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The complete mitochondrial DNA sequence of the pantropical earthworm *Pontoscolex corethrurus* (Rhinodrilidae, Clitellata): Mitogenome characterization and phylogenetic positioning

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Abstract

Pontoscolex corethrurus (Müller, 1857) plays an important role in tropical soil ecosystems and has been widely used as an animal model for a large variety of ecological studies, in particular due to its common presence and generally high abundance in human-disturbed tropical soils. In this study we describe the complete mitochondrial genome of the peregrine earthworm *P. corethrurus*. This is the first record of a mitochondrial genome within the Rhinodrilidae family. Its mitochondrial genome is 14 835 bp in length containing 37 genes (13 protein-coding genes (PCG), 2 rRNA genes and 22 tRNA genes). It has the same gene content and structure as in other sequenced earthworms, but unusual among invertebrates it has several overlapping open reading frames. All genes are encoded on the same strand. Most of the PCGs use ATG as the start codon except for ND3, which uses GTG as the start codon. The A+T content of the mitochondrial genome is 59.9% (31.8% A, 28.1% T, 14.6% G, and 25.6% for C). The annotated genome sequence has been deposited in GenBank under the accession number KT988053.

Keywords

Pontoscolex corethrurus, mitochondria, mitochondrial genome, Rhinodrilidae, earthworm, Azores, peregrine species

Introduction

Excluding a few aquatic taxa, earthworms (Annelida: Clitellata) are mostly terrestrial and include ca. 5,500 species (Blakemore et al. 2006). Believed to have originated in the Guyana Shield (Righi 1984), the earthworm *Pontoscolex corethrurus* (Müller, 1857) is a globally distributed species found in most tropical regions. It mainly occurs in human-disturbed areas and can be used as an indicator of ecosystem disturbance (Brown et al. 2006), and is commonly used in ecotoxicological studies (e.g. Buch et al. 2011; Buch et al. 2013; Da Silva et al. 2016). The species formerly belonged in the Glossoscolecidae family, but was recently allocated to the Rhinodrilidae family by James (2012), following the phylogeny of James and Davidson (2012). It is also the most well-known earthworm species in the humid tropics, frequently used in ecological and agronomic studies (Bhattacharjee and Chaudhuri 2002; Buch et al. 2013; Chapuis-Lardy et al. 2010; Dupont et al. 2012; Hamoui 1991; Marichal et al. 2010). Being a geophagous endogeic species, *P. corethrurus* shows high plasticity regarding its tolerance to soil physicochemical characteristics, including variable moisture, high temperatures, exceptionally high carbon dioxide and low oxygen levels, and is capable of inhabiting nutrient-poor soils (Cunha et al. 2014; Hamoui 1991; Lavelle et al. 1987), as well as rotten logs (Buch et al. 2011).

Molecular data have become increasingly important in recent years. In animals, the mitochondrial DNA (mtDNA) typically contains 37 genes, encoding 13 proteins for the enzymes required for oxidative phosphorylation, the two ribosomal RNA units (rRNA), and 22 transfer RNAs (tRNAs) necessary for the translation of the proteins encoded by mtDNA (Anderson et al. 1981; Boore 1999; Zhao et al. 2015). Remarkable progress has been made over the past several years in the field of the molecular systematics of annelids. Compared with individual genes, the mitochondrial genome is still a promising tool for inferring phylogenetic relationships due to its high content of information, and has been applied in some phylogenetic studies involving earthworms (Zhang et al. 2015; Zhang et al. 2016b).

In this study, we sequenced the complete mtDNA sequence of *P. corethrurus* for the first time and analyzed its structure. Additionally, we conducted phylogenetic analyses based on the mitochondrial sequence data available elsewhere with the purpose of investigating the phylogenetic position of *P. corethrurus* within Clitellata. The information reported in this article will facilitate further investigations of phylogenetic relationships of different Annelida species.

Material and methods

Sample collection and DNA extraction

A group of clitellate (adult) *P. corethrurus* was collected in São Miguel Island (Azores, Portugal) inside pineapple greenhouses (Locality: Fajã de Baixo, 37°45'12.2"N, 25°38'21.3"W) during January 2015. Animals were euthanized in 10% ethanol and preserved in 96% ethanol for later work. A piece of body wall tissue was used for genomic DNA extraction using standard phenol/chloroform (Sambrook and Russell 2001) procedure followed by ethanol precipitation and kept at 4°C for subsequent use.

Mitochondrial DNA amplification

The complete *P. corethrurus* mitogenome was amplified using seven sets of primers (Table 1) designed based on sequences retrieved from a previous study (Cunha et al. 2014).

Long PCR targets were amplified using different combinations of the primer sets, and initially sequenced with the same forward or reverse primers. Subsequent primer walking method was used to close the sequencing gaps. To ensure the accuracy of the sequence, every two contiguous segments overlapped by at least 80 bp. PCRs were performed using ~40 ng of DNA and 0.4 μM forward and reverse primers, 0.2 mM dNTP mix and 1.25 U Platinum HiFi DNA polymerase buffered with 1X Mg-free buffer (Thermofisher Scientific, UK). PCR amplification buffer was supplemented with $MgCl_2$ to achieve a final concentration of 1.75 mM in a total volume of 25 μl reaction mixture. The reaction was denatured at 95°C for 3 min, cycled 35 times at 95°C for 30 s, 30 s at the required primer annealing temperature and 72°C for 1 min per 1000 bp depending on target fragment length. Negative controls were included in all PCR amplifications to confirm the absence of contaminants. Before sequencing, PCR cleanups were performed using Exo-SAP-IT (Amersham Pharmacia, UK) reagents. Exonuclease 1 (0.25 μl) and Shrimp Alkaline Phosphatase (0.5 μl) were mixed with the PCR product (10 μl) and incubated at 37°C for 45 min followed by 80°C for 15 min. DNA was sequenced using ABI PRISM® BigDye v3.1 Terminator Sequencing technology (Applied Biosystems, USA) on an ABI PRISM® 3100 DNA automated Sequencer.

Sequence editing and analysis

Sequence trace files were corrected and aligned with the MEGA v.7 (Kumar et al. 2016). The sequence overlap and mitogenome assembly were performed using CLC main workbench v.6 (Qiagen). The annotation of the 13 protein-coding genes and two rRNA genes were determined using the MITOS v.2 web server (Bernt et al. 2013) and

Table 1. Details of the primers used to amplify the mitochondrial DNA of *P. corethrurus*.

Primer code	Orientation	Annealing position (bp)	Nucleotide sequence (5’-3’)	Melting Temperature (°C)
FP_1	Forward	2154..2175	CTCTACTATGTACCCAGGAGTG	57.46
RP_1	Reverse	2758..2775	GCGGCCAAGATAAAGCAC	57.67
RP_2	Reverse	3740..3762	TAGAGGCGGTAAGGAGAAAGTAT	58.61
RP_3	Reverse	5691..5708	CAGAGGCGAGGTAAATTC	53.85
RP_4	Reverse	6356..6373	TGTTCAGGGCTAGGATTG	54.99
FP_5	Forward	7983..8004	ACTAGTGTCACTTACAACAACC	57.16
RP_5	Reverse	8649..8670	TGATAAGGGGGAAAGTCTGATC	56.84
FP_6	Forward	8766..8787	AGTAGCCGCTATAATAGTCCTT	57.91
RP_6	Reverse	10328..10349	TGATTTGGGGTCAGAGCCGTAG	61.59
FP_7	Forward	10459..10478	AAAGCTTGGCGGTGCTTCAC	63.23
RP_7	Reverse	11242..11263	CCTAGTGTGTGTCAGGACGCTT	64.75

Table 2. Representative Clitellata species included in this study for comparison.

Species	Family	Length (bp)	GenBank accession number
Pontoscolex corethrurus	Rhinodrilidae	14,835	**Present study**
Tonoscolex birmanicus	Megascolecidae	15,170	KF425518
Amynthas gracilis	Megascolecidae	15,161	NC_027258
Duplodicodrilus schmardae	Megascolecidae	15,156	NC_029867
Metaphire guillemi	Megascolecidae	15,174	NC_029869
Perionyx excavatus	Megascolecidae	15,083	NC_009631
Lumbricus terrestris	Lumbricidae	14,998	NC_001673
Drawida japonica	Moniligastridae	14,648	NC_028050
Hirudo nipponia	Hirudinidae	14,414	NC_023776

manually curated using other published annelid mitogenomes as shown in Table 2, whereas the tRNA genes were identified using the program tRNAscan-SE 1.21 (Lowe and Eddy 1997). The annotated genome sequence was deposited in GenBank under accession number KT988053.

Phylogenetic analyses

To clarify the phylogenetic position of *P. corethrurus* within the Clitellata, the complete mitogenome sequences of eight representative Clitellata species (Table 2) were incorporated together with the presently obtained *P. corethrurus* mitogenome sequence for phylogenetic analysis. Phylogenetic analyses were based on 13 protein-coding genes and the two rRNA units, which were aligned separately using MEGA v.7 (Kumar et al. 2016) with minor manual adjustments and then concatenated. The possible bias of substitution saturation at each codon position of protein-coding genes and two rRNA genes was investigated using DAMBE v.4.5.57 (Xia and Xie 2001) and MEGA v. 7 (Kumar et al. 2016).

Two different methods, Bayesian inference (BI) and maximum likelihood (ML) were used to construct the phylogenetic tree. Bayesian analyses were undertaken with MrBayes v.3.1.2 (Ronquist and Huelsenbeck 2003) under the best-fit model of nucleotide evolution selected in MrModeltest v.2.3 (Nylander 2004), using the Assignment Index Criterion (AIC). Analyses were run for 1,000,000 generations, and sampled every 100 generations to assess convergence. Trees that produced non-stationary log-likelihood values were discarded as part of a burn-in procedure and combined the remaining trees that resulted in convergent log-likelihood scores from both independent searches. These trees were used to construct a consensus tree.

Maximum likelihood analysis (ML) was performed with MEGA v.7 (Kumar et al. 2016). Initial tree(s) for the heuristic search were obtained automatically by applying Neighbour-Joining and BioNJ algorithms to a matrix of pairwise distances estimated using the Maximum Composite Likelihood (MCL) approach and then selecting the topology with superior log likelihood value. A discrete Gamma distribution was used to model evolutionary rate differences among sites (5 categories (+G, alpha parameter = 0.9143)). The rate variation model allowed for some sites to be evolutionarily invariable ([+I], 17.7596% sites). The analysis involved nine mitogenome sequences (Table 2). All positions containing gaps and missing data were eliminated. The bootstrap consensus tree inferred from 1000 replicates was taken to represent the evolutionary history of the taxa analysed (Felsenstein 1985).

Results and discussion

Mitochondrial genomic structure

The mitochondrial genome of *P. corethrurus* was determined to be 14 835 bp in length, comprising 13 protein-coding genes (PCGs), 22 transfer RNAs (tRNAs), two ribosomal RNAs (rRNAs), and one putative control region with a length of 318 bp (Figure 1).

The mitochondrial genome structure is detailed in Table 3. Gene order and orientation are similar to the previous earthworm mitochondrial genomes (Boore and Brown 1995; Zhang et al. 2015) but slightly smaller and more condensed (with several intergenic overlaps, see Table 3). The gene organization is similar to other earthworm species (e.g. *Lumbricus terrestris*: Boore and Brown 1995).

The nucleotide composition is asymmetric (31.9% A, 27.9% T, 14.9% G, and 25.3% for C) with an overall A+T content of 59.9%. One remarkable trait of metazoan mitogenomes is the strand-specific bias in nucleotide composition (Hassanin et al. 2005; Reyes et al. 1998). Such bias is measured as G/C-skew (G%-C%)/(G%+C%) and A/T-skew (A%-T%)/(A%+T%), respectively (Perna and Kocher 1995). The overall GC- and AT-skews of the H-strand of *P. corethrurus* mitogenome were -0.258 and 0.066, respectively, indicating a compositional bias associated with an excess of C over G nucleotides and a slight excess of A over T nucleotides on the H-strand.

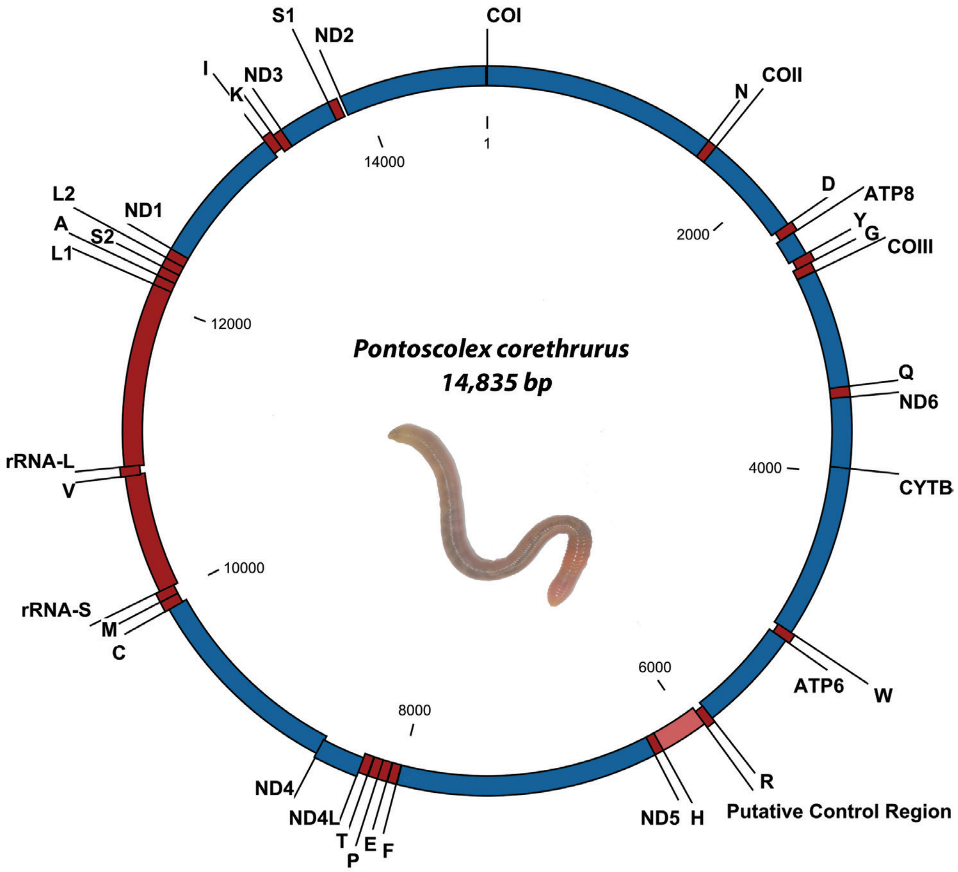

Figure 1. The mitochondrial genome of *Pontoscolex corethrurus* (Müller, 1857). Gene order and positions are shown, including the putative control region. IUPAC single letter codes are used to identify transfer RNA. The L1, L2, S1, and S2 transfer RNAs are differentiated on the basis of their anti-codons TAG, TAA, TCT, and TGA, respectively.

Protein-coding genes

The *P. corethrurus* genome contained the expected 13 protein-coding genes with a total of 11,131 bp in size, accounting for 75.03% of the whole mitogenome. Most of the PCGs are initiated with ATG codons, except for ND3 gene, which uses GTG as the initiation codon. Six PCGs (COX1, ATP8, COX3, ND6, ND3, and ND2) are terminated with an incomplete codon T or TA, which could be completed to TAA by polyadenylation post-transcriptionally (Ojala et al. 1981). COX2 and ND1 use TAG as a termination codon.

Nucleotide composition and codon usage frequencies were calculated from a concatenated sequence of all protein-coding genes on the H-strand. The base composition of protein-coding genes revealed a negative bias for A (14.4%), especially at second codon positions (12.9%, Table 4). For all protein genes, T was the most frequent nucleotide at the first and third positions whereas G was most frequent at the second position.

Table 3. Organisation and structure of the *P. corethrurus* mitochondrial genome.

Gene	Direction	From	To	Size (bp)	Start	Stop	Anti-codon	Intergenic bases (bp)
COX1	+	1	1540	1540	ATG	T--		0
tRNA-$^{\mathrm{Asn}}$	+	1541	1602	62			GTT	0
COX2	+	1603	2289	687	ATG	TAG		-1
tRNA-$^{\mathrm{Asp}}$	+	2289	2351	63			GTC	2
ATP8	+	2354	2513	160	ATG	T--		0
tRNA-$^{\mathrm{Tyr}}$	+	2514	2576	63			GTA	-1
tRNA-$^{\mathrm{Gly}}$	+	2576	2638	63			TCC	3
COX3	+	2642	3419	778	ATG	T--		0
tRNA-$^{\mathrm{Gln}}$	+	3420	3488	69			TTG	0
ND6	+	3489	3954	466	ATG	T--		0
Cytb	+	3955	5094	1140	ATG	TAA		-2
tRNA-$^{\mathrm{Trp}}$	+	5092	5154	63			TCA	1
ATP6	+	5156	5851	696	ATG	TAA		-2
tRNA-$^{\mathrm{Arg}}$	+	5850	5910	61			TCG	0
Putative Control Region	+	5911	6228	318				
tRNA-$^{\mathrm{His}}$	+	6229	6288	60			GTG	0
ND5	+	6289	8010	1722	ATG	TAA		3
tRNA-$^{\mathrm{Phe}}$	+	8014	8073	60			GAA	4
tRNA-$^{\mathrm{Glu}}$	+	8078	8141	64			TTC	0
tRNA-$^{\mathrm{Pro}}$	+	8142	8204	63			TGG	4
tRNA-$^{\mathrm{Thr}}$	+	8209	8272	64			TGT	0
ND4L	+	8273	8569	297	ATG	TAA		-7
ND4	+	8563	9921	1359	ATG	TAA		-2
tRNA-$^{\mathrm{Cys}}$	+	9920	9986	67			GCA	1
tRNA-$^{\mathrm{Met}}$	+	9988	10050	63			CAT	-1
s-rRNA	+	10050	10838	789				-7
tRNA-$^{\mathrm{Val}}$	+	10832	10894	63			TAC	-2
l-rRNA	+	10893	12104	1212				0
tRNA-$^{\mathrm{Leu\ 1}}$	+	12105	12166	62			TAG	2
tRNA-$^{\mathrm{Ala}}$	+	12169	12230	62			TGC	1
tRNA-$^{\mathrm{Ser\ 2}}$	+	12232	12293	62			TGA	1
tRNA-$^{\mathrm{Leu\ 2}}$	+	12295	12360	66			TAA	0
ND1	+	12361	13290	930	ATG	TAG		-1
tRNA-$^{\mathrm{Ile}}$	+	13290	13354	65			GAT	0
tRNA-$^{\mathrm{Lys}}$	+	13355	13417	63			TTT	0
ND3	+	13418	13770	353	GTG	TA-		-1
tRNA-$^{\mathrm{Ser\ 1}}$	+	13770	13832	63			TCT	0
ND2	+	13833	14835	1003	ATG	T--		0

Ribosomal and transfer RNA genes

Like other mitochondrial genomes (Inoue et al. 2000; Zardoya et al. 1995), twenty-two tRNA genes were identified (Supplementary figure 1). The tRNA genes were scattered throughout the mitochondrial genome and ranged in size from 60 to 67 bp

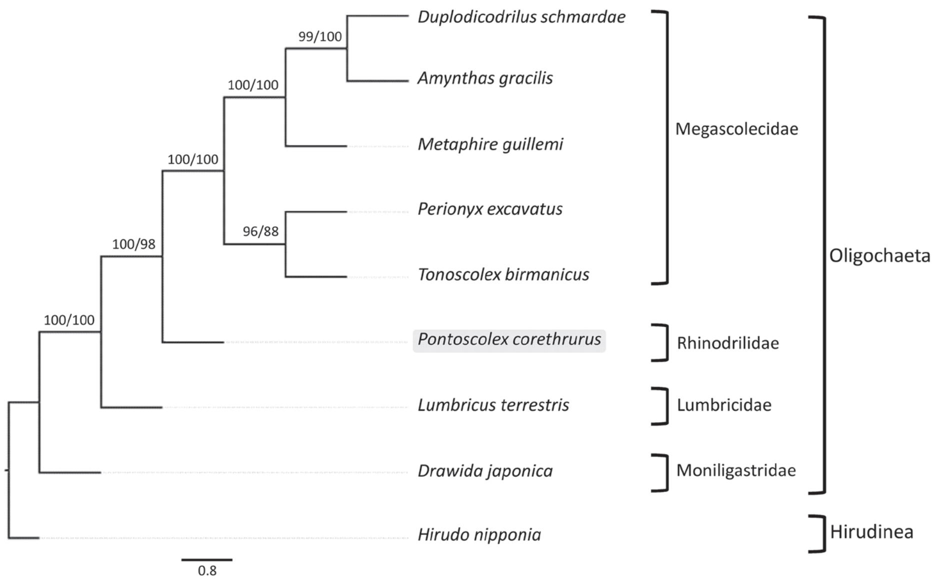

Figure 2. Phylogenetic relationships among phylum Annelida based on the combined 13,416 bp nucleotide positions. Total alignment length is greater than the combined *P. corethrurus* protein coding and rRNA sequence lengths due to overlapping protein coding sequences that are subsequently concatenated, and indel regions in the alignment. The posterior probability value of BI analyses and bootstrap support values of ML analyses (in the order: BI, ML) are indicated near the branches.

(Table 3). The *P. corethrurus* mitogenome also contained a small subunit of rRNA and a large subunit of rRNA, which were 789 bp and 1212 bp in length, respectively. As in other Clitellata genomes, these genes were located between the tRNA^{Met} and tRNA^{Val} genes and between tRNA^{Val} and tRNA^{Leu} genes, respectively (Zhang et al. 2016b).

Non-coding regions

As shown in Table 3, there are 22 intergenic spacer regions, ranging in size from -7 to 4 bp observed in *P. corethrurus*.

As in most Clitellata, the major non-coding region in *P. corethrurus* mitochondrial genome was located between tRNA-^{Arg} and tRNA-^{His}. It was determined to be 318 bp in length, less than other reported Clitellata species (Zhang et al. 2016a), and it had a base composition that was rich in A and T (A+T=67.6%).

Phylogenetic analyses within the Clitellata

The phylogenetic trees (the 50% majority-rule consensus tree is shown in Figure 2) were highly consistent regardless of the analytic method used, and were statistically

Table 4. Base composition for protein-coding, tRNA, and rRNA genes of *P. corethrurus* mitogenome.

Gene/Region	Base composition (%)				A+T (%)	Size (bp)
	T	C	A	G		
COX1	27.4	26.7	27.9	18.1	55.3	1,540
COX2	25.8	24.3	34.9	15.0	60.7	687
ATP8	24.4	26.9	36.9	11.9	61.3	160
COX3	27.3	27.5	25.7	19.5	53.0	778
ND6	28.3	26.0	30.3	15.5	58.6	466
Cytb	28.0	27.1	29.7	15.3	57.6	1,140
ATP6	29.6	29.2	30.6	10.6	60.2	696
ND5	27.7	26.7	31.7	13.9	59.4	1,722
ND4L	25.9	28.3	33.0	12.8	58.9	297
ND4	27.5	27.5	32.3	12.7	59.8	1,359
ND1	28.4	25.8	29.8	16.0	58.2	930
ND3	32.6	26.1	27.2	14.2	59.8	353
ND2	30.0	28.2	30.7	11.1	60.7	1,003
Protein Coding						
1st	30.2	25.4	17.4	27.0	47.6	3,710
2st	24.9	27.6	12.9	34.6	37.8	3,710
3st	36.1	27.9	13.8	22.3	49.8	3,710
Total	30.4	27.0	14.7	28.0	45.1	11,131
tRNA	30.5	17.6	34.9	17.0	65.5	1,391
rRNA	24.1	22.0	37.6	16.3	61.7	2,001
Putative Control Region	31.5	18.9	36.2	13.5	67.6	318
Overall	28.1	25.6	31.8	14.6	59.9	14,835

supported by high posterior probability and intermediate bootstrap values. This phylogenetic analysis represented the first investigation of *P. corethrurus* relationships within the Clitellata based on the complete mitogenome. As indicated by the tree, different species from the same family clustered together (Megascolecidae: *M. guillemi, D. schmardae, A. gracilis, P. excavatus and T. birmanicus*), and the species from Lumbricidae and the *P. corethrurus* formed a monophyletic group. The species *D. japonica* belongs to the Moniligastridae, the sister group to Crassiclitellata (earthworms), which explains its phylogenetic position. The Moniligastridae are not Crassiclitellata because they have a single cell layer in the clitellum.

Conclusion

For the first time, the sequencing, annotation and analysis of the mitochondrial genome of a member of Rhinodrilidae was completed. The mitogenome of *P. corethrurus* was found to be 14,835 bp in length and showed a similar composition in size, low GC content and gene order to earthworm mitogenomes already available. The complete mitogenome reported here is expected to allow for further studies of the *P. corethrurus* phylogeny and for analyses on the taxonomic status of the family Rhinodrilidae.

Declaration of interest section

The authors report no conflicts of interest and are responsible for the content and writing of the paper.

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Supplementary material 1

Inferred secondary structure of 22 tRNA genes in the mitochondrial DNA of the pantropical earthworm *Pontoscolex corethrurus* (Rhinodrilidae, Clitellata).

Authors: Ana C. Conrado, Hugo Arruda, David W.G. Stanton, Samuel W. James, Peter Kille, George Brown, Elodie Silva, Lise Dupont, Shabnam Taheri, Andrew J. Morgan, Nelson Simões, Armindo Rodrigues, Rafael Montiel8, Luis Cunha

Data type: molecular data

Link: https://doi.org/10.3897/zookeys.688.13721.suppl1

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Contribution to the knowledge of Neanurinae of Vietnam with description of three new species (Collembola, Neanuridae)

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Abstract

Detailed and illustrated descriptions of three new species belonging to the tribe Lobellini from Vietnam are given. *Lobellina weinerae* **sp. n.** is the most similar to *L. minuta* (Lee, 1980) and *L. musangensis* Yosii, 1976, but differs from them in chaetotaxic details and the number of mandibular teeth. *Lobellina pomorskii* **sp. n.** differs from *L. perfusionides* (Stach, 1965) in chaetotaxic details and the number of tubercles on Abd.V. *Yuukianura deharvengi* **sp. n.** is superficially similar to *Y. halophila* Yosii, 1955, but it differs in the build of the maxilla, the size of eyes and an inner tooth on the claw, and in chaetotaxic details. Furthermore, some remarks on the characteristics and the peculiarity of the Vietnamese fauna of the subfamily, and the key to all species from the country, are included.

Keywords

Lobellina pomorskii sp. n., *Lobellina weinerae* sp. n., Lobellini, springtails, taxonomy, *Yuukianura deharvengi* sp. n.

Introduction

Vietnam, in spite of its relatively small area (*ca*. 320,000 km^2, 65th in the world), is commonly known for its unique and extremely high biological diversity. This extraordinary level of biodiversity is associated with several factors, like the notable altitudinal gradient, the extreme north-south extension (8°N – 24°N), the geological complexity, the absence of larger catastrophic events in the Cenozoic Era, the tropical or subtropical climate, and the presence of precious remnants of many natural environments. Nonetheless, regarding research on the fauna, the country is among the most underrepresented on the continent. The knowledge of many groups of animals in Vietnam, especially invertebrates, seems to be still in an initial phase. One of such poorly known groups are undoubtedly springtails (Collembola) belonging to primitive and wingless Hexapoda. Among Collembola living in tropics, members of the subfamily Neanurinae are probably most spectacular and conspicuous due to their relatively large body size and vivid colours.

The study of Vietnamese Neanurinae has improved notably during the two last decades, with several new taxa described and recorded from both the southern and the northern parts of the country (Nguyen Tri Tien 1995, Deharveng and Bedos 2000, Bedos and Deharveng 2000, Deharveng and Smolis 2002, Smolis and Deharveng 2003, 2005, 2006a, b, Smolis 2007). At present, considering old (Denis 1934, 1948, Stach 1965) and new contributions, the fauna of the subfamily in the country includes 18 species classified into 3 tribes (Neanurini, Paleonurini, and Lobellini) and 12 genera, namely: *Neanura* MacGillivray, 1893; *Vietnura* Deharveng & Bedos, 2000; *Womersleya* Denis, 1948; *Rambutanura* Deharveng, 1988; *Blasconura* Cassagnau, 1983; *Vitronura* Yosii, 1969; *Pronura* Delamare Deboutteville, 1953; *Paleonura* Cassagnau, 1982; *Paralobella* Cassagnau & Deharveng, 1984; *Lobellina* Yosii, 1956; *Sphareonura* Cassagnau, 1983; and *Deuterobella* Yoshii & Suhardjono, 1992.

In the present contribution, three new species of Lobellini are reported, from one of the six tribes established within the subfamily (Cassagnau 1989). This large tribe currently encompasses more than 130 species and 15 genera, distributed primarily in the Oriental and the Australian regions (Bellinger *et al.* 2017). The Lobellini are defined by the following combination of features: the presence of 3+3 eyes or the ocelli absent, four labral chaetae positioned in two rows, the absence of a blue hypodermic pigment on the body, the separateness of tubercles An and Fr on the head, and a bilobate last abdomen (Cassagnau 1983, 1989, Deharveng 1983). Two new species, presented in this paper, belong to the genus *Lobellina* Yosii, 1956, while the third one to *Yuukianura* Yosii, 1955. Their detailed descriptions and suggestions about their close affinities are included. Additionally, general remarks on Vietnamese Neanurinae and a key to all species from the country are provided.

Materials and methods

The specimens were cleared in potassium hydroxide and chloral phenol, then mounted on slides in Swan's medium (distilled water, chloral hydrate, glacial acetic acid, glucose, Arabic gum) and studied using a Nikon Eclipse E600 phase contrast microscope. Figures were drawn with camera lucida and prepared for publication using Adobe Photoshop CS3.

Institutions of depository of materials

DIBEC	Department of Invertebrate Biology, Evolution and Conservation, Institute of Environmental Biology, University of Wrocław, Poland
MNHN	Muséum national d'Histoire naturelle in Paris, France

Terminology for the description follows that of Deharveng (1983, with rationale for the definition of chaetae categories), Deharveng and Weiner (1984), Greenslade and Deharveng (1990), Smolis and Deharveng (2006) and Smolis (2008).

Abbreviations used in text, tables and figures

General morphology:

Abd.	abdomen,
Ant.	antenna,
AOIII	sensory organ of antennal segment III,
Cx	coxa,
Fe	femur,
Scx2	subcoxa 2,
T	tibiotarsus,
Th.	thorax,
Tr	trochanter,
VT	ventral tube.

Groups of chaetae:

Ag	antegenital,
An	chaetae of anal lobes,
ap	apical,
ca	centroapical,
cm	centromedial,
cp	centroposterior,
d	dorsal,
Fu	furcal,
vc	ventrocentral,
Ve or **ve**	ventroexternal,
Vea	ventroexternoanterior,
Vem	ventroexternomedial,
Vep	ventroexternoposterior,
Vel	ventroexternolateral,
Vec	ventroexternocentral,
Vei	ventroexternointernal,
Vi or **vi**	ventrointernal,
Vl	ventrolateral.

Tubercles:

An	antennal
Fr	frontal,
Cl	clypeal,
De	dorsoexternal,
Di	dorsointernal,
Dl	dorsolateral,
L	lateral,
Oc	ocular,
So	subocular.

Types of chaetae:

Ml	long macrochaeta,
Mc	short macrochaeta,
me	mesochaeta,
mi	microchaeta,
ms	sensory microchaeta,
S or **s**	sensory chaeta,
bs	sensory chaeta on Ant. IV,
miA	microchaetae on Ant. IV,
iv	ordinary chaetae on ventral Ant. IV,
or	organite of Ant. IV,
brs	border s-chaeta on Ant. IV,
i	ordinary chaeta on Ant. IV,
mou	cylindrical s–chaetae on Ant. IV,
L'	ordinary lateral chaeta on Abd.V,
B4, **B5**	ordinary chaetae on tibiotarsi.

Taxonomy

Lobellina weinerae sp. n.

http://zoobank.org/53BC9AF3-8792-4FEF-B546-6F3990014A51
Figs 1–8; Table 1

Type material. Holotype: male on slide: Vietnam, ca. 70 km northwest of Hanoi, top of Tam Dao mountain, ca. 1300 m a.s.l., leaf-litter in shrubs, Berlese-Tullgren extraction, 10.IV.1997, leg. R.J.Pomorski (housed in DIBEC). Paratype: female on slide, same data as holotype (MNHN).

Etymology. The species is named in honour of Prof. Wanda Maria Weiner, for her important contribution to the knowledge on Collembola.

Diagnosis. Habitus typical of the genus *Lobellina*. Dorsal tubercles present and well developed. 3+3 medium eyes. Color of body alive yellow. Mandible with seven teeth. Head with chaetae A, B, C, D, E and O. Tubercle Oc with two chaetae on head. Tubercles Di on Th. II and III with 3 chaetae. Abd. V with 2+2 tubercles. Abd. V with 2+2 chaetae Di. Claw with inner tooth. Tibiotarsi with chaetae B4 and B5 short and pointed.

Description. *General* (Figs 1, 8). Body length (without antennae): 1.55 to 1.70 mm (holotype: 1.55 mm). Habitus elongate, parallel and slightly dorsoventrally flattened. Cuticular granulations fine, tubercles well developed on dorsal side of body, without reticulations. Color yellow alive and white in alcohol. 3+3 medium black eyes, anterior ocelli not on tubercle Oc.

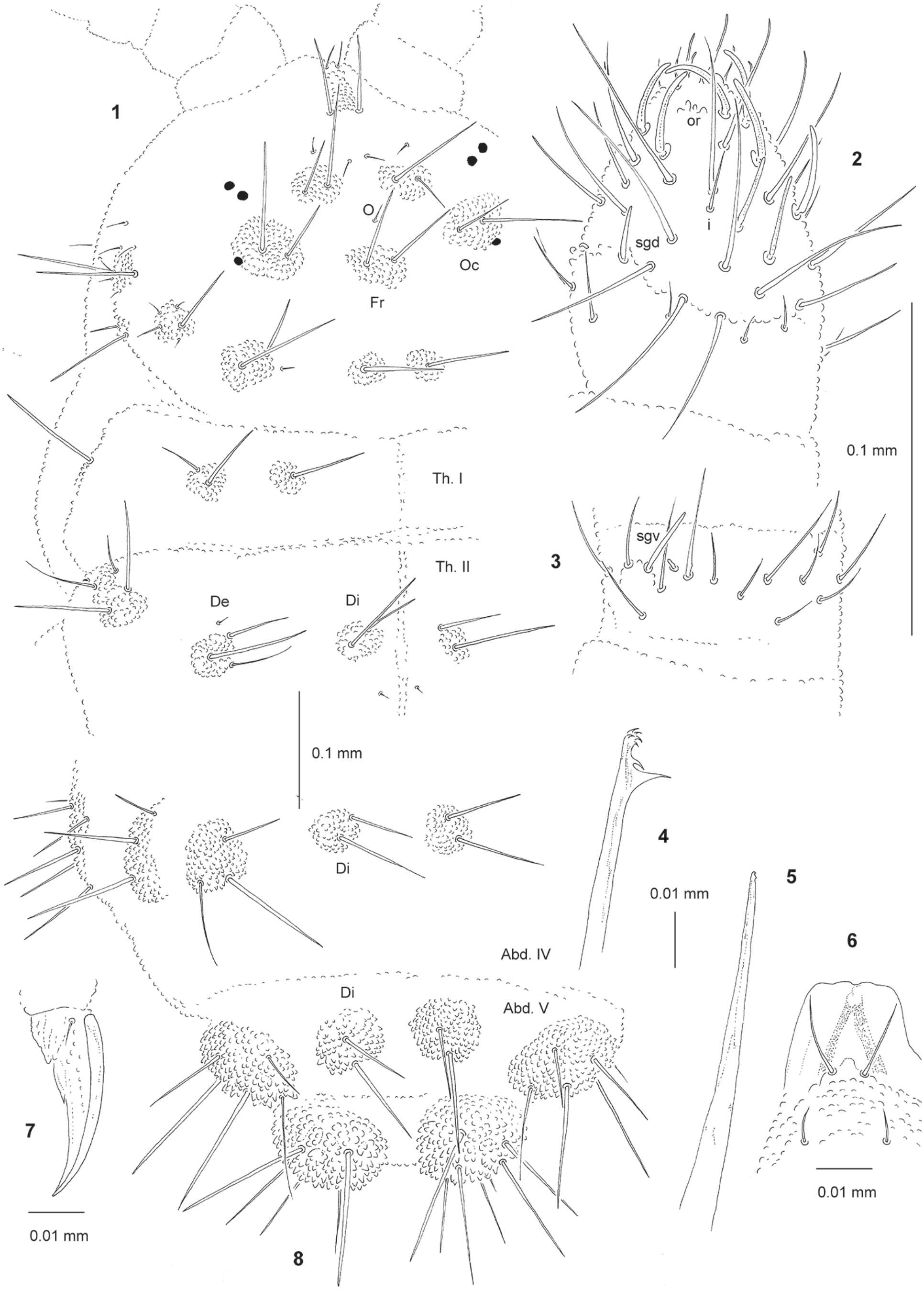
Chaetal morphology (Figs 1, 8). Dorsal ordinary chaetae of four types: Ml, Mc, me, and mi. Macrochaetae Ml moderately long, thin, straight, narrowly sheathed, smooth and pointed at apex. Macrochaetae Mc morphologically similar to long macrochaetae, but shorter. Mesochaetae similar to ventral chaetae, thin, smooth, and pointed. Microchaetae similar to mesochaetae, but apparently short. S-chaetae of tergites thin, smooth, and slightly shorter than nearby Ml.

Antennae (Figs 2, 3; Table 1b). Typical of the genus. S-chaetae of Ant. IV of medium length and moderately thickened. Apical vesicle trilobed. Sensillum sgd shorter and thinner than S-chaetae, not migrated distally.

Mouthparts (Figs 4–6). Buccal cone relatively short and wide with labral sclerifications non-ogival (Fig. 6), labral formula: 0/2,2. Labium with four basal, three distal and four lateral chaetae, papillae x absent. Maxilla styliform (Fig. 5), mandible with seven teeth, five minute apical and two large basal (Fig. 4).

Dorsal chaetotaxy and tubercles (Figs 1, 8; Tab. 1a, c). Chaetotaxy and arrangement tubercles of head as in Fig. 1 and Tab. 1a. Chaeta O present, not integrated with tubercle Fr. Chaetotaxy of Th. and Abd. as Figs 1, 8 and Table 1c. Abd. V with 2+2 tubercles, s–chaeta integrated with tubercle Dl. On Abd. V, chaetae Di3 absent (Fig. 8).

Ventral chaetotaxy (Tab. 1c). On head, groups Vea, Vem and Vep with 4, 3, 4 chaetae respectively. Group Vi on head with 6 chaetae. On Abd. IV, furca rudimentary without microchaetae. On Abd. V, chaeta Vl present. Male without modified chaetae.



Figures 1–8. *Lobellina weinerae* sp. n.: **1** dorsal chaetotaxy of head and Th. I, II **2** dorsal chaetotaxy of Ant. III–IV **3** ventral chaetotaxy of Ant. III **4** mandible **5** maxilla **6** labrum **7** claw **8** dorsal chaetotaxy of Abd. IV–VI.

Table 1a. Chaetotaxy of *Lobellina weinerae* sp. n.: cephalic chaetotaxy of dorsal side.

Chaetal group	Tubercle	Number of chaetae	Types of chaetae	Names of chaetae
Cl (unpaired)	+	4	Mc me	F G
An	+	4	Ml Mc mi	B C D, E
Fr (unpaired)	+	3	Ml mi	A O
Oc	+	2	Ml Mc	Ocm Ocp
Di	+	2	Ml mi	Di1 Di2
De	+	2	Ml Mc	De1 De2
Dl	+	5	Ml, Mc, 3 mi	Chaetal homology uncertain
1/2L	+	2	Ml, Mc	Chaetal homology uncertain
1/2L+So	+	7-8	2 Ml, 4 me, 1-2 mi	Chaetal homology uncertain

Table 1b. Chaetotaxy of *Lobellina weinerae* sp. n.: chaetotaxy of antennae.

Segment, Group	Number of chaetae	Segment, Group	Number of chaetae adult
I	7	IV	or, 8 S, i, 12 mou, 6 brs, 2 iv
II	12		
III	5 sensilla AO III		
ve	5	ap	8 bs, 5 miA
vc	4	ca	2 bs, 3 miA
vi	4	cm	3 bs, 1 miA
d	5	cp	8 miA, 1 brs

Table 1c. Chaetotaxy of *Lobellina weinerae* sp. n.: postcephalic chaetotaxy.

	Terga				Legs				
	Di	De	Dl	L	Scx2	Cx	Tr	Fe	T
Th. I	1	2	1	-	0	3	6	12	19
Th. II	3	3+s	3+s+ms	3	2	7	6	11	19
Th. III	3	3+s	3+s	3	2	8	6	10	18
					Sterna				
Abd. I	2	2+s	2	4	TV: 4				
Abd. II	2	2+s	2	4	Ve: 4 Ve1 absent				
Abd. III	2	2+s	2	4	Vel: 4	Fu: 3-4 me, 0 mi			
Abd. IV	2	2+s	3	6	Vl: 5	Vel: 3	Vec: 2	Vei: 1	
Abd. V	2	5+s		4	Ag:3	Vl: 1			
Abd. VI	7				Ve: 13	An: 2 mi			

Legs (Fig. 7, Tab. 1c). Claw with internal tooth. On tibiotarsi, chaeta M present and chaetae B4 and B5 short and pointed.

Remarks. As presently understood the genus *Lobellina* includes 13 species distributed mostly in East and Southeast Asia (Deharveng and Weiner 1984, Ma and Chen 2008, Wang et al. 2006). Interestingly, five of all known species were described from the Korean Peninsula (Lee 1980, Deharveng and Weiner 1984). *Lobellina weinerae* sp. n. is morphologically most similar to *L. minuta* (Lee, 1980) (from South Korea) and *L. musangensis* Yosii, 1976 (from Malaysia), resembling those species in having smooth body macrochaetae, similar length of body, tubercle Oc with 2 chaetae on head and Abd. V with 2+2 tubercles. Nevertheless, they are readily distinguished by a number of characters: body color alive (in *weinerae* yellow, in *minuta* red, in *musangensis* unknown), presence/absence of chaeta O on head (in *weinerae* present, in *minuta* and *musangensis* absent), number of mandibular teeth (in *weinerae* 7, in *minuta* 5, in *musangensis* 8), number of chaetae Di on Th. II–III (in *weinerae* 3, in *minuta* and *musangensis* 2), number of ordinary chaetae De on Th. II–III (in *minuta* 4, in *weinerae* and *musangensis* 3) and number of chaetae Di on Abd. V (in *minuta* 3, in *weinerae* and *musangensis* 2).

Lobellina pomorskii sp. n.

http://zoobank.org/F9B7C5B8-17F8-46F3-9989-4EF899D41DEB
Figs 9–15; Table 2

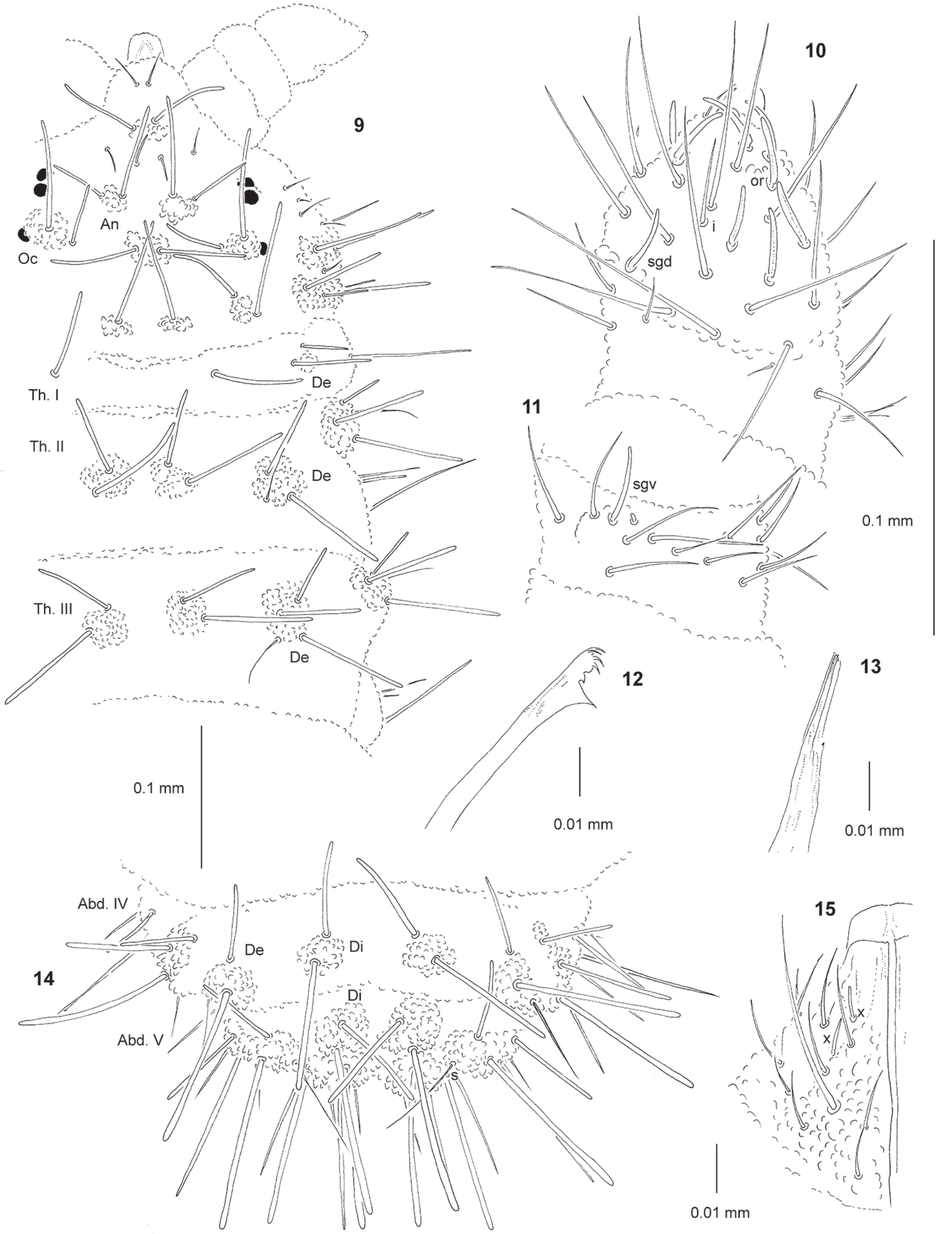
Type material. Holotype: female on slide: Vietnam, Do son near Haiphong, communities of grasses on sea rocks, Berlese-Tullgren extraction, 12.IV.1997, leg. R.J.Pomorski (housed in DIBEC). Paratypes: 2 females and 3 juveniles on slides, same data as holotype (DIBEC and MNHN).

Etymology. The species is named in honour of Prof. Romuald Jacek Pomorski who has contributed so very much to the knowledge of Collembola.

Diagnosis. Habitus typical of the genus *Lobellina*. Dorsal tubercles present and well developed. 3+3 large eyes. Color of body alive red. Mandible with six teeth. Head with chaetae A, B, C, D and E, chaeta O absent. Tubercle Oc with two chaetae on head. Tubercles Di on Th. II and III with 2 chaetae. Abd. V with 2+2 tubercles. Abd. V with 2+2 chaetae Di. Claw with inner tooth. Tibiotarsi with chaetae B4 and B5 short and pointed.

Description. *General* (Figs 9, 14). Body length (without antennae): 0.55 (juvenile) to 1.70 mm (holotype: 0.95 mm). Habitus elongate, parallel and slightly dorsoventrally flattened. Cuticular granulations fine, tubercles well developed on dorsal side of body, with subcuticular reticulations. Color red alive and white in alcohol. 3+3 large black eyes, anterior ocelli not on tubercle Oc.

Chaetal morphology (Figs 9, 14). Dorsal ordinary chaetae of four types: Ml, Mc, me, and mi. Macrochaetae Ml long, moderately thickened, straight, narrowly sheathed, smooth and rounded apically. Macrochaetae Mc morphologically similar to



Figures 9–15. *Lobellina pomorskii* sp. n.: **9** dorsal chaetotaxy of head and Th. **10** dorsal chaetotaxy of Ant. III–IV **11** ventral chaetotaxy of Ant. III **12** mandible **13** maxilla **14** dorsal chaetotaxy of Abd. IV–VI **15** labium.

Table 2a. Chaetotaxy of *Lobellina pomorskii* sp. n.: cephalic chaetotaxy of dorsal side.

Chaetal group	Tubercle	Number of chaetae	Types of chaetae	Names of chaetae
Cl (unpaired)	+	4	Ml me	F G
An	+	4	Ml Mc mi	B C D, E
Fr (unpaired)	+	2	Ml	A
Oc	+	2	Ml Mc	Ocm Ocp
Di	+	1	Ml	Di1
De	+	2	Ml Mc	De1 De2
Dl	+	4	2 Ml, Mc, me	Chaetal homology uncertain
(L+So)	+	7	2 Ml, 4 me, 1 mi	Chaetal homology uncertain

Table 2b. Chaetotaxy of *Lobellina pomorskii* sp. n.: chaetotaxy of antennae.

Segment, Group	Number of chaetae	Segment, Group	Number of chaetae adult
I	7	IV	or, 8 S, i, 12 mou, 6 brs, 2 iv
II	11		
III	5 sensilla AO III		
ve	5	ap	8 bs, 5 miA
vc	4	ca	2 bs, 3 miA
vi	4	cm	3 bs, 1 miA
d	5	cp	8 miA, 1 brs

Table 2c. Chaetotaxy of *Lobellina pomorskii* sp. n.: postcephalic chaetotaxy.

	Terga				Legs				
	Di	De	Dl	L	Scx2	Cx	Tr	Fe	T
Th. I	1	2	1	-	0	3	6	13	19
Th. II	2	2+s	3+s+ms	3	2	7	6	12	19
Th. III	2	3+s	3+s	3	2	8	6	11	18
					Sterna				
Abd. I	2	2+s	2	4	TV: 4				
Abd. II	2	2+s	2	4	Ve: 4 Ve1 absent				
Abd. III	2	2+s	2	4	Vel: 4	Fu: 3-4 me, 0 mi			
Abd. IV	2	2+s	3	6	Vl: 5	Vel: 3	Vec: 2	Vei: 1	
Abd. V	2	5+s		4	Ag:3	Vl: 1			
Abd. VI	7				Ve: 13	An: 2 mi			

long macrochaetae, but shorter. Mesochaetae similar to ventral chaetae, thin, smooth and pointed. Microchaetae similar to mesochaetae, but apparently short. S-chaetae of tergites thin, smooth and distinctly shorter than nearby Ml.

Antennae (Figs 10, 11; Tab. 2b). Typical of the genus. S-chaetae of Ant. IV of medium length and moderately thickened. Apical vesicle trilobed. Sensillum sgd not migrated distally (Fig. 10).

Mouthparts (Figs 12, 13, 15). Buccal cone relatively short and wide with labral sclerifications non-ogival, labral formula: 0/2,2. Labium as in Fig. 15, papillae x present and relatively large. Maxilla styliform (Fig. 13), mandible with 6 teeth, 4 apical and 2 basal (Fig. 12).

Dorsal chaetotaxy and tubercles (Figs 9, 14; Tab. 2a, c). Chaetotaxy and arrangement of tubercles of head as in Fig. 9 and Tab. 2a. Chaeta O absent. Chaetotaxy of Th. and Abd. as in Figs 9, 14 and Tab. 2c. Abd.V with 2+2 tubercles, s–chaeta integrated with tubercle Dl. On Abd. V, chaetae Di3 absent.

Ventral chaetotaxy (Tab. 2c). On head, groups Vea, Vem and Vep with 4, 3, 4 chaetae respectively. Group Vi on head with 6 chaetae. On Abd.IV, furca rudimentary without microchaetae. On Abd.V, chaeta Vl present. Male without modified chaetae.

Legs (Tab. 2c). Claw with internal tooth. On tibiotarsi, chaeta M present and chaetae B4 and B5 short and pointed.

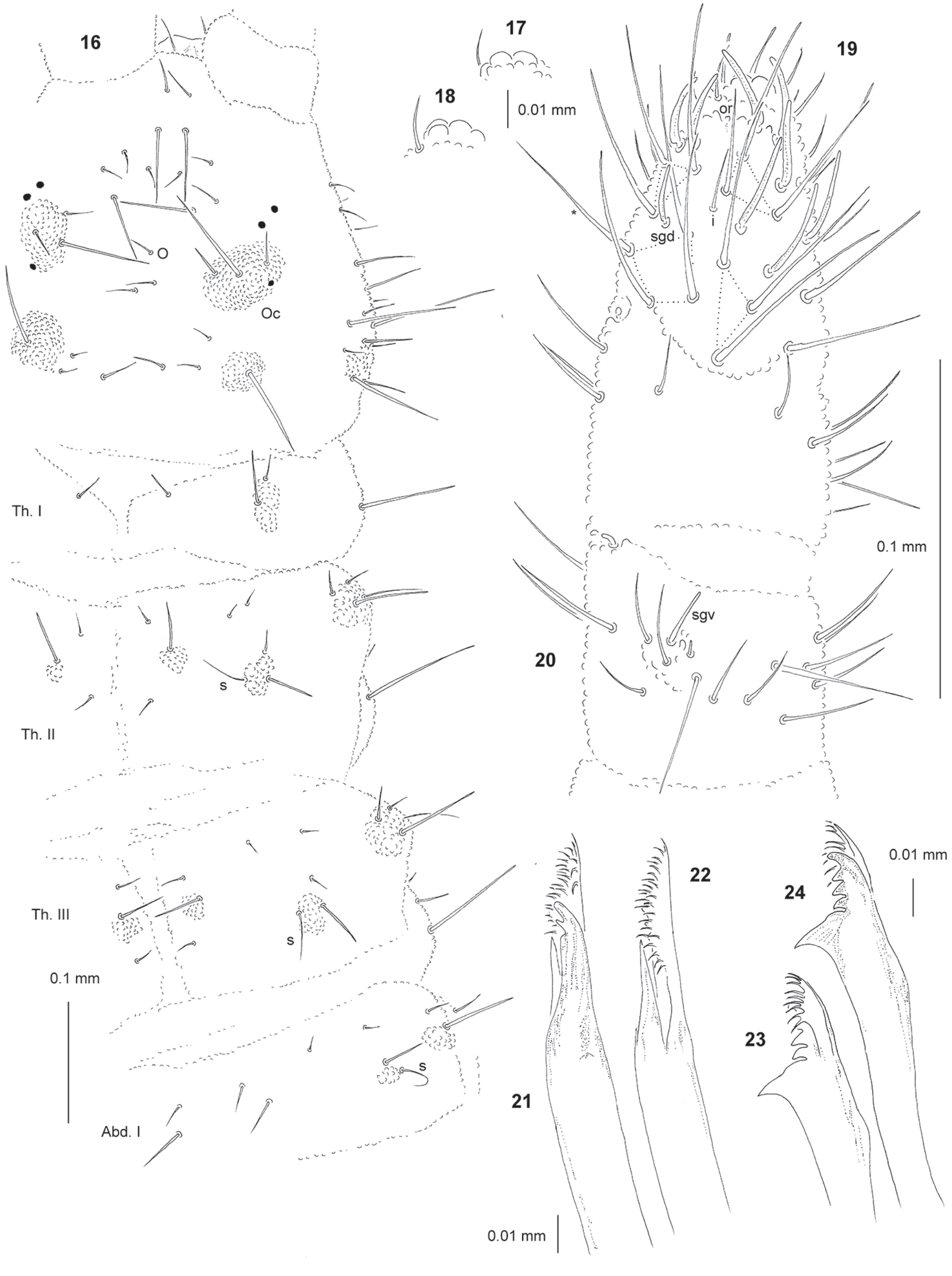
Remarks. *Lobellina pomorskii* sp. n. strongly resembles another Vietnamese species of the genus, *L. perfusionides* (Stach, 1965). However, these species can be distinguished by the following features: shape of dorsal long macrochaetae (in *pomorskii* cylindrical, in *perfusionides* flattened and extended apically), number of chaetae in tubercles An on head (in *pomorskii* 8, in *perfusionides* 6), number of chaetae De on Th. I (in *pomorskii* 2, in *perfusionides* 1), number of ordinary chaetae De on Th. III (in *pomorskii* 3, in *perfusionides* 2), number of ordinary chaetae De on Abd.IV (in *pomorskii* 2, in *perfusionides* 1) and number of tubercles on Abd. V (in *pomorskii* 2+2, s–chaetae integrated with tubercles Dl; in *perfusionides* 3+3, s–chaetae not integrated with tubercles Dl). Furthermore, the new species was found in communities of grasses on sea rocks (Northeastern Vietnam) while type material of *L. perfusionides* was collected from “moss growing on a tree” (mountain region of Northern Vietnam, Stach 1965).

***Yuukianura deharvengi* sp. n.**

http://zoobank.org/D6295100-18A5-4022-B2DE-62FE60F3B7D1
Figs 16–29; Table 3

Type material. Holotype: male on slide: Vietnam, Do son near Haiphong, marine littoral zone, by hand, 12.IV.1997, leg. R.J.Pomorski (housed in DIBEC). Paratypes: 2 females on slides, same data as holotype (DIBEC and MNHN).

Etymology. The species is named in honour of Prof. Louis Deharveng, for his important contribution to the knowledge on Collembola.



Figures 16–24. *Yuukianura deharvengi* sp. n.: **16** dorsal chaetotaxy of head Th. and Abd. I **17** apical bulb, ventral view **18** apical bulb, dorsal view **19** dorsal chaetotaxy of Ant. III–IV **20** ventral chaetotaxy of Ant. III **21** maxilla, dorsal view **22** maxilla, ventral view **23** mandible, ventral view **24** mandible, dorsal view.

Diagnosis. Habitus typical of the genus *Yuukianura*. Dorsal tubercles present but poorly developed. 3+3 small eyes. Color of body alive yellow. Mandible with five teeth. Ventral lamella of maxilla with 20–25 cilia. Head with chaetae A, B, C, D and E, chaeta O absent. Tubercle Oc with three chaetae on head. Tubercles Di on Th. II and III with 3 chaetae. Abd. V with 2+2 tubercles. Abd. V with 3+3 chaetae Di. Claw with small inner tooth. Tibiotarsi with chaetae B4 and B5 short and pointed.

Description. *General* (Figs 16, 27, 29). Body length (without antennae): 1.60 to 1.70 mm (holotype: 1.65 mm). Habitus elongate, narrow, parallel-sided and slightly dorsoventrally flattened. Cuticular granulations fine, tubercles inconspicuous or poorly developed, without visible subcuticular reticulations. Color yellow alive and white in alcohol. 3+3 small black eyes (Figs 16, 27), anterior ocelli outside tubercle Oc.

Chaetal morphology (Figs 16, 29). Dorsal ordinary chaetae of four types: Ml, Mc, me and mi. Macrochaetae Ml long, moderately thickened, straight, narrowly sheathed, feebly scaled and rounded apically. Macrochaetae Mc morphologically similar to long macrochaetae, but shorter. Mesochaetae similar to ventral chaetae, thin, minutely scaled and pointed. Microchaetae similar to mesochaetae, but apparently short. S-chaetae of tergites thin, smooth and equal or slightly shorter than closest Ml.

Antennae (Figs 17–20, Tab. 3b). Typical of the genus. S-chaetae of Ant. IV of medium length and moderately thickened. Ant. IV with one additional s-chaeta mou (Fig. 19). Apical vesicle not elevated and multilobed (Figs 17, 18). Sensillum sgd migrated distally. Sensillum sgv short and straight (Fig. 20).

Mouthparts (Figs 21–26). Buccal cone relatively short, wide and truncated, with labral sclerifications non-ogival (Fig. 26), labral formula: 0/2,2. Labium as in Fig. 25, papillae x present and relatively large. Maxilla well developed with 2 teeth and 2 lamellae, inner ventral lamella dagger-like and not fringed, outer ventral lamella fringed with 20–25 cilia arranged in 2–3 rows (Figs 21, 22). Mandible thick with five teeth, four apical and one strong basal, and one ventral lamella with 7–9 cilia in one row (Figs 23, 24).

Dorsal chaetotaxy and tubercles (Figs 16, 29; Tab. 3a, c). Chaetotaxy and arrangement of tubercles of head as in Fig. 16 and Tab. 3a. Chaeta O present. Tuberle Oc with three chaetae. Chaetotaxy of Th. and Abd. as in Figs 16, 29 and Tab. 3c. Abd. IV with 3 chetae Di. Abd. V with 2+2 tubercles, tubercles Di not fused to (De+Dl).

Ventral chaetotaxy (Tab. 3c). On head, groups Vea, Vem and Vep with 4, 3, 4 chaetae respectively. Group Vi on head with 6 chaetae. On Abd.IV, furca rudimentary without microchaetae. On Abd.V, chaeta Vl present. Male without modified chaetae.

Legs (Fig. 28, Tab. 3c). Claw with small internal tooth. On tibiotarsi, chaeta M absent and chaetae B4 and B5 short and pointed (Fig. 28).

Remarks. Taxonomy of the genus *Yuukianura* is controversial and problematic mostly due to insufficient descriptions of some species (Deharveng et al. 2017). The majority of species live in littoral zones of streams and seashore of many Pacific regions, from Russian Far East to Hawaiian Island and North Australia. *Yuukianura deharvengi* sp. n. seems to be most similar to *Y. halophila* Yosii, 1955, found in the Nakanoshima Island belonging to the Ryukyu Archipelago (Southern Japan). They differ in a few

Table 3a. Chaetotaxy of *Yuukianura deharvengi* sp. n.: cephalic chaetotaxy of dorsal side.

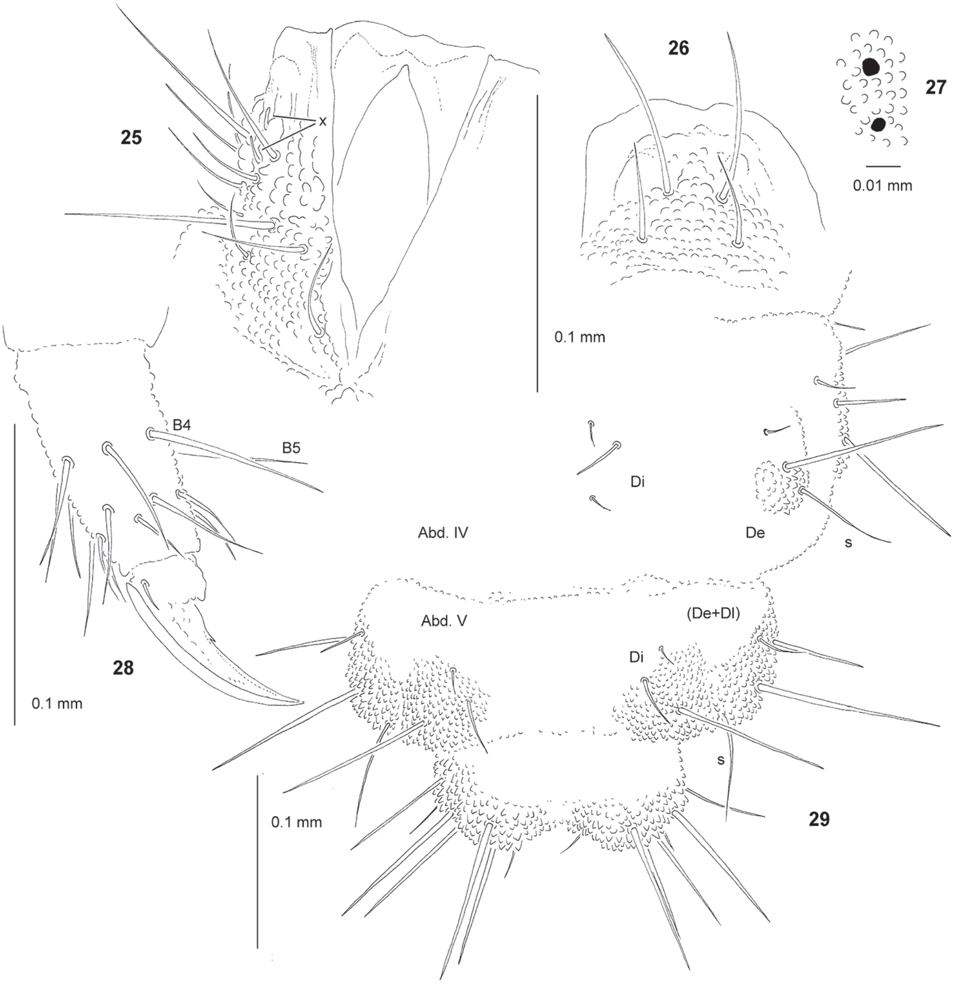
Chaetal group	Tubercle	Number of chaetae	Types of chaetae	Names of chaetae
Cl (unpaired)	-	4	Ml me	F G
An	-	4	Ml me	B C, D, E
Fr (unpaired)	-	3	me	A, O
Oc	+	3	Ml Mc me	Ocm Ocp Oca
Di	-	2	Mc me	Di1 Di2
De	+	2	Ml me	De1 De2
Dl	+	5	Ml, Mc, 3 me	Chaetal homology uncertain
(L+So)	-	8	2 Ml, 2 Mc, 4 me	Chaetal homology uncertain

Table 3b. Chaetotaxy of *Yuukianura deharvengi* sp. n.: chaetotaxy of antennae.

Segment, Group	Number of chaetae	Segment, Group	Number of chaetae adult
I	7	IV	or, 8 S, i, 13 mou, 6 brs, 2 iv
II	11		
III	5 sensilla AO III		
ve	5	ap	8 bs, 5 miA
vc	4	ca	2 bs, 3 miA
vi	4	cm	3 bs, 1 miA
d	5	cp	8 miA, 1 brs

Table 3c. Chaetotaxy of *Yuukianura deharvengi* sp. n.: postcephalic chaetotaxy.

	Terga				Legs				
	Di	De	Dl	L	Scx2	Cx	Tr	Fe	T
Th. I	1	2	1	-	0	3	6	12	18
Th. II	3	4+s	3+s+ms	3	2	7	6	11	18
Th. III	3	4+s	3+s	3	2	8	6	10	17
					Sterna				
Abd. I	2	3+s	2	3	TV: 4				
Abd. II	2	3+s	2	3	Ve: 5-6 Ve1 absent				
Abd. III	2	3+s	2	3	Vel: 6	Fu: 6 me, 0 mi			
Abd. IV	3	2+s	3	7-8	Vl: 5	Vel: 3	Vec: 2	Vei: 1	
Abd. V	3	4+s		4	Ag:3	Vl: 1			
Abd. VI	7				Ve: 12-13	An: 2 mi			



Figures 25–29. *Yuukianura deharvengi* sp. n.: **25** labium **26** labrum **27** anterior ocelli **28** claw and TIII, dorsolateral view **29** dorsal chaetotaxy of Abd. IV–VI.

subtle but distinctive and important features: shape of maxilla (in *deharvengi* with one ciliated lamella, in *halophila* lamellae without cilia), size of eyes (in *deharvengi* small, with diameter not longer than twice of diameter of closest granules; in *halophila* large, with diameter at least three times longer than diameter of closest granules), number of chaetae Di on Abd. IV (in *deharvengi* 3 chaetae, in *halophila* 2 chaetae), number of tubercles on Abd. V (in *deharvengi* 2+2, in *halophila* 1+1 tubercles), and position and size of inner tooth on claw (in *deharvengi* small and situated in one third of inner edge, in *halophila* large and in half of inner edge).

Discussion

Presently, the Neanurinae fauna in Vietnam includes 21 species in the following genera: *Neanura* MacGillivray, 1893 – 1, *Vietnura* Deharveng & Bedos, 2000 – 1, *Womersleya* Denis, 1948 – 1, *Rambutanura* Deharveng, 1988 – 2, *Blasconura* Cassagnau, 1983 – 3, *Vitronura* Yosii, 1969 – 2, *Pronura* Delamare Deboutteville, 1953 – 2, *Paleonura* Cassagnau, 1982 – 2, *Paralobella* Cassagnau & Deharveng, 1984 – 1, *Lobellina* Yosii, 1956 – 3, *Sphareonura* Cassagnau, 1983 – 1, *Deuterobella* Yoshii & Suhardjono, 1992 – 1 and *Yuukianura* Yosii, 1955 – 1. Nevertheless, the Vietnamese fauna of this subfamily is expected to be surely much richer and can include at least 100 taxa. This potential number seems to be likely and adequate to the biological diversity of Vietnam and the knowledge of the subfamily in other Asian countries. For comparison, the Neanurinae fauna of North Korea, a country nearly three times smaller than Vietnam and located far norther, currently comprises 23 species (Deharveng and Weiner 1984).

Despite the still initial phase of the knowledge of this subfamily in Vietnam, a comparison with the data on the Neanurinae diversity in other countries, well or similarly documented in this respect, in East Asia (e.g. North Korea, China) and Southeast Asia (e.g. Thailand, Malaysia) indicates many similarities between these areas but also some peculiarities of Vietnam's fauna. These similarities are strongly manifested in the presence of many genera, e.g. *Blasconura*, *Vitronura*, *Pronura*, *Paleonura*, *Paralobella*, *Lobellina*, *Sphareonura*, *Deuterobella*, *Yuukianura* and *Rambutanura*, widely distributed and common in East Asia or Southeast Asia, or both. Interestingly, Vietnam has some of the most spectacular Neanurinae known, members of the genus *Rambutanura*. This genus, probably endemic for Southeast Asia, currently contains four species: *R. dawydofii* (Denis, 1934) (from Vietnam), *R. malayana* (Yosii, 1976) (Malayasia), *R. yoshiiana* Deharveng, 1988 (Thailand) and *R. carcharia* Smolis, 2007 (Vietnam). Most Neanurinae taxa are small to medium-sized, reach maximum 2.5 mm in length, and are rather drab in color. *Rambutanura*, however, is much larger (up to 7 mm), more colorful, and its body is covered by numerous extremely long finger-like projections. Additionally, these unusual springtails can also be interesting for the whole scientific community, because *R. yoshianna* is characterized by extremely large polytene chromosomes in its salivary glands (Deharveng 1988).

The largest peculiarities in the Neanurinae fauna of Vietnam are the *Vietnura* genus and the species of *Pronura pomorskii* Smolis & Deharveng, 2006. Biogeographically, *Vietnura* is one of the most interesting genera in the world, as the localities of *V. caerulea* Deharveng & Bedos, 2000 are the most southern (12° N) records of Neanurini (Deharveng and Bedos 2000). Until its discovery, excluding a few Neanurini species introduced by humans outside their natural range limit, this large and diversified tribe was known exclusively from the Palearctic and Nearctic Regions (e.g. Fjellberg 1985, Babenko and Fjellberg 2006, Deharveng *et al.* 2015, Mayvan *et al.* 2015). *Pronura pomorskii*, in turn, is unique among all Neanurinae due to presence of tubercles on the border between terga; normally, if present, these cuticular structures are located on tergites only (Smolis and Deharveng 2006a).

Considering the present stage of knowledge on Neanurinae, notable absences from the Vietnamese fauna are *Paranura* Axelson, 1902, *Siamanura* Deharveng, 1987 and *Blasconurella* Deharveng & Bedos, 1992, genera that are species-rich and widespread on the continent. Nevertheless, as the fauna of Vietnam becomes better explored, we will probably discover these speies also there and see more similarities with the adjacent countries' fauna. It is also likely that most of the described species will be endemic to the country. To sum up, a great deal of work is needed regarding the taxonomy of this group in the country, particularly to describe the unknown diversity, sort out the taxonomy, and resolve relationships among the species.

Key to Neanurinae species from Vietnam

The key is based partially on Deharveng and Bedos (2000). It should be noted that the published records of some taxa from Vietnam are not well-documented (species marked below by asterisks); therefore, they are in need of verification and confirmation.

1 Blue pigmentation present on body **2**
– Blue pigmentation absent on body.. **3**
2 2+2 ocelli, tubercles Af and Oc fused on head, head with complete fusion of lateral tubercles .... ***Vietnura caerulea* Deharveng & Bedos, 2000**
– 3+3 ocelli, tubercles Af and Oc separate on head, head with incomplete fusion of lateral tubercles....................... ***Neanura muscorum* (Templeton, 1835)***
3 Ocelli absent...................................***Deuterobella murphyi* (Yosii, 1976)***
– Ocelli present.. **4**
4 2+2 ocelli.. **5**
– 3+3 ocelli.. **16**
5 Tubercles well developed on body, most of them in form of long digitations.... **6**
– Tubercle present or absent on body but never in form of long digitations **7**
6 Mandible tridentate, tubercles De and Dl digitate in form on Abd. I–III...... .... ***Rambutanura carcharia* Smolis, 2007**
– Mandible with larger number of teeth, tubercles De and Dl not digitate in form on Abd. I–III .... ***Rambutanura dawydoffi* (Denis, 1934)**
7 Abd. V with tubercles Di positioned laterally and fused with tubercles (De+Dl)... **8**
– Abd. V with tubercles Di not positioned laterally and not fused with tubercles (De+Dl).. **9**
8 Labium with 5+5 chaetae, tubercles present between terga of Th. I–Abd. IV... ....***Pronura pomorskii* Smolis & Deharveng, 2006**
– Labium with 9+9 chaetae, tubercles absent between terga of Th. I–Abd. IV ....***Pronura bidoup* Deharveng & Smolis, 2002**

9 Tubercles well developed over all body **10**

– Tubercles not well or poorly developed on body **15**

10 Tubercles Di and De fused on head and on Abd. V .... ***Womersleya vicina*** **(Denis, 1934)**

– Tubercles Di and De separate on head and on Abd. V **11**

11 Tubercles An and Fr separate on head **12**

– Tubercles An and Fr fused complete or partially on head **13**

12 Tubercle Oc on head with 3 chaetae, labrum non-ogival .... ***Vitronura giselae*** **(Gisin, 1950)***

– Tubercle Oc on head with 1 chaeta, labrum ogival .... ***Vitronura mascula*** **Smolis & Deharveng, 2006**

13 Head with fusion of two tubercles An, tubercle Fr alone .... ***Blasconura separata*** **(Denis, 1934)**

– Head with fusion of two tubercles An and tubercle Fr in one mass **14**

14 Ant. I with 7 chaetae, Th. II–III with 2 chaetae Di .... ***Blasconura batai*** **Bedos & Deharveng, 2000**

– Ant. I with 9 chaetae, Th. II–III with 3 chaetae Di .... ***Blasconura hirtella*** **(Börner, 1906)***

15 S-chaetae on Th. II–III and Abd. I–V distinctly longer than nearby macrochaetae Ml, macrochaetae Ml on Abd. I–VI not clavate in form .... ***Paleonura tenuisensillata*** **Smolis & Deharveng, 2005**

– S-chaetae on Th. II–III and Abd. I–V clearly shorter than nearby macrochaetae Ml, macrochaetae Ml on Abd. I–VI claviform .... ***Paleonura epiphytica*** **Smolis & Deharveng, 2003**

16 Body with strong plurichaetosis .... ***Sphareonura bornensis*** **(Schött, 1925)***

– Body without plurichaetosis **17**

17 S-chaetae present on tubercle L of Abd. II–IV .... ***Paralobella perfusa*** **(Denis, 1934)**

– S-chaetae absent on tubercle L of Abd. II–IV **18**

18 Abd. V with tubercles Di positioned laterally towards tubercles (De+Dl) .... ***Yuukianura deharvengi*** **sp. n.**

– Abd. V with tubercles Di not positioned laterally **19**

19 Cephalic chaeta O present, Th. II–III with 3 chaetae Di .... ***Lobellina weinerae*** **sp. n.**

– Cephalic chaeta O absent, Th. II–III with 2 chaetae Di **20**

20 Tubercles An on head with 6 chaetae, Abd. V dorsally with 3+3 tubercles .... ***Lobellina perfusionides*** **(Stach, 1965)**

– Tubercles An on head with 8 chaetae, Abd. V dorsally with 2+2 tubercles .... ***Lobellina pomorskii*** **sp. n.**

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Description of a new species of *Lamellothyrea* Krikken (Coleoptera, Scarabaeidae, Cetoniinae) from the iSimangaliso Wetland Park, KwaZulu-Natal (South Africa)

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Abstract

Recent data and material obtained from northern KwaZulu-Natal (South Africa) and Maputo Bay (Mozambique) have provided support for the description of a new species of the genus *Lamellothyrea* Krikken, 1980. The genus previously included only one species, *L. descarpentriesi,* with uncertain and poorly defined type locality, i.e. "Transvaal". It is now evident that two different species are actually involved, *L. descarpentriesi* with currently known distribution limited to the coastal area north of Maputo, and *L. isimangaliso* **sp. n.** with a known distribution range virtually restricted to the iSimangaliso Wetland Park, in north-eastern KwaZulu-Natal. The two species appear to be separated by a substantial discontinuity in southern Mozambique and can be easily separated on the basis of their clypeal structure, extent of white dorsal tomentum and shape of aedeagal parameres. Both species appear to be restricted to the coastal belt, with *L. isimangaliso* sp. n. occupying almost exclusively dune forest habitats. In this species, adult activity depends on rainfall and shows two peaks, one at the onset of summer and the second in autumn.

Keywords

Lamellothyrea, Cetoniinae, new species, South Africa, Mozambique

Introduction

The genus *Lamellothyrea* was described by Krikken in 1980 on the basis of a single male specimen reportedly from the "Transvaal" in South Africa. It has until now included only one species, *L. descarpentriesi*, with a reported distribution restricted to the coastal area of northern KwaZulu-Natal (KZN) and a vague type locality. It has now emerged, however, that two specimens recently collected along the Mozambique coast, north of Maputo Bay represent a different species to that occurring in northern KZN. An analysis of these specimens has revealed that they exhibit remarkable similarity with the holotype and conform well to the original description of Krikken (1980).

On the other hand, the numerous material now available for the KwaZulu-Natal population shows that this is distinct in many respects from the Mozambique and holotype specimens. This distinction appears to be best expressed at the level of the clypeal armour and in the parameres of the male genitalia. Thus, two separate species are involved, with the KwaZulu-Natal species here described as *L. isimangaliso* sp. n.

Substantial ecological data and observations have also become available from the recent studies undertaken in the iSimangaliso Wetland Park in KwaZulu-Natal, within its "Rare, Threatened and Endemic Species Project" (Combrink and Kyle 2006). This has now provided some key information on the distribution, relative abundance, habitat characteristics and general biology of the new species.

Materials and methods

Since the original description of *Lamellothyrea* (Krikken 1980), numerous specimens from this genus have been collected in the iSimangaliso Wetland Park during the period leading up to, and following, its proclamation as UNESCO World Heritage Site (Combrink and Kyle 2006). Specimens were obtained mainly through deployment of aerial fruit-baited traps, inspection of flowers and fruits of waterberry trees (*Syzygium cordatum*) and direct search on the ground. Given the scarcity of museum/collection material, one observation record of *L. descarpentriesi* from Mozambique located on the citizen science platform iSpot (Silvertown et al. 2015) was also included in this study.

For the description of morphological characters, the terminology used by Krikken (1984) and Holm and Marais (1992) is followed in this study. Specimen total length and maximum width were measured using a Vernier calliper, from the anterior margin of the clypeus to the apex of the pygidium and at the widest point of the elytra, respectively. Photos of specimen dorsal and ventral habitus were taken with a Nikon CoolPix S9700 digital camera with macro setting, while photos of the male genitalia were obtained using a Nikon DigitalSight DS-Fi2 camera attached to a Nikon SMZ25 dissecting microscope. The background was removed from the photos using Microsoft Word 2010 (Picture Tools), in order to increase clarity of resolution. The Combine ZP Image Stacking Software by Alan Hadley (alan@micropics.org.uk) was used to obtain z-stacking composite images.

Repositories are abbreviated as follows

BMPC	Jonathan Ball and Andre Marais Private Collection, Cape Town, South Africa;
CDPC	Cyril Di Gennaro Private Collection, Arcueil, France;
DMPC	Daniel Moore Private Collection, Oro Valley, USA;
DMSA	Durban Natural Science Museum, Durban, South Africa;
EPPC	Ernest Pringle Private Collection, Bedford, South Africa;
GBPC	Gerhard Beinhundner Private Collection, Euerbach, Germany;
ISAM	Iziko South African Museum, Cape Town, South Africa;
PCPC	Renzo Perissinotto and Lynette Clennell Private Collection, Port Elizabeth, South Africa;
RMNH	Museum Naturalis, Leiden, The Netherlands;
SANC	South African National Collection of Insects, Pretoria, South Africa;
SRPC	Sébastien Rojkoff Private Collection, Lyon, France;
TGPC	Thierry Garnier Private Collection, Montpellier, France;
TMSA	Ditsong National Museum of Natural History (formerly Transvaal Museum), Pretoria, South Africa.

Data on distribution and period of adult activity were also obtained from the literature (Rigout and Allard 1992; Sakai and Nagai 1998) and the original description of *L. descarpentriesi* (Krikken 1980).

Taxonomy

Lamellothyrea descarpentriesi Krikken, 1980

Lamellothyrea descarpentriesi Krikken, 1980: 185–187; Holm and Marais 1992: 126–127; Rigout and Allard 1992: 85, 92; Sakai and Nagai 1998: 332, 395.

Known material. Holotype (♂): Transvaal (RMNH). Other material: 1♂, Mozambique, Marracuene 10 m, 50 km N of Maputo, S25°46'14", E32°40'11", 23–26 Aug 2001, AK Brinkman leg (BMPC); 1♀: Mozambique, Praia Do Bilene (25°28'63"S, 33°25'71"E) 27 Oct 2015, found dead on the beach, Andrew Deacon (https://www.ispotnature.org/node/747181, accessed on 25 May 2016).

Description of female. While a detailed description of the holotype male of this species, complete with quality drawings, is provided in Krikken (1980), the female has remained unknown until the recent posting of a photo of a specimen by A. Deacon on the iSpot site. The specimen was found freshly dead on the beach of Praia do Bilene and the photo is of sufficiently high resolution to ascertain that it belongs to this sex and to recognise its key characters. On the basis of this, the female is essentially identical to the male in general appearance (Figs 1A, 2A), with the exception that it exhibits

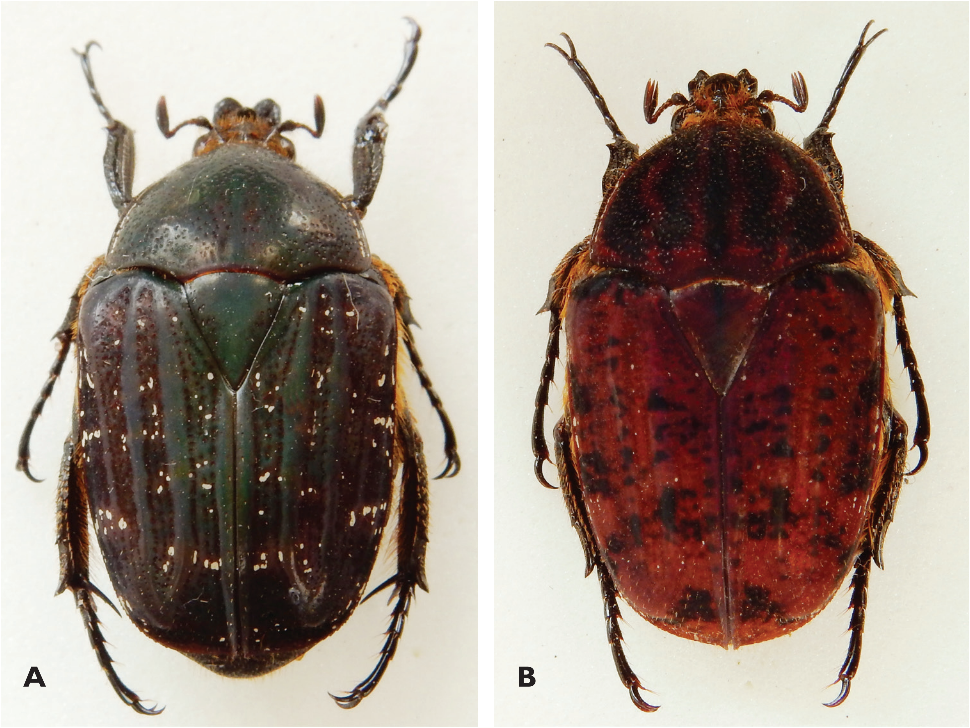

Figure 1. Dorsal habitus of **A** *Lamellothyrea descarpentriesi* (Marracuene, Mozambique) and **B** *Lamellothyrea isimangaliso* sp. n. (St Lucia, South Africa).

convex abdominal sternites without the medial vertical groove, which is typical of the male. The protibiae are also significantly broader anteriorly than in the male, and visibly tridentate.

Lamellothyrea isimangaliso sp. n.

http://zoobank.org/B0BBF1C9-AFA0-4D89-80AA-5B17DC87A409

Diagnosis. The two species of *Lamellothyrea* can easily be separated on the basis of their key differences at the level of the clypeal armour, parameres of the male genitalia and the general body colour and ornamentation. In particular, both clypeal horns and longitudinal blade are more pronounced and developed in *L. isimangaliso* sp. n. than in *L. descarpentriesi*. The clypeal horns of *L. isimangaliso* are narrower but projected further forward than those of *L. descarpentriesi*; they are also sharper and form a distinct point at the internal apex. The total body length of *L. descarpentriesi* appears to be slightly shorter than that of *L. isimangaliso* and the tomentose maculation on the dorsal surface is much more developed in the former than in the latter species. *Lamellothyrea descarpentriesi* is also more uniform in its background colouration, which is

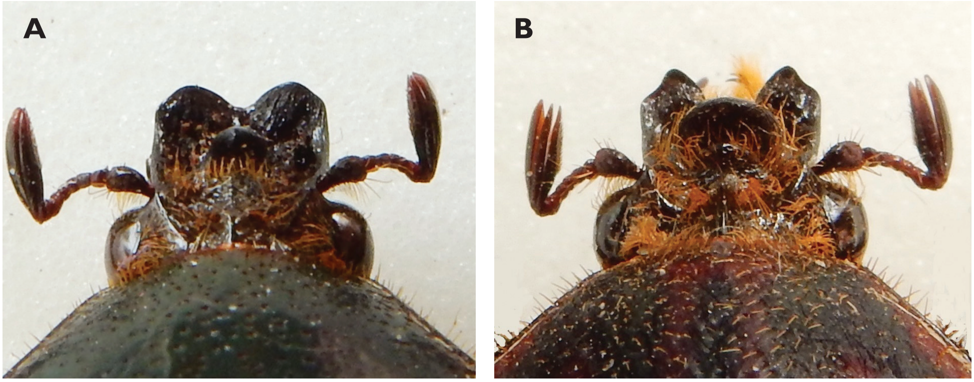

Figure 2. Clypeal armour of **A** *Lamellothyrea descarpentriesi* (Marracuene, Mozambique) and **B** *Lamellothyrea isimangaliso* sp. n. (St Lucia, South Africa).

consistently dark green, while a purple sheen and even brown-green dominance generally prevails in *L. isimangaliso*. Finally, the aedeagal parameres are slightly longer in *L. isimangaliso*, with their dorsal lobes narrowing substantially in the middle and then forming a sinuate apex with a visible protuberance on each side. In *L. descarpentriesi*, the dorsal lobes are more compact and virtually lack both central constriction and apical protuberances.

Description of holotype male (Figs 1B, 2B, 3C–D, 4). *Size*. Length 20.3; width 11.0 mm.

Body. Dark green to purple, with blackish maculation on elytra and residual tomentum on pronotal sides and elytral surface (Figs 1B, 2B, 4); velutinous, with remarkable sheen when exposed to direct light (Fig. 4).

Head. Black to dark green towards vertex; clypeus deeply concave and sharply upturned at anterior margin, where two symmetrical lateral horns with external indentation project forward (Fig. 2B); frons with one major transverse round lamina, one minor frontal lamina and one longitudinal ridge extending to posterior end of vertex; numerous long, yellow to orange setae emerging from all cavities; frontal cavity forming secondary, shorter horns at lateral margins; entire surface covered in coarse, round punctures or geminate striae, except on clypeal ridges and supra-ocular tubercle (Figs 1B, 2B); antennal clubs dark brown, of normal cetonid length, of approximately the same length of flagellum; pedicel dark brown to black, flagellum brown, both bearing scattered but long, erected yellow setae.

Pronotum. Octagonal with angles smoothly rounded; lateral margins carinate and exhibiting white cretaceous band often interrupted, particularly near base and apex; posterior margin trisinuate with pre-scutellar arch marked; purple to brown, with widespread iridescence and five longitudinal black to dark-green bands (narrow one at center and two broader ones on each side); matt with dense crescent to semi-crescent sculpture, except on pre-scutellar arch; short, scattered yellow setae present throughout surface, longer at margins but absent on pre-scutellar arch (Figs 1B, 4).

Figure 3. Dorsal (**A**) and lateral (**B**) views of aedeagal parameres of *Lamellothyrea descarpentriesi* (Marracuene, Mozambique); dorsal (**C**) and lateral (**D**) views of same for *Lamellothyrea isimangaliso* sp. n. (St Lucia, South Africa).

Scutellum. Purple to brown and even black at base; generally smooth, with few scattered punctures and setae at margins only; broadly triangular with sharp apex and without lateral grooves (Fig. 1B).

Figure 4. *Lamellothyrea isimangaliso* sp. n. in its natural habitat at St Lucia Estuary (Photo: Lynette Clennell, March 2009).

Elytron. With moderately elevated sutural, discal, humeral and lateral costae; colour varying from purple to brown, with dark spots and bands particularly developed on apical half, on lateral declivity and above humeral callus; residual tomentose marking noticeable only along lateral declivity; both humeral and apical calluses pronounced; apical margin smoothly rounded, bearing a short proximal spine; crescent to horseshoe punctures on all interstrial surfaces, with very short yellow setae scattered throughout but becoming longer on lateral and apical declivities (Figs 1B, 4).

Pygidium. Triangular and remarkably convex; black to dark green, without any tomentum; with dense layered to wrinkled sculpture throughout and short yellow setae at centre, becoming much longer on apico-lateral margins.

Legs. Slender and elongate, with apical tarsal segments hypertrophic; protibia effectively bidentate, with third tooth obsolete and well-developed longitudinal grooves, with sparse short yellow setae, becoming longer and denser on inner margin; meso- and metatibia with longer and denser yellow setae, with striolate surfaces and mid spine on outer carina, distal margin bi- and tridentate respectively; spurs slender and sharply pointed, twice as long in metatibia than in mesotibia (Fig. 1B).

Figure 5. Typical habitat of *Lamellothyrea isimangaliso* sp. n. in the coastal forest on the Eastern Shores of Lake St Lucia (Photo: Nicola Carrasco, May 2010).

Ventral surface. Shiny dark brown to green; exhibiting small and sparse crescent sculpture throughout surface; with long and dense yellow setae throughout surface, except on metasternum, metafemora and abdominal sternites; mesometasternal lobe very round and broadly expanded anteriorly; abdominal sternites with visible concavity at centre.

Aedeagus. Parameres with blunt apex in both lateral and dorsal view (Fig. 3C); dorsal lobes narrower than ventral lobes and sharply constricted at mid length, then expanded again at apex; apex broad with sinuations and small sutural projections at centre (Fig. 3C–D).

Derivatio nominis. The name *L. isimangaliso* sp. n. reflects its known distribution range, which, with the exception of the southern locality of Lake Nhlabane, falls entirely within the iSimangaliso Wetland Park, South Africa's first UNESCO World Heritage Site.

Description of female. There is little sexual dimorphism in this species. The main difference lies in the female exhibiting a tridentate and more enlarged protibia than the male. The general body shape of the female also appears more globose than that of the male, particularly at the level of the abdominal sternites, which bulge out quite significantly to impart a convex shape. The length of the antennal clubs is slightly shorter than in the male. Finally, the female metatibial spurs are more blunt, concave

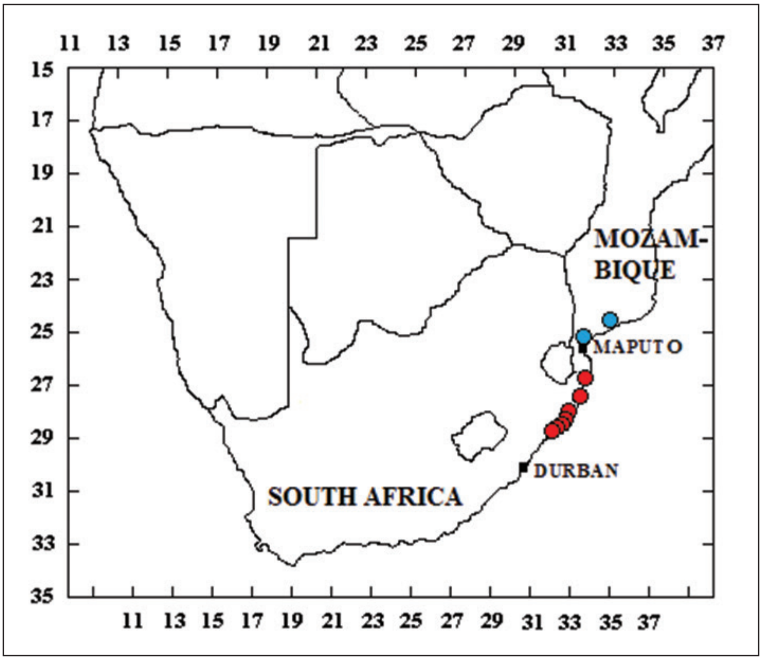

Figure 6. Known distribution of *Lamellothyrea descarpentriesi* (blue circles) and *L. isimangaliso* sp. n. (red circles).

and laterally expanded, assuming the shape of a typical fossorial organ used to burrow into the dune sand (Fig. 3B).

Distribution. The species appears to be virtually restricted to the coastal dune forest of the iSimangaliso Wetland Park. The only known record outside the Park is from Lake Nhlabane, which is located about 10 km southeast of its southern boundary.

Conservation status. Although there are indications that coastal dune mining maybe negatively impacting the population of *L. isimangaliso* at the southernmost end of its distribution range, there are currently no threats to the species, as its habitat within the iSimangaliso Wetland Park is entirely under statutory protection. The Park was proclaimed as South Africa's first UNESCO World Heritage Site in 1999, and is particularly recognised for its exceptional biodiversity and as a center of endemism (Porter 2013).

Biology. Although its larval stages remain unknown, *L. isimangaliso* is a typical coastal forest dweller. The period of adult activity mirrors the pattern of rainfall in the region, with a bimodal distribution and peaks in November-December and February-March. Both sexes have been repeatedly observed feeding on flowers and fruits of dune waterberry trees, *Sizygium cordatum* and have occasionally been trapped using aerial devices baited with fermenting fruits (Combrink and Kyle 2006).

Remarks. Within the type series, the size ranges as follows: ♂ length 20.1–21.4 mm, width 11.0–11.4 mm (n = 21); ♀ length 21.2–21.8 mm, width 11.3–12.0 mm (n = 11).

There is substantial variability in the background colouration of the numerous specimens of *L. isimangaliso* sp. n. examined, ranging from light-brown to purple and dark-green. The velutinous purple sheen is, however, the most dominant chromatic form in both sexes. The residual tomentose spots on the elytral and pronotal margins are always poorly noticeable and can virtually disappear completely in some specimens. The vittate pronotum seems to be a consistent feature, but the longitudinal bands range in colour from black to dark-green and even brown. The pygidial background colour is invariably dark green, but occasionally it exhibits residual tomentum near the basal margin.

Type material examined. Holotype (♂): South Africa, KZN, St Lucia, 19–20 Feb 2000, R Perissinotto & L Clennell (TMSA). Paratypes: 2♂♂, as above (PCPC); 1 ♀, as above, but 13 Jan 2001; 1♂, as above, but 17 Feb 2001 (PCPC); 1♀, as above, but 12–13 Feb 2000; 1♀, as above, but 01 Jan 1999 (PCPC); 2♂♂, as above, but 28 Feb 99 (PCPC); 1♂, as above, but 30 Oct 1999; 4♂♂, 4♀♀, as above, but 20–23 Mar 2004 (ISAM, DMSA); 1♂, but False Bay, 4 Apr 2012 (PCPC); 1♂, KZN, iSimangaliso, Dec 2002, X. Combrink leg (PCPC); 1♂, KZN, Kosi Mouth, 21/10/2003, R Kyle leg (PCPC); 1♂, Natal, Mapelane, 12/93, I.R. Willem (PCPC); 14♂♂ + 5♀♀, South Africa KwaZulu-Natal, 6 km N of St. Lucia Bay, S27°38'16", E32°26'24" 20 m a.s.l., 22–30 Dec 1990, P. Stobbia *leg.* (PCBM); 12♂♂ + 7♀♀, South Africa, KwaZulu-Natal, St. Lucia Bay S28°22'13", E32°24'50" 23 m a.s.l., 12 Dec 1994, A.P. Marais *leg.* (PCBM); 1 ♀, South Africa KZN, Sodwana Bay 27.33S, 32.40E 30 m, 1987/03/05, Reavell PE leg., Coastal dune forest, Feeding on fruit (unspecified) (SANC, COLS16846); 8♂♂ + 6♀♀, South Africa KZN, near Lake Nhlabane, 25 km NE of Richards Bay 28.38S 32.16E, 1991/02/03, Vogt M leg., Coastal dune forest, Hanging fermenting fruit bait trap, Specimen from University of Pretoria research programme into post-mining dune rehabilitation at Richards Bay Minerals, In rehabilitating dune forest 12 years post mining (SANC, COLS16847); 1♀, South Africa KZN, near Lake Nhlabane, 25 km NE of Richards Bay 28.38S, 32.16E, 1992/03/16 to 1992/03/20; Vogt M leg., Coastal dune forest, Hanging fermenting fruit bait trap, Specimen from University of Pretoria research programme into post-mining dune rehabilitation at Richards Bay Minerals (SANC, COLS16848); 1♂, South Africa KZN, Sodwana Bay 27.33S, 32.40E, 1988/01/05 to 1988-01-06, E Holm E & E Marais leg., Hanging fermenting fruit bait trap (SANC, COLS16849); 10 ind., South Africa, Zululand Natal, Sodwana Bay 5 km S, 27.35S, 32.39E, 23/11/1992, Endrody-Younga E-Y: 2845, Ground traps, 4 days, with banana bait (TMSA, CPH6477); 14 ind., South Africa, Zululand Natal, Sodwana Bay 5km S, 27.35S, 32.39E, 23/11/1992, Endrödy-Younga leg. E-Y: 2846, Hanging fruit traps (TMSA, CPH6478); 1 ind., South Africa, Zululand Natal, Sodwana Bay 5km S, 27.35S, 32.39E, 23/11/1992, Endrödy-Younga leg. E-Y: 2847, Grass netting (TMSA, CPH6479); 2 ind., South Africa, Natal, Sodwana Bay 27.32S, 32.42E, 16/1/1992, M. Vogt & E. Holm leg. (TMSA, CPH6480); 1 ind., South Africa, Natal, Sodwana Bay, 4–12–1988, HP Terblanche leg., Waterbessie (TMSA, CPH6481); 1 ind., South Africa, Zululand Natal, Sodwana Bay, 28/2/1982

(TMSA, CPH6482); 1 ind., South Africa, Natal, Sodwana Bay Nat. Res., 27.32S 32.4E, 1/1988, O. Bourquin leg., Baited forest trap (TMSA, CPH6483); 1 ind., South Africa, Natal, Sodwana Bay Nat. Park Cmpgrd., 9–13/11/1986, Evans, d'Hotman & Nel leg. (TMSA, CPH6484); 1 ind., South Africa, Natal, Cape Vidal Park Cmpgrd., 28.07S, 32.33E, 13–15/11/1986, Evans, d'Hotman & Nel leg. (TMSA, CPH6485); 1 ind., South Africa, Natal, Cape Vidal, St. Lucia Nat. Res., 28.08S 32.33E, 1–4/1/1988, E. Holm & E. Marais leg., Bait traps (TMSA, CPH6486); 2 ind., South Africa, KZN, St Lucia, 22 Mar 2004, R Perissinotto & L Clennell (DMSA, COL35760, COL35760); 1 ind., as above but 26 Oct 2004 (DMSA, COL35762); 3 ind., as above but 1 Oct 2008 (DMSA, COL35763, COL35764, COL35765); 2♂♂ + 1♀, RSA KwaZulu-Natal, St Lucia, 20–22/03/2004, Perissinotto & Clennell (TGPC); 2♂♂ + 2♀♀, RSA N. Natal, Sodwana Bay, 03/1994 (TGPC); 3♂♂ + 2♀♀, South Africa KZN, St Lucia, 20–22 Mar 2004, R Perissinotto & L Clennell (CDPC); 3 ind., South Africa, KwaZulu-Natal, St. Lucia –28.37.500S, 32.42694E, 20–22/3/1904 (most likely 2004), (ISAM, COL-A059118); 3♂♂ + 2♀♀, S-Africa Natal, Sta Lucia, 1–91, Ex collection Dr. Vincent Allard received from Christophe Allard 21.II.2015 (GBPC); 1♂, R.S.A. Zululand, Sodwana Bay, II-1987. P. Stobbia, Ex collection Dr. Vincent Allard received from Christophe Allard 21.II.2015 (GBPC); 1♀, R.S.A. Zululand, Sodwana Bay, 7–XI–1987. P. Stobbia, Ex collection Dr. Vincent Allard received from Christophe Allard 21.II.2015 (GBPC); 1♂ + 1♀ , RSA Natal, St. Lucia, 1.1991, coll. V. Allard (GBPC); 1♂ + 1♀, No data, Ex collection Dr. Vincent Allard received from Christophe Allard 21.II.2015 (GBPC); 1♀, RSA Natal, St. Lucia, 3.1992, coll. Owen (GBPC); 1♂, South Africa, , KZN, St Lucia 31 Oct 2008, R. Perissinotto & L. Clennell leg. (SRPC); 1♀, 11–XII–2008, South Africa, KZN, 11 Dec 2008, St Lucia R. Perissinotto & L. Clennell leg. (SRPC).

Discussion

Holm and Marais (1992) interpreted the different clypeal horn exhibited by the type specimen of *Lamellothyrea descarpentriesi* (used by Krikken in his description), compared to the other specimens from KwaZulu-Natal (KZN), as a result of wearing off of the apical angles. In the absence of other specimens of the true *L. descarpentriesi*, presumably they believed that the type specimen was an old individual, while all other specimens represented freshly emerged beetles. However, the recent availability of a further fresh specimen and photographs posted on the citizen science platform iSpot (Silvertown et al. 2015) of the true *L. descarpentriesi* have allowed a somehow unexpected resolution to their interpretation. Two separate species are obviously involved in this genus, with one distributed in Mozambique in an area of unknown extension to the north of Maputo Bay, and the other restricted to the Maputaland coast of KZN, from Kosi Bay in the north to Lake Nhlabane in the south. In fact, with the exception of the latter locality, which falls a few kms outside the borders of the iSimangaliso Wetland Park, the entire range of *L. isimangaliso* sp. n. is essentially inside the park itself.

All specimens reported in the illustrations contained in Holm and Marais (1992), Rigout and Allard (1992), and Sakai and Nagai (1998) belong unequivocally in *L. isimangaliso*. Interestingly, the known distribution of the leaf chafer species *Asthenopholis rex* Harrison, 2009 and *A. adspersa* (Boheman 1857) is almost identical to this, and they are also coastal dune endemics (Harrison 2009).

The relatively short gap in distribution between the two species, i.e. about 130 km, is surprising to some extent, but actually involves a major climatic and vegetation transition from wetter coastal forests in the south to the drier swamp forests and Indian Ocean coastal belt vegetation in the north-east (Mucina and Rutherford 2006). Indeed, records of *L. isimangaliso* to the north of the coastal forests around Sodwana Bay and Lake Sibayi are few, and Kosi Bay itself has only one confirmed record thus far. On the other hand, the distance between the South African border inland of Maputo (formerly the Transvaal Province) to the Mozambican capital city itself is only about 60 km. Thus, it is possible that the vague locality of "Transvaal" associated with the holotype described by Krikken (1980) may represent a simple mislabelling error by a collector travelling from the South African province to the popular holiday destinations on the Mozambique coast. It is also plausible though that the holotype specimen may represent an unusual struggler occurrence of a specimen on a random dispersal trajectory.

While the only two dated records available for *L. descarpentriesi* show that this species is active at least from late winter (August) to early spring (October), in northern KZN adults of *L. isimangaliso* appear to be especially active during the rainy months, from October through May. In accordance with what was previously reported by Holm and Marais (1992), this species shows a population peak in November/December. However, records suggest it flies well beyond February, actually reaching a second lower peak in abundance around March. This follows the pattern of rainfall along the north coast of KwaZulu-Natal, with summer rains beginning in late September to early October, escalating in November, then subsiding in December/January to increase again in February/March and eventually ending in May.

Unfortunately, virtually nothing is yet known about the biology/ecology of *L. descarpentriesi*, as no other information is provided on the data labels that accompany the few specimens that are currently known. On the other hand, *L. isimangaliso* shows a preference for dense forest clumps and the immediate outskirts of forests. It has mostly been collected using fruit-baited traps containing either fermenting banana, pineapple, or a fruit-wine-sugar mixture. It has also been observed and captured hovering above, and feeding on, fruits and flowers of *Syzigium cordatum* (waterberry tree) and on sap of *Ziziphus* sp. (cf. specimen data labels). This species has so far only been observed in a narrow coastal forest belt, and appears to avoid moving further inland. This restricted distribution suggests that it is linked to a very specific microclimate and possibly able to tolerate only a narrow range of environmental variability. In this context, the protection afforded by the special status of the iSimangaliso Wetland Park, as an UNESCO World Heritage Site, is crucial towards the survival of this species in the long term.

Acknowledgements

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RESEARCH ARTICLE

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A review of the genus *Lordiphosa* Basden in India, with descriptions of four new species from the Himalayan region (Diptera, Drosophilidae)

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Abstract

All Indian species of the genus *Lordiphosa* Basden are reviewed, with descriptions of four new species, *L. curva* Fartyal & Toda, **sp. n.** of the *denticeps* species group and *L. ayarpathaensis* Kandpal & Singh, **sp. n.**, *L. makaibarensis* Pradhan & Chatterjee, **sp. n.** and *L. srinagarensis* Sati & Fartyal, **sp. n.** of the *nigricolor* species group. Two of the new species, *L. ayarpathaensis* and *L. makaibarensis*, were found visiting flowers of *Hedychium spicatum* and *Datura suaveolens*, respectively. This is the first record of flower visitation in *Lordiphosa* flies. In addition, *L. parantillaria* (Kumar & Gupta, 1990), **syn. n.** is synonymized with *L. antillaria* (Okada, 1984). Supplementary and revised descriptions for *L. antillaria* and *L. neokurokawai* (Singh & Gupta, 1981) and a key to all Indian species of *Lordiphosa* are provided.

Keywords

Darjeeling, key, *Lordiphosa denticeps* species group, *Lordiphosa nigricolor* species group, new synonymy, redescription, Uttarakhand

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* These authors contributed equally to the present study.

Introduction

The genus *Lordiphosa* Basden is a moderately-sized genus of the family Drosophilidae, currently comprising 57 species (Brake and Bächli 2008). The taxonomy of this genus had once been confused by assignment of some species to the subgenera *Sophophora* Sturtevant (Kikkawa and Peng 1938, Okada 1956, 1966, 1974, 1977, Lee 1959, Takada and Okada 1960, Bock and Wheeler 1972), *Hirtodrosophila* Duda (Okada and Sasakawa 1956, Okada 1966, 1967, 1971, 1988, Singh and Gupta 1981), or *Drosophila* Fallén (Duda 1935) of the genus *Drosophila*. However, some revisional works (Laštovka and Máca 1978, Okada 1984, 1990) reclassified those species into the subgenus *Lordiphosa* of the genus *Drosophila*. Grimaldi (1990) elevated *Lordiphosa* to the generic rank according to morphological characters. Furthermore, Hu and Toda (2001) showed, by a cladistic analysis focusing on *Lordiphosa*, that the *tenuicauda* species group, initially included in *Lordiphosa* (Toda 1983, Hu et al. 1999), is remotely related to the *Lordiphosa* proper, and Hu and Toda (2002) transferred all species of the *tenuicauda* group to the revised genus *Dichaetophora* Duda. A molecular phylogenetic study by Gao et al. (2011) has revealed that *Lordiphosa* is the sister group to the Neotropical *Sophophora* consisting of the *Drosophila saltans* and *D. willistoni* species groups.

The genus *Lordiphosa* is distributed from the tropics of Oriental Region (Okada 1984, 1988, Toda unpublished data) to the subarctics of Palearctic Region (Toda et al. 1996, Bächli et al. 2004), with the highest species richness in the subtropics of Oriental Region (Okada 1966, 1984, Zhang 1993a, b, Zhang and Liang 1992, 1994, Quan and Zhang 2001, 2003). However, this genus has been poorly represented in India: only seven species have been recorded (Dwivedi and Gupta 1980, Singh and Gupta 1981, Kumar and Gupta 1990, Gupta and Gupta 1991, De and Gupta 1996, Gupta 2005, Upadhyay and Singh 2007).

Four new species of *Lordiphosa* have been discovered from India. Two of them were found visiting flowers of *Hedychium spicatum* Smith (Zingiberaceae) in Kumaon, Uttarakhand and of *Datura suaveolens* (Humb. & Bonpl. exWilld.) Bercht. & J. Presl (Solanaceae) in Darjeeling, West Bengal. Until now, *Lordiphosa* flies were known for breeding on herbage plants, and their larvae feeding on decayed tissues of leaves and stems (Kimura et al. 1977, Shorrocks 1982, Toda et al. 1984) or living tissue of leaves as leaf minors (Okada and Sasakawa 1956). This paper describes the four new species, and reviews all known Indian species of *Lordiphosa* with supplementary and revised descriptions for some species, and provides a key to all Indian species of *Lordiphosa*.

Materials and methods

Specimens used for the present study were collected from four different hill stations of the Himalayan region in India: Chopta (2,700 m a.s.l.; 30°29'N, 79°10'E) in Rudraprayag district; Ayarpatha (2,278 m a.s.l.; 29°23'N, 79°27'E) in Nainital district; Kurseong subdivision of Darjeeling hills (1,458 m a.s.l.; 26°53'N, 88°17'E) in West Bengal; and HNBGU Forestry nursery (560 m a.s.l.; 30°13'N, 78°47'E) at Chauras Srinagar Garhwal in Uttarakhand. These localities are covered with dense mixed-deciduous subtropical forests, under extremely moist condition due to heavy rainfall during the summer monsoon season. The temperature ranges approximately from 3°C to 24°C. Specimens were collected by net sweeping or directly from flowers of *Hedychium spicatum* and *Datura suaveolens* by an aspirator, and preserved in 70% ethanol. In addition, some specimens collected from China were examined to give supplementary and revised descriptions for some known species.

External morphology of adult flies was examined under a stereomicroscope and metric characters were measured with an ocular micrometer. To observe detailed structures, the male and female terminalia and some other organs were detached from the body, cleared by warming in 10% KOH solution at approximately 100°C for several minutes, mounted in a droplet of glycerin on a cavity slide, examined under a light microscope, and some samples imaged using a DinoLite® Digital Eyepiece Camera.

The morphological terminology and the definition of measurements and indices mostly follow McAlpine (1981), Zhang and Toda (1992) and Hu and Toda (2001). All the holotypes and some paratypes of new species are deposited in the Department of Zoology, H.N.B Garhwal University, Chauras Campus, Srinagar-Garhwal, Uttarakhand, India (**DZHNBGU**), some paratypes in Museum of Zoological Survey of India, Kolkata, India (**MZSIK**) and the remaining paratypes in the Systematic Entomology, Hokkaido University Museum, Hokkaido University, Sapporo, Japan (**SEHU**).

Systematic accounts

Genus *Lordiphosa* Basden

Lordiphosa Basden, 1961: 186 (as a subgenus of *Drosophila*); Laštovka and Máca 1978: 404; Okada 1984: 571. Type species: *Drosophila fenestrarum* Fallén, 1823.

Lordiphosa: Grimaldi, 1990: 121 (new status as genus); De and Gupta 1996: 131; Bächli et al. 2004: 250.

Diagnosis. Prementum thicker in ventral than in dorsal portion from lateral view (Figs 2B, 3C, 4A), ventrally more or less expanded in posterior view (Figs 2C, 4B). Paramere ventrobasally articulated with hypandrium (Figs 1D, 2H, 3E, 4E, F, 7D). Hypandrium lacking paramedian setae.

Lordiphosa denticeps species group

Drosophila (*Hirtodrosophila*) *denticeps* species-group, Okada, 1967: 3.

Lordiphosa denticeps species-group: Zhang, 1993b: 144.

Diagnosis. Male foreleg tarsomeres I to III often with sex combs (Figs 1B, 2D). Surstylus with numerous recurved setae on ventral portion of inner surface in addition to primary prensisetae on caudobasal margin (Figs 1C, 2F). Ventral margin of cercus tapering or truncated, fringed with a row of stout spines (Figs 1C, 2F). Aedeagus membranous, hirsute apically, fused with posterior, roof-like gonopod (Figs 1E, 2H). Oviscapt with numerous lateral ovisensilla (Figs 1F, 2I).

Remarks. Okada (1967) proposed the *denticeps* group as a new species group of *Hirtodrosophila* (a subgenus of *Drosophila* at that time), including two species so far described, *denticeps* Okada & Sasakawa, 1956 and *tripartita* Okada, 1966. However, it had been noticed that these two species have aberrant morphological characters inconsistent with the definition of *Hirtodrosophila*. Three more species were subsequently added to this species group (Okada 1971, Singh and Gupta 1981). Then Okada (1990) transferred the members of this species group to *Lordiphosa* (a subgenus of *Drosophila* at that time), but considered that the *denticeps* group was synonymous with the *nigricolor* group proposed by Laštovka and Máca (1978). Then, Grimaldi (1990) elevated *Lordiphosa* to the generic rank, and Zhang (1993b) resurrected and redefined the *denticeps* group as a species group independent from the *nigricolor* group in the genus *Lordiphosa*.

Key to Indian species of the *denticeps* group

1 Arista with one ventral branch (except terminal fork) **2**

– Arista with two ventral branches .... ***tripartita* (Okada, 1966)**

2 Setae of all, approximately 15 TBRs (Transverse Bristle Rows; Baumina and Kopp 2007) on tarsomere I of ♂ foreleg thick, forming sex combs (Fig. 1B); gonopod not concaved proximally on posterior margin in lateral view (Fig. 1E); ♀ abdominal tergite VIII without setae (Fig. 1F); oviscapt with approximately 100 lateral ovisensilla (Fig. 1F); spermathecal capsule apically not indented (Fig. 1G)...................***neokurokawai* (Singh & Gupta, 1981)**

– Setae of only distal most TBR on tarsomere I of ♂ foreleg thick, forming sex comb (Fig. 2D); gonopod concaved proximally on posterior margin in lateral view (Fig. 2H); ♀ abdominal tergite VIII dorsally with approximately two setae per side (Fig. 2I); oviscapt with approximately 35 lateral ovisensilla (Fig. 2I); spermathecal capsule apically indented (Fig. 2J)............................ .... ***curva* Fartyal & Toda, sp. n.**

***Lordiphosa neokurokawai* (Singh & Gupta)**

Fig. 1

Drosophila (*Hirtodrosophila*) *neokurokawai* Singh & Gupta, 1981: 207.
Lordiphosa neokurokawai: Zhang, 1993b: 145.

Specimens examined. CHINA: 1♂, Sichuan, Mt. Emei, 2,000 m a.s.l., 19 July 1992; 1♂, 1♀, Yunnan, Kunming, 22 March 2005 (all in SEHU).

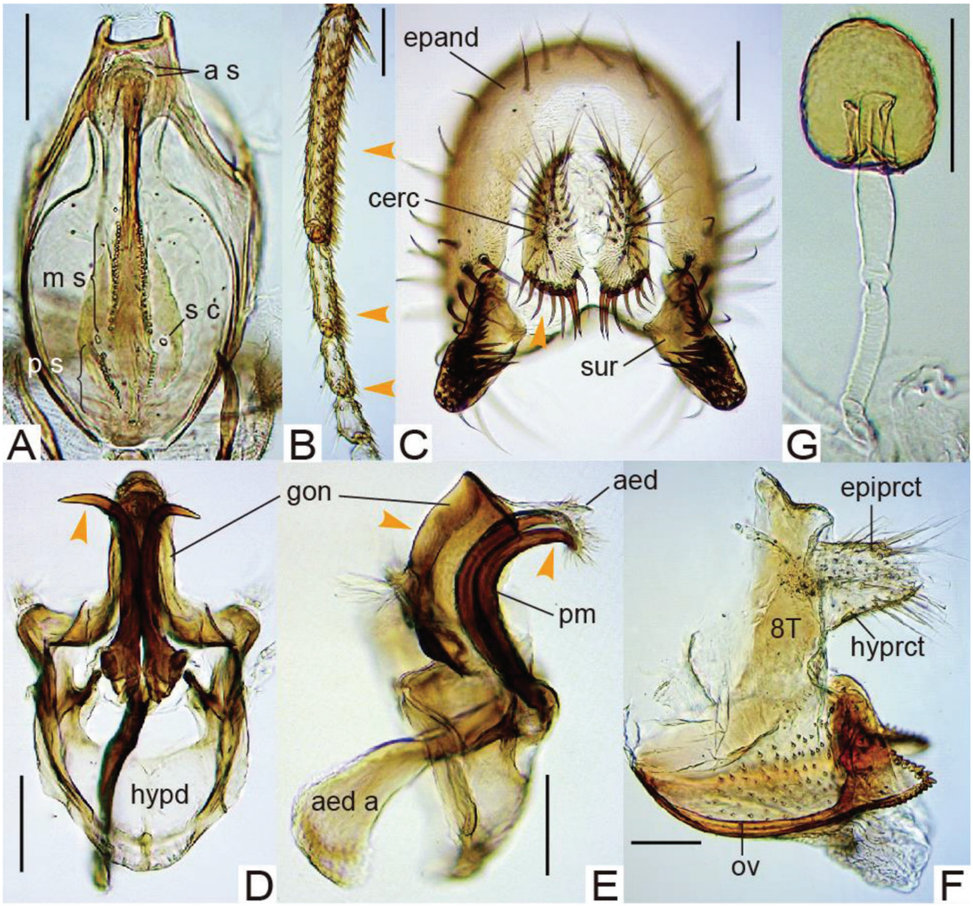

Figure 1. *Lordiphosa neokurokawai* (Singh & Gupta, 1981) (♂♀ from Kunming, Yunnan, China): **A** cibarium: anterior sensilla (a s), medial sensilla (m s), posterior sensilla (p s) and sensilla campaniformia (s c) (dorsal view) **B** foreleg tarsus **C** periphallic organs: epandrium (epand), cercus (cerc) and surstylus (sur) (caudal view) **D, E** phallic organs: aedeagus (aed), aedeagal apodeme (aed a), gonopods (gon), hypandrium (hypd) and paramere (pm) (**D** ventral view **E** lateral view) **F** ♀ terminalia: tergite VIII (8T), epiproct (epiprct), hypoproct (hyprct) and oviscapt (ov) **G** spermatheca. Arrowheads indicate the diagnostic characters. Scale bars 0.1 mm.

Diagnosis. Sex combs composed of thick setae of all, approximately 15 TBRs on anteroventral surface of tarsomere I, of four distal TBRs on tarsomere II and of two distal TBRs on tarsomere III (Fig. 1B). Cercus ventrally broadest, nearly horizontally truncated, with approximately seven large, stout spines on ventral margin (Fig. 1C). Gonopod not concaved proximally on posterior margin in lateral view (Fig. 1E). Paramere basally with strong knob, subapically without spinule (Fig. 1D, E). Female abdominal tergite VIII without setae (Fig. 1F). Oviscapt with approximately 100 small, trichoid lateral ovisensilla (Fig. 1F). Spermathecal capsule apically not indented (Fig. 1G).

Description (supplementary and revised). **Adult male.** *Head*. Eye with interfacetal setulae. Approximately 15 supracervical setae thin, apically more or less curved and pointed; postocular setae approximately 18; occipital setae 30–31, including medial tiny ones. Dorsolateral arms of tentorial apodeme divergent, nearly straight, reaching to fronto-orbital plate; dorsomedial arm 1/3 as long as dorsolateral arm. Interspace between antennal sockets narrower than half of socket width; first flagellomere with only one small invaginated pouch ("sacculus" called by earlier taxonomists: Ferris 1965); arista with 4–5 dorsal and one ventral branches in addition to terminal fork. Facial carina slightly elevated, narrower and shorter than first flagellomere, without setulae below. Subvibrissal seta distinctly shorter than vibrissa; additional row of oral setulae present above marginal row on anterior portion. Palpus with one prominent terminal and several short, subapical to lateroventral setae, without setulae on basal lobe. Cibarium (Fig. 1A) thickened on anterior margin, not dilated laterad in anterior portion; anterolateral projections shorter than half width of anterior margin; dorsal sclerite pear-shaped in dorsal view, anteriorly convex in lateral view; anterior sensilla two pairs, widely arranged in square behind anterior margin of hypopharynx; 23–26 medial sensilla arranged in anteriorly slightly convergent rows; two sensilla campaniformia; posterior sensilla very long, trichoid, gently curved forward, approximately 17 arranged in anteriorly divergent rows; somewhat sclerotized, thickened (in lateral view), anterior portion of hypopharynx shorter than 1/5 length of cibarium. Prementum ventrally slightly expanded. Labellum with five pseudotracheae per side.

Thorax. Postpronotal lobe with two prominent setae. Posterior dorsocentral seta situated nearer to anterior margin of scutellum than to anterior dorsocentral seta. Prescutellar setae absent. One or a few acrostichal setulae in lines with and anterior to dorsocentral setae thicker and longer than others. Mid katepisternal seta shorter than anterior katepisternal seta; anterior katepisternal seta thicker than aristal branches; no setula present anteriorly to anterior katepisternal seta.

Wing hyaline. Veins light brown; crossveins not clouded; bm-cu crossvein absent; R_{2+3} nearly straight; R_{4+5} and M_1 nearly parallel. Two C_1 setae unequal in size.

Legs. Foreleg tarsus with neither tuft of dense, soft hairs nor long setae. Foreleg tarsomere I as long as three succeeding tarsomeres together; midleg one slightly longer than three succeedings together; hindleg one slightly longer than rest together.

Abdomen. Setigerous sternite VI present.

Terminalia (Fig. 1C–E). Epandrium shallowly and widely notched on posterior mid-dorsal margin (Fig. 1C), nearly entirely pubescent except antero-lateral to -ventral margin, gently curved on caudosubmedial margin near articulation to surstylus, ventrally narrowing but apically somewhat roundish and not sclerotized, with approximately16 setae on medial to dorsal portion, approximately six setae on ventral lobe and unpubescent, inward fold on caudoventral margin. Surstylus articulated to epandrium, simple plate longer than wide and apically round, with 8–9 prensisetae on caudodorsal margin and 42–43 recurved setae on ventral portion of inner surface but neither pubescence nor peg-like setae on outer surface (Fig. 1C). Cercus separated from epandrium, nearly entirely pubescent except for lateral margin, with 32–33 setae (Fig. 1C). Membrane between cercus and epandrium pubescent dorsally. Lateral lobe of tenth sternite larger than median lobe. Hypandrium anteriorly fringed with arched apodeme, slightly pubescent on caudolateral plates fused to gonopod, with a pair of narrow sclerotized processes connecting between bases of parameres and lateral margins of hypandrium (Fig. 1D). Paramere long, sclerotized process curved ventrad medially and outward apically (Fig. 1D, E). Aedeagal basal processes degenerated. Gonopods fused with each other, forming roof-like plate posteriorly surrounding aedeagus (Fig. 1E).

Adult female. Head, thorax, wings and legs as in male, except for absence of sex combs on foreleg tarsus.

Terminalia (Fig. 1F, G). Tergite VIII entirely narrow, pubescent only on posterior portion (Fig. 1F). Epiproct and hypoproct entirely pubescent and setigerous (Fig. 1F). Oviscapt subapically broadest, apically triangular, with approximately 13 and 9 apically blunt, peg-like ovisensilla on apico-ventral and -dorsal margins, respectively; subapical, trichoid ovisensillum as long as largest, marginal one (Fig. 1F). Spermathecal capsule ellipsoidal; introvert half as deep as capsule height; outer duct not wrinkled in distal 1/3 (Fig. 1G).

Distribution. Southwestern China (Sichuan*, Yunnan), India (West Bengal) [* new record].

Remarks. This species was first described by Singh and Gupta (1981) based on three male specimens collected from Darjeeling, West Bengal, India. Later, Zhang (1993b) reported this species from southwestern China, based on some male and female specimens collected from Kunming, Yunnan, but did not describe the female characters. Here, the description of the female is provided, with a supplementary and revised description for male based on the specimens collected from southwestern China.

Lordiphosa curva Fartyal & Toda, sp. n.

http://zoobank.org/F7A1A1E5-DBE8-4D82-879A-112056A9710E
Fig. 2

Type material. *Holotype.* ♂: INDIA: Uttarakhand, Rudraprayag District, Chopta Forest. 30°27.560'N, 79°15.234'E, 2,700 m a.s.l., 31 August 2010, R. S. Fartyal leg. (DZHNBGU).

Paratypes. INDIA: 1♂, 1♀, same data as the holotype except 1 September 2010 (MZSIK, SEHU).

Diagnosis. Sex combs composed of thick setae of only distal most TBR on tarsomeres I–III (Fig. 2D). Cercus ventrally somewhat obliquely truncated, with approximately eight large, stout spines on ventral margin (Fig. 2F). Gonopod concaved proximally on posterior margin in lateral view (Fig. 2H). Paramere basally curved ventrad, apically much narrow, subapically with spinule (Fig. 2G, H). Female abdominal tergite VIII dorsally with approximately two setae (Fig. 2I). Oviscapt with approximately 35 small, lateral ovisensilla (Fig. 2I). Spermathecal capsule apically indented (Fig. 2J).

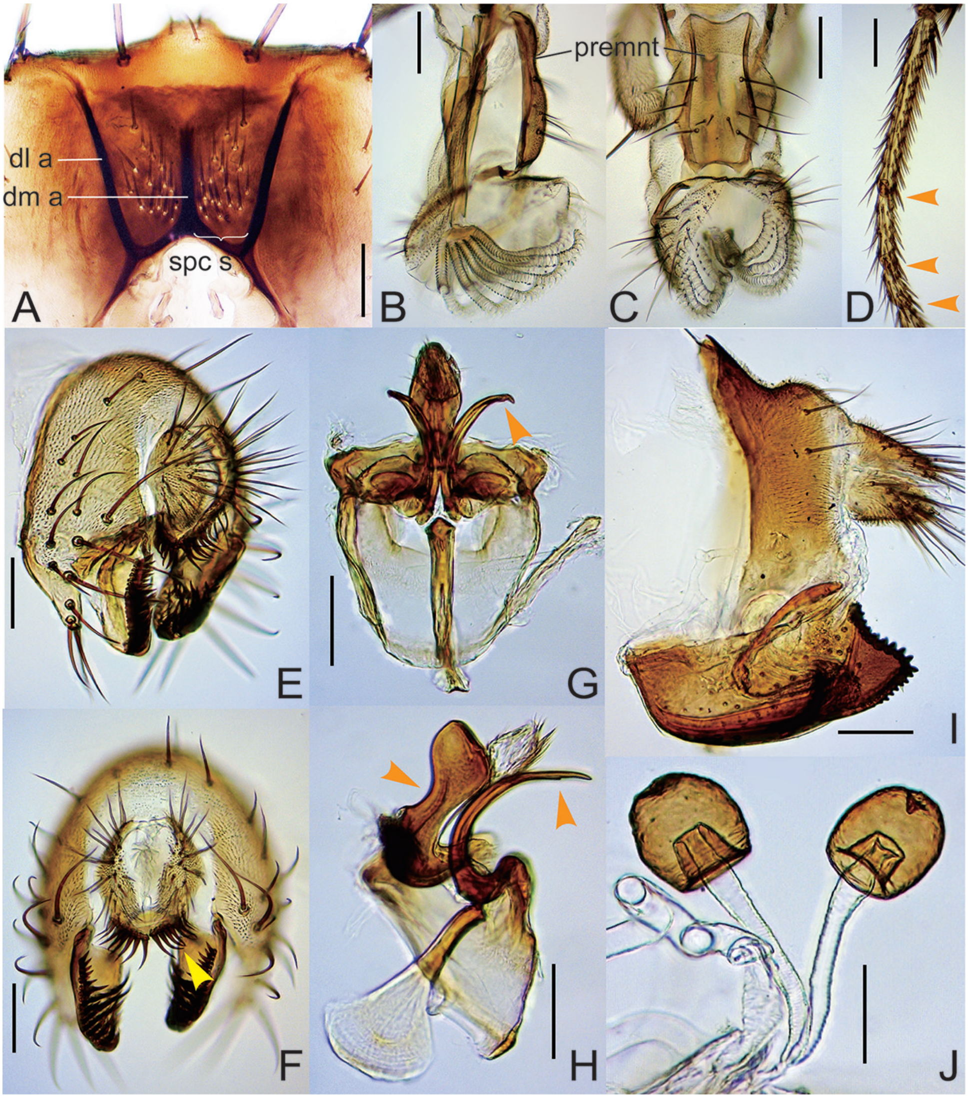

Figure 2. *Lordiphosa curva* Fartyal & Toda, sp. n. (♂♀ paratypes from Chopta Forest, Uttarakhand, India): **A** occiput: dorsolateral arms (dl a) and dorsomedial arm (dm a) of tentorial apodeme and supracervical setae (spc s) **B, C** proboscis: prementum (premnt) (**B** lateral view **C** posterior view) **D** foreleg tarsus **E, F** periphallic organs (**E** caudolateral view **F** caudal view) **G, H** phallic organs (**G** ventral view **H** lateral view) **I** ♀ terminalia **J** spermathecae. Scale bars 0.1 mm.

Description (not referring to characters commonly seen in the foregoing species, *L. neokurokawai*). **Adult male.** *Head.* Eye with dense, interfacetal setulae. Supracervical setae 16–18 (Fig. 2A); postocular setae approximately 19; occipital setae 21–25. Dorsolateral arms of tentorial apodeme divergent, apically curved outward; dorsomedial arm half as long as dorsolateral arm (Fig. 2A). Occiput orange yellow, medially dark brown; ocellar triangle and fronto-orbital plates glossy, orange yellow; frontal vittae mat, greyish orange. Pedicel greyish orange yellow; first flagellomere grey; arista with 3–4 dorsal and one ventral branches in addition to terminal fork. Face orange yellow. Gena orange yellow but dark brown on anteroventral margin. Clypeus orange brown. Palpus yellow. Cibarium: anterolateral projections longer than half width of anterior margin; medial sensilla 20–21; posterior sensilla approximately 20. Prementum ventrally slightly expanded, thicker in ventral than in dorsal portion from lateral view (Fig. 2B, C). Labellum with five pseudotracheae per side (Fig. 2B).

Thorax. Postpronotal lobe grey yellow, with two prominent setae: upper one 0.8 as long as lower one. Scutum and scutellum grey yellow but grey brown medially. Thoracic pleura grey yellow, with dark grey patches. Acrostichal setulae in six rows. Basal scutellar setae divergent; apicals cruciate.

Wing. C_1 setae two, subequal in size. Halter opaque white.

Legs grey yellow; tarsomere Vs of all legs darker. Foreleg femur with 4–6 long setae in two rows on outer side. Fore- and mid-leg tarsomere Is longer than three succeeding tarsomeres together; hindleg one longer than rest together. Preapical, dorsal setae present on tibiae of all legs; apical setae on tibiae of fore- and mid-legs.

Abdomen. Tergites grey yellow, each posteriorly darker. Sternites yellow.

Terminalia (Fig. 2E–H). Epandrium with 11–14 setae on medial to dorsal portion and approximately four setae on ventral lobe (Fig. 2E). Surstylus with 6–7 apically pointed prensisetae arranged along caudobasal margin and 40–42 recurved setae on ventral portion of inner surface (Fig. 2E, F). Cercus nearly entirely pubescent except for lateral to ventral margin, with 21–24 setae (Fig. 2E, F). Hypandrium caudolaterally pubescent and fused to gonopod, with a pair of inward extended plates apically articulated to bases of parameres (Fig. 2G).

Measurements (holotype / 1♂ paratype, in mm). BL (straight distance from anterior edge of pedicel to tip of abdomen) = 2.65 / 2.77, ThL (distance from anterior notal margin to apex of scutellum) = 1.30 / 1.42, WL (distance from humeral cross vein to wing apex) = 3.47 / 3.67, WW (maximum wing width) = 1.40 / 1.54.

Indices (holotype / 1♂ paratype, in ratio). FW/HW (frontal width / head width) = 0.51 / 0.55, ch/o (maximum width of gena / maximum diameter of eye) = 0.28 / 0.27, prob (proclinate orbital seta / posterior reclinate orbital seta in length) = 0.70 / 0.81, rcorb (anterior reclinate orbital seta / posterior reclinate orbital seta in length) = 0.32 / 0.35, vb (subvibrissal seta / vibrissa in length) = 0.52 / 0.50, dcl (anterior dorsocentral seta / posterior dorsocentral seta in length) = 0.65 / 0.64, sctl (basal scutellar seta / apical scutellar seta in length) = 1.18 / 1.17, sterno (anterior katepisternal seta / posterior katepisternal seta in length) = 0.57 / 0.50, orbito (distance between proclinate and posterior reclinate orbital setae / distance between inner vertical and posterior reclinate orbital setae) = 0.54 / 0.53, dcp (distance between ipsilateral dorsocentral setae / distance between anterior dorsocentral setae) = 0.55 / 0.57, sctlp (distance between ipsilateral scutellar setae / distance between apical scutellar setae) = 1.09 /1.03, C (2nd costal section between subcostal break and R_{2+3} / 3rd costal section between R_{2+3} and R_{4+5}) =3.70 / 3.21, 4c (3rd costal section between R_{2+3} and R_{4+5} / M_1 between r-m and dm-cu) = 0.62 / 0.69, 4v (M_1 between dm-cu and wing margin / M_1 between r-m and dm-cu) = 1.62 / 1.62, 5x (CuA_1 between dm-cu and wing margin / dm-cu between M_1 and CuA_1) = 1.64 / 1.70, ac (3rd costal section between R_{2+3} and R_{4+5} / distance between distal ends of R_{4+5} and M_1) = 2.08 / 2.51, M (CuA_1 between dm-cu and wing margin / M_1 between r-m and dm-cu) = 0.49 / 0.50, C3F (length of heavy setation in 3rd costal section + length of light setation in 3rd costal section) = 0.50 / 0.58.

Adult female. Head, thorax, wings, and legs as in male, except for absence of sex combs on foreleg tarsus.

Terminalia (Fig. 2I, J). Tergite VIII pubescent laterally to dorsally (Fig. 2I). Epiproct and hypoproct entirely pubescent and setigerous (Fig. 2I). Oviscapt broad from basal to subapical portion, apically triangular, with 9–11 apically blunt, stout, peg-like ovisensilla on apicodorsal margin and 13–15 ones proximally reducing in size and increasing in interspace on ventral margin (Fig. 2I). Spermathecal capsule ellipsoidal, basally horizontally wrinkled; introvert 2/5 as deep as capsule height (Fig. 2J).

Measurements (1♀ paratype, in mm). BL = 2.84, ThL = 1.39, WL = 3.54, WW = 1.44.

Indices (1♀ paratype, in ratio). FW/HW = 0.50, ch/o = 0.28, prorb = 0.76, rcorb = 0.34, vb = 0.38, dcl = 0.66, sctl = 1.26, sterno = 0.48, orbito = 0.57, dcp = 0.54, sctlp = 1.20, C = 3.30, 4c = 0.69, 4v = 1.64, 5x = 1.66, ac = 2.64, M = 0.50, C3F = 0.46.

Etymology. The specific name curva = “curved” refers to the paramere basally curved ventrally.

Distribution. India (Uttarakhand).

Remarks. This species closely resembles *L. neokurokawai* in having only one ventral branch of arista, the long, apically pointed paramere curved ventrad medially and outward apically, and the epandrium and the surstylus nearly identical in morphology, but can be clearly distinguished from it by the diagnostic characters.

Lordiphosa tripartita (Okada)

Drosophila (*Hirtodrosophila*) *tripartita* Okada, 1966: 78.
Lordiphosa tripartita: Zhang, 1993b: 145; Upadhyay and Singh 2007: 185.

Distribution. Nepal, India (Uttarakhand).

Lordiphosa nigricolor species group

Drosophila nigricolor species group, Laštovka & Máca, 1978: 418.
Lordiphosa nigricolor species group: Bächli et al., 2004: 264.

Diagnosis. Acrostichal setulae in line with and anterior to dorsocentrals as long as others. Male foreleg tarsomeres without sex combs. Epandrium more or less projected or expanded on caudosubmedial margin near surstylus (Figs 3D, 4C, D, 6A, B, 7B).

Key to Indian species of the *nigricolor* group

1 Epandrium with large flap covering surstylus on caudosubapical margin (Figs 4C, D, 6A, B); oviscapt medially broad and humped in lateral view, distally narrowing and curved ventrad, with large, sclerotized perineal plate between them (Figs 4G, H, 6E, F)..**2**

– Epandrium without large flap on caudosubapical margin (Figs 3D, 7B); oviscapt neither medially humped in lateral view nor distally curved ventrad, without sclerotized perineal plate between them (Fig. 3G)........................**3**

2. Epandrial, caudosubapical flap serrate on dorsal margin (Fig. 4C, D) .... ***ayarpathaensis* Kandpal & Singh, sp. n.**

– Epandrial, caudosubapical flap not serrate on dorsal margin (Fig. 6A, B)...... .... ***makaibarensis* Pradhan & Chatterjee, sp. n.**

3 Paramere apically not hirsute, unevenly bifurcated (Fig. 3F) .... ***antillaria* (Okada, 1984)**

– Paramere apically hirsute (Fig. 7C, D) **4**

4 Hypandrium not shorter than twice of width (Fig. 7C) **5**

– Hypandrium shorter than twice of width...**6**

5 Ventral branches of parameres symmetric in length, apically truncated; arista with five dorsal and two ventral branches in addition to terminal fork......... .... ***nigrovesca* (Lin & Ting, 1971)**

– Ventral branches of parameres asymmetric in length, apically pointed (Fig. 7C, D); arista with 6–7 dorsal and 3–4 ventral branches (Fig. 7A)........ .... ***srinagarensis* Sati & Fartyal, sp. n.**

6 Epandrial, ventral lobe not extending beyond distal end of surstylus............ .... ***coei* (Okada, 1966)**

– Epandrial, ventral lobe much elongated, extending beyond distal end of surstylus **7**

7. Hypandrium triangular, anteriorly narrowing; dm-cu crossvein somewhat clouded..***himalayana* (Gupta & Gupta, 1991)**

– Hypandrium quadrate; dm-cu crossvein clear ....***peniglobosa* (Kumar & Gupta, 1990)**

***Lordiphosa antillaria* (Okada)**

Fig. 3

Drosophila (*Lordiphosa*) *antillaria* Okada, 1984: 565.
Lordiphosa antillaria: Zhang et al., 1996: 349.
Drosophila (*Lordiphosa*) *parantillaria* Kumar & Gupta, 1990: 27. **Syn. n.**

Specimens examined. INDIA: 5♂, 3♀, West Bengal, Assam, Bagdogra, 29 November 1981 (NSMT: National Museum of Nature and Science, Tsukuba, Japan; SEHU); 7♂,

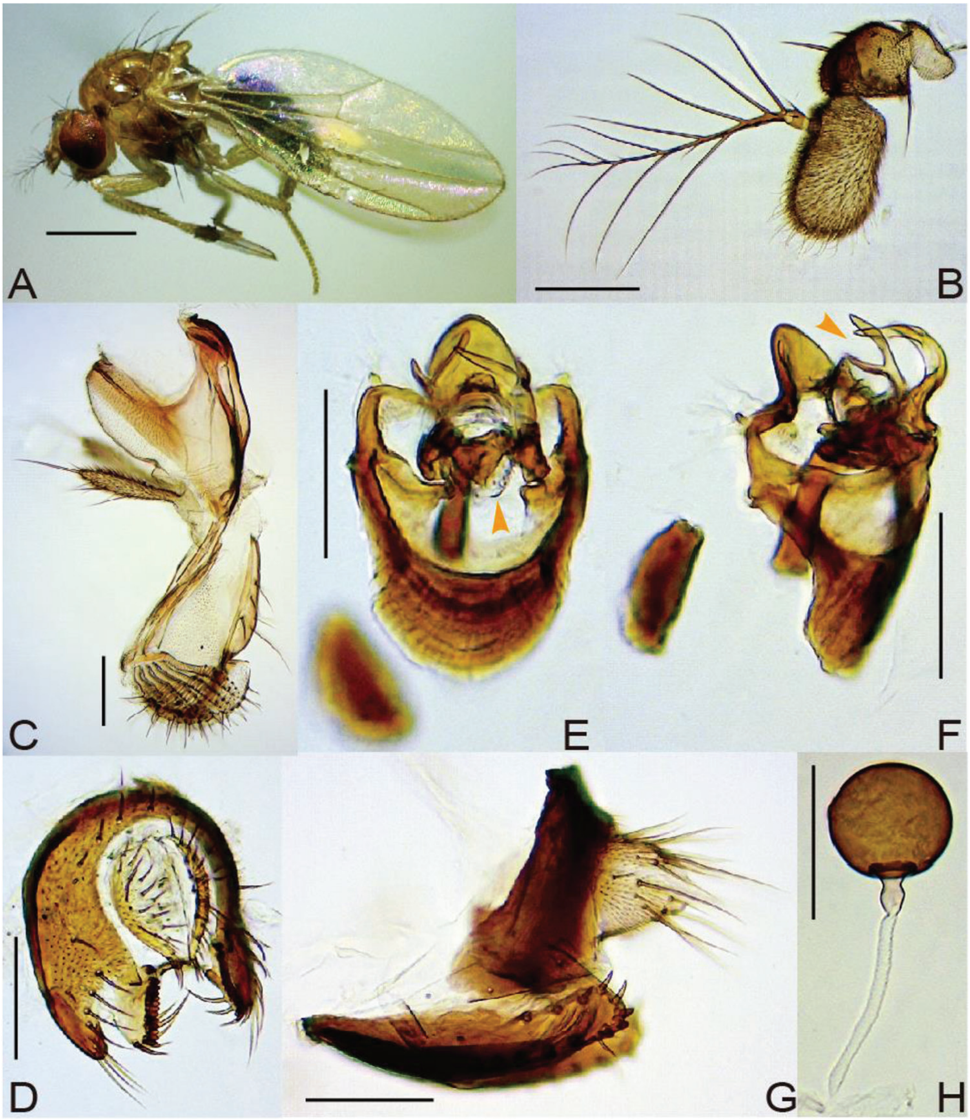

Figure 3. *Lordiphosa antillaria* (Okada, 1984) (♂♀ from Bagdogra, Assam, West Bengal, India): **A** ♂ left lateral habitus **B** antenna **C** mouthparts (lateral view) **D** periphallic organs (caudolateral view) **E, F** phallic organs (aedeagal apodeme broken) (**E** ventral view **F** ventrolateral view) **G** ♀ terminalia **H** spermatheca. Scale bars 0.5 mm **A**; 0.1 mm **B–H**.

1♀, Uttarakhand, Srinagar-Pauri Garhwal, Develgarh, 26 January 2011 (DZHNB-GU, MZSIK, SEHU). MYNMAR: 1♂, Pyin Oo Lwin, 30 December 1981 (SEHU). TAIWAN: 1♂, Chitou, 20 January 1982 (SEHU); 11♂, 10♀, Chitou, 8 January 2008 (SEHU); 1♂, Fushan, 17 April 1997 (SEHU).

Diagnosis. Paramere apically unevenly bifurcated (Fig. 3F). Aedeagus membranous, with numerous spinules (Fig. 3E).

Description (supplementary and revised). **Adult male.** *Head*. Eye with sparse, interfacetal setulae. Occiput dark brown in upper half, pale yellow in lower half. Approximately 13–18 supracervical setae thin, apically more or less curved and pointed; postocular setae 12–18; occipital setae 7–11, including medial tiny ones. Dorsolateral arms of tentorial apodeme divergent, apically curved outward, reaching to fronto-orbital plate; dorsomedial arm half as long as dorsolateral arm. Interspace between antennal sockets narrower than half of socket width; first flagellomere grey, fringed with sparse, somewhat curved and long hairs on distal, outer margin, with only one small invaginated pouch; arista with 5–7 dorsal and 3–4 ventral branches in addition to terminal fork (Fig. 3B). Facial carina only slightly elevated, without setulae below. Subvibrissal seta distinctly shorter than vibrissa; additional row of oral setulae present above marginal row on anterior portion. Palpus with one prominent terminal and 3–4 short, subapical to lateroventral setae, without setulae on basal lobe (Fig. 3C). Cibarium thickened on anterior margin, not dilated laterad in anterior portion; anterolateral corners almost not projected; dorsal sclerite pear-shaped in dorsal view, anteriorly convex in lateral view; anterior sensilla two pairs, widely arranged in square behind anterior margin of hypopharynx; 28–37 medial sensilla arranged in anteriorly convergent rows; sensilla campaniformia two; posterior sensilla long, trichoid, anteriad curved, 20–22 arranged in anteriorly slightly convergent rows; somewhat sclerotized, thickened (in lateral view), anterior portion of hypopharynx 1/4 as long as cibarium (Fig. 3C). Labellum with five pseudotracheae (Fig. 3C).

Thorax. Posterior dorsocentral seta nearly equidistant from anterior margin of scutellum and anterior dorsocentral seta. Prescutellar setae absent. Anterior katepisternal seta as thin as aristal branches; no setula present anteriorly to anterior katepisternal seta.

Wing. Veins grey yellow; crossveins clear; bm-cu crossvein absent (Fig. 3A). C_1 setae two, unequal in size.

Legs. Foreleg femur with approximately nine long setae in two rows on outer side; tarsus with neither tuft of dense, soft hairs on ventral side nor long setae.

Abdomen. Sternites pale grey; V and VI darker; VI setigerous.

Terminalia (Fig. 3D–F). Epandrium smoothly curved on posterior mid-dorsal margin, folded inward on ventral margin, pubescent except anterolateral margin and ventral lobe, triangularly pointed at insertion of surstylus (Fig. 3D). Surstylus articulated to epandrium, somewhat semicircular plate with neither pubescence nor trichoid setae on outer surface; prensisetae apically blunt (Fig. 3D). Cercus separated from epandrium, more or less sclerotized along anterior margin, nearly entirely pubescent except for posterior margin (Fig. 3D). Membrane between cercus and epandrium not pubescent (Fig. 3D). Lateral lobe of tenth sternite smaller than median lobe. Hypandrium dark brown, pubescent on small patches near caudolateral corners, approximately 1.5 times as long as wide, with a pair of inward extended plates apically articulated to ventral apices of parameres (Fig. 3E). Paramere distally curved posteriad, with 3–5 tiny sensilla in a row on proximal portion (Fig. 3F). Aedeagal basal process sclerotized, small, half as short as aedeagus, posteriorly connected through arch-shaped membrane bearing numerous tiny spinules to gonopod (Fig. 3F). Gonopods fused, forming somewhat semicircular plate (Fig. 3E).

Measurements (range in 6♂, in mm). BL = 1.40–1.72, ThL = 0.65–0.75, WL = 1.74–1.98, WW = 0.65–0.81.

Indices (range in 6♂, in ratio). FW/HW = 0.53–0.61, ch/o = 0.11–0.25, prorb = 0.40–0.73, rcorb = 0.07–0.27, vb = 0.30–0.63, dcl = 0.67–0.80, sctl = 1.36–1.54, sterno = 0.38–0.50, sterno2 (mid katepisternal seta / posterior katepisternal seta in length) = 0.10–0.33, orbito = 0.60–0.75, dcp = 0.25–0.43, sctlp = 1.27–1.33, C = 2.64–3.08, 4c = 0.80–1.00, 4v = 1.57–2.00, 5x = 1.40–1.88, ac = 2.40–3.25, M = 0.47–0.54, C3F = 0.27–0.45.

Adult female. Head, thorax, wings, and legs as in male.

Terminalia (Fig. 3G, H). Tergite VIII dark brown, ventrally broadened, pubescent only on caudodorsal margin, with 2–3 small setae near ventral margin (Fig. 3G). Epiproct and hypoproct pale greyish yellow; nearly entirely pubescent and setigerous (Fig. 3G). Oviscapt with approximately 12 marginal ovisensilla (proximal most and dorsal two trichoid but the others peg-like), approximately four lateral trichoid ones, ventro-subterminal trichoid one as long as dorsal marginal ones and approximately three apical small setae (Fig. 3G). Spermathecal capsule dark brown, spherical, smooth; introvert very shallow, 1/10 as deep as capsule height (Fig. 3H).

Measurements (1♀, in mm). BL = 1.79, ThL = 0.81, WL = 2.11, WW = 0.81.

Indices (1♀, in ratio). FW/HW = 0.56, ch/o = 0.11, prorb = 0.50, rcorb = 0.17, vb = 0.60, dcl = 0.56, sctl = 1.00, sterno = 0.63, sterno2 = 0.25, orbito = 0.75, dcp = 0.50, sctlp = 1.33, C = 3.00, 4c = 0.81, 4v = 1.75, 5x = 1.33, ac = 4.33, M = 0.50, C3F = 0.31.

Distribution. Taiwan, China (Guangdong), Myanmar*, India (Sikkim, West Bengal*, Uttarakhand*) [* new record].

Remarks. Kumar and Gupta (1990) described *L. parantillaria* (under the subgenus *Lordiphosa* of the genus *Drosophila*), based on 3♂ and 1♀ specimens collected from Ranipool, Gangtok district, Sikkim, India, distinguishing it from *L. antillaria* by the following characters: "arista branches 6/2 (6/5 in *antillaria*), 5X-index 1.6 (2.5 in *antillaria*) and anterior gonapophysis with 3 sensilla on basal half (with 9–10 sensilla on entire margin in *antillaria*)". However, examining the specimens collected from Bagdogra, West Bengal, approximately 60 km south of Ranipool, in comparison with the specimens from Chitou, Taiwan (the type locality of *L. antillaria*), we found no significant differences in these and other characters between them (Fig. 3, Suppl. material 1). Thus, we here synonymize *Lordiphosa parantillaria* (Kumar & Gupta, 1990) with *Lordiphosa antillaria* (Okada, 1984). This species closely resembles *Lordiphosa subantillaria* (Okada, 1984) from Java and *Lordiphosa ramipara* (Zhang & Liang, 1992) in having the distally posteriad curved and bifurcated paramere, but can be distinguished from them by the diagnostic characters:

in *subantillaria*, the paramere has short branch submedially and the aedeagus lacks spinules (Okada 1984: "Fig. 4"); in *ramipara*, the paramere has the longer branches equal in size (Zhang and Liang 1992: "Figs 2, 3").

Lordiphosa coei (Okada)

Drosophila (*Sophophora*) *coei* Okada, 1966: 82; Dwivedi and Gupta 1980: 88.
Lordiphosa coei: Wheeler, 1981: 54; Zhang et al. 1996: 349.
Drosophila (*Sophophora*) *angusi* Okada, 1977: 369.

Specimens examined. CHINA: 8♂, 11♀, Sichuan, Mt. Emei, 550 m a.s.l., 16 July 1992; 14♂, 12♀, same data, except for 18 July 1992, 700–800 m a.s.l.; 3♂, 1♀, Hubei, Shennongjia, 26 July 1992 (SEHU).

Distribution. China (Hubei*, Sichuan*, Guangdong), Nepal, India (West Bengal) [* new record].

Lordiphosa himalayana (Gupta & Gupta)

Drosophila (*Lordiphosa*) *himalayana* Gupta & Gupta, 1991: 123.

Distribution. India (Sikkim).

Lordiphosa nigrovesca (Lin & Ting)

Drosophila (*Phloridosa*) *nigrovesca* Lin & Ting, 1971: 25 (as *nigrovescum*).
Lordiphosa nigrovesca: Zhang et al., 1996: 352.
Drosophila (*Lordiphosa*) *aurantifrons* Okada, 1984: 568.
Lordiphosa aurantifrons: De & Gupta, 1996: 131.

Distribution. Taiwan, India (West Bengal).

Lordiphosa ayarpathaensis Kandpal & Singh, sp. n.

http://zoobank.org/E1222A9A-7A33-42E0-A456-2840AEE17747
Fig. 4

Type material. *Holotype*. ♂: INDIA: Uttarakhand, Kumaon, Nainital district, Ayarpatha, 29°23'N, 79°27'E, 2,278 m a.s.l., 20–23 August 2009, M. C. Kandpal leg. (DZHNBGU).

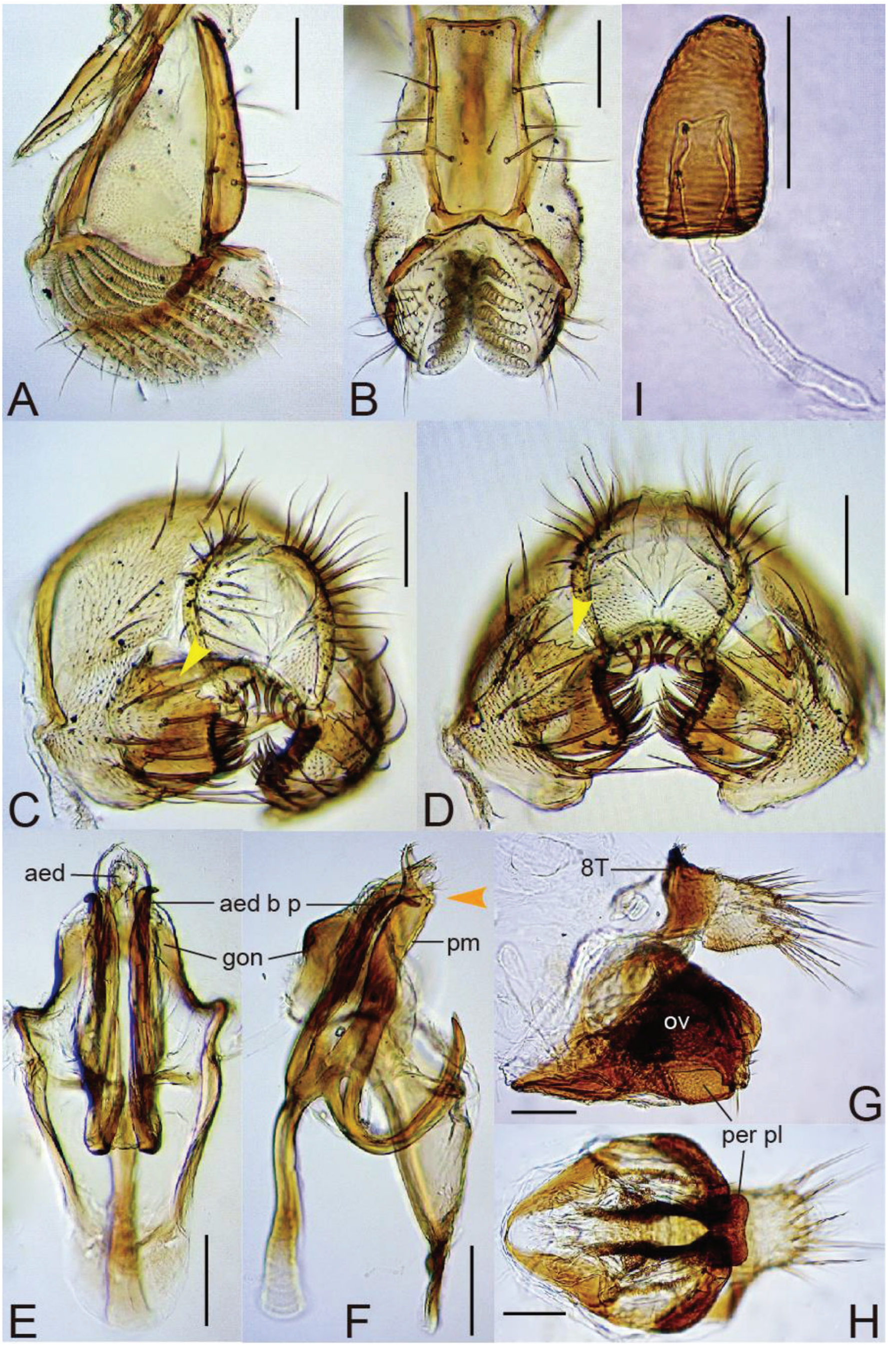

Figure 4. *Lordiphosa ayarpathaensis* Kandpal & Singh, sp. n. (♂♀ paratypes from Ayarpatha, Nainital district, Kumaon, Uttarakhand, India): **A, B** proboscis (**A** lateral view **B** posterior view) **C, D** periphallic organs (**C** caudolateral view **D** caudoventral view) **E, F** phallic organs: aedeagal basal processes (aed b p) (**E** ventral view **F** lateral view) **G, H** ♀ terminalia: perineal plate (per pl) (**G** lateral view **H** ventral view) **I** spermatheca. Scale bars 0.1 mm.

Paratypes. INDIA: 5♂, 5♀, same data as the holotype; 10♂, 6♀, same data as the holotype except 3–5 September 2010 (DZHNBGU, SEHU).

Diagnosis. Epandrium caudosubapically with large flap pointed apically, serrate on dorsal margin and covering largely surstylus (Fig. 4C, D). Paramere broader than aedeagal basal process, apically truncate, serrated; narrow, inward recurved, apically slightly pubescent elongation present at dorsal corner; sensilla 3–5, spaced in a longitudinal row (Fig. 4F).

Description. Adult male. *Head*. Eye dark red, with sparse, interfacetal setulae. Supracervical setae 15–18, thin, apically more or less curved and pointed; postocular setae 16–19; occipital setae approximately 18, including medial tiny ones. Dorsolateral arms of tentorial apodeme divergent, nearly straight, reaching to fronto-orbital plate; dorsomedial arm half as long as dorsolateral arm. Occiput, ocellar triangle and fronto-orbital plates black; frontal vittae mat, light orange. Interspace between antennal sockets narrower than half of socket width; pedicel yellowish brown; first flagellomere grey, with only one small invaginated pouch; arista with 3–4 dorsal and two ventral branches in addition to terminal fork. Facial carina slightly elevated, narrower and shorter than first flagellomere, without setulae below. Gena and clypeus light brown. Subvibrissal seta distinctly shorter than vibrissa; additional row of oral setulae present above marginal row on anterior portion. Palpus with one prominent terminal and several short, subapical to lateroventral setae, without setulae on basal lobe. Cibarium thickened on anterior margin, not dilated laterad in anterior portion; anterolateral corners almost not projected; dorsal sclerite pear-shaped in dorsal view, anteriorly convex in lateral view; anterior sensilla two pairs, widely arranged in square behind anterior margin of hypopharynx; 32–33 medial sensilla arranged in mostly parallel but anteriorly convergent rows; sensilla campaniformia two; posterior sensilla very long, trichoid, gently curved forward, approximately 22, arranged in anteriorly divergent rows; somewhat sclerotized, thickened (in lateral view), anterior portion of hypopharynx shorter than 1/5 length of cibarium. Prementum slightly thicker in ventral than in dorsal portion from lateral view (Fig. 4A), nearly parallel-sided in posterior view (Fig. 4B). Labellum with five pseudotracheae per side (Fig. 4A).

Thorax. Postpronotal lobe grey yellow, with two prominent setae: lower one longer. Scutum and scutellum glossy, light brown. Thoracic pleura greyish brown. Posterior dorsocentral seta situated nearer to anterior dorsocentral seta than to anterior margin of scutellum. Prescutellar setae absent. Acrostichal setulae in six rows. Basal scutellar setae parallel or convergent; apicals cruciate. Anterior katepisternal seta thicker than aristal branches; no setula present anteriorly to anterior katepisternal seta.

Wing hyaline. Veins light brown; crossveins not clouded; bm-cu crossvein absent; R_{2+3} nearly straight; R_{4+5} and M_1 nearly parallel. C_1 setae two, unequal in size. Halter opaque white.

Legs light brown; last two tarsomeres of all legs darker. Foreleg femur with approximately eight long setae in two rows on ventral and outer surfaces; tarsus without any sexual ornamentation. Foreleg tarsomere I as long as three succeeding tarsomeres together; mid-leg one slightly longer than three succeeding tarsomeres together; hindleg one slightly shorter than rest together. Preapical, dorsal setae present on tibiae of all legs; apical setae on tibiae of fore- and mid-legs.

Abdomen. Tergites I to IV medially, widely yellow, laterally brown; V and VI nearly entirely dark brown; each tergite with small setae in approximately three rows and large setae on posterior margin. Sternites light brown; setigerous VI present.

Terminalia (Fig. 4C–F). Epandrium smoothly curved on posterior mid-dorsal margin, folded inward on caudoventral margin, nearly entirely pubescent except anterolateral margin, lower portion of ventral lobe and apical portion of caudo-subapical flap, with approximately seven setae on medial to dorsal portion, 10–11 setae on ventral lobe and sclerotized process at caudoventral apex (Fig. 4C, D). Surstylus articulated to epandrium, distally narrowing and apically truncate, pubescent medially on outer surface; distal margin with a row of 17–18 stout, trichoid prensisetae on dorsal portion and two or three irregular rows of such setae on ventral portion; outer surface lacking peg-like seta.(Fig. 4C, D). Cercus separated from epandrium, nearly entirely pubescent, with 24–25 setae medially to dorsally, ventro-apically truncate and with 3–4 prominent, curved setae on margin and small, apically round projection at anterior corner (Fig. 4C, D). Membrane between cercus and epandrium not pubescent (Fig. 4C). Lateral lobe of tenth sternite smaller than median lobe. Hypandrium anteriorly narrowing, with a pair of narrow plates connecting between bases of parameres and lateral margins of hypandrium (Fig. 4E). Paramere very long, sclerotized process, articulated to basal part of aedeagus, basally much elongated and curved like hook; basal elongation apically pointed, subapically articulated to tip of hypandrial, lateral plate (Fig. 4F). Aedeagus membranous, apically shaped like "funnel" with hirsute margin, apicodorsally connected with gonopod by membrane, basally fused to apodeme; basal process strongly sclerotized, slightly shorter than aedeagus, apically hamate and pointed; apodeme rod-like, as long as aedeagus (Fig. 4E, F). Gonopods fused, forming plate situated dorsally to aedeagus (Fig. 4E, F).

Measurements (holotype / range in 6♂ paratypes, in mm). BL = 2.59 / 2.04–2.41, ThL = 1.22 / 0.96–1.11, WL = 2.96 /2.48–2.96, WW = 1.15 / 0.85–1.26.

Indices (holotype / range in 6♂ paratypes, in ratio). FW/HW = 0.50 / 0.44–0.67, ch/o = 0.11 / 0.10–0.22, prorb = 0.60 / 0.50–0.89, rcorb = 0.20 / 0.17–0.40, vb = 0.40 / 0.50–0.67, dcl = 0.55 / 0.36–0.60, sctl = 1.33 / 1.18–1.56, sterno = 0.33 / 0.25–0.40, sterno2 = 0.22 / 0.13–0.33, orbito = 1.00 / 0.50–1.00, dcp = 0.44 / 0.30–0.57, sctlp = 1.20 / 1.20–1.33, C =3.64 /2.87–3.33, 4c = 0.67 / 0.67–0.75, 4v = 1.67 / 1.14–1.55, 5x = 1.43 / 1.17–1.67, ac = 2.00 / 2.14–2.50, M = 0.48 / 0.37–0.48, C3F = 0.23 / 0.11–0.21.

Adult female. Head, thorax, wings, and legs as in male.

Terminalia (Fig. 4G–I). Tergite VIII short, ventrally tapering, without setae, pubescent only on dorsocaudal portion (Fig. 4G). Oviscapt medially broad and humped in lateral view, distally narrowing and curved ventrad, with three stout, peg-like ovisensilla and ventro-subterminal, trichoid one on apical margin, and approximately six trichoid, lateral ones on distal surface (Fig. 4G). Large, sclerotized perineal plate present between oviscapts (Fig. 4G, H). Spermathecal capsule elongated dome-shaped,

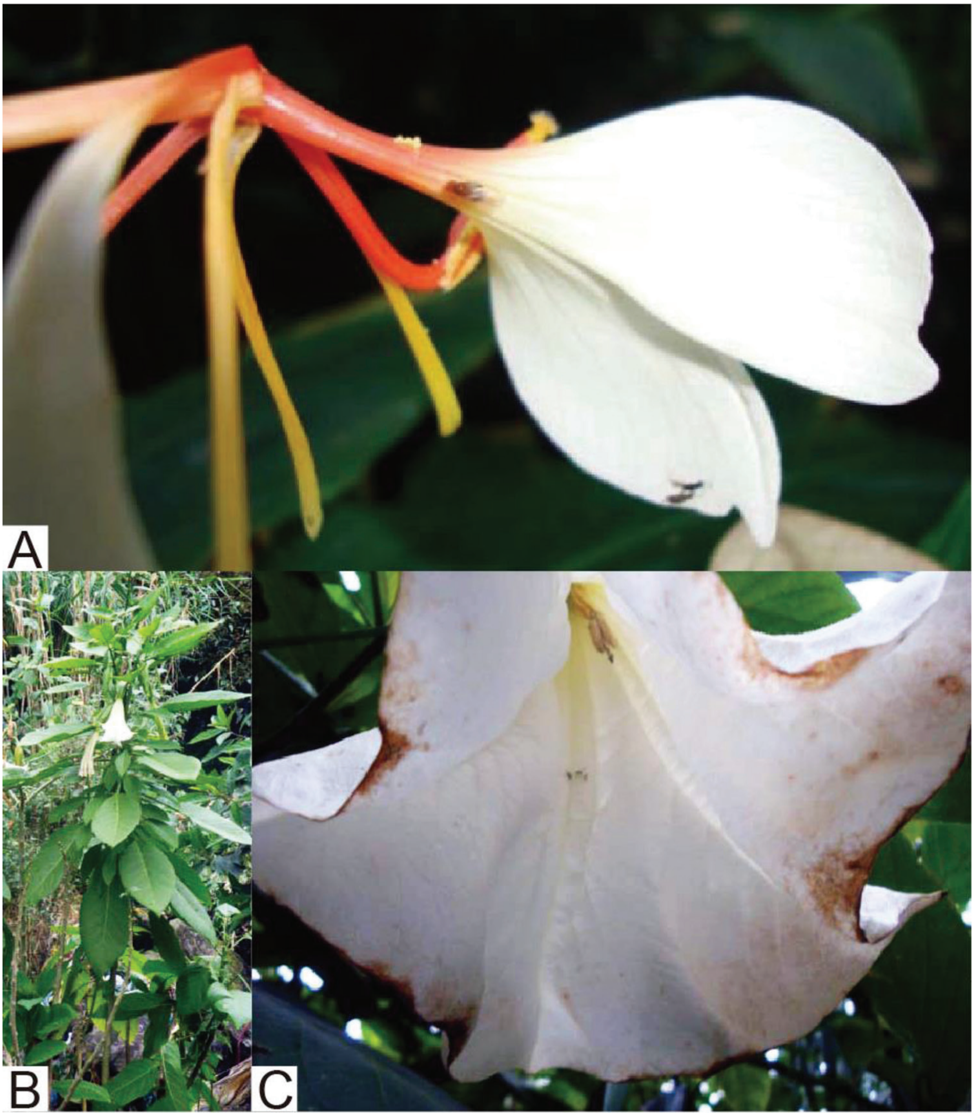

Figure 5. Flowers visited by *Lordiphosa* flies: **A** *Hedychium spicatum* (Zingiberaceae) visited by *L. ayarpathaensis* sp. n. **B, C** *Datura suaveolens* (Solanaceae) visited by *L. makaibarensis* sp. n.

with horizontal wrinkles basally and somewhat irregular ones on apical surface; introvert half as deep as capsule height (Fig. 4I).

Measurements (range in 5♀ paratypes, in mm). BL = 2.22–2.52, ThL = 1.04–1.41, WL = 2.85–3.15, WW = 1.04–1.37.

Indices (range in 5♀ paratypes, in ratio). FW/HW = 0.50–0.65, ch/o = 0.10–0.30, prorb = 0.50–0.70, rcorb = 0.17–0.33, vb = 0.33–0.67, dcl = 0.46–0.67, sctl = 1.20–1.36, sterno = 0.25–0.60, sterno2 = 0.13–0.33, orbito = 0.67–1.33, dcp =

0.40–0.50, sctlp = 0.80–1.25, C = 3.13–3.85, 4c = 0.60–0.67, 4v = 1.38–1.67, 5x = 1.17–1.80, ac = 2.17–2.67, M = 0.33–0.45, C3F = 0.20–0.27.

Etymology. Pertaining to type locality.

Distribution. India (Uttarakhand).

Flower visitation. Adult flies were collected from flowers of *Hedychium spicatum* (local name: Haldu, Kapur Kachri or Sand harlika; English common name: Spiked Ginger Lily; Fig. 5A), a smallish, hardy, perennial herb, belonging to the family Zingiberaceae, with fleshy rhizomes, green, broadly lanceolate leaves, straight stem (up to approximately 1 m high) and large orange and white flowers. It grows throughout subtropical Himalaya in the Indian states of Assam, Arunachal Pradesh and Uttarakhand, with an altitudinal range of 1,000 m to 3,000 m.

Remarks. This species somewhat resembles *L. nigrovesca* in having the sclerotized, apically pointed process at caudoventral apex of epandrium and the paramere apically truncated, but differs from it in the color of thorax and abdomen (glossy black in *nigrovesca*), the caudosubapical, large flap of epandrium (absent; Okada 1984: "Fig. 8", De and Gupta 1996: "Fig. 6"), the position of apically pubescent process on the paramere (subapical; Okada 1984: "Fig. 9", De and Gupta 1996: "Fig. 7"), the shape of oviscapt (distally broad; De and Gupta 1996: "Fig. 8") and the number of ovisensilla (11 marginals and six laterals; De and Gupta 1996: "Fig. 8"). Molecular data of this species are available from Sarswat et al. (2016).

Lordiphosa makaibarensis Pradhan & Chatterjee, sp. n.

http://zoobank.org/794AE213-2170-4321-A9D1-DD3B65119051
Fig. 6

Type material. *Holotype*. ♂: INDIA: West Bengal, Darjeeling, Kurseong, 26°53'N, 88°17'E, 1,458 m a.s.l., 1 September 2010, S. Pradhan leg. (DZHNBGU).

Paratypes. INDIA: 5♂, 5♀, same data as the holotype (DZHNBGU, SEHU).

Diagnosis. Epandrial, caudosubapical, large flap not serrate on dorsal margin (Fig. 6A, B). Paramere as broad as aedeagal basal process, apically pointed, sclerotized and without pubescence, subapically not serrate; sensilla approximately four, arranged relatively compactly in an irregular row on submedial portion (Fig. 6C, D).

Description (not referring to characters commonly seen in the foregoing species, *L. ayarpathaensis*). **Adult male.** *Head*. Supracervical setae 10–15; postocular setae 12–13; occipital setae 11–13. Occiput, ocellar triangle and fronto-orbital plates brownish yellow. Antennal pedicel light brownish yellow; first flagellomere light grey; arista with 3–4 dorsal and 1–2 ventral branches in addition to terminal fork. Gena and clypeus brownish yellow. Cibarial medial sensilla 22–23; posterior sensilla approximately 16.

Thorax light brownish yellow. Posterior dorsocentral seta situated nearer to anterior dorsocentral seta than to anterior margin of scutellum.

Wing. Veins greyish yellow.

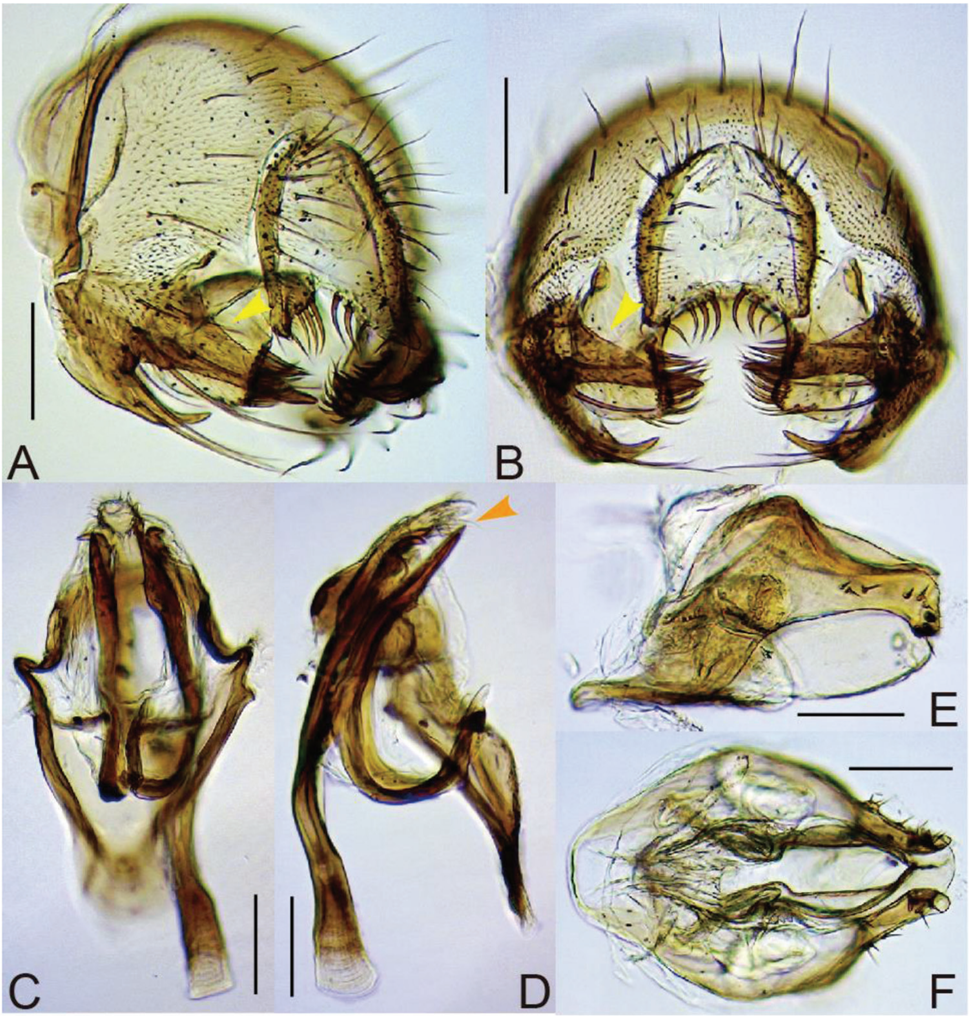

Figure 6. *Lordiphosa makaibarensis* Pradhan & Chatterjee, sp. n. (♂ paratype from Kurseong, Darjeeling, West Bengal, India): **A, B** periphallic organs (**A** caudolateral view **B** caudoventral view) **C, D** phallic organs (**C** ventral view **D** lateral view) **E, F** oviscapt (**E** lateral view **F** ventral view). Scale bars 0.1 mm.

Legs light brownish yellow. Foreleg femur with approximately five long setae in two rows on ventral and outer surfaces.

Abdomen. Tergites nearly entirely yellow; each tergite with small setae in approximately two rows and large setae on posterior margin. Sternites off-white.

Terminalia (Fig. 6A–D). Epandrium with 6–9 setae on medial to dorsal portion and approximately nine setae on ventral lobe (Fig. 6A). Surstylus with approximately 15 stout, trichoid prensisetae in a single row dorsally but in two or three irregular rows ventrally on distal margin (Fig. 6B). Cercus with 16–17 setae medially to dorsally, ventro-apically truncate and with approximately four prominent, curved setae on margin and small, apically somewhat pointed projection at anterior corner (Fig. 6A, B).

Measurements (holotype / range in 5♂ paratypes, in mm). BL = 1.78 / 1. 48–1.81, ThL = 0.81 / 0.74–0.85, WL = 2.22 / 2.22–2.44, WW = 0.74 / 0.81–0.85.

Indices (holotype / range in 3♂ paratypes, in ratio). FW/HW = 0.50 / 0.44–0.53, ch/o = 0.13 / 0.10–0.31, prorb = 0.83 / 0.63–0.78, rcorb = 0.17 / 0.22–0.44, vb = 0.40 / 0.31–0.50, dcl = 0.61 / 0.56–0.71, sctl = 1.38 / 1.17–1.35, sterno = 0.25 / 0.30–0.50, sterno2 = 0.13 / 0.10–0.38, orbito = 0.25 / 0.48–0.80, dcp = 0.57 / 0.47–0.62, sctlp = 1.00 / 0.75–0.97, C = 3.40 / 2.91–3.18, 4c = 0.77 / 0.67–0.79, 4v = 1.69 / 1.39–1.69, 5x = 1.00 / 1.10–1.75, ac = 2.00 / 2.00–2.75, M = 0.31 / 0.34–0.47, C3F = 0.30 / 0.17–0.27.

Adult female. *Terminalia*. Oviscapt with approximately five trichoid, lateral ovisensilla (Fig. 6E, F).

Etymology. Partaining to "Makaibari tea estates". Makaibari was the first tea factory in the world, established in 1859, in Kurseong, Darjeeling, West Bengal.

Distribution. India (West Bengal).

Flower visitation. Adult flies of this species were collected from flowers of *Datura suaveolens* (local name: Dhokrey; English common name: Angel trumpet or Devils trumpet; Fig. 5B, C), an exotic plant belonging to the Solanaceae. It was introduced from South America and is now found growing along riverbeds or forest edges at moist places almost all over India.

Remarks. This species closely resembles the foregoing species, *L. ayarpathaensis*, in having the large flap on caudosubapical margin of epandrium, the oviscapt medially broad and humped in lateral view and distally narrowing and curved ventrad, and the large, sclerotized perineal plate present between oviscapts, but can be distinguished from it by the smaller size and paler color of the body and the diagnostic characters.

Lordiphosa peniglobosa (Kumar & Gupta)

Drosophila (*Lordiphosa*) *peniglobosa* Kumar & Gupta, 1990: 25.

Distribution. India (West Bengal).

Lordiphosa srinagarensis Sati & Fartyal, sp. n.

http://zoobank.org/117C76F6-4F6B-44FF-9659-6D94DB4C19E0
Fig. 7

Type material. *Holotype*. ♂: INDIA: Uttarakhand, Srinagar Garhwal, Tehri district, HNBGU Forestry Nursery Chauras Campus, 30°13'N, 78°47'E, 560 m a.s.l., 16 December 2010, R. S. Fartyal leg. (DZHNBGU).

Paratypes. INDIA: 3♂, same data as the holotype (DZHNBGU, MZSIK, SEHU).

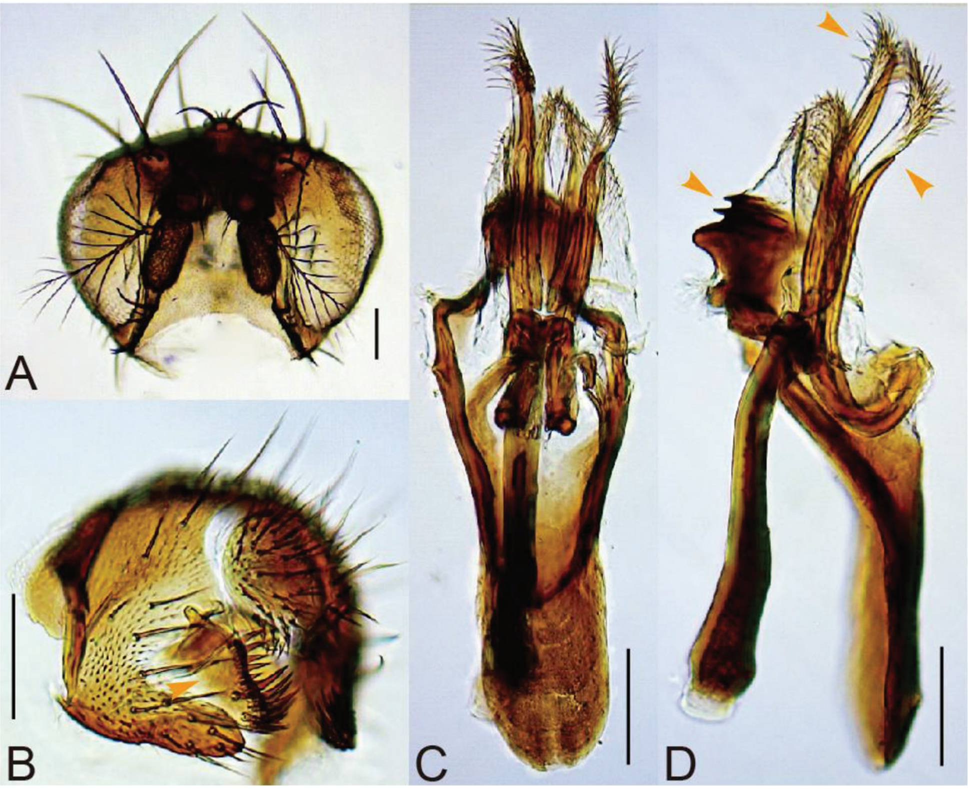

Figure 7. *Lordiphosa srinagarensis* Sati & Fartyal, sp. n. (♂ paratype from HNB Garhwal University, Srinagar, Garhwal, Uttarakhand, India): **A** head (frontal view) **B** periphallic organs (caudolateral view) **C, D** phallic organs (**C** ventral view **D** lateral view). Scale bars 0.1 mm.

Diagnosis. Surstylus with neither pubescence nor stout setae on outer surface (Fig. 7B). Paramere distally bifurcated into ventral, sclerotized, apically pointed branch and dorsal, less sclerotized, apically hirsute branch; ventral branches asymmetric in length: left one longer (Fig. 7C, D). Gonopods with medial broad and two apical narrow ridges in lateral view (Fig. 7D).

Description. Adult male. *Head*. Eye red, with sparse interfacetal setulae (Fig. 7A). Occiput glossy, dark brown in dorsal half, grey yellow in ventral half. Supracervical setae 14–19, thin, apically more or less curved and pointed; postocular setae 14–16; occipital setae 12–13, including tiny medial ones. Dorsolateral arms of tentorial apodeme divergent, apically curved outwards, reaching to fronto-orbital plate; dorsomedial arm half as long as dorsolateral arm. Frons grey yellow except for dark brown upper portion of fronto-orbital plate and medial portion of ocellar triangle. Interspace between antennal sockets narrower than half of socket width; pedicel grey brown; first flagellomere grey, fringed with sparse, somewhat curved and long hairs on distal, outer margin, with only one small invaginated pouch; arista with 6–7 dorsal and 3–4 ventral branches in addition to terminal fork (Fig. 7A). Face grey-yellow; carina only slightly elevated, without setulae below (Fig. 7A). Gena grey-yellow, with dark brown, medial patch and ventral margin; subvibrissal seta distinctly shorter than vibrissa; additional row of oral setulae present above marginal row on anterior portion. Clypeus grey-brown. Palpus dark grey, with one prominent terminal and several short subapical to lateromedian setae, without setulae on basal lobe. Cibarium thickened on anterior margin, not dilated laterad in anterior portion; anterolateral corners almost not projected; dorsal sclerite pear-shaped in dorsal view, anteriorly convex in lateral view; anterior sensilla two pairs, widely arranged in square behind anterior margin of hypopharynx; 24–29 medial sensilla arranged in anteriorly convergent rows; sensilla campaniformia two; posterior sensilla long, trichoid, nearly straight, approximately 27, arranged in nearly parallel rows; somewhat sclerotized, thickened (in lateral view) anterior portion of hypopharynx 1/4 as long as cibarium. Labellum with five pseudotracheae.

Thorax. Nearly entirely brownish black. Posterior dorsocentral seta nearly equidistant from anterior margin of scutellum and anterior dorsocentral seta. Prescutellar setae absent. Acrostichal setulae in six rows. Basal scutellar setae parallel; apicals cruciate. Anterior katepisternal seta as thin as aristal branches; no setula present anteriorly to anterior katepisternal seta.

Wing slightly fuscous; veins grey brown; cross veins clear; bm-cu crossvein absent; R_{2+3} nearly straight; R_{4+5} and M_1 nearly parallel. C_1 setae two, unequal in size. Halter opaque white.

Legs grey yellow. Foreleg femur with approximately six long setae in two rows on outer side; tarsus without any sexual ornamentation. Foreleg tarsomere I as long as three succeeding tarsomeres together; mid- and hind-leg ones as long as rest together. Preapical, dorsal setae present on tibiae of all legs; apical setae on tibiae of fore- and mid-legs.

Abdomen. Tergites entirely glossy, brownish black, each with setae arranged in roughly four transverse rows: those in last row longest. Sternites pale to dark grey; setigerous VI present.

Terminalia (Fig. 7B–D). Epandrium brown, smoothly curved on posterior mid-dorsal margin, nearly entirely pubescent except anterolateral margin and caudosubmedial portion, expanded on caudosubmedial margin at insertion of surstylus, caudoventrally developed into ventral lobe extended posteriad and apically slightly pointed, with about 5–7 setae on medial to dorsal portion, 9–12 setae on ventral lobe and unpubescent, inward fold on ventral margin (Fig. 7B). Surstylus articulated to epandrium, wide, somewhat triangular plate, with 9–10 apically pointed prensisetae reducing in size below in a row on distal margin and 13–16 recurved setae on caudoventral, inner portion (Fig. 7B). Cercus separated from epandrium, pubescent only medially, with 20–24 long setae medially to dorsally and 13–14 short ones on ventral portion (Fig. 7B). Membrane between cercus and epandrium unpubescent (Fig. 7B). Lateral lobe of tenth sternite larger than median lobe. Hypandrium dark brown, completely unpubescent, approximately thrice as long as wide, with a pair of inward extended plates apically articulated to ventral apices of parameres (Fig. 7C). Paramere longer than aedeagus, basally curved ventrad and U-shaped in lateral view, with approximately three minute sensilla in a row proximally (Fig. 7D). Aedeagal basal processes distally fused to membranous aedeagus and membrane posteriorly connected to gonopod; this composite distally hirsute (Fig. 7C, D). Gonopods fused with each other, forming roof-like plate (Fig. 7C, D).

Measurements (holotype / range in 2♂ paratypes, in mm). BL = 1.69 / 1.66–1.69, ThL = 0.65 / 0.65–0.75, WL = 2.05 / 1.95–2.05, WW = 1.04 / 0.81–1.04.

Indices (holotype / range in 2♂ paratypes, in ratio). FW/HW = 0.50 / 0.50–0.53, ch/o = 0.17 / 0.17–0.22, prorb = 0.71 / 0.57–0.71, rcorb = 0.33 / 0.33–0.57, vb = 0.50 / 0.50–0.63, dcl = 0.55 / 0.55–0.70, sctl = 1.50 / 1.44–1.50, sterno = 0.88 / 0.56–0.88, sterno2 = 0.50 / 0.44–0.50, orbito = 0.80 / 0.50–0.80, dcp = 0.43 / 0.43–0.50, sctlp = 0.63 / 0.63–0.80, C = 2.64 / 2.64–2.92, 4c = 0.88 / 0.88–0.92, 4v = 1.63 / 1.63–1.77, 5x = 1.60 / 1.33–1.60, ac =2.33 / 2.33–2.40, M = 0.50 / 0.50–0.62, C3F = 0.29 / 0.25–0.29.

Etymology. Referring to the type locality.

Distribution. India (Uttarakhand).

Remarks. This species closely resembles *Lordiphosa penicilla* (Zhang, 1993) from southwestern China in the morphology of male terminalia, but can be distinguished from it by the diagnostic characters: in *L. penicilla*, surstylus medially pubescent and with several stout setae on outer surface; sclerotized ventral branches of parameres symmetric in length; gonopod with single medial ridge in lateral view (Zhang 1993a: “Figs 4–6”).

Discussion

In this study, it was found that *L. neokurokawai* has a special type of sex comb composed of thick setae of approximately 15 TBRs along the entire length of tarsomere I of male foreleg (Fig. 1B), which was overlooked in its original description by Singh and Gupta (1981). This finding is important for considering the evolution of sex comb in the genus *Lordiphosa*. The sex comb is a male-specific morphological structure composed of thickened setae (“teeth”) that develops on the foreleg tarsus of adult male in the Drosophilidae. This male-specific character is seen only in *Sophophora* and *Lordiphosa* (Hu and Toda 2001), and is used variously in tactile interactions between males and females during courtship and mating behavior (Spieth 1952; see also Kopp 2011 for a review of sex comb functions). Likely in relation to its use as an important component of mating behavior, the sex comb varies in structure even between closely related species, implying that its rapid diversification would have been driven by sexual selection (Markow et al. 1996, Kopp 2011). Three major patterns are recognized in the sex comb structure: (i) “transverse” sex comb comprising TBR(s) of thickened setae on the distal portion of tarsomere; (ii) “oblique” one of row(s) more or less rotated and moderate in length on the distal portion of tarsomere; and (iii) “longitudinal” one aligned along the nearly entire length of tarsomere (Kopp and True 2002, Atallah et al. 2009). All four known species of the *Lordiphosa miki* species group have extended “longitudinal” sex combs of the last type (Laštovka and Máca 1978, Okada 1984, Kopp 2011). The phenotypically identical “longitudinal” sex combs are present in the *melanogaster* and *obscura* species groups of the subgenus *Sophophora* (Kopp 2011, Atallah et al. 2012), explaining why members of the *miki* group had once been assigned to the subgenus *Sophophora* (Kikkawa and Peng 1938, Okada 1956, Lee 1959, Bock and Wheeler 1972). Species of the *L. denticeps* group possess the “transverse” sex combs on the foreleg tarsomeres I to III (Kopp 2011, Atallah et al. 2012). However, probably because the sex comb teeth of the *denticeps* group are less prominent than those of *Sophophora*, this structure had been overlooked in earlier descriptions of *denticeps*-group species until Zhang (1993b) first recognized it. The two other species groups, i.e., *fenestrarum* and *nigricolor* ones, of *Lordiphosa* lack sex combs. Interestingly, the Neotropical *Sophophora* comprising the *saltans* and the *willistoni* groups, which is the sister clade of *Lordiphosa* (Gao et al. 2011), has no sex comb either. This character distribution pattern across *Lordiphosa* and *Sophophora* suggests two hypotheses for the evolution of sex comb. One is the “single-origin” hypothesis: the sex comb was acquired in the common ancestor of *Lordiphosa* and *Sophophora*, and secondarily lost in several lineages. The other is the “multiple-origin” hypothesis: the sex comb evolved independently on several lineages. To date, any approach from the phylogenetic analysis has not succeeded in distinguishing between these two hypotheses. Another possible way is to elucidate the real homology of similar phenotypes by studying the molecular processes underlying their development. Recent evo-devo studies have succeeded in revealing that similar phenotypic structures in sex comb result from different developmental mechanisms (Atallah et al. 2009, 2012, Tanaka et al. 2009, Kopp 2011). For instance, the “longitudinal” sex combs seen in the *melanogaster* and *obscura* groups develop, under similar regulation by the same key genes, through different cellular mechanisms. In some species, such as *Drosophila rhopaloa* Bock & Wheeler, 1972 of the *melanogaster* group and *Drosophila guanche* Monclus, 1976 of the *obscura* group, the “longitudinal” sex comb originates from one or a few distal, transverse rows of bristle-precursor cells that are homologous to those for female TBRs but subsequently rotate 90° and align to form a longitudinal row (Tanaka et al. 2009, Atallah et al. 2012). In species of the *montium* subgroup and *Drosophila ficusphila* Kikkawa & Peng, 1938 of the *melanogaster* group, however, the sex comb arises from male-specific precursor cells aligned in a longitudinal row on the presumptive region (Tanaka et al. 2009, Atallah et al. 2012). Furthermore, Atallah et al. (2012) found the third developmental mode of “longitudinal” sex comb in *Lordiphosa magnipectinata* (Okada, 1956) of the *miki* group: the sex comb development starts from the ancestral, sexually monomorphic arrangement of TBR precursor cells; then, most of such short, transverse rows of precursors rotate independently of each other and eventually assemble into a contiguous, longitudinal row. In relation to this developmental process of “longitudinal” sex comb in the *miki* group, the sex comb of *L. neokurokawai* of the *denticeps* group, which consists of multiple transverse combs arranged along the entire length of tarsomere I, may represent an intermediate stage, i.e., before rotation of TBRs, of the sex comb development in the *miki* group. Taken together these results support a common origin for sex combs in *Lordiphosa*.

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Supplementary material 1

Figure S1

Authors: Rajendra S. Fartyal, Pradeep C. Sati, Sushmika Pradhan, Mukul C. Kandpal, Masanori J. Toda, Rabindra N. Chatterjee, Birendra K. Singh

Data type: multimedia

Explanation note: *Lordiphosa antillaria* (Okada, 1984) (♂♀ from the type locality: Chitou, Taiwan).

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RESEARCH ARTICLE

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Description of a new species of *Alburnus* Rafinesque, 1820 (Actinopterygii, Cyprinidae, Leuciscinae) from the Kolpa River in the Sava River system (upper Danube drainage), with remarks on the geographical distribution of shemayas in the Danube

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Abstract

Alburnus sava, new species, is described from the Kolpa River. The Kolpa is a tributary of the Sava, a major tributary of the Danube River, in the Black Sea basin. *Alburnus sava* is distinguished from its congeners in the Danube drainage, *A. mento* and *A. sarmaticus*, by having 23–27, usually 24–26, gill rakers; the ventral keel usually completely covered by scales (scaleless part maximum 15% of the keel length); 15–16, mode 15, branched pectoral-fin rays; the length of the gill raker at the junction of the arch limbs 65–70% of the length of the opposite outer gill filament; and a relatively long lower jaw (37–40% HL or 112–130% interorbital width). *Alburnus sava* is a large-sized potamadromous shemaya known to occur in the entire Sava drainage. The taxonomic status of *A. mento* and *A. sarmaticus* is confirmed. *Alburnus danubicus* is discussed and as there are no new arguments, it is kept as a valid species. New details on the distribution of shemayas in the Danube drainage are presented.

Keywords

Freshwater and anadromous shemayas, taxonomy, morphology, *Alburnus sarmaticus*, *Alburnus mento*, Black Sea-Sea of Azov basin

Introduction

Shemaya is a Russian vernacular name based on the Persian name *shah-mahi*, King's fish, used for *A. chalcoides* Gueldenstaedt, 1772 in the Caspian Sea basin. It is also commonly applied to a number of nominal species and subspecies in the Caspian and the Black seas since Borodin (1904) and Berg (1905, 1916). Shemayas were later assigned to a distinct genus *Chalcalburnus* Berg, 1932 and discriminated from *Alburnus* by a usually scaled (almost completely or completely covered by scales) ventral keel between the pelvic fins and the anus (vs. usually scaleless in *Alburnus*) and an increased number of lateral-line scales, usually over 55 (vs. usually less then 55 in *Alburnus*, with the exception of *A. filippii* Kessler, 1877 with up to 63) (Berg 1932, 1933). Recent morphological studies (Freyhof and Kottelat 2007a,b, Özuluğ and Freyhof 2007) supported the idea that shemayas form a distinct morphological group of species though included in the genus *Alburnus* Rafinesque, 1820. Not all shemaya species have been studied using molecular markers but preliminary data support the conclusion that they form a monophyletic group (Perea et al. 2010, Geiger et al. 2014).

Earlier authors (e.g., Kessler 1877, Berg 1916, 1923, 1932, 1933, 1949, Abdurakhmanov 1962, Tscherbukha 1965, Elanidze 1983, Movchan and Smirnov 1983) emphasized an extremely high similarity of shemayas in the few characters used in their discrimination – usually only the number of branched anal-fin rays and gill rakers, and some body measurements. For example, a reliable range of the branched anal-fin rays based on large samples examined of the Caspian shemaya is 12–17 (=12½–17½ according to the method of counting by Kottelat and Freyhof (2007) with means 14.6 (Berg 1932) and 14.7 (Abdurakhmanov 1962). The Black Sea forms are characterised by higher numbers of rays, averaging 15.6 in the Don drainage (Berg 1932), 15.9 in the South Bug (Tscherbukha 1965), 15.2 in the Berda River, Sea of Azov basin (Loshakov 1973) with a range of 14–17 (=14½–17½) (i.e. within the range in the Caspian shemaya). However, there are also local forms in the Black Sea basin with a lower number of branched anal-fin rays. For example, the Colchis (or Georgian) shemaya from Western Transcaucasia has a range of branched anal-fin rays of 12–16 (=12½–16½), usually 13–15, and a mode of 14 (Berg 1949, Elanidze 1983). However, later studies (Freyhof and Kottelat 2007a,b, Özuluğ and Freyhof 2007), based on a larger set of characters, discriminated 16 species of shemayas. They consider the following nominal taxa as valid in the Black Sea basin (clockwise from the Danube): *A. danubicus* Antipa, 1909 (anadromous), *A. mento* (Heckel, 1836) (landlocked), *A. sarmaticus* Freyhof & Kottelat, 2007b (anadromous and potamadromous), *A. mentoides* Kessler, 1859 (resident), *A. leobergi* Freyhof & Kottelat, 2007b (originally anadromous, also landlocked populations in dammed rivers), *A. derjugini* Berg, 1923 (resident), *A. istanbulensis* Battalgil, 1941 (resident), *A. schischkovi* (Drensky, 1943) (anadromous), and *A. mandrensis* (Drensky, 1943) (probably, landlocked). Shemayas distributed outside of the Black Sea basin include *A. volviticus* Freyhof & Kottelat, 2007a and *A. vistonicus* Freyhof & Kottelat, 2007a, both from lakes in eastern Greece, Aegean Sea basin (landlocked); two species from lakes in the Sea of Marmara basin in Turkey, *A. carinatus* Battalgil, 1941 (landlocked) and *A. nicaeensis* Battalgil, 1941 (landlocked, possibly extinct); two species in the Aegean Sea basin in western Turkey, *A. attalus* Özuluğ & Freyhof, 2007 (resident) and *A. battalgilae* Özuluğ & Freyhof, 2007 (resident), and *A. chalcoides* from the Caspian Sea basin (anadromous).

However, this taxonomic scheme still does not answer all remaining questions for the group. For example, it is not clear which species occurs between the ranges of *A. derjugini* and *A. istanbulensis* (e.g., in Sakarya, Kızılırmak, and Yesilirmak rivers). It is still to be clarified if there is (was) a single species in the Çoruh as supposed by Berg (1923) or there occurred two species or morphs, resident and anadromous as found by Deryugin (1899). Furthermore, the identification of shemaya in coastal lakes and limans in Bulgaria and Romania, other than Lake Madra, remains unknown.

Current knowledge of the morphology of the Black Sea shemayas is inadequate as the keys offered to distinguish them (Kottelat and Freyhof 2007) are often not helpful since most character ranges overlap. This may be the reason why a taxonomic compromise was offered (Parin et al. 2014) to consider only two shemaya species as valid, the Caspian *A. chalcoides* and the Black Sea *A. mento*.

As to the Danube drainage, as indicated above, Freyhof and Kottelat (2007b), based on literature and their own data, assumed an occurrence at least in former times, of two sympatric anadromous shemayas, one lake-dwelling species landlocked in a number of subalpine lakes, and one probable resident river species. The latter was only known from the Kolpa [Kupa] River and only three specimens were available for examination for Freyhof and Kottelat (2007b: 223). These authors found the Kolpa shemaya morphologically similar to *A. sarmaticus,* but differing in the number of gill rakers (27 vs. 28–34 in 28 individuals from the South Bug and the lower Danube). A morphological analysis of additional material from the Kolpa is important in resolving the taxonomy of the Danubian shemayas. Herein, we examine morphological variation of shemayas and provide a critical review of their historical distributions in the Danube.

Material and methods

The triangular-shaped symphisis of the lower jaws is referred as the chin. The ventral keel is defined as the distance between the base of the anus and the level of the posterior ends of the pelvic-fin bases. The dorsal-fin insertion is the posterior-most point where the last dorsal-fin ray connects with the body. All measurements were made point-to-point with a dial caliper and recorded to the nearest of 0.1 mm. Methods for counting rays, lateral-line scales and scales along the scaleless part of the ventral keel, and for most measurements follow Kottelat and Freyhof (2007). Additional measurements of the cranium, jaws, and operculum as defined in Tables 1 and 3 were made point to point from the anteriormost extremity to the posteriormost extremity (lengths), from the uppermost extremity to the lowermost extremity (depths), and between the lateralmost extremities (widths). Length of the cranial roof was measured from the anterior margin of the supraethmoid to the base of the supraoccipital crest. Vertebral counts are given according to Naseka (1996). Standard length was measured from the tip of the upper jaw to the posterior margin of the hypurals. Head length was measured from the anteriormost extremity of the head (either tip of the upper jaw or the projected symphysis of the lower jaw) to the posterior opercular margin that includes the skin fold. In total, 49 morphometric indices were used for descriptions and statistical analyses as in Tables 1 and 3. These also included the length of the scaleless portion of the ventral keel relative to the keel length (Bogutskaya et al. 2010, fig. 1) and length of a gill raker at the junction of the lower and the upper limbs of the first gill arch (the 6–8th, usually the 7th, gill raker) relative to the opposite outer gill filament (Kottelat and Freyhof 2007, fig. 42). The last two rays in the dorsal and anal fins based on a single pterygiophore were counted as 1½ rays. Numbers of vertebrae and fin rays were counted from radiographs. In total, 16 meristic characters were examined (nine as given in Table 2 and numbers of branched dorsal-fin rays, branched pelvic-fin rays, intermediate vertebrae, total lateral scales, total lateral-line scales, scales above the lateral line, and scales below the lateral line). All characters were obtained from specimens of both sexes and combined in analyses and tables.

Abbreviations: SL, standard length; HL, lateral head length; HDBI, Croatian Biological Research Society; MNCN, Museo Nacional de Ciencias Naturales (Madrid, Spain); NMW, Naturhistorisches Museum Wien (Vienna, Austria); vs., versus; ZM NASU, Zoological Museum of National Academy of Sciences of Ukraine.

Cluster Analysis (CA), Multidimensional Scaling (MDS), Principal Component Analysis (PCA), Discriminant Function Analysis (DFA), and a Kruskal-Wallis test which is helpful for comparison of three and more groups with a presumably non-parametric distribution of variables, were performed using STATISTICA 6.0 and PRIMER v6.1.9 to identify the most important characters that contribute to the differentiation of samples and visualise the degree of morphological separation among the new species, *A. mento*, *A. sarmaticus*, and *A. leobergi*.

Results

***Alburnus sava* sp. n.**
http://zoobank.org/AEE0CFF9-6F12-4DA6-BCF6-A4B6C61B3961
Figs 1–2, 3a

Holotype. MNCN 291345, 173.6 mm SL, female, Kolpa River at Griblje (45.58°N 15.30°E), Slovenia, 3 Oct 2013, coll. B. Levai.

Figure 1. *Alburnus sava* sp. n., **a** MNCN 291345, holotype, 173.6 mm SL, before preservation **b** MNCN 291345, paratype, 151.5 mm SL, formaldehyde-preserved specimen.

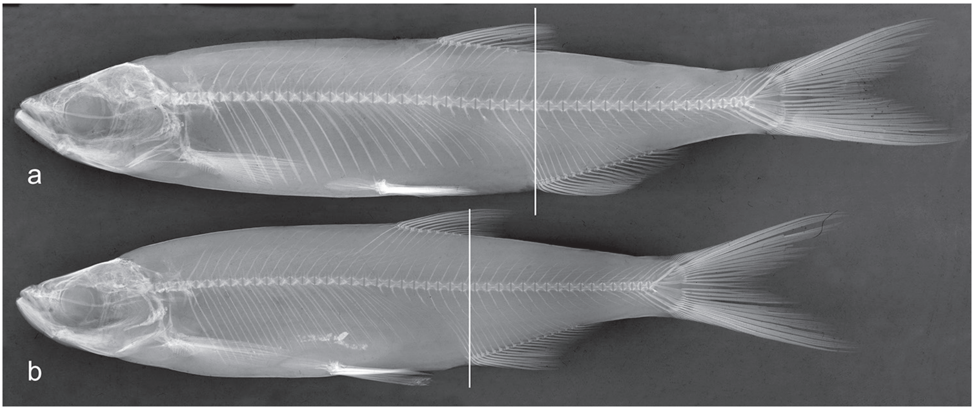

Figure 2. Radiograph of *Alburnus sava* sp. n., same specimens as in Fig. 1; vertical lines showing origin of anal fin located on (**b**) or slightly behind (**a**) vertical of dorsal-fin insertion.

Paratypes. MNCN 291346-53, 8, 105–151.5 mm SL, same data as holotype; HDBI 255, 218 mm SL, Kupa [Kolpa] River at Ozalj (45.62°N 15.47°E), Sept 2011, Croatia, coll. D. Jelić; HDBI 1224, 3, 62.9–79.8 mm SL, same data as HDBI 255.

Diagnosis. *Alburnus sava* sp. n. is distinguished from all other species of *Alburnus* in the Danube drainage by having 23–27, usually 24–26, gill rakers; the ventral keel usually completely scaled (scaleless maximum 15% of the keel length); 15–16 (mode = 15) branched pectoral-fin rays; the length of gill raker 65–70% of the length of the opposite outer gill filament; and a relatively long lower jaw (37–40% HL, 112–130% interorbital width).

Description. The general appearance of *Alburnus sava* sp. n. can be seen in Figure 1. Relative measurements are provided in Table 1. Variation in ten (of the 16) examined meristic characters is provided in Table 2. The largest specimen, a spent female, is 218 mm SL.

Table 1. Morphometric data of *Alburnus sava* sp. n. Most influential characters (as discussed in text) given in bold.

	MNCN 291345, holotype	MNCN 291345, holotype and paratypes, n=9				HDBI 255	HDBI 1224, small-sized, n=3				All speciemens of *A. sava* sp. n., n=13			
		min	max	mean	sd		min	max	mean	sd	min	max	mean	sd
SL, mm	173.6	105	173.6	131.3		218	62.9	79.8	70.9		62.9	218		
Body depth at dorsal-fin origin (% SL)	21.3	19.9	23.3	21.4	1.1	26.6	20.7	21.5	21.2	0.4	19.9	26.6	21.8	1.7
Depth of caudal peduncle (% SL)	9.0	9.0	9.2	9.1	0.1	9.8	8.9	9.1	9.0	0.1	8.9	9.8	9.1	0.2
Depth of caudal peduncle (% length of caudal peduncle)	50.9	45.9	53.1	50.8	2.5	59.9	48.1	54.0	51.1	3.0	45.9	59.9	51.6	3.4
Body width at dorsal-fin origin (% SL)	9.8	9.7	11.6	10.5	0.7	11.6	7.6	8.6	8.2	0.5	7.6	11.6	10.1	1.3
Caudal peduncle width (% SL)	5.1	4.8	5.6	5.2	0.3	3.5	2.6	3.1	2.8	0.3	2.6	5.6	4.5	1.1
Predorsal length (% SL)	56.4	54.1	57.4	56.0	1.0	58.3	56.6	57.9	57.1	0.7	54.1	58.3	56.5	1.1
Postdorsal length (% SL)	36.5	34.3	38.4	35.6	1.2	35.3	34.2	35.1	34.5	0.6	34.2	38.4	35.3	1.1
Prepelvic length (% SL)	48.2	46.3	49.3	48.5	1.0	47.8	49.4	50.8	49.9	0.8	46.3	50.8	48.8	1.1
Preanal length (% SL)	71.3	67.5	71.3	69.5	1.1	69.6	68.5	71.1	69.6	1.3	67.5	71.3	69.5	1.1
Pectoral – pelvic-fin origin length (% SL)	25.8	23.1	25.8	24.4	0.9	27.7	24.5	27.1	25.4	1.5	23.1	27.7	24.9	1.3
Pelvic – anal-fin origin length (% SL)	21.7	20.5	23.9	21.7	1.0	24.1	19.2	21.1	20.1	0.9	19.2	24.1	21.5	1.4
Caudal peduncle length (% SL)	17.7	16.9	19.5	18.0	0.8	16.4	16.7	18.6	17.7	1.0	16.4	19.5	17.8	0.9
Dorsal-fin base length (% SL)	10.8	9.0	11.2	10.2	0.7	11.4	9.5	10.8	10.1	0.6	9.0	11.4	10.3	0.7
Dorsal-fin depth (% SL)	15.6	15.6	17.7	17.0	0.6	18.2	17.7	18.4	18.0	0.4	15.6	18.4	17.3	0.7
Anal-fin base length (% SL)	16.4	16.4	18.1	17.0	0.5	17.7	16.1	17.8	17.0	0.8	16.1	18.1	17.1	0.6
Anal-fin depth (% SL)	12.3	9.8	12.4	11.7	0.8	13.6	13.5	14.1	13.9	0.3	9.8	14.1	12.3	1.2
Pectoral-fin length (% SL)	17.7	17.7	21.5	20.1	1.2	20.6	20.7	21.1	21.0	0.2	17.7	21.5	20.3	1.1
Pelvic-fin length (% SL)	14.4	14.4	16.0	15.3	0.5	15.2	14.9	15.6	15.3	0.4	14.4	16.0	15.3	0.5
Head length (% SL)	22.7	22.7	24.8	23.8	0.7	22.7	25.4	26.4	25.8	0.5	22.7	26.4	24.2	1.2
Head length (% body depth)	106.7	99.0	122.5	111.2	7.4	85.3	117.9	124.3	122.1	3.6	85.3	124.3	111.7	11.1
Head depth at nape (% SL)	15.4	15.3	16.3	15.7	0.4	16.5	14.8	16.7	15.5	1.0	14.8	16.7	15.7	0.6
Head depth at nape (% HL)	67.8	61.7	69.4	66.1	2.7	72.7	56.5	65.6	59.9	5.0	56.5	72.7	65.1	4.6
Head depth through eye (% HL)	47.2	45.0	52.6	48.8	2.3	54.4	41.8	48.6	45.4	3.4	41.8	54.4	48.4	3.3
Maximum head width (% SL)	10.7	10.7	11.9	11.3	0.3	11.6	11.0	11.4	11.2	0.2	10.7	11.9	11.3	0.3
Maximum head width (% HL)	47.2	45.7	51.5	47.4	1.9	51.2	42.3	44.7	43.2	1.3	42.3	51.5	46.7	2.8

	MNCN 291345, holotype	MNCN 291345, holotype and paratypes, n=9				HDBI 255	HDBI 1224, small-sized, n=3				All speciemens of *A. sava* sp. n., n=13			
		min	**max**	**mean**	**sd**		**min**	**max**	**mean**	**sd**	**min**	**max**	**mean**	**sd**
Snout length (% SL)	6.7	6.6	7.5	6.9	0.3	6.1	7.4	7.9	7.7	0.3	6.1	7.9	7.0	0.5
Snout length (% HL)	29.7	27.4	30.4	28.9	0.9	26.9	28.9	30.7	29.9	0.9	26.9	30.7	29.0	1.1
Eye horizontal diameter (% SL)	5.7	5.7	6.7	6.3	0.4	5.1	7.2	7.7	7.5	0.3	5.1	7.7	6.5	0.7
Eye horizontal diameter (% HL)	25.3	24.1	28.0	26.4	1.3	22.5	27.2	30.3	29.0	1.6	22.5	30.3	26.7	2.1
Eye horizontal diameter (% interorbital width)	78.7	78.7	90.6	83.7	4.0	66.6	87.6	94.1	91.0	3.2	66.6	94.1	84.1	7.1
Postorbital distance (% HL)	50.6	46.9	51.3	49.2	1.3	52.2	44.7	46.4	45.7	0.9	44.7	52.2	48.6	2.2
Interorbital width (% SL)	7.3	7.3	7.8	7.5	0.2	7.6	8.2	8.3	8.2	0.1	7.3	8.3	7.7	0.4
Interorbital width (% HL)	32.1	30.0	32.7	31.5	0.9	33.7	31.1	32.4	31.9	0.7	30.0	33.7	31.8	1.0
Length of upper jaw (% HL)	28.0	27.8	30.6	29.1	1.0	29.1	28.2	29.9	29.0	0.9	27.8	30.6	29.1	0.9
Length of upper jaw (% SL)	6.4	6.4	7.3	6.9	0.3	6.6	7.4	7.6	7.5	0.1	6.4	7.6	7.0	0.4
Length of lower jaw (% SL)	8.9	8.9	9.6	9.2	0.3	8.5	9.9	10.2	10.0	0.2	8.5	10.2	9.3	0.5
Length of lower jaw (% HL)	39.1	37.2	39.8	38.6	1.0	37.6	37.6	40.3	38.9	1.4	37.2	40.3	38.6	1.1
Length of lower jaw (% interorbital width)	121.8	116.6	129.6	122.7	4.4	111.6	119.3	125.3	121.9	3.1	111.6	129.6	121.6	4.9
Length of lower jaw (% depth of operculum)	98.2	96.7	107.6	100.9	3.9	90.7	102.3	109.6	107.1	4.2	90.7	109.6	101.5	5.6
Length of lower jaw (% cranium roof length)	63.8	59.7	67.1	62.5	2.8	62.4	62.7	64.0	63.2	0.7	59.7	67.1	62.8	2.2
Length of lower jaw (% cranium width between margins of pterotics)	99.9	94.4	104.6	99.1	3.2	85.8	100.0	100.5	100.2	0.3	85.8	104.6	98.4	4.6
Cranium roof length (% SL)	13.9	13.6	15.9	14.7	0.9	13.7	15.5	16.3	15.9	0.4	13.6	16.3	14.9	1.0
Cranium width between margins of pterotics (% cranium roof length)	63.9	59.5	68.6	63.2	3.0	72.7	62.7	63.7	63.1	0.5	59.5	72.7	63.9	3.6
Cranium width between margins of sphenotics (% cranium roof length)	55.2	49.0	57.3	53.8	2.4	65.6	56.9	60.0	58.9	1.8	49.0	65.6	55.9	4.2
Cranium width between margins of lateral ethmoids (% cranium roof length)	19.7	19.7	23.4	21.8	1.4	23.4	16.6	18.0	17.3	0.7	16.6	23.4	20.9	2.4
Cranium width between margins of lateral ethmoids (% cranium width between margins of pterotics)	30.9	30.9	38.4	35.0	1.9	32.1	26.6	28.6	27.5	1.0	26.6	38.4	32.7	3.6
Depth of operculum (% HL)	39.9	36.3	39.9	38.3	1.4	41.5	35.3	36.8	36.3	0.9	35.3	41.5	38.1	1.8

	MNCN 291345, holotype	**MNCN 291345, holotype and paratypes, n=9**				**HDBI 255**	**HDBI 1224, small-sized, n=3**				**All speciemens of *A. sava* sp. n., n=13**			
		min	**max**	**mean**	**sd**		**min**	**max**	**mean**	**sd**	**min**	**max**	**mean**	**sd**
Ratios														
Interorbital width/eye horizontal diameter	1.3	1.1	1.3	1.2	0.1	1.5	1.1	1.1	1.1	0.0	1.1	1.5	1.2	0.1
Snout length/eye horizontal diameter	1.2	1.0	1.2	1.1	0.1	1.2	1.0	1.1	1.0	0.1	1.0	1.2	1.1	0.1
Head depth at nape/eye horizontal diameter	2.7	2.3	2.7	2.5	0.1	3.3	1.9	2.2	2.1	0.1	1.9	3.2	2.5	0.3
Head length/caudal peduncle depth	2.5	2.5	2.8	2.6	0.1	2.3	2.8	2.9	2.9	0.1	2.3	2.9	2.6	0.2
Length of caudal peduncle/caudal peduncle depth	2.0	1.9	2.2	2.0	0.1	1.7	1.9	2.1	2.0	0.1	1.7	2.2	1.9	0.1
Length of lower jaw/caudal peduncle depth	1.0	1.0	1.1	1.0	0.0	0.9	1.1	1.1	1.1	0.0	0.9	1.1	1.0	0.1
Pectoral fin length/pectoral – pelvic-fin origin distance	0.7	0.7	0.9	0.8	0.1	0.7	0.8	0.9	0.8	0.1	0.7	0.9	0.8	0.1
Predorsal length/head length	2.5	2.3	2.5	2.4	0.1	2.6	2.1	2.3	2.2	0.1	2.1	2.6	2.3	0.1

Table 2A. Meristic data for *Alburnus sava* sp. n. and three species used for comparisons. Counts in holotype marked with *.

	Branched anal-fin rays						**Branched pectoral-fin rays**				
	13½	**14½**	**15½**	**16½**	**17½**	**Mean (+½)**	**15**	**16**	**17**	**18**	**Mean**
A. sava sp. n., n=13			4*	9		**15.7**	8*	5			**15.4**
A. sarmaticus, South Bug, n=5		1	3		1	**15.2**	2	3			**15.6**
A. sarmaticus, Danube, n=15		1	9	5		**15.3**	5	7	3		**15.9**
A. leobergi, n=6			2	3	1	**15.8**	1	5			**15.8**
A. mento, n=62 for anal-fin rays, n=50 for pectoral-fin rays	2	16	25	14	5	**15.1**	1	11	26	12	**17.0**

Table 2B.

	Predorsal abdominal vertebrae					**Abdominal vertebrae**				**Caudal vertebrae**				**Total vertebrae**				
	15	**16**	**17**	**18**	**mean**	**23**	**24**	**25**	**mean**	**20**	**21**	**22**	**mean**	**43**	**44**	**45**	**46**	**mean**
A. sava sp. n., n=13		7*	6		**16.5**	8	5*		**23.4**	1*	12		**20.9**		9*	4		**44.3**
A. sarmaticus, South Bug, n=5		5			**16.0**	4	1		**23.2**	1	3	1	**21.0**	1	2	2		**44.2**
A. sarmaticus, Danube, n=15	1	9	5		**16.3**	7	7	1	**23.6**	5	8	2	**20.8**		10	4	1	**44.4**
A. leobergi, n=6		5		1	**16.3**	5	1		**23.2**		3	3	**21.5**		2	4		**44.7**
A. mento, n=60	2	35	22	1	**16.4**	30	27	3	**23.6**	7	31	22	**21.3**	1	20	29	10	**44.8**

Table 2C.

	Lateral-line scales to posterior hypural margin														
	54	**56**	**57**	**58**	**59**	**60**	**61**	**62**	**63**	**64**	**65**	**66**	**69**	**71**	**mean**
A. sava sp. n., n=13			1	1	3*	5	1	1							**59.6**
A. sarmaticus, South Bug, n=5						2			1	2					**62.2**
A. sarmaticus, Danube, n=15		1	1	1	1	3	2	2	3		1				**60.7**
A. leobergi, n=6		1		1		2	1	1							**59.5**
A. mento, n=50	1		1	3	11	9	7	5	2	7		2	1	1	**61.1**

Table 2D.

	Gill rakers																
	19	**20**	**21**	**22**	**23**	**24**	**25**	**26**	**27**	**28**	**29**	**30**	**31**	**32**	**33**	**34**	**mean**
A. sava sp. n., n=13					**1**	**4**	**3**	**4***	**1**								**25.0**
A. sarmaticus, South Bug, n=5												**1**	**3**			**1**	**31.4**
A. sarmaticus, Danube, n=15										**1**	**3**	**3**	**3**	**4**	**1**		**30.6**
A. leobergi, n=6							**1**			**1**		**2**	**1**	**1**			**29.3**
A. mento, n=50	**1**	**1**	**1**	**8**	**11**	**15**	**10**	**2**	**1**								**23.6**

Table 2E.

	Scales along scaleless portion of ventral keel														
	0	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**mean**
A. sava sp. n., n=13	**8***	**1**	**4**												**0.7**
A. sarmaticus, South Bug, n=5		**3**	**2**												**1.4**
A. sarmaticus, Danube, n=15		**1**	**5**	**5**	**2**	**2**									**2.9**
A. leobergi, n=6		**1**	**4**			**1**									**2.3**
A. mento, n=50				**1**	**5**	**7**	**6**	**9**	**11**	**3**	**3**	**2**	**2**	**1**	**7.2**

As the examined samples are rather small in number of specimens and contain individuals with a wide range in SL, Table 1 also presents the range and mean for the holotype and size groups separately. Body depth at the dorsal-fin origin in the 218 mm-long specimen represented 27% SL and considerably exceeded the range in body depth of smaller specimens (20–23% SL). The same is found for the depth of the caudal peduncle, 10% SL (60% length of caudal peduncle or 1.7 times in its length) vs. 9% (46–54% length of caudal peduncle or 1.9–2.2 times in its length), respectively. However, head length and eye diameter are clearly negatively allometric. The head length mean in the small-size group (63–80 mm SL) is 25.8% SL vs. 22.7% SL in specimens 174 and 218 mm SL; in smaller specimens the head length considerably exceeds the body depth while in the largest specimen it is much smaller than the latter. The eye diameter mean is 7.5% SL (29% HL) in the small-sized group (63–80 mm) vs. 5.1% SL (22.5% HL) in 218 mm long specimen.

The mouth is upturned and the mouth cleft is straight. The tip of the mouth is about at a level with the upper margin of the pupil. The lower jaw is long, its length

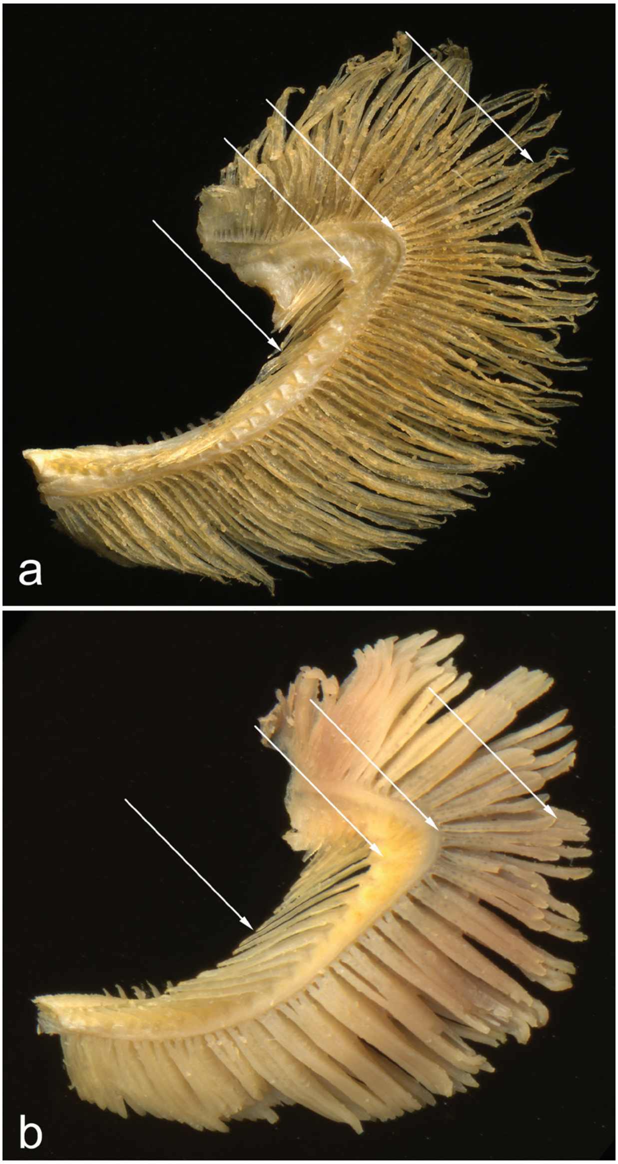

Figure 3. First gill arch in **a** *Alburnus sava* sp. n., HDBI 255, 218 mm SL (length of gill raker is 70% of opposite gill filament length) and **b** *A. mento*, NMW 79592, 184 mm SL (length of gill raker is 120% of opposite gill filament length).

112–130% interorbital width. The chin is variably developed (Fig. 1). The holotype has a well developed chin while the chin of the paratype is smoothed and slightly projected.

The ventral keel between the pectoral-fin bases and the anus is well pronounced but not sharp and usually completely covered by scales (in 8 specimens, including the holotype) or scaleless (exposed) for 1–2 scales only (Table 2), reaching up to 15% of the keel.

Figure 4. Map of distribution of shemayas in the Danube drainage: ⁙ *A. mento*, ■ *A. sava* sp. n., ▨ distribution of potamadromous shemaya (supposedly *A. sava* sp. n.), ▥ anadromous *A. sarmaticus* and/or *A. danubicus*.

Dorsal fin with 3 unbranched and 8½ branched rays. Anal fin with 3 unbranched and 15½ or 16½ branched rays (Table 2). Origin of anal fin located on (in three specimens) or slightly behind the vertical of the dorsal-fin insertion (Fig. 2).

Number of gill rakers 23–27, mode 24–26 (Table 2). In two specimens examined (80 and 218 mm SL) length of gill raker 65% and 70% (Fig. 3a) the length of the opposite gill filament in the outer row. Pharyngeal teeth 2.5-5.2 (n =2 paratypes).

Total lateral-line scales number (61)63–64, mode 63; lateral-line scales to the posterior margin of hypurals 57–62, mode 60. Total vertebrae 44–45, 23–24 abdominal and 20–21 caudal (Table 2; Fig. 2).

No nuptial tubercles in the examined material. Four dissected individuals were females. Overall colouration is silvery with no orange or red pigment at fin bases and no faint dark midlateral stripe in both freshly caught and preserved specimens.

Distribution and habitat. The species is currently known from the Kolpa River drainage, a tributary of the Sava River in the upper Danube drainage, Black Sea basin (Fig. 4). *Alburnus sava* sp. n. is a potamadromous species, occurring in streams and rivers with moderate to rapid current and a gravel and cobble bottom; in spring, during the spawning season, the species migrates upstream to smaller tributaries to shallow riffles where they spawn.

Etymology. The species name refers to the Sava River. A noun in apposition.

Vernacular name. Local names are bucov or velika pliska in Croatian and Serbian, pegunica in Slovene.

Comparisons

The new species belong to the *Alburnus mento* group of shemayas (former genus *Chalcalburnus*), which includes *A. mento*, *A. sarmaticus*, and *A. leobergi*, as defined by Freyhof and Kottelat (2007b) to have 57–62 lateral-line scales to the posterior margin of hypurals and 15–16½ branched anal-fin rays. The maximum size of *Alburnus sava* sp. n. in examined materials is 218 mm SL (adult female), which is about the body size of mature adults of migratory shemayas in the Black Sea basin, including the Danube – ca. 200–300 mm (vs. 100–130 mm in resident small-sized *A. derjugini* and *A. mentoides*) (Drensky 1943, Berg 1949, Tscherbukha 1965, Movchan and Smirnov 1983).

Alburnus sava sp. n. does not demonstrate a distinct difference in most morphometric characters from all or either of the other Danubian species and *A. leobergi* (Tables 1, 3; discussed below regarding statistical analysis). The same is true for numbers of vertebrae (total and in vertebral regions), branched anal-fin rays, and scales in the lateral row, total lateral line and the lateral line to the posterior margin of hypurals) (Table 2).

However, *Alburnus sava* sp. n. can be clearly distinguished from *A. sarmaticus* (Table 2) using two meristic characters. First, the number of gill rakers is 23–27 in *A. sava* sp. n. vs. 28–34 in *A. sarmaticus*; this same range for *A. sarmaticus* was found by Freyhof and Kottelat (2007b: table 3). A wider range in number of gill rakers, 26–33 (mean = 30), was reported by Tscherbukha (1965) for specimens from the South Bug. Second, the ventral keel of *A. sava* sp. n. is usually completely covered by scales, while in *A. sarmaticus* the typical state is a scaleless keel along about 10–15% its length (i.e., 1–3 scales exposed (Fig. 5a) found in 16 of 20 specimens). Among specimens of *A. sarmaticus* examined, neither a completely scaled keel nor a keel exposed along 6 scales (4–6 indicated by Freyhof and Kottelat (2007b) was found.

Though measurements based on the limited material examined cannot be used for taxonomic purposes with regard to being variable depending on season, sex, size and other factors, some relative measurements may have some taxonomic value as reflecting basic morphological differences between these species. *Alburnus sava* sp. n. differs from *A. sarmaticus* by a longer upper jaw, 28–31% HL (vs. 24–29% HL); a longer lower jaw, 37–40% HL or 112–130% interorbital width (vs. 34–38% HL or 89–113% interorbital width); and a longer cranial roof, 14–16% SL (vs. 12–15% SL) (Tables 1, 3).

Freyhof and Kottelat (2007b) described *A. sarmaticus* as having 17½ or fewer branched anal-fin rays as an anadromous shemaya in the Danube; they described *A. danubicus,* a purportedly extinct species from the Danube, as having 17–20½ branched anal-fin rays; this was based on their opinion of data of Antipa (1909) and Drensky (1943) for an anadromous shemaya from the lower Danube. However, no specimens have been reported since then with 18½ or more branched anal-fin rays and some authors (Halasi-Kovacs and Harka 2012, Stefanov and Trichkova 2015) continue to use *danubicus* for the Danubian shemaya as the only anadromous species in the Danube. Materials collected by Shishkov [=Chichkoff] and Drensky, still deposited in the National Museum of Natural History in Sofia (Bulgaria), should be examined to clarify the problem.

When compared to *A. mento*, *A. sava* sp. n. with 15½ or 16½ branched anal-fin rays do not differ from the former species possessing 13–18½ branched anal-fin rays (Table 2, Fig 7; Freyhof and Kottelat 2007b). Three more counts are overlapping and only statistically (see below) different in *Alburnus sava* sp. n. and *A. mento*. First, the

Table 3. Morphometric data for *Alburnus sarmaticus*, *A. mento*, and *A. leobergi*. Most influential characters (as discussed in text) given in bold.

	***Alburnus sarmaticus*, South Bug; n=5**				***Alburnus sarmaticus*, Lower Danube and delta; n=15**				***Alburnus mento*, Bavaria, Traunsee, Mondsee; n=10**				***Alburnus leobergi*, NMW Yasenskiy Bay, Sea of Azov, n=6**			
	min	**max**	**mean**	**sd**	**min**	**max**	**mean**	**sd**	**min**	**max**	**mean**	**sd**	**min**	**max**	**mean**	**sd**
SL, mm	170.7	204.5	184.5		157.5	209	183.2		134.3	204.5	147.9		154.8	193.1	176.6	
Body depth at dorsal-fin origin (% SL)	25.4	27.2	26.0	0.8	23.9	27.3	25.8	1.0	19.8	24.0	22.0	1.6	22.2	26.2	24.9	1.5
Depth of caudal peduncle (% SL)	9.2	10.3	9.5	0.5	8.8	10.5	9.6	0.4	7.9	9.7	9.0	0.6	9.5	10.1	9.7	0.2
Depth of caudal peduncle (% length of caudal peduncle)	48.3	61.0	53.8	6.0	49.3	64.9	58.4	4.8	41.5	59.2	51.0	5.9	57.4	75.8	64.1	7.9
Body width at dorsal-fin origin (% SL)	11.4	14.7	12.6	1.2	10.1	13.8	11.5	1.2	8.7	13.2	11.4	1.8	11.6	12.6	12.1	0.3
Caudal peduncle width (% SL)	3.2	4.9	3.8	0.7	3.4	4.7	4.1	0.4	3.4	8.7	6.1	2.4	3.8	4.7	4.2	0.3
Predorsal length (% SL)	53.5	55.8	54.3	1.0	52.7	57.3	55.8	1.1	52.8	57.2	55.5	1.5	55.6	59.7	57.0	1.5
Postdorsal length (% SL)	35.6	39.4	36.6	1.6	34.7	38.9	36.1	1.0	35.1	37.9	36.3	1.0	30.3	36.4	34.5	2.5
Prepelvic length (% SL)	44.5	49.4	47.8	2.0	46.4	49.0	47.8	0.7	46.1	50.6	48.2	1.6	46.3	50.4	47.5	1.5
Preanal length (% SL)	65.4	69.8	68.0	1.6	66.9	72.8	70.1	1.7	66.4	70.4	68.3	1.4	68.4	73.3	70.1	1.7
Pectoral – pelvic-fin origin length (% SL)	22.6	27.4	25.4	2.0	25.0	27.9	26.3	0.8	24.8	27.5	26.4	1.0	24.2	26.1	25.0	0.7
Pelvic – anal-fin origin length (% SL)	21.6	22.9	22.5	0.5	21.5	27.2	24.3	1.7	19.3	23.4	21.5	1.2	22.6	26.1	24.1	1.3
Caudal peduncle length (% SL)	16.5	19.0	17.9	1.1	14.6	18.9	16.6	1.3	16.2	19.8	17.7	1.2	13.3	16.9	15.3	1.6
Dorsal-fin base length (% SL)	11.7	12.1	11.9	0.2	9.6	12.4	11.1	0.7	9.0	11.6	10.6	0.7	10.4	11.7	11.0	0.5
Dorsal-fin depth (% SL)	17.8	21.6	19.1	1.5	16.5	20.5	18.5	1.4	14.8	17.4	16.1	0.8	17.9	21.4	19.0	1.3
Anal-fin base length (% SL)	18.4	19.4	18.7	0.4	16.3	18.7	17.6	0.8	14.2	19.6	17.1	1.6	17.2	19.1	17.8	0.7
Anal-fin depth (% SL)	11.3	14.0	12.8	1.0	10.3	15.0	13.1	1.3	10.2	12.5	11.2	0.7	12.3	14.4	13.3	0.7
Pectoral-fin length (% SL)	18.9	21.3	20.2	1.0	19.6	22.3	20.4	0.7	17.1	19.8	18.7	0.8	19.8	21.4	20.4	0.6
Pelvic-fin length (% SL)	14.6	16.6	15.4	0.8	14.3	17.3	15.4	0.7	14.0	15.5	14.6	0.5	15.0	16.2	15.6	0.5
Head length (% SL)	22.3	24.4	23.5	0.9	21.9	24.0	23.0	0.5	20.6	24.1	22.7	1.1	22.3	25.3	23.4	1.0
Head length (% body depth)	84.8	96.0	90.4	4.4	83.8	95.9	89.4	4.1	97.6	110.5	103.4	5.0	86.7	106.2	94.5	7.2
Head depth at nape (% SL)	13.9	15.8	15.0	0.7	14.3	16.4	15.4	0.5	14.0	16.4	15.1	0.9	15.3	16.5	15.9	0.5
Head depth at nape (% HL)	62.7	65.7	63.9	1.3	62.7	71.2	67.2	2.1	63.7	71.3	66.6	2.4	65.4	69.6	67.8	1.9
Head depth through eye (% HL)	45.8	51.8	47.8	2.3	46.6	51.2	49.2	1.4	45.8	52.9	48.4	2.2	47.3	51.5	49.9	1.5
Maximum head width (% SL)	10.6	11.7	11.4	0.5	10.5	12.1	11.2	0.5	10.1	12.4	11.2	0.9	11.1	11.9	11.4	0.3

	***Alburnus sarmaticus*, South Bug; n=5**				***Alburnus sarmaticus*, Lower Danube and delta; n=15**				***Alburnus mento*, Bavaria, Traunsee, Mondsee; n=10**				***Alburnus leobergi*, NMW Yasenskiy Bay, Sea of Azov, n=6**			
	min	**max**	**mean**	**sd**	**min**	**max**	**mean**	**sd**	**min**	**max**	**mean**	**sd**	**min**	**max**	**mean**	**sd**
Maximum head width (% HL)	47.5	50.8	48.4	1.4	44.2	52.8	48.8	2.5	46.2	52.6	49.4	2.1	45.4	51.4	48.9	2.2
Snout length (% SL)	6.6	7.2	6.9	0.2	6.0	6.8	6.4	0.2	5.6	7.6	6.5	0.7	6.0	6.9	6.6	0.3
Snout length (% HL)	28.1	30.7	29.4	1.3	26.9	29.2	27.6	0.8	26.2	32.1	28.3	1.9	25.6	29.6	28.0	1.5
Eye horizontal diameter (% SL)	5.3	6.0	5.6	0.3	5.0	6.7	5.7	0.5	4.9	6.0	5.6	0.4	4.6	5.5	5.0	0.3
Eye horizontal diameter (% HL)	23.1	24.5	23.8	0.6	22.1	29.0	24.7	2.2	23.0	27.0	24.8	1.2	20.7	22.0	21.4	0.6
Eye horizontal diameter (% interorbital width)	57.6	76.5	66.2	7.1	64.4	86.1	71.6	6.7	70.9	87.5	77.5	6.2	58.3	66.6	63.5	3.6
Postorbital distance (% HL)	49.2	51.4	50.2	0.9	47.5	53.8	50.8	1.5	46.2	52.0	48.7	1.7	49.4	52.8	51.6	1.2
Interorbital width (% SL)	7.8	9.3	8.5	0.6	7.2	8.5	7.9	0.4	6.7	7.9	7.3	0.4	7.5	8.4	7.9	0.4
Interorbital width (% HL)	32.0	40.2	36.2	2.9	32.1	37.2	34.5	1.5	29.5	33.6	32.0	1.2	32.7	35.5	33.7	1.2
Length of upper jaw (% HL)	23.8	27.1	25.2	1.5	24.4	28.7	26.9	1.1	24.6	29.8	27.4	1.6	23.9	27.2	26.3	1.2
Length of upper jaw (% SL)	5.5	6.6	5.9	0.4	5.6	6.6	6.2	0.3	5.3	6.9	6.2	0.6	5.4	6.8	6.2	0.4
Length of lower jaw (% SL)	7.7	9.1	8.4	0.5	8.1	8.9	8.4	0.3	7.7	9.2	8.5	0.6	7.8	9.0	8.4	0.4
Length of lower jaw (% HL)	33.8	37.6	35.6	1.5	34.6	38.2	36.6	1.0	36.3	38.8	37.4	0.9	34.3	37.3	35.8	1.3
Length of lower jaw (% interorbital width)	89.2	106.5	98.8	7.4	96.6	113.4	106.5	5.7	111.8	124.0	116.9	3.6	103.9	112.8	106.4	3.4
Length of lower jaw (% depth of operculum)	89.0	102.1	97.3	5.6	89.0	104.7	97.2	4.5	93.2	114.4	100.6	7.1	87.1	93.9	91.4	2.8
Length of lower jaw (%cranium roof length)	55.3	67.8	61.1	4.8	59.7	73.5	64.9	3.5	63.2	73.8	68.0	3.9	60.1	68.3	63.9	2.7
Length of lower jaw (% cranium width between margins of pterotics)	79.3	90.4	85.7	4.5	83.2	93.3	88.8	3.1	86.8	96.5	90.9	3.6	85.1	91.0	88.5	2.6
Cranium roof length (% SL)	12.6	14.9	13.7	1.0	12.1	13.8	13.0	0.5	11.7	13.8	13.0	4.4	12.5	14.1	13.1	0.7
Cranium width between margins of pterotics (% cranium roof length)	69.6	76.2	71.3	2.8	69.3	84.2	73.1	3.6	66.1	77.6	72.0	0.7	69.4	75.4	72.3	2.7
Cranium width between margins of sphenotics (% cranium roof length)	60.5	70.4	63.8	3.9	62.4	78.2	67.3	3.9	53.6	65.1	59.0	4.0	64.5	68.7	66.7	1.6
Cranium width between margins of lateral ethmoids (% cranium roof length)	20.5	23.3	22.6	1.2	21.2	27.0	23.9	1.7	17.7	23.0	20.4	1.9	21.8	26.5	24.0	1.6
Cranium width between margins of lateral ethmoids (% cranium width between margins of pterotics)	29.4	33.4	31.7	1.8	29.7	39.0	32.8	2.3	23.7	31.0	27.4	3.2	29.2	36.0	33.2	2.8
Depth of operculum (% HL)	33.6	40.8	36.7	2.6	34.4	40.2	37.7	1.4	34.0	39.7	37.3	1.9	36.9	40.7	39.2	1.4

	***Alburnus sarmaticus*, South Bug; n=5**				***Alburnus sarmaticus*, Lower Danube and delta; n=15**				***Alburnus mento*, Bavaria, Traunsee, Mondsee; n=10**				***Alburnus leobergi*, NMW Yasenskiy Bay, Sea of Azov, n=6**			
	min	**max**	**mean**	**sd**	**min**	**max**	**mean**	**sd**	**min**	**max**	**mean**	**sd**	**min**	**max**	**mean**	**sd**
Ratios																
Interorbital width/eye horizontal diameter	1.3	1.7	1.5	0.2	1.2	1.6	1.4	0.1	1.1	1.4	1.3	0.1	1.5	1.7	1.6	0.1
Snout length/eye horizontal diameter	1.1	1.3	1.2	0.1	0.9	1.3	1.1	0.1	1.0	1.3	1.1	0.1	1.2	1.4	1.3	0.1
Head depth at nape/eye horizontal diameter	2.6	2.8	2.7	0.1	2.2	3.0	2.7	0.2	2.4	2.8	2.7	0.1	3.0	3.3	3.2	0.1
Head length/caudal peduncle depth	2.2	2.6	2.5	0.1	2.2	2.6	2.4	0.1	2.4	2.8	2.5	0.1	2.3	2.5	2.4	0.1
Length of caudal peduncle/caudal peduncle depth	1.6	2.1	1.9	0.2	1.5	2.0	1.7	0.1	1.7	2.4	2.0	0.2	1.3	1.7	1.6	0.2
Length of lower jaw/caudal peduncle depth	0.8	0.9	0.9	0.1	0.8	0.9	0.9	0.0	0.9	1.1	1.0	0.0	0.8	0.9	0.9	0.0
Pectoral fin length/pectoral – pelvic-fin origin distance	0.7	0.9	0.8	0.1	0.7	0.8	0.8	0.0	0.7	0.8	0.7	0.0	0.8	0.9	0.8	0.0
Predorsal length/head length	2.2	2.4	2.3	0.1	2.3	2.5	2.4	0.1	2.3	2.6	2.4	0.1	2.3	2.6	2.4	0.1

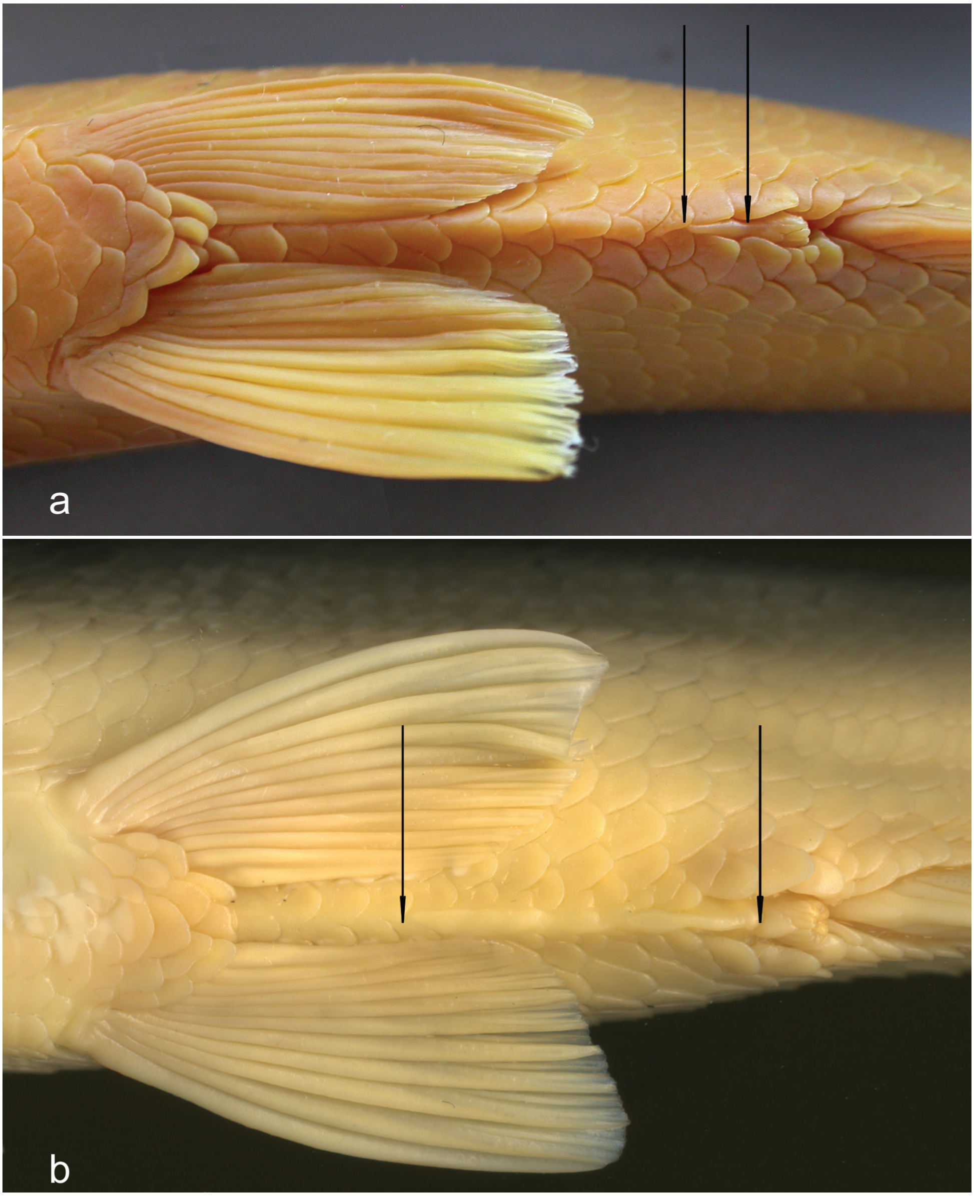

Figure 5. Ventral keel. Arrows showing anterior margin of anus base and beginning of scaleless portion of keel in **a** *Alburnus sarmaticus,* ZM NASU 4145, South Bug, 184.2 mm SL, 2 scales along scaleless portion of ventral keel; and **b** *A. mento*, NMW 79592, Mondsee, 135.1 mm SL, 11 scales along scaleless portion of ventral keel.

new species has 23–27, usually 24–26, gill rakers (vs. 19–27, usually 22–25 in *A. mento*, Table 2). Second, 8½ branched dorsal-fin rays (vs. 7–8½, 7½ found in 14 from 62 examined specimens; *A. mento* is the only Danubian species with often 7½

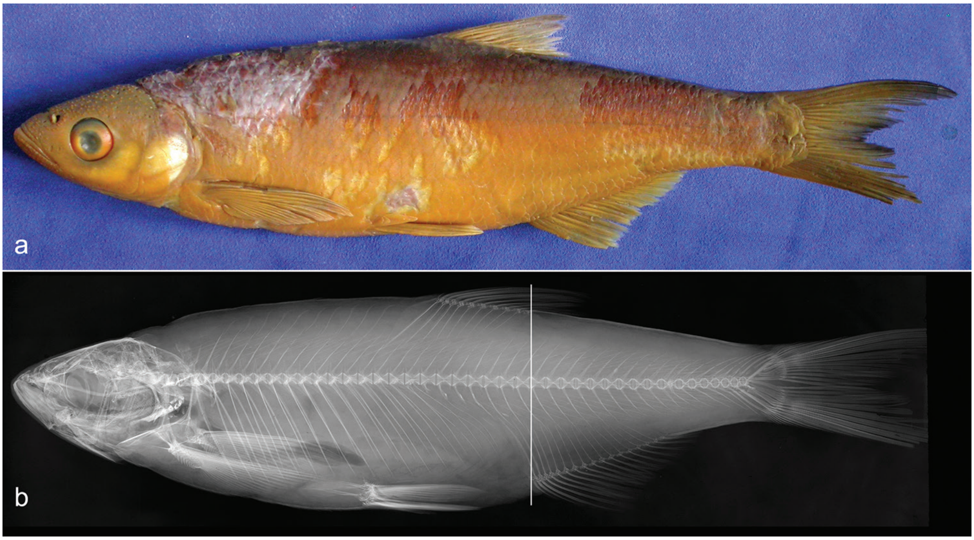

Figure 6. *Alburnus sarmaticus* ZM NASU 4145, South Bug, 184.2 mm SL; general appearance (**a**) and radiograph (**b**). Vertical line showing origin of anal fin located on vertical of dorsal-fin insertion.

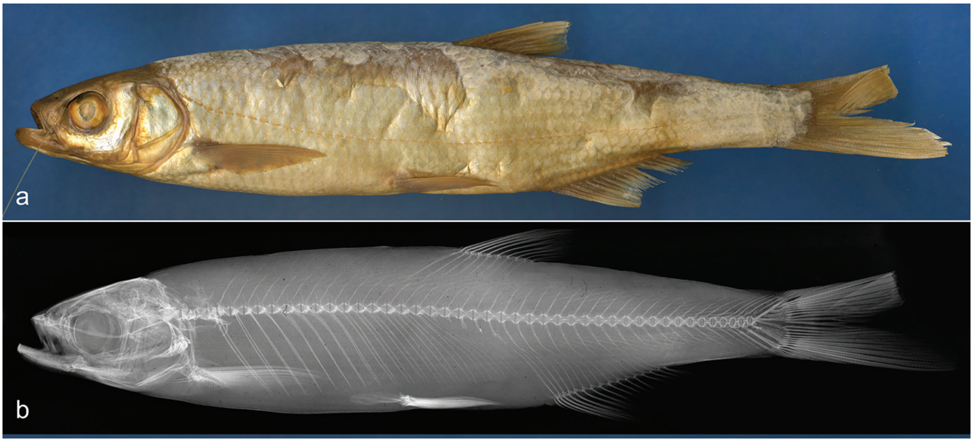

Figure 7. *Alburnus mento* NMW 55629, general appearance and radiograph, Kremsmünster, 144 mm SL.

branched dorsal-fin rays). Third, 15–16, with a mode of 15, branched pectoral-fin rays that is the lowest count among the examined samples (Table 2) (vs. the highest count, (15)16–18, mode = 17, found in *A. mento*).

Alburnus sava sp. n. and *A. mento* are distinguished in a much clearer way by the length of the scaleless portion of the ventral keel and the relative length of a gill raker. As shown by Freyhof and Kottelat (2007a, b), these characters have a considerable taxonomic value in the genus *Alburnus*. In *A. sava*, the ventral keel is completely scaled or there is a very short scaleless portion of the keel which is only 1 or 2 scales long, maximum 15% of the keel length (the shortest keel among the examined species,

Table 2). In contrast, *A. mento* has a very long scaleless portion of the keel which is 3 to 13, usually 4–12, scales long (Fig. 5b) that is 25–85% of the keel length (up to 90% according to Freyhof and Kottelat (2007b).

In *A. sava* sp. n. (Fig. 3a), the length of the gill raker is 65–70% of the opposite outer gill filament. In *A. sarmaticus*, it is similar, 50–93%, usually 60–75%, averaging 68% (examined in 20 specimens). In contrast, in *A. mento* (Fig. 3b), the length of the gill raker is 75–126%, usually 90–120%, averaging 103%, of the length of the opposite gill filament (examined in 20 specimens).

Alburnus sava sp. n. further differs from *A. mento* by the shape of the symphysial part of the jaws. Neither a pronounced chin nor a knob formed by the symphysis of the lower jaws were found in the new species. In contrast, *Alburnus mento* of a different size have a very prominent chin and a strong knob on the lower jaw entering a corresponding notch formed at the symphysis of the upper jaws, a feature also typical of the asp *Leuciscus aspius.*

Alburnus sava sp. n. differs from geographically close *A. mandrensis*, *A. schischkovi,* and *A. istanbulensis* (data from Freyhof and Kottelat (2007a) and Özuluğ and Freyhof (2007) by having the ventral keel completely scaled or exposed for 1 or 2 scales (vs. 6–12) and 15–16½ branched anal-fin rays (vs. 13–15½). The new species further differs from *A. mandrensis* by the length of the gill raker 65–70% of the opposite outer gill filament (vs. 45–55%) and 23–27, usually 24–26, gill rakers (vs. 25–35) and from *A. schischkovi* by having 57–62 lateral-line scales to posterior margin of hypurals (vs. 63–67). *Alburnus sava* sp. n. is also distinguished from *A. istanbulensis* by having the length of the gill raker 65–70% of the opposite outer gill filament (vs. 30–55%) and 23–27, usually 24–26, gill rakers (vs. 24–35, usually 26–32).

Note on syntypes of *A. mento*. A comment should be given clarifying the status of the specimens labelled as syntypes of *A. mento* in NMW. The original description (Heckel 1836: 225–226) clearly indicates three samples of specimens it was based on. All three samples are still present among specimens labeled as syntypes in NMW. The set of Acquisition Sheets (bound in a number of books at present) is the most reliable source of primary original information which accompanied the samples at the moment of their accession in the collection.

1. Specimens collected by Heckel in September 1824 in Lake Traun («... at Gmunden, .. especially abundant under the Traun bridge»). Heckel's specimens collected in September 1824 are registered under the acquisition number 1824.II.10: «Traun, … Heckels Reise durch Oberösterreich… Nr. 80». Two specimens are still in NMW (16261 and 16441), and one was sent to the Paris Museum. The two NMW specimens have a standard length (139.8 mm and 134 mm, respectively) which corresponds to a total length equaling "Spanne" [Handspanne] (the distance between the end of the little finger and the end of the thumb that is about 18–22 cm), mentioned in the original description.
2. Specimens later [than 1824] received from Agassiz. These specimens are most probably those registered under the acquisition number 1830.II.3. The acquisi-

tion 1830.II contains 7 entries in total (e.g., 1830.II.1 is for *Gobio uranoscopus*) and reads «Bavaria. November 1829. Von Herrn Leopold Fitzinger durch Kauf». This acquisition is made by Jos. Natterer and 1830.II.3 refers to *Leuciscus macroramphus* Agassiz with the name *Aspius Heckelii* Fitz. handwritten later by Heckel. "4-6" Individual: (?) 4 were sent somewhere on exchange [in Tauch]. The labels for NMW 50440, 55650, and 55652 (with the acquisition number 1830.II.3) reading «Durch Agassiz aus München» appeared later, at Steindachner's time, and are most probably based on information from the Heckel's description of *Aspius mento* Agassiz as a synonym of *Aspius heckelii* Fitzinger (Heckel 1836: 225): «Später erhielt das hiesige Museum durch die Güte des Herrn Professor Agassiz sehr schöne Exemplare seines *Aspius Mento* aus München; ich habe nun diese Exemplare auf das sorgfältigste mit jenen aus der Traun verglichen, …» [«Later, the local museum received by the generosity of Professor Agassiz very beautiful specimens of his *Aspius Mento* from Munich; I have now most carefully compared these specimens with those from the Traun...»]. So, the exact locality is not given but Bavaria in a general way, and this does not exclude Bavarian lakes, e.g., Starnberger See, closest to Munich in the south-wets still inhabited by *A. mento*.

3. One specimen (9 Viennese inches long) from the Danube near Vienna. This specimen was registered under the acquisition number 1836.I.19: «Danube at Vienna. November 1835».

The accession information for one more sample labeled as syntypes, NMW 55629, does not match the original description. It contains four specimens registered under the acquisition numbers 1835.VI.1 (2 specimens) and 1835.VI.1a (2 specimens) which say that the specimens were received by exchange from [Dr] Schreibers in 1832 and 1834, respectively, from near Stift Kremsmünster (Kremsmünster Abbey; in the town of Kremsmünster in the district of Kirchdorf an der Krems, Upper Austria, 29 km from Gmunden on Traunsee). However, the source of the acquisition may mean any water body in the surroundings of Kremsmünster where an astronomical and geophysical observatory ("Mathematical tower"), the first in the world, was located which included a natural history museum with botanical and zoological collections (still existing as a weather station). However, the label (from Steindachner's time) says «Traunsee» (probably just based on the original description). As Kremsmünster was not mentioned in the original description by Heckel (1836), even if the specimens are from Lake Traun, they do not belong to the Traunsee sample collected by Heckel in 1824. So, the NMW 55629 specimens do not belong to the type series and the designation of the 150 mm long (SL) specimen from this sample as lectotype by Freyhof and Kottelat (2007b: 217) is invalid.

Statistical analysis

Results of PCA and MDS analyses applied to 16 meristic variables outlined three clusters (Fig. 8). *A. sava* sp. n. is most distant from *A. mento*. *Alburnus sarmaticus* and *A. leobergi*

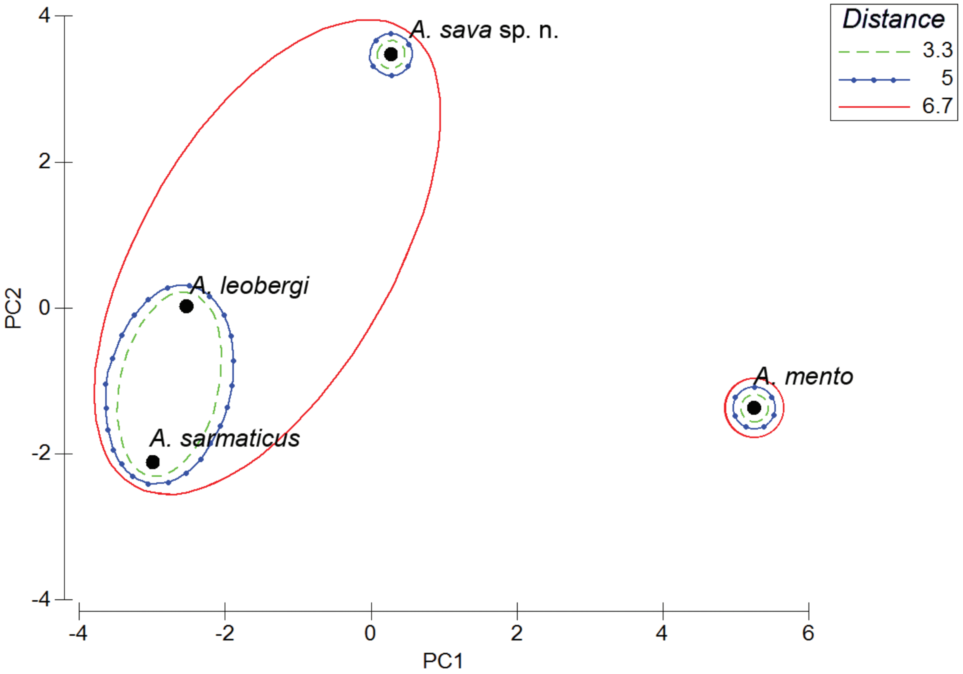

Figure 8. Results of PCA and MDS (Complete Linkage, Euclidean distances) realised in Primer v6 on meristic characters of *Alburnus sava* sp. n., *A. sarmaticus*, *A. mento*, and *A. leobergi*.

are comparatively close; specimens of these two species cannot be distinguished based on their meristic characters.

Based on the PCA, the most influential variables are the number of gill rakers and the number of scales along the scaleless portion of the ventral keel–these variables' contributions based on covariances are over 0.24 (Factor 1: 0.668885, Factor 2: 0.316944 and Factor 1: 0.241753, Factor 2: 0.434431, respectively) vs. less than 0.1025 for all other variables. A Kruskal-Wallis test revealed four (including the two mentioned above) characters different on a statistically significant (0.01%) level: the number of scales above the lateral line (H (3, 48)=18.0313487 p=0.0004); the number of branched pectoral-fin rays (H (3, 48)=19.2995932 p=0.0002); the number of gill rakers (H (3, 48)=34.8078209 p=0.0000001), and scales along the scaleless portion of the ventral keel (H (3, 48)=29.1477545 p=0.00002).

To reduce the number of morphometric indices (47 in total, as in Tables 1 and 3) to be used in a DFA, a Kruskal-Wallis test was applied and 10 characters were excluded as not significantly different (p>0.01%) between the samples. Among other 37 characters, a PCA revealed 15 variables whose contribution to either of Factors 1 and 2 or both was the highest (over an arbitrary fixed threshold of 0.01 based on the number of the used variables and their variance); these characters are given in bold in Tables 1 and 3. These 15 indices were used for a DFA (Fig. 9) that revealed three groups corresponding to *A. sava* sp. n., *A. mento*, and *A. sarmaticus* + *A. leobergi*.

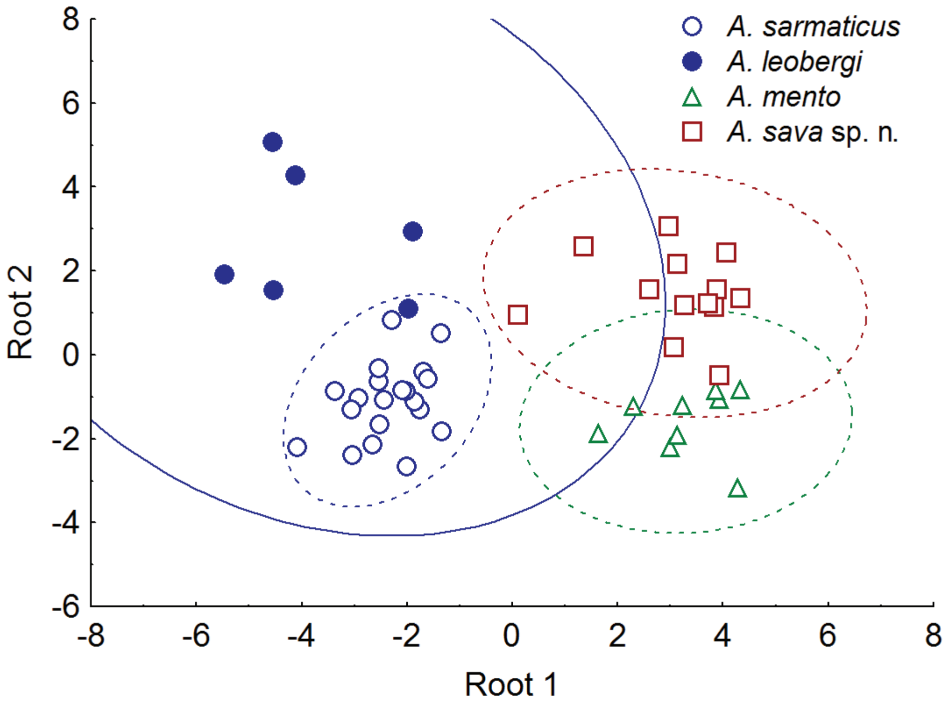

Figure 9. Result of DFA carried out on morphometric characters to discriminate *Alburnus sava* sp. n., *A. sarmaticus*, *A. mento,* and *A. leobergi*.

As a further step, the samples of *A. sarmaticus* and *A. leobergi* were combined as being indistinguishable by the studied morphometric and meristic characters (Figs 8, 9) and a DFA was performed based on 19 most contributing characters (4 meristic and 15 morphometric ones as discussed above) (Fig. 10); predicted classifications for *A. sava* sp. n., *A. mento*, and *A. sarmaticus* + *A. leobergi* were 100%-correct. DFA statistics values are as follows: Wilks' Lambda 0.00618, approx. F (38, 54) =16.661, p<0.0000, which indicate almost perfect discrimination. *Alburnus sava* sp. n. is the most distant from *A. mento* (Squared Mahalanobis Distance equals 103.99); *A. sarmaticus*+*A. leobergi* is much closer (54.11) but still significantly distant. The most removed, by the characters considered, are *A. mento* and *A. sarmaticus*+*A. leobergi* (107.02). Partial Lambdas demonstrate the unique contribution of the respective variable to the discriminatory power of the whole model, and the most significant (Partial Lambda <0.9) for discrimination of the samples under consideration were number of scales along scaleless portion of ventral keel (0.481705), cranium width between margins of sphenotics (% cranium roof length) (0.545483), cranium width between margins of pterotics (% cranium roof length) (0.803940), number of gill rakers (0.810206), number of branched pectoral-fin rays (0.830673), number of scales above lateral line (0.845142), length of lower jaw (% interorbital width) (0.878840), and body depth at dorsal-fin origin (% SL) (0.886565).

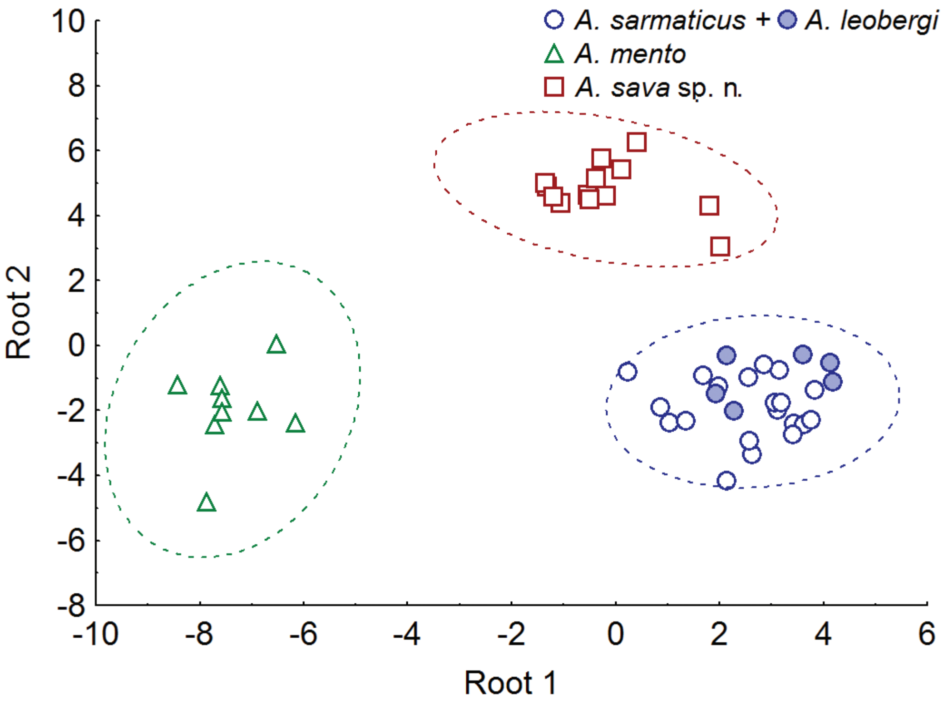

Figure 10. Result of DFA performed on 19 (4 meristic and 15 morphometric) most contributing characters to discriminate *A. sava* sp. n., *A. mento*, and *A. sarmaticus* + *A. leobergi*.

Discussion on distribution of shemayas in the Danube and adjacent areas

Shemayas are reported in the Danube from the upper reaches down to the delta (Fig. 4).

In the upper Danube, a shemaya, commonly identified as *A. mento*, was known to occur (or still occurs) in lakes in Germany in systems of the Isar (Starnberger See [Würmsee]) and the Inn (Chiemsee, Simsee, and Waginger See), in Austria in the Traun River system (Traunsee, Attersee, Mondsee, Hallstättersee, Grundsee, Wolfgangsee, probably Fuschlsee and Irrsee) and the Drava [Drau] system (Wörthersee [Vrba]), and in Slovenia in the upper Sava (Bled [Veldeser See, Blejsko Jezero]) (Freyhof and Brunken 2004, Freyhof and Kottelat 2007b, Glowacki 1896, Heckel 1836, Honsig-Erlenburg and Petutschnig 2002, Patzner et al. 1993). Most of these lakes belong to Danubian tributaries on the northern slope of the Alps. Lake Wörthersee is located much further south, on the northern slope of the Karawankas mountain ridge (one of the south-eastern ridges of the Alps) and belongs to the Gurk [Krka] River in the upper Drava system. *Alburnus mento* was found in the lake, in the inflowing Reifnitzbach and the outflowing Glanfurt River (Honsig-Erlenburg and Petutschnig 2002). Freyhof and Kottelat (2007b) found no morphological difference between *A. mento* inhabiting Lake Wörthersee and *A. mento* in the lakes of the Isar and Inn river systems. This means that *A. mento* has a rather geographically fragmented range and it cannot be excluded that a shemaya which was historically present in Lake Bled (Glowacki 1896, Munda 1926) in the very upper reach of the Sava River belonged to

A. mento. No museum samples of the Bled shemaya are known to exist to check this hypothesis. The lake morph (lake phenotype) from different lakes likely have a polyphyletic origin as found for lake *Coregonus* (e.g., Kottelat 1997, Douglas et al. 1999, Østbye et al. 2006). However, a problem of distinguishing between single (homology) and multiple (homoplasy of ecomorphological traits) origins of the distinct lake phenotype (= *A. mento*) and its taxonomic implication is beyond the scope of this paper.

In the Danube in Germany, Austria, Hungary, and Croatia downstream to the confluence with the Drava [Drau], one collection specimen (now lost) was known from near Vienna (NMW 55630, a syntype); this specimen may represent the anadromous or potamadromous Danubian species rather than the landlocked *A. mento* (Berg 1932, Freyhof and Kottelat 2007b). We examined the other NMW specimen labeled as *A. mento* from the Danube at Lobau near Vienna (NMW 54831) but it is a *Leuciscus aspius*. Shemayas were also known to be found occasionally in the Hungarian section of the Danube downstream of the Austrian section (Hankó 1931) but are extirpated (Halasi-Kovacs and Harka 2012). It was also mentioned as a fish occurring in a lake in the Maros [Mureș] river system (a tributary of the Tisza) (Herman 1887).

Based on records of Glowacki (1896) and Munda (1926), most subsequent authors gave the upper Sava and the upper Drava as areas of historical distribution of shemaya (e.g., Taler 1953, Sabioncello 1967, Povž 2012, Povž et al. 2015). Povž and Sket (1990) supposed its occurrence in the Mura [Mur] (a tributary of Drava), the entire Drava, and the lower course of the Sava. Povž et al. (1998) presented a first reliable record (with voucher specimens and a photo) of a shemaya from the Sava system. Several specimens were collected in the Kolpa [Kupa] River, a right tributary of the Sava, between Dol and Sodevci on August 11, 1996, between Dečina and Radenci on September 10, 1996, and up and below the Radenci dam on September 11, 1996.

In Croatia, a shemaya is reported from the Drava, Sava, Kupa [Kolpa] and their tributaries (Bogut et al. 2006, Mrakovčić et al. 2006, Jelić et al. 2016) but no voucher specimens are known except those from the Kolpa. However, it cannot be excluded that the authors based their statements on reliable data from local fishery sources. Further south and eastwards, in Bosnia and Herzegovina, a shemaya is found in the lower Sava and its tributaries Una, Sana, Vrbas, Ukrina, Tinja, Bosna (upper section with tributaries and from Žepče to the confluence with the Sava), and Drina (at Ljubovija and from Loznica to the confluence with the Sava) (Vuković and Ivanović 1971, Vuković 1977, 1982, Kosorić et al. 1980, Hađiselimović and Hamzić 1999, Mikavica and Savić 1999, Drešković et al. 2011, Simonović et al. 2015, Jelić et al. 2016). In Serbia, it is reported from the Danube down to the Iron Gates [Đerdap] at the border with Romania (Janković 1996, Simonović 2001) and from the Zapadna Morava River between the Ovčar Banja and Međuvršje reservoirs (Veljović et al. 1985) and in its tributary Ibar (Šorić 2009).

Formally, the name *A. sava* sp. n. is applied herein only for the shemaya found in the Kolpa but we suppose that this potamadromous fish inhabits (or inhabited in the past) the entire Sava River system and, probably, the Danube upstream from the Iron Gates being geographically separated from the anadromous shemaya of the lower Danube (*A. danubicus* and *A. sarmaticus*).

Right below the Iron Gates (a 134-m-long gorge on the main stream of the Danube dammed in 1964 and 1977), shemaya were known in Romania (Bănărescu 1964) and the entire Bulgarian stretch of the Danube with tributaries (e.g., Iskyr, Vit, Osym, Yantra) where it still occurs though rare (numerous publications reviewed by Stefanov and Trichkova (2015). Records of shemaya in the lower section of the Danube (downstream from Cernavodă) in Romania and Ukraine were also numerous (e.g., Drensky 1943, Bănărescu 1961, 1964, Movchan and Smirnov 1983).

So, historically, shemaya in the Danube were distributed in almost the entire drainage. The length of the Danube and its major tributaries makes it reasonable to suppose that the drainage was historically populated by an anadromous shemaya (entering the river for spawning from the sea) and a resident potamadromous shemaya. At present, it is difficult to assume how far the anadromous form(s) or species used to migrate upstream in the Danube. All anadromous shemayas in the Black Sea and the Sea of Azov basins spend most of the year foraging in coastal sea areas, estuaries, and limans with salinity up to 10–12‰ and wintering in deep places of lower reaches of rivers. Before damming of rivers, they migrated upstream to reach spawning grounds at a distance from tens to hundreds of kilometers depending on the size of the river and availability of grounds suitable for spawning (riffles with gravel bottom and rapid flow). Shemaya were known to migrate up to Pervomaysk in the South Bug, the Dnieper River Rapids (upstream of the town of Zaporizhia) in the Dnieper, and the Oskol River in the Siversky Donets system of the Don River (Movchan and Smirnov 1983). It looks quite likely that the anadromous shemaya in the main Danube migrated upstream to at least the Iron Gates gorges. Our data support a taxonomic conclusion of previous authors (Bănărescu 1961, 1964, Tscherbukha 1965, Loshakov 1973, Freyhof and Kottelat 2007b) that the South Bug and Dnieper and the Danube are populated by one and the same species.

No materials were available for us to clarify a border between the ranges of *A. danubicus*, *A. sarmaticus*, and *A. sava* sp. n. This question can only be solved when specimens from the mainstream Sava and its tributaries in Croatia, Bosnia and Herzegovina, and Serbia are studied as well as specimens from the Danube below its confluence with the Sava. This task cannot be easily implemented because of the rarity of the fish; though, in some tributaries of the Sava in Bosnia and Herzegovina it is still often recorded (Jelić et al. 2016).

Comparative material

Alburnus sarmaticus ZM NASU 4145, 5, 170.7–204.5 mm SL, Ukraine, South Bug River, 2 km downstream of Yuzhno-Ukrainsk, 7–11.05.1989; ZM NASU 2529, 6, 157.5–209 mm SL, Ukraine, Danube, 1922; NMW55507, 3, 178–208.8 mm SL, Lower Danube, Galatz, 1910; NMW55509, 5, 169.4–202.8 mm SL, Lower Danube, Cernavodă, 1910; NMW 55654,1, 175.3 mm SL, Delta of Danube, Vilkovo, 08.1924.

Alburnus leobergi NMW 6, 154.8–193.1 mm SL, Yasenskiy Bay, Sea of Azov.

Alburnus mento NMW 16261, 1, syntype, 139.8 mm SL, Traunsee; NMW 16441, 1, syntype, 134 mm SL, Traunsee; 50440, 1, syntype, 220.4 mm SL, Bavaria; NMW 50441, 1, 89.2 mm SL, Wörther-See; NMW 55628, 7, 123.8–147.4 mm SL, Gilgen, Wolfgangsee; NMW 55629, 2, 129.2–144 mm SL, Kremsmünster; NMW 55634, 17, 103.3–156.4 mm SL, Traunsee; NMW 55636, 9, 97.1–122.7 mm SL, Attersee; NMW 55642, 4, 148–162.9 mm SL, Gilgen, Wolfgangsee; NMW 55646, 1, 104.4 mm SL, Wörther See; NMW 55648, 2, 112,2–112.9 mm SL, Attersee; NMW 56652, 1, syntype, 210.9 mm SL, Bavaria; NMW 79592, 5, 134.3–143.3 mm SL, Mondsee; NMW 79593, 1, 214.3 mm SL, Mondsee; NMW 80138, 1, 221.2 mm SL, Mondsee; NMW 80622, 1, 191.2 mm SL, Mondsee; NMW 80623, 7, 170.4-220.5 mm SL, Mondsee.

Key to *Alburnus* species in the Danube drainage

1 Ventral keel between pectoral bases and anus completely scaleless. Lateral-line scales to posterior margin of hypurals 42–50 .... ***A. alburnus***

– Ventral keel between pectoral bases and anus completely scaled or covered by scales at least at anterior part of keel. Lateral-line scales to posterior margin of hypurals 52–71 .... **2**

2 Branched anal-fin rays 17–20½ .... ***A. danubicus***

– Branched anal-fin rays 13–17½, usually 14–16½ **3**

3 Gill rakers 28–34 .... ***A. sarmaticus***

– Gill rakers 19–27 .... **4**

4 Branched pectoral-fin rays 15–16, usually 15. Ventral keel between pectoral bases and anus scaleless along 1–2 scales (up to 15% of keel length) or completely scaled .... ***A. sava***

– Branched pectoral-fin rays (15)16–18, usually 17. Ventral keel between pectoral bases and anus scaleless along 3–13 scales (25–90% of keel length) ..... ***A. mento***

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RESEARCH ARTICLE

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Stranding survey as a framework to investigate rare cetacean records of the north and north-eastern Brazilian coasts

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Abstract

Marine mammal stranding events are used as an important tool for understanding cetacean biology worldwide. Nonetheless, there are vast gaps of knowledge to be filled in for a wide range of species. Reputable information is required regarding species from large baleen whales to sperm and beaked whales, as well as pelagic dolphins. This paper describes new cetacean records from north and north-eastern Brazil, which are both the least surveyed areas regarding aquatic mammals. Regular beach surveys were conducted to recover cetacean carcasses along the coast of Pará beginning November 2005. At the coasts of the Maranhão and Piauí states, the surveys were conducted between 2003 and 2013. From 2003 to 2014, 34 strandings of cetaceans were registered. The study provides four additional species records' in the area based on strandings (*Balaenoptera borealis, Balaenoptera physalus, Peponocephala electra,* and *Pseudorca crassidens*). A mass stranding of Guiana dolphins (*Sotalia guianensis*, N = 12), the most common species for the region, was reported for the first time. The records presented herein are of special concern, since they expand the knowledge on cetaceans from the Brazilian coast. In addition, this study conducted an analysis to verify the similarity between cetacean compositions described for north and north-eastern Brazil and the southern Caribbean region. The results showed a high similarity between these regions, proving the connection with the Caribbean cetacean fauna.

Keywords

Amazon, Balaenopteridae, beach survey, cluster analysis, Delphinidae, marine mammals distribution, Physeteridae, South America

Introduction

The Amazon Coastal Zone (ACZ), between 4°N and 4°S, presents a singular environment, characterized by the immense discharge of the Amazon River. ACZ is an unique aquatic ecosystem where the largest continuum of mangrove belt of the world is found (Souza-Filho et al. 2005). These characteristics combined with the recent coral reef ecosystem discovered in the mouth of the Amazon (Moura et al. 2016) make this region a top priority for conservation among Brazilian coastal environments. In this vast coastal area cetacean records have been documented only in the last decade. The north and north-eastern Brazil have been one of the least surveyed areas regarding aquatic mammals. Only one report made a compilation of cetacean records from this area (Siciliano et al. 2008) bringing the number of recorded species to 22, including large whales and dolphins. This study is an effort to increase this information after abovementioned study. Stranding events is one of the best ways to access data on biology and ecology of marine mammals (Norman et al. 2004, Evans et al. 2005, Pyenson 2010, Santos et al. 2010, Peltier et al. 2012, Covelo et al. 2015).

The cetacean fauna of Brazil has been subject to an extensive number of groups dedicated to conservation and research issues since the 1980's. Because of this, a considerable amount of new information on cetacean distribution, biology and ecology has arisen (e.g. Pinedo et al. 2001; Zerbini et al. 2004). In 2005, with the establishment of the 'Grupo de Estudos de Mamíferos Aquáticos da Amazônia (GEMAM)' at the Museu Paraense Emílio Goeldi, the first systematic studies on aquatic mammals were initiated in the region.

Nevertheless, there are vast gaps of knowledge to be filled in for a wide range of species. From large baleen whales to sperm and beaked whales, as well as pelagic dolphins, reputable information is required. This is due, in part, to the lack of inventories over large areas of the Brazilian coast.

Studies on the occurrence of cetaceans around the world are made using different methodologies such as: strandings, historical records, opportunistic sightings, and dedicated surveys (Maldini et al. 2005, Lucena 2006, Toledo and Langguth 2009, Ramos et al. 2010, Authier et al. 2014, Prado et al. 2016). In other words, cetacean composition in a specific area could be assessed through strandings when other sources of information are not available (Maldini et al. 2005, Byrd et al. 2014, Jung et al. 2015). In this sense, Pyenson (2010) validated that cetacean death assemblages is a faithful method to document taxonomic richness and relative abundance of living cetaceans.

The main goals of this report are to: (1) present a variety of new cetacean records along the northern Brazilian coast, (2) increase baseline information on cetacean occurrence over 11 years (2003–2014); and (3) compare the cetacean composition in distinct areas along the north and north-eastern Brazilian coasts with southern Caribbean region, investigating the similarity between these sectors. The results of this study could open a promising new window for understanding the cetacean community structure in this vast stretch of coast.

Materials and methods

Study sites

The northern Brazilian coast has Cabo Orange as its limits to the north (05°N, 51°W), and São José Bay to the south (02°S, 44°15'W) and represent the Atlantic coastal sector. This vast coastal area extends for approximately 2250 km composed of different systems: mangrove forests, salt marshes, tidal sand flats, chenier sand ridges, coastal dunes, beach ridge barriers, and ebb tidal delta (Souza-Filho et al. 2008). The semi-diurnal macro-tidal regime with variations of approximately 4 m in Marajó Bay and 7.5 m in São José Bay (DHN 2010) is another characteristic. Also, the ecosystem in this region is strongly influenced by rain season (high precipitation, low salinity, and high turbidity) and dry season (low precipitation, high salinity, and low turbidity) (Moraes et al. 2005). The freshwater input along this coastline is different. In the northern the influence of the enormous discharge of the Amazon River dominates, while in the eastern coast of Maranhão and in the coastline of Piauí, the principal effect comes from the discharge of Parnaíba River (Szczygielski et al. 2014).

The study site of this report is divided into three sampling sectors: (1) Marajó Bay (MB), (2) Eastern Pará state (EP) and (3) Maranhão/Piauí coastline (MA/PI), encompassing part of Parnaíba Delta (Fig. 1).

Marajó Bay is located on the eastern coast of Marajó Island (Fig. 1, frame 1). This bay is formed mainly by the discharges of Pará and Tocantins rivers and Guajará Bay and is influenced by oceanographic processes such as superficial saline intrusion during dry season under low river discharges.

In the eastern coast of Pará (Fig. 1, frame 2) and north-west coast of Maranhão the coastline is designated as Amazon macrotidal coastal zone. This region encompass almost 70% of Brazilian mangrove forests, possess low relief (0 to 80 m), broad coastal plain (with up to 70 km wide) and large continental shelf (200 km approximately). This coastline is extremely irregular and forms numerous bays and small estuaries, the area is influenced for a macrotidal regime (Souza-Filho 2005).

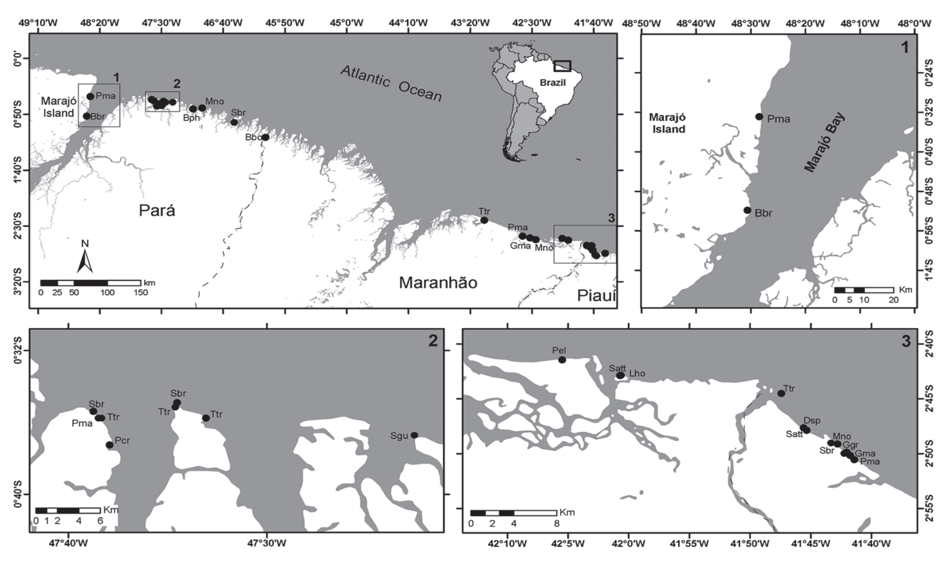


Figure 1. Study sites. Surveyed sectors on north and north-eastern Brazilian coast: **1** Marajó Bay **2** Eastern Pará state and **3** Maranhão and Piauí coastlines encompass part of Parnaíba Delta. Black dots representing stranding locations. *Physeter macrocephalus* (Pma), *Balaenoptera brydei* (Bbr), *Balaenoptera physalus* (Bph), *Megaptera novaeangliae* (Mno), *Steno bredanensis* (Sbr), *Balaenoptera borealis* (Bbo), *Tursiops truncatus* (Ttr), *Globicephala macrorhynchus* (Gma), *Pseudorca crassidens* (Pcr), *Sotalia guianensis* (Sgu), *Peponocephala electra* (Pel), *Stenella attenuata* (Sat), *Lagenodelphis hosei* (Lho), *Delphinus* sp. (Dsp) and *Grampus griseus* (Ggr).

Maranhão and Piauí coastlines, encompassing the Parnaíba Delta (Fig. 1, frame 3), are considered a semiarid region. Parnaíba River discharge into the Atlantic Ocean forming a delta with five bays: Tutóia, Caju, Melancieiras, Canárias and Igaraçu (Moreira and Mavigner 2007). Parnaíba Delta comprises a mesotidal coast with beaches with up to 200 m wide and large areas of mangrove forests (Szczygielski et al. 2014).

Stranding events in these areas were regularly monitored between 2005 and 2014 in MB and EP sampling sectors, and from 2003 to 2013 in MA/PI sector (Fig. 1, see frame 1 and 2). Every two-week period, the vast stretch of coastline is reached by boat and four-wheel vehicle or, alternatively, monitored by foot. At least three field monitors take part in the field and on dedicated surveys the same routes were covered.

On occasion, the team was called to respond to live strandings or carcasses washed ashore. These events, as they are exclusive in their nature and circumstances, are validated with voucher samples, photographs, and other sources of original information.

The carcasses were identified in the field following specific features (e.g. colouration, shape of the head and fins/flippers, number of teeth). In such specific cases as *Delphinus* sp. and *Megaptera novaeangliae*, the identification was made posteriorly through cranial measures and scapular features, respectively.

Complete and incomplete carcasses were recovered and their skeletons and soft tissues, after preparation, are deposited at the Mammal Collection from Museu Paraense Emílio Goeldi (MPEG), located in Belém, Pará, Brazil. Table 1 lists the records of cetaceans stranded from 2003 to 2014 on the northern and north-eastern Brazilian coast according to the three sampling sectors, including voucher numbers from the abovementioned collection.

Data source and analysis

The similarity between the cetacean composition described in this study and the most representative surveys conducted in the north (Siciliano et al. 2008) and part of the north-eastern Brazilian coasts (Alves-Júnior et al. 1996, Motta et al. 2008, Meirelles et al. 2009), in addition to the Caribbean region (Romero and Creswell 2005, Luksenburg 2014a, 2014b), was analyzed.

Similarities between the stranding and sighting records of cetaceans reported in seven articles and the present study was accomplished through an analysis of similarity by the Jaccard index based on the presence or absence of species. A similarity profile (SIMPROF) test was performed to detect the significantly different groups using the default of 1.000 permutations for the mean similarity profile and 999 permutations for the simulated profile, with a significance level of 0.05. Multivariate analyses were performed using PRIMER 6.0 (PRIMER-E Ltd., Plymouth, U.K) (Clarke and Gorley 2006).

Results

Species richness and diversity

The present study recorded rare strandings of some cetacean species (e.g. mass stranding of *Sotalia guianensis*), and the first occurrence of these cetaceans in northern coast: 1. Sei whale, *Balaenoptera borealis* Lesson, 1828, 2. Fin whale, *Balaenoptera physalus* (Linnaeus, 1958), 3. Melon-headed whale, *Peponocephala electra* (Gray, 1846) and 4. False killer whale, *Pseudorca crassidens* (Owen, 1846).

Species richness was represented for 15 taxa, within three distinct families: Balaenopteridae (*B. borealis*, *B. physalus*, *B. brydei*, *M. novaeangliae*), Physeteridae (*P. macrocephalus*) and Delphinidae (*Delphinus* sp., *G. macrorhynchus*, *G. griseus*, *Lagenodelphis hosei*, *P. electra*, *P. crassidens, S. guianensis*, *S. attenuata, S. bredanensis, T. truncatus*) (Table 1).

Spatial distribution

In these sampling sectors (MB = Marajó bay; EP = Eastern Pará; MA/PI = Maranhão/Piauí coastline) stranding events were distributed unequally. The spatial distribution among

Table 1. Cetacean records from 2003 to 2014 on the northern Brazilian coast.

Species by Family	Category	TL (m)	Sex	Sector	Date	Voucher specimens
Balaenopteridae						
Balaenoptera borealis	LS	10.32	F	EP	13/09/2008	MPEG 39691
Balaenoptera brydei	CA	4.20	NI	MB	15/09/2012	MPEG 42154
Balaenoptera physalus	LS	14.90	M	EP	21/01/2010	MPEG 39690
Megaptera novaeangliae	CA	16.70	F	MA/PI	15/01/2003	-
	CA	-	NI	MA/PI	2005	-
	CA	10.00*	M	EP	08/10/2008	MPEG 39692
	CA	13.00*	NI	MA/PI	22/05/2009	MPEG 42184
Physeteridae						
Physeter macrocephalus	CA	11.80	F	MA/PI	02/02/2010	MPEG 42088
	CA	-	F	MA/PI	14/05/2010	MPEG 42173
	LS	10.50	F	EP	07/04/2014	MPEG 42177
	CA	4.22	NI	MB	08/08/2014	MPEG 42178
Delphinidae						
Delphinus sp.	CA	2.06	NI	MA/PI	12/08/2011	MPEG 42095
Globicephala macrorhynchus	CA	-	NI	MA/PI	2009	-
	CA	-	NI	MA/PI	15/01/2009	MPEG 42128
Grampus griseus	CA	-	NI	MA/PI	13/04/2011	MPEG 42130
Lagenodelphis hosei	LS	-	M	MA/PI	03/04/2009	MPEG 42080
Peponocephala electra	CA	-	NI	MA/PI	2007	MPEG 42067
	CA	-	NI	MA/PI	19/06/2008	MPEG 42069
Pseudorca crassidens	CA	3.30	NI	EP	20/04/2012	MPEG 42132
Sotalia guianensis	LS	-	NI	EP	31/01/2013	-
Stenella attenuata	CA	-	NI	MA/PI	14/03/2009	MPEG 42077
	CA	1.59	M	MA/PI	10/12/2009	MPEG 42085
Steno bredanensis	CA	-	NI	EP	19/04/2009	MPEG 39635
	CA	-	NI	MA/PI	15/08/2011	MPEG 42096
	CA	-	NI	EP	20/11/2011	MPEG 42066
	CA	-	NI	EP	20/11/2011	MPEG 42102
	CA	2.68	M	EP	04/04/2012	MPEG 42131
	CA	-	NI	MA/PI	23/08/2013	MPEG 42176
Tursiops truncatus	CA	-	NI	MA/PI	24/04/2009	MPEG 42129
	CA	-	NI	MA/PI	26/07/2009	MPEG 42081
	CA	-	NI	MA/PI	05/03/2010	-
	CA	-	NI	EP	12/03/2010	MPEG 39612
	CA	3.17	M	EP	13/02/2013	MPEG 42174
	CA	2.87	F	EP	04/03/2013	MPEG 42175

Species were divided by family, details of specimens and stranding. Abbreviations: TL= total length in meters; Sex= female, male and not identified (NI); Category of stranding - live (LS) and carcass (CA), Date, sector and location of events; MB= Marajó bay; EP= Eastern Pará state; MA/PI= Maranhão/Piauí coastline

* represents the estimated TL (specimens missing the skull)

sectors showed that the majority of strandings occurred at the Maranhão/Piauí coastline 54.5% (N = 18), on the north-eastern coast of Brazil. The EP occupies the second place, with 39.4% (N = 13) and MB with only 6.1% (N = 2) of rare cetacean records.

Differences in strandings by family and sector were analyzed in order to evaluate patterns along the distinct sectors of this coastline. Members of the Delphinidae family were the most representative in the frequency of strandings, comprising 66.7% (N = 22), followed by Balaenopteridae 21.2% (N = 7) and Physeteridae 12.1% (N = 4).

Cetacean Composition

Balaenopteridae

Balaenoptera borealis Lesson, 1828

Sei whale

On 13 September 2008 a large whale stranded alive on the eastern Pará state (EP). The specimen (MPEG 39691) had features characteristically attributed to Sei whales: a dark body with patches of lighter gray, both sides of head evenly dark, head slightly arched, throat grooves ending just behind the flippers, grey-black baleen plates with a metallic sheen, lighter plates near the front of the mouth and a sickle-shaped dorsal fin. After unsuccessful trials of pushing the whale back to the sea, it died (Fig. 2d). The necropsy revealed a large amount of mud in the stomach, probably originated from the mangrove sediments nearby the stranding site.

Balaenoptera brydei Olsen, 1913

Bryde's whale

On 15 September 2012 a decomposed carcass of a newborn *B. brydei* washed ashore on the rocky shore of Praia de Água Boa, Salvaterra, Marajó bay (MB), Pará (Fig. 2b). Partial skeleton and tissue samples were collected (MPEG 42154).

Balaenoptera physalus (Linnaeus, 1958)

Fin whale

On January 2010, a male Fin whale *B. physalus* was reported stranded on the eastern Pará state (EP) in Baía do Japerica, São João de Pirabas municipality. Fishermen and residents sighted the live specimen entering the estuary but a few days later the carcass was found stranded in a different location. The fresh specimen had a large callosity on the top of the head (Fig. 2a). The nearly complete skeleton, including skull and mandibles, is deposited in the mammal collection (MPEG 39690).

Figure 2. Stranding events: large baleen whales. **a** Fin whale *Balaenoptera physalus* stranded at Baía do Japerica, São João de Pirabas municipality (0°45'S, 47°4.2'W), eastern coast of the Pará state. Note the good condition of the carcass and the strange swelling on the top of the head (see white arrow) (photo by GEMAM/MPEG) **b** A newborn Bryde's whale *Balaenoptera brydei* stranded on the east coast of Marajó island (0°51.6'S, 48°30'W), Pará on 15 September 2012 (photo by GEMAM/MPEG) **c** Humpback whale *Megaptera novaeangliae* stranded at Praia do Rio Novo, Tutóia municipality, Maranhão state (2°42.6'S, 42°26.4'W), note the absence of skull on the carcass (photo by A.F. Costa) **d** Sei whale *Balaenoptera borealis* stranded at Fernandes Belo, Viseu municipality (1°10.8'S, 46°5.4'W), the particular coloration of the baleen plates and slightly arched head are diagnostic characters to identify the species (photo by DEMA/PA).

Megaptera novaeangliae (Borowski, 1781)

Humpback whale

The first evidence of a Humpback whale *M. novaeangliae* in Piauí is represented by a female stranded at Praia da Pedra do Sal, Parnaíba on 15 January 2003. Subsequently, in 2005, locals collected a scapula at Praia da Carnaubinha, Luís Correia municipality. On May 2009, a decomposed carcass missing the skull was found stranded in the Praia do Rio Novo, Tutóia municipality, voucher No MPEG 42184 (Fig. 2c).

Figure 3. Stranding events: sperm whales. **a** Sperm whale *Physeter macrocephalus* (MPEG 42166) stranded at Praia do Crispim, Marapanim municipality (0°34.8'S, 47°38.4'W) eastern coast of Pará state few hours after death (photo by A.F. Costa) **b** The same specimen (MPEG 42166) still alive with local people trying to help (internet file) **c** A newborn Sperm whale (MPEG 42178) stranded at the east coast of Marajó island, Praia do Camburupy (0°32.4'S, 48°28.2'W) on 2014, note the absence of teeth characteristic of a very young specimen (photo by A.F. Costa) **d** A female Sperm whale (MPEG 42088) stranded at Praia da Pedra do Sal, Parnaíba municipality, Piauí state coastline (2°49.8'S, 41°41.4'W) on 2010 (photo by A.F. Costa).

Physeteridae

Physeter macrocephalus Linnaeus, 1758

Sperm whale

On 02 February 2010 a female with TL = 11.80 m (MPEG 42088) stranded at Praia da Pedra do Sal, Parnaíba municipality, Piauí state (Fig. 3d). The carcass was moderately fresh, had numerous bites of cookie-cutter shark (*Isistius* sp.) over the body and evidence of endoparasites in the blubber. The remains of a second specimen, also a female, stranded at Praia do Baixo da Boia, Paulino Neves municipality, Maranhão state on 14 May 2010. Some vertebrae and chevrons with deformations were recovered (MPEG 42173). On 07 April 2014, a live-stranded Sperm whale was recorded for the first time along the Pará coastline. The specimen was a female with TL = 10.50 m (MPEG 42166) that came ashore at Praia do Crispim (Fig. 3a, b), had a large amount of squid beaks in its stomach and intestines. On 08 August 2014, a very young specimen (TL = 4.22 m) stranded at east coast of the Marajó Island in Praia do Camburupy, Soure municipality. Sex was not determined due to the advanced decomposition of the carcass (Fig. 3c).

Delphinidae

Oceanic species

Delphinus sp. Gray, 1828

Common dolphin

On 12 August 2011 a decomposed delphinid carcass was found at Praia da Pedra do Sal, Parnaíba municipality, Piauí (MA/PI coastline) (MPEG 42095). This is the very first record of a Common dolphin for this portion of the Brazilian coast.

Lagenodelphis hosei Fraser, 1956

Fraser's dolphin

On the coast of the Maranhão state, at Praia da Barrinha, Canárias Island, the carcass of a Fraser's dolphin *Lagenodelphis hosei* was found fresh by fishermen on 03 April 2009, after it presumably live-stranded on the beach. The carcass was divided up at the village of Carnaubeira, Araioses municipality (Fig. 4a) and consumed locally (meat slices were found drying on clothes-line in villagers' houses).

Stenella attenuata (Gray, 1846)

Pantropical spotted dolphin

The Pantropical spotted dolphin *S. attenuata* is represented by two records from the MA/PI sector, both from 2009. Bone remains were found at the Canárias Island, Praia da Barrinha, Maranhão (MPEG 42077) and a decomposed carcass of a male was found at Praia da Pedra do Sal, Parnaíba municipality, Piauí (MPEG 42085), with marks of human interaction.

Figure 4. Stranding events: small and medium-sized cetaceans. **a** The carcass of a Fraser's dolphin, *Lagenodelphis hosei* was cut down on the village of Carnaubeira, Araioses municipality (2°42.6'S, 42°0.6'W) and consumed locally (photo PROCEMA files) **b** A group of 12 live Guiana dolphins *Sotalia guianensis* were found trapped (photo showed just one specimen) in a tidal channel alongside Corvina beach, Salinópolis municipality, eastern Pará (0°36'S, 47°22.2'W) **c** A False killer whale *Pseudorca crassidens* stranded on the north-eastern coast of Pará, Praia de Marudá, Marapanim municipality (photo GEMAM files) **d** A Short-finned pilot whale *Globicephala macrorhynchus* stranded in advanced stage of decomposition at Praia da Pedra do Sal, Parnaíba municipality (2°40.8'S, 42°31.2'W), the shape of the head and number of teeth were diagnostic to identify the species (photo PROCEMA files).

Blackfish

A Short-finned pilot whale *Globicephala macrorhynchus* Gray, 1846 was found stranded in an advanced state of decomposition in the Parnaíba municipality, Piauí (MA/PI), on 15 January 2009 (MPEG 42128). Identification was made possible through a combination of head features and tooth counts (Fig. 4d). In August 2009, during beach surveys at the Maranhão coast, the photographic record of a mounted skeleton on display in a beach tent was confirmed as *G. macrorhynchus* at the locality of Paulino Neves (MA/PI).

An incomplete Risso's dolphin skull *Grampus griseus* (G. Cuvier, 1812) (MPEG 42130) found buried was recovered at Praia da Pedra do Sal, Parnaíba municipality, Piauí, MA/PI sector, on 13 April 2011.

Two stranding records of Melon-headed whale *Peponocephala electra* (Gray, 1846) were reported for the Ilha do Caju and Delta do Parnaíba (MA/PI). Only bone remains were recovered (MPEG 42067, MPEG 42069).

The first record of a False killer whale *Pseudorca crassidens* (Owen, 1846) on the Pará coastline occurred on 20 April 2012. The carcass was found in the mudflats at Praia de Marudá (Fig. 4c), Marapanim municipality, eastern Pará (EP). The specimen (MPEG 42132) had marks of human interaction; the peduncle was cut-off and most of the teeth were removed.

Coastal species

Sotalia guianensis (Van Bénedén, 1864)

Guiana dolphin

On 31 January 2013, 12 Guiana dolphins *S. guianensis* were reported as trapped in a tidal channel alongside Praia da Corvina, Salinópolis municipality, eastern Pará state (Fig. 4b, see Suppl. materials 1 and 2). They were all rescued by life savers and locals, who carried the dolphins along 700 m to the receding waters during the low tide. This event could be considered a mass stranding of Guiana dolphins, so far the first reported for the northern coast of Brazil, and presumably in the country.

Steno bredanensis (G. Cuvier in Lesson, 1828)

Rough-toothed dolphin

On 04 April 2012, the carcass of a mature male (MPEG 42131) Rough-toothed dolphin *S. bredanensis* stranded in a moderate state of decomposition showed bites and a huge scar on the caudal peduncle, which could indicate interaction with long line fisheries. New records of rough-toothed dolphins were confirmed from EP, MPEG 39635 (Praia da Princesa, Algodoal Island, Maracanã municipality), MPEG 42066/MPEG 42102 (Praia do Crispim, Marapanim municipality) and from MA/PI coastline, MPEG 42096/MPEG 42176 (Praia da Pedra do Sal, Parnaíba municipality).

Tursiops truncatus (Montagu, 1821)

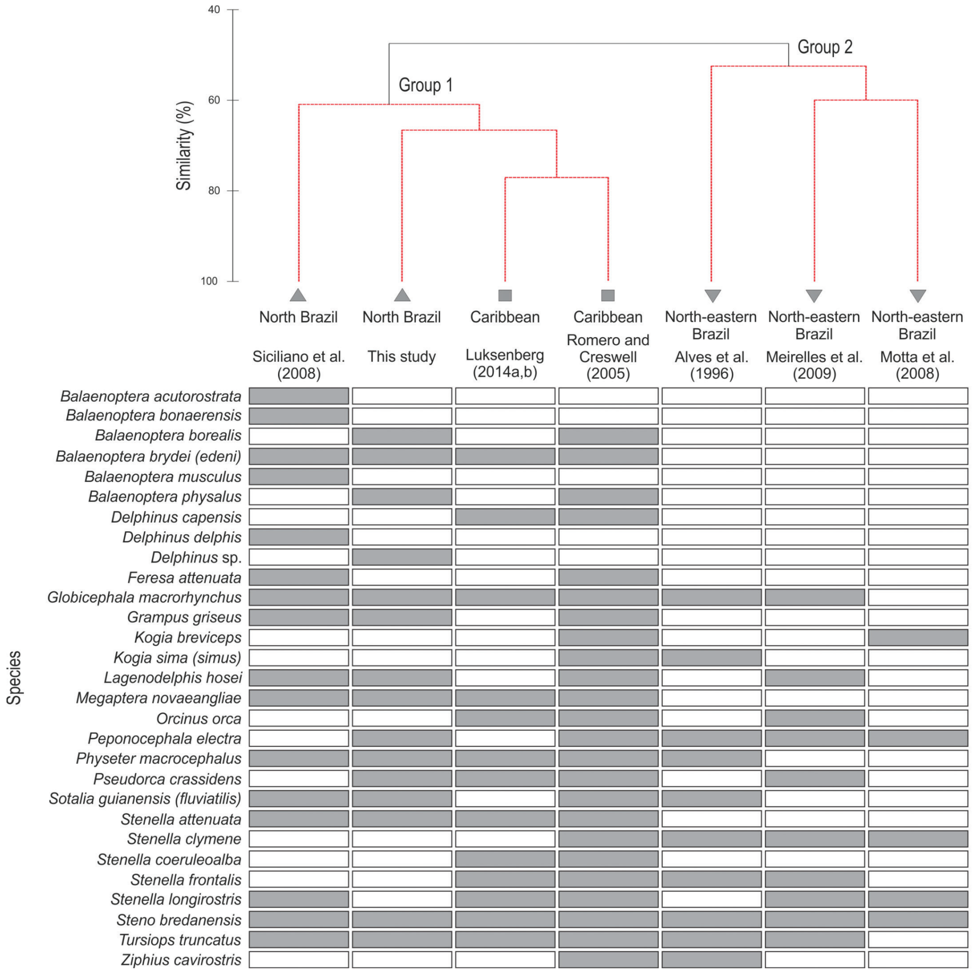
Bottlenose dolphin

Five new records of Bottlenose dolphin *T. truncatus* were reported after the previous review by Siciliano et al. (2008) (see Table 1 for complete information). On 5 March 2010, in Praia da Travosa, Santo Amaro municipality, Maranhão (MA/PI) (See Suppl. material 3), a young Bottlenose dolphin *T. truncatus* was found live-stranded on a beach and was rescued by bathers, locals and fishermen.

Cluster analysis

The cluster analysis indicated that the cetacean composition from the Caribbean and north Brazilian coast form a single group (Group 1) that differs significantly from the group formed at north-eastern Brazil (Group 2) (Fig. 5).

The richness of species varied from 13 to 24, with 29 species in totality. Group 2 was defined by adding stranding information from north-eastern Brazil (Alves-Júnior et al. 1996, Motta et al. 2008, Meirelles et al. 2009). Species richness ranged from 5 to 10, with 15 species in total.



	North Brazil Siciliano et al. (2008)	North Brazil This study	Caribbean Luksenberg (2014a,b)	Caribbean Romero and Creswell (2005)	North-eastern Brazil Alves et al. (1996)	North-eastern Brazil Meirelles et al. (2009)	North-eastern Brazil Motta et al. (2008)
Balaenoptera acutorostrata	■						
Balaenoptera bonaerensis	■						
Balaenoptera borealis		■		■			
Balaenoptera brydei (edeni)	■	■	■	■			
Balaenoptera musculus	■						
Balaenoptera physalus		■		■			
Delphinus capensis			■	■			
Delphinus delphis	■						
Delphinus sp.		■					
Feresa attenuata	■			■			
Globicephala macrorhynchus	■	■	■	■	■	■	
Grampus griseus	■	■		■			
Kogia breviceps				■			■
Kogia sima (simus)				■	■		
Lagenodelphis hosei	■	■		■		■	
Megaptera novaeangliae	■	■	■	■			
Orcinus orca			■	■		■	
Peponocephala electra		■		■	■	■	■
Physeter macrocephalus	■	■	■	■	■		
Pseudorca crassidens		■	■	■		■	
Sotalia guianensis (fluviatilis)	■	■		■	■		
Stenella attenuata	■	■	■	■			
Stenella clymene				■	■	■	■
Stenella coeruleoalba			■	■			
Stenella frontalis			■	■	■	■	
Stenella longirostris	■		■	■		■	■
Steno bredanensis	■	■	■	■	■	■	■
Tursiops truncatus	■	■	■	■	■	■	
Ziphius cavirostris				■	■		

Figure 5. Cluster analysis of cetacean stranding and sighting records. Diagram of cetaceans reported from the north-eastern (inverted triangle) and north coast of Brazil (triangle) and southern Caribbean (square), reported in seven articles and the present study. The two distinct groups formed (Group 1 and 2) are indicated. A similarity profile (SIMPROF) permutation test highlights dashed clusters that show significant internal structure. Shaded cells indicate occurrence of species.

Discussion

Stranding data are an important source to understanding the biology and ecology of marine mammals (Geraci and Lounsbury 1993, Moura and Siciliano 2012, Covelo et al. 2015). Indeed, the records presented herein are of particular importance, as they clarify the composition and richness of the cetacean community in a vast stretch of the Brazilian coast. Current data, with the addition of four species to the previous list (Siciliano et al. 2008), expanded this number to 26 aquatic mammal species. This study is in accordance to (Pyenson 2010) which suggests that stranding records at extensive latitude gradients (>1000 km) and for long time periods, provide an useful and faithfully method to understand richness and cetacean community in a particular area than the sighting surveys (e.g. line-transect method). Maldini et al. (2005) analyzing the composition of cetaceans of the Hawaiian Islands suggested that the stranding data reflects the species composition found in living animals surveys and defends the usefulness use of stranding data as an important source of information in the absence of other data. Marine mammalogists assumed that stranding events could be affected by oceanographic features such as bottom topography, tides, currents, winds and seasonal patterns (Norman et al. 2004). Carcasses can be moved by currents and winds along the coast and the oceanographic differences observed in this vast area of the north and north-eastern Brazilian coast should be considered important factors on stranding distributions. This coastline is singular and considered a continuous belt of mangroves with approximately 7591km^2 (Souza-Filho 2005) which complicates access and, consequently the rescue and reports of stranded marine mammals. The macrotidal characteristic observed in the Marajó Bay and Eastern Pará sectors is another factor that should explain the low frequency of strandings especially of small and medium-sized cetaceans. The dynamic of macrotidal regime may in a few hours carry the carcass of a small cetacean without it could be seen in these vast uninhabited areas.

The spatial distribution of species in the three sectors (MB, EP and MA/PI coastline) provides relevant information on their occurrence through stranding notifications. Small pelagic and neritic-pelagic cetaceans have been recorded in higher numbers along the EP and MA/PI sectors. In contrast, the MB area and adjacent coastline are influenced by the Amazon and Pará/Tocantins Rivers discharges, thus presumably limiting the occurrence and subsequent stranding of pelagic species. It should be considered that areas of difficult access and sparsely populated in the Amazon coastal zone, as the eastern Pará and the western Maranhão states, could represent potential circumstances for the low stranding notifications in these sectors. Although the systematic monitoring effort was lower at Maranhão/Piauí coastline, this area resulted to be singular due to the exclusive cetacean records of pelagic and neritic-pelagic species (e.g. *Lagenodelphis hosei*, *Peponocephala electra*). Despite the surveys were more dedicated in the MB sector, the frequency of uncommon strandings (e.g. oceanic species) were lower. However, single strandings of *Sotalia guianensis*, *Inia geoffrensis* and *I. araguaiaensis* in MB are not included in this report as they are commonplace and will be analyzed separately.

Table 2. Localities of marine mammal strandings and other localities mentioned in this study.

Locality	Municipality	Lat	Long	Description (species, others)
Cabo Orange	Oiapoque	5°N	51°W	northern Brazilian coast to the west
Ponta do Tubarão	Baía de São José	4°00'S	43°00'W	northern Brazilian coast to the east
Fernandes Belo	Viseu	1°10.8'S	46°5.4'W	*Balaenoptera borealis*
Praia de Água Boa	Salvaterra	0°51.6'S	48°30'W	*Balaenoptera brydei*
Baía do Japerica	São João de Pirabas	0°45'S	47°4.2'W	*Balaenoptera physalus*
Praia da Pedra do Sal	Parnaíba	2°49.15'S	41°42.8'W	*Megaptera novaeangliae*
Praia da Carnaubinha	Luís Correia	2°54.77'S	41°30.66'W	*Megaptera novaeangliae*
Praia do Rio Novo	Tutóia	2°42.6'S	42°26.4'W	*Megaptera novaeangliae*
Praia de Fora	Quatipurú	0°44.4'S	46°57'W	*Megaptera novaeangliae*
Praia da Pedra do Sal	Parnaíba	2°49.8'S	41°41.4'W	*Physeter macrocephalus*
Praia do Baixo da Boia	Paulino Neves	2°37.2'S	42°38.4'W	*Physeter macrocephalus*
Praia do Crispim	Marapanim	0°34.8'S	47°38.4'W	*Physeter macrocephalus*
Praia do Camburupy	Soure	0°32.4'S	48°28.2'W	*Physeter macrocephalus*
Praia da Pedra do Sal	Parnaíba	2°47.4'S	41°45'W	*Delphinus* sp.
Praia da Barrinha	Araioses	2°42.6'S	42°0.6'W	*Lagenodelphis hosei*
Praia da Barrinha	Araioses	2°42.6'S	42°0.6'W	*Stenella attenuata*
Praia da Pedra do Sal	Parnaíba	2°47.4'S	41°45'W	*Stenella attenuata*
Praia da Pedra do Sal	Parnaíba	2°49.8'S	41°41.4'W	*Globicephala macrorhynchus*
Paulino Neves	Paulino Neves	2°40.8'S	42°31.2'W	*Globicephala macrorhynchus*
Praia da Pedra do Sal	Parnaíba	2°49.8'S	41°42'W	*Grampus griseus*
Delta do Parnaíba	-	-	-	*Peponocephala electra*
Ilha do Caju	Araioses	2°41.4'S	42°5.4'W	*Peponocephala electra*
Praia de Marudá	Marapanim	0°37.2'S	47°37.8'W	*Pseudorca crassidens*
Praia da Corvina	Salinopólis	0°36'S	47°22.2'W	*Sotalia guianensis*
Praia de Ajuruteua	Bragança	0°49.2'S	46°36'W	*Steno bredanensis*
Praia da Princesa	Maracanã	0°34.2'S	47°34.8'W	*Steno bredanensis*
Praia do Crispim	Marapanim	0°34.8'S	47°38.4'W	*Steno bredanensis*
Praia do Crispim	Marapanim	0°34.8'S	47°38.4'W	*Steno bredanensis*
Praia da Pedra do Sal	Parnaíba	2°49.2'S	41°42.6'W	*Steno bredanensis*
Praia da Pedra do Sal	Parnaíba	2°48'S	41°43.8'W	*Steno bredanensis*
Praia da Princesa	Maracanã	0°34.2'S	47°34.8'W	*Tursiops truncatus*
Praia da Moita Verde	Araioses	2°43.8'S	41°48'W	*Tursiops truncatus*
Furo Velho	Maracanã	0°35.4'S	47°33'W	*Tursiops truncatus*
Porto do Sossego	Marapanim	0°36.6'S	47°37.8'W	*Tursiops truncatus*
Praia da Travosa	Santo Amaro	2°21'S	43°15'W	*Tursiops truncatus*
Baía do Capim	-	1°76'S	44°83'W	Sperm whale stranding at Maranhão coast

Earlier records of balaenopterids on the Amazon coastal zone were performed by Siciliano et al. (2008) and Silva et al. (2013). Previous revised information on stranding and sighting records of baleen whales along the Brazilian coast post-1997 (Siciliano et al. 2011) emphasizes the relevance of the current records. They are the very first evidence of the Fin and Sei whales occurrence along the northern Brazilian coast. The stranding intervals in each case could be informative of their movements and migration periods. The Sei whale was stranded in September 2008, during the peak migration period of southward migration (August–October) (sensu Best and Lockyer 2002). In accordance to these authors, the Viseu specimen (MPEG 39691), TL = 10.32 m in length, is acceptable as the size of immature females caught off South Africa (range 10.7–13.7 m). The Fin whale stranding demands a more complex interpretation, since the specimen became stranded in January, by the time most large whales have already reached Antarctic and sub-Antarctic waters. Siciliano et al. (2011) reported on a Fin whale sighted in January 2004 off the Santos Basin. Our present record poses an intriguing question, whether this whale was of a southern hemisphere origin or truly representative of a northern hemisphere stock.

The newborn Bryde's whale recorded in Praia da Água Boa (MB) on 15 September 2012 is a remarkable record, since very limited information exists on the pregnancy and time of birth of this tropical balaenopterid and, in Brazil, information on the calving season is almost non-existent (Santos et al. 2010). Moura and Siciliano (2012) report on two newborns stranded only a few months apart in south-eastern Brazil (August and September). Our record was in September, agreeing with these authors' previous observations and, thus, suggesting the same period of the year as a calving season for Bryde's whales off the northern Brazilian coast. Records of Humpback whales herein expands significantly the presence of the species westwards the north-eastern Brazilian coast. Pretto et al. (2009) reported the first occurrence of *M. novaeangliae* for eastern coast of Pará at Praia de Fora, Quatipuru municipality.

In relation to odontocetes, four additional Sperm whales records are included from the Pará state and Maranhão/Piauí coastlines. One of them is of a very young specimen (TL = 4.22 m) stranded at east coast of the Marajó Island. This record strongly suggests the existence of calving off in the northern Brazilian coast. Sperm whales are a regular component of cetaceans found stranded on the north-eastern coast of Brazil. For example, Meirelles et al. (2009) provided 26 records for the Ceará state alone over the period from 1993 to 2005. The sample includes specimens of all sizes, from a 3.53 m newborn to a 18.10 m bull male. It was assumed that the strandings took part mostly in spring, summer, and winter months, influenced by strong north-western winds, when carcasses have more chance of coming ashore. Magalhães et al. (2008) have contributed with two records of Sperm whales for the Maranhão coastline, during their short-term surveys from 2004 to 2006. Other records for the north-eastern coast include a newborn stranded near of Baía do Capim (1°76'S, 44°83'W), Maranhão coast (S3 File), and four other specimens reported by Tosi et al. (2006). In a broader perspective, it is highly recommended to conduct future surveys in order to estimate Sperm whale density and abundance off the north and north-eastern Brazilian coasts. The structure of this Sperm whale population in relation to more southerly aggregations in Brazil deserves further studies and agreed with literature which report that females and immature males inhabit lower latitudes in subtropical and tropical oceans (Whitehead 2009).

Blackfish records were represented by the False killer whale, Melon-headed whale, Short-finned pilot whale, and Risso's dolphin recovered in distinct areas. These specimens improve the knowledge on blackfish occurrence off the northern Brazilian coast. The False killer listed in this study is noteworthy; this is the first record of this species in the region. The specimen (MPEG 42132) had marks of human interaction; the peduncle was cut-off and most of the teeth were removed. This strongly suggests interactions with the pelagic fishery activity. Such marks were also observed in a rough-toothed dolphin stranded in eastern Pará (MPEG 42131) and on a pantropical spotted dolphin found on Piauí (MPEG 42085), suggesting that this kind of negative interaction may be more common than previously thought. Luksenburg (2014a) has informed on injuries found in live small cetaceans off Aruba, in the southern Caribbean. The author reported that of the 18 False killer whales individuals presenting injuries, six (33.3%) showed wounds that were probably caused by human activities. Two of these injuries were most likely caused by fishing gear and two others were most likely the result of a propeller hit. Further investigation on the impact of such fishing operations off northern and north-eastern Brazil on False killer whales and other smaller pelagic delphinids is highly needed.

The records of Bottlenose, Rough-toothed, and Common dolphins indicate their regular presence in neritic waters off northern Brazil, where then are referred by the fishermen as 'tuninas'. The Common dolphin specimen recorded at Praia da Pedra do Sal is certainly of special interest as so little is known of this population inhabiting the north-eastern coast (see Tavares et al. 2010 for more details).

The evidence of consumption of a fresh Fraser's dolphin denotes further investigation on the use of marine bushmeat along small fishing communities of north-eastern Brazil. Tosi et al. (2008) have reported a similar episode involving Fraser's dolphin consumption in the coast of Maranhão. Even if these represent fortuity events, dolphin and whale meat consumption should be more deeply investigated in such villages in future surveys.

Regarding coastal species, a mass stranding of Guiana dolphins was reported for the first time, based on an incident recorded in Salinópolis (EP), on January 2013. The coastline of Pará possesses certain unique features, such as very strong tides that cause accelerated movement of the sand banks and tidal channels. It seems plausible to believe in a natural condition characterized by this muddy beach and fast lowering tide acting as a trap. Although Guiana dolphins are familiar with flat waters and tide dynamism, the particular location of the Praia da Corvina could have acted as a natural trap. Nonetheless, the singularity of this event is worth mentioning and adds a new feature to the natural behavior of Guiana dolphins.

The cetacean fauna off the north and north-eastern Brazilian coast is much richer and diverse that previously thought, including both residents of tropical oligotrophic waters and highly-migrating baleen whales. Species associated to productive environments are also included, such as Bryde's whales and the Common dolphin. As pointed out by the present survey, the connection with the southern Caribbean cetacean fauna seems plausible and deserves further investigation. This is particularly relevant for understanding the affinity of north Brazilian Bryde's whale and Common dolphin populations with the adjacent Venezuela regional aggregations of these species (Galindo

2007). There is a lack of information on countries of Wider Caribbean Region as Guyana, Suriname, and French Guiana, however, surveys developed at Guiana's coastal province supply important records on cetacean composition (Manocci et al 2013, Boer 2015). Such a relationship is highly expected and could potentially connect the north Brazilian cetacean fauna with that from the southern Caribbean.

Conclusions

Strandings of uncommon cetacean species and a live beached Sperm whale are recorded for the first time along the north coast of Brazil. Balaenopterids records (*Balaenoptera borealis* and *B. physalus*) presented herein are of particular importance and include the very first of its category on the northern coast of Brazil providing new insights on migration movements on the southern hemisphere.

Evidence of anthropogenic injuries inflicted to cetaceans and also the consumption of marine bushmeat is reported to the north-eastern coast of Brazil. Although the proximity of the northern and the north-eastern Brazilian coasts, results showed that cetacean fauna in northern is more similar with the Caribbean region. Further investigation on movements and distribution patterns should be encouraged.

In this context, beach surveys and stranding network definitely provide relevant information on marine mammals' richness and diversity is this vast region. The cetacean fauna of north and north-eastern Brazil combines different elements from tropical marine ecosystems under the influence of the most powerful river of the world. Such dynamism requires a proportional research effort to better evaluate and understand its own complexity.

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Supplementary material 1

S1 Movie

Authors: Alexandra Fernandes Costa, Salvatore Siciliano, Renata Emin-Lima, Bruna Maria Lima Martins, Maura Elisabeth Moraes Sousa, Tommaso Giarrizzo, José de Sousa e Silva Júnior

Data type: MOV file

Explanation note: Dolphins trapped at Praia da Corvina, Salinópolis (MOV).

Link: https://doi.org/10.3897/zookeys.688.12636.suppl1

Supplementary material 2

S2 Movie

Authors: Alexandra Fernandes Costa, Salvatore Siciliano, Renata Emin-Lima, Bruna Maria Lima Martins, Maura Elisabeth Moraes Sousa, Tommaso Giarrizzo, José de Sousa e Silva Júnior

Data type: MOV file

Explanation note: Rescue Guiana dolphins trapped at Praia da Corvina, Salinópolis (MOV).

Link: https://doi.org/10.3897/zookeys.688.12636.suppl2

Supplementary material 3

S3 Movie

Authors: north-eastern Brazilian coasts
Alexandra Fernandes Costa, Salvatore Siciliano, Renata Emin-Lima, Bruna Maria Lima Martins, Maura Elisabeth Moraes Sousa, Tommaso Giarrizzo, José de Sousa e Silva Júnior
Data type: MOV file
Explanation note: Dolphin stranded at Praia da Travosa, Santo Amaro (MOV)

Link: https://doi.org/10.3897/zookeys.688.12636.suppl3

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RESEARCH ARTICLE

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Reinstatement of Eschatoporiini Blaisdell, 1906, a unique tribe of blind cavernicolous Tenebrionidae from California, with a new species from Napa County (Coleoptera, Tenebrionidae, Lagriinae)

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Abstract

The tribe Eschatoporini Blaisdell, 1906 is reinstated, based on molecular and morphological data, and the spelling corrected as Eschatoporiini. The tribe currently includes only the cave-dwelling genus Eschatoporis Blaisdell, 1906 from California, which is associated with underground aquifers. A second species of Eschatoporis is described from a cave in Napa County, California. The phylogenetic placement of Eschatoporiini within the Lagriinae is examined, and notes on the biology of Eschatoporis are provided.

Keywords

Eschatoporis, Blind, Subterranean, Cave, endemicity

Introduction

Historical background

Blaisdell (1906) described *Eschatoporis nunenmacheri*, a new genus and species of blind tenebrionid collected from under a rock next to a spring. He compared this species to *Eulabis* Eschscholtz, 1829 and *Cerenopus* LeConte, 1851, at that time placed in the

Scaurini. Blaisdell suggested that the tribe be expanded to include *Eschatoporis* or that it be placed in a new tribe which he named Eschatoporini. Lacordaire (1859) placed *Eulabis*, *Centrioptera* Mannerheim, 1843, and *Cryptoglossa* Solier, 1836 in his tribe Scaurides Billberg, 1820. *Eulabis* was placed in "Groupe III: Nyctoporides" (Lacordaire 1859: 131) while *Centrioptera* and *Cryptoglossa* were placed in "Groupe IV: Centrioptérides" along with *Cerenopus* (Lacordaire 1859: 135). This is perhaps why Gebien in both his catalogs (1910, 1937) placed *Eschatoporis* in the Cryptoglossini, which now includes the genera *Cryptoglossa* (= *Centrioptera*) and *Asbolus* LeConte, 1851 (= *Cryptoglossa*) see Aalbu (1985, 2005). Despite LeConte's removal of *Cerenopus* and *Eulabis* from the Cryptoglossini (LeConte 1862), subsequent catalogs listed *Eschatoporis* in the Cryptoglossini.

Aalbu (1985: 50) moved *Eschatoporis* from the Cryptoglossini (subfamily Pimeliinae) to the subfamily Lagriinae, based on morphological data (see discussion below), but placed it as *incertae sedis* within the lagriine tribal classification due to the lack of specimens to dissect at that time. Doyen (1994: 445–446) later tentatively placed *Eschatoporis* in the tribe Goniaderini (Lagriinae). This placement was accepted by Aalbu et al. (2002). Later, Aalbu (2005) placed *Eschatoporis* in the Laenini based on the lack of defensive gland reservoirs and the presence of multiple non-glandular spermathecal tubules. The placement of *Eschatoporis* in the Goniaderini by Aalbu and Smith (2014), as pointed out by Kanda (2016), was an accidental error. At that time, *Eschatoporis* should have remained in the Laenini as pointed out Matthews et al. (2010: 577). These errors, as well as the shuffling of *Eschatoporis* between various tribes, were recently summarized (Kanda 2016), and helped emphasize that a reevaluation of the placement of this genus was overdue.

Over the past decade material belonging to a new species of *Eschatoporis* has been collected from a cave in Napa County, California; thus allowing for a representative of the genus to be sequenced and analyzed within the context of a large pre-existing molecular dataset for the Lagriinae (Kanda et al. 2015). This new species, *Eschatoporis styx*, is described below.

Materials and methods

For this study, material was borrowed from the following individuals and institutions. These persons (in parentheses) are gratefully acknowledged for loan of their materials:

ADSC	Aaron Smith Collection, Flagstaff, Arizona, USA (Aaron D. Smith)
CASC	California Academy of Sciences, San Francisco, California, USA (Dave Kavanaugh).
CDFA	California State Collection of Arthropods, Sacramento, CA, USA. (Andrew R. Cline)
NSDA	Nevada State Department of Agriculture, Reno, Nevada, U.S.A. (Robert Bechtel)
OSAC	Oregon State Arthropod Collection, Corvallis, Oregon, USA. (David R. Maddison)
RLAC	Rolf L. Aalbu Collection, El Dorado Hills, California, USA. (Rolf L. Aalbu)

Morphological methods

Measurements were taken using digital calipers or an optical micrometer attached to a Leica MZ16 APO stereomicroscope. Images were taken using a Passport Imaging system (R. Larimer, www.visionarydigital.com). Montaged images were assembled using Zerene Stacker (zerenesystems.com/stacker/) and backgrounds were cleaned up in Adobe Photoshop CS6. Internal structures were cleared with warm 10% KOH and stained with either Chlorazol Black E or Mercurochrome stains.

Molecular methods

DNA was extracted from a specimen of *Eschatoporis styx* sp. n. collected from the type locality (Clay Cave), using a Qiagen DNeasy Blood and Tissue kit. Four gene fragments were amplified: 28S nuclear ribosomal DNA (28S), arginine kinase (ArgK), carbamoyl phosphate synthetase domain of the rudimentary gene (CAD), and *wingless* (*wg*). These gene fragments were previously sequenced for the Lagriinae sampled in Kanda et al. (2015). Polymerase chain reactions (PCRs) were performed on either an Eppendorf Mastercycler ProS or Mastercycler gradient Thermal Cycler using Ex Taq DNA polymerase (TaKaRa) and basic protocols recommended by the manufacturers. Primer pairs and cycler profiles are described in Kanda et al. (2015). PCR products were cleaned, quantified, and sequenced at the University of Arizona's Genomic and Technology Core Facility using a 3730 XL Applied Biosystems automatic sequencer. Assembly of multiple chromatograms of each gene fragment and initial base calls were made with Phred v. 0.020425.c (Green and Ewing 2002) and Phrap v. 0.990319 (Green 1999) as orchestrated by Mesquite's Chromaseq v. 1.12 package (Maddison and Maddison 2014a, 2014b) with subsequent modifications by Chromaseq and manual inspection. Final sequences are available on GenBank (accessions MF370333–MF370336).

Sequences were incorporated into matrices from Kanda et al. (2015). The final matrix (http://insectbiodiversitylab.org/data/) includes 31 Lagriinae spanning all currently recognized tribes and five outgroup taxa from other subfamilies of Tenebrionidae.

Ribosomal 28S gene fragments were aligned using MAFFT v. 7.130b (Katoh and Standley 2013) and the L-INS-i algorithm. ArgK sequences were manually aligned, asthere were no indels among our sampled taxa. CAD and *wg* were first translated to amino acid sequences, which were aligned using MAFFT v. 7.130b (Katoh and Standley 2013) and the L-INS-i algorithm with default parameter values. The nucleotide sequences were then mapped onto the amino acid alignment using Mesquite (Maddison and Maddison 2014b). The 28S, CAD, and *wg* alignments contained regions with numerous indels. These poorly aligned regions were identified using the server version of Gblocks (Castresana 2000; Talavera and Castresana 2007) with all options for less stringent block selection chosen. For CAD and *wg*, the "Codon" option was selected to maintain the triplet codons in the alignment.

Phylogenetic analyses were performed on a concatenated dataset of all four genes using maximum likelihood (ML), Bayesian (MB), and parsimony (MP) methods. For ML and MB analyses, optimal dataset partitions and substitution models were identified using the BIC implemented in PartitionFinder v.1.1.1 (Lanfear et al. 2012) from initial schemes based on genes and codon position. Two analyses were conducted, first restricting examined models to only those available in RAxML (ML) and then restricting models to only those available in MrBayes (MB). The inferred optimal data partition for ML analyses grouped first and second codon positions of all genes in the first partition, codon position three of ArgK and *wg* in the second partition, codon position three of CAD in the third partition, and 28S in the fourth partition. GTR+I+G was identified as the optimal substitution model for all partitions. The optimal partitioning scheme for MB analyses was the same, but SYM+I+G was identified as the optimal substitution model for the fourth partition.

Maximum Likelihood (ML) analyses were performed using RAxML v. 8.2.9 (Stamatakis 2014) implemented through the Zephyr v. 1.1 package (Maddison and Maddison 2015) in Mesquite (Maddison and Maddison 2014b). Five hundred independent searches for the maximum likelihood tree and 1000 bootstrap replicates were run on all datasets. Bayesian analyses were conducted using MrBayes v. 3.2.2 (Ronquist et al. 2012) on servers maintained by the CIPRES Scientific Gateway (Miller et al. 2010). Analyses were run for 36.8 million generations using default search parameters (two independent runs each with one cold chain and three hot chains). The two runs were considered to have converged when the standard deviation of split frequencies fell below 0.01 and the estimated sample size (ESS) for all parameters was greater than 200, suggesting adequate mixing between the two independent runs. ESS was calculated using Tracer v. 1.6 (Rambaut et al. 2014).

Taxonomy

Recently, one of us (Kanda 2016) observed what was thought to be tergal defensive gland reservoirs between tergal segments 7 and 8 in *Eschatoporis*, which would be the first example of this reservoir placement in tenebrionids. Whether these cuticular sacs (Kanda 2016, fig. 10) are defensive or not remains unclear as evidence of any defensive secretion was not observed while collecting live specimens. It is possible these may serve another function not as yet determined. Regardless of their function, these cuticular sacs seem to be unique in Tenebrionidae.

Tschinkel and Doyen (1980) examined defensive gland reservoirs, ovipositors, and female genital tubes within Tenebrionidae. In examining female genital tubes, they considered the Adeline lineage the most "primitive" (Tschinkel and Doyen 1980: 337). They found that this condition, where the primary bursa copulatrix gives rises to multiple apical spermathecae and a spermathecal accessory gland was present in all species of both the Adeliini and Pycnocerini (both tribes within Lagriinae) specimens examined. Later, Matthews (1998), in his revision of the genera of Adeliini, found that in some adeliine genera, such as *Isopteron* Hope, 1840, the spermatheca and spermathecal accessory gland are subapical (Matthews 1998: 786). The female reproductive tract of *Eschatoporis* (Fig. 4) can easily fit within the range of the Adeliini in the configuration of both the female internal tract and that the external genitalia lack any of what they termed "advanced" characters. Whether the subapical spermathecae and spermathecal accessory gland represent a small secondary bursa copulatrix (see Matthews 1998: 699) is debatable, but both these characters are found to occur within the Adeliini. However *Eschatoporis* differs from both the Pycnocerini and the Adeliini in lacking sternal defensive glands (Pycnocerini: between segments 7 and 8 or Adeliini: between segments 8 and 9).

This lack of sternal defensive glands, the lack of eyes in some species, as well as the plesiomorphic state of the external female genitalia tract, might place *Eschatoporis* in the Laenini, as some Laenini lack defensive glands. Doyen and Tschinkel (1982: 159) mention that "in Lupropini and Laenini, glands are similar to those of Lagriini and open between sternites 7 and 8" so glands may be present in some Laenini. In any case, the female internal tract of *Laena* (Laenini) differs from *Eschatoporis* in that the spermathecae are few and subapical and the spermathecal accessory gland is apical.

Maximum Likelihood analyses of the 4-gene concatenated dataset recovered *Eschatoporis* sister to a monophyletic Adeliini (Fig. 1). Bootstrap analysis showed moderately high support for this clade (BP = 82). There is no support for the inclusion of *Eschatoporis* in a clade with Laenini. Bayesian analyses converged after 5.85 million generations. The majority rule consensus of post-burn-in trees largely agrees with the ML results. *Eschatoporis* was again recovered as sister to Adeliini (PP=0.94), with no support for its inclusion in Laenini.

Redefinition of Eschatoporiini

Eschatoporiini Blaisdell, 1906

Eschatoporiini Blaisdell, 1906 (Tenebrionidae, Lagriinae)

Eschatoporini Blaisdell, 1906: 78 [stem: Eschatopori-]. Type genus: *Eschatoporis* Blaisdell, 1906 (type species: *Eschatoporis nunenmacheri* Blaisdell, 1906, by monotypy). Comment: incorrect original stem formation, not in prevailing usage (See Bouchard et al. 2011: 398).

Remarks. The Eschatoporiini are very similar to the Adeliini and mostly fit within the description and range of the characters as described by Matthews (1998: 701). However, the Eschatoporiini differ from the Adeliini in some key characteristics. The following characters/character states will separate the Eschatoporiini from the Adeliini: Head with basal membrane of labrum exposed, eyeless, but occasionally with a remnant eye scar; maxillary palps with apical segment oblique, not strongly triangular; tentorial bridge present, not arched, sides of tentorium broad, subparallel, continuing to submentum as low ridges; mesepisternum and mesosternum greatly expanded ante-

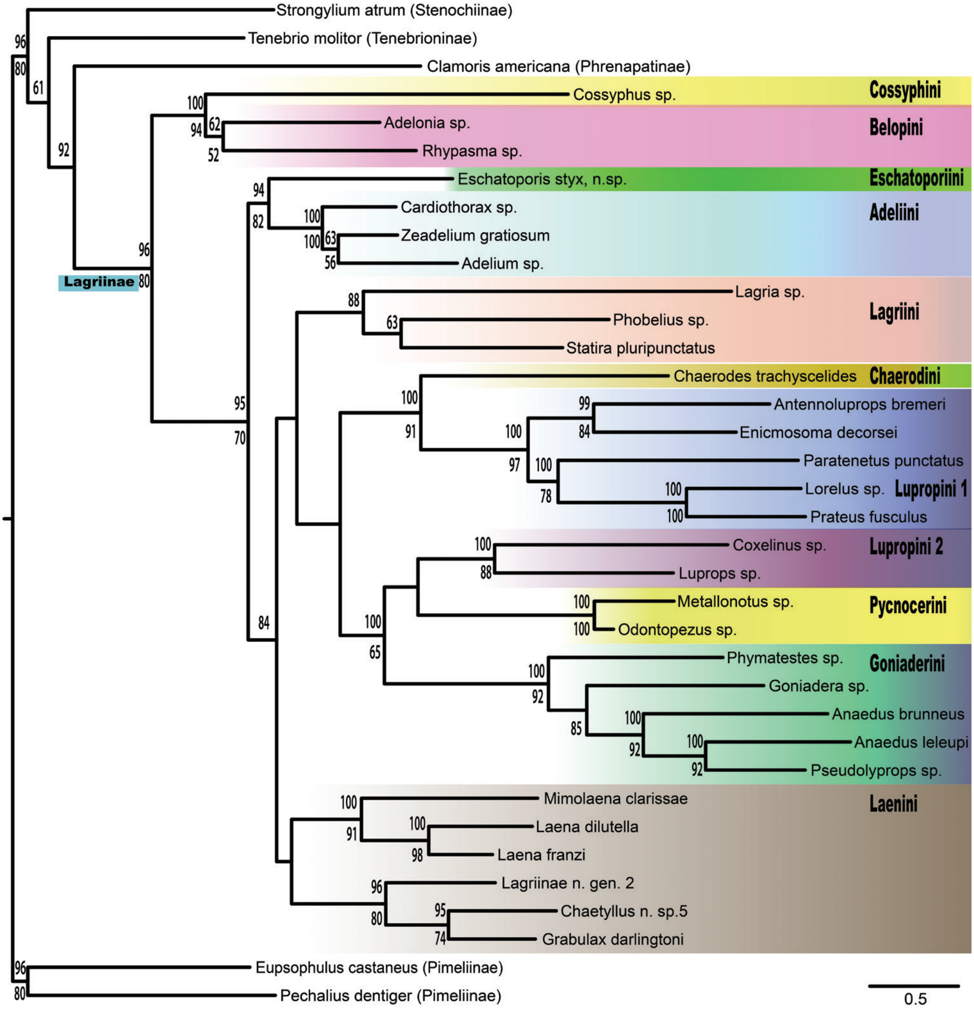

Figure 1. Maximum Likelihood tree from RaxML. Posterior probability values above branches and bootstrap values below. Clades colored according to tribe.

riorly forming a neck-like process between thorax and abdomen; scutellum very large; sternal defensive glands absent on all sternites and tergal cuticular sacs present between tergites 7 and 8.

Eschatoporis styx Aalbu, Kanda & Smith, sp. n.

http://zoobank.org/CEBC2164-969A-4ADC-BB00-BAE5CF85A7FA
Figs 2–4

Description. *Holotype male:* Length 5.5 mm. width 1.5 mm. greatest width at mid-elytra. Integument reddish brown, luster slightly shining (Fig. 2).

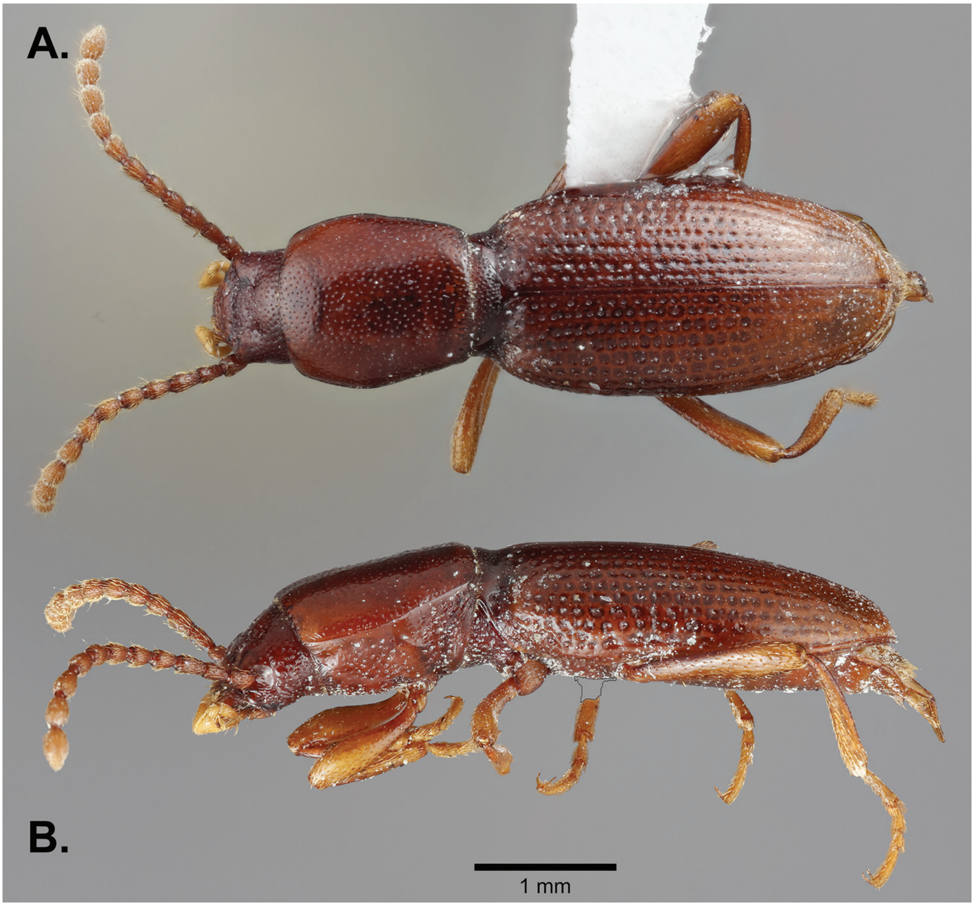

Figure 2. Habitus of *Eschatoporis styx* sp. n.: **A** Dorsal **B** Lateral.

Body elongate, semi-cylindrical, apterous.

Head prognathous, widest near base, vertex flattened; surface bearing 1–2 long setae dorso-laterally and few short setae laterally, longer setae moderately long, yellow, approximately twice length of clypeus anteriorly; surface punctuate; distance between punctures about equal to puncture diameter or more, moderate in size, moderately shallow in form; clypeus anteriorly rounded, posteriorly somewhat sinuate, broad, about 4 × as wide as long, bearing two long yellow setae on mid-lateral surface; labrum produced, rectangular, about 1.5 × as broad as long, flattened, with membrane exposed between clypeus and labrum; frons with gena only very slightly produced anteriorly above antennal insertions; eyes absent; mentum square-trapezoid in shape, slightly wider anteriorly; ligula kite shaped, maxillary palps elongate, nearly as long as first four antennomeres, with apical palpomere triangular but hollow apically, interior of apex bearing numerous short setae, ratio of segment lengths 20:10:20:12:21; antennae long and slender, filiform-moniliform, apical segments reaching elytra, eleventh segment longest; ratio of segment lengths 20:16:15:16:15:15:16:17:16:15:26.

Pronotum narrower than elytra, subquadrate, slightly arcuate laterally, slightly inflated, widest anterior to middle: anterior margin slightly rounded, posterior surface punctuate, punctures small in size, separated by 1 to 3× puncture diameter, surface glabrous.

Scutellum very large, visible, triangular.

Elytra only slightly convex, surface punctate-striate, punctures set in 10 even striae on disc, punctures, shallow, moderate in size on disk; distance between punctures approximately equal to puncture diameter; apically, punctures smaller; surface glabrous except few long yellow hairs on apical declivity; three near apex and 1-2 subapically, setae often worn off in older specimens; epipleurae indistinct at base, forming basal part of elytra, only becoming distinct behind metacoxae where elytra abruptly narrows, then gradually narrowing but reaching apex.

Ventral surface: prosternal process narrow, convex between procoxae, flattened and slightly expanded posteriorly; mesepisternum and mesosternum greatly expanded anteriorly forming a neck-like process between thorax and abdomen; mesosternum not excavate, distant from prosternal process; mesotrochantin hidden; metacoxae separated by about equal distance between meso-metacoxae; mesocoxae separated by width of coxae; surface of thoracic pleura punctate, interspaced with few moderate sized yellow setae; intercoxal process of abdomen parallel with rounded apex; surface of first visible sternite punctate apically and centrally, punctures becoming smaller more sparse laterally and apically, with few moderate sized yellow setae; second visible abdominal ventrite sparsely, minutely punctate, rest of visible sterna nearly impunctate, with few, small, sparse, shallow punctures; apical sternite with few medium length yellow setae along apex; sternal ratios (anterior to posterior midline) 40:31:25:14:18. Seventh sternite with groove along lateral margin.

Legs moderate in length, slender, profemur slightly inflated; leg ratios (femur: tibia) pro. 45:40; meso. 47:37; meta. 65:49; tibiae, tarsi with ventral surface bearing sparse long spine-like setae, femora sparsely setose. Tarsal length ratios as follows (base to apex): protarsus 12:7:5:5:18; mesotarsus 12:10:9:7:21; metatarsus 30:14:9:22.

Male genitalia: Aedeagus (Fig. 3) length 1.27 mm., width 0.2 mm. Basal piece elongate, arcuate, with sides not inflected; flange present at base but very small. Parameres short, flat, apex rounded, alae separate, 0.33 mm. Median lobe flat, apex rounded length 0.75 mm, width 0.2 mm.

Allotype female genitalia (Fig. 4) Ovipositor length 0.5 mm., coxites with segments elongate, slightly longer than paraprocts, gonostyle long and thin. Internal tract with two vaginal sclerites; spermatheca, multiple; spermathecal accessory gland very long and thin, 0.53 mm; spermatheca, spermathecal accessory gland positioned subapically. Bursa copulatrix apical.

Holotype: (male) CALIF., Napa Co., White (Clay) Cave, nr. Deer Park, II-26-2005, R. L. Aalbu col. Holoype deposited at CASC.

Allotype: (female) CALIF., Napa Co., White (Clay) Cave, nr. Deer Park, II-10-2007, R. L. Aalbu col. Allotype deposited at RLAC.

Paratypes: CALIF., Napa Co., White (Clay) Cave, nr. Deer Park, IV-24-2004, R. L. Aalbu col., RLAC (2); same except II-27-2007 (2); same except II-26-2005 (7); same except II-10-2007 (1); same except IV-12-2008 (8); same except VIII-16-2004 (1); same

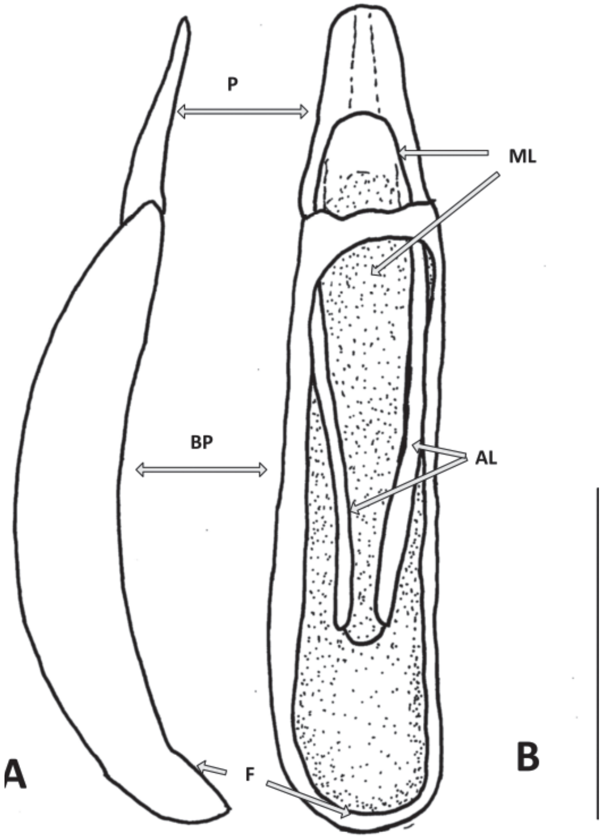

Figure 3. Aedeagus of *Eschatoporis styx* sp. n.: **A** Lateral **B** Ventral. **P** parameres; **AL** ala; **BP** basal piece; **F** flange of basal piece; **ML** median lobe. Scale bar: 0.5 mm.

except II-20-2011 (2); same except V-3-2014 (3); same except II-12-2017 (4); same except IV-24-2004, kept alive, found dead VIII-16-2004 (2); same location, collected by K. Kanda and R. L. Aalbu,V-3-2014; Voucher specimen or DNA extraction KKDNA0329.

Other material examined (parts/ condition of specimens not adequate for paratype designation)**.** CALIF., Napa Co. 9 mi. E St. Helena, White Cave, IV-10-1951, Hugh Leech col., CASC (abdomen only) (1); same except White (Clay) Cave, nr. Deer Park, II-26-2005, R. L. Aalbu col., RLAC (1); same except II-10-2007 (5); same except IV-12-2008 (17); same except II-20-2011 (6); same except III-28-2004 (7); same except III-9-2004 (9).

Larvae: unknown.

The two species of *Eschatoporis* can easily be separated by the clearly different setation patterns on the elytra. While in *E. nunenmacheri*, the elytra are covered with short setae (Fig. 5), in *E. styx*, (Fig. 2) the elytra are glabrous except for a few long, hair-like setae near and at the base of the elytra. *Eschatoporis styx*, also lacks any eye "scar" which is found in various sizes in *E. nunenmacheri.*

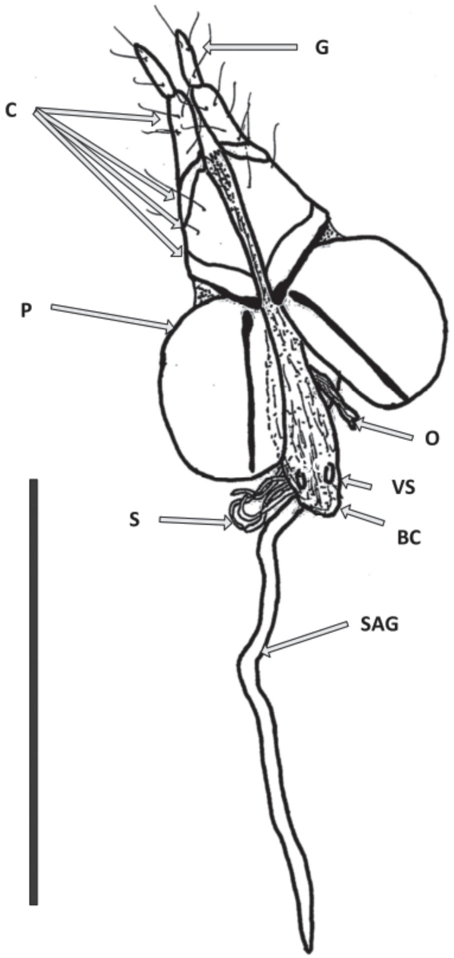

Figure 4. External and internal female genitalia of *Eschatoporis styx* sp. n. Dorsal view. Ovipositor: **G** gonostyle; **C** Coxites; **P** paraproct. Internal genital tract: **O** oviduct; **VS** vaginal sclerites; **S** spermatheca; **BC** bursa copulatrix; **SAG** spermathecal accessory gland. Scale bar: 0.5 mm.

Eschatoporis nunenmacheri Blaisdell, 1906

Fig. 5

Material examined. CALIF., Marin Co. Mill Valley, I-18-1948 (CDFA, 1); same except V-3-1947, E. S. Ross, In rock crack 4' below surface, *Eschatoporis nunenmacheri* det. Aalbu, 2004 ((NSDA, 1); CALIF., Marin Co. Fairfax, IV-6-1919, Van Dyke Colln. (CASC, 1); same except V-25-1919, (CASC, 1); CALIF., Marin Co. Samuel P. Taylor St. Pk. II-3-1958 J. Helfer (CASC, 1); same except XII-13-1954, *Eschatoporis nunenmacheri* det. Boddy, 1955, (1); same except South Entrance, XI-3-1953 G. A. Marsh, R. O. Schuster cols., *Eschatoporis nunenmacheri* det. Boddy, 1955, (2).

Figure 5. Dorsal habitus of *Eschatoporis nunenmacheri* Blaisdell.

It is unclear from Blaisdell's description (1906) where the Holotype (California, Marin Co., Fairfax, June, collected by Nunenmacher while digging on a ledge near a spring) was deposited. Checks of the CASC, Philadelphia Academy of Sciences, Museum of Comparative Zoology, Harvard University and Smithsonian did not locate the type. However, from Blaisdell's description and drawing, it is clear that the holotype is the same as the other specimens of *Eschatoporis nunenmacheri*.

Discussion and notes on biology

Clay Cave is located in oak woodland in the California wine country adjacent to the northern margin of San Francisco Bay, California. Known since the 1870s, the cave formed as a soil pipe cave in an ash flow of the Miocene Sonoma Volcanics, a continental packet of rhyolitic to andesitic volcanoclastic sediments and tephras. The cave consists of 229 m of linear passage with several small rooms floored with a seasonal stream (see Elliott et al., in press: fig.17). It appears that this cave originated along root casts in the bedded volcanic sediments that are mostly altered to smectite clay locally stained with iron oxides. Subsequent invasion by seasonal streams has integrated the initial fist-sized soil pipes into vadose canyon passages. The cave has at least two seeping springs. Clay Cave also has a rich biota, including some unusual terrestrial invertebrates, and is ranked fourth in the most bio-diverse caves of California (Elliott et al. in press).

Repeated attempts to find larvae in the cave or acquire larvae from adults in the lab yielded no results.

Species of the tribe Eschatoporiini seem to be associated with deep interstitial layers in rocky soils or underground water flows. Specimens of the tribe are either collected in deep rocky soil layers or in caves, both near springs. In Clay Cave, most *Eschatoporis styx* were collected under rocks rather than walking freely. Sometimes specimens have been found dead in standing small pools water from spring seepage in the cave. Specimens of Eschatoporiini remain very rare in collections. For instance, as far as we know *Eschatoporis nunenmacheri* has not been recollected since 1958.

Eschatoporis species are very similar in appearance and biology to the laenine genus *Hypolaenopsis* (Masumoto, 2001), which was originally placed in Adeliini but subsequently transferred to Laenini (see Schawaller 2008). Some *Hypolaenopsis* species are superficially very similar to *Eschatoporis*, differing only in size. The species *H. nanpingica* (originally described in *Laena* by Schawaller 2001), which is blind with only an eye scar remaining (as in *E. nunenmacheri*), could be mistaken for a large species of *Eschatoporis* based on external morphology. Other *Hypolaenopsis* species also have reduced eyes. Even the species-rich genus *Laena* contains taxa with reduced eyes; such as *L. subcoeca* Kaszab, 1973 and *L. sherpa* Schawaller, 2002 (both from forest litter in Nepal), and *L. deplanata* Weise, 1878 from Turkey in which the eyes are reduced to single facets (Schwaller, personal communication).

Masumoto (2001) mentioned that all specimens of *Hypolaenopsis nanpingica* were taken from the upper hypogean zone by digging soil mingled with gravel beneath large stones to the depth of 20–30 cm, about five or six meters above a stream.

Acknowledgments

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In our recent revision of the genus *Prespelea* Park, we neglected to explicitly designate a repository for the holotype of *Prespelea enigma* Caterino & Vásquez-Vélez **sp. n.** This omission invalided its description, and that species name is not yet available. We herein rectify this problem.

Introduction

Caterino and Vásquez-Vélez (2017) revised the Pselaphine genus *Prespelea* Park, redescribing two known species, and describing ten new ones from southern Appalachia. In one of these species descriptions we neglected to designate a type repository for the holotype of *Prespelea enigma* Caterino & Vásquez-Vélez, which, under ICZN Article 16.4.2, renders this a nomen nudum. We herein resolve this problem.

Repositories

Specimens came from our own collections, and through loans from several institutions:

CUAC	Clemson University Arthropod Collection, Clemson, SC
FMNH	The Field Museum, Chicaco, IL
UNHC	University of New Hampshire Arthropod Collection, Durham, NH

Taxonomy

Tribe Speleobamini Park 1951: 51
Genus *Prespelea* Park, 1953: 251

Prespelea enigma Caterino & Vásquez-Vélez, sp. n.

Type material. Holotype male: USA: NC: Macon Co., Jones Gap, 35.0785°N, 83.2923°W, S. Myers, vii.22.2015, sifted litter (CUAC000026531, DNA Extract MSC-2403); deposited in FMNH. **Other material:** 4 males & 6 females, NC: Macon Co., 11 mi. SW Franklin, Back Country info center, VIII-17/21-1990, hardwood litter nr. dead logs, S. O'Keefe; UNHC, FMNH, CUAC. 1 female: NC: Macon Co. Highlands, vi.8.1973, Coker Rhododendron Trail, litter under rhododendron, W. Suter; FMNH.

Diagnosis. This species is externally indistinguishable from *P. copelandi* except in the following male characters: metaventrite elevated anteromedially to form small but distinct median tubercle about one-fourth metaventral length behind mesocoxae (Fig. 21), metaventrite moderately flattened behind; posteroapical corner of male metatrochanter produced to form short, incurved flange (Fig. 32), the whole trochanter being somewhat parallelogram-shaped; aedeagus with sides weakly sinuate toward apex, apicodorsal ridges weakly divergent to apical corners; apical margin subtruncate to weakly emarginate; internal sac with broad band of about 18 short, sclerotized teeth. Female not definitely associated. TL 1.76–1.88mm; Max. width (EW) 0.61–0.71mm.

Acknowledgments

We appreciate Al Newton's calling our attention to this problem, and advising us on how to correct it.