

Supporting Information

Unexplored Character Diversity in

Onychophora (Velvet Worms): a Comparative

Study of Three Peripatid Species

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Supporting Figures

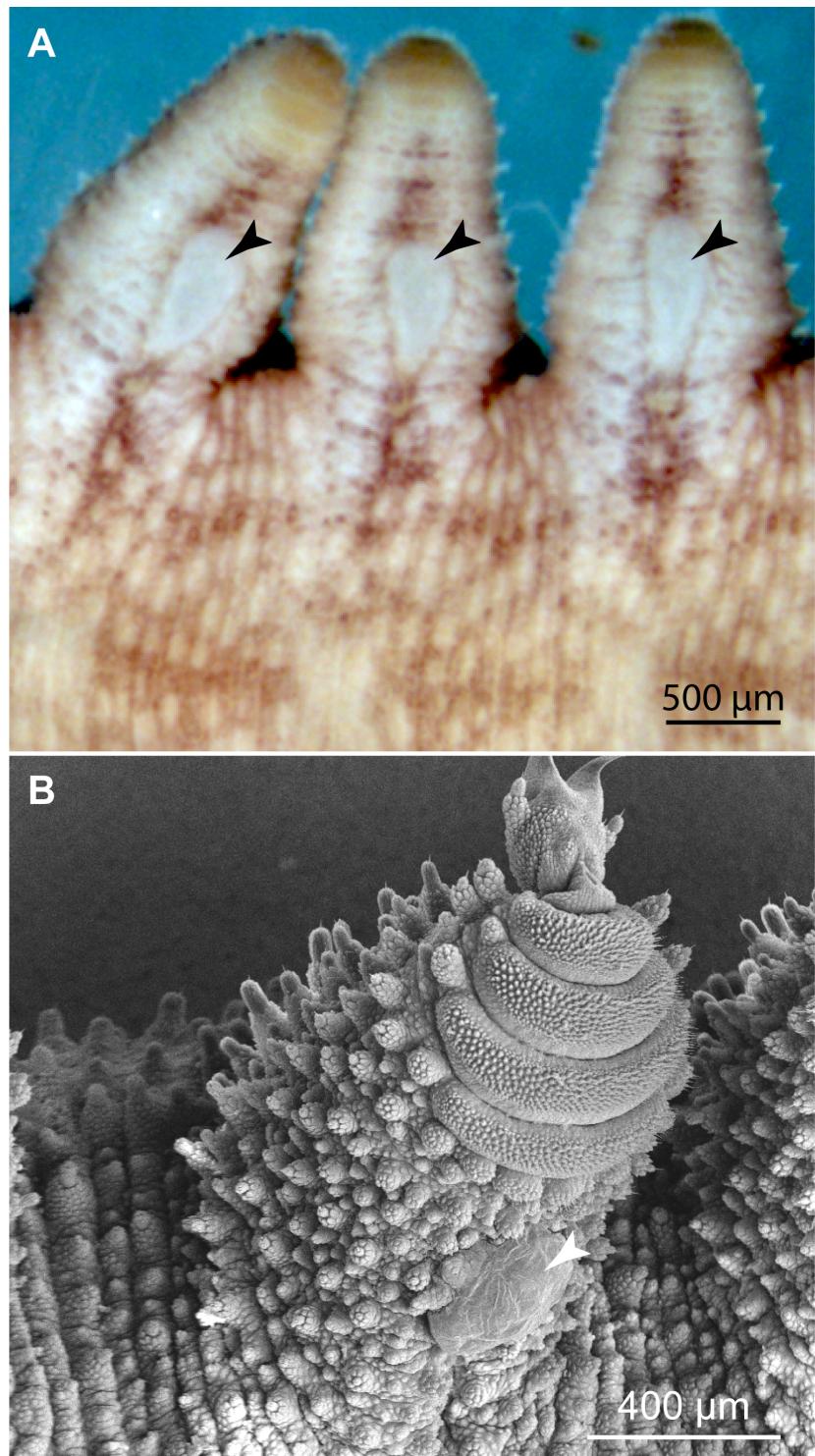


Figure S1. Eversible coxal vesicles (arrowheads). (A) *Principapillatus hitoyensis* gen. et sp. nov.. Light micrograph of ventral leg surface. (B) *Eoperipatus* sp.. Scanning electron micrograph of ventral leg surface. Anterior is right in both images.

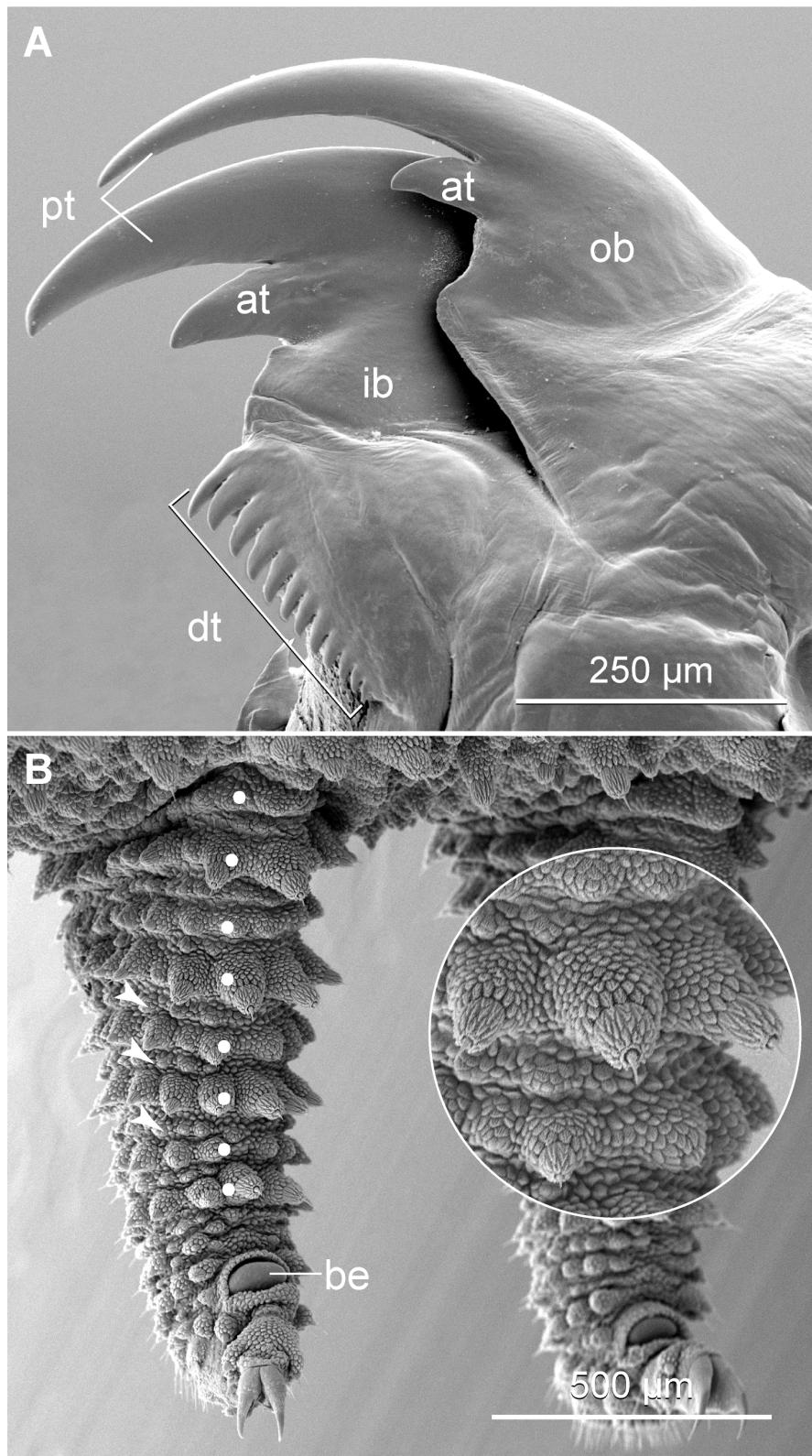


Figure S2. Additional features of *Principapillatus hitoyensis* gen. et sp. nov.. (A) Characteristics of the inner and outer jaw blades. (B) Arrangement of transverse rings on legs. Anterior is left. Note the presence of thin semi-rings (arrowheads) between the complete rings (white dots). Circular inset shows an enlarged primary papilla. Abbreviations: at, accessory tooth; be, bean-shaped papillae; dt, denticles; ib, inner jaw blade; ob, outer jaw blade; pt, principal tooth.

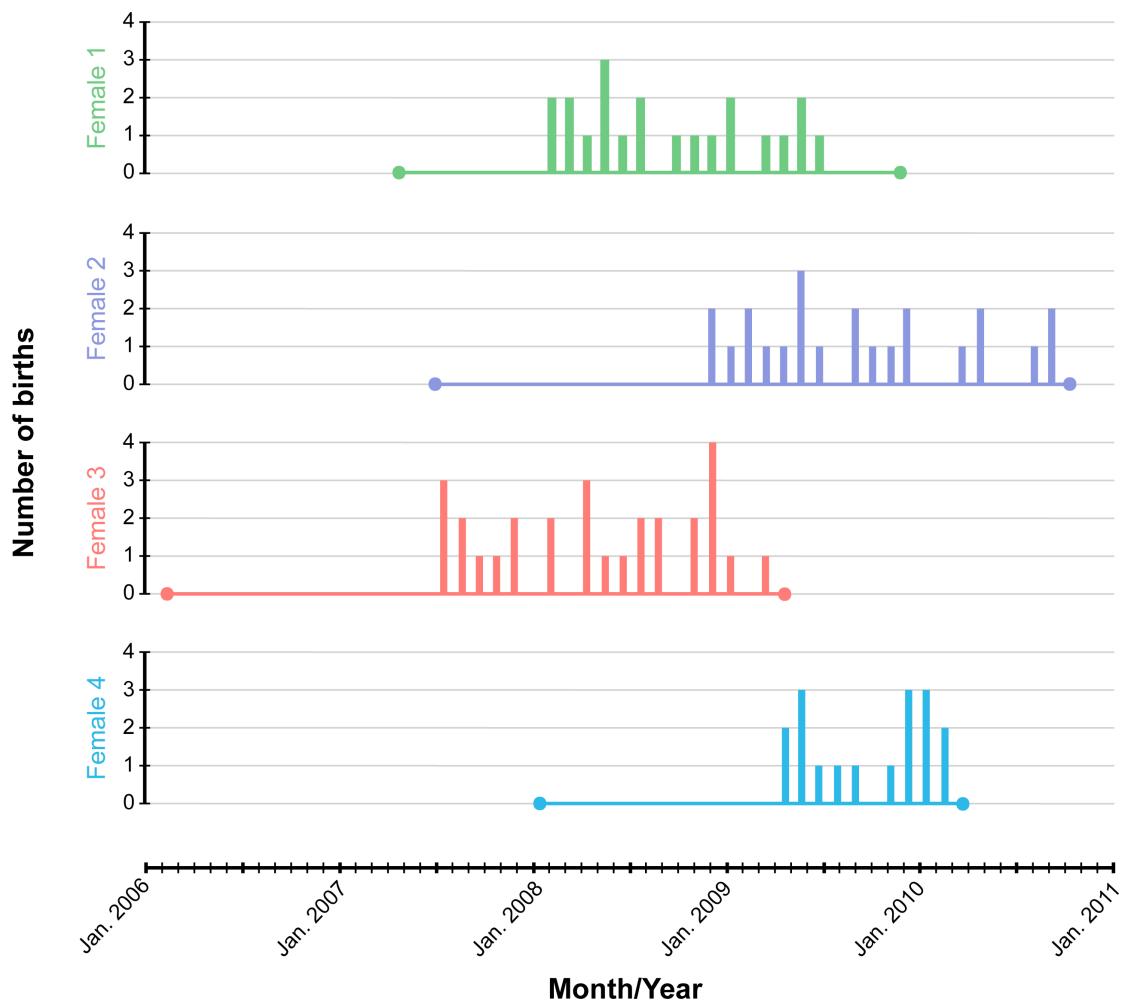


Figure S3. Number of births during the lifespan in four females of *Principapillatus hitoyensis gen. et sp. nov.* Lifespan is represented by horizontal lines; number of births is illustrated by vertical bars. The left and right filled circles associated with horizontal lines indicate birth and death of each female, respectively.

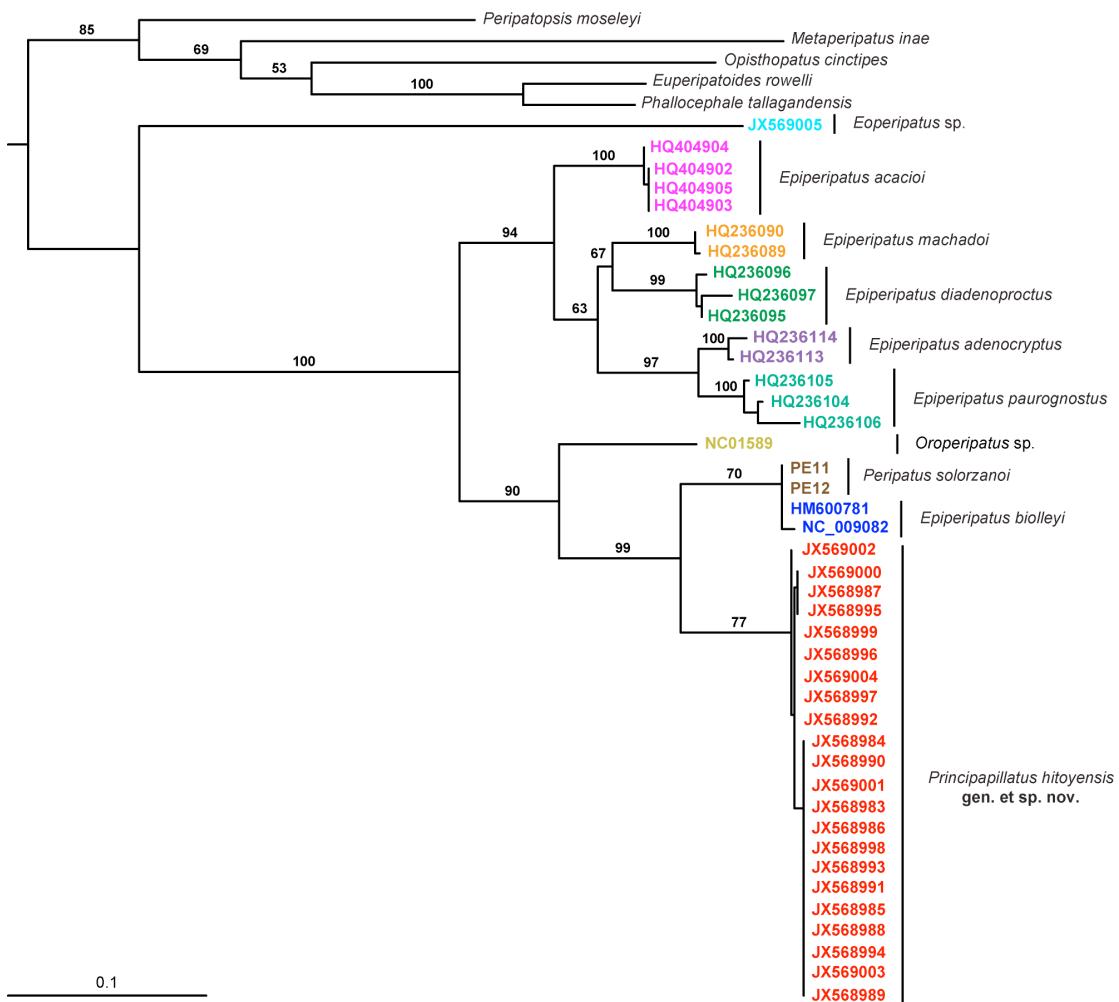


Figure S4. Maximum Likelihood topology illustrating the phylogenetic relationships of several species of Peripatidae. Combined analysis of nucleotide sequences of 12S rRNA and translated aminoacids of *COI*, with five peripatopsid species as an outgroup. Bootstrap values lower than 50 are not shown. Abbreviations correspond to the accession numbers of the *COI* sequence in GenBank.

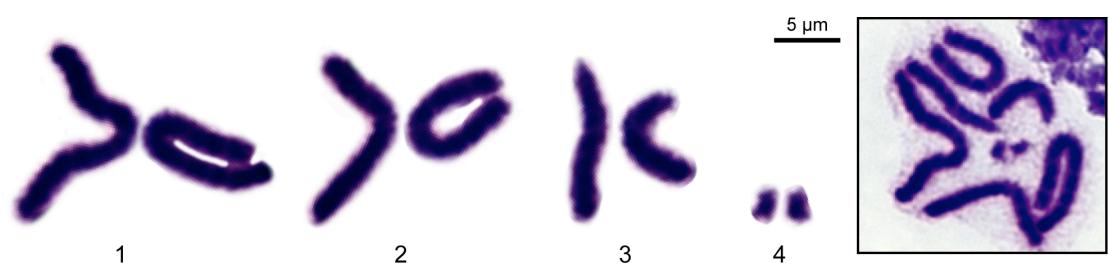


Figure S5. Karyotype of *Eoperipatus* sp.. Inset shows a light micrograph of the original preparation of mitotic chromosomes from a testis stained with Giemsa. Note three pairs of large and one pair of small chromosomes.

Supporting Tables

Table S1. Number of specimens analysed using each method. Numbers are provided for specimens of different sexes and ages, including embryos.

Method	Number of specimens analysed		
	<i>Principapillatus hitoyensis</i> gen. et sp. nov.	<i>Eipiperipatus biolleyi</i>	<i>Eoperipatus</i> sp.
Analyses of colour pattern	1109	16	66
Counting of leg pairs	1109	16	66
Light microscopy	~20	~5	~10
Scanning electron microscopy	14	9	6
Karyology	5	–	3
Molecular analyses	21	–	1

Table S2. Current inconsistencies with the diagnostic features used for the peripatid genera. Symbols used are as follows: (+) present, (−) absent, (+/−) found only in some species of the genus, (?) data unavailable. Grey shading highlights potentially unique features for each genus (note that most proposed diagnostic features are not unique and have to be revised).

Diagnostic features of each genus, as suggested in the original descriptions and subsequent revisions (see References below)	Occurrence of the diagnostic features among the genera, as found in the descriptions of species assigned to each genus (see References listed below)									
	<i>Eoperipatus</i>	<i>Epiperipatus</i>	<i>Heteroperipatus</i>	<i>Macropiperipatus</i>	<i>Mesoperipatus</i>	<i>Oroperipatus</i>	<i>Peripatus</i>	<i>Plicatoperipatus</i>	<i>Speleoperipatus</i>	<i>Typhloperipatus</i>
<i>Eoperipatus</i> Evans, 1901										
Four or five spinous pads per leg	+	+	+	+	−	+	+	+	+	+
Nephridial tubercle in the middle of the 4 th pad or on its proximal side	+/-	−	−	−	−	−	−	−	−	−
4 th and 5 th leg pairs with only four spinous pads	+	+	+	+	−	+/-	+	+	+	+
One anterior and one posterior distal foot papillae	+	−	−	−	−	−	−	−	−	+
One or more bristles on each setiform ridge	+	+	+	+	+	+	+	+	+	+
Inner jaw blade with a diastema	+	+	+	+	+	+	+	+	+	+
Two accessory teeth on outer jaw blade	+	+/-	+/-	+/-	+/-	+/-	+/-	+/-	−	+/-
Up to three accessory teeth on inner jaw blade	+	−	−	−	−	+/-	−	−	−	+
Male genital opening cross-shaped	+	+/-	?	+	+	+	+	+	?	+
Single transverse opening on female genital pad	+	−	−	−	+	−	−	−	−	−
Ductus ejaculatorius long, forming a loop	+	+	?	+	+	+	+	+	?	+
Ovarian funnels (=“receptacula ovarum”) and receptacula seminis present	+	+	?	+	+/-	+	+	+	?	+
Ovary large and spreads out over the organs	+	−	?	−	−	−	−	−	?	+
Ova large, full of yolk and exogenous	+	−	?	−	−	−	−	−	?	+
Embryos without placental structures	+	−	?	−	−	−	−	−	?	+
Single and medial anal gland opening situated between the last pair of legs	+	−	?	−	−	−	−	−	?	−

<i>Epiperipatus</i> (Clark, 1913)	<i>Eoperipatus</i>	<i>Epiperipatus</i>	<i>Heteroperipatus</i>	<i>Macroperipatus</i>	<i>Mesoperipatus</i>	<i>Oroperipatus</i>	<i>Peripatus</i>	<i>Plicatoperipatus</i>	<i>Speleoperipatus</i>	<i>Typhloperipatus</i>
Twelve dorsal plicae per segment separated by continuous furrows	+	+	+	+	+	+	+	-	+	+
Incomplete or bifurcated dorsal plicae in the space between two legs	+	+/-	+	+	-	+/-	+	+	-	-
Dorsal primary papillae all of one type	-	+	?	+/-	+	-	-	-	-	?
Dorsal primary papillae with rounded bases	+	+	+	-	+	+	+	+	+	+
Dorsal primary papillae intergraded through all stages in medium-sized and large specimens, while certain ones are markedly prominent in small specimens	+/-	+	?	+/-	+	+/-	+	+	+	?
Dorsal primary papillae close together, although accessory papillae may occur between them	+	+	+	+	+	+/-	-	+	-	?
Accessory papillae very diversely developed	+	+	?	-	+	+/-	+/-	-	?	?
Crural tubercles present only in two pregenital leg pairs in males	+	+	?	+	+	+	+/-	+	?	+
Apical piece of dorsal primary papillae with 2-3 scale ranks	+/-	+/-	?	+/-	?	-	-	+/-	?	?
<i>Heteroperipatus</i> Zilch, 1954	<i>Eoperipatus</i>	<i>Epiperipatus</i>	<i>Heteroperipatus</i>	<i>Macroperipatus</i>	<i>Mesoperipatus</i>	<i>Oroperipatus</i>	<i>Peripatus</i>	<i>Plicatoperipatus</i>	<i>Speleoperipatus</i>	<i>Typhloperipatus</i>
27-28 leg pairs in males	-	+/-	+	+/-	-	+/-	+/-	-	-	-
31-32 leg pairs in females	-	+	+	+	-	+	+	-	-	-
Three anterior and one posterior distal foot papillae	-	-	+	-	-	-	-	-	-	-
Two anterior and two posterior distal foot papillae	-	-	+ ¹	-	-	+	-	-	-	-
Four or five spinous pads	+	+	+	+	-	+	+	+	+	+
Nephridial tubercle in the 4 th and 5 th leg pairs below the 3 rd spinous pad	+/-	+	+	+	+	-	+	+	+	-
Nephridial tubercle "more or less" (Zilch 1954b) separated from the 3 rd spinous pad	+/-	+	+	+	+	-	+	+	+	-
<i>Macroperipatus</i> (Clark 1913)	<i>Eoperipatus</i>	<i>Epiperipatus</i>	<i>Heteroperipatus</i>	<i>Macroperipatus</i>	<i>Mesoperipatus</i>	<i>Oroperipatus</i>	<i>Peripatus</i>	<i>Plicatoperipatus</i>	<i>Speleoperipatus</i>	<i>Typhloperipatus</i>
Twelve dorsal plicae per segment separated by continuous furrows	+	+	+	+	+	+	+	-	+	+
Incomplete or bifurcated dorsal plicae in the space between two leg pairs	+	+/-	+	+	-	+/-	+	+	-	-
Dorsal primary papillae with quadrangular bases	-	-	-	+/-	-	-	-	-	-	-
Dorsal primary papillae separated by longitudinal grooves parallel to the main body axis	-	-	-	+	-	+/-	-	-	-	-
Accessory papillae "well reduced in size and few in number" (Peck 1975).	-	+/-	-	+	-	+/-	+/-	-	-	-
Crural tubercles present in two pregenital leg pairs in males (at least in <i>M. perrieri</i>)	+	+	?	+	+	+	+/-	+	?	+

<i>Mesoperipatus</i> Evans, 1901	<i>Eoperipatus</i>	<i>Epiperipatus</i>	<i>Heteroperipatus</i>	<i>Macropiperipatus</i>	<i>Mesoperipatus</i>	<i>Oroperipatus</i>	<i>Peripatus</i>	<i>Plicatoperipatus</i>	<i>Speleoperipatus</i>	<i>Typhloperipatus</i>
Three spinous pads per leg	-	-	-	-	+ ²	-	-	-	-	-
Nephridial tubercle indenting the third pad	+/-	+/-	-	+/-	+	-	+/-	+	+	-
22–25 leg pairs	+	+/-	-	-	+	+/-	-	-	+	-
Single accessory tooth on both jaw blades	+/-	+/-	+	+/-	+	+/-	+/-	+/-	+/-	-
Inner jaw blade with a long row of denticles	+	+	+	+	+	+	+	+	+	+
Dorsal plicae complete	-	+/-	-	-	+	-	-	-	+	+
A pair of receptacula seminis in females	+	+	?	+	+	+	+	+	?	+
Presumable lack of ovarian funnels	-	-	?	-	- ³	-	-	-	?	-
Male anal gland openings in front of the anus in a common groove	-	-	?	-	+	-	-	-	?	-
Two anterior and one posterior distal foot papillae	-	+	-	+	+	-	+	+	+	-
<i>Oroperipatus</i> Cockerell, 1908	<i>Eoperipatus</i>	<i>Epiperipatus</i>	<i>Heteroperipatus</i>	<i>Macropiperipatus</i>	<i>Mesoperipatus</i>	<i>Oroperipatus</i>	<i>Peripatus</i>	<i>Plicatoperipatus</i>	<i>Speleoperipatus</i>	<i>Typhloperipatus</i>
Two distinct anal gland openings in front of the anus	-	-	?	+	-	-	-	-	-	-
4–7 distal foot papillae	-	-	+	-	-	+	-	-	-	-
At least four spinous pads	+	+	+	+	-	+	+	+	+	+
Nephridial tubercles inserted in the third pad	-	-	-	-	-	+	-	-	-	-
<i>Peripatus</i> Guilding, 1826	<i>Eoperipatus</i>	<i>Epiperipatus</i>	<i>Heteroperipatus</i>	<i>Macropiperipatus</i>	<i>Mesoperipatus</i>	<i>Oroperipatus</i>	<i>Peripatus</i>	<i>Plicatoperipatus</i>	<i>Speleoperipatus</i>	<i>Typhloperipatus</i>
Dorsal primary papillae varying in size in all ages	-	+	?	+/-	+	+/-	+	-	+	?
Primary papillae predominantly cylindrical in shape	-	-	?	-	-	+/-	+	-	?	?
Conical accessory papillae smaller than the primary papillae	-	-	?	-	-	+/-	+	-	?	?
Usually three accessory papillae between two primary papillae	+/-	+/-	+/-	-	-	+/-	+	-	-	?
Primary papillae separated by rather broad intervals with accessory papillae	-	-	-	-	-	+/-	+	-	?	+
Crural tubercles in males usually present in more than two pregenital leg pairs	-	-	-	-	-	-	+	-	-	-
Apical piece of primary papillae with four or more scale ranks	+/-	-	?	-	?	+	+	-	?	?

<i>Plicatoperipatus</i> (Clark, 1913)								
	<i>Eoperipatus</i>	<i>Epiperipatus</i>	<i>Heteroperipatus</i>	<i>Macroperipatus</i>	<i>Mesoperipatus</i>	<i>Oroperipatus</i>	<i>Peripatus</i>	<i>Plicatoperipatus</i>
24 plicae per segment	-	-	-	-	-	-	-	+
Plicae very indistinct, with numerous and irregular anastomoses	-	-	-	-	-	-	-	-
Dorsal primary papillae small and “subequal” (Clark 1913)	-	+/-	?	+/-	-	-	-	-
Accessory papillae rare or absent	-	+/-	-	+/-	-	+/-	-	-
Crural tubercles present in two pregenital leg pairs in males	+	+	?	+	+	+	+	+
<i>Speleoperipatus</i> Peck, 1975								
22 or 23 leg pairs	+	<i>Eoperipatus</i>	<i>Epiperipatus</i>	<i>Heteroperipatus</i>	<i>Macroperipatus</i>	<i>Mesoperipatus</i>	<i>Oroperipatus</i>	<i>Peripatus</i>
One posterior and two anterior distal foot papillae	-	+	-	+	+	-	+	+
Nephridial tubercle broadly attached to and indenting the 3 rd spinous pad	+/-	+/-	-	+/-	-	-	+/-	-
Fourth spinous pad crescent-shaped	+	+	+	+	-	+	+	+
Fifth spinous pad absent	-	+/-	-	+/-	+	-	+/-	-

<i>Typhloperipatus</i> Kemp, 1913	<i>Eoperipatus</i>	<i>Epiperipatus</i>	<i>Heteroperipatus</i>	<i>Macropiperipatus</i>	<i>Mesoperipatus</i>	<i>Oroperipatus</i>	<i>Peripatus</i>	<i>Plicatoperipatus</i>	<i>Speleoperipatus</i>	<i>Typhloperipatus</i>
19 or 20 leg pairs	—	—	—	—	—	—	—	—	—	+
Number of leg pairs variable	+	+	+	+	+	+	+	+	+	+
Inner jaw blade with a diastema and a row of denticles	+	+	+	+	+	+	+	+	+	+
Four spinous pads per leg	+	+	—	+	—	+/-	+	+	+	+
Nephridial tubercle on the third pad	—	—	—	—	—	+	—	—	—	+
One anterior and one posterior distal foot papillae	+	—	—	—	—	—	—	—	—	+
Genital pad between the penultimate leg pair	+	+	+	+	+	+	+	+	+	+
Receptacula seminis present, with two ducts opening into the oviducts	+	+	?	+	+	+	+	+	?	+
Ovarian funnels present	+	+	?	+	—	+	+	+	?	+
Oviducts united for a long distance in front of the ovary	—	—	?	—	—	—	—	—	?	+
Ovaries completely fused, with a single cavity	+	—	?	—	—	—	—	—	?	+
Ovaries closely pressed against but not directly attached to the floor of the pericardium to which they are connected posteriorly by means of a ligament (=“funiculus”)	+	+	+	+	^{—4}	+/- ⁴	+	+	?	+
Ovary exogenous	+	—	?	—	—	—	—	—	?	+
Ova large and heavily charged with yolk	+	—	?	—	—	—	—	—	?	+
Ova about 5 mm in their longest diameter	+	—	?	—	—	—	—	—	?	+
Embryo without placental structures (referred to as “trophic vesicle” in the original description)	+	—	?	—	?	—	—	—	?	+
Uterine embryos of about the same age	—	—	?	—	—	—	—	—	?	+ ⁵
Spermatophores long, with horny coat and cap	+	+	?	?	?	?	?	?	?	+
Skin pigment brown, soluble in alcohol	+	+/-	+/-	+/-	+	+/-	+/-	+	—	+
Welldeveloped coxal vesicles	+	+	?	+	+	+	+	+	?	+
Single crural gland per leg in the two pre-genital leg pairs in males	—	+/-	?	—	—	—	—	—	?	+
Two distinct anal gland openings in males, found anteriorly to the anus	—	+	?	+	—	+	+	+	?	+

¹Feature found in *Heteroperipatus clarki*, which might not belong to this genus (Oliveira *et al.* 2012).

²Feature, which is also found in peripatopsid species. However, the structure of the spinous pads is clearly different between Peripatidae and Peripatopsidae, as they are not socle-shaped and the second pad is always larger than the first and the third pads in the species of Peripatopsidae (see Read 1988a).

³The presence of ovarian funnels in this taxon has been shown by Bouvier (1905), Kemp (1914) and Reid (1996).

⁴The lack of the anterior ligament might be a dissection artifact since it is present in most Peripatidae and Peripatopsidae taxa (Mayer & Tait 2010). Only in representatives of *Metaperipatus* and *Peripatopsis* the ovary is fused along its entire length with the pericardial septum (Mayer 2007; Mayer & Tait 2010).

⁵Note that this feature has also been reported from different species of Peripatopsidae (Purcell 1899; Kemp 1914).

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