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Research article

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Sechelleptus arborivagus sp. nov., a new arboreal spirostreptid millipede (Diplopoda, Spirostreptidae) endemic to Mayotte Island (Comoros Archipelago), Indian Ocean

Didier VANDENSPIEGEL^{1,*}, Arnaud HENRARD² & Aurore MATHYS³

^{1,2,3}Biological Collection and Data Management Unit,
Royal Museum for Central Africa, Tervuren, Belgium.

³Royal Belgian Institute of Natural Sciences, Vautierstraat 29, B-1000 Brussels, Belgium.

* Corresponding author: didier.van.den.spiegel@africamuseum.be

² Email: arnaud.henrard@africamuseum.be

³ Email: aurore.mathys@africamuseum.be

¹[urn:lsid:zoobank.org:author:CE8C3D01-28AD-43F7-9D4F-04802E68CB1A](https://zoobank.org/author:CE8C3D01-28AD-43F7-9D4F-04802E68CB1A)

²[urn:lsid:zoobank.org:author:E1B02E6E-D91C-43FE-8D8C-CD102EFEE3B4](https://zoobank.org/author:E1B02E6E-D91C-43FE-8D8C-CD102EFEE3B4)

³[urn:lsid:zoobank.org:author:0C719566-2901-471D-B88E-CE3EBB476172](https://zoobank.org/author:0C719566-2901-471D-B88E-CE3EBB476172)

Abstract. A new millipede species of the genus *Sechelleptus* Mauriès, 1980 is described and illustrated from Mayotte Island, Indian Ocean. This new species, *S. arborivagus* sp. nov., found on trees, looks particularly similar to the sympatric *S. variabilis* VandenSpiegel & Golovatch, 2007, but is much larger and has a very different ecological behavior. Phylogenetic analyses based on a concatenated dataset of the COI and 16S rRNA genes and including nine species of Spirostreptidae (including *Sechelleptus*, *Doratogonus* Attems, 1914, *Bicoxidens* Attems, 1928 and *Spirostreptus* Brandt, 1833), strongly support the monophyly of *Sechelleptus*. Despite the similarity of their genitalia, the molecular analyses also reveal a clear-cut genetic divergence between *S. arborivagus* sp. nov. and *S. variabilis* (22.55% for COI and 6.63% for 16SrRNA) and further suggest the presence of a higher diversity within the genus *Sechelleptus* on Mayotte.

Keywords. Comoros archipelagos, new species, phylogeny, taxonomy.

VandenSpiegel D., Henrard A. & Mathys A. 2021. *Sechelleptus arborivagus* sp. nov., a new arboreal spirostreptid millipede (Diplopoda, Spirostreptidae) endemic to Mayotte Island (Comoros Archipelago), Indian Ocean. *European Journal of Taxonomy* 755: 1–21. <https://doi.org/10.5852/ejt.2021.755.1395>

Introduction

The genus *Sechelleptus* Mauriès, 1980, a senior synonym of *Rubanostreptus* Krabbe, 1982 (Jeekel 1999), was erected by Mauriès (1980) to reassign *Iulus seychellarum* originally described by Desjardins (1835) from a specimen collected in the Seychelles. The genus had been considered as monotypic until the extension of the generic concept by Golovatch & Korsós (1992) to include a large group of spirostreptids from Madagascar. First supposed to be restricted to Madagascar and some adjacent islands, the genus

appears to have a wider distribution ranging from East Africa to Madagascar and species of the same genus have been observed in Tanzania (Enghoff *et al.* 2016), Mauritius and Zanzibar (Jeekel 1999), and the Comoros (VandenSpiegel & Golovatch 2007). In the latter study, the authors described a new species of *Sechelleptus*, i.e., *S. variabilis* VandenSpiegel & Golovatch, 2007, and mentioned the presence of another putative congener. However, they were unable to assign the single female specimen found to a formal species. Based on its appearance and peripheral characters, the unknown female was at least assigned to the genus *Sechelleptus* (VandenSpiegel & Golovatch 2007).

In 2019, at the initiative of DEAL Mayotte, a new visit was organized to Mayotte. The visit was effectuated in November, during the rainy season, and allowed to collect, for the first time, two mature males of a large spirostreptid, corresponding to the unknown females mentioned in VandenSpiegel & Golovatch (2007). The study of the newly collected material shows that this species and *S. variabilis* are morphologically quite similar. At the first glance, even the gonopods of the large specie look like a giant form of those of *S. variabilis*. Nevertheless, a closer observation of the specimens reveals morphological differences, which are corroborated by a molecular study. In this paper, a morphological description of the new species is provided and its phylogenetic affinities are discussed.

Material and methods

Taxon sampling and morphological examination

This study is mainly based on material from Mayotte Island, collected in 2019 by the first and third authors. Some additional samples were obtained from the Royal Museum for Central Africa (RMCA), Tervuren, Belgium.

All samples are stored in 70% ethanol. Specimens for scanning electron microscopy were air-dried, mounted on aluminum stubs, coated with gold and studied using a JEOL JSM-6480LV scanning electron microscope.

The terminology used to describe the gonopod structures follows that of Hoffman (2008). All measurements are in mm unless otherwise indicated.

Abbreviations

bp	=	base pair(s)
DEAL	=	Direction de l'Environnement, de l'Aménagement et du Logement
MB	=	Bayesian inference performed with MrBayes
ML	=	Maximum Likelihood analysis
PB	=	Bayesian inference performed with Phylobayes
RMCA	=	Royal Museum for Central Africa, Tervuren, Belgium
SEM	=	Scanning Electron Microscopy

DNA extraction, amplification and sequencing

A few legs of seven freshly collected diplopod specimens, plus one older sample from the RMCA collection, all from Mayotte, were detached for molecular analysis (Table 1). The DNA of each sample was extracted with a Macherey-Nagel NucleoSpin tissue kit, slightly adapted from the manufacturer's protocol. Partial fragments of two mitochondrial markers, the cytochrome c oxidase subunit I (COI) and the large subunit ribosomal RNA (16S rRNA), were amplified with polymerase chain reactions (PCRs) using the primers displayed in Appendix 1. All amplifications were performed in a 20 µL reaction mixture containing 2 µL of extracted DNA (regardless of initial concentrations), 2 µL of 10X buffer, 0.5 mM MgCl₂, 0.2 mM dNTP, 0.8 µM of each primer, and 0.02 units/µL of Platinum™ Taq DNA Polymerase (Invitrogen™, Waltham, MA, USA). PCR conditions comprised an initial denaturation at

Table 1. List of the sequenced specimens of *Sechelleptus* Mauriès, 1980, with DNA sample ID, Type specimen (Ht = Holotype; Pt = Paratype; NT = non type), Museum and collection information, sex stage (F = female; M = male; SubF = sub-adult female) and GenBank accession number for COI and 16S rRNA markers.

Sample ID	Species	Type	Specimen Ref.	Locality	Date	M/F	COI	16S
DB1	<i>S. arborivagus</i> sp. nov.	NT	BE_RMCA_MYR.Dip.17917	Mt Benara	2004	F	–	MW148621
DB2	<i>S. arborivagus</i> sp. nov.	Ht	BE_RMCA_MYR.Dip.22874	Mt Tchaourembo	2019	M	MW168813	MW148622
DB3	<i>S. arborivagus</i> sp. nov.	Pt	BE_RMCA_MYR.Dip.22875	Mt Tchaourembo	2019	F	MW168814	MW148623
DB4	<i>S. arborivagus</i> sp. nov.	Pt	BE_RMCA_MYR.Dip.22876	Mt Tchaourembo	2019	F	MW168815	MW148624
DS1	<i>S. variabilis</i>	NT	BE_RMCA_MYR.Dip.22877	Mapouera	2019	M	MW168816	MW148625
DS2	<i>S. variabilis</i>	NT	BE_RMCA_MYR.Dip.22878	Merereni	2019	F	MW168817	MW148626
DS3	<i>S. variabilis</i>	NT	BE_RMCA_MYR.Dip.22879	Merereni	2019	F	MW168818	MW148627
DU1	<i>Sechelleptus</i> sp.	NT	BE_RMCA_MYR.Dip.22880	Mt Combani	2019	SubF	MW168819	MW148628

94°C for 3 min followed by 35 cycles with, per cycle, a denaturation at 94°C for 45 s followed by an annealing step of 45 s at 48°C and an extension step at 72°C (1 min) and with a final extension at 72°C for 10 min followed by 10 min at 4°C. PCR products (and negative controls) were checked on a 1.5% agarose gel containing 0.03% of MidoriGreen™ Direct (NIPPON Genetics Europe, Dueren, Germany) using a UV transilluminator. Positive amplifications were purified using the ExoSAP-IT™ protocol (following manufacturer's instructions) and then sequenced in both directions, using the same couples of primers (0.05 mM each), by Macrogen Inc. (Seoul, South Korea). The sequences were checked using Geneious R11 (Biomatters Ltd., Auckland, New Zealand): paired bi-directional strands were trimmed, assembled, edited and consensus sequences were extracted for each specimen and each DNA marker. The COI sequences obtained for the three specimens of *Sechelleptus arborivagus* sp. nov. (DB2, DB3 and DB4) were further manually edited as they presented two additional nucleotides generating a frameshift in the predicted coding region and numerous stop codons. For DB2 and DB4 sequences, one thymine was removed from position 345–346 and another for position 426–427. For DB3 sequence, the same nucleotides were removed from position 315–316 and position 396–397. As control, consensus sequences were compared against the Identification System of BOLD (www.boldsystems.org) and the BLAST web application of GenBank (<https://blast.ncbi.nlm.nih.gov/Blast.cgi>).

Phylogenetic analyses

For phylogenetic analyses, a few additional Spirostreptidae Brandt, 1833 sequences from GenBank and BOLD (see Appendix 2) were selected based on BOLD and BLAST's comparative results. Although this set of taxa does not represent an exhaustive list, we estimate it sufficient to evaluate the relationships of the new species. In addition, some representatives of the family Harpagophoridae Attems, 1909 (*Thyropygus* spp.) were chosen as outgroup. All sequences of each marker were aligned with MAFFT ver. 7 implemented online (Katoh & Standley 2013) with default settings. Both COI and 16S alignments were cured (by removing ambiguous sites and trimming datasets) using the least stringent settings in Gblocks 0.91b online (Castresana 2000; Talavera & Castresana 2007; Gblocks Server available online at http://molevol.cmima.csic.es/castresana/Gblocks_server.html). Finally, a combined COI-16S alignment was created with Mesquite ver. 3.5 (Maddison & Maddison 2018) by concatenating the Gblocks curated alignments of the two markers. Best partition scheme and best-fit substitution models (see Appendix 3) were estimated using PartitionFinder 2 (Lanfear *et al.* 2016) on the basis of one partition for 16S and three for COI (protein coding gene partitioned into single codon positions).

Phylogenetic reconstructions were evaluated using statistical approaches including maximum likelihood (ML) using GARLI ver. 2.01 (Zwickl 2006) and Bayesian inferences, the latter utilizing MrBayes ver. 3.2.7a (Ronquist *et al.* 2012) (MB) and Phylobayes MPI ver. 1.5a (Lartillot *et al.* 2009) (PB), all performed on the CIPRES Science Gateway ver. 3.3 (Miller *et al.* 2010). For the MB analysis, two parallel runs (with four chains each) were executed for ten million generations. Parameters were estimated independently for each partition using the following command: unlink statefreq = (all) revmat = (all) shape = (all) pinvar = (all) tratio = (all). Trees were sampled every 1000th generations and were used to reconstruct a 50% majority rule consensus tree after having discarded the first 25% as ‘burn-in’. Analysis in PB was conducted under the CAT+GTR+Gamma substitution model (with maxdiff" value set to 0.1 Minimum Effective Size to 300 and excluding the 1000 first of cycles from convergence checks). Analyses using the ML method were conducted in GARLI with 1000 bootstrap replicates. Values were then summarized on the best ML tree using SumTree ver. 4.0.0 (Sukumaran & Holder 2015) (run in DendroPy ver. 4.0.0, Sukumaran & Holder 2010).

In addition, estimations of the average evolutionary divergences for each marker (based on a MAFFT alignments of the sequenced specimens only) were calculated in MEGA-X (Kumar *et al.* 2018; Stecher *et al.* 2020) as the number of base differences per site (P-distance) from between sequences and species group with the option ‘Pairwise deletion’.

Results

Phylogeny

Amplification and sequencing provided a fragment length of about 730–900 bp for COI (partial sequence of 200 bp only for two samples, DS1 and DS2, could be retrieved) and about 500 bp for 16S. The combined COI–16S final dataset comprised 1051 sites (561 bp for COI and 490 bp for 16S).

All phylogenetic analyses provided congruent results with identical tree topologies. The Figure 1 presents the tree resulting from the MB analysis on which the support values of PB and ML are also summarized. The genus *Sechelleptus* represented here by *S. variabilis*, *S. arborivagus* sp. nov. and an undetermined species (i.e., DU1, a sub-adult female collected at Mont Combani on Mayotte) is strongly recovered as monophyletic in all analyses and appears sister to an unidentified diploplod from Madagascar (for which the COI sequence was retrieved from BOLD, see Appendix 2). Both *S. variabilis* and *S. arborivagus* sp. nov. are recovered with high support, but their relationship with DU1 is not clearly resolved.

The relationships among the outgroups were not the primary focus of the present study, but are consistent with previous studies (Mwabvu *et al.* 2013, 2015; Tinago *et al.* 2017) that showed paraphyletic groups suggesting the presence of cryptic species (i.e., *Bicoxidens* spp.) and possible identification errors in the GenBank database.

Genetic distances

The MAFFT alignment of the sequenced specimens used for calculations in MEGA-X provided datasets of 1007 positions for COI and 502 positions for 16S. Little genetic variation is observed between the sequenced individuals of *Sechelleptus arborivagus* sp. nov. compared to *S. variabilis* (see Table 2). For *S. arborivagus* sp. nov., the within mean group distance calculated in MEGA-X is very low: 0.1% for COI and 0.2% for 16S. These values are much higher within *S. variabilis*, being 4.9% and 1.5% respectively.

The genetic distance between *S. arborivagus* sp. nov. (DB) and *S. variabilis* (DS) is about 22.6% for COI and 6.9% for 16S (Table 3). The undetermined *Sechelleptus* (DU1) from Mont Combani also showed the same range of genetic differences between DB and DS: respectively 17.5% and 14% for COI and 7% and

Table 2. Estimates of Evolutionary Divergence between Sequences. The number of base differences per site between sequences are shown. DB1–DB4 = *Sechelleptus arborivagus* sp. nov.; DS1–DS3 = *Sechelleptus variabilis* VandenSpiegel & Golovatch, 2007; DU1 = *Sechelleptus* sp. Molecular analyses were conducted in MEGA X (Kumar *et al.* 2018; Stecher *et al.* 2020).

COI	16S	DB1	DB2	DB3	DB4	DS1	DS2	DS3	DU1
DB1			0.40%	0.00%	0.00%	7.01%	7.20%	6.41%	7.00%
DB2		–		0.40%	0.40%	7.21%	7.40%	6.61%	7.00%
DB3		–	0.14%		0.00%	7.01%	7.20%	6.41%	7.00%
DB4		–	0.00%	0.14%		7.01%	7.20%	6.41%	7.00%
DS1		–	25.00%	22.03%	25.00%		1.80%	1.00%	8.62%
DS2		–	25.52%	25.22%	25.52%	6.34%		1.60%	8.00%
DS3		–	18.27%	18.07%	18.33%	3.90%	4.46%		8.42%
DU1		–	17.51%	17.35%	17.58%	14.90%	15.61%	11.60%	

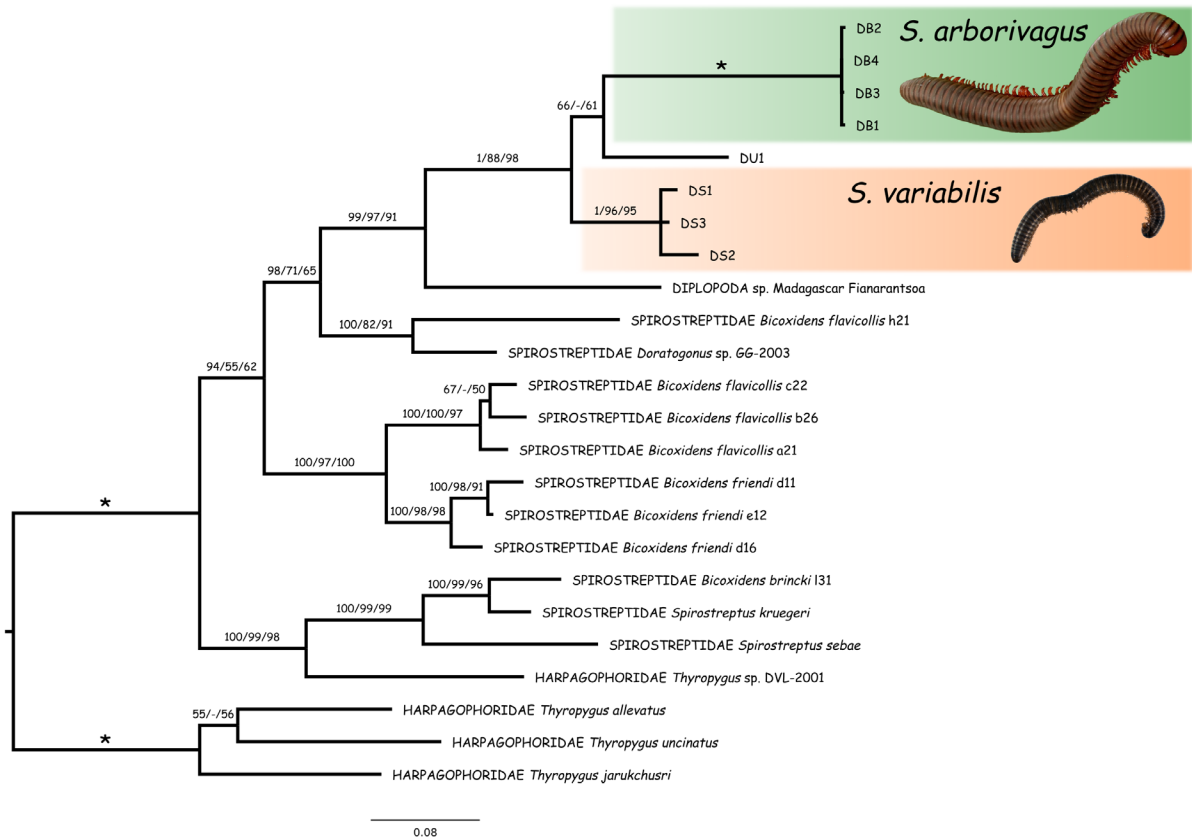


Fig. 1. Bayesian Inference tree resulting from the MB analysis based on the COI–16S dataset, showing relationships between *Sechelleptus arborivagus* sp. nov. and other relatives of the family Spirostreptidae Brandt, 1833 and outgroups Harpagophoridae Attems, 1909. The tree is congruent in its topology with the PB and ML analyses of the same dataset. Nodal support values (MB and PB posterior probabilities and ML Bootstrap) are indicated as following: MB/PB/ML. Stars designate absolute supports (i.e., 100 in all three analyses), and hyphens indicate collapsed nodes in the PB tree.

Table 3. Estimates of Evolutionary Divergence over Sequence Pairs between Groups. The number of base differences per site averaging over all sequence pairs between groups are shown. DB = *Sechelleptus arborivagus* sp. nov. (DB2–DB4 in COI dataset, DB1–DB4 in 16S dataset); DS = *Sechelleptus variabilis* VandenSpiegel & Golovatch, 2007 (DS1–DS3); DU = *Sechelleptus* sp. (DU1). Molecular analyses were conducted in MEGA X (Kumar *et al.* 2018; Stecher *et al.* 2020).

COI	16S	DB	DS	DU
	DB		6.93%	7.00%
	DS	22.55%		8.34%
	DU	17.48%	14.04%	

8.3% for 16S (Table 3). The overall mean distances within *Sechelleptus* (calculated as the estimates of average evolutionary divergence over all sequence pairs) is 14.9% for COI and 5.1% for 16S (Tables 2–3).

Systematics

Class Diplopoda De Blainville in Gervais, 1844

Order Spirostreptida Brandt, 1833

Suborder Spirostreptidea Brandt, 1833

Family Spirostreptidae Brandt, 1833

Genus *Sechelleptus* Mauriès, 1980

Sechelleptus Mauriès, 1980: 147.

Rubanostreptus Krabbe, 1982: 183, synonymized by Golovatch & Korsós (1992).

Sechelleptus – Golovatch & Korsós 1992: 24.

Type species

Iulus seychellarum Desjardins, 1835

Diagnosis (adapted from Mauriès 1980 and Golovatch & Korsós 1992)

A genus of moderate to large spirostreptid millipedes (up to 120 mm long) characterized by the rather simple gonocoxite and the long, slender, ribbon-shaped gonotelopodite with a spine arising well distad of the knee and a small free solenomerite arising just near the apex.

Distribution

Tanzania, Zanzibar, Comoros archipelago, Seychelles, Madagascar, Mauritius.

Species included (adapted from Millibase: Sierwald & Spelda 2021)

Sechelleptus aberrans (Brölemann, 1923); <http://www.millibase.org/aphia.php?p=taxdetails&id=1044974>

S. anulatus (Attems, 1914); <http://www.millibase.org/aphia.php?p=taxdetails&id=999985>

S. argus (Attems, 1896); <http://www.millibase.org/aphia.php?p=taxdetails&id=998239>

S. betaminena (De Saussure & Zehntner, 1902); <http://www.millibase.org/aphia.php?p=taxdetails&id=1044975>

S. confusus (Attems, 1950); <http://www.millibase.org/aphia.php?p=taxdetails&id=1044977>

S. coriaceus (De Saussure & Zehntner, 1901); <http://www.millibase.org/aphia.php?p=taxdetails&id=998243>

S. dauphini (De Saussure & Zehntner, 1902); <http://www.millibase.org/aphia.php?p=taxdetails&id=998244>

- S. fulgens* (De Saussure & Zehntner, 1901); <http://www.millibase.org/aphia.php?p=taxdetails&id=998237>
S. gonospinosus (Attems, 1910); <http://www.millibase.org/aphia.php?p=taxdetails&id=1044978>
S. kalobaptus (Attems, 1914); <http://www.millibase.org/aphia.php?p=taxdetails&id=1044979>
S. krabbae Jeekel, 1999; <http://www.millibase.org/aphia.php?p=taxdetails&id=940073>
S. lambertoni (Brölemann, 1923); <http://www.millibase.org/aphia.php?p=taxdetails&id=1155959>
S. lobifer (Attems, 1951); <http://www.millibase.org/aphia.php?p=taxdetails&id=1044980>
S. macilentus (De Saussure & Zehntner, 1897); <http://www.millibase.org/aphia.php?p=taxdetails&id=998247>
S. metazonalis (De Saussure & Zehntner, 1901); <http://www.millibase.org/aphia.php?p=taxdetails&id=998238>
S. moramangae (De Saussure & Zehntner, 1897); <http://www.millibase.org/aphia.php?p=taxdetails&id=998249>
S. multiporus (Attems, 1951); <http://www.millibase.org/aphia.php?p=taxdetails&id=1246528>
S. nigrinus (De Saussure & Zehntner, 1897); <http://www.millibase.org/aphia.php?p=taxdetails&id=998250>
S. obscuratus (Attems, 1914); <http://www.millibase.org/aphia.php?p=taxdetails&id=1248577>
S. obscurus (Attems, 1951); <http://www.millibase.org/aphia.php?p=taxdetails&id=1248590>
S. piesthopygus (Attems, 1914); <http://www.millibase.org/aphia.php?p=taxdetails&id=999987>
S. praepolitus (Attems, 1910); <http://www.millibase.org/aphia.php?p=taxdetails&id=1248594>
S. procerus (Attems, 1951); <http://www.millibase.org/aphia.php?p=taxdetails&id=1249715>
S. punctatulus (Attems, 1910); <http://www.millibase.org/aphia.php?p=taxdetails&id=1249718>
S. pyrhoneus (Gerstäcker, 1873); <http://www.millibase.org/aphia.php?p=taxdetails&id=998242>
S. scabricollis (De Saussure & Zehntner, 1897); <http://www.millibase.org/aphia.php?p=taxdetails&id=998252>
S. seychellarum (Desjardins, 1835); <http://www.millibase.org/aphia.php?p=taxdetails&id=947475>
S. speculorbis (Attems, 1910); <http://www.millibase.org/aphia.php?p=taxdetails&id=1249894>
S. sulcicollis (De Saussure & Zehntner, 1897); <http://www.millibase.org/aphia.php?p=taxdetails&id=999880>
S. unilineatus Golovatch & Korsós, 1992; <http://www.millibase.org/aphia.php?p=taxdetails&id=940074>
S. variabilis VandenSpiegel & Golovatch, 2007; <http://www.millibase.org/aphia.php?p=taxdetails&id=1024527>
S. arborivagus sp. nov.

Key to species of *Sechelleptus*

Waiting a complete revision work of the genus, only the following additional lines to the key of Jeekel (1999) is given here:

22. Metaplica (posterior blade) with a small latero-distal uncus. Number of body rings 49–52, width 7.0–8.0 mm *S. sulcicollis* (De Saussure & Zehntner, 1897)
 – Metaplica without latero-distal uncus. Number of body rings usually higher 22A
- 22A. Metaplica simply widened. Number of body rings 59, width 12.0 mm
 *S. macilentus* (De Saussure & Zehntner, 1897)
 – Metaplica widened and a little higher than proplica (anterior blade) 22B
- 22B. Proplica with a lateral finger-shaped lobe. Number of body rings 50–60, width 3.0–5.0 mm
 *S. variabilis* VandenSpiegel & Golovatch, 2007
 – Proplica ending apically in a more or less spiniform mesapical projection. Number of body rings 57–62, width 7.0–9.0 mm *S. arborivagus* sp. nov.

Sechelleptus variabilis VandenSpiegel & Golovatch, 2007

Fig. 2

Material examined

Holotype

UNION OF THE COMOROS • ♂; Mohéli, Fomboni; 12°15' S, 043°45' E; 21 May 2003; dead wood; R. Jocqué and D. VandenSpiegel leg.; BE_RMCA_MYR.Dip.21733.

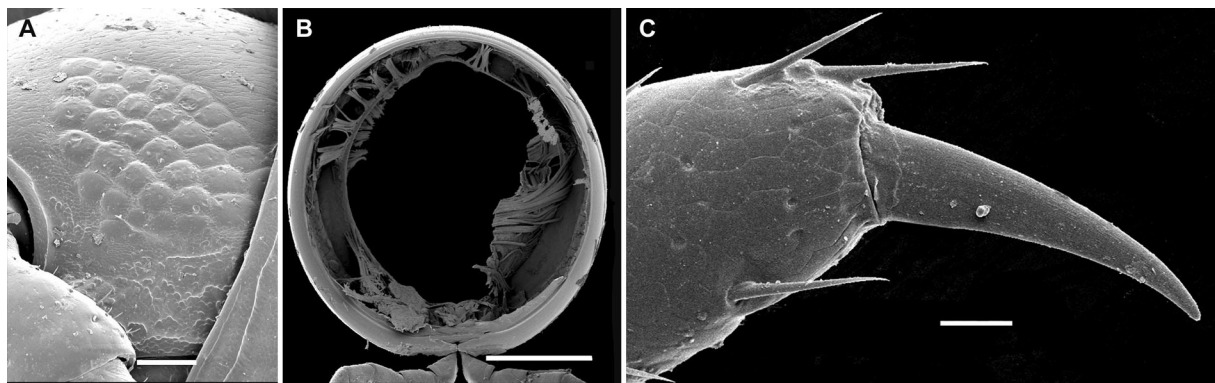


Fig. 2. *Sechelleptus variabilis* VandenSpiegel & Golovatch, 2007 (BE_RMCA_MYR.Dip.22883). SEM views. **A.** Ommatidia, lateral view. **B.** Transversal section of midbody, frontal view. **C.** Tarsal claw, lateral view. Scale bars: A = 100 µm; B = 1 mm; C = 20 µm.

New material

FRANCE – **Department of Mayotte** (Comoros archipelago) • ♂; Mereni, back of mangrove; 19 Nov. 2019; hand collecting; D. VandenSpiegel and A. Mathys leg.; BE_RMCA_MYR.Dip.22878 • 1 ♂, 1 ♀; same collection data as for preceding; 21 Nov. 2019; BE_RMCA_MYR.Dip.22879 • 1 ♂; same collection data as for preceding; BE_RMCA_MYR.Dip.22983 • 1 ♂, 1 ♀; Mont Benara; 12°52' S, 045°09' E; 21 Nov. 2019; hand collecting & sieving, litter; D. VandenSpiegel and A. Mathys leg.; BE_RMCA_MYR.Dip.22883 • 1 ♂; Mont Combani, 500 m before Lodge; 12°47' S, 045°09' E; 15 Nov. 2019; sieving; litter; D. VandenSpiegel and A. Mathys leg.; BE_RMCA_MYR.Dip.22930.

Remarks

In the original description of the species, the length unit given for the specimens' sizes is wrong and should have been cm instead of mm. Correct measurements are: length ♂♂: 3.4–8.0 cm; ♀♀: 5.0–7.0 cm; midbody width 0.34–0.5 cm and 0.3–0.5 cm, respectively.

Taking this modification into account, the recently collected specimens agree with the description (VandenSpiegel & Golovatch 2007).

Sechelleptus arborivagus sp. nov.

[urn:lsid:zoobank.org:act:0F7B3368-17F8-4CAD-8B11-C34B564FE8DE](https://zoobank.org/urn:lsid:zoobank.org:act:0F7B3368-17F8-4CAD-8B11-C34B564FE8DE)

Figs 3–8

Diagnosis

A medium-sized arboreal millipede with relatively long legs, particularly similar to *S. variabilis* by sharing the structure of the male first leg and rather simple gonopods with the metaplica widened and a little higher than proplica, the latter without lateral cone. The two species differ by the gonotelopodite being apically divided in two branches in *S. arborivagus* sp. nov. and simple in *S. variabilis*.

Etymology

Referring to the ecology of the species, which has always been observed climbing trees.

Material examined

Holotype

FRANCE – **Department of Mayotte** (Comoros archipelago) • ♂; Mt. Tchaourembo; 12°52'14" S, 045°08'44" E; 540–550 m a.s.l.; 25 Nov. 2019; D. VandenSpiegel and A. Mathys leg.; on tree; by hand; GenBank accession numbers: MW168813 (COI), MW148622 (16S rRNA); BE_RMCA_MYR.Dip.22874.

Paratypes

FRANCE – **Department of Mayotte** (Comoros archipelago) • 1 ♂, 1 ♀; same collection data as for holotype; GenBank accession numbers: MW168814 (COI), MW148623 (16S rRNA); BE_RMCA_MYR.Dip.22875 • 9 ♀♀; same collection data as for holotype; GenBank accession numbers: MW168815 (COI), MW148624 (16S rRNA); BE_RMCA_MYR.Dip.22876.

Additional material

FRANCE – **Department of Mayotte** (Comoros archipelago) • 1 ♀; Mt. Benara; 12°52' S, 045°11' E; 23 Jan. 1999; R. Jocqué and G. De Smet leg.; forest; by hand; GenBank accession numbers: MW148621 (16S rRNA); BE_RMCA_MYR.Dip.17917.

Description

Holotype

With 57 body rings (plus telson, no apodous rings); ca 100 mm long, 7 mm wide.

LIVE COLORATION (Fig. 3). Head, collum, antennae, telson, anal valves and legs uniformly light brownish to dark brownish. Metazonae light brown to red-brown. Posterior margin of metazonites dark brown.

HEAD. Smooth. Each eye patch with circa 60 ommatidia arranged in seven horizontal rows (Fig. 4A), Labrum with three smoothly rounded teeth and a single row of 21 short labral setae (Fig. 4G). Clypeus with four supra-labral setae, two on each side (Fig. 4G). Antennae moderately long (Fig. 3), protruding back to ring 2. Relative length of antennomeres: 1>2>3=4=5>6. Terminal antennomere (disc) with four large sensory cones located together inside a membranous area. Each of antennomeres 5 and 6 apicolaterally with a field of narrow and long sensilla basiconica (Fig. 4B). Gnathochilarium, usual for spirostreptideans (Fig. 4D). Prementum (pm) smooth and straight, not depressed. Mentum (me) smooth.

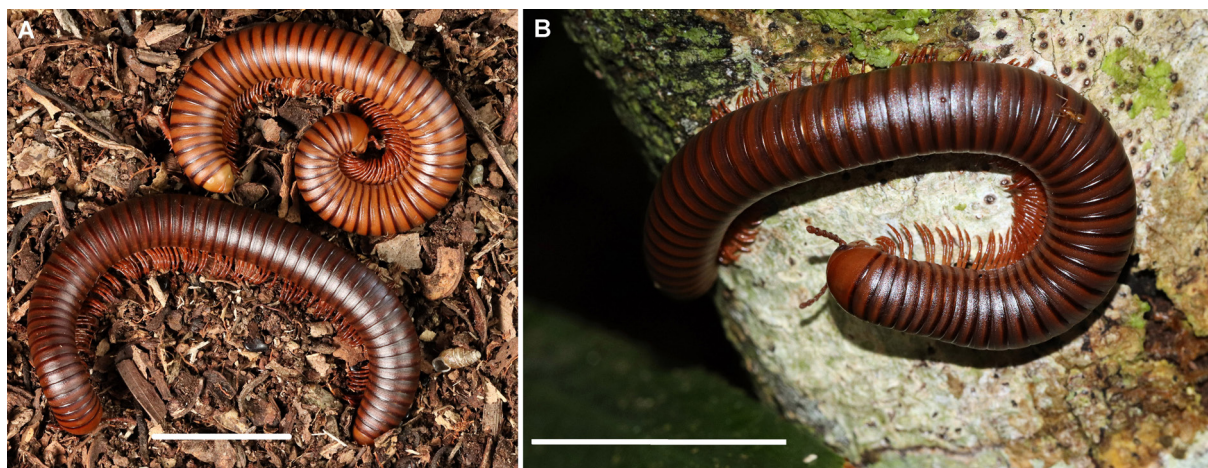


Fig. 3. *Sechelleptus arborivagus* sp. nov., in vivo habitus. **A.** Two females showing different color. **B.** Female on tree. Scale bars = 3 cm.

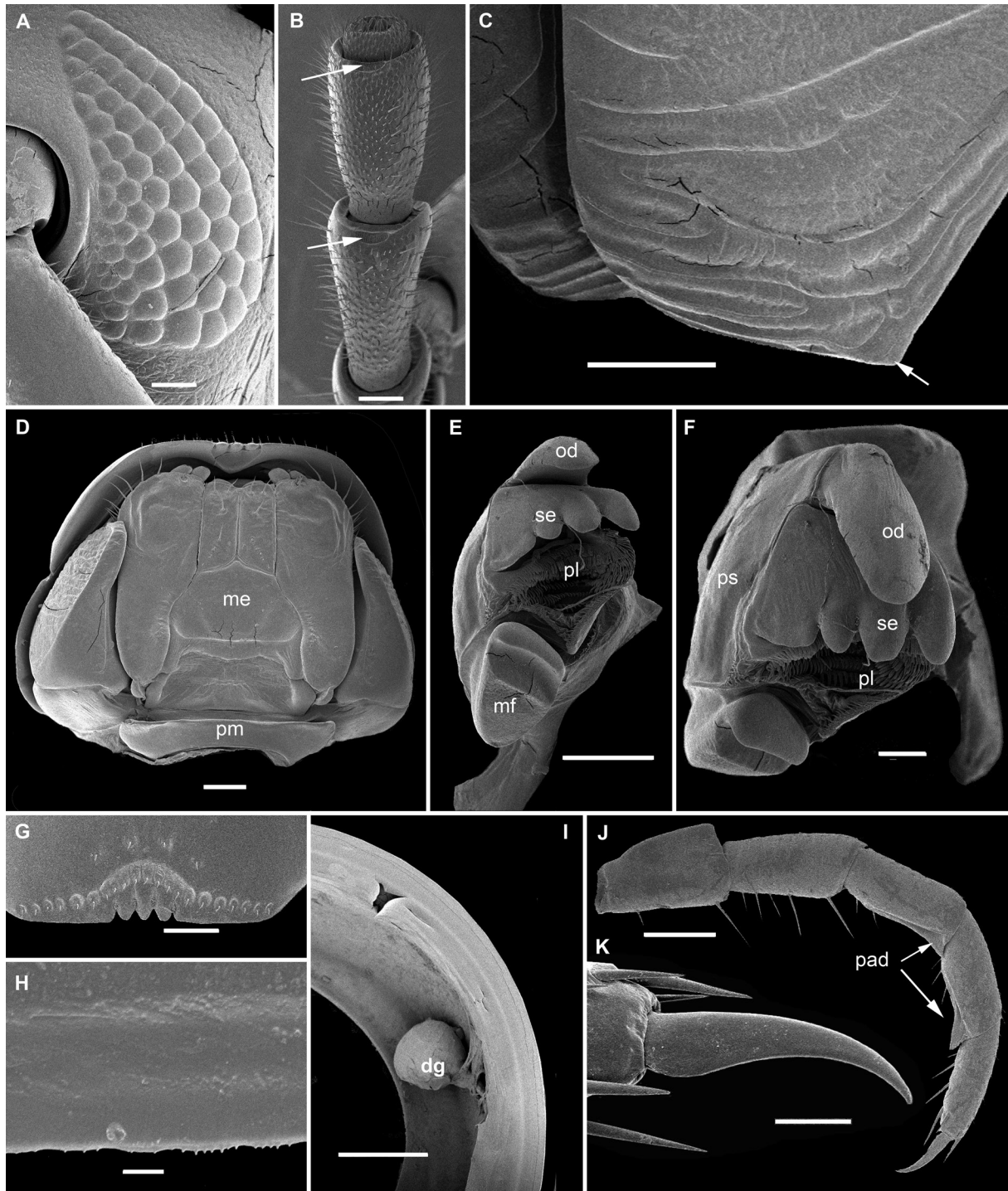


Fig. 4. *Sechelleptus arborivagus* sp. nov., paratype, ♂ (BE_RMCA_MYR.Dip.22875). SEM views. **A.** Ommatidia, frontal view. **B.** Antennae, ventral view, arrows pointing to sensilla basiconica. **C.** Lateral view of collum, arrows pointing to the anteroventral angle 80–90°. **D.** Head, ventral view. **E.** Mandible, ventral view. **F.** Mandible, frontal view (F). **G.** Clypeus, frontal view. **H.** Limbus. **I.** Defensive gland. **J.** Walking leg, lateral view. **K.** Tarsal claw, lateral view. Abbreviations: dg = defensive gland; me = mentum; od = odontomere; pl = pectinate lamellae; pm = prementum; pad = tibial pad; ps = psectomere; se = sectile edge. Scale bars: A–B, F = 200 µm; C–E, G, J = 500 µm; H = 5 µm; I = 1 mm; K = 100 µm.



Fig. 5. *Sechelleptus arborivagus* sp. nov., paratype, ♂ (BE_RMCA_MYR.Dip.22875). SEM views. **A.** First pair of legs, frontal view. **B.** Apical part of gonopod telopodite. **C.** Gonocoxite, oral view. **D.** Gonocoxite, caudal view. **E.** Gonopod, lateral view. Abbreviations: al = apicolateral lamellose lobe; ats = antetorsal process; mp = metaplica; mpp = mesapical projection; pfp = prefemoral process; pp = proplica; px = paracoxite; sl = solenomere; st = sternite; tlp = telopodite. Scale bars: A = 500 μ m; B = 100 μ m; C–E = 1 mm.

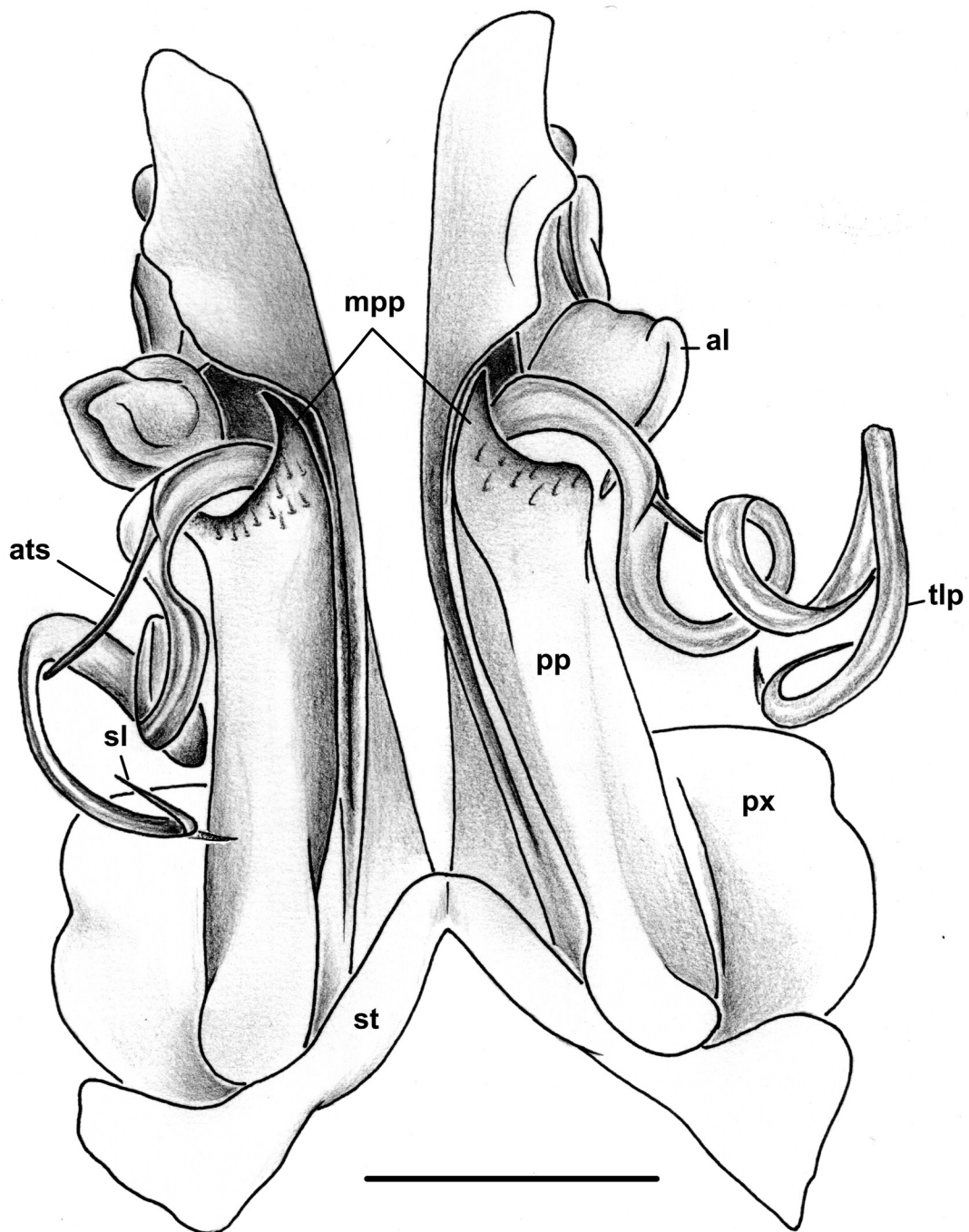


Fig. 6. *Sechelleptus arborivagus* sp. nov., paratype, ♂ (BE_RMCA_MYR.Dip.22875). Drawing of the gonopods, oral view. Abbreviations: al = apicolateral lamellose lobe; ats = antetorsal process; mpp = mesapical projection; pp = proplica; px = paracoxite; sl = solenomere; st = sternite; tlp = telopodite. Scale bar = 1 mm.

Lamellae linguales each with two strong apical setae, one equally strong seta behind these, plus, basally, an oblique line of four setae. Stipites with a basal longitudinal field of setae, lateral margin in distal half with a row of setae; one isolated, subapical, stout seta or sensillum; cardo small, kidney-shaped. Mandibles (Fig. 4E, F) with stipes devoid of differentiation. Odontomere (od) long, moveable. Sectile edge (se) of psectromere (ps) with four lobes; eight pectinate lamellae (pl). One wide molar furrow (mf).

COLLUM. Smooth, ventrally with six longitudinal furrows, anteroventral angle 80–90°.

BODY RINGS. Prozonae smooth. Metazonae with longitudinal striae ventrally from ca $\frac{2}{3}$ ring length below ozopore. Ozopores located on metazonae, starting with ring 6, located close to, but not touching the suture between pro- and metazonae. Limbus simple (Fig. 4H). Defensive glands well-developed (Fig. 4I).

TELSON. Preanal ring with a shallow submarginal depression. Anal valves smooth, without submarginal depression. Hypoproct, small, widely triangular.

LEGS. Length 0.45–0.5 × body diameter, postfemoral and tibial pads (Fig. 4J) from third male leg-pair until beyond midbody, pads decreasing in size posteriorly; claw large, curved (Fig. 4K). First pair of male legs with a well-developed prefemoral process ending in an inward curved tip (Fig. 5A). Coxosternum with a laterobasal field of four strong setae on anterior side.

GONOPODS (Figs 5B–E, 6). Sternum (st) triangular, not reaching as far distad as paracoxite (px). Metaplica (mp) higher than proplica, rounded apically (Fig. 5C; mp). Proplica with straight sides, in apical part with scattered short setae, ending apically in a more or less spiniform mesapical projection (Fig. 5C; mpp) and a well-developed, apicolateral, lamellose lobe (Fig. 6C; al); telopodite (Fig. 5B, E; tlp) long and slender, without a distinct demarcation between femoral and postfemoral parts, femorite with a small and pointed antetorsal process (Fig. 5E; ats), postfemorite spiralled, ribbon-shaped, broad and long, with a divided tip, the longer branch carrying the terminal opening of the solenomere (Fig. 5B; sl).

Paratypes

Male similar to holotype.

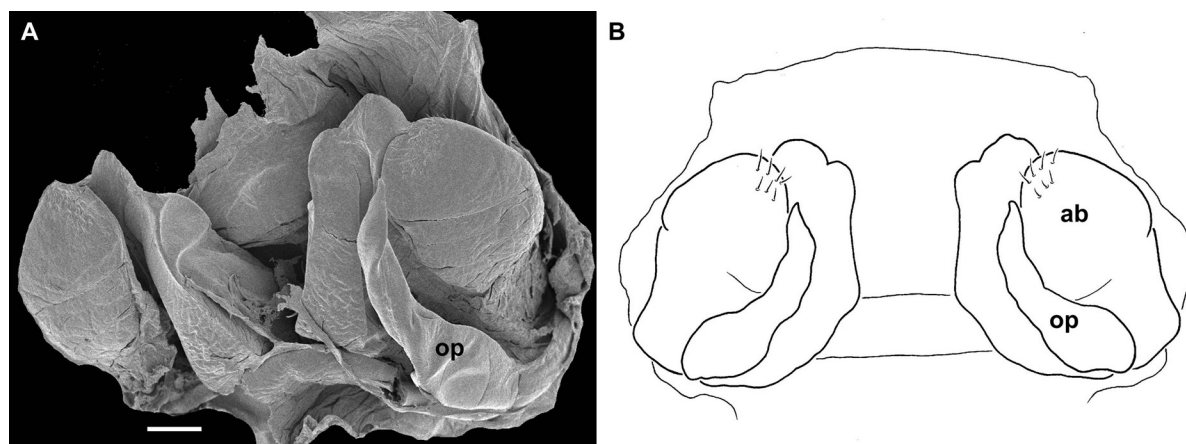


Fig. 7. *Sechelleptus arborivagus* sp. nov., paratype, ♀ (BE_RMCA_MYR.Dip.22876). Vulva. **A.** SEM of the vulva removed from the body, caudal view, slightly lateral. **B.** Drawing of the vulva, caudal view. Abbreviations: ab = aboral valve; op = opercula. Scale bar = 100 µm.

Female coloration as in male, but generally larger in size than male (up to 120 mm long 9 mm wide (58–61 body rings plus telson, no apodous rings). Vulvae located in membranous pouches attached to coxae 2 and 3 and to the inner lateral margin of ring 1, simple, consisting of two simple, subequally-sized, moderately sclerotized valves, the aboral valve with an apical cluster of setae; ridge between valves covered with a lateral longitudinal operculum (Fig. 7).

Distribution

The species seems endemic to Mayotte (Fig. 8).

Affinities

On the basis of the gonopod structure having the telopodite with a spine arising well distad of the knee, a ribbon-shaped distal part, and a small free solenomerite arising just near the apex, the new species is manifestly a new member of the large genus *Sechelleptus*. Following the key published by Jeekel in 1999, *arborivagus* keys out close to *sulcicollis* and *macilentus*. Indeed the three species have a rather simple gonocoxite with a distally widened metaplica without a strong lateral cone but the new species do not show the small lateral uncus present on the metaplica of *sulcicollis* and possess a more or less spiniform mesapical projection on the proplica which is not present in *sulcicollis* neither in *macilentus*. By the overall shape of the male first leg and gonocoxite, the new species seems to be especially close to *S. variabilis*, also from the Comoros, but it differs strikingly by the structure of the gonotelopodite (in *S. variabilis* the gonotelopodite has a simple and pointed tip carrying the terminal opening of the seminal groove whereas in the new species the gonotelopodite has a divided tip, the longer branch carrying the terminal opening of the seminal groove) as well as by the larger body size and the longer and curved claws (Fig. 4K vs Fig. 2C). Other important differences concern the defensive glands, large in *S. arborivagus* sp. nov. (Fig. 4I) (vs inconspicuous in *S. variabilis* (Fig. 2A)), and the size of eyes: in the new species the eyes are larger and include 60 ± 5 ommatidia ($n = 10$) arranged in 12 rows; whereas in *S. variabilis* the eyes, smaller, include 34 ± 3 ($n = 10$) ommatidia arranged in 9 rows.

Natural history

Most of the specimens belonging to the new species were collected on Mt Tchaourembo (see Fig. 8) in a forest fragment at 500–550 m a.s.l. All specimens were seen in trees and never in pairs, the males being rare (sex ratio $> 1/6$). The species possesses enlarged ommatidia, relatively long legs with strongly curved tarsal claws, as well as a tendency for specimens to secrete extremely copiously from their defensive glands when irritated. Such modifications are considered by several authors as an adaptation to tree climbing and to arboreal life (Enghoff & Enghoff 1976; Hoffman & Howell 1983; VandenSpiegel 2001).

Discussion

Millipede systematics is mainly based on male gonopods because they use to be species-specific (Bond *et al.* 2003). However, studies based on DNA have demonstrated that molecular divergence in different millipede groups may not reflect divergence in morphology-based identifications and may hide considerable variation (Bond & Sierwald 2002; Bond *et al.* 2003; Adams *et al.* 2009; Mwabvu *et al.* 2013, 2015; Tinago *et al.* 2017). Although our relatively small taxon sampling, the phylogenetic analysis strongly recovers *Sechelleptus* as monophyletic and discriminates at least two or three different groups. Furthermore, the mean inter-specific distance values (14.9% for COI and 5.1% for 16S) were remarkably similar to previous studies that reported the presence of high genetic divergence among population of different spirostreptid species (Mwabvu *et al.* 2013, 2015), suggesting the existence of more than one species in those taxa. It is argued that high level of divergence between identified spirostreptid species may indicate that changes in genital morphology occur rather slowly relative to

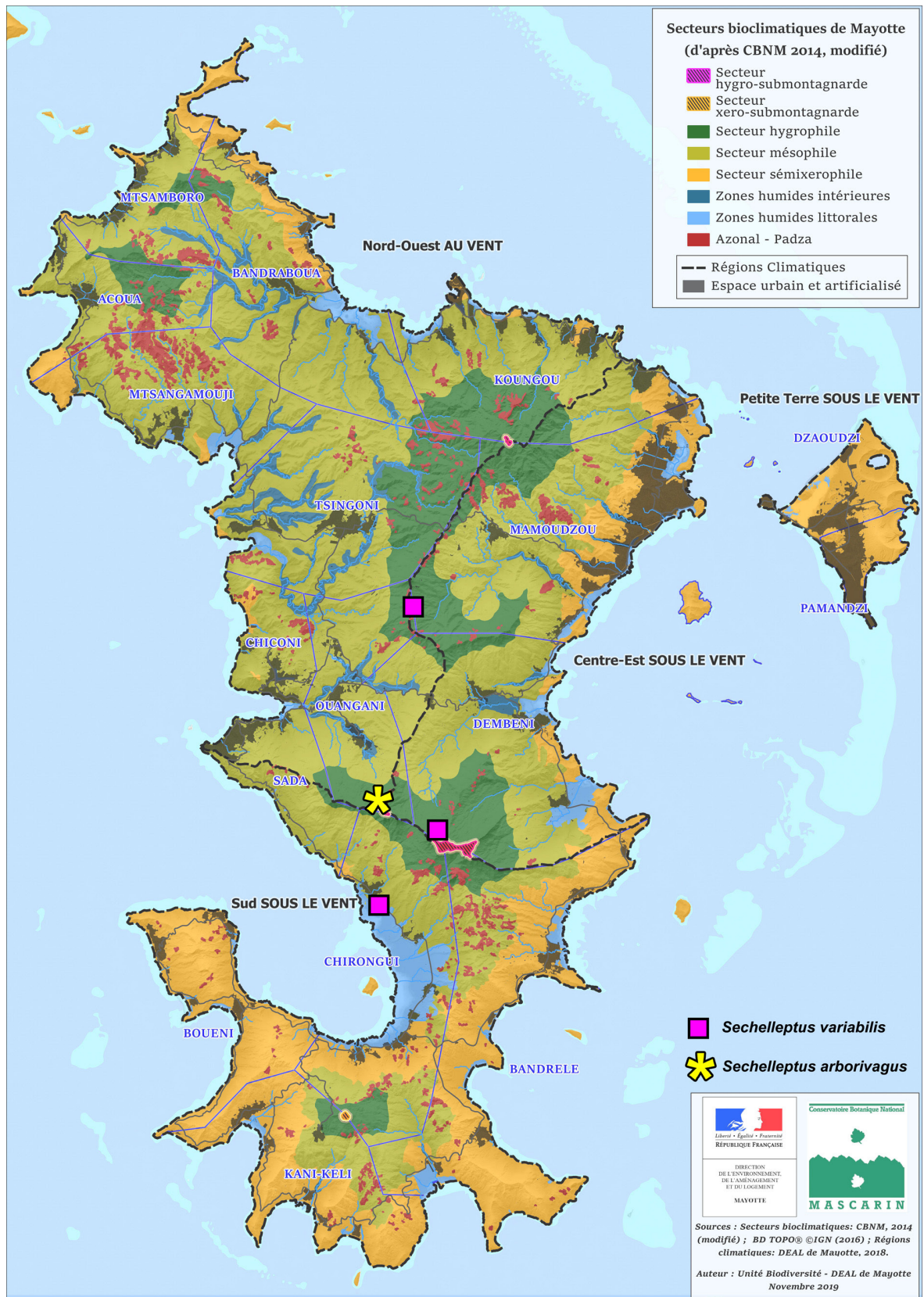


Fig. 8. Distribution of *Sechelleptus arborivagus* sp. nov and *S. variabilis* VandenSpiegel & Golovatch, 2007 on Mayotte.

the high rate of substitution in mitochondrial sequences (especially for COI), and may underestimate species diversity. This also appears to be the case among the different forms of Mayottan *Sechelleptus*, which also share strongly similar gonopods. At the first glance, the new species of *Sechelleptus* seems to be a giant form of *S. variabilis*. However, although only subtle morphological differences are observed within the gonopods, the comparatively large body size and the behavior of *S. arborivagus* sp. nov. are remarkable. These observations finally corroborate our molecular analyses that clearly show sufficient genetic difference between the different *Sechelleptus* species collected on Mayotte (22.6% for COI and 6.6% for 16S between *S. arborivagus* sp. nov. and *S. variabilis*).

The genetic analyses also suggest the presence of another different species, i.e., DU1, although its phylogenetic position remains unresolved. This unique specimen found at Mont Combani is a sub-adult female that could not allow a formal identification, but, judging from its general appearance, appears to be an intermediate form between the two *Sechelleptus* species collected on Mayotte. The genetic divergences, along with adaptations to arboreal life observed in the novel species, may indicate an “adaptive micro-radiation” on Mayotte Island or even the Comoros. However, the inclusion of more specimens, including adult males, in phylogenetic analyses is needed to test this hypothesis and evaluate the status of that putative new species.

Acknowledgments

This work was supported by the ‘Ministère de la transition écologique et solidaire - France’. Present sampling was performed in the framework of the program “Acquisition de connaissances sur les myriapodes de Mayotte” (convention N° 2019-003/DEAL/SEPR) organized by the DEAL at Mayotte. We are grateful to Antoine Rouillé for his help in the organization of the sampling and Miguel Lamalfa-Diaz who guided us on the Mt. Tchaourembo. We are grateful to Alain Reygel for the drawings and Dr Sergei Golovatch for his kind advice on an early draft.

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Manuscript received: 28 October 2020

Manuscript accepted: 12 April 2021

Published on: 16 June 2021

Topic editor: Nesrine Akkari

Desk editor: Pepe Fernández

Printed versions of all papers are also deposited in the libraries of the institutes that are members of the *EJT* consortium: Muséum national d'histoire naturelle, Paris, France; Meise Botanic Garden, Belgium; Royal Museum for Central Africa, Tervuren, Belgium; Royal Belgian Institute of Natural Sciences, Brussels, Belgium; Natural History Museum of Denmark, Copenhagen, Denmark; Naturalis Biodiversity Center, Leiden, the Netherlands; Museo Nacional de Ciencias Naturales-CSIC, Madrid, Spain; Real Jardín Botánico de Madrid CSIC, Spain; Zoological Research Museum Alexander Koenig, Bonn, Germany; National Museum, Prague, Czech Republic.

Appendices

Appendix 1. List of primers used to amplify the COI and 16S rRNA markers.

Marker	Primer name	Direction	Primer sequence	References
COI	C1-J-1718-spider	F	5'-GGNGGATTTGGAAATTGRITTRGTTCC-3'	Vink <i>et al.</i> (2005)
	C1-N-2568	R	5'-GCTACAACATAATAAGTATCATG-3'	Hedin & Maddison (2001)
16S rRNA	LR-N-13398 (16Sar)	F	5'-CGCCTGTTTAACAAAAACAT-3'	Simon <i>et al.</i> (1994)
	LR-J-12887 (16Sbr)	R	5'-CCGGTCTGAACTCAGATCACGT-3'	Simon <i>et al.</i> (1994)

Appendix 2. List of the COI and 16S rRNA sequences included on the phylogenetic analyses and obtained from GenBank and BOLD databases. Stars indicate unpublished sequences, but available online.

Species	Family	Sample ID	COI	16S	Database	Locality	Reference
<i>Thyropygus allevatus</i>	Harpagophoridae	CUMZ_D00018	KC519484	KC519557	GenBank	Thailand	Pimvichai <i>et al.</i> 2014
<i>Thyropygus jarukchusri</i>	Harpagophoridae	CUMZ_D00053	KC519516	KC519592	GenBank	Thailand	Pimvichai <i>et al.</i> 2014
<i>Thyropygus uncinatus</i>	Harpagophoridae	CUMZ_D00039	KC519503	KC519578	GenBank	Thailand	Pimvichai <i>et al.</i> 2014
<i>Thyropygus</i> sp.	Harpagophoridae	DVL-2001	AY055728 (region 1-1533)	AY055728 (region: 12796-11547)	GenBank	–	Lavrov <i>et al.</i> 2002
<i>Bicoxidens brincki</i>	Spirostreptidae	l31	KM982528	KM982488*	GenBank	Zimbabwe	Tinago <i>et al.</i> 2017
<i>Bicoxidens flavicollis</i>	Spirostreptidae	a21	KM982490	KM982455*	GenBank	Zimbabwe	Tinago <i>et al.</i> 2017
<i>Bicoxidens flavicollis</i>	Spirostreptidae	b26	KM982499	KM982463*	GenBank	Zimbabwe	Tinago <i>et al.</i> 2017
<i>Bicoxidens flavicollis</i>	Spirostreptidae	c22	KM982501	KM982465*	GenBank	Zimbabwe	Tinago <i>et al.</i> 2017
<i>Bicoxidens flavicollis</i>	Spirostreptidae	h21	KM982520	KM982483*	GenBank	Zimbabwe	Tinago <i>et al.</i> 2017
<i>Bicoxidens friendi</i>	Spirostreptidae	d11	KM982505	KM982469*	GenBank	Zimbabwe	Tinago <i>et al.</i> 2017
<i>Bicoxidens friendi</i>	Spirostreptidae	d16	KM982510	KM982474*	GenBank	Zimbabwe	Tinago <i>et al.</i> 2017
<i>Bicoxidens friendi</i>	Spirostreptidae	e12	KM982512	KM982476*	GenBank	Zimbabwe	Tinago <i>et al.</i> 2017
<i>Doratogonus</i> sp.	Spirostreptidae	GG-2003	AY288738	AY288715	GenBank	Swaziland	Edgecombe & Giribet 2004
<i>Spirostreptus kruegeri</i>	Spirostreptidae	Sk	KF219556	MT114492*	GenBank	South Africa	Mwabvu <i>et al.</i> 2015
<i>Spirostreptus sebae</i>	Spirostreptidae	Ss	KF219557	MT114491*	GenBank	Zambia	Mwabvu <i>et al.</i> 2015
<i>Diplopoda</i> sp.	–	XMAD_134	RNOCF124-17*	–	BOLD	Madagascar	–

Appendix 3. Selection model estimated using PartitionFinder 2 (Lanfear *et al.* 2016) and based on one partition for 16S and three for COI (partitioned into single codon positions).

Settings used

alignment: ./COI_16S_GBLOCKS.phy
 branchlengths: linked
 models: JC, K80, SYM, F81, HKY, GTR, JC+G, K80+G, SYM+G, F81+G, HKY+G, GTR+G, JC+I, K80+I, SYM+I, F81+I, HKY+I, GTR+I, JC+I+G, K80+I+G, SYM+I+G, F81+I+G, HKY+I+G, GTR+I+G
 model_selection: bic
 search: greedy

Best partitioning scheme

Scheme Name: start_scheme
 Scheme lnL: -6422.05841064
 Scheme BIC: 13324.1841399
 Number of params: 69
 Number of sites: 1051
 Number of subsets: 4

Subset | Best Model | # sites | subset id | Partition names

1 | SYM+G | 187 | 555f317e2b0e658f989fe7dcbaff9c08 | COI_1
 2 | F81 | 187 | d014815e8c9674f9db22782683dcefb2 | COI_2
 3 | HKY+I+G | 187 | c8108630a819b5a05188ac5b43a5da04 | COI_3
 4 | HKY+I+G | 490 | 855453fb15f46ffa6d1fc11c5a2c8afd | 16S

Nexus formatted character sets used for GARLI

```
begin sets;
  charset Subset1 = 1-561\3;
  charset Subset2 = 2-561\3;
  charset Subset3 = 3-561\3;
  charset Subset4 = 562-1051;
  charpartition PartitionFinder = Group1:Subset1, Group2:Subset2, Group3:Subset3, Group4:Subset4;
end;
```

MrBayes block for partition definitions

```
begin mrbayes;
  charset Subset1 = 1-561\3;
  charset Subset2 = 2-561\3;
  charset Subset3 = 3-561\3;
  charset Subset4 = 562-1051;
  partition PartitionFinder = 4:Subset1, Subset2, Subset3, Subset4;
  set partition=PartitionFinder;
  lset applyto=(1) nst=6 rates=gamma;
  prset applyto=(1) statefreqpr=fixed(equal);
  lset applyto=(2) nst=1;
  lset applyto=(3) nst=2 rates=invgamma;
  lset applyto=(4) nst=2 rates=invgamma;

  prset applyto=(all) ratepr=variable;
  unlink statefreq=(all) revmat=(all) shape=(all) pinvar=(all) tratio=(all);
end;
```