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Authors: Bernard , Rafał, Felska , Magdalena, and Małol , Joanna

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Erythraeid larvae parasitizing dragonflies in Zambia—description of *Leptus (Leptus) chingombensis* sp. nov. with data on biology and ecology of host-parasite interactions

RAFAŁ BERNARD¹, MAGDALENA FELSKA² & JOANNA MAŁKOL^{2,3}

¹ Department of Nature Education and Conservation, Adam Mickiewicz University, Umultowska 89, 61-614 Poznań, Poland, e-mail: rbernard@amu.edu.pl

² Department of Invertebrate Systematics and Ecology, Wrocław University of Environmental and Life Sciences, Koźuchowska 5b, 51-631 Wrocław, Poland, email: magdalena.felska@upwr.edu.pl, joanna.makol@upwr.edu.pl

³ corresponding author

Abstract

A survey of odonate fauna in Zambia (Central Province, Luano District) resulted in discovery of ectoparasitic larvae of *Leptus* (L.) *chingombensis* sp. nov. (Trombidiformes: Parasitengona, Erythraeidae) on four species of dragonflies (Odonata) representing four different families assigned to Zygoptera and Anisoptera. The morphological characteristics of the new species is supported with DNA barcode sequence. Despite some intra-group variation related to relatively large sample, the morphological and genetic consistence confirm the common specific identity of the material. A brief comparison of *Leptus* spp. hitherto known from the Afrotropic as larvae is given. Supplementary data to the descriptions of *Leptus* (L.) *bicristatus* Fain et Elsen, 1987, *Leptus* (L.) *aldonae* Haitlinger, 1987 and *Leptus* (L.) *soddagus* Haitlinger, 1990, based on examination of type material, are provided.

In the case of *L. (L.) chingombensis* sp. nov., the parasite load reached high, previously not recorded for Odonata–terrestrial Parasitengona association values, attaining at 44 and 49 larvae. Clear topic preferences towards the ventral side of the host's body were recorded, with an additional tendency to distal parts of synthorax and the ventral depression of the abdomen.

We hypothesize that the infestation did not take place synchronously at dragonflies emergence, but consisted in repeated infestation events during the recurrent appearance of dragonflies in the contact microhabitat occupied by *Leptus*.

The very local character of the finding along with the regular appearance of larvae parasitizing dragonflies, obviously favoured by specific habitat conditions, no doubts confirms the non-accidental nature of the phenomenon.

Key words: Erythraeidae, Odonata, attachment site, morphology, DNA barcode, Africa

Introduction

The nominate subgenus *Leptus* of *Leptus* Latreille, 1796 comprises 281 species (Małkol & Wohltmann 2012; Haitlinger & Šundić 2014; Khademi *et al.* 2015; Šundić & Haitlinger 2015; Haitlinger *et al.* 2017; Šundić *et al.* 2017; Saboori *et al.* 2018), the majority (227) of those are known exclusively from larvae and for only 12 species, both larvae and postlarval forms have been described. In addition, three other species based on larvae from Pakistan have been informally described by Kamran (2009) in the manuscript of his PhD thesis and their names remain unavailable (see also Halliday *et al.* 2018).

The number of described species varies between the biogeographic realms. The highest diversity, amounting to 30% of the 239 *Leptus* spp. known from larvae or from larvae and active

postlarval forms have been reported for the Palaearctic, 25%—for the Australian region, 14%—the Afrotropic, 13%—the Neotropic, 11%—the Oriental Region, and 7%—the Nearctic.

Leptus spp. in tropical Africa are poorly known. Thirty-seven species (*c.* 14% of all *Leptus* spp.), including 35 known exclusively from larvae and two known only from active postlarval forms, have been described from the Afrotropic to date (Małol & Wohltmann 2012).

Larvae of *Leptus* spp., like most other parasitengone species, are known for their parasitic life-style, whereas active postlarval forms (deutonymphs and adults) are predators. The host group, or at least one host named at the species level, has been identified for about 83% of known *Leptus* spp. larvae. Members of the genus tend to display relatively low host specificity and for several species the host range encompasses various orders (e.g. Łaydanowicz & Małol 2010; Kerry 2013). However, despite the increasing interest paid to the studies of host-parasite interactions, the knowledge of host spectrum is hindered due to: a) the lack of ecological studies focused on particular species of parasites, b) usually low, limited number of specimens representing parasitic species, significantly affecting the inference on intraspecific variation, but also c) insufficient efforts to identify the hosts species.

Terrestrial Parasitengona, contrary to aquatic ones (Hydrachnidia) (e.g. Åbro 1979; Grant & Samways 2007; López Salmeron & Mendoza-Cuenca 2010), have been rarely reported to parasitize dragonflies. The hitherto records apply to members of two erythraeid genera, *Charletonia* and *Leptus*. *Charletonia edytiae* Haitlinger, 1987 was collected from Odonata indet. in Madagascar (Haitlinger 1987), *C. hunanensis* Zheng, 1996—from Libellulidae indet and Megapodagriidae indet. in China (Zheng 1996), *C. rageaui* Southcott, 1966—from Libellulidae indet. in New Caledonia (Southcott 1966), *C. rocciai* Treat et Flechtmann, 1979—from Anisoptera indet. in Brazil (Rosa & Flechtmann 1980), and *C. sp.*—from Zygoptera indet. in Ivory Coast (Welbourn 1983). *Leptus* (*L.*) *draco* Southcott, 1984 was reported as parasite of Odonata indet. in New Guinea (Southcott 1999), *Leptus* sp.—from *Orthetrum coerulescens* (Libellulidae) in Switzerland (Juillerat & Wildermuth 2006) and *Leptus* sp. from *Ischnura senegalensis* (Coenagrionidae) in South Africa (Grant & Samways 2007). The widest array of odonate hosts, known for *Leptus* (*L.*) *killingtoni* Turk, 1945, includes *Orthetrum coerulescens*, *Pyrrhosoma nymphula*, *Ceragrion tenellum*, *Coenagrion puella*, *Coenagrion mercuriale*, *Lestes sponsa*, *Enallagma cyathigerum*, *Cordulegaster boltonii*, *Anax imperator*, *Ischnura hastata*, *Ischnura pumilio* and *Sympetrum fonscolombii* recorded in Great Britain and in the Azores, Portugal (Turk 1945; Killington & Bathe 1946; Lorenzo-Carballa *et al.* 2011; Kerry & Baker 2012; Kerry 2013; Cordero-Rivera *et al.* 2018).

In the course of studies on dragonfly fauna of Zambia, carried out from 2013 to 2017, the non-incidental cases of parasitism of *Leptus* larvae on odonates were observed. The substantial material collected during the survey allowed the extensive analysis of parasite taxonomy, backed with the characteristics of ecology and of host-parasite interactions.

Material and methods

The extensive field studies of dragonflies were carried out by R. Bernard (RB) and B. Daraż in Central Province of Zambia, mostly in broad environs of Chingombe (Luano District) and marginally in environs of Kabwe, during three expeditions between 2013 and 2017 (Bernard & Daraż 2018). The main study area combined the bottom of the rift valley (the Luano Valley), its mountain slopes (the Muchinga Escarpment) and the adjacent upland plateau.

Fifty-seven localities, covering diverse habitats (rivers, temporary and permanent streams, standing temporary waters) were studied. The collected *c.* 800 individuals of 105 species of Odonata, preserved as dry or in ethyl alcohol (the collection of RB in the Nature Collections of the Faculty of Biology, Adam

Mickiewicz University in Poznań, Poland), were searched by RB for the presence of ectoparasitic parasitengone larvae. However, the discovery of ectoparasitic mites, despite their number and frequency of observations, should be regarded as a side effect of the odonate-focused project.

The assessment of attachment site preferences of larvae was based on the examination of body regions of hosts (according to various criteria of body division), followed by reckoning of larval specimens. Due to various usefulness of body divisions in inference on topic preferences, we have referred to four different systems of partitioning (for details see tab. 3 in the Results section).

Ectoparasitic parasitengone larvae preliminarily assigned to *Leptus* were removed with the entomological pin and transferred to EtOH. For purpose of morphological examination, 30 larvae were stained with Chlorazol Black (Sigma Aldrich) and then mounted on microscopic slides in Faure's liquid. Measurements were taken in compound microscope Nikon Eclipse E600, equipped with DIC and DS-Fi1 camera, using the NIS-Elements BR software. Damaged or ambiguously positioned structures were excluded from the sample. Examination of quantitative characters, followed by preparation of drawings, was carried out in Nikon Eclipse 80i, equipped with DIC and camera lucida. The maximum length of dorsal setae (DS max) was calculated based on the five longest setae arising at idiosoma dorsum, the mean length (DS mean)—for ten randomly selected setae, and the length of ventral setae (VS)—for five randomly selected VS. For purpose of diagnostic comparison, the type material of three *Leptus* spp., selected based on the distributional criterion and the overall similarity to the newly collected larvae, was examined. The terminology and abbreviations follow Grandjean (1947), Southcott (1961, 1992) and Wohltmann *et al.* (2007).

Twelve non-pooled samples (each containing one specimen), taken from four different host species were designed for molecular analysis. Total genomic DNA was extracted from a single specimen using DNeasy Blood and Tissue Kit (Qiagen, Düsseldorf, Germany). The mites were transferred from 96% ethanol to ATL lysis buffer with Proteinase K and incubated for three days at 56°C. After digestion, the lysis buffer containing nucleic acids was transferred to a new Eppendorf tube and subjected to DNA isolation according to the manufacturer's protocol. Amplification of the DNA barcode region (cytochrome c oxidase subunit 1) was performed using degenerate forward primers bcdF01 (5'-CATTTCCHACTAAYCATAARGATATTGG-3') or bcdF04 (5'-CATTTCCHACTAAYCATAARGATATTGG-3') and reverse bcdR04 (5'-TATAAACYTCDGGATGNCCAAAAA-3') (Dabert *et al.* 2010) in the following thermocycling conditions: 95°C/3 min—initial denaturation; 95°C/30 sec, 50°C/30 sec, 72°C/45 sec—40 cycles; 72°C/7 min—final extending. The PCR reaction (25 µl) was performed using following PCR mix: 4 µl of genomic DNA, 10mM Tris-HCl, 50mM KCl, 1.5 mM MgCl₂, 200 µM of each dNTP, 150 pmol of each primer and 2 units of Taq polymerase (EurX). The bcdF04 and bcdR04 pairs of primers, used at the first step of analysis, gave unsatisfactory results in most cases, thus the PCR reaction and sequencing were repeated with bcdF01 and bcdR04, which produced much better results compared to initial sequencing. In some cases, however, the 3' end of the sequence was not reliable due to the presence of double peaks. The amplification product was purified using QIAquick PCR purification kit (Qiagen, Düsseldorf, Germany) and sequenced in both directions (Genomed S.A., Poland). The COI sequences were deposited in the GenBank under the following accession numbers (collection numbers of larvae are given in brackets): MK496500 [DISE206], MK496501 [DISE210], MK496502 [DISE211], MK496503 [DISE208], MK496504 [DISE209], MK496505 [DISE207], MK496506 [DISE215], MK496507 [DISE216], MK496508 [DISE217].

The multiple alignment was performed using CLUSTAL W implemented in MEGA 7.0 package (Kumar *et al.* 2016) with default parameters (gap opening penalty—15, gap extension penalty—6.66, both for pairwise and multiple alignment). Pair-wise sequence alignment and cutting resulted in obtaining a compact 529 bp data block. Distance calculation between sequences was performed using a Kimura two parameter model (K2P) implemented in MEGA 7.0 (Kumar *et al.* 2016). DNA sequence polymorphism was analyzed with DnaSP software v.6.12.01 (Rozas *et al.* 2017). For purpose of comparison, three COI sequences (acc. no. ACG5338—voucher BIOUG05863-A04 and ABW2695—vouchers BIOUG01951-

A10, BIOUG07022-H08), of *Leptus* spp. and of Erythraeidae sp. were retrieved from the GenBank. Phylogenetic tree was constructed using maximum likelihood (ML) estimation, based on the Kimura 2-parameter model and bootstrap analysis with 1000 replicates in MEGA 7.0.

Results

Ecology

Dragonflies infested by *Leptus chingombensis* sp. nov. were found on the Muchinga Escarpment, at four close localities along the upper reaches of Mikwa stream, in the span of 2 km below the stream source, 1180–1060 m a.s.l., 6.7–8.3 km W of Chingombe catholic mission, between 14°25'16"S, 29°53'23"E and 14°24'45"S, 29°54'18"E.

The upper course of this permanent mountain stream is narrow and mostly deeply shaded by a gallery forest, with small clearings and locally with adjacent open or semi-open habitats on steep slopes covered with herbaceous vegetation. As an effect, the mosaic of light and thermal conditions occurs at this locality.

No cases of *Leptus* parasitism on dragonflies were recorded at other 53 localities in the study area, including four localities in the lower reaches of the Mikwa stream and places with the presence of the same potential hosts (Tab. 1).

The *Leptus* larvae were recorded on dragonflies in 2015 and 2017 (with a break in field studies in 2016), along the same stream section. Ectoparasitic larvae (n = 134) at different levels of engorgement, were discovered on nine host specimens assigned to four species of four distinct families and two suborders of Odonata, out of the total number of 53 dragonflies assigned to 11 species and collected at the same four localities (Tab. 1 and 2).

The occurrence of larvae on host was recorded in the peak of the rainy season (late January, 2017) and at the very end of it (late April, 2015). Higher mean intensity of infestation (36 vs 4.3) was observed at the end of the rainy season (Tab. 2). Host specimens comprised eight males and one female.

The 50% incidence was observed for larvae infesting *Orthetrum julia* (for 12 hosts from the upper reaches of the stream) and the mean intensity of infestation for this host species was 20.5.

One hundred twenty-eight *Leptus* larvae were localised on hosts according to several host body divisions (Tab. 3); six other larvae detached during manipulation and preservation. A half of larvae occurred on the host's synthorax and almost the next one fourth on abdomen while legs were less frequently and only in their basal parts occupied. Most larvae were attached on the ventral side of the host's body. Most larvae were attached to places with softer cuticle, such as sutures and intersegmental cuticle, and they occurred to a lesser extent on heavily sclerotized areas.

Taxonomy

Erythraeidae Robineau-Desvoidy, 1828

Leptinae Billberg, 1820

***Leptus* Latreille, 1796**

subgenus *Leptus* Latreille, 1796

Type species: *Acarus phalangii* de Geer, 1778

For generic diagnosis see Małol *et al.* 2012.

***Leptus (L.) chingombensis* sp. nov.**

Diagnosis

Larva. One seta on palp femur and palp genu. Four setae 3a (intercoxalae) behind the level of coxae II (setae not extending past the posterior margin of coxae III). Anterior 3a₁ setae 1.4–1.9 times shorter than the posterior 3a₂ setae. Tibia I = 227–267. ISD = 60–72. Ti III/AW = 3.2–3.6. DS max = 67–79. Scutum L/W = 0.9–1.0. Ti III = 277–319. Vestigiala (κ) absent from tibia II.

Deutonymph and adult. Not known.

Description (compiled from holotype and paratypes, unless stated otherwise)

Larva (Figs 1–8A, holotype). For morphometric data (except for those provided in the description) see Table 4.

Gnathosoma (Fig. 1). Chelicera composed of basal segment and movable, hook-like claw. Gnathosoma with a pair of nude setiform adoral setae *cs* (18, in holotype) placed antero-dorsally and a pair of peg-like supracoxal setae *elcp* (2, in holotype) in dorso-lateral position, at gnathosoma base. On gnathosoma venter, a pair of short and nude acicular setae *as* (11, in holotype) and a pair of longer subcapitular setae *bs* (47, in holotype), the latter with a few barbs. Pedipalp formula: 0-B-B-BBB-NNBBBBωζ. Palp femur and palp genu each with one setulose seta. Palp genu with or without a sclerotized, longitudinal bar of different length. Palp tibia with three barbed setae. Odontus simple. Palp tarsus with six normal setae, of which four are covered with setules and two are smooth, one solenidion (ω) located in proximal part of the segment and one eupathidium (ζ), placed distally.

TABLE 1. Feedback of parasitic larvae of *Leptus (L.) chingombensis* sp. nov. in localities surveyed for the presence of Odonata along the Mikwa stream, central Zambia.

area	Dragonflies (total)			Parasitism by <i>Leptus (L.) chingombensis</i> sp. nov.		
	number of specimens collected	number of species	number of localities	number of host specimens parasitized	number of host species parasitized	number of localities with recorded cases of parasitism
upper Mikwa stream	53	11	4	9	4	4
lower Mikwa stream	60 (incl. 7 <i>Orthetrum julia</i>)	26	4	–	–	–
other localities in the stream basin	13	6	3	–	–	–
total	126	33 ¹	18	9	4	4

¹ includes the repeated species

TABLE 2. Host specification, collection sites and parasite load for larvae of *Leptus (L.) chingombensis* sp. nov. (n = 134).

host	locality no. / coordinates	coll. date	host's sex	parasite load
<i>Pseudagrion spernatum</i> Selys, 1881 (Zygoptera: Coenagrionidae)	1/14°25'16"S, 29°53'23"E	27.01.2017	m	3
<i>Heliaeschna fuliginosa</i> Selys, 1883 (Anisoptera: Aeshnidae)	1/14°25'16"S, 29°53'23"E	28.01.2017	f	2
<i>Paragomphus cognatus</i> (Rambur, 1842) (Anisoptera: Gomphidae)	1/14°25'16"S, 29°53'23"E	26.01.2017	m	6
<i>Orthetrum julia</i> Kirby, 1900 (Anisoptera: Libellulidae)	1/14°25'16"S, 29°53'23"E	26.01.2017	m	8
<i>O. julia</i> Kirby, 1900	1/14°25'16"S, 29°53'23"E	26.01.2017	m	6
<i>O. julia</i> Kirby, 1900	1/14°25'16"S, 29°53'23"E	26.01.2017	m	1
<i>O. julia</i> Kirby, 1900	2/14°25'09"S, 29°53'31"E ¹	28.04.2015	m	49
<i>O. julia</i> Kirby, 1900	3/14°25'02"S, 29°53'40"E	28.04.2015	m	44
<i>O. julia</i> Kirby, 1900	4/14°24'45"S, 29°54'18"E	25.04.2015	m	15

¹ type locality

TABLE 3. Distribution of larvae of *Leptus* (*L.*) *chingombensis* sp. nov. translated into attachment preferences of parasites towards different regions of host body according to four criteria of body division.

body division criterion	parts of the body considered	number of <i>Leptus</i> larvae	percentage (%) of total (n = 128) number of parasites
I. tagma	head	0	0
	thorax (thorax-legs-wings)	99 (69-24-6)	77.3 (53.9-18.7-4.7)
	abdomen	29	22.7
II. morphological sectors	head	0	0
	small prothorax with forelegs	6	4.7
	wide and massive complex of synthorax (with basal parts of middle- and hind legs and wings) and anterior segments (S1, S2) of abdomen	103	80.4
	narrow, remaining parts of abdomen (S3-S10)	19	14.9
III. functional morphology	head	0	0
	prothorax	2	1.6
	synthorax	67	52.3
	legs ¹	24	18.8
	wings ²	6	4.7
	anterior segments (S1, S2) of abdomen, partly involved in reproduction	10	7.8
	subsequent, usually narrowed segments of abdomen (S3-S8) ³	19	14.8
	posterior segments of abdomen (S9-10), incl. appendages; involved in reproduction	0	0
IV. body planes	ventral ⁴	103	80.5
	lateral ⁵	15	11.7
	dorsal ⁶	10	7.8

¹ only proximal leg segments, mostly coxae and their basal parts (19 larvae), less frequently femora (5 larvae)

² only the hind wings, 3 larvae—at wing bases and 3—in the most proximal parts of main wing veins (subcosta, cubitus): close to the wing base, still before the first Ax or in thickened basal part of the vein

³ all specimens sheltered on the ventral side of the abdomen

⁴ mainly the ventral surface of synthorax, with special preference to the area behind legs, but also basal parts of legs and ventral side of the abdomen (with a depression)

⁵ lateral parts of synthorax (in its posterior part) and first abdominal segments

⁶ wing bases (incl. proximal parts of veins), middorsal carina

Dorsal idiosoma (Figs 2–3). Scutum with indistinct punctations on the entire surface. The medial part of the sclerite, more strongly sclerotized, forming the hourglass-shape structure, extending between the anterior and posterior margin of scutum and encompassing the bases of trichobothria. Reticulate pattern of varying distinctness, asymmetrical with respect to the shape and distribution, present at postero-lateral margins of the ‘hourglass’, in the lower stratum of the sclerite. Setae AL comparable with respect to the shape and length to PL. Anterior pair of trichobothria (ASens) shorter than the posterior pair (PSens), both with setules distributed along the distal half of the stem, more distinct towards the stem termination. Single eyes at the level of postero-lateral margins of scutum. Dorsal setae uniform in shape, like AL and PL, densely setulated along the entire stem, parallel-sided and rounded (or only indistinctly narrowed) at termination (Fig. 8A). *fD* = 74–86 (n = 7, incl. holotype: 86).

TABLE 4. Morphometric data on larvae of *Leptus* (L.) *bicristatus*, *L. (L.) aldonae*, *L. (L.) soddagus* and *L. (L.) chingombensis* sp. nov.

	<i>Leptus</i> (L.) <i>bicristatus</i> Fain et Elsen, 1987	<i>Leptus</i> (L.) <i>aldonae</i> Haitlinger, 1987	<i>Leptus</i> (L.) <i>soddagus</i> Haitlinger, 1990	<i>Leptus</i> (L.) <i>chingombensis</i> sp. nov.		
	holotype ¹	holotype ¹	holotype ¹	holotype	sample size (incl. holotype)	range / mean / SD
Gnathosoma L	210 (198)	138 (128–146)	208 (200)	217	29	214–253 / 231 / 9.99
Pa Tr	37	24	26	43	34	37–44 / 40 / 2.01
Pa Fe	62	38	75	73	34	64–76 / 71 / 3.56
Pa Ge	67 (69)	48	69	68	34	59–72 / 66 / 3.47
Pa Ti	27	11	18	25	34	17–28 / 22 / 2.13
Pa Ta	25	14	21	22	34	18–26 / 22 / 1.83
Odo	30	17	34	30	34	27–37 / 30 / 2.56
IL	1366 (1500 ²)	516 (560–728 ³)	480 (792 ²)	521	33	424–976 / 645 / 146.3
IW	903 (900)	339 (272–384)	300 (296)	340	33	267–787 / 434 / 118.71
IL/IW	1.5	1.5	1.6	1.5	33	1.2–1.9 / 1.5 / 0.16
L	102 ³	78 (34–84 ⁴)	105 (106)	115	33	103–120 / 113 / 4.02
W	135 (129)	68 (72–82 ⁴)	128 (128)	120	34	110–126 / 119 / 3.90
L/W	0.7 ³	1.1	0.8 (0.8)	0.9	33	0.9–1.0 / 1.0 / 0.04
ASens	50 ([?] 40)	31 (24–30)	[?] 31 ([?] 30)	55	33	45–55 / 49 / 2.57
SBa	15 (14)	8 (8)	13 (14)	16	33	12–17 / 14 / 1.19
PSens	62 ([?] 60)	64 (66–70)	78 (80)	69	19	60–79 / 74 / 3.17
SBp	17 (18)	12 (12–14)	18 (18)	18	32	16–21 / 18 / 1.22
AL	73 ([?] 64)	37 (40–42)	–	82	31	71–84 / 79 / 3.70
AW	100 (103)	49 (52–60)	94 (100)	87	33	82–92 / 88 / 2.41
PL	82 (78)	48 (48–52)	74 (72)	84	29	75–88 / 83 / 3.51
PW	122 (120)	62 (66–76)	115 (120)	109	33	90–114 / 108 / 4.91
ISD	62	41 (40–44)	71 (72)	67	32	60–72 / 67 / 2.48
AW/AL	1.2	0.8	–	1.1	30	1.0–1.2 / 1.1 / 0.05
AW/ISD	1.6	1.2	1.3 (1.4)	1.3	32	1.2–1.5 / 1.3 / 0.05
AL/PL	1.0	0.8	–	1.0	27	0.9–1.1 / 1.0 / 0.05
PW/AW	1.2	1.3	1.2 (1.2)	1.2	33	1.1–1.3 / 1.2 / 0.04
DS max	77 (60–73 ⁵)	45–48 (52)	59–62 (60–64 ⁵)	76	34	67–79 / 73 / 3.11
DS mean	67 ⁶	42	58	67	34	57–72 / 67 ⁶ / 2.92
VS	52–60 (42–58)	26–33 (30–44)	40–51	52	33	47–59 / 54 / 2.65
1a (St I)	[?] 32	52 (54)	51 (56)	60	29	50–63 / 58 / 3.44
2a (St II)	45	34 (36–40)	–	53	30	46–62 / 52 / 3.56
3a ₁ ⁷	27	25	–	28	31	27–35 / 32 / 2.46
3a ₂ ⁷	45	32	–	50	31	46–54 / 50 / 2.25
3a ₂ /3a ₁	1.7	1.3	–	1.8	31	1.4–1.9 / 1.6 / 0.14
1b	85 (82)	63	73 (76)	100	33	87–101 / 93 / 3.77
2b	35 (34)	27	– (42)	45	31	39–59 / 44 / 3.57
3b	55 (52)	31	–	57	33	52–66 / 58 / 2.98
Cx I	77	41 (44–48)	74 (68)	69	34	64–82 / 74 / 3.86
Tr I	55	28 (40)	50 (54)	56	34	55–69 / 62 / 4.28
bFe I	115	46 (44)	103 (120)	121	34	113–134 / 122 / 4.63
tFe I	112	40 (50)	94 (100)	127	34	108–130 / 121 / 5.68
Ge I	160 (157)	66 (72–82)	151 (162)	176	33	158–191 / 176 / 7.69
Ti I	237 (219)	89 (90–94)	219 (234)	259	33	227–267 / 250 / 10.42
Ta I	172 (154)	80 (76–82)	169 ⁸	178	33	156–180 / 170 / 7.08
Ta I (W)	25	34 (26–30)	29	32	33	26–35 / 32 / 1.83

.....continued on the next page

TABLE 4. (Continued)

	<i>Leptus (L.) bicristatus</i> Fain et Elsen, 1987	<i>Leptus (L.) aldonae</i> Haitlinger, 1987	<i>Leptus (L.) soddagus</i> Haitlinger, 1990	<i>Leptus (L.) chingombensis</i> sp. nov.		
	holotype ¹	holotype ¹	holotype ¹	holotype	sample size (incl. holotype)	range / mean / SD
leg I	928 (810 ^b)	390	860	986	33	900–1030 / 975 / 30.78
Cx II	102	63 (64–66)	94 (84)	94	34	73–104 / 94 / 6.94
Tr II	62	29 (40–42)	51 (54)	63	34	56–70 / 64 / 3.57
bFe II	95	37 (40–44)	84 (92)	104	34	89–111 / 102 / 5.53
tFe II	92	44 (42)	87 (86)	109	34	96–115 / 107 / 4.97
Ge II	125	61 (54–70)	130 (130)	144	33	128–151 / 141 / 6.05
Ti II	192	73 (76–80)	182 (196)	225	33	205–232 / 219 / 7.54
Ta II	150	69 (68–72)	137 (140)	159	33	141–162 / 151 / 5.98
Ta II (W)	25	33 (26–30)	30	30	33	27–36 / 31 / 2.29
leg II	818	376	765 (782)	898	33	825–908 / 878 / 24.19
Cx III	97	63 (64)	59 (80)	90	34	82–108 / 93 / 5.74
Tr III	65	41 (40)	52 (50)	74	34	60–74 / 68 / 3.53
bFe III	125	49 (52)	111 (114)	126	34	111–135 / 126 / 5.15
tFe III	122	51 (52)	106 (110)	134	33	121–141 / 132 / 5.22
Ge III	145	69 (70)	138 (150)	163	32	143–170 / 160 / 6.75
Ti III	280	102 (108)	256 (264)	302	32	277–319 / 300 / 11.01
Ta III	170	76 (76–82)	150 (152)	167	32	156–175 / 165 / 4.23
Ta III (W)	20	31 (26–30)	18	27	32	23–29 / 26 / 1.35
leg III	999 (825 ^b)	451	872 (920)	1056	32	981–1093 / 1044 / 29.57
IP	2745	1211	2497	2940	32	2715–3024 / 2900 / 79.18
Ti I/AW	2.4	1.8	2.3 (2.3)	3.0	33	2.6–3.0 / 2.8 / 0.10
Ti I/Ge I	1.5	1.3	1.4 (1.4)	1.5	33	1.4–1.5 / 1.4 / 0.03
Ti II/PW	1.6	1.2	1.6 (1.6)	2.1	33	1.9–2.4 / 2 / 0.10
Ti II/Ge II	1.5	1.2	1.4 (1.5)	1.6	33	1.5–1.8 / 1.6 / 0.05
Ti III/AW	2.8	2.1	2.7 (2.6)	3.4	32	3.2–3.6 / 3.4 / 0.10
Ti III/Ge III	1.9	1.5	1.9 (1.8)	1.8	32	1.8–2.0 / 1.9 / 0.04
Ti III/Ti I	1.2	1.1	1.2 (1.1)	1.2	32	1.1–1.2 / 1.2 / 0.02

¹ based on reexamination of holotype (present study); data in parentheses refer to measurements included in the original descriptions (Fain & Elsen 1987, Haitlinger 1987, 1990) and, in case of *L. (L.) aldonae*, follow also (Haitlinger 2001)

² body length, incl. gnathosoma

³ anterior margin of scutum probably indistinctly folded beneath the shield

⁴ distinct variation in scutum length and width observed by Haitlinger (1987) for holotype (34x72) and two paratypes (76x82, 84x74)

⁵ range for DS

⁶ mean for 10 setae measured in one specimen

⁷ anterior (3a₁) and posterior (3a₂) intercoxalae III

⁸ separated from other segments of leg I and overimposed on idiosoma

⁹ measured [?] with or [?] without coxae

Ventral idiosoma (Fig. 4). *fCx* = 1-1-1. Peg-like supracoxal seta *elc I* present on dorsal rim of coxa I. Setae 1b, 2b, 3b located on coxal plates I, II, III, respectively. Setae 1a (= sternalae I), 2a (= sternalae II) placed between the coxal plates, at the level of coxae I and II, respectively. Four intercoxalae (setae 3a₁ and 3a₂) located between the level of coxae II and III; 3a₁ distinctly shorter than 3a₂. *fV* = 20–22 (n = 7, incl. holotype: 20). Ventral setae similar in shape to dorsal ones but slightly shorter. The total number of setae in *fD* and *fV* formula (NDV) = 94–106 (n = 7, incl. holotype: 106).

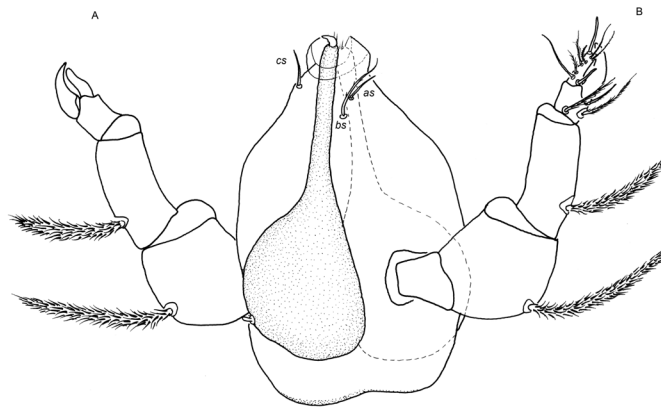


FIGURE 1. *Leptus (L.) chingombensis* sp. nov. Gnathosoma. A. Dorsal view (chaetotaxy of palp tibia and palp tarsus omitted). B. Ventral view.

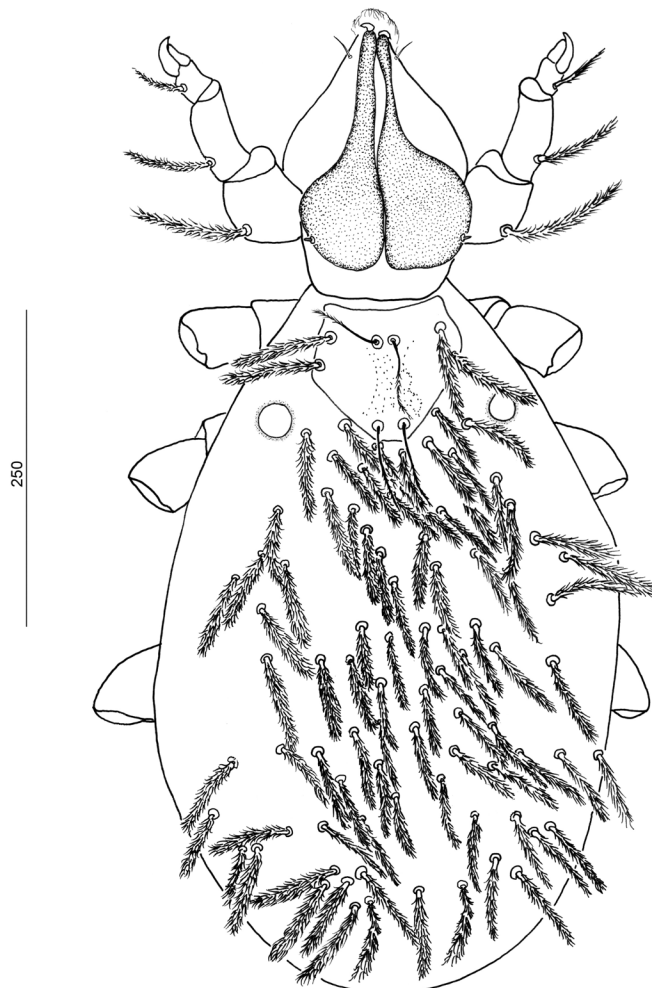


FIGURE 2. *Leptus (L.) chingombensis* sp. nov. Gnathosoma and idiosoma, dorsal view (chaetotaxy of palp tibia and palp tarsus partly or completely omitted; legs omitted beyond trochanters).

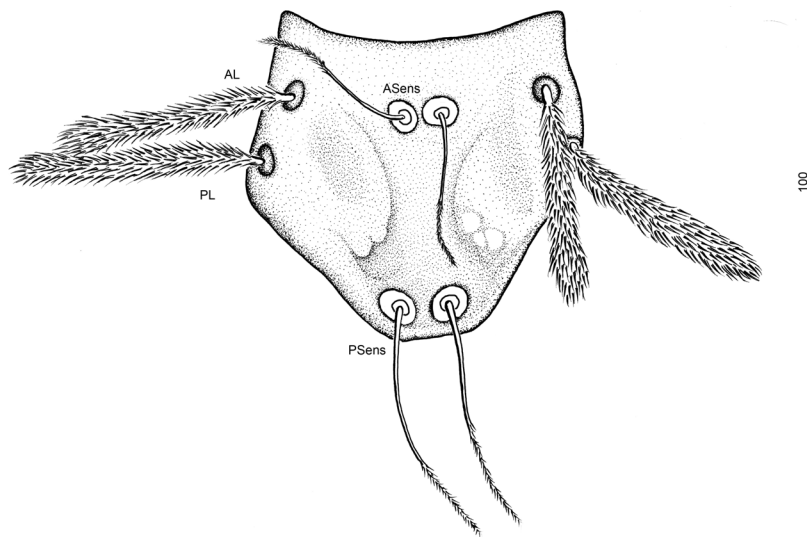


FIGURE 3. *Leptus (L.) chingombensis* sp. nov. Scutum.

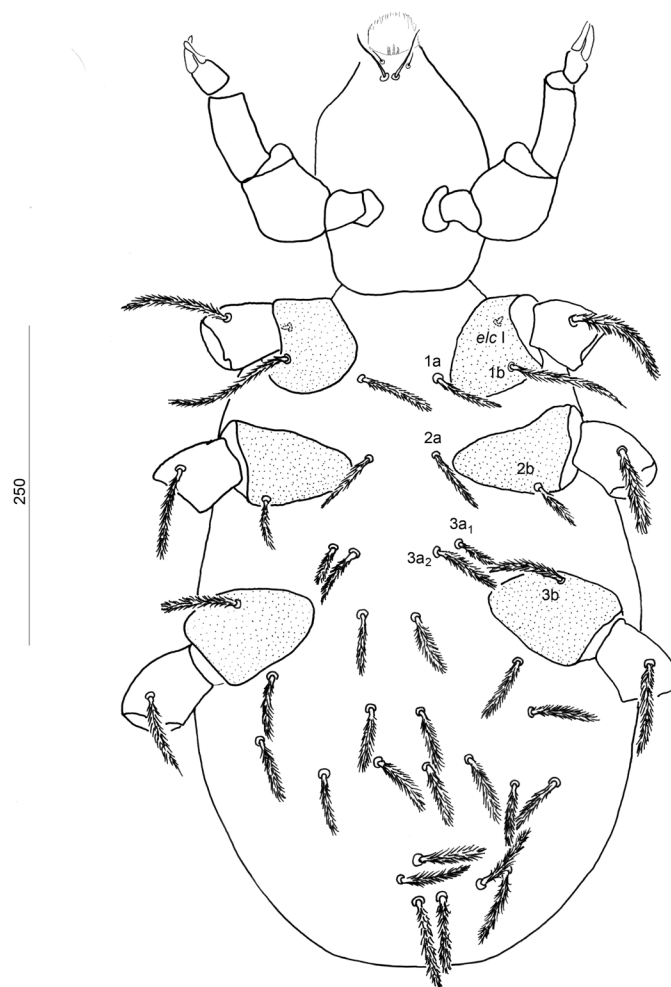


FIGURE 4. *Leptus (L.) chingombensis* sp. nov. Gnathosoma and idiosoma, ventral view (chaetotaxy of palps omitted; legs omitted beyond trochanters). *elc* I arises at dorsal rim of coxa I.

Legs (Figs 5–7). Leg segmentation formula: 7-7-7. For leg chaetotaxy see Table 5. Cuticle on legs, except for small, triangular, insertions of normal setae, arranged in transverse folds, especially distinct on tarsi. Normal setae on legs densely setulated. Slender setae, usually narrowing apically, are found on the proximal leg segments (Cx—bFe), whereas shorter stout setae are either parallel-sided or widened terminally and are located on distal leg segments (tFe—Ta). Famulus on tarsus I located distal to solenidion (ω). Subterminal eupathidia on tarsi I–III covered with delicate fimbriae. Another eupathidium, with slightly longer fimbriae present at tarsus I and II termination. All tarsi terminated with paired claws and empodium. Anterior claw only slightly shorter and thicker than empodium, both with very short onychotrichs. Posterior claw the shortest, densely covered with distinct onychotrichs.

Type material

Holotype, larva (H7804/10). Paratypes, 29 larvae (H7804/1–9, H7804/11–30).

Type locality

Upper reaches of the Mikwa stream, Muchinga Escarpment, 1160 m a.s.l., 8.0 km W of Chingombe catholic mission, 14°25'09"S, 29°53'31"E (see also Tab. 2).

Type host

Orthetrum julia Kirby, 1900.

Etymology

The specific name is derived from the name Chingombe, the village situated in proximity to type locality.

Type material deposition

The holotype (ID: H7804/10) and five paratypes (ID: H7804/11–15) in the Museum of Natural History, Wrocław University, Wrocław, Poland; three paratypes (ID: H7804/16, H7804/18, H7804/20)—in the Nature Collections, Faculty of Biology, Adam Mickiewicz University, Poznań, Poland and the remaining 21 paratypes (ID: H7804/1–9, H7804/17, H7804/19, H7804/21–30)—in the Department of Invertebrate Systematics and Ecology (DISE), Wrocław University of Environmental and Life Sciences, Wrocław, Poland.

Other material

Larvae ex *Orthetrum julia* (18 LV from locality 14°25'09"S, 29°53'31"E, including three vouchers [DISE206, DISE207, DISE215] after DNA extraction; 44 LV from locality 14°25'02"S, 29°53'40"E; 15 LV from locality 14°24'45"S, 29°54'18"E; 8 LV from locality 14°25'16"S, 29°53'23"E, including one voucher [DISE216] after DNA extraction; 6 LV from locality 14°25'16"S, 29°53'23"E, including one voucher [DISE217] after DNA extraction; 1 LV from locality 14°25'16"S, 29°53'23"E), larvae ex *Pseudagrion spernatum* (3 LV from locality 14°25'16"S, 29°53'23"E, including two vouchers [DISE208, DISE209] after DNA extraction), larvae ex *Heliaeschna fuliginosa* (2 LV from locality 14°25'16"S, 29°53'23"E, including one voucher [DISE210] after DNA extraction) and larvae ex *Paragomphus cognatus* (6 LV from locality 14°25'16"S, 29°53'23"E, including one voucher [DISE211] after DNA extraction) in the Department of Invertebrate Systematics and Ecology, Wrocław University of Environmental and Life Sciences, Wrocław, Poland. Larva ex *Orthetrum julia* (1 LV from locality 14°25'09"S, 29°53'31"E) in the Nature Collections, Faculty of Biology, Adam Mickiewicz University, Poznań, Poland.

Distribution. Zambia.

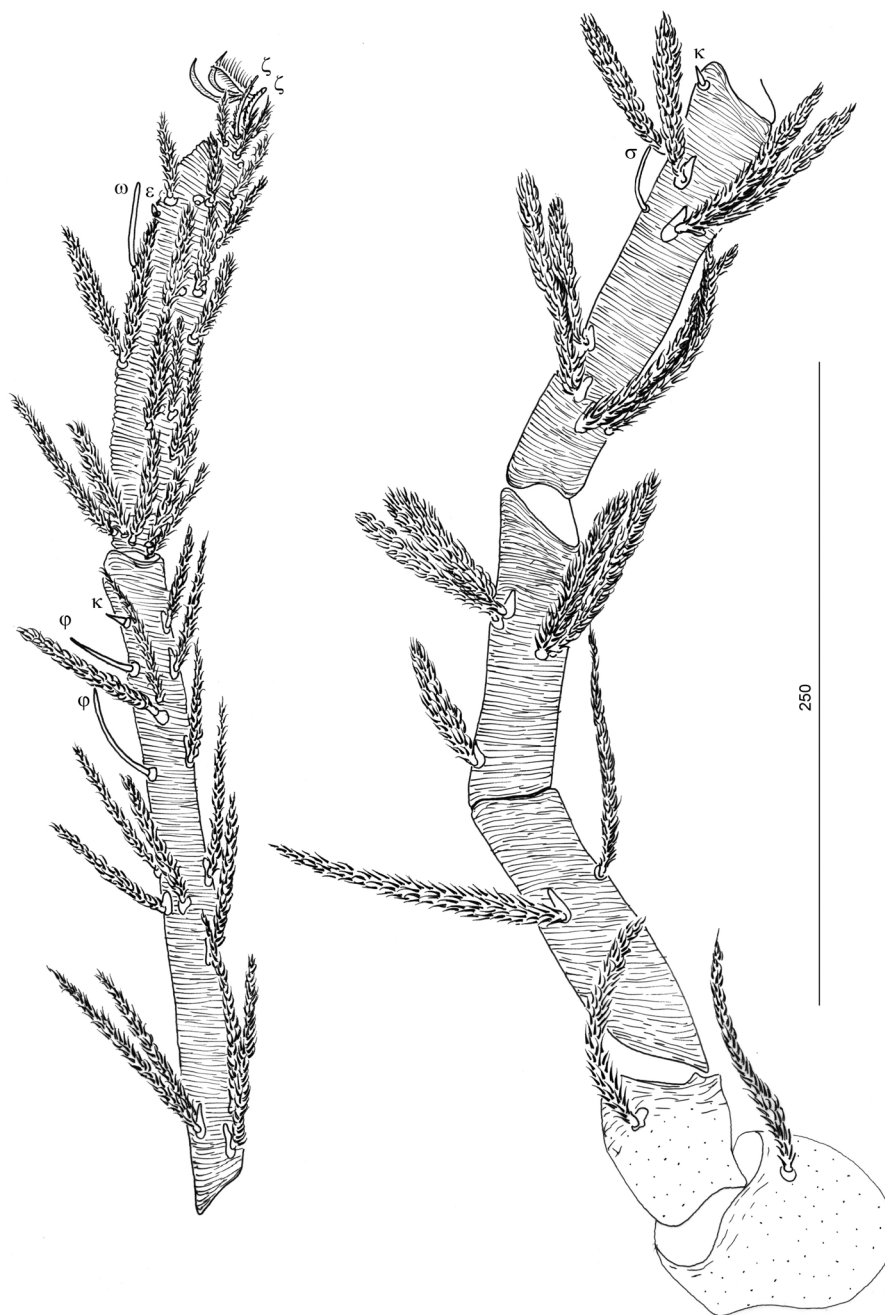


FIGURE 5. *Leptus (L.) chingombensis* sp. nov. Leg I.

Remarks

A comparison of larval species level diagnostic traits, distributions and hosts of *Leptus* spp. known from the Afrotropic is provided in Table 6. *Leptus (L.) chingombensis* sp. nov. belongs to the group of species having one seta each on the palp genu and palp femur and two pairs of 3a setae between coxae II and III. Afrotropical species, most similar to the new species (according to the data contained in the original descriptions and considering the available set of diagnostic traits), include: *L. (L.) aldonae*, *L. (L.) bicristatus* and *L. (L.) soddagus*. In order to proceed with comparative

analysis and in search for character states not included in original descriptions, the type material of all species in question was examined (Tab. 4–5).

Leptus (*L.*) *aldonae* departs from other species in the lower value of majority of measurable traits, with special reference to AW, PW and IP, in the presence of two solenidia on genu I (2 σ) and three on tibia I (3 ϕ), as well as the presence of solenidion on genu II (1 σ).



FIGURE 6. *Leptus* (*L.*) *chingombensis* sp. nov. Leg II.

The metric values for *L.* (*L.*) *bicristatus* and *L.* (*L.*) *soddagus*, each described from a specimen, mostly fall within or near the range of values of *L.* (*L.*) *chingombensis* sp. nov. The most substantial difference between all three species in question pertains to Ti III/AW ratio (Tab. 4), being clearly higher in *L.* (*L.*) *chingombensis* sp. nov. (3.2–3.6) than in both relatives (2.7–2.8), a somewhat lesser extent, also to Ti II/PW ratio, amounting to 1.9–2.4 in *L.* (*L.*) *chingombensis* sp. nov. (1.6—in *L.* (*L.*) *bicristatus* and in *L.* (*L.*) *soddagus*). Moreover, the verified value of fD and fV in *L.* (*L.*) *bicristatus* (Tab. 6) is another character that distinguishes between *L.* (*L.*) *bicristatus* (52/16) and *L.* (*L.*)

chingombensis sp. nov. (74–86/20–22). The presence of two cuticular bars on palp genu in *L. (L.) bicristatus* (character state varies between 0 and 1 in *L. (L.) chingombensis* sp. nov.), due to unrecognized variation, should be considered as an auxiliary character, until the larger sample of *L. (L.) bicristatus* can be examined. The new species can be also distinguished from *L. (L.) soddagus* by the dorsal idiosomal setae that are slightly widening terminally in *L. (L.) soddagus*, and nearly parallel-sided in *L. (L.) chingombensis* sp. nov. (see also Fig. 8).

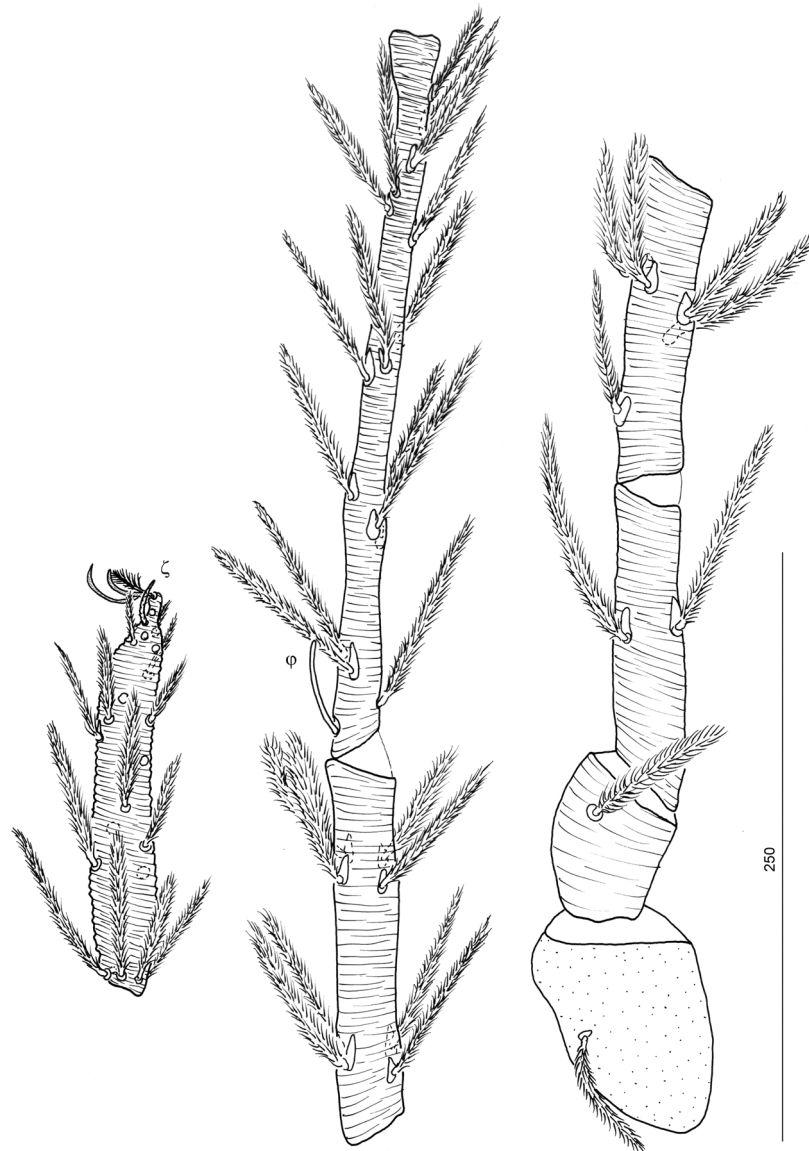


FIGURE 7. *Leptus (L.) chingombensis* sp. nov. Leg III.

The cuticular ornamentation of the scutum differs from what was reported by Fain and Elsen (1987) for *Leptus* spp., described from Central Africa, in the presence of mesh-like or reticular asymmetrical pattern extending between the bases of the trichobothria, along the medial line of scutum. This character may be of taxonomic value; however its usefulness should be verified through the detailed examination of scutum structure in other *Leptus* spp.

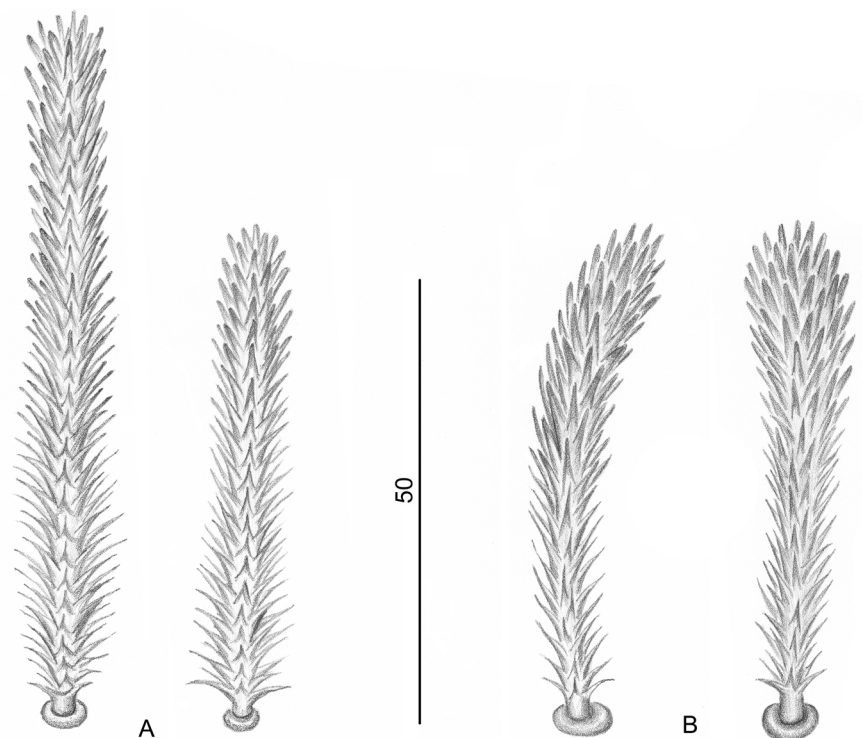


FIGURE 8. Dorsal opisthosomal setae. A. *Leptus* (*L.*) *chingombensis* sp. nov. B. *L.* (*L.*) *soddagus*.

The insight into variability range of characters provided in the description of *L.* (*L.*) *chingombensis* sp. nov. resulted also in the discovery of some asymmetries, which are recognized here as anomalous states. One specimen had three anterior trichobothria (ASens), in another specimen there was an anomalous shape of right coxa I (subrectangular instead of subtriangular). Also, the inconsistency in the number of setae on basifemur II (three setae on one side of symmetry axis in one specimen, vs two setae considered as a normal state) and on basifemur III (two setae on one side of symmetry axis in two specimens vs one seta considered as a normal state) was recorded.

Complementary data to the description of *L.* (*L.*) *bicristatus*, *L.* (*L.*) *aldonae* and *L.* (*L.*) *soddagus*

Leptus (*L.*) *bicristatus* Fain et Elsen, 1987

The original description of species was based on a single specimen and contained the general set of diagnostic traits. Here we provide the supplementary data to species characteristics, based on re-examination of the holotype.

Two cuticular ridges (bars) extending between proximal and distal part of palp genu. $fD = 52$, $fV = 16$. Dorsal setae parallel-sided in outline or only slightly narrowing apically, covered with relatively short setules. Four intercoxalae present between coxae II and III ($3a_1$ shorter than $3a_2$). Vestigial seta (κ) (5) is present on at least the left tibia II. For complementary set of character states see also Tables 4–6.

Leptus (*L.*) *aldonae* Haitlinger, 1987

$fD = 92$, $fV = 24$ (holotype). Dorsal setae slightly widened distally, due to the presence of longer setules than those covering the proximal half of the stem (setae similar in shape to those which are

present in *L. (L.) soddagus*). Tarsi I–III expanded and distinctly (about 2x) shorter than the respective leg segments in *L. (L.) chingombensis* sp. nov., *L. (L.) bicristatus* and *L. (L.) soddagus*.

The condition of the holotype does not allow to confirm the presence of vestigiala (κ) on genu and tibia of the first leg and on genu II. For complementary set of character states see also Tables 4–6.

Leptus (L.) soddagus Haitlinger, 1990

About 106 setae present in NDV formula. Dorsal setae (Fig. 8B) expanded distally (setules arising at distal half of the seta shaft longer than those covering the proximal part of the shaft). For complementary set of character states see also Tables 4–6.

TABLE 5. Leg chaetotaxy in larvae of *Leptus (L.) bicristatus*, *L. (L.) aldonae*, *L. (L.) soddagus* and *L. (L.) chingombensis* sp. nov.

	<i>Leptus (L.) bicristatus</i> Fain et Elsen, 1987	<i>Leptus (L.) aldonae</i> Haitlinger, 1987	<i>Leptus (L.) soddagus</i> Haitlinger, 1990	<i>Leptus (L.)</i> <i>chingombensis</i> sp. nov.
	holotype ¹	holotype ¹	holotype ¹	(n = 15)
Cx I	1n, 1 supracoxal seta	1n, 1 supracoxal seta	1n, 1 supracoxal seta	1n, 1 supracoxal seta
Tr I	1n	1n	1n	1n
bFe I	2n	2n	2n	2n
tFe I	5n	5n	5n	5n
Ge I	8n, 1 σ , 1 κ	8n, 2 σ , [?] κ	8n, 1 σ , 1 κ	8n, 1 σ , 1 κ
Ti I	14n, 2 ϕ , 1 κ	14n, 3 ϕ , [?] κ	14n, 2 ϕ , 1 κ	14n, 2 ϕ , 1 κ
Ta I	[?]22n, 2 ζ , 1 ω , 1 ϵ	c. 25n, 2 ζ , 1 ω , 1 ϵ	[?]n ² , 2 ζ , 1 ω , 1 ϵ	22–25n ³ , 2 ζ , 1 ω , 1 ϵ
Cx II	1n	1n	1n	1n
Tr II	1n	1n	1n	1n
bFe II	2n	2n	2n	2(3) ⁴ n
tFe II	5 n	5n	5n	5n
Ge II	8n, 1 κ	8n, 1 σ , [?] κ	8 n, 1 κ	8n, 1 κ
Ti II	14n, 2 ϕ , 1 κ	15n, 2 ϕ , 1 κ	15n, 2 ϕ , 1 κ	14–15n ⁵ , 2 ϕ
Ta II	[?]21n, 2 ζ , 1 ω	21n, 2 ζ , 1 ω	[?]n, [?]2 ζ , 1 ω	21–24n ⁶ , 2 ζ , 1 ω
Cx III	1n	1n	1n	1n
Tr III	1n	1n	1n	1n
bFe III	1n	1n	1n	1(2) ⁷ n
tFe III	5n	5n	5n	5n
Ge III	8n	8n	8n	8n
Ti III	15n, 1 ϕ	15n, 1 ϕ	15n, 1 ϕ	15n, 1 ϕ
Ta III	[?]22n, 1 ζ	25n, 1 ζ	[?]22n, 1 ζ	22–25n ⁸ , 1 ζ

¹ based on reexamination of holotype (present study)

² the segment overimposed on idiosoma (number of normal setae impossible to ascertain)

³ 22n in holotype

⁴ the value provided in the parenthesis refers to asymmetrical state observed in one specimen

⁵ 15n in holotype

⁶ 21n in holotype

⁷ the value provided in the parenthesis refers to asymmetrical state observed in two specimens

⁸ 23n in holotype

TABLE 6. *Leptus* spp. (larvae) known from the Afrotropic.

species	number of setae on palp genu	number of setae in fD / fV formula	3a setae	sample size (original description)	distribution	host	source
<i>Leptus</i> (<i>L.</i>) <i>abrafoicus</i> Haitlinger, 2007	1	47 / 10	4	1	Ghana	unknown	Haitlinger 2007
<i>Leptus</i> (<i>L.</i>) <i>aggoratus</i> Haitlinger, 1990	2	c. 60 / c. 30	4	1	Zambia	Tenebrionidae indet. (Coleoptera)	Haitlinger 1990
<i>Leptus</i> (<i>L.</i>) <i>aldonae</i> Haitlinger, 1987	1	92 ¹ / 24 ¹	4	3	Madagascar	unknown	Haitlinger 1987, 2001; present study
<i>Leptus</i> (<i>L.</i>) <i>assagasicus</i> Haitlinger, 2001	2	c. 48 / 26	4	1	South Africa	unknown	Haitlinger 2001
<i>Leptus</i> (<i>L.</i>) <i>atticolus</i> Lawrence, 1940	1	40–45 / c. 24	4	8	South Africa	<i>Saitis</i> sp. (Araneae: Salticidae)	Lawrence 1940; Fain and Elsen 1987; Fain and Jocqué 1996
<i>Leptus</i> (<i>L.</i>) <i>aureliani</i> Fain et Elsen, 1987	2	c. 100 / 40	4	1	Rwanda	unknown	Fain and Elsen 1987
<i>Leptus</i> (<i>L.</i>) <i>benzaliensis</i> Fain et Elsen, 1972	2	72 / (50 on idiosoma venter) ²	[?] 4	8	Congo, Kenya, Ivory Coast	<i>Glossina fuscipes quanzensis</i> , <i>G. pallidipes</i> , <i>G. palpalis</i> (Diptera: Glossinidae), <i>Chaetadoretus rusticus</i> (Coleoptera: Scarabeidae)	Fain and Elsen 1987, 1987
<i>Leptus</i> (<i>L.</i>) <i>bertoldi</i> Haitlinger, 1993	2	c. 90 / [?]	c. 30	2	Ghana	Tenebrionidae indet. (Coleoptera)	Haitlinger 1993
<i>Leptus</i> (<i>L.</i>) <i>bicristatus</i> Fain et Elsen, 1987	1	52 ³ / 16	4	1	Malawi	<i>Cristina lettowi</i> (Opiliones: Phalangidae)	Fain and Elsen 1987; present study
<i>Leptus</i> (<i>L.</i>) <i>bogoriacus</i> Haitlinger, 2001	2	c. 196 / c. 58	c. 12	1	Kenya	Tenebrionidae indet. (Coleoptera)	Haitlinger 2001
<i>Leptus</i> (<i>L.</i>) <i>carpenteri</i> Fain et Elsen, 1972	1	51 / (c. 30 on idiosoma venter) ²	[?] 4	2	Congo	<i>Glossina fuscipes quanzensis</i> (Diptera: Glossinidae)	Fain and Elsen 1972, 1987
<i>Leptus</i> (<i>L.</i>) <i>cavernicola</i> Fain et Elsen, 1987	1	50 / [?] 21	4	1	Rwanda	Nematocera indet. (Diptera)	Fain and Elsen 1987
<i>Leptus</i> (<i>L.</i>) <i>chingombensis</i> sp. nov.	1	74–86 / 20–22	4	34 ⁴	Zambia	<i>Pseudagrion spernatum</i> (Odonata: Coenagrionidae), <i>Heliaeschna fuliginosa</i> (Odonata: Aeshnidae), <i>Paragomphus cognatus</i> (Odonata: Gomphidae), <i>Orthetrum julia</i> (Odonata: Libellulidae)	present study
<i>Leptus</i> (<i>L.</i>) <i>dalicus</i> Haitlinger, 2006	1	46 / 30	4	1	Ethiopia	Carabidae indet. (Coleoptera)	Haitlinger 2006
<i>Leptus</i> (<i>L.</i>) <i>dinekaicus</i> Haitlinger, 2006	2	c. 85 / 30	4	1	Ethiopia	unknown	Haitlinger 2006
<i>Leptus</i> (<i>L.</i>) <i>elminus</i> Haitlinger, 2007	2	55 / 20	4	1	Ghana	unknown	Haitlinger 2007
<i>Leptus</i> (<i>L.</i>) <i>glossinarum</i> Fain et Elsen, 1972	2	51 / (30 on idiosoma venter) ²	4	1	Congo	<i>Glossina fuscipes quanzensis</i> (Diptera: Glossinidae)	Fain and Elsen 1972, 1987
<i>Leptus</i> (<i>L.</i>) <i>jocquei</i> Fain et Elsen, 1987	2	54 / (28 on idiosoma venter) ²	4	9	Malawi	<i>Cristina lettowi</i> (Opiliones: Phalangidae)	Fain and Elsen 1987
<i>Leptus</i> (<i>L.</i>) <i>korneli</i> Haitlinger, 2009	2	c. 113 / 35	10	2	Cape Verde	unknown	Haitlinger 2009
<i>Leptus</i> (<i>L.</i>) <i>leleupi</i> Fain et Elsen, 1987	1	c. 60 / (34 on idiosoma venter) ²	4	1	Tanzania	<i>Oreoparnus</i> sp. (Coleoptera: Staphylinidae)	Fain and Elsen 1987
<i>Leptus</i> (<i>L.</i>) <i>lovaniensis</i> Fain et Elsen, 1987	1	60 / (26 on idiosoma venter) ²	[?] 4	1	Congo	Scarabeidae indet. (Coleoptera)	Fain and Elsen 1987
<i>Leptus</i> (<i>L.</i>) <i>madagascariensis</i> André, 1941	1	c. 57 / [?] 20	4	[?] 1	Madagascar	<i>Stenopirates longipes</i> (Hemiptera: Enicocephalidae)	André 1941
<i>Leptus</i> (<i>L.</i>) <i>maranaensis</i> Haitlinger, 1987	1	46–56 / [?] 18	4	3	Madagascar	Orthoptera indet.	Haitlinger 1987
<i>Leptus</i> (<i>L.</i>) <i>maringensis</i> Fain et Elsen, 1972	1	50–60 / (35–40 on idiosoma venter) ²	[?] 1	1	Congo	<i>Glossina fuscipes fuscipes</i> (Diptera: Glossinidae)	Fain and Elsen 1972, 1987
<i>Leptus</i> (<i>L.</i>) <i>olamukijacus</i> Haitlinger, 2001	2	c. 150 / c. 44	c. 52	1	Kenya	Orthoptera indet.	Haitlinger 2001
<i>Leptus</i> (<i>L.</i>) <i>pasopaicus</i> Haitlinger, 1990	2	c. 100 / c. 56	16	1	Namibia	Tenebrionidae indet. (Coleoptera)	Haitlinger 1990
<i>Leptus</i> (<i>L.</i>) <i>pelebinus</i> Haitlinger, 2007	2	c. 180 / 56	27	1	Benin	Orthoptera indet.	Haitlinger 2007
<i>Leptus</i> (<i>L.</i>) <i>polythrix</i> Fain et Elsen, 1987	2	> 200 / (106 on idiosoma venter) ²	19	9	Malawi	<i>Cristina lettowi</i> (Opiliones: Phalangidae)	Fain and Elsen 1987; Cokendolpher 1993
<i>Leptus</i> (<i>L.</i>) <i>puylaerti</i> Fain et Elsen, 1987	2	72 / (46 on idiosoma venter) ²	4	5	Malawi	<i>Cristina lettowi</i> (Opiliones: Phalangidae)	Fain and Elsen 1987
<i>Leptus</i> (<i>L.</i>) <i>rwandae</i> Fain et Jocqué, 1996	1	50 / 20	4	2	Rwanda	<i>Systemoplacis</i> sp. (Araneae: Zodariidae)	Fain and Jocqué 1996
<i>Leptus</i> (<i>L.</i>) <i>salicus</i> Haitlinger, 2009	1	c. 110 / 25	10	1	Cape Verde	Orthoptera indet.	Haitlinger 2009
<i>Leptus</i> (<i>L.</i>) <i>similis</i> Fain et Elsen, 1987	2	50–60 / (c. 40 on idiosoma venter) ²	[?] 1	1	Congo	<i>Chlorion ciliatum</i> var. <i>maxillae</i> (Hymenoptera: Sphaecidae)	Fain and Elsen 1987
<i>Leptus</i> (<i>L.</i>) <i>soddagus</i> Haitlinger, 1990	1	(c. 106) ⁶	[?]	1	Tanzania	Elateridae indet. (Coleoptera)	Haitlinger 1990; present study
<i>Leptus</i> (<i>L.</i>) <i>sudanensis</i> (Oudemans, 1911)	2	c. 148 / c. 25	[?] 7	1	Sudan	<i>Adesmia dilatata</i> (Coleoptera: Tenebrionidae)	Oudemans 1911, 1912

¹ based on reexamination of holotype (present study); according to Haitlinger (1987, 2001) 84 setae (holotype), 64, 76 setae (paratypes) present in fD formula and 40 setae (holotype), 38, 32 setae (paratypes)—in fV formula (respective values of fD provided by Haitlinger (2001) reduced by 8 setae, i.e. sternalae I, II and intercoxalae II–III)

² number of ventral setae provided in the description (may refer to fV or to fV plus sternalae I, II and intercoxalae II–III)

³ based on reexamination of the holotype (present study); 72 dorsal and 30 ventral setae reported by Fain and Elsen (1987)

⁴ 34 specimens (incl. vouchers after DNA extraction) measured and additionally 100 specimens with confirmed identity

⁵ in the Type material section (Haitlinger 2001) only the holotype is listed, whereas in the measurements data for paratype are also provided

⁶ data on NDV (fD + fV) based on reexamination of holotype (present study), dorsal and ventral setae superimposed and difficult to discern; a total of c. 86 setae reported by Haitlinger (1990) in fD formula

[?] denotes uncertain or missing (due to the condition of the material) character states

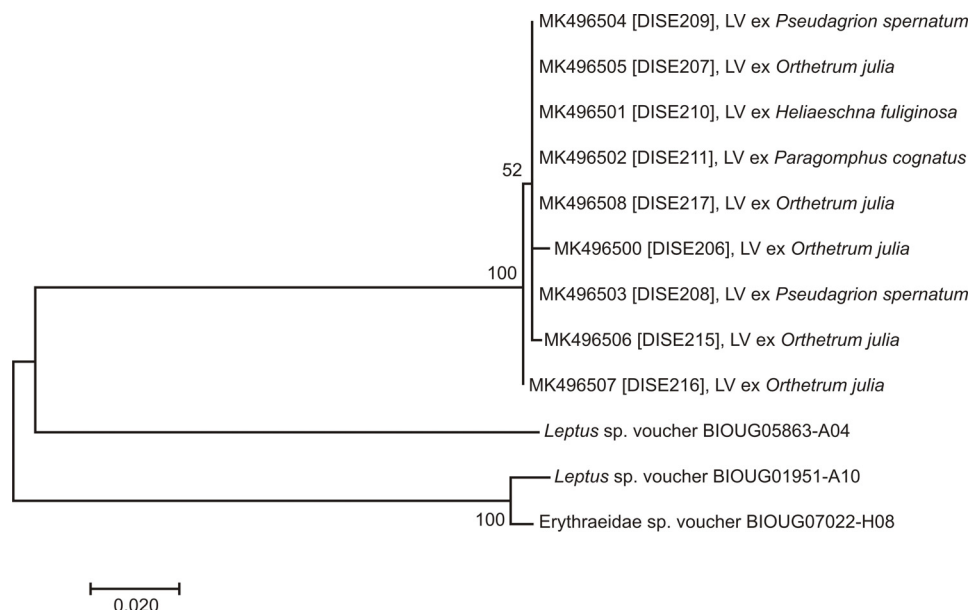


FIGURE 9. COI bootstrap original tree for nine sequences of *Leptus (L.) chingombensis* sp. nov. and three selected sequences of other *Leptus* species, analysis by ML method, sequences 529 bp long. Accession numbers (GenBank) followed by ID of larva (DISE) and host name.

Of 12 samples designed for molecular analyses we obtained nine COI sequences (for three specimens the analyses failed). Five sequences were obtained from larvae which parasitized *O. julia* (DISE206, DISE207, DISE215, DISE216, DISE217), two—from larvae collected from *P. sparnatum* (DISE208, DISE209), one—from *H. fuliginosa* (DISE210) and one—from *P. cognatus* (DISE211).

Four haplotypes were recorded (Fig. 9). Six sequences (DISE207, DISE208, DISE209, DISE210, DISE211, DISE217), including those obtained from larvae collected from four host species, were identical and three other ones (DISE206, DISE215, DISE216), all from larvae which parasitized *O. julia*, slightly departed from the main group. DISE206 differed by two synonymous nucleotide substitutions, whereas for DISE215 and DISE216 a single but different nucleotide was substituted. The genetic distance (K2P) between sequences was 0–0.6%.

Discussion

The taxonomy of *Leptus* ssp. is based predominantly on measurable traits. Several meristic characters, and among them the number of setae on palp femur and palp genu, number of setae on idiosoma dorsum (fD), but also the number of intercoxal setae (1a, 2a, 3a), with special reference to 3a (= intercoxalae of Southcott (1992)) have been used in species grouping/identification. Contrary to the latter, the number of setae on leg segments displays relatively high consistency at the genus level (see also Tab. 5). Nevertheless, the actual knowledge of intraspecific variation of metric, meristic and qualitative traits is limited, due to the low numbers of specimens used in descriptions. For majority of the genus members, single or few specimens (see also Tab. 6) constituted the base for description. The insufficiency of diagnosable traits, together with scarcity of material examined and knowledge of distribution, frequently limited to single localities, make it difficult to refer to statistical methods applied in species discrimination. The application of molecular methods followed

by the detailed insight in variation of morphological traits in larvae should be thus of priority value in step by step verification of specific level taxa. In view of limited number of species known from both larvae and active postlarval forms it may contribute, via future molecular matching, to a comprehensive characteristics of species.

Considering the problems described above, the large size of the studied Zambian sample and its morphological and genetic consistence make both the species description and ecological inference reliable. A comparison of the new species with other, similar specific level taxa, supported by the overall knowledge and validity of diagnostic traits in *Leptus* spp., refrained us from translating the observed differences only into interpopulation variability within the previously described congeners. In view of the more extensive data on intraspecific variation recorded within the new Zambian species, we can conclude that some characters (e.g. the number of setae on basifemora) seem to be prone to malformations, thus their usefulness for species diagnosing is highly limited.

Another crucial problem in studies of *Leptus* spp. is the absence of data on host spectrum for most parasitic larvae that is correlated to the knowledge of species limits. Multi-faceted analyses should involve the comprehensive studies on biology and ecology, of both the parasite and its host. The present discovery of conspecific larvae on four species of dragonflies representing different and not closely related families contributes to the earlier presumption of relatively wide host range known for *Leptus* species (see e.g. Killington & Bathe 1946; Kerry 2013). The comparatively frequent and numerous occurrences of *Leptus* larvae on *Orthetrum julia* might have been an effect of greater numbers and availability of this species in comparison with three other host species. However, as only one or two individuals of *P. spernatum*, *H. fuliginosa* and *P. cognatus* were collected, no host preferences can be reliably assessed. It is noteworthy, that in Switzerland *Leptus* sp. was found several times on *Orthetrum coerulescens*, belonging to the same genus as *O. julia*, but not on other species of dragonflies (Juillerat & Wildermuth 2006; Wildermuth 2007). Considering the above, we agree with Wildermuth *et al.* (2015), that the species of dragonfly is not as crucial for host choice as the habitat conditions of the area where infestation takes place. Thus, the occurrence of larvae on particular hosts but also the intensity of infestation probably results mainly from the availability of dragonfly species in microhabitats inhabited by *Leptus* larvae.

Orthetrum julia collected in Zambia commonly used the lower strata of vegetation (herbaceous vegetation and low bushes) as resting sites and frequently did it in insolated open places on slopes. The remaining three host species may also use, at least partly, these vegetation layers. Several other dragonfly species, collected at the same Zambian localities, but not parasitized by *Leptus*, such as *Chlorocypha consueta*, *Allocnemis marshalli* and *Phyllomacromia monoceros*, were mostly related to bush and tree branches and foliage, and were predominantly observed at the stream and in its immediate surroundings. Thus, we hypothesize that the infestation did not take place just after the emergence of dragonflies on stream banks, but later, at the resting phase of host activity in the lower strata of vegetation. Such scenario is supported not only by the absence of larvae on several potential host species, but also by the fact that the parasitism was recorded on sexually mature dragonflies that, with special reference to individuals recorded in April, most probably emerged at least several days before being infested. This appears clear as a long time of larval attachment to the host, having its onset at dragonfly's emergence, seems unlikely in the light of overall knowledge of parasitism duration in *Leptus* spp. larvae. For example, the expected mean attachment time of *L. (L.) killingtoni* on *Ischnura hastata* (Odonata) was calculated at 6.7 days and many individuals remained on hosts for only 1 or 2 days (Cordero-Rivera *et al.* 2018). In England, both mature and immature individuals of *Ceriagrion tenellum* were parasitized by *L. (L.) killingtoni*, however, the proportion of the former was significantly higher. According to Killington and Bathe (1946), the host-dragonfly may be newly emerged or mature and the period of attachment is indefinite and variable, which implies the more casual nature of getting in contact with the host.

The different levels of engorgement in conspecific larvae on the same host specimen in our study in Zambia suggests an extended appearance of larvae in the field, partly confirmed also by collection time span (January–April) and translating into asynchronous infestation events. Similar observations were made in the case of *L. (L.) killingtoni* on *Ceriagrion tenellum* in England (Kerry 2013). The asynchronous infestation has been discussed in relation to trombiculid mites associated with vertebrate hosts (Wohltmann *et al.* 2007; Moniuszko & Mąkol 2016), however has not been extensively examined for arthropod-associated terrestrial Parasitengona. For the latter group, the differences in size of larvae, reflected also in the size of deutonymph instar, may correspond to the amount of food taken by specimen (Husband & Wohltmann 2011), however no evidence for asynchronous infestation has been hitherto provided. The observed variation in size of larvae of *L. (L.) chingombensis* sp. nov. may reflect repeated infestation events during the recurrent appearance of dragonfly in the contact microhabitat occupied by *Leptus*, contrary to single and synchronous infestation that could occur just after the dragonfly emergence.

The infestation of freshly emerged dragonflies seems less probable also due to the high risk for *Leptus* larvae, that would be associated with the search for hosts just at stream banks, i.e. in a flood-prone zone, influenced by repeated, sudden increase in stream water level during the rainy season. The latter points also to most likely difference in time and place of getting in contact with host, observed between terrestrial Parasitengona and water mites. Water mite larvae enter the parasitic phase on newly emerged dragonflies (e.g. Åbro 1979; Rolff 2000), which is obviously facilitated by cohesion of microhabitats occupied by these mites and serving also for dragonfly emergence.

The parasite load, i.e. the number of *Leptus* larvae per infested dragonfly specimen was variable, reaching a high of 44 and 49 larvae. In earlier studies, low or moderate parasite load, ranging between 1–12 larvae was recorded, with the most frequent situations of only one or two larvae (Killington & Bathe 1946; Juillerat & Wildermuth 2006; Kerry 2013; Wildermuth *et al.* 2015; Cordero-Rivera *et al.* 2018). The male-biased sex ratio of Zambian hosts was certainly an effect of the fact that dragonfly males were, as usually, more numerous and more frequently collected at the studied localities. In a very systematic and season-long study in Devon, England, damselfly (*Ceriagrion tenellum*) females were more frequently infested than males by larvae of *L. (L.) killingtoni* (Kerry 2013).

The analysis of attachment sites of larvae revealed their clear preference for the ventral side of the host's body. A slight preference to distal parts of synthorax, between basal parts of hind legs and anterior part of abdomen, as well as to the depression on the ventral side of the abdomen was also observed. The reasons for such strategy likely lie, at least partly, in the avoidance of exposed and strongly insolated places on hosts dorsum, posing the risk of overheating and dehydration in Afrotropical weather conditions. The ventral surface of the thorax (and especially between the legs) was the most frequently (25.3%) infested part of the dragonfly body also by larvae of *L. (L.) killingtoni* in Devon, England (Kerry 2013). However, Kerry interpreted this location as areas more difficult to groom by hosts. The attachment site preferences of larvae recorded in Zambia differ from those described by Killington and Bathe (1946), Juillerat and Wildermuth (2006), Kerry and Baker (2012), Wildermuth *et al.* (2015), and Cordero-Rivera *et al.* (2018), where dragonfly legs were much more frequently infested with terrestrial Parasitengona (*Leptus* sp.) and constituted the most frequently infested parts of the host's body. However, the results obtained by Wildermuth *et al.* (2015) might have been partly affected by the method applied, i.e. searching for Parasitengona larvae on photographs, where legs are well visible, but ventral parts of the synthorax and abdomen almost invisible. Nevertheless, the occurrence of terrestrial Parasitengona larvae on tibia and other very exposed parts of the body (Wildermuth *et al.* 2015) as well as high percentages of *Leptus* larvae on legs in other European studies (Killington & Bathe 1946; Juillerat & Wildermuth 2006; Cordero-Rivera *et al.* 2018) suggest less limiting influence of local weather conditions (e.g. of insolation

strength) on the attachment site selection in temperate Europe than in the Afrotropic. The tendency of *L. (L.) chingombensis* sp. nov. larvae to attach to places with softer cuticle has been rarely reported for *Leptus*. Flechtmann (1980) observed the *Leptus* sp. larvae attached to intersegmental membranes on abdomen of *Apis mellifera*. Contrary to the latter observations, Kerry (2013) did not record such a preference: larvae of *L. (L.) killingtoni* occurred on both weakly and heavily sclerotized body areas of *Ceriagrion tenellum* in England. Also, Southcott (1992) considered larvae of *Leptus* spp. as selecting the more sclerotized surfaces of their hosts. The detailed sequence of events displayed by larva at entering parasitic phase (getting in contact with host) remains largely unknown.

The new Zambian records of *Leptus* parasitising dragonflies confirm the earlier data on the rare occurrence of this phenomenon (see Wildermuth *et al.* 2015). The discovery of the new species only locally, in a small area, and in the limited number of localities out of all surveyed, may point to its spatial isolation, however more comprehensive data on other members of the genus but also on the other potential host taxa from the same study area would be needed to confirm this hypothesis. A specific combination of habitat conditions in the narrow mountain valley, fostering the interaction between dragonflies and terrestrial *Leptus* larvae, supports the hypothesis on the very local nature of the phenomenon. On the other hand, the local character of the finding along with the regular appearance of larvae parasitizing dragonflies, obviously favoured by specific habitat conditions, no doubts confirm the non-accidental nature of the phenomenon.

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