

## Speciation without changes in genital shape: A case study on Brazilian pholcid spiders (Araneae: Pholcidae)

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### Abstract

Speciation in arthropods is usually coupled with marked changes in genital morphology, which explains the usefulness of genitalia in distinguishing closely related species. The present paper describes specimens that are assigned to separate species based on extreme size differences and colour pattern differences, but the shape of the genitalia is essentially identical. We argue that such cryptic species may be more common than currently assumed, but if marked morphological (non-genital) differences are missing, traditional taxonomic methodology is biased against discovering them. The two new species from the Brazilian Atlantic Forest are tentatively assigned to the genus *Psilochorus*: *Psilochorus itagyruusu* n. sp. and *Psilochorus ybytyriguara* n. sp.

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### 1. Introduction

In several groups of arthropods, including most insects and spiders, taxonomists heavily rely on genital morphology to distinguish species (review in Eberhard 1985). On one hand, genitalia tend to evolve more rapidly than other structures, probably due to sexual selection (Eberhard 1985, 2004; Hosken and Stockley 2004; Huber 2005). On the other hand, genitalia tend to show low levels of variation within species, probably due to selection to fit the opposite sex (Eberhard et al. 1998; see also Arnold 1986; Teder 1998; Palestini et al. 2000;

Tatsuta et al. 2001). Both mechanisms result in genitalia usually being reliable indicators of species limits.

However, in the last years, evidence has been accumulating suggesting that discontinuities in genital morphology may not always indicate species limits, and species limits need not necessarily be reflected in discontinuities in genital morphology. For example, genital dimorphisms have been documented in several species of insects and spiders (review in Huber and Pérez 2001b), and many cases of considerable variation of copulatory organs within species have been described (e.g., Lucas and Bücherl 1965; Levi 1968, 1974; Sierwald 1983; Kraus and Kraus 1988; Pérez-Miles 1989; Huber 2000; see also Goulson 1993; Hribar 1994; Johnson 1995; Tanabe and Shinohara 1996; Tanabe et al. 2001). On the other side, morphologically cryptic species have

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been discovered even though traditional methodology makes discovery of cryptic species almost impossible, and speciation in the absence of conspicuous divergence of genitalia has been documented (Hollander and Dijkstra 1974; Costa and Francescoli 1991; Bond et al. 2003). Furthermore, cases have been described of species differing in their non-genital morphology (as well as ecology), but not in their genitalia (Huber 1998b).

The present paper describes a further case of putative speciation without changes in genital shape. A species limit is inferred from the extreme size difference as well as some colour patterns that vary in a way that usually reflects species limits.

## 2. Materials and methods

This study is based on material deposited in the collections of the Instituto Butantan, São Paulo (IBSP). Style of descriptions is as in Huber (2000). Measurements are in mm unless indicated otherwise. For cladistic analysis see Relationships below.

## 3. Results

### 3.1. Taxonomy

#### Genus *Psilochorus* Simon, 1893

#### *Psilochorus itaguyrussu*, new species

(Figs. 1, 2, 5a, 6a and 7–9)

**Type:** Male holotype from Gruta dos Crioulos (22°42'S, 45°37.5'W), Campos do Jordão, São Paulo, Brazil; February 23, 1993 (P. Gnaspini), in IBSP (31892).

**Etymology:** The specific name is a noun in apposition taken from the Tupi-Guarani Indian language, meaning grotto and referring to the habitat.

**Diagnosis:** Easily distinguished from most known Brazilian pholcids by the male chelicerae densely covered with hairs but without further modification (Fig. 9); from *Psilochorus ybytyriguara* by the larger size (Figs. 5 and 6, and measurements below) and the pattern on the carapace (Fig. 1).

**Male (holotype):** Total length 6.6 (6.7 with clypeus), carapace width 2.6. Leg 1: 76.4 (20.1+1.2+20.4+31.5+3.2), tibia 2: 15.9, tibia 3: 12.9, tibia 4: 14.3. Tibia 1 L/d: 82. Habitus as in Figs. 1 and 2. Prosoma ochre with slightly darker mark medially, ocular area and clypeus also light brown; sternum pale, almost whitish, with darker margins; legs ochre to light brown, without dark rings; abdomen bluish-grey with black pattern, ventrally with light brown mark around gonopore and smaller mark between epigastric furrow and spinnerets.

Ocular area elevated, thoracic furrow distinct, very deep behind ocular area; distance PME–PME 220 µm, diameter PME 215 µm, distance PME–ALE 180 µm, distance AME–AME 55 µm, diameter AME 100 µm. Clypeus unmodified. Sternum wider than long (1.8/1.5), unmodified. Chelicerae as in Fig. 9, densely covered with hairs but without apophyses. Palps as in Figs. 7 and 8; coxa with retrolateral apophysis, trochanter without projection, femur with prominent unsclerotized projection proximally and hooked apophysis distally, procurus very simple rod, bulb with prominent dorsal inflation and terminal hooked apophysis. Retrolateral trichobothrium of tibia 1 at 4%; prolateral trichobothrium present on all tibiae; legs without spines, with curved hairs on tibiae and metatarsi (mostly on legs 1 and 2), with vertical hairs on femora in slightly higher density than usual, especially on femur 3; femur 3 slightly thicker than others; tarsus 1 with over 20 pseudosegments, about 15 distally fairly distinct.

**Female:** Unknown.

**Distribution:** Known from type locality only.

**Material examined:** Only holotype above.

#### *Psilochorus ybytyriguara*: new species

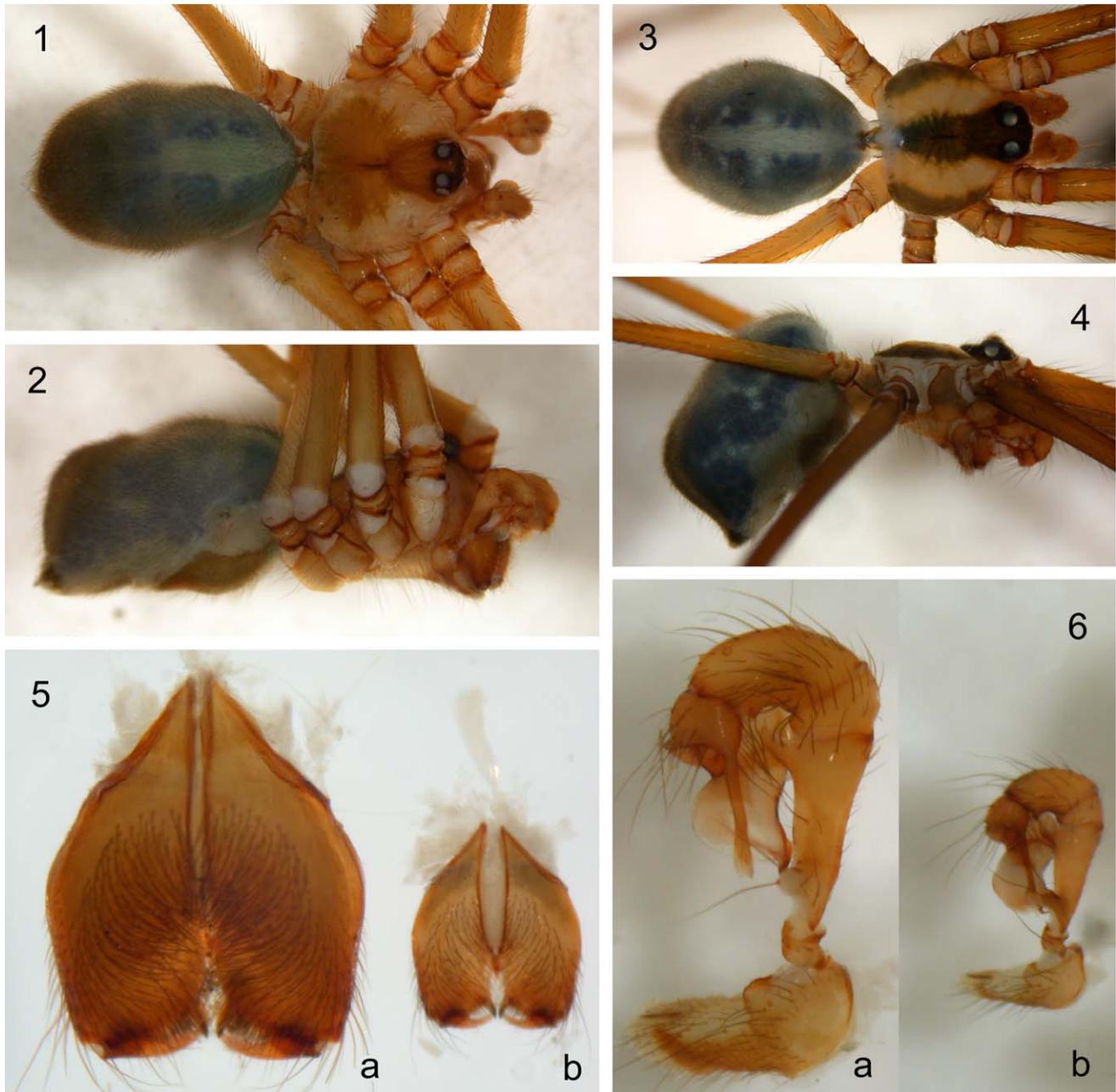
(Figs. 3, 4, 5b, 6b)

**Type:** Male holotype from Estação Biológica do Alto da Serra (23°46.7'S, 46°18.6'W), 750–890 m a.s.l., Paranaipiacaba, São Paulo, Brazil; December 14–15, 2003 (B. A. Huber), in IBSP (41216).

**Etymology:** The specific name is a noun in apposition taken from the Tupi-Guarani Indian language, meaning mountain inhabitant and referring to the type locality.

**Diagnosis:** Easily distinguished from most known Brazilian pholcids by the male chelicerae densely covered with hairs but without further modification; from *Psilochorus itaguyrussu* by the smaller size and the pattern on the carapace (Figs. 3, 5b, 6b, and measurements below), genital shape almost identical (see description below).

**Male (holotype):** Total length 3.9 (4.0 with clypeus), carapace width 1.6. Leg 1: 30.9 (8.6+0.7+8.4+11.1+2.1), tibia 2: 6.1, tibia 3: 4.6, tibia 4: 5.4. Tibia 1 L/d: 50. Habitus as in Figs. 3 and 4. Prosoma pale ochre with black median and lateral stripes, ocular area also black, clypeus not darker; sternum pale, almost whitish, margins and median area darker; legs brown with distinct black rings on femora (subdistally) and tibiae (proximally and subdistally); abdomen bluish-grey with black pattern, ventrally with light brown mark around gonopore and smaller mark between epigastric furrow and spinnerets. Ocular area elevated, thoracic furrow distinct, very deep behind ocular area; distance PME–PME 150 µm, diameter PME 150 µm, distance PME–ALE 160 µm, distance AME–AME 35 µm, diameter AME 60 µm. Clypeus unmodified. Sternum wider than long (1.0/0.9), unmodified. Chelicerae similar to



**Fig. 1–6.** *Psilochorus itaguyrussu* and *P. ybytyriguara*, holotypes. **1, 2.** *P. itaguyrussu*, dorsal and lateral views. **3, 4.** *P. ybytyriguara*, dorsal and lateral views. **5, 6.** Male chelicerae and left palps of *P. itaguyrussu* (a) and *P. ybytyriguara* (b) at the same scales.

*P. itaguyrussu*, with similar density of hairs, but much smaller size (Fig. 5b). Palps much smaller than those of *P. itaguyrussu* (Fig. 6b) but almost identical in shape (cf. Figs. 7 and 8); minimal differences in the shapes of the distal femur apophysis (slightly less curved) and the unsclerotized tip of the procurus. Retrolateral trichobothrium of tibia 1 at 5%; prolateral trichobothrium present on all tibiae; legs without spines, with curved hairs on tibiae and metatarsi of legs 1–3, vertical hairs in usual low density; femur 3 slightly thicker than others; tarsus 1 with about 20 pseudosegments, about 15 distally quite distinct.

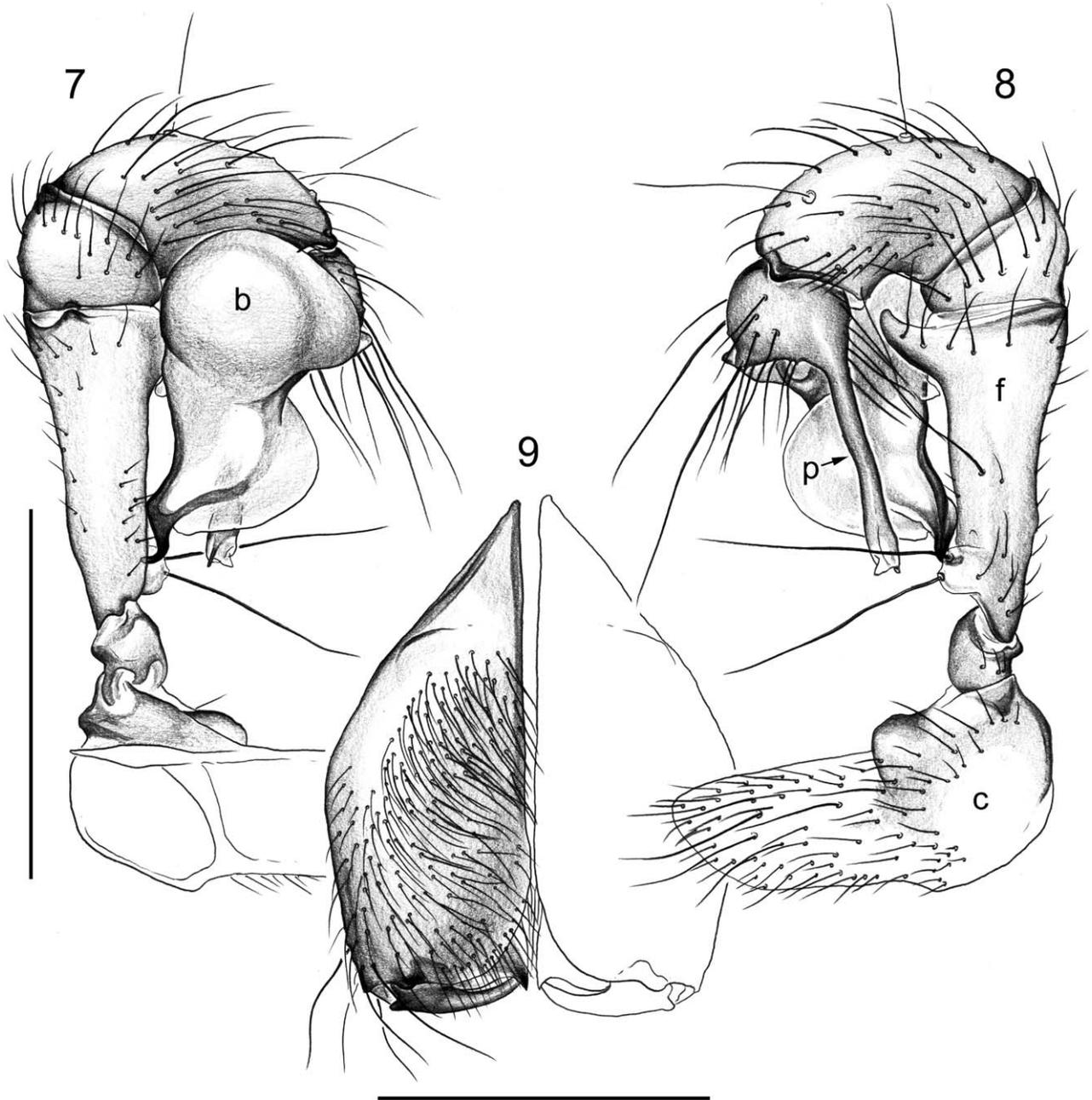
**Female:** Unknown.

**Distribution:** Known from type locality only.

**Material examined:** Only holotype above.

### 3.2. Relationships

The preliminary phylogenetic placement of the new species is based on cladistic analysis. As a basis, the matrix used in Huber and Pérez (2001a) was taken and the new species *P. itaguyrussu* was added (codings see Appendix 1). Using the command string



**Fig. 7–9.** *Psilochorus itagyurussu*, male holotype. **7, 8.** Left palp in prolateral and retrolateral views. **9.** Chelicerae, frontal view. Abbreviations: b, bulb; c, coxa; f, femur; and p, procurus. Scale lines: 1 mm.

<amb-; hold5000; hold/50; mult\*100> in NONA (version 2.0; Goloboff 1993) resulted in 104 trees of length = 184 (CI: 37, RI: 77). Deletion of longer trees due to collapsing of unsupported nodes (using Winclada, version 0.9.9+, Nixon 1999; see also Coddington and Scharff 1994) left 74 most parsimonious trees, all of which placed the new species in a clade consisting of *Modisimus* and *Psilochorus*, representing the *Modisimus* group (sensu Huber 1998a). Synapomorphies of this group were the curved hairs on the legs and the curved apophysis distally on the male palpal femur. Within this

clade, the new species was sister to *Modisimus*, based on the vertical hairs on the male walking leg femora.

## 4. Discussion

### 4.1. Speciation without genital evolution

We consider the differences in the specimens above as indications of species status. Several points support

this assumption. First, the extreme size differences in the genitalia are in strong contrast with allometric studies showing that within species, allometric values of genitalia tend to be low, i.e., genitalia tend to be of rather uniform size within species (Eberhard et al. 1998; Teder 1998; Palestini et al. 2000; Tatsuta et al. 2001). This probably reflects the functional constraint to fit the corresponding structures of the opposite sex, meaning that the size difference is a likely indicator of reproductive isolation, a main criterion in several species concepts. The size difference, as well as some further differences in colour patterns on carapace, clypeus, and legs, suggest that diagnosability is also given, satisfying the main criterion of the phylogenetic species concept sensu Wheeler and Platnick (2000). Clearly, sample sizes in this study are at the absolute minimum, but comparison with other pholcids strongly supports the conclusion that the colour pattern differences are not extremes of a continuum.

The probably most surprising result of this study is the near-identical shape of the genitalia in the two supposed species. There is a wide consensus that genitalia and other sexual contact structures commonly evolve more rapidly than other characters (reviews in Eberhard 1985; Hosken and Stockley 2004). This relatively rapid evolution, coupled with a relative uniformity of genitalia within populations and supposed reproductive communities, explains the importance of genitalia in species discrimination in many animal taxa, including spiders (Huber 2005). Using genitalic shape as the main criterion to distinguish species biases results against finding speciation without changes in genitalic shape. Most cases that have been documented were discovered either by chance or because some non-genitalic character suggested species limits. Cryptic species are more likely to be discovered using behavioral and molecular data, but few studies have used this approach combined with morphological study of the genitalia (Hollander and Dijkstra 1974; Costa and Francescoli 1991; Bond et al. 2003).

## 4.2. Generic placement

Ideally, new species should be analysed cladistically before assignment to a genus, at least if the assignment is not obvious. The prerequisite for this is a more or less stable hypothesis of relationships based on a matrix with adequate representation of taxa. Unfortunately, this is still not the case in pholcid spiders. The matrices published so far were designed to solve the basic (mainly subfamily) clades within the family, and taxon sampling is inadequate for most questions requiring finer resolution. Thus, the analysis above left

no doubt about the inclusion of the new species in the New World clade (sensu Huber 2000), and within that clade in the *Modisimus* group (sensu Huber 1998a), but within the *Modisimus* group, relationships remain obscure. The analysis above placed the new species closer to the Central and Northern American genera *Modisimus* and *Psilochorus* rather than to South American genera. The character supporting this position are the curved hairs on the legs, a character with substantial homoplasy (Huber 2000). The sister-group relationship with *Modisimus* is suggested by the vertical hairs on the femora, but these occur in only one of the species newly described, and in much lower density than in typical *Modisimus* (cf. Huber 1998b). The synapomorphy of *Modisimus* is a turret-like ocular area, a character state not present in the new species. Thus, inclusion in *Modisimus* is not supported. The genus *Psilochorus* lacks any known synapomorphies, but apart from the larger sizes (especially of *P. itaguyrussu*), the genitalia are strikingly similar. The only Brazilian Atlantic Forest genus possibly included in the *Modisimus* group is *Tupigea* (Huber 2000), but most species of *Tupigea* are less than 2 mm long, and the male palpal patella is unusually long ventrally in most species. Thus, we conclude that the new species cannot presently be assigned with confidence to any existing genus, but based on similarity we prefer tentative assignment to *Psilochorus*, a solution to be tested in the future.

It should be noted that several Brazilian pholcids were originally described as *Psilochorus*. Most have been transferred to other genera, but three remain in *Psilochorus*, in all cases due to poor knowledge of the species concerned: *P. bruneocyaneus* Mello-Leitão, 1941, *P. sectus* Mello-Leitão, 1939 (both known from the female only) and *P. taperae* Mello-Leitão, 1929. The types of all these species are apparently lost (Huber 2000), but the descriptions suggest that none of them is conspecific with any of the specimens described herein.

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## Appendix 1

Codings of *Psilochorus itaguyrussu* for cladistic analysis (characters and codes are described in Huber 2001).

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      10      20      30      40      50      60
      |      |      |      |      |      |
0111010101 00111----- 1010101101 1111010000 0000--1010 010000-002-000-

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