia, P. shikueii* and *P. wilsoni*) in Taiwan and adjacent coastal waters based on paragnath numbers and arrangement and the ratio between dorsal cirrus length and dorsal ligule in the anterior and posterior parapodia.

Paragnaths have been widely used to quantify variation within and among Nereididae populations and species (Ben-Eliahu, 1987; Glasby & Hsieh, 2006; Bakken *et al.*, 2009). In a study conducted with the species *Hediste diversicolor* Müller, 1776 (Garcia-Arberas & Rallo, 2000), the paragnaths numbers of Areas I and III of the proboscis contributed the most to among-population variation; whereas in a study with *Neanthes succinea* Leuckart, 1847, resurrected by Sato (2013), from the Brazilian coast (Clímaco, 2013) Areas III, IV, V and VII–VIII were reported as the most important in order to separate populations. Here, the number of paragnaths of Areas I, II, III, IV, V and VII–VIII of the proboscis contributed most to among-population variation in *P. ponteni*; and the number of paragnaths of Areas I, II, III, IV, VI and VII–VIII contributed most to among-population variation in *P. anderssoni*. Moreover, the paragnaths differ from arrangement between species. Nevertheless, it was suggested that the use of the number of paragnaths as a taxonomic character may be inappropriate because its intra-specific variability could result from phenotypic plasticity as a response to different environments, biotic constraints or differences in gene expression (Wilson, 1993; Garcia-Arberas & Rallo, 2000; Maltagliati *et al.*, 2001).

Many studies described inter-population variation based on number of paragnaths of Areas of proboscis trying to associate variation with size, sex and different habitat conditions, such as sediment type, seasonal fluctuations in salinity, diet differences and dominant mode of feeding (Barnes & Head, 1977; Barnes, 1978; Gillet, 1986, 1990; Wilson, 1993; Garcia-Arberas & Rallo, 2000; Maltagliati *et al.*, 2006). Barnes (1978), for example, did not find a correlation between paragnaths numbers and salinity and sediment. Gillet (1986) attributed variation found in *Nereis*

diversicolor Müller, 1776, to ecological factors, especially the sediment. However, in 1990, the same author discarded salinity as the cause of variation and concluded that granulometry could not be considered to be a determining factor. Also, number of paragnaths is not related either to size or sex (Barnes & Head, 1977; Clímaco, 2013). Nevertheless, the relation between number of paragnaths and environmental differences is beyond the scope of this study. However, the similarities of our collecting sites, being intertidal regions on similar hard substrates and salinity, could explain the absence of differentiation among-population within-species. Our results indicate that the number and arrangement of paragnaths demonstrate a pattern of variation that effectively differs and could be used to discriminate these two *Perinereis* species. These differences are more significant than the differences observed between geographic regions, thus making them useful morphological markers.

The discrimination among geographic populations within each species was very tenuous. The lack of geographic distinction was also reported for *H. diversicolor* across 14 sites in Europe (Maltagliati *et al.*, 2006) and for *P. ponteni* on the Brazilian coast (Silva, 2014). In contrast, Clímaco (2013) was able to demonstrate a geographic separation, based on paragnaths, for *N. succinea* from the northern/north-eastern and south-eastern/southern regions. The same pattern of southern/northern differentiation was reported for *P. anderssoni* by Silva (2014), based on a phylogeographic analysis.

The morphometric analyses performed in this study allowed the recognition and separation of *P. anderssoni* and *P. ponteni* and demonstrated that these species really belong to distinct taxa, with notopodial cirri length from chaetigers 30 and 45 and the number of paragnaths in Areas I and V being the most useful morphological and morphometric markers for discrimination of these two *Perinereis* species. *Perinereis ponteni* showed little variation among-populations, which suggests there is a single species for the Brazilian coast. This distinction was confirmed by the restricted among-population variability within each species, even when populations that are geographically very distant from each other were considered.

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