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Integrative taxonomy reveals a new genus and new species of Philosciidae (Crustacea: Isopoda: Oniscidea) from Neotropical region

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**Integrative taxonomy reveals a new genus and new species of Philosciidae (Crustacea: Isopoda: Oniscidea) from Neotropical region.**

B.L. Zimmermann, I.S. Campos-Filho, and P.B. Araujo.

**Abstract:** Although new methods and data are conquering space in the field of taxonomy, such as integrative taxonomy, most of the terrestrial isopod species are still described based only on morphology. Species of the genus *Atlantoscia* Ferrara & Taiti, 1981 were the first and are the unique terrestrial isopods from the Neotropics for which a molecular phylogeny was already conducted. Previous results indicated that this genus could be paraphyletic, and a more detailed analysis would be required. Our aim was to reconstruct the phylogeny of *Atlantoscia* using mitochondrial and nuclear markers and test its monophyly by integrating molecular and morphological data. We observed that, indeed, *Atlantoscia* is paraphyletic. *Atlantoscia ituberasensis* Campos-Filho, Lisboa & Araujo, 2013 and *Atlantoscia rubromarginata* Araujo & Leistikow, 1999 were placed in a new genus of terrestrial isopods, *Paratlantoscia* gen. nov., together with a new species described in the present study, *Paratlantoscia robusta* sp. nov. The new genus is defined by the presence of specialized respiratory areas in the pleopod exopods and its validity is highly corroborated by molecular analyses and by biogeographic information. This study highlights the importance of multiple and complementary perspectives as a way to improve the quality of species hypothesis and associated descriptions.

**Keywords:** *Atlantoscia*, Brazilian Atlantic Forest, isopods phylogeny, Neotropics, *Paratlantoscia* gen. nov., *Paratlantoscia robusta* sp. nov.,
**Introduction**

Species are a cornerstone of biology, ecology, and conservation (Petit and Excoffier 2009). Hence, identifying the boundaries between a set of species has been a largely discussed issue in the last years in the field of systematic biology (e.g., De Queiroz 2007; Knowles and Carstens 2007; Aguilar et al. 2013; Grummer et al. 2014). Due to practical and historical reasons most species were primarily described based on morphology for most part of our history (Padial et al. 2010). Nonetheless, new methods and data are conquering space in taxonomy. For example, assigning groups of organisms to species is one of the most challenging aspects of phylogenetic studies (Coyne and Orr 2004).

One of these new approaches is integrative taxonomy. It integrates information from different sources (usually from morphological and molecular data) to delimit and describe taxa. The result is improved rigor in species delimitation, which is its ultimate goal as well (Marshall et al. 2006; Schilick-Steiner et al. 2010; Castalanelli et al. 2017). Integrative taxonomy has gained many supporters in recent years, especially because this approach has been proved to be effective in solving systematic issues in a variety of groups (e.g., Castro-Romero et al. 2016; Decker et al. 2016; Ekimova et al. 2016; Hernandez-Orts et al. 2017; Mazancourt et al. 2017), thus improving the quality of species hypothesis and associated descriptions (Pante et al. 2015). In this context, terrestrial isopods (Oniscidea) are an interesting group, since the majority of the species are still described based only on morphology, even though in many cases morphological characters do not provide a clear taxonomic resolution (Schmalfuss 2003). Terrestrial isopods comprise the most successful colonizers of terrestrial environments among crustaceans and show morphological, physiological, and behavioral adaptations to this lifestyle (Richardsone and Araujo 2015). For example, the development of the respiratory structures on the pleopod exopods was a key factor in
the success of terrestrial colonization in woodlice. Variations in these structures represent both convergence and functional constraints. The complexity of respiratory structures is generally linked to different levels of environment adaptation (Richardson and Araujo 2015) and could potentially be used in the species delimitation.

Among the main representatives of terrestrial isopods we can highlight the family Philosciidae Kinahan, 1857, one of the most important groups in tropical and wetlands habitats (Leistikow 2001; Schmalfuss 2003; Campos-Filho et al. 2014). Within Philosciidae, the genus *Atlantoscia* Ferrara & Taiti, 1981 consists of seven described species, mainly recorded in Brazil, except for the widely distributed species *A. floridana* Van Name, 1940 (Campos-Filho et al. 2013). Members of this genus differ in few morphological characteristics and are defined based mainly on the shape of the male pleopods (Campos-Filho et al. 2013). Recently, Zimmermann et al. (2015) tried to overcome these taxonomic problems within *Atlantoscia*. The authors demonstrated that using integrative taxonomy by coupling morphology and DNA sequences is an efficient strategy for delimitation of *Atlantoscia* species and to investigate their relationships. However, some issues still persist. For instance, *Atlantoscia rubromarginata* Araujo & Leistikow, 1999 and *Atlantoscia ituberasensis* Campos-Filho, Lisboa & Araujo, 2013 are quite different from the other congeneric species and thus might not belong to this genus (Zimmermann et al. 2015). Specifically, they are genetically and geographically distant and possess unique and specialized morphologies, with well developed respiratory areas in the pleopods (Campos-Filho et al. 2013).

Taking into account that populations that are considered to be candidate species will typically exhibit some evidence of genetic isolation, either in the form of phenotypic/genetic differences or geographic separation (Jackson et al. 2017), our aim was to reconstruct the phylogeny of the species in *Atlantoscia* using mitochondrial and nuclear markers and test its monophyly by integrating molecular, morphological and biogeographical data. We observed, in this study, that *Atlantoscia* is paraphyletic. *Atlantoscia ituberasensis* and *A. rubromarginata* were grouped with a new species
described in the present study and all of them should be placed in a new genus of terrestrial isopods. Information from morphology, genetic and biogeography corroborate these findings.

Material and Methods

Material studied

Terrestrial isopods used in this study come from samples deposited in scientific collections of Museu de Zoologia (MZUSP), Universidade de São Paulo, São Paulo and Coleção de Carcinologia (UFRGS), Universidade Federal do Rio Grande do Sul, Porto Alegre. Individuals from an undescribed species sampled in the Brazilian state of Bahia and possibly related to the Atlantoscia genus (due to morphological similarities) were also examined.

Morphological Analysis: description of the new genus and new species

The specimens were dissected and the appendages and pereonites were mounted on slides. Drawings were prepared using a camera lucida. The noduli laterales were measured and illustrated as in Vandel (1962). The terminology for the setae follows Campos-Filho et al. (2013). The descriptions are based on the paratypes mounted on slides. The material was deposited in the same museums mentioned in the previous session.

Molecular Analysis: DNA extraction and PCR amplification

Dissections were conducted as described by Bouchon et al. (1998). Total DNA was extracted using a PureLink Genomic DNA kit (Invitrogen/K1820-01). PCR amplifications were performed using
the *LCO*/*HCO* primer set (Folmer et al. 1994), which amplifies an approximately 700-bp fragment of the mitochondrial *COI* gene (*COI*) and using primers *SSU_04F*/*SSU_R22* (Blaxter et al. 1998) which amplifies an approximately 500-bp fragment of the nuclear *18S rRNA* gene (*18S*). Standard polymerase chain reaction (PCR) was run and PCR products were checked by agarose gel electrophoresis (see Zimmermann et al. 2015). All DNA purification (performed with ExoSAP-IT PCR Product Cleanup Reagent) and sequencing was carried out by Macrogen (Seoul, South Korea), using BigDye technology, with each sample being sequenced in both the forward and reverse directions using the same primers as those used in amplification. All sequences generated in this study were deposited in the GenBank database (http://www.ncbi.nlm.nih.gov/). We also used *COI* mitochondrial haplotypes obtained by Zimmermann et al. (2015) for phylogenetic analysis. The analyzed terrestrial isopods and GenBank accession numbers are shown in Table 1. All applicable international, national, and/or institutional guidelines for the care and use of animals were followed.

**Outgroup choice**

Outgroup can affect reconstructed phylogenies due to heterogeneity of evolutionary rates and variation on base composition among taxa (Brinkmann and Philippe 2008; Caravas and Friedrich 2010). One way to overcome this problem is to choose species that are closely related to the ingroups and include multiple outgroups (Brinkmann and Philippe 2008). We chose ten different outgroups for the mitochondrial tree and five for the nuclear tree. It is worth mentioning that terrestrial isopods, and in particular the family Philosciidae, are a group in which studies with molecular biology are still scarce. Thus, few species have DNA sequences available for constructing molecular phylogenies. The sequences of all specimens used as outgroup species were downloaded from GenBank.
Phylogenetic Analysis

Consensus sequences were aligned with Muscle (Edgar 2004), implemented in MEGA v. 6.0 (Tamura et al. 2013). Phylogenetic analyses were conducted for mitochondrial and nuclear sequences data sets using Bayesian (BI) and maximum likelihood (ML) methods. The best-fit models of sequence evolution for each gene were selected with JModeltest 2.1.10 (Darriba et al. 2012). The GTR + I + G model was selected for both mitochondrial and nuclear sequences. Bayesian analyses were performed using Monte Carlo Markov Chain (MCMC) implemented in BEAST version 1.8.0 (Drummond et al. 2012). The Bayesian analysis was run for 50 million chains and sampled every 1,000 generations. The species-tree prior assumed a Yule process. Posterior probabilities were calculated with a burn-in of 5 million states and checked for convergence using Tracer version 1.6 (Rambaut et al. 2014). ML analyses were performed with raxmlGUI 1.5 (Silvestro and Michalak 2012) using a nodal support estimated by 1,000 bootstrap replicates. Divergence times, i.e. time to the most recent common ancestor (TMRCA), among mitochondrial haplotypes were also estimated with BEAST with four gamma categories and the strict molecular clock. The COI substitution rate employed was 0.0164 ± 0.008 substitutions per site per Myr, an estimative used for other terrestrial isopods (Poulakakis and Sfenthourakis 2008; Lee et al. 2014). All results were visualized and checked with FigTree 1.4.2 (Rambaut 2014). Pairwise genetic distances of COI and 18S sequences were calculated using p-distance model in MEGA 6.0 (Tamura et al. 2013). The average values of the intraspecific and interspecific distances were obtained with 1000 bootstrap replicates.

Testing species models hypotheses
We attempted to test the monophyly of *Atlantoscia* by comparing species models hypotheses using a Bayes Factor (BF) approach for model selection. The tested models were: Model 1 = constrained tree considering *Atlantoscia* as monophyletic (i. e., one main clade with all species included); and Model 2 = constrained tree considering *Atlantoscia* as paraphyletic (i.e., two distinct clades, according to the tree generated in this study). BF calculates the ratio of the marginal likelihood of two models, which has the advantage of taking into account priors used in Bayesian analysis (Xie et al. 2011). The marginal likelihood values of these competing models were estimated using stepping-stone sampling (SS, Xie et al. 2011) in BEAST package and run for 10 million generations of 50 path-steps. The better model was chosen when twice the natural logarithm of the Bayes-factor testing statistic ($2 \ln B_f$) was greater than 2 (Kass and Raftery 1995). A value greater than 10 was assumed to indicate decisive support for distinguishing between competing species-delimitation hypotheses (Grummer et al. 2014). All parameters were setup as described in the Section Phylogenetic Analyses. This allows for the direct comparison of the two models considering both the topology and the branch lengths of species trees.

**Results**

**Taxonomy**

*Paratlantoscia* B. L. Zimmermann, I. S. Campos-Filho & P. B. Araujo, gen. nov.

Type species: *Paratlantoscia robusta*, sp. nov. by present designation.
Species included: *Paratlantoscia ituberasensis* (Campos-Filho et al., 2013), *Paratlantoscia robusta* sp. nov., and *Paratlantoscia rubromarginata* (Araujo & Leistikow, 1999).

Genus diagnosis

Body convex; cephalon with supra-antennal line, without frontal line, lateral lobes not developed; telson triangular with lateral margin sinuous; antennal flagellum of three articles; mandibles with molar pecinil dichotomized; maxillula outer endite of 4+6 teeth; maxilla bilobate; dactylar organ longer than outer claw; uropod endopod inserted proximally; pleopod 1-5 exopods with well-developed uncovered lungs.

Remarks

The genus *Atlantoscia* was erected by Ferrara and Taiti (1981) to allocate *A. alceui* from Ascension Island (currently synonym of *A. floridana*). The authors distinguished the genus from the Mediterranean genus *Chaetophiloscia* Verhoeff, 1908 by having: pleopod 1 exopod with uncovered lung, and distinct *noduli laterales* coordinates. Araujo and Leistikow (1999) described the second species of the genus, *A. rubromarginata* from the state of Sergipe. This species was distinguished from *A. floridana* by the pleopod exopods (all of them) with respiratory areas partially covered. Campos-Filho et al. (2012) described one new species from the State of Rio Grande do Sul, *Atlantoscia petronioi* Campos-Filho et al. 2012. Subsequently, Campos-Filho et al. (2013) described *Atlantoscia sulcata* Campos-Filho et al. 2013 from the state of São Paulo, and *A. ituberasensis* from the state of Bahia. Based on author’s descriptions the last two species show well developed respiratory areas on all pleopod exopods (especially *A. ituberasensis*), characteristic shared with *A. rubromarginata*. Recently, Zimmermann et al. (2015) based on molecular and morphological evidences described *Atlantoscia inflata* Campos-Filho & Araujo, 2015 from the state of Rio Grande do Sul, and *Atlantoscia meloi* Campos-Filho & Araujo, 2015 from the state of Santa Catarina. The
authors also found some molecular divergences within *Atlantoscia*. In one of those divergences *A. ituberasensis* and *A. rubromarginata* were recovered in a clade with close relationships and with high molecular divergence from the main clade of *Atlantoscia* (see Fig. 5 on Zimmermann et al. 2015). As mentioned by the authors, within *Atlantoscia*, only these species have well-developed respiratory areas on pleopod exopods (see also Araujo and Leistikow 1999; Campos-Filho et al. 2013) - these structures were previously recognized and classified as uncovered lungs by Ferrara et al. (1994). The authors mentioned that the molecular divergence between the clades could have resulted from geographic isolation, and the presence of specialized respiratory organs could be related to an adaptation to high temperatures and/or dry conditions.

*Atlantoscia ituberasensis* and *A. rubromarginata* are recorded in the Brazilian Atlantic Forest of the states of Bahia and Sergipe, northeastern Brazil (see distribution map in Campos-Filho et al. 2013). This region is inserted in the Equatorial Rainforest (Af) or Equatorial Savannah climate classifications (Aw), characterized by high temperatures, dry and concentrated precipitations periods along the year (Kottek et al. 2006; Peel et al. 2007).

*Paratlanoscia* gen. nov. most resembles the genus *Atlantoscia* in almost all morphological characteristics, but it can be distinguished by having well-developed uncovered lungs on all pleopod exopods. *Paratlanoscia* gen. nov. is supported in the integrative view using molecular, morphological and biogeographical evidences.

*Paralantoscia robusta* B. L. Zimmermann, I. S. Campos-Filho & P. B. Araujo, *gen. et sp. nov.*

Figs. 1 - 4

Zoobank: urn:lsid:zoobank.org:pub:0E1E3881-E48E-4123-9458-6DBB6DB6C3A0.


Etymology: The new species name refers the robust shape of the animal.

Description

Maximum body length: ♂ 5.2 mm, cephalon width 1.0 mm; ♀ 5.3 mm, cephalon width 1.2 mm. Color light brown; cephalon with irregular unpigmented spots; antennae with third article and distal half portion of fifth article of peduncle and second article of flagellum unpigmented; pereonites 1-7 epimera strongly pigmented, unpigmented longitudinal spots on median and paramedian areas, epimera 5-7 unpigmented on posterior corners; pleonites 2, 4 and 5 strongly pigmented, pleonites 1-3 with median longitudinal unpigmented area; telson with three unpigmented spots (Fig. 1A). Dorsum smooth covered with sparse short scale-setae. Cephalon (Figs. 1B-C) with supra-antenal line slightly bent downwards in the middle; eyes of about 11-13 ommatidia arranged in rows. Pleon (Fig. 1A) narrower than pereon; neopleurae 3-5 well-developed,
posterior corners acute and directed backwards. Telson (Fig. 1D) triangular, distal portion concave. *Noduli laterales* (Figs. 1E-G) flagelliform, d/c coordinates reaching maximum on pereonite 4 relative to lateral margin of pereonites, b/c coordinates gradually decreasing relative to posterior margin of pereonites. Antennula (Fig. 1H) with distal article longest, eight aesthetascs arranged in three rows plus apical pair. Antenna (Fig. 1I) when extended posteriorly reaches posterior margin of fourth pereonite; flagellum with distal article longest; apical organ short, about half of length of distal article of flagellum. Mandibles with molar penicil of about five branches and dense cushion of setae, left mandible (Fig. 2A) with 2+1 penicils, right mandible (Fig. 2B) with 1+1 penicils. Maxillula (Fig. 2C) inner endite with two slender penicils, distal margin rounded; outer set of outer endite with slender seta and accessory tooth, inner set with five teeth cleft at apex, one of them trifid. Maxilla (Fig. 2D) outer lobe twice as wide as inner endite, distal margin rounded covered with thin setae; inner lobe rounded covered with thick setae. Maxilliped (Fig. 2E) base rectangular bearing sparse piliform scale-setae, cuticle scaled proximally and distal margin slightly prominent bearing slender fringe of thin setae; endite sub-rectangular covered with thin setae on distal portion, distal margin rounded bearing two hook-like setae, median seta not surpassing the distal margin. Pereopods (Figs. 3A-C) rather slender bearing sparse long setae along sternal margin of merus and carpus; carpus 1 with transverse antennal-grooming brush and distal seta with hand-like apex; dactylus with inner claw reaching distal margin of outer claw, ungual seta simple not surpassing distal margin of outer claw, dactylar organ with lanceolate apex. Uropod (Fig. 4A) protopod grooved on outer margin, exopod and endopod with sparse setae along inner and outer margins; exopod longer than endopod, grooved on outer margin and bearing five setae on apex, endopod with three setae on apex.

Male: Genital papilla with triangular ventral shield and subapical orifices with thin and short setae (Fig. 4B). Pleopod 1 (Fig. 4C) exopod heart-shaped, outer margin sinuous with three setae; endopod 1 robust, slightly bent outwards, distal part pointed with slightly inflated apex. Pleopod 2
(Fig. 4D) exopod triangular, outer margin concave with two setae, distal portion elongated; endopod 2 slender, reaching fourth pleopod. Pleopod 3 triangular, outer margin with 5 setae, distal portion elongated (Fig. 4E). Pleopod 4 subtriangular, outer margin with with 4 setae near the distal portion (Fig. 4F). Pleopod 5 exopod (Fig. 4G) triangular with transverse plumose fringe, outer margin slightly sinuous bearing two setae (one on tip), distal portion elongated and acute.

Molecular Analyses

All the specimens utilized in the phylogenetic analysis are described in Table 1. We obtained COI sequences for two individuals of Paratlantoscia robusta sp. nov. (640 bp unambiguous alignment), and nuclear 18S sequences for 30 individuals belonging to nine species (434 bp aligned, including gaps). No intraspecific variation was observed in the 18S sequences. The number of parsimony informative sites was 162 for COI and 20 for 18S sequences. Tree topologies were congruent between maximum likelihood and Bayesian analyses. Besides that, the phylogenies generated with both mitochondrial (Fig. 5) and nuclear (Fig. 6) sequences were congruent regarding the taxa of interest; that is, both markers support the monophyly of Paratlantoscia. The results observed confirm the paraphyly of Atlantoscia, justifying the new genus Paratlantoscia with the inclusion of the new species described P. robusta plus P. ituberasensis and P. rubromarginata, positioned in a distinct and well supported clade. The other species of Atlantoscia, A. floridana, A. inflata, A. meloi, A. petronioi and A. sulcata also comprise a well-supported clade and can be considered a monophyletic group.

The average mtDNA genetic distance among species of Atlantoscia was 15.7%, ranging from 13.6 to 18.3%, and among species of Paratlantoscia was 16.5%, ranging from 13.1 to 18.4% (Table S1). The mean mtDNA genetic divergence between genera was 20.8%. In relation to the nuclear sequences, the average genetic distance between genera was 5.4%, a considerably higher value than
that observed among species of *Atlantoscia* (0.7%) and *Paratlantoscia* (1.7%) (Table 2).

Interestingly, *Atlantoscia* species appear to be more related to the specimen of *Balloniscus sellowii* (Brandt, 1833) than to the *Paratlantoscia* species (Figs. 5 and 6). However, despite this supposed closeness, *B. sellowii* is also genetically distant from the *Atlantoscia* species (e.g., they show a genetic divergence of 4.1% among the nuclear gene sequences).

Bayes Factor model selection results show that SS marginal likelihood estimators prefer Model 2 over Model 1 (-5,668.73 versus -5,681.05; $2\ln \text{BF} = 24.64$), suggesting the paraphyly of *Atlantoscia*. Estimates of divergence times show that *Atlantoscia* and *Paratlantoscia* seem to have diverged at about 22.93 Mya. The TMRCA for *Atlantoscia* and *Paratlantoscia* haplotypes was estimated at 14.02 and 16.09 Mya, respectively. Divergence times among congeneric species are more recent, most of them dating from Pleistocene (between 11 and 8 Mya).

**Discussion**

**A new genus of terrestrial isopods described by means of integrative taxonomy**

This study demonstrated that *Atlantoscia* constitute a paraphyletic assemblage. Additionally, a new genus and a new species of terrestrial isopod were described, *Paratlantoscia robusta* gen. nov et sp. nov., and other two species of *Atlantoscia* were placed within this new genus. The validity of this new taxonomic entity was supported both by morphological and molecular data, reinforcing the merits of integrative taxonomy to delimit species and other taxa boundaries.

As pointed out previously, *Atlantoscia* species are morphologically similar and the diagnostic characteristics traditionally used to delimit species are few and subtle (Campos-Filho et al. 2013; Zimmermann et al. 2015). Despite this similarity, the respiratory areas on the pleopod exopods of
*Paratlantoscia* are considerably more developed than in *Atlantoscia*. The various stages of the evolution of respiratory areas in Oniscidea are considered a key factor for their terrestrial colonization (for a more comprehensive overview see Hornung 2011; Richardson and Araujo 2015). Specifically, specialized respiratory areas are observed typically in derived taxa presumably as an adaptation to life in xeric environments (Paoli et al. 2002). Furthermore, all representatives of *Paratlantoscia* are found only in northeastern Brazil, a region characterized by high temperatures and dry climate (Kottek et al. 2006). Therefore, since the presence of well develop respiratory area is one of the main diagnostic features of *Paratlantoscia*, the inclusion of *A. ituberasensis* and *A. rubromarginata* into the new genus is justified: they present more developed respiratory areas than what is expected for *Atlantoscia*.

In addition to the morphological evidence, molecular analysis with both mitochondrial and nuclear genes also showed that the *Atlantoscia* and *Paratlantoscia* comprise distinct and well supported clades with a high genetic divergence between them. Levels of sequence divergence found in this study are consistent with other studies conducted for congeneric species of terrestrial isopods (e.g., Rivera et al. 2002; Cooper et al. 2008; Sicard et al. 2014; Javidkar et al. 2016; Karasawa 2016), corroborating results of the morphological analysis and the effectiveness of an integrated approach to solve taxonomic issues.

We should mention that while the development of new concepts and approaches are indispensable, more taxonomists and more funding for taxonomy are also urgently needed. A stronger focus on actually publishing systematic revisions and species descriptions should be part of a strategy to help overcoming the taxonomic impediment (Evenhuis 2007). In most cases, when new species are discovered, they are not systematically described (Pante et al. 2015). Taxonomic studies are the foundation of biological research and naming newly delimited species should be strongly stimulated.
Molecular clock and biogeography aspects

Geographic distribution and divergence times of *Atlantoscia* and *Paratlantoscia* provide additional evidence for the existence of these two genera. *Atlantoscia* species are mainly distributed in the southeast- and southern Brazil (i.e., they are included in the southern component of the Brazilian Atlantic Forest - BAF), except the widespread *A. floridana*, recorded from USA, Brazil, Argentina, and Ascension and St. Helena Islands (Campos-Filho et al. 2013). On the other hand, *Paratlantoscia* gen. nov. occurs only in northeastern Brazil, the northern component of the BAF (Araujo and Leistikow 1999; Campos-Filho et al. 2012, 2013; Hoffmesiter and Ferrari 2016).

One of the main geographic barriers associated to the general historical patterns of taxa distribution in the BAF is the Doce River, which separates the Northern and Central components from the Southern component (DaSilva et al. 2015; Hoffmesiter and Ferrari 2016). Considering that the divergence between *Atlantoscia* and *Paratlantoscia* was estimated in the Late Miocene (ca. 23 Mya), and based on their distribution patterns, past events as marine transgressions and/or tectonic activities, Doce River probably have drove the split of *Atlantoscia* and *Paratlantoscia* gen. nov. (see also Almeida and Carneiro 1998; Mohriak 2003; DaSilva et al. 2015; Thomaz et al. 2015). Large rivers are clear geographic barriers for the terrestrial invertebrate fauna not adapted to fly (Dantas et al. 2011), and similar results were reported for other groups in the BAF during the Miocene (e.g., Pellegrino et al. 2005; Fouquet et al. 2012; Roxo et al. 2014). On the other hand, climate fluctuations and tectonic events during the Pliocene-Pleistocene would have led to the radiation of *Atlantoscia* and *Paratlantoscia* species. It is interesting to highlight that representatives of *Paratlantoscia* have larger sizes than those of *Atlantoscia* (data not shown). In an evaluation of biogeography of body size in terrestrial isopods, the authors concluded that climatic factors alone do not define the differential response of body size among isopod species. This variation, on the other hand, seems to be best predicted by generic affiliation (Karagkouni et al. 2016).
Contrary to other species of *Atlantoscia*, the biogeographic history of the widely distributed *A. floridana* is quite hard to trace. As previously mentioned, *A. floridana* is recorded from coastal regions of Florida, USA until north of Argentina, and Ascension and St. Helena islands. Along this wide distribution range many historical barriers are well known (e. g., Nihei and Carvalho 2007; Morrone 2014; Silva and Noll 2014; DaSilva et al. 2015). Thus, the distribution of *A. floridana* is probably the result of human introductions (see also Ferrara and Taiti 1981). This argument is supported by molecular comparisons between sequences of *A. floridana* specimens from Florida and south Brazil, which demonstrated high levels of similarity (based on DNA Barcoding http://www.boldsystems.org/, data not shown). In addition, *A. floridana* is referred as an abundant species (Lopes et al. 2005; Bugs et al. 2014) and is classified as *r*-strategist, which explains the high success to adapt and colonize distinct environments along its distribution range (Quadros and Araujo 2007; Quadros et al. 2009).

Recently, many efforts have been made to describe the diversity of the terrestrial isopods from Neotropics (e. g., Schmidt, 2007; Campos-Filho et al. 2013, 2014, 2015; Souza et al. 2015; López-Orozco et al. 2017). Unfortunately, few studies had focused in the BAF (e. g., Campos-Filho et al. 2013, 2015; Zimmermann et al. 2015). Many surveys were made in the last years and many specimens still wait for recognition and formal descriptions (e. g., Fernandes et al. 2015; Silva and Ferreira 2015). The identification of this hidden diversity provides very useful information to conduct evolutionary and conservation works, giving a better understanding about the species distribution patterns in the Atlantic forest.

In summary, in this study we used evidence from different sources to demonstrate that *Atlantoscia* is a paraphyletic group. Previously, morphological variations in the complexity of respiratory structures in *Atlantoscia* species were not considered a strong enough argument to challenge the validity of this genus. However, molecular and biogeographic data reinforced and corroborated the hypothesis that *A. rubromarginata* and *A. ituberasensis* (with their specialized
morphologies) compose a new genus, *Paratlantoscia*. In addition, a new species of *Paratlantoscia* was described, which also exhibits well developed respiratory areas in the pleopods. We hope that the current popularity of integrative taxonomy will instigate researchers to use this approach, thus enhancing the quality of taxonomic descriptions and facilitating taxa delimitation.

**Acknowledgments**

We are grateful to Profs. Marcos Tavares and Maria José from MZUSP for the assistance in material deposit and collection analysis process; to Coordenação de Aperfeiçoamento de Pessoal de Nível Superior - CAPES for PNPD scholarship to ISC-F (201713705-5) and BLZ, to Conselho Nacional de Desenvolvimento Científico e Tecnológico - CNPq for scholarships to ISC-F during his Ph.D. and postdoctoral, and productivity fellowship to PBA. Special thanks to Alexandre V. Palaoro for providing helpful comments and suggestions on the manuscript. This work was supported by CNPq (grant numbers 562202/2010-2, 470286/2011-3 and 204468/2014-0).

**References**


https://mc06.manuscriptcentral.com/cjz-pubs


Table 1. Data on specimens used for this study. Terrestrial isopods species and GenBank accession numbers. Accession numbers in bold represent sequences obtained in this study.

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<thead>
<tr>
<th>Species</th>
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**Outgroup**

*Anchiphiloscia pilosa* AF191112
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Table 2. Pairwise genetic divergence (p-distance) among terrestrial isopods species (outgroup in bold) using nuclear (18S rRNA) gene sequences.

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Figure captions

Figure 1. *Paratlantoscia robusta* sp. nov., male paratype MZUSP 24750. (A) habitus; (B) cephalon, dorsal view; (C) cephalon, frontal view; (D) Telson; (E) d/c noduli laterals coordinates; (F) b/c noduli laterales coordinates; (G) epimeron 1; (H) antennule; (I) antenna. Scale bars: (A) = 1 mm; (B – I) = 0.1 mm

Figure 2. *Paratlantoscia robusta* sp. nov., male paratype MZUSP 24750. (A) Left mandible; (B) right mandible; (C) maxillula; (D) maxilla; (E) maxilliped. Scale bars = 0.1 mm

Figure 3. *Paratlantoscia robusta* sp. nov., male paratype MZUSP 24750. (A) pereopod 1; (B) pereopod 7; (C) dactylus of pereopod 1. Scale bars = 0.1 mm

Figure 4. *Paratlantoscia robusta* sp. nov., male paratype MZUSP 24750. (A) uropod; (B) genital papilla; (C) pleopod 1; (D) pleopod 2; (E) pleopod 3 exopod; (F) pleopod 4 exopod; (G) pleopod 5 exopod. Scale bars = 0.1 mm; (C), detail of endopod apex = 0.05 mm

Figure 5. ML tree based on COI mitochondrial sequences of terrestrial isopods. Numbers above branches represent Bayesian posterior probabilities (bold) and bootstrap support, respectively. Only posterior probabilities ≥ 0.95 and bootstrap values ≥ 70% are shown

Figure 6. ML tree based on 18S rRNA nuclear sequences of terrestrial isopods. Numbers above branches represent Bayesian posterior probabilities (bold) and bootstrap support, respectively. Only posterior probabilities ≥ 0.95 and bootstrap values ≥ 70% are shown
Figure 1. Paratiantosca robusta sp. nov., male paratype MZUSP 24750. (A) habitus; (B) cephalon, dorsal view; (C) cephalon, frontal view; (D) Telson; (E) d/c noduli laterals coordinates; (F) b/c noduli laterales coordinates; (G) epimeron 1; (H) antennule; (I) antenna. Scale bars: (A) = 1 mm; (B – I) = 0.1 mm.
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279x394mm (300 x 300 DPI)
Figure 3. *Paratlantoscia robusta* sp. nov., male paratype MZUSP 24750. (A) pereopod 1; (B) pereopod 7; (C) dactylus of pereopod 1. Scale bars = 0.1 mm

261x369mm (300 x 300 DPI)
Figure 4. *Paratlantoscia robusta* sp. nov., male paratype MZUSP 24750. (A) uropod; (B) genital papilla; (C) pleopod 1; (D) pleopod 2; (E) pleopod 3 exopod; (F) pleopod 4 exopod; (G) pleopod 5 exopod. Scale bars = 0.1 mm; (C), detail of endopod apex = 0.05 mm

217x237mm (300 x 300 DPI)
Figure 5. ML tree based on COI mitochondrial sequences of terrestrial isopods. Numbers above branches represent Bayesian posterior probabilities (bold) and bootstrap support, respectively. Only posterior probabilities $\geq 0.95$ and bootstrap values $\geq 70\%$ are shown.
Figure 6. ML tree based on 18S rRNA nuclear sequences of terrestrial isopods. Numbers above branches represent Bayesian posterior probabilities (bold) and bootstrap support, respectively. Only posterior probabilities ≥ 0.95 and bootstrap values ≥ 70% are shown.

308x426mm (300 x 300 DPI)