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Litoporus iguassuensis Mello-Leitão, 1918 (Araneae, Pholcidae): Camouflaged retreat, sexual dimorphism, female color polymorphism, intra-specific genital variation, and description of the male

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ABSTRACT

We describe the previously unknown male of *Litoporus iguassuensis* Mello-Leitão, 1918 and document the exceptional biology of this species: (1) females and juveniles but not adult males construct camouflaged retreats; (2) there is a strong sexual dimorphism involving coloration, leg length, and behavior; (3) females at some localities are color polymorphic, i.e. discontinuously variable within the population. The species, previously only known from Rio de Janeiro, is widely distributed in the Brazilian Atlantic Forest. Among some of the localities studied it shows genital and male cheliceral variation that is here interpreted as being intraspecific. Preliminary molecular data (16S, H3) reveal low *p*-distances and no significant grouping information, leading us to hypothesize a single variable species rather than two or more species. Finally, we argue that Mello-Leitão's original assignment to *Litoporus* (instead of *Tupigea*) may be correct, and discuss the implications: (1) *Litoporus* may be nested within *Mesabolivar* and represent a case of evolutionary shift among microhabitats with the correlated morphological changes; (2) extreme sexual dimorphism may be common in *Litoporus* and may explain the fact that females continue to be unknown in several species (because they were not recognized as being conspecific with the males).

1. Introduction

Mello-Leitão's (1918) description of *Litoporus iguassuensis* was based on a single female specimen and a juvenile male from "Nova Iguassú" (=Nova Iguaçu), Rio de Janeiro, Brazil. No figure accompanied the original description, and the species did not reappear in the literature until Huber (2000) provided a brief redescription and figures of the female genitalia based on the female type specimen. Since no new material and no male were available at that time, the generic position was considered unclear. The species was tentatively transferred to the genus *Tupigea* Huber, 2000 based on general morphological similarity and geographic distribution (*Tupigea* is endemic to the Brazilian Atlantic Forest while *Litoporus* Simon, 1893 is largely restricted to northern South America), but this transfer was considered preliminary, pending "eventual generic placement" (Huber, 2000).

When males finally were discovered and collected during the last years, this tiny and at first sight inconspicuous species turned out to be surprisingly exceptional among pholcids in various ways. First, females and juveniles were found to live in camouflaged retreats, a behavior that has not been documented in any other pholcid spider. Camouflaged retreats are also rare among the closest relatives of Pholcidae (e.g., Nuessly and Goeden, 1984 on Diguetidae), while they are not uncommon in the more distantly related entelegyne spiders (e.g., Scharff and Coddington, 1997; Eberhard et al., 2008). Second, males were found to live outside the retreats in the domed sheets, and this behavioral difference probably explains the strong sexual size and color dimorphism that is also rare in pholcids (Huber, 2005a). Third, females were found to be color polymorphic, a phenomenon that is equally uncommon in pholcids (Huber and Hopf, 2004; Huber, 2011).

In addition to being thus exceptional, Mello-Leitão's species continues to defy easy classification, both at the levels of genus and species. The most recent molecular analysis (Dimitrov et al., 2012) placed *L. iguassuensis* in *Mesabolivar* González-Sponga, 1998 (Fig. 1; adapted from Dimitrov et al., 2012). However, no unambiguous

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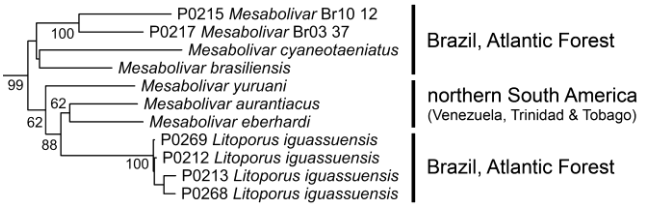


Fig. 1. Detail of the cladogram published in Dimitrov et al. (2012) (ML tree based on analysis of seven molecular markers; with bootstrap support), suggesting that *L. iguassuensis* is more closely related to northern South American representatives of *Mesabolivar* than to Brazilian species.

Litoporus species was available for sequencing, meaning that a simple transfer of Mello-Leitão's species to *Mesabolivar* would only touch the surface of the problem. The new data below and previously published data about *Litoporus* lead us to the conclusion that Mello-Leitão's original assignment is not only the best hypothesis but also generates a number of exciting testable predictions.

The first prediction relates to the fact that females remain unknown in all three closest relatives of the type species *Litoporus aerius* Simon, 1893 (i.e., in *Litoporus dimona, Litoporus saul, Litoporus secoya*; all described in Huber, 2000). We predict that, just as in *L. iguassuensis*, their females are so different from the males that they were separated from them when the material was initially sorted to species.

The second prediction concerns the phylogenetic relationship between *Litoporus* and *Mesabolivar*. If the placement of *L. iguassuensis* in *Litoporus* is correct, then this means that *Litoporus* is nested within a paraphyletic *Mesabolivar*, eventually requiring splitting or synonymization of *Mesabolivar*.

At the species level, we initially assumed to be dealing with up to five species. Morphological differences weakly pointed in that direction. However, molecular markers failed to provide congruent grouping information, so we decided that with the current evidence it is more parsimonious to argue for a single variable species than for two or more species.

2. Materials and Methods

The spiders studied herein were mostly collected by the first and second authors during recent expeditions to the Brazilian Atlantic Forest and are deposited at the Museu Nacional de Rio de Janeiro (MNRJ), and the Zoologisches Forschungsmuseum Alexander Koenig, Bonn (ZFMK).

Morphological descriptions follow the style of recent publications (e.g., Huber, 2011, 2012). Measurements are in mm (± 0.02 mm if two decimals are given) unless otherwise noted. Eye measurements are ± 5 µm. Drawings were done with a camera lucida on a Leitz Dialux 20 compound microscope. Cleared epigyna were stained with chlorazol black. Photos were made with a Nikon Coolpix 995 digital camera (2048 x 1536 pixels) mounted on a Nikon SMZ 1500 dissecting microscope.

Molecular data were analyzed for 14 specimens of *L. iguassuensis* (Table 1) in order to provide an independent dataset to strengthen or reject putative morphological species boundaries. Since Dimitrov et al.'s (2012) large sample of Modisiminae genera had shown that *L. iguassuensis* is deeply nested within *Mesabolivar*, only two outgroups were used (*Carapoia genitalis* (Moenkhaus, 1898), *Mesabolivar* sp.; Table 1). To compare the results with previous studies on Pholcidae where this gene had shown a high suitability for pholcid taxonomy (Astrin et al., 2006; Huber and Astrin, 2009; Huber et al., 2010), we sequenced part of the mitochondrial ribosomal 16S gene. However, 16S amplified in only 10 of 14 specimens, and sequencing of cytochrome oxidase subunit 1 (COI) failed for the majority of specimens both with the standard LCO1490 and HCO2198 barcoding primers (Hebert et al., 2003; Huber et al., 2010; working well for many other pholcids) and their widely applicable variation Lep-F1 and Lep-R1 (Hebert et al., 2004). For these reasons we added the nuclear protein-coding histone 3 (H3) gene, which provided sequences in all 14 specimens but allows only limited comparisons with other pholcid taxa (but see Dimitrov et al., 2012). H3, usually known for lower genetic variation, has shown an elevated variability in the taxon (Dimitrov et al., 2012) and did not lead into problems of intraspecific variability as did ITS2 in many pholcids (JJA, pers. obs.).

Total genomic DNA was extracted from female or juvenile prosomata (in small individuals whole body) using the BioSprint96 magnetic bead extractor by Qiagen (Hilden, Germany). Extracted DNA is deposited at the Biobank of the ZFMK.

Polymerase Chain Reaction was carried out in total reaction mixes of 20 µl, including 1.2–2 µl of undiluted DNA template, 1.6 µl of each primer (10 pmol/µl), 2 µl of 'Q-Solution' and 9.5 µl of 'Multiplex PCR Master Mix' (reactions run individually), containing hot start Taq DNA polymerase and buffers. The latter components are available in the 'Multiplex PCR' kit from Qiagen (Hilden, Germany). Primers used for amplification and sequencing were: 16s1471-mod: 5'-GCCTGTTTAWCAAAAACAT-3' with 16sbr-H-mod: 5'-CCGGTYTGAACTCARATCAYGT-3' (Astrin et al., 2006) and H3aF 5'-ATGGCTCGTACCAAGCAGACVGC-3' with H3aR 5'-ATATCCTTRGGCATRATRGTGAC-3' (Colgan et al., 1998). Thermal cycling was performed on a GeneAmp PCR System 2700 (Applied Biosystems, Foster City, CA, USA) machine, using a generic 'touchdown' protocol for both genes alike: hot start Taq activation: 15 min at 95°; first cycle set (15 repeats): 35 s denaturation at 94°C, 90 s annealing at 60°C (−1°C per cycle) and 90 s extension at 72°C. Second cycle set (25 repeats): 35 s denaturation at 94°C, 90 s annealing at 50°C, and 90 s extension at 72°C. After enzymatic cleanup (Exo/SAP), PCR products were sent to a sequencing facility (Macrogen, Amsterdam, NL) for double stranded sequencing. Sequence length was 328 bp for H3 and 446 bp for 16S (aligned). These partitions were concatenated into a single alignment (available from the authors or as download from the journal's website). DNA sequence alignment was performed using the MUSCLE ver. 3.6 program (Edgar, 2004), run with default parameters.

The sequence data of the two genes was used in parallel Bayesian Markov chain Monte Carlo (MCMC) analyses, as implemented in MrBayes ver. 3.1.2 (Ronquist and Huelsenbeck, 2003). Within the ingroup, no gaps occurred in the alignment. Gaps due to the outgroup were treated as missing characters. We applied a GTR+I+Γ model of nucleotide substitution, but adopted uninformative prior distributions for the parameter values. Parameters were unlinked among partitions and also between the 3rd versus 1st plus 2nd codon positions in H3. Analyses were run for 2 million generations (using the default chain number and temperatures). Every 1000th tree was sampled (4000 trees retained for the two – converging – parallel runs). Negative log-likelihood score stabilization was determined graphically. Accordingly, we retained 3968 trees. These were used for building a 50%-majority rule consensus dendrogram (with posterior probabilities). PAUP* ver. 4.0b10 (Swofford, 2002) was used to calculate uncorrected (*p*-)distances of the genes, without excluding positions (cf. Astrin et al., 2006). Low molecular signal, as assessed from the tree support values, and the reduced number of samples available for study, prevented the application of further methods (see below).

3. Results

3.1. Molecular data

We found relatively low *p*-distances among *L. iguassuensis* specimens from different localities: 0.0-3.5% in 16S and 0.0-6.1% in

Table 1
Specimens sequenced, GenBank accession numbers, and DNA voucher numbers (bold: new data); for more detailed information, see Section 3.3.6 and/or the respective GenBank records.

Vial number	Species	Locality	Voucher	16S	H3	DNA voucher
P0205	*Carapoia genitalis*	SP: Paranapiacaba	Br03-20	JX023897	JX023656	ZFMK-DNA-100446401
P0215	*Mesabolivar* sp. Br10-12	SC: Reserva Volta Velha	Br10-63	JX023905	JX023666	ZFMK-DNA-100446398
P0212	*L. iguassuensis*	ES: Vargem Alta	Br10-87	JX023902	JX023663	ZFMK-DNA-100446408
P0213	*L. iguassuensis*	RJ: Santa Maria Madalena	Br10-72	JX023903	JX023664	ZFMK-DNA-100446409
P0268	*L. iguassuensis*	RJ: Santa Maria Madalena	Br10-72	JX023935	JX023699	ZFMK-DNA-100437895
P0269	*L. iguassuensis*	ES: Vargem Alta	Br10-87	JX023936	JX023700	ZFMK-DNA-100437894
P0289	*L. iguassuensis*	RJ: Santa Maria Madalena	Br10-72	**JX393865**	**JX393855**	**ZFMK-DNA-100446117**
P0290	*L. iguassuensis*	RJ: Macaé, PN Jurubatiba	Rio 14	na	**JX393856**	**ZFMK-DNA-100446385**
P0291	*L. iguassuensis*	RJ: Macaé, PNM Atalaia	Rio 17	na	**JX393857**	**ZFMK-DNA-100449156**
P0292	*L. iguassuensis*	ES: Sooretama, site 1	Br11-124	na	**JX393858**	**ZFMK-DNA-100449061**
P0293	*L. iguassuensis*	ES: Sooretama, site 2	Br11-132	na	**JX393859**	**ZFMK-DNA-100449149**
P0294	*L. iguassuensis*	ES: Córrego do Veado, site 1	Br11-138	**JX393866**	**JX393860**	**ZFMK-DNA-100449059**
P0295	*L. iguassuensis*	ES: Córrego do Veado, site 2	Br11-143	**JX393867**	**JX393861**	**ZFMK-DNA-100449150**
P0296	*L. iguassuensis*	BA: Res. Michelín	Br11-185	**JX393868**	**JX393862**	**ZFMK-DNA-100449057**
P0297	*L. iguassuensis*	BA: Res. Biol. Una	Br11-175	**JX393869**	**JX393863**	**ZFMK-DNA-100449056**
P0299	*L. iguassuensis*	ES: Presidente Kennedy	Rio 13	**JX393870**	**JX393864**	**ZFMK-DNA-100449054**

H3. In the case of H3, *p*-distances among specimens from the same locality were sometimes larger than those among localities. For example, specimens from Santa Maria Madalena (the most southern population sequenced) differed among each other by 1.5–4.6% while their distance to the specimen from Reserva Michelín (the most northern population known) ranged from only 0.3–3.7%. A similar situation occurred with specimens from Sooretama (distance within population: 4.5%; to other populations: 0.6–6.1%).

In the case of 16S, two groups could be delimited, one comprising Santa Maria Madalena and Presidente Kennedy (0.0–0.2%), the other comprising Reserva Michelín, Res. Biol. Una, and Vargem Alta (0.5–0.9%). All other distances among localities were significantly higher (1.9–3.5%). Both groups are in clear conflict with morphological data that suggest a closer relationship between Presidente Kennedy and Vargem Alta on one side and specimens from all other localities studied on the other side (see below).

The Bayesian analysis resulted in an almost entirely unresolved dendrogram (therefore not shown) with only two nodes supported by posterior probability values above 95%: the entire ingroup; and specimens P0289 (Santa Maria Madalena) and P0291 (Macaé, Atalaia).

3.2. Natural history

The web of *L. iguassuensis* is a typical pholcid sheet that is slightly domed and at some point connected to the underside of a leaf. What makes the web unique among pholcids is a sac-like silken retreat that is attached to the leaf and that is camouflaged with small particles of plant detritus (Figs. 3, 4, 6 and 7). Some webs, presumably very fresh ones, lack the particles. It is not clear how and where the spiders collect the particles. Females and juveniles are usually found within their retreats, while males hang from the apex of the dome as usual in pholcids (Fig. 2). The sizes of the retreats and male leg lengths (about twice as long as female legs; see below) suggest that adult males do not fit into the retreats.

Males and females react differently to disturbance. Females hide in the retreat; males swing their bodies at high amplitude but at a much lower frequency than the usual pholcid vibration (see video clip at http://www.pholcidae.de). The high amplitude is facilitated by the extremely long legs (leg 1 is ~20 x body length; see below and Fig. 2). The third pair of legs (which is the shortest as usual in pholcids) looses contact with the web during swinging and is held sideways.

3.3. Taxonomy

Litoporus iguassuensis Mello-Leitão, 1918 orig. comb. (Figs. 2–44).

Litoporus iguassuensis Mello-Leitão, 1918: 94–95. Dimitrov et al., 2012.

Tupigea iguassuensis: Huber, 2000: 327, figs. 1313–1314.

Tupigea sp. (*iguassuensis*?, "Br09-32"): Huber and Rheims, 2011: 282, fig. 3G.

3.3.1. Types

Female lectotype (designated in Huber, 2000) and juvenile male paralectotype from Brazil, Rio de Janeiro, Nova Iguaçu [22°46.1'S, 43°28.0'W], without date, B. de Freitas leg., in MNRJ (852), examined (Huber, 2000).

3.3.2. Diagnosis

Easily distinguished from most other pholcids by strong sexual dimorphism (males with much longer legs and prosoma without dark pattern; compare Figs. 2 and 7; 8 and 11), by armature of male chelicerae (two pairs of apophyses; Figs. 27-38), by shape of simple procursus (Fig. 39), by embolus with pair of distal apophyses (Figs. 40-44), and by female internal genitalia (median sac-like structure and perpendicular pore plates at varying angle; Figs. 23-26). *Litoporus pakitza* Huber, 2000 is extremely similar but has only one pair of cheliceral apophyses (Huber, 2000; fig. 1221), shorter distal apophyses on embolus (Fig. 45), and no carapace pattern in females.

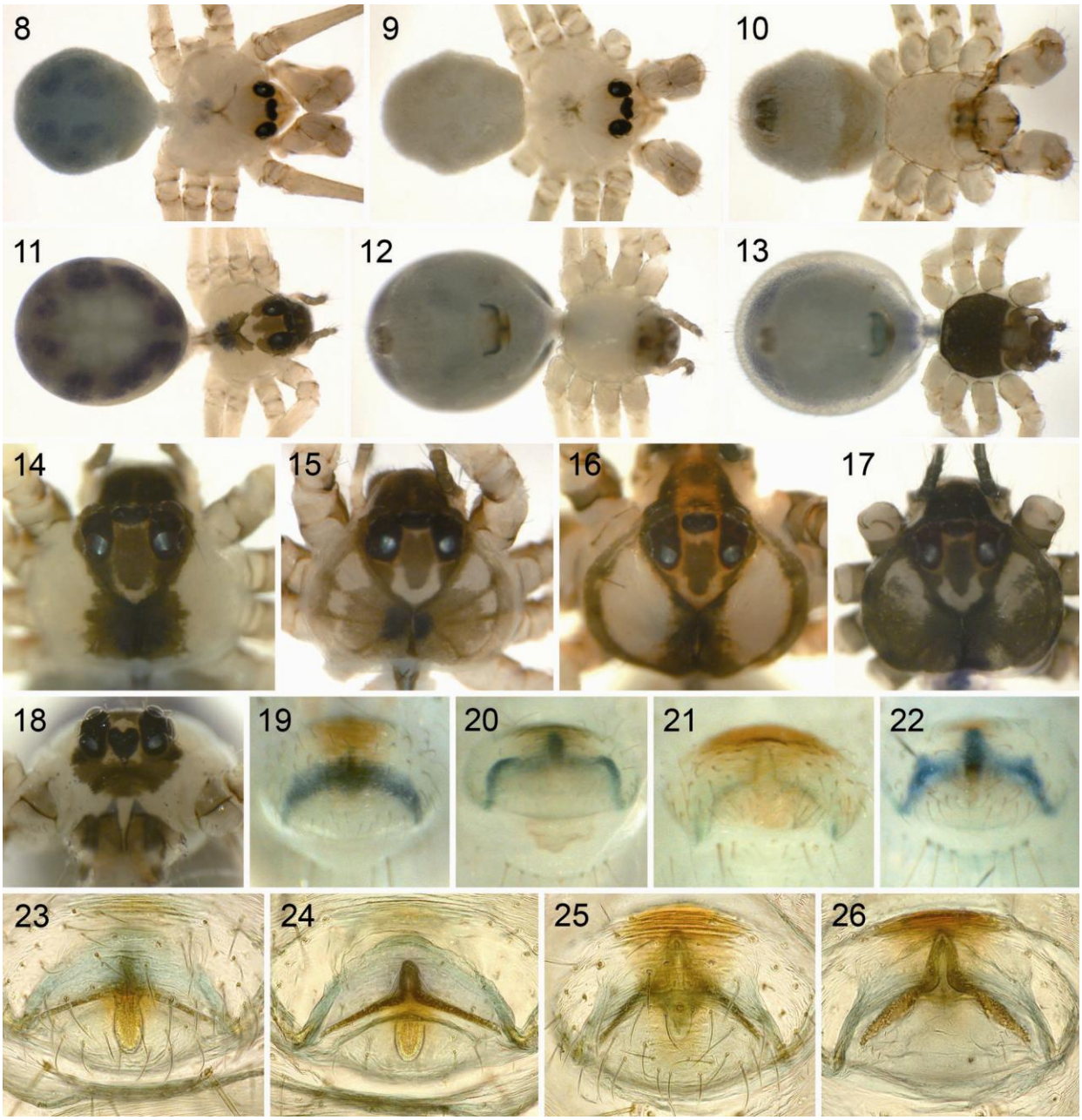
3.3.3. Description (Santa Maria Madalena)

Male. Total body length 1.8; carapace width 0.83. Leg 1: 36.5 (10.2 + 0.3 + 8.1 + 16.3 + 1.6), tibia 2: 5.9, tibia 3: 4.3, tibia 4: 5.4, tibia 1 length/diameter: 114. Habitus as in Figs. 2 and 8–10; entire prosoma monochromous ochre-yellow, legs slightly darker, femora and tibiae with whitish tips, metatarsi distally and tarsi whitish, abdomen pale gray. Distance PME-PME 175 µm, diameter PME 70 µm, distance PME-ALE 60 µm, distance AME-AME 40 µm, diameter AME 55 µm. Ocular area slightly elevated, thoracic furrow shallow, clypeus unmodified. Chelicerae with two pairs of frontal apophyses (Figs. 27 and 28). Sternum unmodified. Palps as in Fig. 39, coxa with retrolateral apophysis, trochanter with rounded ventral projection, femur widening distally, with retrolatero-ventral apophysis proximally and prolatero-ventral apophysis in median part, tibia relatively small, procursus S-shaped, very simple, bulb with wide embolus carrying two apophyses distally (Fig. 40). Legs

Figs. 2-7. *Litoporus iguassuensis* in natural habitat. (2) Male (Sooretama). (3 and 4) Females and retreats (Córrego do Veado, Santa Maria Madalena). (5) Female with eggsac (Macaé). (6 and 7) Females and retreats (Sooretama, Santa Maria Madalena). Photos: BAH and APG.

without spines and curved hairs, with few vertical hairs; retrolateral trichobothrium on tibia 1 at 1%; prolateral trichobothrium present on all tibiae; tarsus 1 with >20 pseudosegments, proximally indistinct.

Female. Total body length 2.1; carapace width 0.80. Leg 1: 18.1 (5.0 + 0.3 + 4.3 + 7.2 + 1.3), tibia 2: 2.7, tibia 3: 1.8, tibia 4: 2.3, tibia 1 length/diameter: 61. Habitus similar to male but with larger and higher abdomen; carapace with brown pattern, clypeus and sternum brown, legs with four dark rings each (femur and tibia subdistally, patella, metatarsus proximally), abdomen bluish gray with large dark spots dorsally, no ventral pattern. Ocular area similar to male but triads slightly closer together (distance PME-PME 150 μm). Retrolateral trichobothrium on tibia 1 at 2%; prolateral trichobothrium present on all tibiae; pseudosegments as in male. Epigynum very simple externally, with sclerotized frontal area and internal bluish structures visible through cuticle (Figs. 19–22).

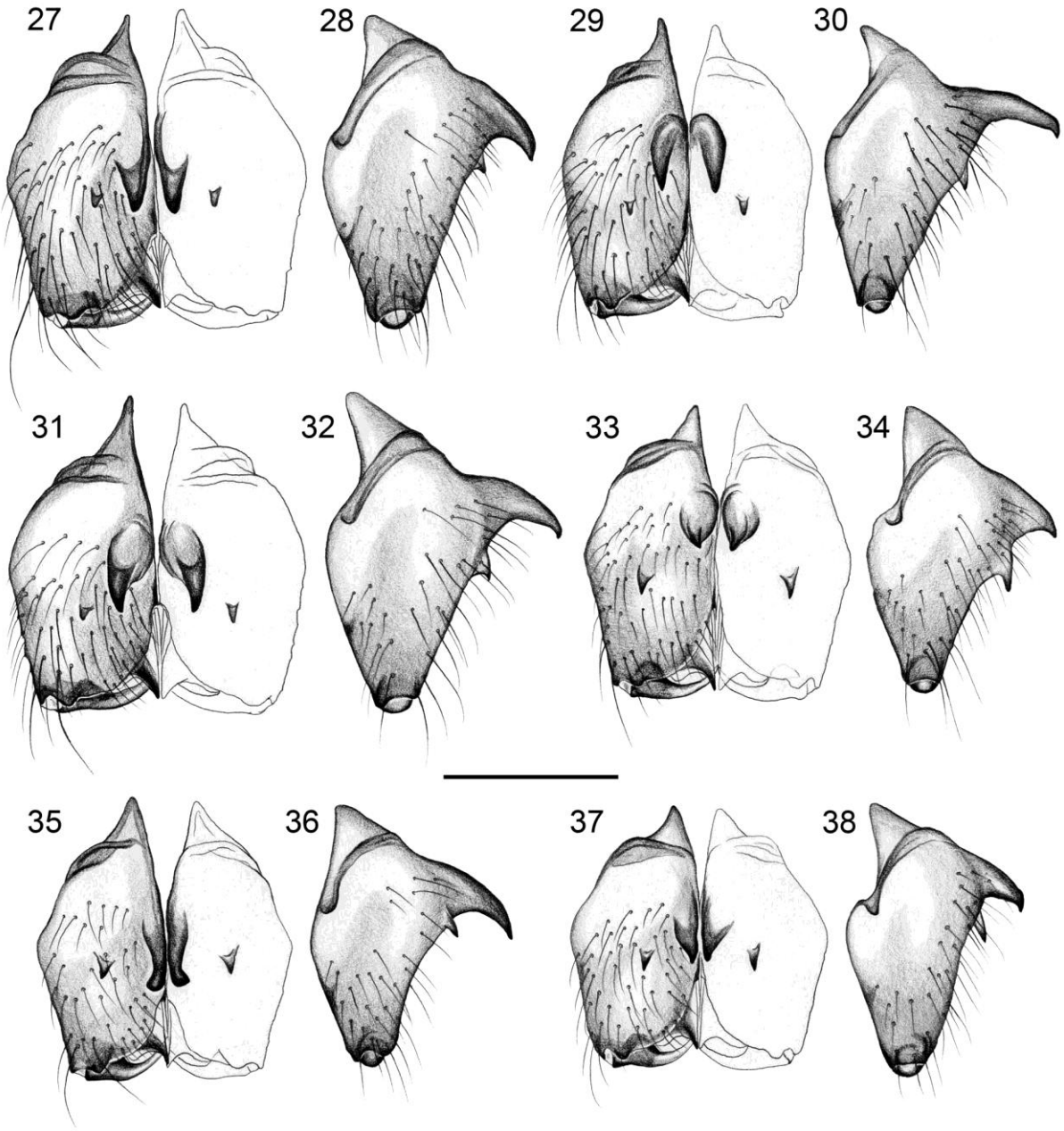


Figs. 8-26. *Litoporus iguassuensis*, color patterns and female genitalia. (8) Male from Cambucí, dorsal view. (9 and 10) Male from Sooretama, dorsal and ventral views. (11 and 12) Female from Córrego do Veado, dorsal and ventral views. (13) Female from Silva Jardim, ventral view. (14-17) Females from Santa Maria Madalena (14 and 15) and Vargem Alta (16 and 17), carapace pattern. (18) Female from Macaé, clypeus pattern. (19-22) Epigyna, ventral views, females from Presidente Kennedy, Macaé, Viçosa, Santa Maria Madalena. (23-26) Cleared epigyna, ventral and dorsal views, females from Guapiaçu (23 and 24), Mimoso do Sul (25 and 26).

3.3.4. Variation

Males never with dark pattern on carapace, only small Y-shaped mark behind ocular area more or less distinct; sternum always pale ochre-yellow (Fig. 10); abdomen more variable, from monochromous pale gray to bluish with dark spots as in female. Male chelicerae and palps variable among populations: larger proximal apophyses of various length, shape, and position (Figs. 27–38), small distal apophyses barely variable. Bulbal apophyses either of same length (Figs. 42–44) or ventral apophysis much longer (Figs. 40 and 41). Leg measurements in 30 males: femur 1: 8.3–10.2 (mean 9.3); tibia 1: 6.4–8.1 (mean 7.3); femur1/tibia 1: 1.23–1.33 (mean 1.28).

Females with extensive color variation: sternum either brown or pale as in male (Figs. 12 and 13); carapace with brown pattern either limited to median part or with lateral components (Figs. 14–17). Both sternum and carapace pattern sometimes variable within populations (Santa Maria Madalena, Cambucí, Vargem Alta), invariable in Macaé population (N=32; sternum always pale, carapace pattern always limited to median part). Clypeus usually brown; in some specimens rather orange surrounded by wide brown margin (Silva Jardim, Viçosa, 4♀ from Vargem Alta); in some specimens brown mark does not reach rim of clypeus (Fig. 18). Angle between pore plates apparently constant within populations but variable among populations: most populations with large angle



Figs. 27-38. Male chelicerae, frontal and lateral views. (27 and 28) Santa Maria Madalena. (29 and 30) Presidente Kennedy. (31 and 32) Vargem Alta. (33 and 34) Viçosa. (35 and 36) Sooretama. (37 and 38) Reserva Michelín. Scale line (same for all): 0.2 mm.

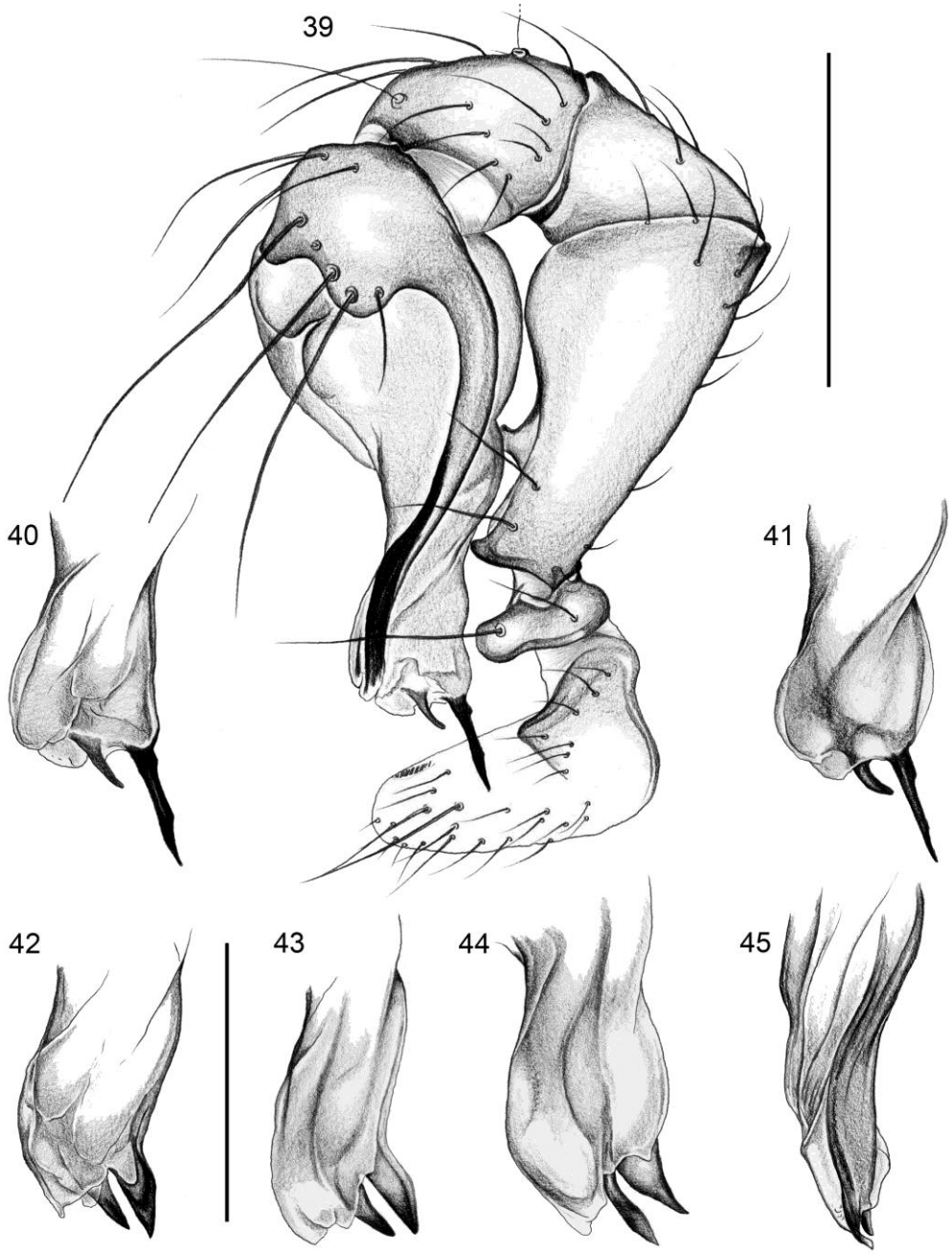
(Figs. 23 and 24), females from southern Espírito Santo (Vargem Alta, Mimoso do Sul, Presidente Kennedy) and Viçosa with smaller angle (Figs. 25 and 26). Leg measurements in 52 females: femur 1: 3.7–5.0 (mean 4.5); tibia 1: 3.2–4.4 (mean 3.8); femur1/tibia 1: 1.14–1.27 (mean 1.18).

3.3.5. Distribution

Known from numerous localities in the Brazilian Atlantic Forest between Rio de Janeiro and Salvador de Bahia (Fig. 46). Intensive collecting in several Atlantic Forest sites southwest of Rio de Janeiro has not produced this species (Huber and Rheims, 2011; B.A. Huber, unpubl. data).

3.3.6. Material examined

Brazil: ***Rio de Janeiro***: Santa Maria Madalena, forest fragment (21°58.9–59.1'S, 41°57.2–57.6'W), 480–590 m a.s.l., 30.ix.–1.x.2010 (B.A. Huber, A. Pérez-G.), 7♂14♀ in ZFMK (Ar 8864); same data, 3♀ in pure ethanol, in ZFMK (Br10-72). Cachoeiras de Macacu, Reserva Ecol. Guapiaçú (22°24.4'–25.3'S, 42°44.2'–44.3'W), 140–300 m a.s.l., 25.ix.2009 (B.A. Huber, A. Giupponi), 1♀ in ZFMK (Ar 8865). Cambucí, floresta frente ao Balneario de Santa Inês (21°32.3'S, 41°56.6'W), 19.–20.vi.2011 (A. Giupponi, G.G. Enne, A. Pérez-G.), 2♂4♀ 5 juvs. in MNRJ. Silva Jardim [22°39.6'S, 42°22.8'W], "BRD 0475", R. Baptista, 1♀ 1 juv. in MNRJ. Macaé (Captação, São Iazaro, Mata Fazenda, Odebei; ~22°16'S, 41°42'W), iii.2010 (M.R.Q. & G.A. da Costa),



Figs. 39-45. *Litoporus iguassuensis* (39-44) and *L. pakitza* (45). (39) Left male palp, retrolateral view, male from Santa Maria Madalena. (40-45) Left male embolus tips, retrolateral views, at same scale, males from Santa Maria Madalena (40), Reserva Michelín (41), Vargem Alta (42), Presidente Kennedy (43), Viçosa (44), Peru (Pakitza; 45). Scale lines: 39 = 0.3 mm; 40-45 = 0.2 mm.

9♂32♀ 20 juvs. in MNRJ. Macaé, Parque Nacional da Restinga de Jurubatiba [22°15.8'S, 41°38.9'W], iii.2011 (A. Pérez-G., G. Cardoso), 5♂3♀ 2 juvs. in pure ethanol, in MNRJ. Macaé, Parque Natural Municipal Atalaia [22°15.8'S, 42°18.6'W], 26.ix.2009 (A. Pérez-G.), 1♂ in MNRJ; same data, 2011 (A. Pérez-G.), 1♀ in pure ethanol in MNRJ. ***Espírito Santo***: Vargem Alta, Fazenda Monte Verde (20°27.6–28.2'S, 40°59.5'–41°00.2'W), 1000–1200 m a.s.l., 2.–3.x.2009 (B.A. Huber, A. Pérez-G.), 2♂8♀ 1 juv. in ZFMK (Ar 8866); same data, 2 juvs. in pure ethanol in ZFMK (Br10-87). Mimoso do Sul, Finca Tacutinga, forest fragment (21°01.4'S, 41°23.4'W), 240 m a.s.l., 4.x.2010 (B.A. Huber, A. Pérez-G.), 1♀ in ZFMK (Ar 8867); same data, 1 juv. in pure ethanol in ZFMK (Br10-95). Presidente Kennedy, Restinga de Praia das Neves (21°16.1'S, 40°58.1'W), 18.–19.vi.2011 (A. Giupponi, G.G. Enne, A. Pérez-G.), 2♂5♀ 5 juvs. in MNRJ; same data, 1♀ in pure ethanol in MNRJ. Reserva Biológica de Sooretama, "site 1" (19°03.3'S, 40°08.8'W), ~90 m a.s.l., 27.ix.2011 (B.A. Huber, A. Pérez-G.), 1♂ in ZFMK (Ar 8868); same data, 2♀ 6 juvs. in pure ethanol, in ZFMK (Br11-124). Reserva Biológica de Sooretama, "site 2" (19°00.7'S, 40°06.5'W), ~80 m a.s.l., 28.ix.2011 (B.A. Huber, A. Pérez-G.), 1 juv. in pure ethanol, in ZFMK (Br11-132). Sooretama, "ponto 2", 8.xi.2009 (D.T. Castro), beating vegetation, 2♂2♀ 9 juvs. in MNRJ. Sooretama ("Soo 0356", no further data), 2♂1♀ in MNRJ. Reserva Biológica Córrego do Veado, "site 1" (18°22.1'S, 40°08.3'W), ~80 m a.s.l., 29.ix.2011 (B.A. Huber, A. Pérez-G.), 1♂7♀ 10 juvs. in ZFMK (Ar 8869); same data, 2♀ in pure ethanol in ZFMK (Br11-138). Reserva Biológica

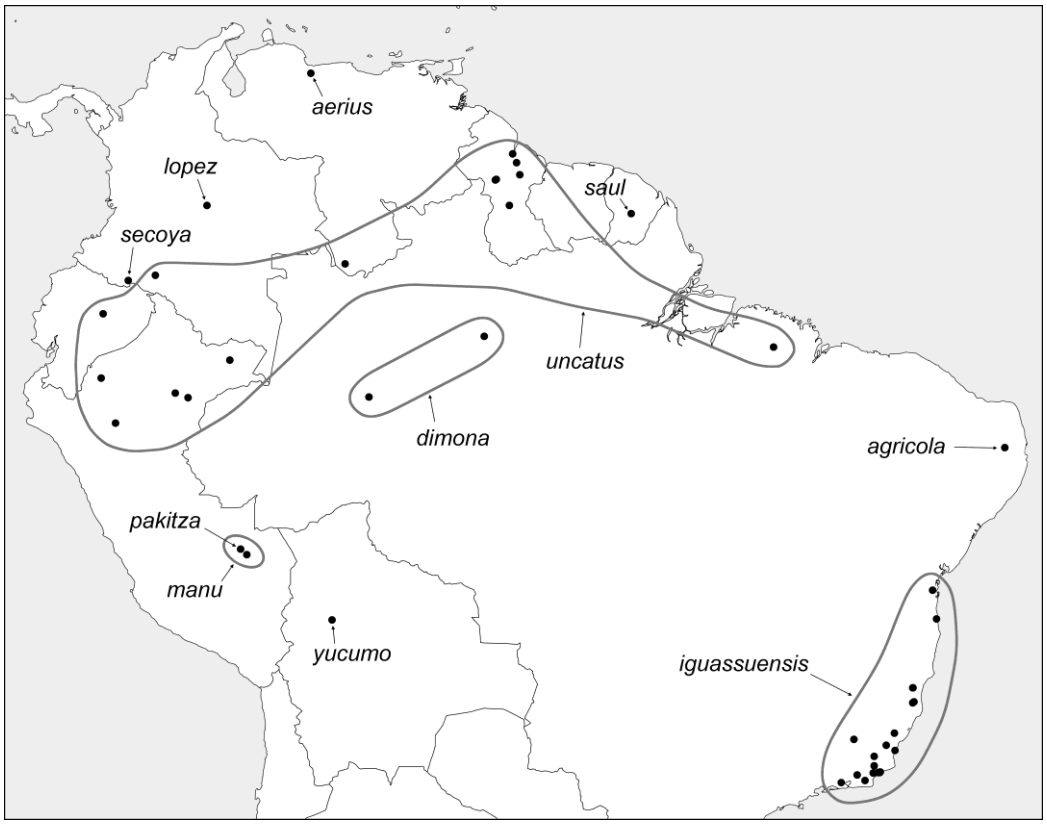


Fig. 46. Known distribution of the genus *Litoporus*. Note that *L. agricola* Mello-Leitão, 1922 is actually considered "incertae sedis" (Huber 2000: 344). Data from: Carvalho et al. (2010), Huber (1997, 2000), Mello-Leitão (1922), and herein.

Córrego do Veado, "site 2" (18°21.7'S, 40°10.0'W), ~90 m a.s.l., 29.ix.2011 (B.A. Huber, A. Pérez-G.), 2♀ 1 juv. in ZFMK (Ar 8870); same data, 2 juvs. in pure ethanol in ZFMK (Br11-143). ***Minas Gerais***: Viçosa [~20°45'S, 42°53'W], R. Baptista leg., no further data, 3♂4♀ 7 juvs. (3 vials) in MNRJ. ***Bahia***: Reserva Ecológica da Michelín, "site 1" (13°49.3'S, 39°11.9'W), ~140 m a.s.l., 5.x.2011 (B.A. Huber, A. Pérez-G., M. Alves Dias), 2♂ in ZFMK (Ar 8871); same data, 1♀ in pure ethanol in ZFMK (Br11-185). Reserva Biológica de Una, sites 1 & 2 (15°10'-11'S, 39°03'-04'W), ~50–100 m a.s.l., 4.x.2011 (B.A. Huber, A. Pérez-G., M. Alves Dias), 1 juv. in pure ethanol in ZFMK (Br11-175).

4. Discussion

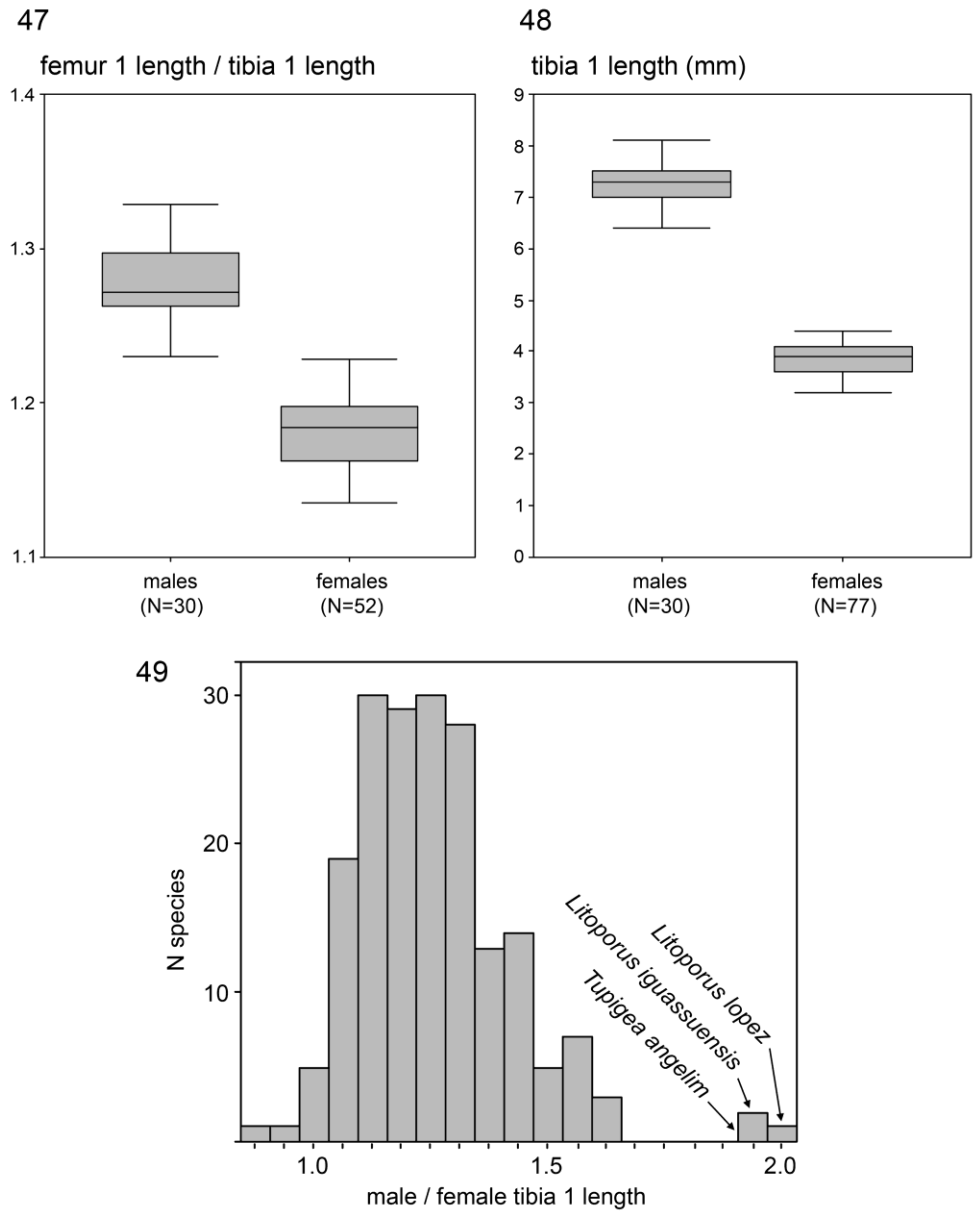
4.1. Intraspecific genital variation and species limits

In spiders, genitalia and other copulatory contact structures provide the most widely used and in most cases exclusive means to distinguish species (Huber, 2004). Intraspecific genital variation, even though not unexpected in theory, presents a recurring phenomenon and challenge (Huber and Pérez González, 2001; Eberhard and Huber, 2010). In a recent revision of *Pholcus* and close relatives (Huber, 2011) genital variation was interpreted as being intraspecific in 35 of 106 species that were represented by specimens from more than one locality. However, drawing well-supported species boundaries in problematic cases usually requires statistical analyses of large samples or ecological or behavioral observations, most or all of which are unavailable in the majority of invertebrate taxa. The extent to which simple DNA divergence data may help solving this problem has been debated extensively (e.g., Moritz and Cicero, 2004; Meier et al., 2006; Boyer et al., 2007; Johnsen et al., 2010; Lara et al., 2010; Taylor and Harris, 2012). Promising more complex methods like the generalized mixed Yule-coalescent approach (GMYC; Pons et al., 2006) rely on data and sample sizes that are not available for the present study. Furthermore, GMYC is a tree-based method, which precludes its efficient application in this case as the underlying dendrogram lacks reliability.

In pholcids, morphologically 'easy' taxa tend to provide clear 'barcoding gaps' (Astrin et al., 2006; Huber et al., 2010) while morphologically problematic taxa are not either easily understood by using the standard barcoding genes (Huber and Astrin, 2009). The current study reflects these problems, and we conclude that the molecular data do not reject the taxonomic null-hypothesis of all specimens belonging to a single species. First, the groupings suggested by 16S and morphological data contradict each other. Morphologically, the specimens from Presidente Kennedy are very similar to those from Vargem Alta and different from other localities, but the 16S *p*-distances are much lower between specimens from Presidente Kennedy and Santa Maria Madalena (0.0–0.2%) than between Presidente Kennedy and Vargem Alta (3.0–3.2%). Second, 16S distances among localities ranged from 0.0 to 3.5%. This is mostly below the 'barcoding gap' and clearly below the lowest interspecific distances identified for this gene by previous studies on closely related species (Huber and Astrin, 2009; Huber et al., 2010). Third, H3 data partly recovered greater similarity between specimens from different localities than between specimens from the same locality. We conclude that with the data currently available it is not justified to split *L. iguassuensis* into two or more species but we recognize the need for further study.

4.2. Sexual dimorphism and behavior

Some cases of conspicuous sexual dimorphism in spiders have attracted much attention (e.g., extreme size dimorphism; conspicuous color dimorphism; reviewed in Huber, 2005b) but in the large



Figs. 47-49. Leg measurements. (47 and 48) *Litoporus iguassuensis*, relationship between femur 1 and tibia 1 length in males and female and absolute tibia 1 length in males and females (all localities pooled). (49) Tibia 1 length dimorphism in Pholcidae based on over 6000 measured tibiae of 188 species for which at least five specimens of each sex were available. The complete datasheet is available online at http://www.pholcidae.de/matrices.html.

majority of spiders, males and females are not much different. This is also true for pholcids, where males usually have slightly longer legs (Huber, 2005a) and females often have bigger abdomens, but otherwise it is only the sexual organs that differ (gonopore, palps, chelicerae, sometimes also clypeus).

Few exceptions have been documented in Pholcidae, but the biological causes remain mostly unknown. In the Venezuelan *Mecolaesthus longissimus* Simon, 1893, males have much longer abdomens than females and intrasexual selection (male-male fights) has been proposed as a possible but untested explanation (Huber, 2005a). In *Pholcus* and close relatives, male eye triads are usually farther apart than in females and sometimes the male ocular area is highly modified (Huber, 2011). The significance of this dimorphism remains unknown. In some pholcid genera (e.g., *Modisimus* Simon, 1893), some male leg segments are densely covered with short vertical hairs of unknown function (Huber, 2000; Huber et al., 2010). Fairly common but equally unexplained are cases where males are darker or have a more conspicuous dark pattern than females [e.g., *Tupigea teresopolis* Huber, 2000; *Mesabolivar luteus* (Keyserling, 1891); *Pholcus ethagala* Huber, 2011; see Huber and Rheims, 2011; Huber, 2000, 2011]. The case of *L. iguassuensis* seems related to the conspicuously different behavior between males and females. Females with their short legs do not swing but they fit into their retreats, while males with their monochromous pale color may be better protected from visually hunting predators when hanging (and swinging) freely in the web.

4.3. Species abundance and color polymorphism

Another intriguing characteristic of *L. iguassuensis* is its heterogeneous abundance across the Tropical and Subtropical Moist Broadleaf Forests Biome (Atlantic Forest). The species is hard to

find in the highly diverse Submontane and Montane Atlantic Forest habitats, which may explain the scarcity of records and specimens in arachnological collections before our intensive and focused collection effort. Even with an intensive effort, we collected a maximum of 24 specimens in the Rio de Janeiro locality of Santa Maria Madalena. Efforts to collect specimens at the type locality were unsuccessful (A. Pérez-G. and D.T. Castro, unpubl. data). On the other hand, *L. iguassuensis* is a dominant species in the much less diverse pholcid community of the Jurubatiba Restinga (A. Pérez-G. and R.L.C. Baptista, unpubl. data). A Restinga is a distinct type of coastal tropical and subtropical moist broadleaf forest that forms on sandy and nutrient-impoverished soils. Restinga forests vary from shrub vegetation to 15-m-tall forests that are distributed over soil mosaics and gradients from the coastal zone inland (World Wildlife Fund 2007). The climatic conditions are more severe in this sand soil forest than in the "typical" humid Brazilian Atlantic Forest. This may explain the generally less diverse pholcid fauna (only four species; A. Pérez-G. and R.L.C. Baptista, unpubl. data), but it is not clear why it is in this ecosystem that *L. iguassuensis* exhibits its highest abundance (of 117 pholcid specimens collected at the Restinga de Jurubatiba, 74 were *L. iguassuensis*; A. Pérez-G. and R.L.C. Baptista, unpubl. data). Equally unexplained is the finding that females from the Restinga localities (Jurubatiba, Presidente Kennedy) are not color polymorphic.

4.4. Generic placement

Two characters that were not available previously suggest that Mello-Leitão correctly assigned this species to *Litoporus*. First, a high ratio of male femur1/tibia1 (usually >1.15) is a putative synapomorphy of *Litoporus* (Huber, 2000), and *L. iguassuensis* males are consistently above this value (1.23–1.33; Fig. 47). Second, a comparable sexual dimorphism in leg length (male tibia 1/female tibia 1; *L. iguassuensis*: 1.9; Fig 48) is only known from *Litoporus lopez* Huber, 2000 (2.0) and *Tupigea angelim* Huber, 2011 (1.9). In other pholcids, this ratio ranges from 0.9–1.6 (Fig. 49). In *L. pakitza*, the value is 1.5 (Huber, 2000). In the type species *L. aerius* Simon, 1893 (where the assignment of the single known female is uncertain; see next paragraph) the ratio is 1.7 (it is not included in Fig. 49 because of the small sample size). The assignment of *L. iguassuensis* to *Litoporus* creates two testable predictions, one regarding the biology and morphology of other *Litoporus* species, the other regarding the relationship between *Litoporus* and *Mesabolivar* and the implied evolutionary shift of microhabitat.

First, as indicated above, females remain unknown in all three closest relatives of the type species *L. aerius*. Based on the strong dimorphism of *L. iguassuensis* documented above, we predict that their females are so different from the males that they were separated from them when the material was initially sorted to species by the collector or curator. Since Huber (2000) as a matter of principle did not describe females that were not accompanied by males and could not unambiguously be assigned to males, the identity of these females may have remained unrecognized. The predictt ion also extends to the type species itself and sheds new light on the dubious female accompanying the male type specimens. This material was studied by Huber (1997) who doubted the correct assignment of the female because of its darker coloration and larger eyes. Since pholcid males and females often share webs, this prediction can easily be tested by observing *L. aerius* and its closest relatives in the field.

Second, the most recent molecular phylogeny of Pholcidae (Dimitrov et al., 2012) has shown that *L. iguassuensis* is nested within *Mesabolivar* (Fig. 1). Since no unambiguous *Litoporus* was available for that study, the taxonomic consequences remained open. If our (and Mello-Leitão's) placement is correct and *L. iguassuensis* is in fact closely related to the type species of *Litoporus*, then this means that *Litoporus* is nested within a paraphyletic *Mesabolivar*. The fact that *L. iguassuensis* grouped with the northern South American clade of *Mesabolivar* rather than with the Brazilian clade (Fig. 1) also points in this direction (because *Litoporus* is also largely restricted to northern South America). This prediction is also easy to test by incorporating *L. aerius* or one of its closest relatives into the molecular data matrix of Dimitrov et al. (2012). Apart from the taxonomic consequences (splitting or synonymization of *Mesabolivar*), this is interesting from an evolutionary perspective as it implies a shift of microhabitat from large dark spaces near the ground (*Mesabolivar*) to the undersides of green leaves higher up in the vegetation (*Litoporus*). Such shifts have occurred frequently in pholcids and may account substantially for the diversity of the group (Dimitrov et al., 2012; Huber 2011, 2012; Huber et al., 2005, 2010).

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