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Assessment of traditional and geometric morphometrics for discriminating cryptic species of the *Pseudechiniscus suillus* complex (Tardigrada, Echiniscidae)

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Abstract

Tardigrade taxonomy is particularly nebulous in the so-called *Pseudechiniscus suillus* complex, a group of species very difficult to distinguish from one another. This species complex needs complete revision. In this study, traditional morphometric and geometric morphometric relationships among five populations of the *P. suillus* group, from four different Atlantic islands (three in the Azores and S. Tomé) and one from the Portuguese mainland, were investigated to explore their potential for discriminating cryptic species. Seven traditional morphometric variables were used to describe variation by means of principal component analysis. In addition, two traits, claws and dorsal segmental plates, were analysed using a geometric morphometric approach that has the ability to determine very subtle differences in shape. The results of both analyses suggest the occurrence of four morphs exhibiting different sizes and shapes of cirrus A, claws and dorsal plates. The high taxonomic value of those characters joined with some other qualitative aspects, such as the cuticle sculpture, seems to indicate that different species can be distinguished among these morphs. This leads to the conclusion that the geometric morphometric approach is a useful complementary tool to the traditional morphometric approach for discriminating cryptic species of heterotardigrades.

Key words: Tardigrada – *Pseudechiniscus suillus* group – comparative morphometry – geometric morphometrics

Introduction

The taxonomy of limnoterrestrial tardigrades is mainly based on the analysis of morphological characters. Although a few papers have presented some promising results on distinguishing species based on the study of few genes, the molecular approach is still in the early stages (Blaxter et al. 2004; Jørgensen et al. 2007; Schill and Steinbruck 2007; Faurby et al. 2008; Cesari et al. 2009; Guidetti et al. 2009; Guill and Giribet 2009). As stressed by Guill and Giribet (2009), to improve this molecular approach, it is necessary to analyse more genes and to solve difficulties in acquiring DNA samples from single instead of pooled individuals. On the other hand, DNA samples are, in general, obtained from specimens identified by a morphological approach. So, as emphasized by Guidetti et al. (2005), we also believe that in the coming years the taxonomy of tardigrades must still be based on the combination of both morphological and molecular approaches. With the current biodiversity crisis and the consequent urgent need to create a legacy of taxonomic knowledge (Wheeler et al. 2004; Wilson 2005; Dubois 2007; Evenhuis 2007), the use of morphological characters will certainly be dominant in the near future.

The low number of useful taxonomic characters and the remarkable degree of phenotypic plasticity exhibited by a considerable number of species are responsible for great problems in the identification process. Additionally, closely related species can often be distinguished by minor morphological details (Kinchin 1994; Pilato and Binda 2001; Pilato et al. 2007). Taxonomists agree that, to solve this problem, more characters are needed, especially quantitative characters (e.g. Dastych 1984, 2005, 2006; Pilato and Binda 1997/98; Pilato et al. 2002; Guidetti and Bertolani 2005).

The use of morphometric characters has attracted the attention of some of the most eminent tardigradologists such as Higgins (1959), Morgan (1976) and Ramazzotti (1977). Strong correlations have been obtained between body length and buccal tube width in *Paramacrobiotus areolatus* (Ramazzotti 1977) and between body length and buccal tube length in *Dactylobiotus grandipes* (Schuster et al. 1978). Wainberg and Hummon (1981) described relationships between several measurements of the buccal apparatus and the claws in *Isohypsibius saltursus*. An important advance was the introduction of morphometric analysis based on the *pt* indices (relationship between a given structure and the buccal tube length) by Pilato (1981), currently an indispensable tool for the specific diagnosis of eutardigrade species (Biserov 1990a,b; Bertolani and Rebecchi 1993; Dastych 2005). The use of *pt* indices also showed (Bertolani and Rebecchi 1993) that intraspecific variability was much more restricted than previously thought and what was considered a single species is now considered a complex of many species (e.g. *Macrobiotus hufelandi* group). Recently, Dastych (2006) introduced some new quantitative relationships, in addition to buccal tube length indices, based on buccal structures and also on the length of the claws to describe *Ramazzottius nivalis*.

However, as stressed by Kinchin (1994, 1996), the application of morphometric data should be carefully interpreted and it is often seriously restricted. In addition to difficulties in obtaining precise measurements because of the orientation and deformation of small mounted specimens (Kinchin 1994), morphometric relationships could be strongly influenced by (1) the mounting media, which could be responsible for a 6–10% variation in body length (Kinchin 1994); (2) the size of the specimens and the under-representation of juveniles (Kathman and Nelson 1987); (3) the sex of the specimens (Biserov 1994); and (4) the different dimensions of various cytotypes (Bertolani et al. 1987).

All previously discussed advances and limitations of morphometric analysis refer to eutardigrades. Unfortunately, in heterotardigrades (Fam. Echiniscidae), this problem is even more pronounced. Besides the importance given to the quantitative measurements of appendages (Lattes 1974) and

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the pioneer attempts to introduce standard measurements to discriminate Echiniscidae species (Franceschi and Lattes 1964; Lattes and Gallelli 1972), no other remarkable advances have been presented until now. In addition, it must be stressed that in armoured heterotardigrades, the buccal tube is often poorly visible, precluding the use of *pt* indices as in eutardigrades. On the other hand, measurements of several structures are generally presented in recent descriptions of heterotardigrade species, but in the absence of precise standards, the usefulness of this practice can be questioned.

Identification is particularly difficult in the genus *Pseudechiniscus*, emended by Kristensen (1987). This genus, with 37 species described up to now, is very homogeneous. According to Kristensen (1987), two main groups can be distinguished: the *Pseudechiniscus suillus* group, with 28 species, characterized by the absence of trunk cirri and the *Pseudechiniscus victor* group composed of species with trunk cirri. Some of the species of the so-called *P. suillus* complex are very difficult to distinguish from one another. Difficulties with the identification process of these species seem to arise from older and imprecise diagnosis, the use of poor equipment and the omission of important characters. Additionally, some type material is absent or lost, precluding adequate comparisons. In addition, problems arise from the use of characters that are difficult to see, exhibit extreme variability or are strongly affected by deformation after slide mounting. These characters are subject to various interpretations. The presence or absence of faceted cephalic and terminal plates, fine or coarse dorsal granulation and small cones on lateral positions are examples of such characters. As already suggested by several authors (e.g. Maucci 1973–1974, 1986; Dastych 1984; Kathman and Dastych 1990; Pilato and Lisi 2006), a complete revision of the genus is strongly needed.

In this study, we used a traditional and a geometric morphometric approach to analyse both the shape and size of some morphological traits of different populations of species belonging to the *P. suillus* complex. Our main goal is to determine new quantitative taxonomic characters that, together with other qualitative morphological traits, can be used to discriminate cryptic species.

Material and Methods

Characterization of the biological material

This study was based on a set of 155 specimens (two-clawed larvae excluded) belonging to five different populations of the *P. suillus* group of species. The specimens were collected from mosses and lichens, during the 2005–2008 period, on the Portuguese mainland ($n = 17$); on three islands of the archipelago of the Azores: Faial ($n = 10$); S. Miguel ($n = 21$); Pico ($n = 76$); and on the island of S. Tomé (equatorial Africa) in the Democratic Republic of S. Tomé e Príncipe ($n = 31$). The great majority of the specimens were mounted in Hoyer's medium, while a very few were mounted in polyvinyl lactophenol. All of the slides are preserved in the collection of P. Fontoura (Department of Biology, Faculty of Sciences, University of Porto). Examination, identification, measurements (in micrometres, μm) and photomicrographs of the specimens were made under oil immersion, using a Zeiss phase-contrast microscope equipped with digital camera and using Axiovision 4.7.1 Imaging System Software (Carl Zeiss Microimaging GmbH, Jena, Germany).

Traditional morphometrics

In a subset of 75 specimens (8 from Portugal, 9 from Faial, 11 from S. Miguel, 25 from Pico and 22 from S. Tomé) chosen by their adequate orientation and good preservation, the length of the following 14 characters was measured: body length measured from the anterior margin to the end of the body, excluding the hind legs (BOL), and from the anterior margin of the scapular plate to the posterior margin of the pseudosegmental plate, excluding the head and the terminal plate (LUT), according to Lattes and Gallelli (1972); scapular plate (SPL); lateral cirrus A; internal cephalic cirrus; external cephalic cirrus; internal claw on legs I–IV; and external claw on legs I–IV. Some soft structures, either sex dependent or showing low variability such as the cepahalic papilla, the clava and the sensorial papilla of hind legs, were not considered. Structures were measured only if they were undamaged and their orientation was suitable. If different values were obtained when measuring symmetric structures, the larger value was chosen.

To assess variation among the five populations under study, principal component analysis (PCA) was performed on log10 ($x + 1$)-transformed measurements (for details, see Sneath and Sokal 1973). To increase the sample size, only seven variables from specimens with the complete set of measurements ($n = 45$) were used for PCA. The body length (BOL) was also excluded for analysis. We believe that the size of a specimen seems to be better represented by LUT, a measurement less influenced by deformation of the body extremities after slide mounting. Concerning the claws, only the larger values for each specimen of internal and external claws of the second and/or third pair of legs were considered for the PCA.

Morphometric indices *pb* and *psc* (where pb = length of structure × 100/LUT and psc = length of structure × 100/SPL), describing the per cent ratio between the measurement of a given structure and the body length or the Scapular plate length were also computed. Equality of medians among populations was tested by means of the non-parametric method, Kruskal–Wallis one-way analysis of variance by ranks (Siegel and Castellan 1988).

Statistical analyses were performed with software Statistica (StatSoft. Inc 2008).

Geometric morphometrics

Geometric morphometric analysis is landmark based. With this method, the morphology of an object is represented by the coordinates of a set of landmark points (Bookstein 1991). In this study, we focused on two external morphological traits: the claws (internal and external) and the dorsal segmental plates. File data were constructed using the program *tpsUtil* (Rohlf 2008a). Landmarks were placed on photomicrographs (previously aligned and having the same scale) and digitized using the program *tpsDig2* (Rohlf 2008b). Only photomicrographs of undamaged specimens providing clear and suitable oriented traits were chosen. Four landmarks were placed on each external claw (Fig. 1a). From 110 specimens (Portugal, $n = 13$; Faial, $n = 6$; S. Miguel, $n = 16$; Pico, $n = 51$; S. Tomé, $n = 24$), 168 external claws from different legs were analysed. For the analysis of internal claws, two more landmarks were added (Fig. 1a). We digitized 42 internal claws from different legs belonging to 33 specimens (Portugal, $n = 3$; Faial, $n = 3$; S. Miguel, $n = 4$; Pico, $n = 18$; S. Tomé, $n = 5$). Ten landmarks were placed (Fig. 1b) on dorsal segmental plates from 40 specimens (Faial, $n = 1$; S. Miguel, $n = 7$; Pico, $n = 20$; S. Tomé, $n = 12$). The Portuguese specimens were excluded from this analysis because their dorsal plates were indistinctly developed.

The landmarks of each object were submitted to a generalized procrustes analysis (GPA) to remove the non-shape effects of translation, rotation and scale (Bookstein 1991; Rohlf 1999). The GPA iteratively estimates a mean shape by aligning and making the superimposition of all landmark configurations. After superimposition, each landmark configuration corresponds to a single point in a non-Euclidean multidimensional space, the Kendall's shape space. Each point is then projected into a Euclidean space tangential to a reference point (the mean shape) in the shape space (Rohlf 1999). To test whether the data in the tangent space are in an almost perfect approximation with the data in the shape space, the program *tpsSmall* (Rohlf 2003) was used. Multivariate descriptions of the data in the tangent space (the shape variables) were generated by a thin-plate spline (TPS) approach (Bookstein 1991). The results of that statistical analysis that translates differences in shape among the objects were graphically visualized in terms of differences in the TPS deformation

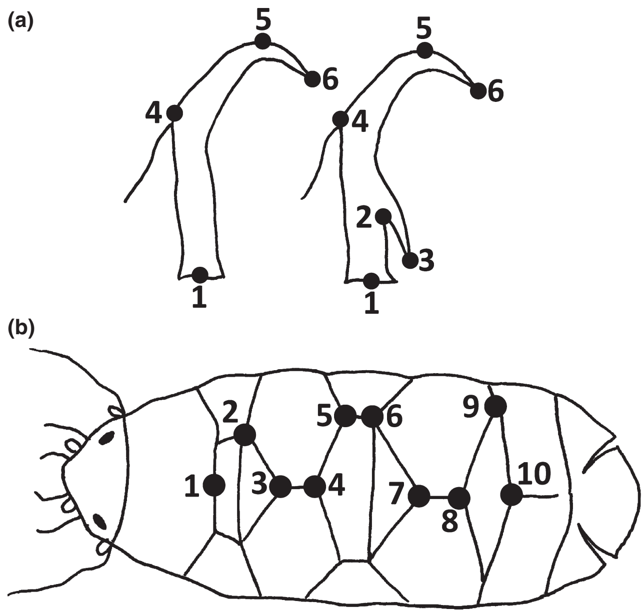

Fig. 1. Location of the landmarks on external and internal claws (a) and on dorsal plates (b)

grids where one object is deformed (or 'warped') into another and also in terms of scatterplots representing the relative warp (RW) analysis. The RWs were computed to summarize variation among the objects with respect to their partial warp scores in a few dimensions. These statistical analyses were performed using the program *tpsRelw* (Rohlf 2008c).

Results

Characterization of populations

All of the specimens were identified as belonging to the *P. suillus* complex. They were all characterized by the absence of trunk cirri (except cirrus A) and other lateral cones or tubercles and by the presence of spurs on internal claws. However, some differences among populations must be emphasized:

The specimens of the Portuguese population had unfaceted cephalic and terminal plates, dorsal cuticle sculptured with very fine dots (density on terminal plate 105–143 dots per 100 μm^2, $n = 7$) and the ventral granulation regularly distributed and not forming a reticular pattern.

The population of S. Miguel showed two peculiar characteristics: (1) the scapular plate was divided by a transverse fold into an anterior portion and a posterior portion; the posterior portion was subdivided into four plates by three longitudinal folds; and (2) the dots of the dorsal cuticle (75–127 dots per 100 μm^2, $n = 11$, on the terminal plate) were joined by very delicate striae. A reticular pattern of the ventral granulation was not visible, and in the majority of the specimens, the cephalic and terminal plates were faceted.

The specimens from Faial and Pico showed a large variability. Cephalic and/or terminal plates seemed unfaceted in the majority of the specimens but faceted in a few others. They were also characterized by having fewer dots (52–94 per 100 μm^2, $n = 26$) on the terminal plate. However, in three other specimens, the density of dots was higher, being between 122 and 159 per 100 μm^2. In some specimens from these two Azorean islands, a reticular pattern of the ventral granulation was visible. Specimens exhibiting barely perceptible dorsal dots joined by very delicate striae were also found.

In the specimens from S. Tomé, the dorsal sculpture was also constituted by dots joined by delicate striae, and ventrally, the granulation formed a reticular design. In some specimens, two triangular projections on the posterior margin of the pseudosegmental plate could be seen. In this population, the density of dots on the terminal plate was between 48 and 79 per 100 μm^2 ($n = 12$).

Traditional morphometrics

When traditional measurements were considered alone, differences between populations were not distinguishable. Actually, except the length of the lateral appendage A from the population of S. Tomé, which was always shorter, the values largely overlapped in all other measured structures (Table S1). However, this population was clearly separated by PCA, plotting PC1 against PC2 (Fig. 2a). The other four populations could not be clearly distinguished, although a slight separation of the S. Miguel and especially the Portuguese specimens (aggregated in an obvious cluster) was perceptible. The population from Pico Island showed a high heterogeneity.

All variables made an important contribution to the overall variability (Fig. 2b) expressed by the first two principal components (78.92%), with the claw lengths making a larger contribution (relative loading based on correlations 0.25 for each external and internal claw) on the first principal component (PC1) and the lengths of cirrus A and external cephalic cirrus with an heavy load on PC2 (relative loading, respectively, 0.45 and 0.43).

With morphometric indices (Table S2), groups with extremely significant different medians ($p < 0.001$) could be distinguished for almost all of the structures. The internal cephalic cirrus (*pb*) and the claws (*pb* – external claws; *psc* – internal and external claws) were the exceptions. Pairwise comparisons showed that the Portuguese population could be clearly separated from all other populations by having a smaller value of the *pb* index of the scapular plate length and a larger value of the *psc* index of cirrus A. On the other hand, a smaller value of both indices for cirrus A and *pb* of the external cephalic cirri discriminated the population of S. Tomé. The morphometric indices failed to distinguish the Azorean populations (Faial, Pico and S. Miguel) from each other.

Geometric morphometrics

When the shape of external claws was considered, RW analysis clearly discriminated the specimens from S. Tomé from all others (Fig. 3). The amount of shape variation of the relative warp 1, responsible for that separation, was considerable (67.06%). Populations from the three Azorean islands and from Portugal could not be discriminated by external claw shape differences. The examination of the TPS deformation grids corresponding to shape changes showed that the claw basal axis was shorter and the distal portion was less curved, forming a wider, more open angle in the specimens from S. Tomé. The relative contribution of landmarks 4 and 5 (0.25 and 0.49, respectively) clearly indicated this shape difference (see Fig. 4b).

With regard to the relative warp analysis of internal claws (Fig. 4a), four clusters were recognized (only 3 objects, from S.

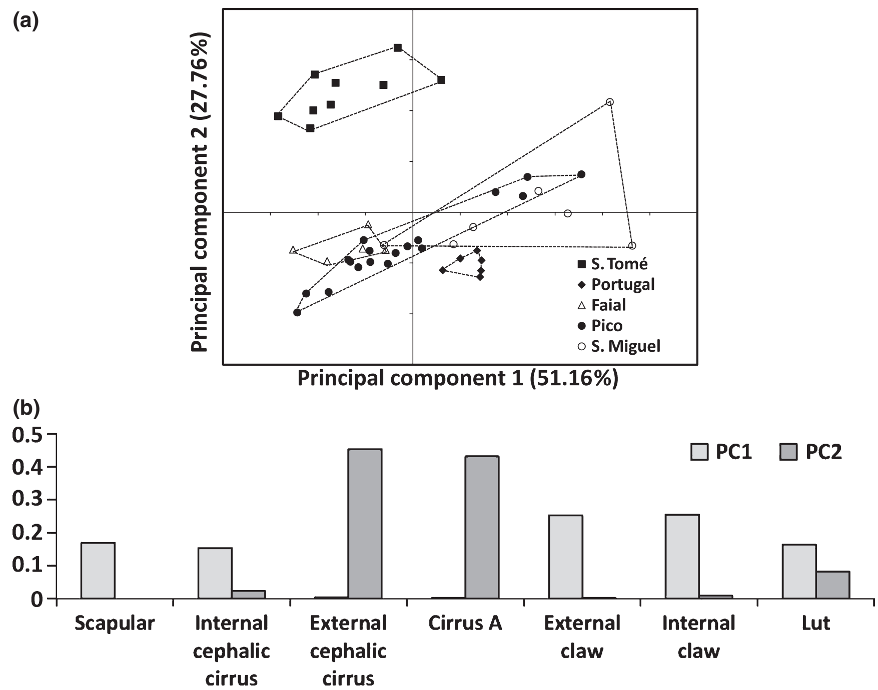

Fig. 2. Traditional morphometrics. (a) Principal component analysis of measurements of body structures of five populations of *Pseudechiniscus suillus*. (b) Relative contributions based on correlations of the variables on PC1 and PC2

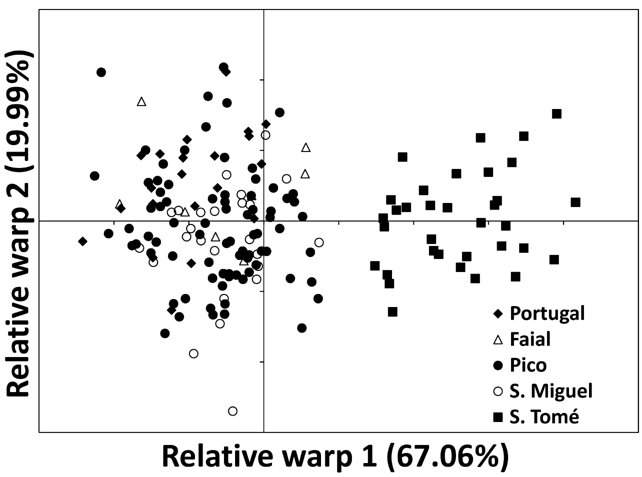

Fig. 3. Geometric morphometrics. Scatterplot of the two first relative warps scores obtained from the analysis of external claws

Tomé, Pico and Faial, do not corroborate this arrangement). On RW1, although explaining a lower amount of shape variation (40.71%) and in addition to the expected and clear separation of the population of S. Tomé with negative values of RW1, the separation of the Portuguese specimens, with the highest positive values and located very near a central cluster with all the Azorean populations, was shown. The specimens of S. Miguel were discriminated from the populations of the other Azorean Islands (Faial and Pico) by shape differences along the relative warp 2 (with a discriminatory power of 30.11%). The TPS deformation grids of internal claws confirmed the overall shape difference already mentioned for external claws. In addition, they indicated the discriminative relevance of the spur (the relative contribution of landmarks 2 and 3 was 0.28 and 0.41, respectively), showing namely in the samples from S. Miguel and Portugal that the spur was different in shape, being much longer than in the samples from Faial and Pico. The distal arc of the claw was much more closed in the Portuguese population than in the others, and the claw tip (assessed by the distance between landmarks 5 and 6) was more pronounced in the specimens from S. Miguel (Fig. 4b).

The shape differentiation among populations on the first two principal warps was also valid for the analysis of dorsal plates (Fig. 5a). Although the relatively low amount of variation explained by RW1 (38.71%), and despite some overlapping, three clusters were evident along a gradient from negative to positive values of the RW1 axis and corresponding to the populations of Pico, S. Miguel and S. Tomé, respectively. Shape differences translated by the examination of the TPS deformation grids are difficult to describe; they could be attributed to landmarks 3, 4, 6, 7 and 8 (together with a relative contribution for shape differentiation of 0.87, landmark 3 having the highest load, 0.22). Apparently, longer median plates 1 and 2 and also a longitudinally less symmetric median plate 1 discriminated the specimens from Pico relative to those of S. Tomé (Fig. 5b).

Discussion

The results obtained by the traditional morphometric approach revealed three morphs (S. Tomé, Portugal and Azores). However, by applying morphometric indices, only the morphotype from Portugal could be clearly discriminated, revealing some limitations of this methodology on the one hand and the importance of the dimensions of the scapular plate, as already suggested by Lattes and Gallelli (1972), on the other hand. As expected, the length of cirrus A and the length of the claws were the other discriminatively powerful characters. In fact, the length of cirrus A is generally accepted as a good specific character. Several authors refer to this character to describe or identify different species of the *P. suillus* complex (e.g. Ramazzotti and Maucci 1983; Maucci 1986; Dastych 1988; Kathman and Dastych 1990; McInnes 1995; Pilato and Lisi 2006). Unfortunately, the usefulness of this

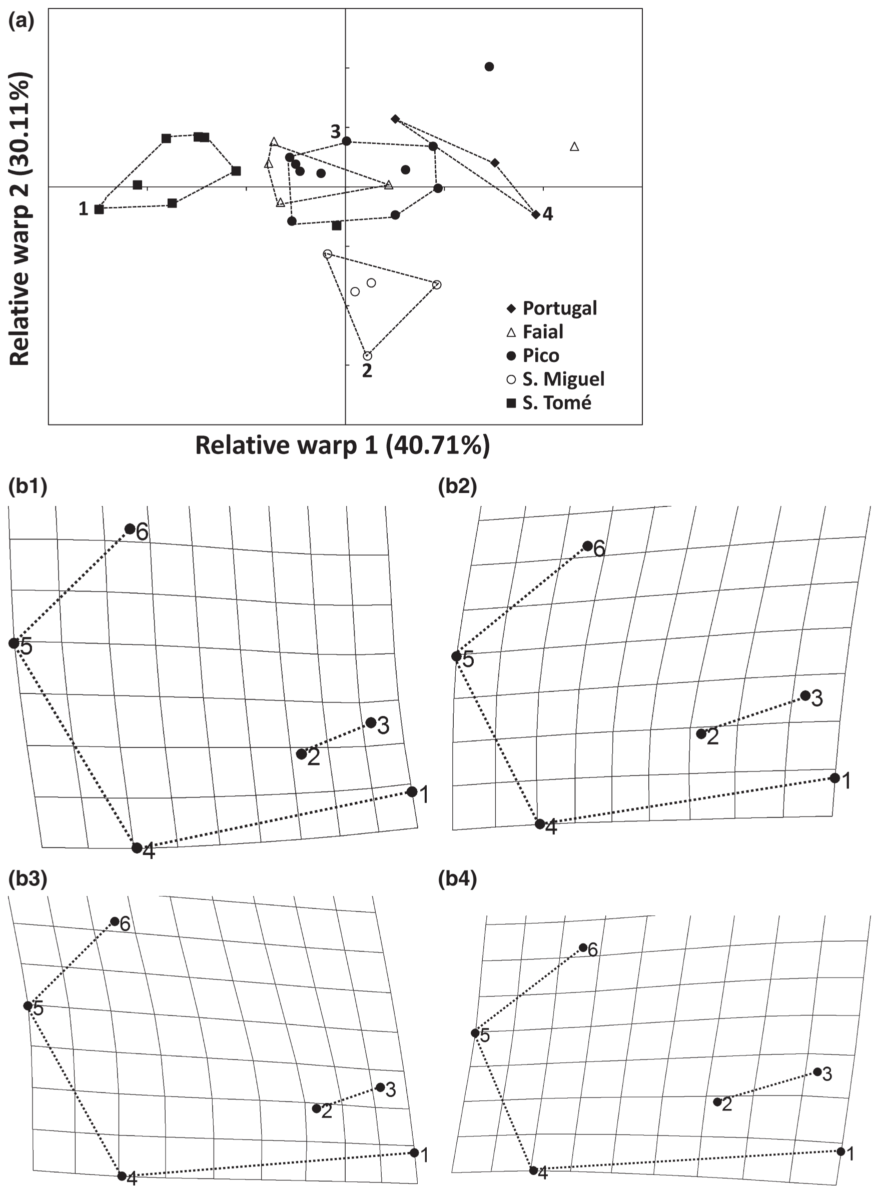

Fig. 4. Geometric morphometrics. (a) Scatterplot of the two first relative warps scores obtained from the analysis of internal claws. (b) Thin-plate deformation grids showing claw shape differences among populations: (b1) refers to S. Tomé; (b2) to S. Miguel; (b3) Pico and Faial; (b4) Portugal, all specimens also identified on the Scatterplot

character when considered alone is limited. When comparing similar species, the length of cirrus A often overlaps as in our results, morph of S. Tomé excluded.

The discriminative power of the geometric morphometric approach must be emphasized. Based on this approach, subtle shape differences in internal and external claws and on dorsal plates were noted and four morphs could be recognized. In addition to the two morphs already distinguished by traditional methods (S. Tomé and Portugal), also a morph from S. Miguel could be clearly separated from the other Azorean islands morph (Pico and Faial). It is interesting to note that no shape differences between claws on different legs were observed. The taxonomic value of the claws and dorsal plates is widely recognized. Several authors have stated the importance of those characters (Kristensen 1987; Guidetti and Bertolani 2005). However, until now the distinction among species has been based on qualitative characters, such as the presence or absence of spurs on the claws, presence of additional dorsal plates, which means that only very different species, not cryptic species, can be distinguished.

The association between some of the qualitative characters and the morphs discriminated by the traditional morphometric and geometric morphometric approaches suggests that there are four different species.

From our point of view, the specimens from S. Tomé belong to a new species that will be described elsewhere. In addition to the substantial traditional morphometric and shape differences observed, the details of the cuticular sculpture, having dorsal dots joined by striae and the presence of a peculiar ventral mesh pattern, are specific characters strong enough to make a clear distinction from other species of the group, including many having two triangular projections on the pseudosegmental plate that are also present in some specimens from S. Tomé.

The specimens constituting the morphotype from Portugal can be ascribed to *P. suillus* (*sensu stricto*). In fact, the length of cirrus A and the presence of a fine dense cuticular granulation without a reticulated ventral pattern are characters that match with the descriptions presented by several authors for this species (e.g. Maucci 1973–1974, 1986; Ramazzotti and Maucci 1983; Dastych 1988).

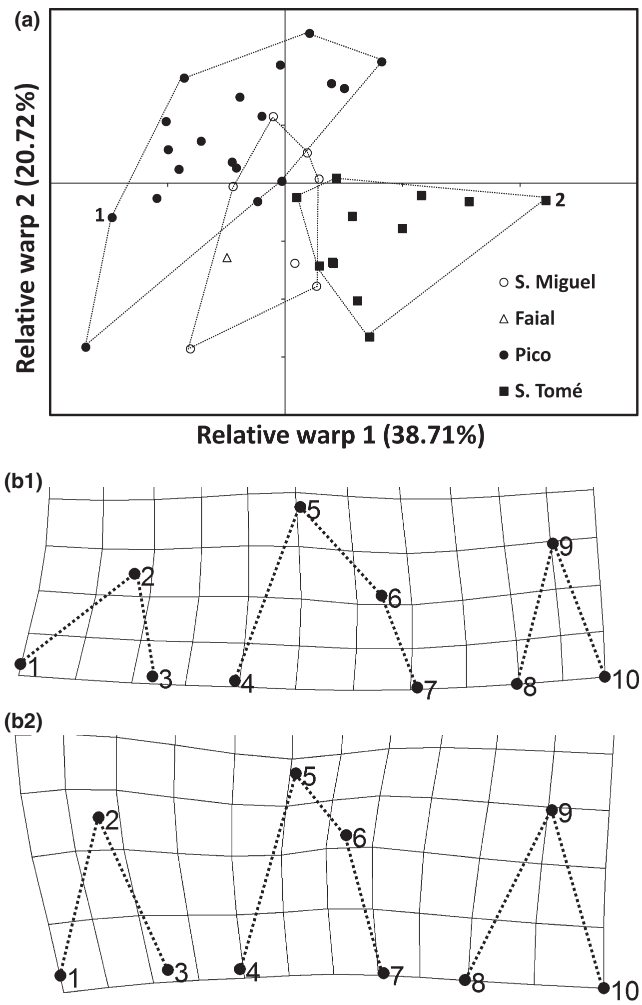

Fig. 5. Geometric morphometrics. (a) Scatterplot of the two first warps scores obtained from the analysis of dorsal plates. (b) Thin-plate deformation grids of extreme specimens identified on the scatterplot, showing plate shape differences among populations: (b1) refers to Pico (b2) to S. Tomé

The two morphotypes from Azores probably also correspond to two different species. The morphs from Pico and Faial are characterized by having a coarse dorsal granulation, a character that, according to Maucci (1973–1974, 1986), is of great importance to distinguish some species. In the current study, we attempted to evaluate the density of dots on the pseudosegmental and terminal plates and considered *Pseudechiniscus facettalis*, *Pseudechiniscus pseudoconifer* and *Pseudechiniscus juanitae* as species with coarse granulation. However, positive identification requires a deeper taxonomic study, not in the scope of this paper, including comparisons with type material of other species of the *P. suillus* complex because of the great morphometric variability observed among the specimens from Pico and Faial (and the possibility of the coexistence of more than one species) and the presence of some particularities in the specimens from S. Miguel (e.g. the dorsal cuticular dots joined by very delicate striae).

The association between the different morphs and different aspects of cuticular sculpture, certainly one of the most important specific characters of the Echiniscidae (e.g. Ramazzotti and Maucci 1983; Kristensen 1987; Kinchin 1994; Guidetti and Bertolani 2005), must be emphasized. Very probably, despite the doubts stated by Dastych (1984), the systematic value of the striation joining the cuticular dots is higher than previously thought. It is interesting to note that Antarctic specimens having this kind of cuticular sculpture identified as *P. suillus* (Dastych 1984; McInnes 1995; Miller et al. 2001) will probably belong to a new species whose description was announced (McInnes 1995) but never published.

Similar conclusions can be drawn concerning the Canadian specimens also attributed to *P. suillus* by Kathman and Dastych (1990) that, as indicated by these authors, could be confused with *P. facettalis*. These Canadian specimens probably belong to the same species of the specimens from S. Miguel (Azores) that we studied. The subdivisions of the scapular plate, faceting of the terminal plate and dorsal plates also sculptured with dots joined by striae are characters shared by both the Canadian and Azorean populations.

Our results also suggest that, in future taxonomic work, special attention must be focused on aspects of the ventral sculpture, a character sometimes neglected, reinforcing the observation of Dastych (1984). This author first described the ventral mesh pattern in *P. suillus* specimens from Antarctica, referring to the presence of this character also in specimens from Europe, Africa and Korea, but not in Himalayan specimens that showed a uniform ventral granulation. A ventral net pattern has been described for other *Pseudechiniscus* species, all of them from the *P. suillus* complex, either in redescriptions of known species (*P. pseudoconifer*, *Pseudechiniscus jiroveci* and *P. juanitae*, made respectively by Maucci 1973–1974; Binda 1984; Pilato and Lisi 2006) or in the descriptions of new species (*Pseudechiniscus brevimontanus* by Kendall-Fite and Nelson 1996; *Pseudechiniscus asper* by Abe et al. 1998; and *Pseudechiniscus spinerectus* by Pilato et al. 2001). As stated by Pilato et al. (2001), different details of the ventral net pattern (lateral meshes elongate or isodiametric) can be distinguished in different species, also indicating the taxonomic value of the ventral sculpture.

The limitations to the use of morphometric approaches stated by Kinchin (1994, 1996) also affected this study. Problems with data acquisition caused by the orientation and deformation of the slide-mounted specimens were responsible for the exclusion of some variables, such as the dimensions of flexible structures, and consequently for both the low number of taxonomic characters and the small sample size that could be used for some analyses. This problem was particularly severe in our attempt to apply the geometric morphometric techniques to the study of the dorsal plates. However, it must be stressed that in tardigrade taxonomic studies the identification process is often already based on small numbers of samples. In addition, the important information that can be acquired by the morphometric approaches compensates some of the referred problems.

In conclusion, both the traditional morphometric approach, including the computation of indices comparing relative dimensions of morphological structures, and the geometric morphometric approach are useful tools for the specific diagnosis of heterotardigrades. Shape analysis, in particular, can open new perspectives for that purpose and can stimulate studies on the much needed revision of the *P. suillus* species complex.

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Résumé

Discrimination d'espèces cryptiques appartenant au groupe Pseudechiniscus suillus *(Tardigrada, Echiniscidae) basée sur la morphométrie traditionelle et la géométrie morphométrique*

La taxonomie des tardigrades pose encore d'énormes difficultés au niveau de l'identification des espèces. Le problème devient particulièrement grave pour un groupe d'espèces très semblables qui composent le complexe *Pseudechiniscus suillus* qui a besoin d'une profonde révision. Dans ce travail on a étudié la morphométrie d'exemplaires appartenant au groupe *P. suillus*, en utilisant non seulement les méthodes traditionnelles, mais aussi les modernes techniques de la géométrie morphométrique. On a cherché des rapports parmi cinq différentes populations provenantes du Portugal et de quatre îles atlantiques, trois aux Azores et l'île de S. Tomé, qui puissent contribuer pour une discrimination objective de quelques espèces cryptiques de ce groupe. L'étude est basée sur la variabilité, mesurée par l'analyse en composantes principales de sept variables morphométriques traditionnelles. En plus, les différences de forme, même que subtils, de deux structures, les griffes et les plaques segmentales dorsales, on été analysées. Les résultats suggèrent que quatre morphotypes caractérisés par différences au niveau de la dimension et forme du cirre A, des griffes et des plaques dorsales, peuvent être considérés. La grande valeur taxonomique de ces caractères et, en même temps, des différences observées en quelques aspects qualitatifs comme la sculpture cuticulaire, semble indiquer que différentes espèces peuvent correspondre à ces morphotypes. Ainsi, peut-on conclure que les techniques de la géométrie morphométrique constituent un complément très intéressant à la morphometrie traditionnelle pour discriminer des espèces cryptiques d'heterotardigrades.

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Table S1. Traditional measurements (mean, minimum-min, maximum-max, and standard deviation-SD, in μm) of some structures on five populations belonging to the *Pseudechiniscus suillus* group of species.

Table S2. Values of morphometric indices (mean, minimum-min, maximum-max, and standard deviation-SD) describing the percent ratio between the measurement of a given structure and the body length (*pb*) or the Scapular plate length (*psc*) on five populations belonging to the *Pseudechiniscus suillus* group of species. Results of the Kruskal-Wallis test (H) are given for the median of each struture (*extreme significance, $p < 0.001$). Results of pairwise comparisons are also indicated; significant different medians are identified by different small letters (a, b, c).

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