

Macroperipatus ohausi: redescription and taxonomic notes on its status (Onychophora: Peripatidae)

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Abstract: Onychophora have undergone a new wave of taxonomic revisions in the last three years. After a recent revision, only two species of *Macroperipatus* are registered from Brazil: *Macroperipatus ohausi* and *Macroperipatus gayi*. The former was only the second species of Onychophora to be described from Brazil, and is herein redescribed, illustrated, and its taxonomic status revised. *Macroperipatus ohausi* had only been collected at the type locality, Petrópolis, state of Rio de Janeiro, but has not been found there since its discovery. In 2009 we collected two specimens of this species in an Atlantic rain forest fragment in Nova Iguaçu in RPPN dos Petroleiros (also state of Rio de Janeiro). The color of the new specimens was interpreted and described based on the NBS/ISCC System of Color Designation, and corresponds only weakly to that described in earlier publications on *M. ohausi*. The identification of *M. ohausi* was based solely on its morphology and compared with its original description. In addition, a female syntype of *M. ohausi* deposited in the Zoologisches Museum (Hamburg) was examined. We reinvestigated its external morphology and concluded that the dorsal papillae have been misinterpreted in *M. ohausi* in previous studies of its integument. The new specimens examined are conspecific with *M. ohausi* mainly by having conical primary papillae, different sized accessory papillae, and by the number of legs. Our material resembles the original description of *M. ohausi*, for example, in spinous pad and nephridial tubercles, even though these features are not represented in the original description and redescription of *M. ohausi*; these features are further detailed here. We concluded that the dorsal papillae morphology of *M. ohausi*, shares diagnostic characters, for example, with representatives of the genera *Peripatus* and *Epiperipatus*. The dorsal papillae are rounded and not square as in the original description and redescription. Another argument that supports our conclusion is that the RPPN dos Petroleiros, the locality where the new samples were collected, belongs to the same mountain chain and Atlantic mosaic as the original material of *M. ohausi*. Furthermore, a male of *M. ohausi* is described here for the first time. Based on our morphological data, we transferred this species to the genus *Epiperipatus*, proposing a new combination, *E. ohausi*. Rev. Biol. Trop. 62 (3): 977-985. Epub 2014 September 01.

Key words: velvet worms, Peripatidae, taxonomy, geographical distribution, Brazil.

Onychophorans, commonly referred to as velvet worms, are a small group of terrestrial invertebrates that are predominantly found in soil, leaf litter, or rotten wood. The species of Onychophora are arranged in two large families, which display a Pangean distribution (Morera-Brenes 2012, Murrienne et al. 2014). Currently 16 species are known from Brazil, belonging to four genera within the family

Peripatidae (*Peripatus* Guilding, 1826, *Epiperipatus* Clark, 1913, *Macroperipatus* Clark, 1913, and *Oroperipatus* Cockerell, 1908). However, Sampaio-Costa, Chagas-Junior & Baptista (2009) recorded 24 morphospecies, of which half are undescribed.

The second oldest species described from Brazil is *Peripatus ohausi* Bouvier, 1900 collected in the city of Petrópolis (state of Rio



de Janeiro). Bouvier (1900) gave a modest description of *P. ohausi*, but singular enough to distinguish it from other species known during that time. Its description was extended in Bouvier (1905) based only on the syntypes. Clark (1913) proposed the subgenus *Macroperipatus* to which *P. ohausi* was allocated. In 1975, Peck revised the New World Onychophora and considered *Macroperipatus* a valid genus of Peripatidae. In 1988, Read studied the integument of *Macroperipatus ohausi* (Bouvier, 1900) and showed that the base of the primary papillae is rounded rather than quadrangular, as in *Macroperipatus torquatus* (von Kennel, 1883), type species of *Macroperipatus*. Furthermore, Read (1988) proposed an approach to understand the morphology of the dorsal papillae using Scanning Electron Microscopy (SEM) in taxonomic studies of Peripatidae. He proposed to subdivide the genera of Peripatidae in three groups based on the scalation and morphology of dorsal papillae.

In a recent study of Brazilian peripatid onychophorans, Oliveira, Wieloch & Mayer (2010) revised two species previously classified as *Macroperipatus* – *M. acacioi* and *M. machadoi* Oliveira & Wieloch, 2005– and also showed that the base of the dermal papillae of these species are rounded rather than quadrangular, according to their original descriptions (Marcus & Marcus, 1955; Oliveira & Wieloch, 2005). In addition, the apical pieces of the primary papillae are well developed and, therefore, they concluded that these two species were not representatives of *Macroperipatus*. Because of the presence of crural tubercles on only two pre-genital segments in males (Clark, 1913; Peck, 1975), the rounded dorsal papillae, and the low number of scale ranks in basal pieces of the primary papilla (Read, 1988), they included these two species in *Epiperipatus*.

As no specimen of *M. ohausi* had been collected in the last thirty years and its taxonomic status has not been reviewed, we redescribed and illustrated in this study its color *in vivo*, and the morphology of the dorsal integument using SEM. Furthermore, its distribution was extended and its taxonomic status revised.

MATERIALS AND METHODS

Material: The two specimens were collected in the Reserva Particular do Patrimônio Natural dos Petroleiros (RPPN dos Petroleiros) (22°35'44.83" S - 43°26'7.62" W), Nova Iguaçu, state of Rio de Janeiro (Brazil), about 28km from Petrópolis (Fig. 1). In respect of syntypes, some characters of the syntype (female) housed in the onychophoran collection of the Zoologisches Museum Hamburg (Hamburg, Germany) were verified by Hilke Ruhberg (personal communication). Repository acronyms are as follows: MNRJ-Museu Nacional/UFRJ, Rio de Janeiro, State of Rio de Janeiro, Brazil and ZMH-Zoologisches Institut und Zoologisches Museum, Hamburg, Germany.



Fig. 1. Political division of Brazilian states. Rio de Janeiro state is amplified and colored gray.

Fixation: Specimen (MNRJ 0056) was fixed directly in alcohol 70%. Specimen (MNRJ 0058) was previously anesthetized in ethyl acetate and then fixed in alcohol 70%, according to the protocol of Monge-Nájera & Morera-Brenes (1994). After fixation, the specimens were sexed and examined under a stereomicroscope and with scanning electron microscope (SEM). SEM methods included: (1) Cleaning:

pieces were rehydrated and cleaned with a 3% solution of Sodium Hypochlorite (NaClO) and distilled water. They were then dehydrated using an increasing alcoholic series: 30%, 50%, 70%, 80%, 90% and 100%. (2) Critical point: all the examined pieces with SEM were critical point dried in model Baltec CPD 030, and then covered by a coat of gold (approximately $200\text{\AA}=2.10^{-10}\text{m}$) in Denton Desk IV gold sputter model.

Nomenclature: Dental formula and descriptive terminology follows Morera-Brenes & Monge-Nájera (2010) and Oliveira et al. (2010), respectively.

Color study: Pictures were taken with a Fuji Finepix S1500 (4-mega pixels) camera. Coloration was described based on living specimens. Following Kury & Orrico (2006), standard names of the 267 Color Centroids of NBS/ISCC Color System (Mundie, 1995) were used in descriptions and given between parentheses.

RESULTS

Family Peripatidae

Genus *Epiperipatus* Clark, 1913

Epiperipatus ohausi (Bouvier, 1900) **new combination**

Peripatus ohausi Bouvier, 1900: 67, 1905: 204.

Peripatus (Macropерipatus) ohausi: Clark, 1913: 17.

Macropерipatus ohausi: Peck 1975: 347; Sampaio-Costa et al. 2009: 558; Oliveira et al. 2012:16.

Fig. 2-12.

Type material: (Syntypes: two females (ZMH and MNHN), BRAZIL, Petrópolis, 31. I. 1900, Dr. Ohaus leg. (Not examined).

Additional material examined: BRAZIL, state of Rio de Janeiro, Nova Iguaçu, Reserva Particular do Patrimônio Natural dos Petroleiros, 23.XII.2009, Guipponi, A.L.; Sampaio-Costa, C. leg. (1 female, MNRJ 0056);

idem, 11.III.2010, Chagas-Jr, A.; Guipponi, A.L.; Kury, A.; Sampaio-Costa, C. leg. (1 male, MNRJ 0058).

Diagnosis: Antennae with 38 to 41 complete rings; outer jaw blade composed of one long curved principal tooth and an additional accessory tooth; inner jaw blade with one principal tooth, one accessory tooth, a diastema, and a series of 12 denticles; dorsal papillae rounded; basal piece of dorsal primary papillae conical; basal and apical pieces of dorsal primary papillae with 7 to 8 and 3 to 4 scale ranks, respectively; leg pairs 27 to 29; ventral organs visible; anal glands of male indistinguishable.

Redescription

Measurements: Female (MNRJ 0056) 54mm long and 5mm wide, and male (MNRJ 0058) 29mm long, 3mm wide. Type (ZMH) 44mm long (syntype).

Color (for living specimens): Body background brownish gray (64) with dorsal light spots (primary papillae) pinkish gray (10). Dorsomedian furrow is pale violet (214), with light olive gray parallel diamonds (112). The head is moderate brown (58) and antennae are grayish to reddish purple (245) (Fig. 2). Dorsal side of legs is grayish to purplish pink (253), ventral side is pale purple (227), and the soles are yellowish white (92). The ventral side is dark pink (6) with light pink (4) ventral organs and light spots pinkish white (9), randomly distributed (Fig. 3). One syntype (ZMH) is totally discolored.

Integument: Twelve plicae per segment, seven of these cross over to ventral side. The incomplete plicae are interrupted over the legs (Fig. 6).

Hyaline organs: On dorsal side, between plicae and parallel to the dorsomedian furrow (Fig. 7).

Dorsomedian furrow: Slender, and starts at mid-point of head and finishes near anus.

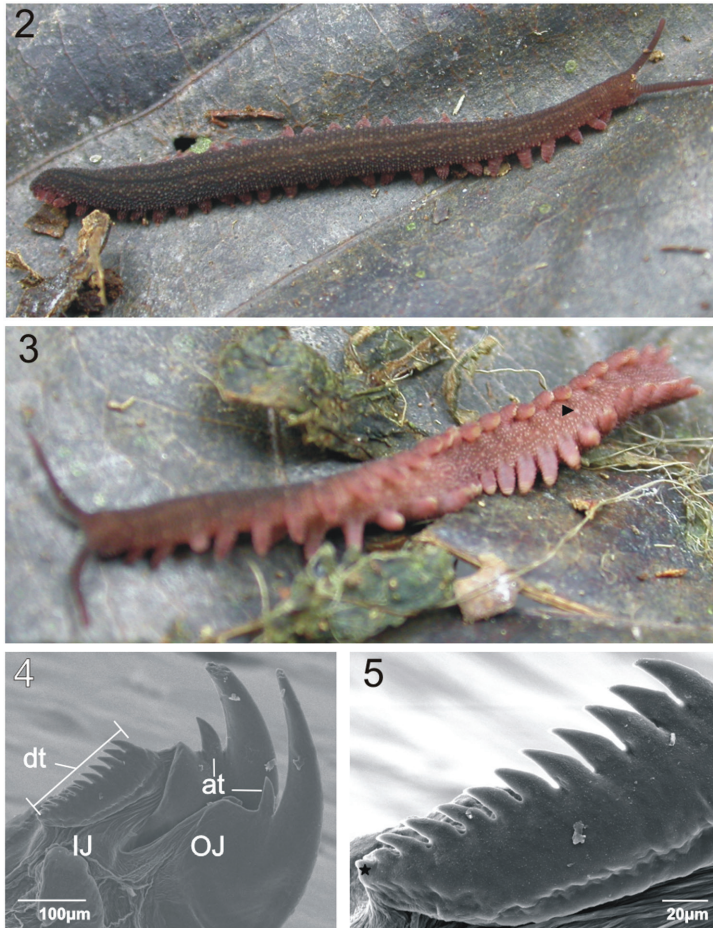


Fig. 2. Living specimen in dorsal habitus. **Fig. 3.** Living specimen in ventral habitus. Ventral organs are visible and pointed with arrowhead (►). **Fig. 4.** Jaws (Female, MNRJ 0056). The principal tooth on both jaws is broken. Outer jaw in foreground and inner jaw in background. Legend. OJ- outer jaw; IJ- inner jaw; at- accessory teeth; dt- denticles. **Fig. 5.** Detail of denticle series (Female, MNRJ 0056; inner jaw,). The last tooth is indicated by the black star.

Dorsal papillae: Female (MNRJ 0056). The dorsal papillae are close to each other and the size varies. Indistinct grooves can be present between two dorsal papillae (Fig. 6). The primary papillae are the biggest dorsal papillae (Fig. 6, 8 and 9); they are rarely in sequence or separated by accessory papillae. The number of accessory papillae varies between two primary papillae. The longest primary papillae are discolored with base diameter larger than plicae. These papillae are always found on alternate plicae in “longitudinal lines” present on different aspects of dorsal side. Depending on its

relative position to the dorsomedian furrow, these lines are composed of a different quantity of the largest primary papillae.

The conical basal piece of the primary papillae has seven (Fig. 8) or nine scale ranks. But nine scale ranks were observed only in the female (MNRJ 0056). These scales are lanceolate and increase in length near the top (Fig. 8). Constriction between the base and top are evident. The apical piece is spherical, asymmetrical and robust. The scales are narrow and as elongated as the taller scales of the base. The scales around the bristle are smaller and

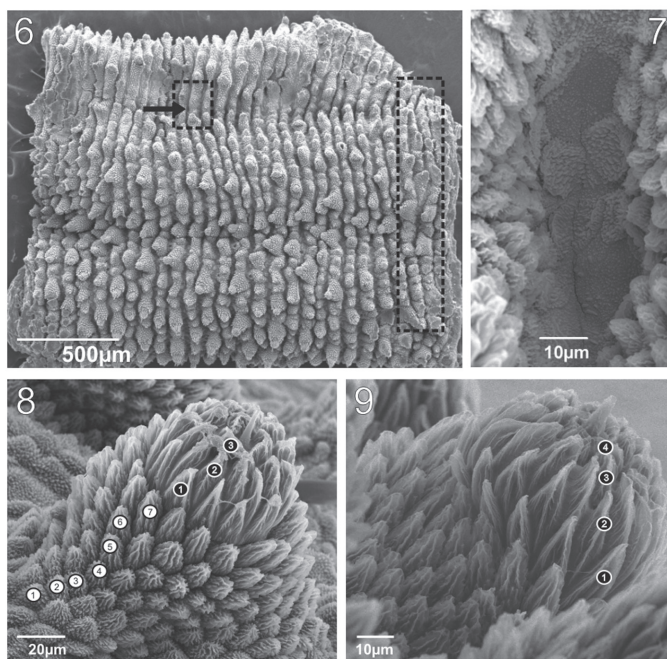


Fig. 6. (Female, MNRJ 0056). Dorsal integument. Dorsomedian furrow is indicated by arrow and dashed square. The dashed rectangle shows the position of incomplete folds between the initial folds on segment. **Fig. 7.** (Female, MNRJ 0056). A pair of hyaline organs on dorsal integument. **Fig. 8.** (Female, MNRJ 0056). Posterior side of Primary papilla. This papilla has seven ranks of scales on base and only three on apical piece. Basal scale (white circles) and apical scale (black circles). **Fig. 9.** (Female, MNRJ 0056). Frontal side view of apical piece with four rank scales. Apical scale (black circles).

truncated (Fig. 8 and 9). The apical piece has three scale ranks on posterior side and four on anterior side.

The accessory papillae are conical and the smallest are the dorsal papillae. Usually the accessory papillae have many in dorsal plicae where the largest primary papillae are present (Fig. 6). The scales on the bottom are more elongated than on top.

Head. Without evident structures. **Antennae.** 38 to 41 complete rings. One syntype (ZMH) has 38 rings. In the collected female (MNRJ 0056) and male (MNRJ 0058) the first seven rings belong to the antennal tip. On both specimens between the 8th and 30th antennal rings there is a sequence of narrow, even and large odd rings. **Frontal organs.** Present at antennal base on ventral side with length about

equal to four fused antennal papillae. **Eyes.** One on each antennal base. **Mouth parts.** Opening surrounded by one anterior oral lobe (unpaired lip) and seven pairs of oral lips. In each pair of oral lip there is a median series of small spines. One syntype (ZMH) has seven pairs of lips. One collected specimen (MNRJ 0056) presents a median series with 9 small spines, whereas the other (MNRJ 0058) presents 7 small spines. **Jaws. (MNRJ 0056). Outer jaw blade.** Composed of one long curved principal tooth and an additional accessory tooth (formula: 1/1) (Fig. 4). The accessory tooth is knife-shaped, curved at the top edge and straight at the bottom edge. **Inner jaw blade.** With one principal tooth, one accessory tooth, a diastema, and a series of 12 denticles (formula: 1/1/12) (Fig. 4 and 5). The accessory tooth of both blades is similar in shape.

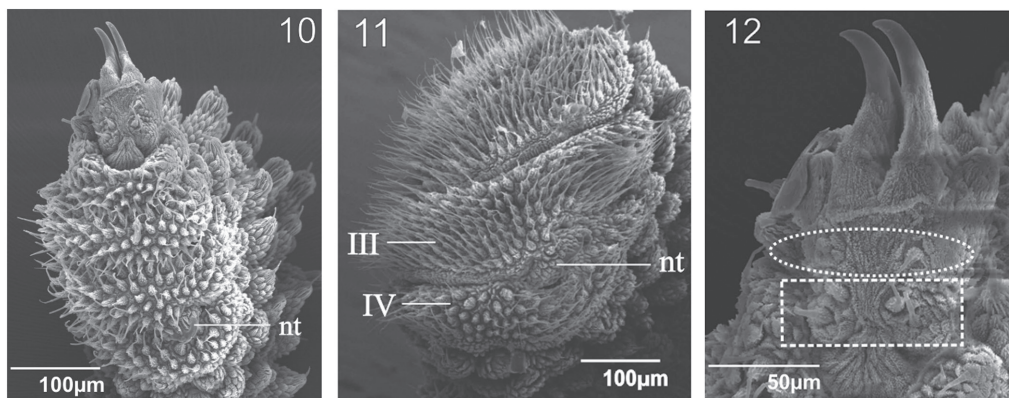


Fig. 10. (Female (MNRJ 0056). Spinous pad and nephridial tubercles. Legend. nt– nephridial tubercles. **Fig. 11.** Detail of nephridial tubercle that is totally connected with the third spinous pad at the top. Legend. The Roman numerals indicate the third and fourth spinous pad of the leg; nt– nephridial tubercles. **Fig. 12.** (Female, MNRJ 0056). Ventral foot surface with indication of bristle areas. Legend. nt- nephridial tubercles. The distal and proximal bristles are indicated by a dashed circle and dashed rectangle, respectively.

Legs: Female with 29 and male with 28 pairs of legs. **Spinous pads.** Fourth and fifth legs have four spinous pads with similar width, and length increases between the first three pads (Fig. 10 and 11). The length of the fourth spinous pad is similar to that of the second. **Nephridial tubercle.** Present on fourth and fifth pairs of legs, between 3rd and 4th spinous pads. It is totally connected with the third pad, articulating with the fourth, but is free from it (Fig. 10 and 11). **Foot papillae.** Feet have two anterior and one posterior foot papillae. Ventral foot surface with six bristles: two pairs in proximal setiform ridge, and at least one bristle on each distal setiform ridge (Fig. 12). **Ventral organs.** They are clearly visible *in vivo*. Post-fixation they are represented by depressions on the ventral integument. **Genital opening.** Located between penultimate pair of legs in both sexes, but the morphology of the genital opening is different among the sexes. The genital opening in female is poorly preserved, but it seems to have two crossed furrows; the transversal one is only weakly visible. Moreover, the male has a single long furrow parallel to the main body.

Male characters: Crural papillae are present in one pre-genital pair of legs (a single

tubercle per leg). Anal glands are only recognized by their corresponding open pores.

Type locality: Petrópolis, state of Rio de Janeiro, Brazil.

Distribution: State of Rio de Janeiro (Petrópolis and Nova Iguaçu, new record), Brazil.

DISCUSSION

The Neotropical Onychophora are traditionally divided into two well-characterized groups named Andean and Caribbean peripatids. Our investigation shows that the two specimens reported in this study are peripatid onychophorans based on simple Caribbean group characteristics: (1) two anterior and one posterior foot papillae; (2) legs IV and V with four spinous pads; and (3) nephridial tubercle openings between the 3rd and 4th pad. These characters are present in all representatives of *Peripatus*, *Epiperipatus*, *Macroperipatus*, *Speleoperipatus* Peck, 1975 and *Plicatoperipatus* Clark, 1913, but the presence of eyes and 12 plicae per segment distinguishes them from *Speleoperipatus* and *Plicatoperipatus*, respectively.

Because of the conical basal piece with a roundish base and the spherical, asymmetrical and robust apical piece, the primary papillae of the analyzed specimens suggest a higher resemblance to *Peripatus* and *Epiperipatus* than to *Macroperipatus*. According to Read (1988), the quadrangular shape of dorsal papillae seen in *M. torquatus* is caused by the outline grooves. In addition, the scales of the basal piece are not arranged in ranks in this species (Oliveira et al., 2010). Therefore analyzed specimens do not belong to *Macroperipatus*.

The dorsal integument of all *Macroperipatus* species is not known in detail, although according to Read (1988) the dorsal primary papillae of *M. acacioi*, *M. ohausi* and *M. guianensis* (Evans, 1903) are rounded and contrast with quadrangular primary papillae of *M. torquatus*. In a recent review of two Brazilian species previously regarded as *Macroperipatus* –*M. acacioi* and *M. machadoi*– Oliveira et al. (2010) also showed that in these species the bases of the dermal papillae are roundish and covered with well-developed conical scales, which are arranged in several ranks. Therefore, Oliveira et al. (2010) transferred both species to the genus *Epiperipatus*. They also suggested that the dorsal papillae should be reinvestigated in other species of *Macroperipatus* by SEM.

Read (1988) registered one *M. ohausi* specimen, collected in 1981 by J. C. Diniz and C. R. Brandão, in Petrópolis. We contacted C. R. Brandão and he informed us of the exact collection point where the specimen was sampled (C. R. Brandão pers. comm.). We returned several times to this locality, but today this area is completely urbanized and we did not find any onychophoran there.

The two new specimens were collected 28km from Petrópolis, type locality of *M. ohausi* (Fig. 1). The characters present in these specimens agree with the description of *M. ohausi sensu* Bouvier (1900, 1905), except for the number of leg pairs. The number of leg pairs is not fixed in Neotropical Peripatidae species (Bouvier, 1905; Campiglia & Lavalard, 1973). The number of leg pairs in our specimens is 28 and 29, and the type is 27.

Bouvier (1900, 1905) poorly described *M. ohausi*, based mainly on the description of the primary papillae morphology. Then, Read (1988) included the number of scale ranks in the description of this species based on recent specimens collected in 1981 by J. C. Diniz and C. R. Brandão in Petrópolis.

The dorsal primary papillae described by Read (1988, figs. A-D) were compared to the specimens from RPPN dos Petroleiros and also agree with them in morphology. The morphology of the dorsal primary papillae and the range of leg pairs show that *Epiperipatus ohausi* is more closely related to the species of *Epiperipatus* from the state of Minas Gerais (Southeastern Brazil): *E. acacioi*, *E. machadoi*, *E. diadenoproctus* Oliveira, Lacorte, Fonseca, Wieloch & Mayer, 2011; *E. adenocryptus* Oliveira et al. 2011; and *E. paurognotus* Oliveira et al. 2011. *Epiperipatus ohausi* has seven or eight scale ranks on the base of the primary papillae, while *E. acacioi*, *E. machadoi*, *E. diadenoproctus*, *E. adenocryptus* have 5-6, 5-8, 5-8 and 5-7, respectively. Four scale ranks on *E. adenocryptus*, three on *E. ohausi* and *E. acacioi*, and two on *E. paurognotus* were counted from the posterior side of the apical piece, but for *E. diadenoproctus* it was not described.

Epiperipatus ohausi, *E. acacioi* and *E. machadoi* have the asymmetrical apical piece in common, though it is spherical in the first species and ovate in latter two (although some *E. acacioi* specimens have a globular shape as well). The sensory bristles usually are needle-shaped and curved posteriorly, as observed in *E. acacioi*, *E. machadoi* and *E. ohausi*. However, it is spine-shaped in the cryptic species from Minas Gerais (Oliveira et al., 2011). The range of leg pairs is different among these six Brazilian Southeastern species. In increasing order, the ranges are: 27 to 29 in *E. ohausi*; 25 to 28 in *E. acacioi* (n=34); 26 to 29 in *E. paurognotus* (n=15, see Oliveira et al., 2011); 26 to 30 in *E. adenocryptus* (n=25, see Oliveira et al., 2011); *E. diadenoproctus* (n=62, see Oliveira et al., 2011), and 27 to 31 in *E. machadoi* (n=21). The anal glands are absent in males of

E. ohausi, but the respective pores are present. These pores are observed on the ventral board of the anus. In *E. acacioi* and *E. machadoi* the anal glands seem also to be absent, while in *E. adenocryptus*, *E. diadenoproctus* and *E. paurognostus* these structures are present in the ventral board of the anus. The SEM primary papillae (Fig. 5, 7 and 8) analysis of *M. ohausi* showed that the base is not square-shaped as in *M. torquatus*, but rounded as in *Peripatus* and *Epiperipatus*.

The new record of *E. ohausi* has prompted us to redescribe and illustrate the color and integument of the species in detail. Furthermore, we reviewed the taxonomic status of this poorly known species.

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RESUMEN

Macroperipatus ohausi: **redescripción y notas taxonómicas sobre su estatus (Onychophora: Peripatidae).**

Onychophora experimentó una nueva serie de revisiones taxonómicas en los últimos tres años. Después de una reciente revisión, sólo dos especies de *Macroperipatus* fueron registradas para Brasil: *Macroperipatus ohausi* y *Macroperipatus geayi*. La primera fue solo la segunda especie de Onychophora en ser descrita en Brasil y aquí es redescrita, ilustrada, y su estatus taxonómico revisado. *Macroperipatus ohausi* sólo se había recolectado en la localidad tipo, Petropolis, Estado de Rio de Janeiro, pero no se ha encontrado allí desde su descubrimiento. En 2009, recolectamos dos ejemplares de esta especie en un fragmento de la 'Mata Atlântica' en Nova Iguaçu en el RPPN de los Petroleiros (también en el estado de Rio de Janeiro). El color de los ejemplares nuevos fue interpretado y descrito, basado en el sistema NBS System/ISCC de designación de colores, y correspondió débilmente al descrito en publicaciones anteriores sobre *M. ohausi*. La identificación de *M. ohausi* se basó únicamente en su morfología, en comparación con la descripción original. Además, se examinó un sintipo hembra de *M. ohausi* depositado en Zoologisches Museum (Hamburgo). Nosotros reinvestigamos su morfología externa y concluimos que las papilas dorsales fueron mal interpretadas en *M. ohausi* en los estudios previos de su integumento. Los nuevos especímenes examinados son coespecíficos con *M. ohausi* principalmente por tener las papilas primarias cónicas, diferente tamaño de las papilas accesorias, y por el número de patas. Nuestro material se asemeja a la descripción original de *M. ohausi*, por ejemplo, la almohadilla espinosa y tubérculos nefridiales, aunque estas características no están representadas en la descripción original y redescrpción de *M. ohausi*, estas características también se detallan aquí. Llegamos a la conclusión de que la morfología de las papilas dorsales de *M. ohausi*, comparte caracteres de diagnóstico, por ejemplo, con los representantes de los géneros *Peripatus* y *Epiperipatus*. Las papilas dorsales son redondeadas y no cuadradas como en la descripción original y redescrpción. Otro argumento que apoya nuestra conclusión es que la RPPN dos Petroleiros, la ubicación en la que se recogieron las nuevas muestras, pertenece a la misma cadena de montañas y al mosaico Atlántico, tal como el material original de *M. ohausi*. Por otra parte, se describe aquí por primera vez un macho de *M. ohausi*. Basado en nuestros datos morfológicos, transferimos esta especie al género *Epiperipatus*, que propone una nueva combinación, *E. ohausi*.

Palabras clave: gusanos de terciopelo, Peripatidae, taxonomía, distribución geográfica, Brasil.

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