

The phylogenetic and geographic limits of Leptodirini (Insecta: Coleoptera: Leiodidae: Cholevinae), with a description of *Sciaphyes shestakovi* sp. n. from the Russian Far East

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> Abstract

The tribe Leptodirini of the beetle family Leiodidae is one of the most diverse radiations of cave animals, with a distribution centred north of the Mediterranean basin from the Iberian Peninsula to Iran. Six genera outside this core area, most notably *Platycholeus* Horn, 1880 in the western United States and others in East Asia, have been assumed to be related to Leptodirini. We studied recently collected specimens of three of these extraterritorial genera, namely *Platycholeus*, *Fusi* Perkovsky, 1989 and *Sciaphyes* Jeannel, 1910, and establish their phylogenetic relationships by analysing a combination of ca. 5 Kb of mitochondrial and nuclear DNA sequences with Bayesian Probability and Maximum Likelihood methods. Our results corroborate the previously proposed hypothesis that *Platycholeus* is the sister group of the remaining Leptodirini, with an estimated age of vicariant separation compatible with the breaking of the Thulean bridge between the Nearctic and the Western Palaearctic in the Early Eocene. We refute close relationship of either *Fusi* or *Sciaphyes* to Leptodirini, with the former genus appearing more closely related to Cholevini, and the latter to Anemadini and warranting a separate tribe, Sciaphyini, in agreement with recent treatments. This phylogenetic position of *Sciaphyes* is in agreement with a parsimony analysis of 28 morphological characters of a representative sample of Cholevinae subgroups. We describe one of the studied *Sciaphyes* species as *S. shestakovi* sp.n. and indicate its remarkable morphological differences from its congeners. We hypothesise that the remaining three extraterritorial monotypic genera of “Leptodirini”, namely *Proleptodirina* Perkovsky, 1998, *Sinobathyscia* Perreau, 1999 and *Coreobathyscia* Szymczakowski, 1975 are unlikely to be closely related to the tribe, which probably has its easternmost geographical limits at Zagros and Alborz (= Elburz) mountains in Iran.

> Key words

Sciaphyes shestakovi sp.n., *Platycholeus*, *Fusi*, Leptodirini, Siberia, Russia, phylogeny, subterranean environment.

1. Introduction

Leiodidae is a family of staphyliniform beetles with world-wide distribution and ca. 3,500 described species. The family is currently divided into six subfamilies, with the highest diversity in Cholevinae

(including the former “Cholevidae” and “Catopidae”) and Leiodinae (NEWTON 2005). There is no available comprehensive formal phylogenetic analysis of Leiodidae, but the monophyly of the subfamilies, in-



Fig. 1. Distribution of *Coreobathyscia*, *Fusi*, Leptodirini (excl. *Platycholeus*), *Sciaphyes*, *Platycholeus*, *Proleptodirina* and *Sinobathyscia*.

cluding that of Cholevinae, is generally accepted (NEWTON 2005). Cholevinae includes seven tribes, among them the most diverse being the Leptodirini, with about 900 known species in ca. 240 genera (PERREAU 2000, 2004).

Leptodirini constitutes, arguably, the most diverse and spectacular radiation of predominantly eyeless subterranean animals. Nearly all Leptodirini, with a few notable exceptions, inhabit caves or deep soil layers in the Mediterranean basin. This area includes the north and east of the Iberian Peninsula, some Mediterranean islands such as Corsica, Sardinia and Sicily, the Southern Alps, Italian and Balkan peninsulas, Carpathian Mountains, southern Russia, the Caucasus, Middle East and Iran (Fig. 1). The monophyletic origin of the tribe's western Palearctic core (Leptodirini excl. *Platycholeina*) is well supported by both morphological (FRESNEDA et al. 2007) and molecular (RIBERA et al. 2010) evidence. There are, however, six inadequately known genera outside the western Palearctic continually attributed to Leptodirini on the basis of a subset of potentially convergent adult morphological features presumably resulting from the subterranean lifestyle. These genera are:

(1) *Platycholeus* Horn, 1880 with eyed and winged species in western North America from British Columbia to California (NEWTON 1998; Figs. 1, 2). This genus was thought to be related to Leptodirini

(= Bathysciinae) by JEANNEL (1910, 1924), who emphasised its similarities with *Sciaphyes* Jeannel, 1910 in the shape of the head and the mesoventral carinae. JEANNEL (1924) particularly favoured the *Platycholeus-Sciaphyes* relationship because the two genera are found on opposite sides of the Pacific Ocean, which might suggest a relict Cretaceous distribution. The genus presently includes termitophilous *P. leptinoides* (Crotch, 1874), myrmecophilous *P. opacellus* Fall, 1909, plus an undescribed species, all of them found under bark of decayed conifer logs (NEWTON 1998). They are currently treated as a subtribe *Platycholeina* of the Leptodirini (NEWTON 1998; PERREAU 2000, 2004).

(2) *Sciaphyes* Jeannel, 1910, originally established for *Bathyscia sibirica* Reitter, 1887 from the Russian Far East (Figs. 1, 6). Two species were subsequently described, *S. kurbatovi* Perreau, 1996 from the Russian Far East (presently a synonym of *S. sibiricus*, see PERREAU 1999) and *S. kawaharai* Hoshina & Perreau, 2008 from Tsushima Island in Japan (Figs. 1, 6). The genus had been attributed to Leptodirini (REITTER 1887; JEANNEL 1907, 1910, 1911, 1924; NEWTON 1998) until PERREAU (2000) proposed a separate tribe *Sciaphyini* for it. Characters emphasised by PERREAU (2000) to justify this decision were the pentamerous female protarsi (tetramerous in Leptodirini), the presence of two setae between the claws on the empo-



Fig. 2. Habitus of *Platycholeus* sp. (voucher number IBE-AF 218).

dium (one seta in Leptodirini) and the structure of the thoracic and abdominal venter. HOSHINA & PERREAU (2008) noted that *Sciaphyini* might be related to Leptodirini or Ptomaphagini based on the presence of the mesoventral carina and reduced male abdominal segment IX. The genus was also notable for having a mixture of morphological characters specific to either of the latter tribes. The few known *Sciaphyes* specimens were found in forest litter or attracted to dog dung placed at the nest entrance of a lesser white-toothed shrew, *Crociodura suaveolens* (Pallas, 1811) (HOSHINA & PERREAU 2008).

(3) *Fusi* Perkovsky, 1989, a monotypic genus established on the basis of a male and a female of *F. nyujwa* Perkovsky, 1989 collected in the “Beliy Dvoretz” (= “White Palace”) cave in the extreme south of the Russian Far East (Figs. 1, 4–6). When described, the genus was included among the “Bathysciini” on the basis of the characters correlating with the subterranean way of life, such as the lack of the eyes and wings, and in spite of the presence of five tarsomeres in the female. No additional specimens of the genus have ever been reported.

(4) *Proleptodirina* Perkovsky, 1998, a monotypic genus known from three eyed type specimens collected,

presumably, from forest leaf litter in the southern part of the Russian Far East (PERKOVSKY 1989; Fig. 1).

(5) *Sinobathyscia* Perreau, 1999, a monotypic genus known from four females collected by sifting litter in a city park in Wuhan, Hubei province, China (PERREAU 1999; Fig. 1).

(6) *Coreobathyscia* Szymczakowski, 1975, a monotypic genus known from two eyeless males collected in a South Korean cave (SZYMCAKOWSKI 1975; Fig. 1).

Recently, we obtained freshly collected specimens of five species representing three of these genera, namely *Platycholeus*, *Fusi* and *Sciaphyes*, including one species of the latter new to science. This offered an opportunity to generate for the first time partial DNA sequences for these poorly-known taxa and use these data in critical testing of their long-assumed relationships with the convincingly monophyletic Mediterranean core of the tribe (RIBERA et al. 2010). We also describe and illustrate a new *Sciaphyes* species and use this new information to test phylogenetic affinities of this genus within the morphology-based Leptodirini phylogenetic framework of FRESNEDA et al. (2007).

2. Material and methods

2.1. Beetle collecting, identification, handling and preservation

Collecting. *Platycholeus* specimens used for this work represent one named and one unnamed species collected by Alfred Newton and Margaret Thayer in western North America and made available to us through José María Salgado. The single *Fusi* specimen was collected using deep-soil traps designed specifically for the endogean mesofauna (Fig. 3) and inspired by THOMPSON’S (1995) paper on the British subterranean Raymondionymidae weevils. Six traps were placed on May 17 and retrieved on July 10, 2008 on Mr. Shestakov’s land in the vicinity of Anisimovka village, Primorsky Krai, Russian Far East. The only obtained *Fusi* specimen was dead when first seen on July 10 and placed directly in 95% ethanol. When examined on July 10, these traps also contained about half a dozen *Sciaphyes* specimens and about the same number of the most peculiar microphthalmic weevil *Alaocybites egorovi* Grebennikov, 2010 (see



Fig. 3. Deep soil trap inspired by THOMPSON (1995) and used in the Russian Far East for the endogean fauna. Both *Fusi* and *Sciaphyes* were collected with this type of trap. This trap, once set up, might be serviced as often as desired by means of retreating the deeply sunk collecting jar with the aid of a screw lid firmly attached to the extended handle. The mesh allowed the beetles to crawl through and fall into the jar filled with glycerol (ethylen glycol or a saturated water solution of table salt are suitable alternatives). It was found that the mesh sleeve is too weak to keep its shape for more than a year and, therefore, a solid plastic sewage pipe some 30–40 cm long with numerous drilled holes should be preferably used. For an alternative strategy of deep soil trapping see GIACHINO & VAI-LATI (2010).

GREBENNIKOV 2010). These two beetle genera, however, were even more commonly collected by sifting forest leaf litter with subsequent arthropod extraction using Winkler funnels. All *Sciaphyes* specimens were placed directly in 95% ethanol.

Identification. Our female *Fusi* specimen was preliminarily identified using the original generic description of PERKOVSKY (1989) and further corroborated by using photographs of the holotype (Figs. 4, 5) specifically requested for this purpose. Lack of male genitalia characters prevented us from making a conclusion on whether our specimen represents the already known or a new species, although the former appears much more likely. External similarity, together with the geographical origin of the specimen, strongly suggests that it could indeed be a third known specimen of *F. nyujwa*. The type locality of this species is about 30–40 km eastwards from where our 2008 specimen was collected, and both

places are within the same rather isolated and comparatively small and east-west oriented Livadiyskiy Mountain Range (Fig. 6). This is a southern fragment of the much larger and north-south oriented Sikhote-Alin Mountain Range, known for its endemic soil organisms and harbouring, among others, arguably the most mysterious extant beetle species, *Sikhotealinia zhiltzovae* Lafer, 1996.

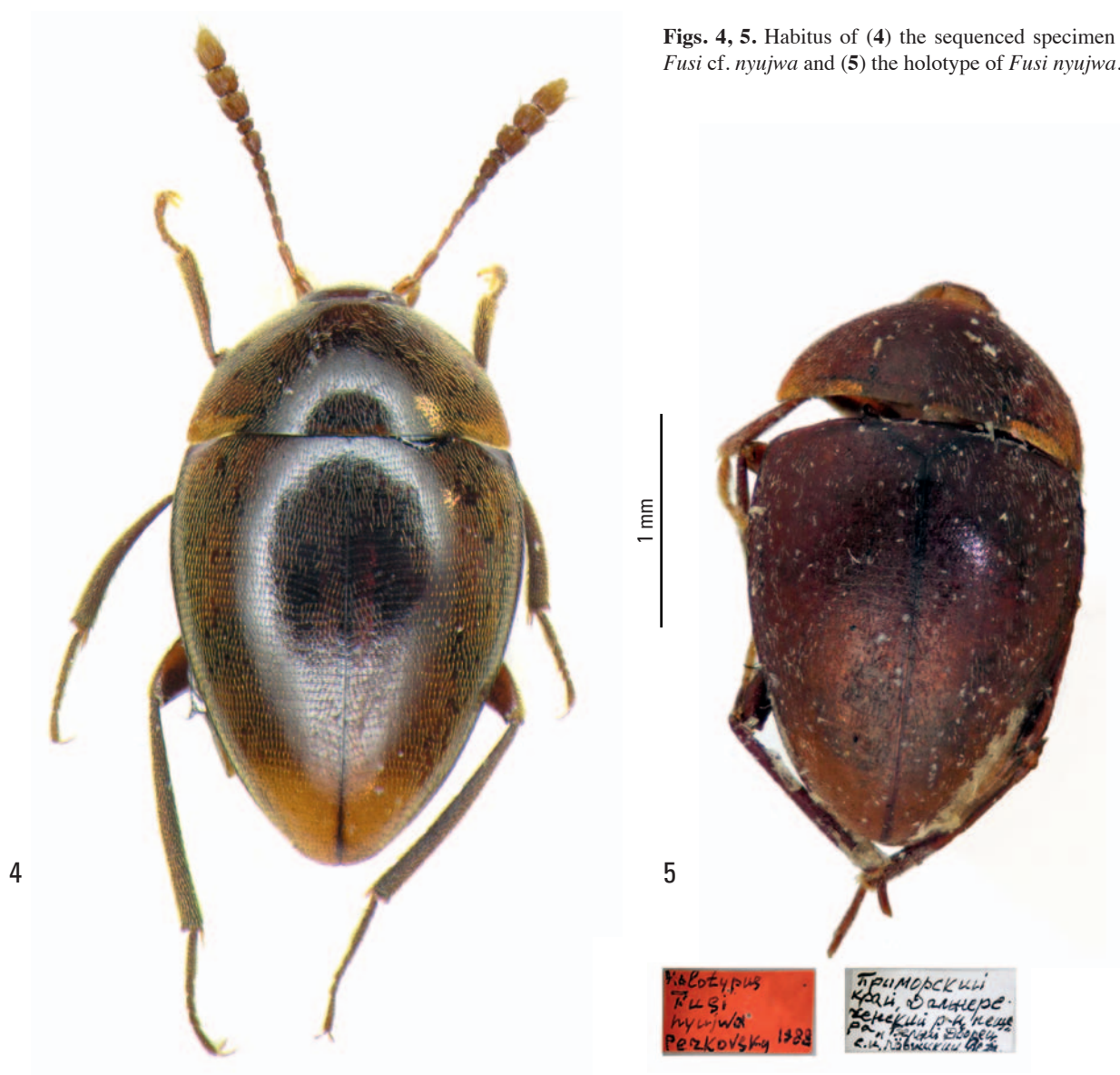
Material of *Fusi* cf. *nyujwa*. Female ‘Russia, Primorsky Kray, Anisimovka vil., N43°07'25" E132°47'43", 09–11.vii.2008, 500 m, soil traps V. Grebennikov’; voucher number IBE-AF67 of Institute of Evolutionary Biology, Barcelona; specimen deposited in collection of J. Fresneda.

Material of *Sciaphyes sibiricus* (Reitter, 1887). Male ‘Russia, Primorsky Kr. / Anisimovka vil., N43°07' / 25" E132°47'43", 09–11. / vii.2008, 500 m, soil / traps V. Grebennikov’ (voucher number IBE-AF68); female, ‘Russia, Primorsky Kray, 5 km S Anisimovka vil., N43°07'30.2" E132°47'43.5", 27.vi.2010, 422 m, hand pick, V. Grebennikov leg.’; male, same locality but from deep soil traps retrieved in 2009 after year-long exposure, specimen mounted in Euparal on microscope slide (voucher number #426) and used for SEM (Figs. 6, 19–24); male ‘Russia, Primorsky Kray, Gamova peninsula, N42°34'47" E131°12'49", 0–20 m, 26–29.vi.2008, V. Grebennikov leg.’. – All material deposited at IBE (Institute of Evolutionary Biology, Barcelona, Spain).

Material of *Platycholeus opacellus* Fall, 1909. 2 specimens ‘USA: CA: Calaveras Co., Stanislaus / N.F., For. Rd. 7N09 to Pumpkin / Hollow, 2103 m, 38°25.301'N, / 120°09.303'W, 11.vi.2006, Abies con- / color forest; under bark conifer logs, / A. Newton & M. Thayer 95% ethanol / FIELD MUSEUM NAT.HIST.’ One specimen used for DNA extraction (voucher number IBE-AF217 of Institute of Evolutionary Biology, Barcelona).

Material of *Platycholeus* sp. 2 specimens ‘USA: CA: Calaveras Co., Stanislaus / N.F., For. Rd. 7N09 to Pumpkin / Hollow, 2103 m, 38°25.301'N, / 120°09.303'W, 11.vi.2006, Abies con- / color forest; under bark conifer logs, / A. Newton & M. Thayer 95% ethanol / FIELD MUSEUM NAT.HIST.’ One specimen used for DNA extraction (voucher number IBE-AF218 of Institute of Evolutionary Biology, Barcelona).

Preparation and pictorial documentation. After DNA extraction (see below) voucher specimens were dry mounted, and the aedeagus and the male genital segment were treated with a 10% solution of potassium hydroxide (KOH) for six hours, then dehydrated in increasing concentrations of ethanol, immersed in xylol (= xylene) for 12 hours, and mounted in Canada balsam on rectangular pieces of transparent plastic pinned under the specimen. Photographs were made with an Olympus szx16 microscope and an Olympus c5060wz camera. Line drawings were traced from digital photographs. Environmental Scanning Electron Microscope (ESEM) images were obtained using uncoated specimens.



Figs. 4, 5. Habitus of (4) the sequenced specimen of *Fusi* cf. *nyujwa* and (5) the holotype of *Fusi nyujwa*.

2.2. DNA extraction and sequencing

DNA was extracted from whole specimens with commercial column kits in a non-destructive manner to preserve voucher specimens for subsequent morphological study. DNA voucher specimens are deposited in the Institute of Evolutionary Biology, Barcelona, Spain (IBE). Five gene fragments were sequenced: three mitochondrial (3' end of cytochrome c oxidase subunit 1, *cox1*; an internal fragment of cytochrome b, *cob*; and 3' end of large ribosomal unit 16S rDNA plus the Leucine transfer RNA gene plus the 5' end of NADH dehydrogenase subunit 1, *rrnL+trnL+nad1*) and two nuclear (5' end of the small ribosomal unit 18S rDNA, *SSU*, and an internal fragment of the large ribosomal unit 28S rDNA, *LSU*). Primers used are given in Appendix 1. For each fragment both forward and reverse sequences were obtained. New sequences have been deposited in GenBank (see El. Supplement).

2.3. Taxon sampling and phylogenetic analyses

2.3.1. DNA-based analyses

We used the dataset from RIBERA et al. (2010), plus newly obtained sequences of various Cholevinae species, including those of *Platycholeus*, *Fusi* and *Sciaphyes* (see El. Supplement). A few representatives of the subfamilies Platypsyllinae and Leiodinae were used as close outgroup taxa (NEWTON 1998; GIACHINO et al. 1998). We also included seven species of various subfamilies of the closely related Staphylinidae (BEUTEL & LESCHEN 2005) to root the tree (see El. Supplement).

Protein coding genes were not length-variable, while the ribosomal genes were aligned with the online version of MAFFT v.6 using the G-INS-i algorithm and default parameters (KATOH & TOH



Fig. 6. Map of Primorsky Krai showing relevant sampling localities of Leiodidae. Localities for *Sciaphyes* in red, for *Proleptodirina* in blue, and for *Fusi* in green. (A) Gamova Peninsula: *Sciaphyes sibiricus*; (B) Kedrovaya Pad' Nature Reserve: type locality of *Sciaphyes kurbatovi*; (C) Vladivostok: type locality of *Sciaphyes sibiricus*; (D) Kamenushka village, the headquarters of the Ussuriyskiy Nature Reserve: type locality of *Proleptodirina kurbatovi* Perkovsky, 1997 and one of *Sciaphyes kurbatovi* paratypes; (E) Anisimovka village: *Sciaphyes sibiricus* (adult SEM image in left lateral view is superimposed) and *Fusi* cf. *nyujwa*; (F) cave 'Beliy Dvoretz': type locality of *Fusi nyujwa*; (G) Verkhnechuguevsky Statsionar: type locality of *Sciaphyes shestakovi* sp.n.

2008). Bayesian analyses were conducted on a combined molecular data matrix with MrBayes 3.1.2 (HUELSENBECK & RÖNQUIST 2001), using six partitions corresponding to the six sequenced genes (the *rrnL*+*trnL* fragment was considered a single partition) and a GTR+I+G model independently estimated for each partition. MrBayes ran for 9×10^6 generations using default values, saving one tree for every 1000th generation. "Burn-in" values were established after visual examination of a plot of the standard deviation of the split frequencies between two simultaneous runs.

We also used Maximum Likelihood as implemented in RAxML v.7.0 (STAMATAKIS et al. 2008) using GTR+G as the evolutionary model and the same six gene partitions. We ran 100 replicas to select the best topology (i.e. that of the tree with the highest likelihood), and then estimated node support with 1,000 fast bootstrap searches (STAMATAKIS et al. 2008).

2.3.2. Morphology-based analyses

To try to establish the phylogenetic placement of the genus *Sciaphyes* using morphological characters we used a selection of 13 species representing different Cholevinae tribes, plus an outgroup composed of Platypsyllinae and Leiodinae, each represented by one species (Appendix 2). We coded a matrix of external morphological characters and of male and female genitalia partly based on that of FRESNEDA et al. (2007). We did not include characters that were known as polymorphic within the lineage represented by our terminal species, including characters related to the subterranean habits of the species (anophthalmy, apterism, depigmentation), as these may vary within the tribes, exemplified here by a limited taxon sampling (unlike in FRESNEDA et al. 2007, in which terminal taxa did not represent any more inclusive group). Whenever character-bearing structures were absent in a particular taxon, or of doubtful homology,

the respective characters were coded as inapplicable ('?'). We used a matrix of 28 unordered characters, most of them binary. An alternative matrix decoupling the multistate characters as binary (with a total of 36) produced the same results (see Discussion). The data matrix was analysed with PAUP 4.0b10 (SWOFFORD 2002) using the parsimony criterion. Shortest trees were heuristically searched with 100 tree-bisection-reconnection (TBR) replications, swapping on all multiple starting trees, and saving all of them. Branches with zero length were collapsed. Node support was measured with non-parametric bootstrap using 1,000 iterations of 30 TBR each, with the "save multiple trees" option not enforced. To obtain a higher resolution, data were successively re-weighted according to the rescaled consistency index (SWOFFORD 2002), and a heuristic search conducted on the initial set of the shortest trees. Character transformation was mapped only for unambiguously optimized changes using McClade 4.0 (MADDISON & MADDISON 2000) on the strict consensus tree obtained with re-weighted characters.

3. Taxonomy

Sciaphyes shestakovi Fresneda, Grebennikov & Ribera sp. n.

Figs. 7–9, 12, 15–16

Differential diagnosis. Besides clearly different male genitalia (see Remarks below) the three *Sciaphyes* species can be easily separated by the characters of the pronotal surface. In *S. shestakovi* sp. n. it is smooth and shiny with a weakly developed and sparse punctation; in *S. kawaharai* it is strongly microreticulated with a weakly developed and sparse punctation (HOSHINA & PERREAU 2008), and in *S. sibiricus* it bears transverse microreticulation and markedly developed punctation. The male protarsomeres are not dilated in *Sciaphyes shestakovi* sp. n. (Fig. 7), slightly dilated although still narrower than the tibial apex in *S. kawaharai* (after HOSHINA & PERREAU 2008: fig. 3), and clearly dilated and subequal in width to, or wider than, the tibial apex in *S. sibiricus*. Abdominal segment IX in dorsal view is regularly arched and with a round apex in *S. shestakovi* sp. n. (Fig. 15), with a spatuliform expansion at the apex in *S. kawaharai* (HOSHINA & PERREAU 2008: fig. 16), and with an acute apex and slightly concave margins in *S. sibiricus* (Fig. 17).



Fig. 7. Holotype of *Sciaphyes shestakovi* sp. n.

Type locality. Russia, Primorsky Krai, Chuguevskiy Rayon, vicinity of the Verkhnechuguevsky field station (= Verkhnechuguevsky Stationar), N44°02' E134°13', 650 m (G in Fig. 6).

Type material. Holotype, male: "RUSSIA, Primorsky / Krai, Chuguevsky r-n. / N44°02' E134°13', 21/–25.v.2008, 650 m, sifting / litter, V. Grebennikov"; aedeagus mounted in Canada balsam on a transparent microslide pinned with the specimen; currently in collection of J. Fresneda, to be eventually deposited in the collection of the Laboratory of Entomology, Institute of Biology and Soil Science (IBSSV), Vladivostok, Russia. **Paratypes,** nine specimens, same data as holotype. Two specimens used for DNA extraction: male, voucher number IBE-AF108, aedeagus mounted in dimethyl hydantoin formaldehyde resin (DMHF) on a transparent microslide pinned with the specimen, deposited in the Museo Nacional de Ciencias Naturales (MNCN, Madrid); male, voucher number IBE-AF66, aedeagus mounted in Canada balsam on a transparent microslide pinned with the specimen, deposited in the MNCN. Other paratypes will be deposited in IBSSV, Canada National Collection (CNC, Ottawa) and collection J. Fresneda. DNA aliquots of the two extracted paratypes are stored in the DNA and tissue collection of the MNCN and IBE (Institute of Evolutionary Biology, Barcelona).

Holotype description. Habitus as in Fig. 7. **Body** length 1.0 mm; ca. 1.7× as long as wide; body oval, with pronotum slightly narrower than base of elytra and forming a weak angle in body outline. Dorsal surface with long yellowish pubescence. **Colouration:** Body pale reddish, appendices paler and yellowish.

Table 1. Antennal formula of the holotype of *Sciaphyes shestakovi* sp. n. (in μm).

Articles	I	II	III	IV	V	VI	VII	VIII	IX	X	XI
Length	50	50	25	25	25	25	38	25	38	38	75
Width	25	25	13	13	25	31	50	38	50	63	63

Head slightly wider than long; surface smooth, very sparsely and minutely punctate; more sparsely pubescent than pronotum and elytra. Eyes and occipital carina absent. Clypeus and labrum sparsely pubescent, smooth, almost impunctate. **Antenna:** total length 0.41 mm; relative length of antennomeres 1–11 as in Table 1. 1st and 2nd antennomeres subequal in size and shape, 2× as long as wide; 3rd to 6th subequal in length; 3rd and 4th cylindrical; 5th spherical; 6th transverse; 7th to 11th forming the loose club typical of Leiodidae, with 7th to 10th antennomeres strongly transverse, 8th shortest and 11th longer than wide, longest of all. Pronotum transverse, ca. 1.8× as wide as long, slightly narrower than elytra, hind lateral corners weakly protruding and forming an acute angle. Pronotal surface smooth and shiny, with fine sparse punctation. **Elytra** not firmly interlocked, ca. 1.2× as long as wide (Fig. 7), lateral margins regularly curved; surface with fine, long pubescence; sculpture formed by strong transverse ridges formed by series of small punctures; parasutural stria absent. **Hind wings** absent. **Scutellar shield** partly visible, small, wide and short. **Legs** with pro- and metatibiae straight, mesotibiae weakly curved. Protarsi with 5 segments, slender, not dilated, distinctly narrower than apex of protibiae. Tibia with apical and lateral row of spines. Empodium of all pretarsi with 2 setae. Ventral surface with anteriorly strongly dented carina extending across mesoventrite, metaventrite and first visible abdominal ventrite. Lateral metaventral sutures well defined and bifurcated anterad. **Male genitalia** with median lobe of aedeagus narrow in dorsal view, triangular in shape; apex round (Fig. 8); in lateral view regularly curved, forming an arc, apically pointed (Fig. 9). Parameres with uniform width through their entire length, inserted in dorsal region of median lobe; basal lobes contiguous ventrally; apex of parameres dilated, with 5 spines (Fig. 12). Internal sac of aedeagus (= endophallus) without sclerotised structures. Abdominal segment IX almost fully developed, with only some reduction in tergite (Figs. 15, 16).

Etymology. The species name is a patronymic derived from the family name of Sergey Revovich Shestakov, the owner of the land near Anisimovka village (Primorsky Krai, Russian Far East) where *Fusi* cf. *nyujwa* and *Sciaphyes sibiricus*, along with

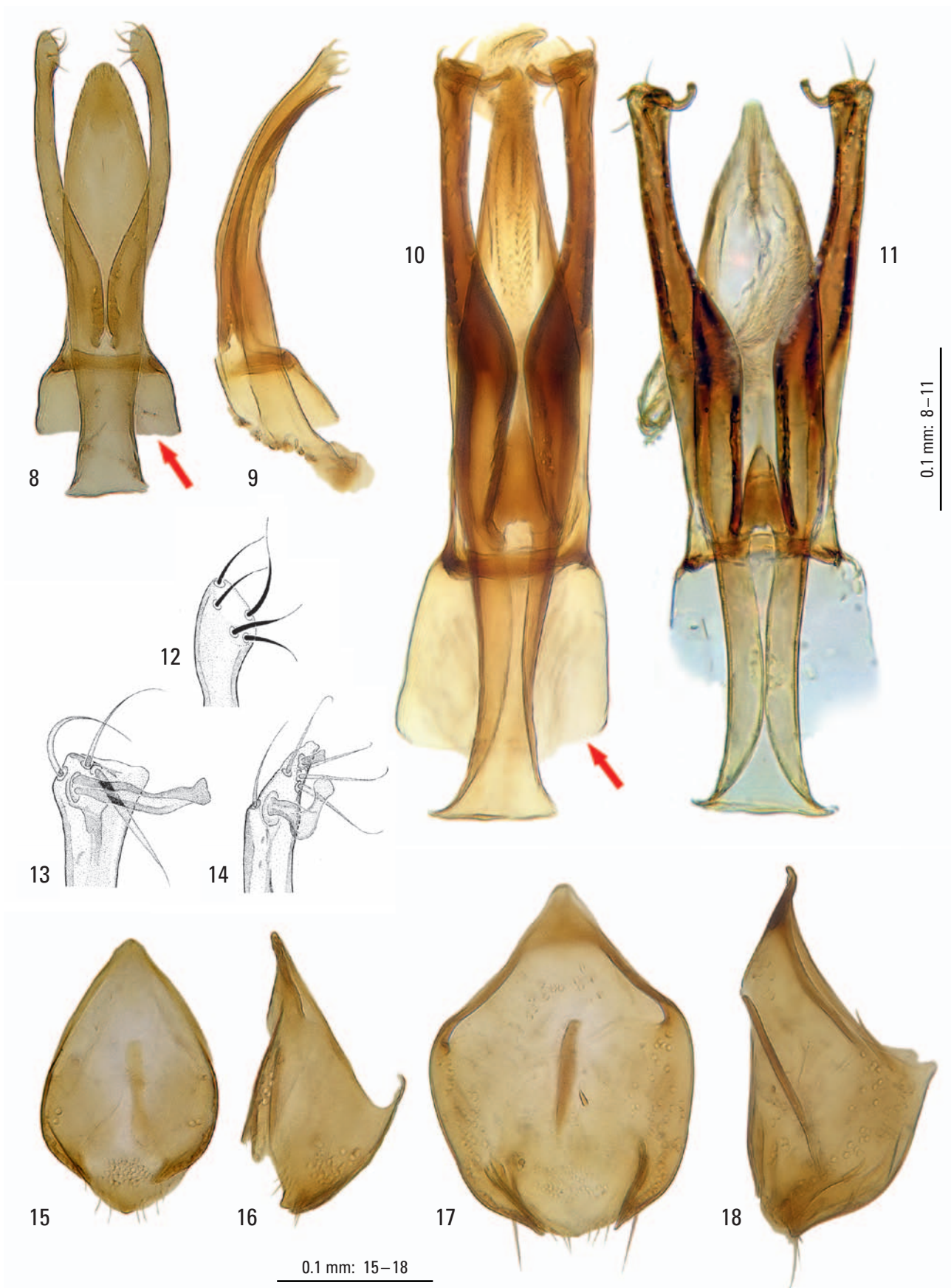
the most peculiar microphthalmic weevil *Alaocybites egorovi*, co-occur. Shestakov variously and consistently supported collecting activities of V. Grebennikov in Primorsky Krai, including permission to sift litter and set deep-soil traps on his property resulting in discovery of these cryptic beetles.

Distribution and Biology. Specimens of *S. shestakovi* sp. n. were collected by sifting forest floor leaf litter in mixed forests on the southern part of the Sikhote-Alin Mountain Range, Primorsky Krai, Russian Far East, at an altitude of 650 m. Immature stages are unknown. No observations were made to suggest species' association with social insects or to indicate preference of coniferous logs (as known for *Platycholeus*), or its association with caves or deeper soil layers (as known for *Fusi* and most Mediterranean Leptodirini). Presently, both mainland *Sciaphyes* species appear as unspecialized inhabitants of the forest leaf litter without any known biological preferences.

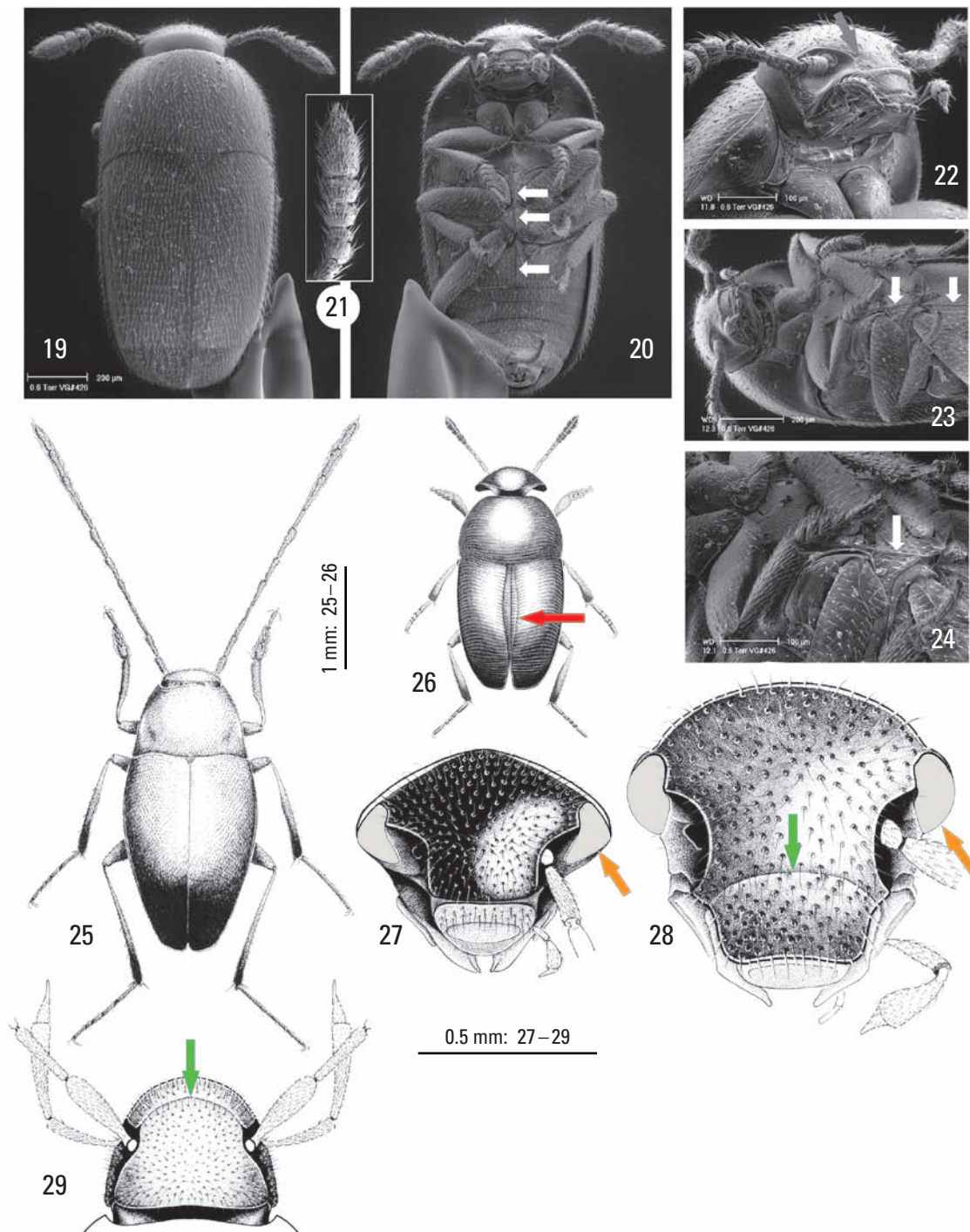
Specimens of *Sciaphyes* were found so far in three quite widely separated localities in Primorsky Krai consistently sampled in 2008 with a sifter and Winkler funnels (Fig. 6). This suggests that the scarcity of the previously known *Sciaphyes* specimens should be attributed to the lack of adequate forest litter sampling and not to the 'rarity' of these species.

Remarks. *Sciaphyes shestakovi* sp. n. shares with the other two species of the genus the general habitus and the small body size (Figs. 7, 19, 20), the shape and distribution of the elytral striae, the shape of the parameres (Figs. 8, 10, 11), robust with an apical widening and inserted in the dorsal part of the median lobe, the well-developed lamella of the tegmen; the long, tubular and narrow basal lamina of the median lobe (Figs. 8, 10, 11; see also HOSHINA & PERREAU 2008: fig. 20), the metaventral and abdominal carinae, the presence of two metaventral sutures, and the same antennal structure (see Table 1 for *S. shestakovi* sp. n.; Fig. 21 for *S. sibiricus*).

Male genitalia have traditionally been used to establish relationships among the Cholevinae genera (e.g. Anemadini: GIACHINO & VAILATI 1993; Leptodirini: FRESNEDA 1998, 1999; FRESNEDA & SALGADO 2000, 2006; SALGADO & FRESNEDA 2003, 2005; SALGADO et al. 2008). The differences in the male genitalic structures among all three *Sciaphyes* species are remarkably pronounced and of an extent similar as otherwise found between different cholevine genera. Thus, parameres of *Sciaphyes sibiricus* bear five setae and two modified spines on the apex (Figs. 13, 14) and there are two bands with spicules in the endophallus (Fig. 10), while *S. kawaharai* has three small parameral setae and an endophallus with scales, and *S. shestakovi* sp. n. has five parameral spines (Fig. 12)



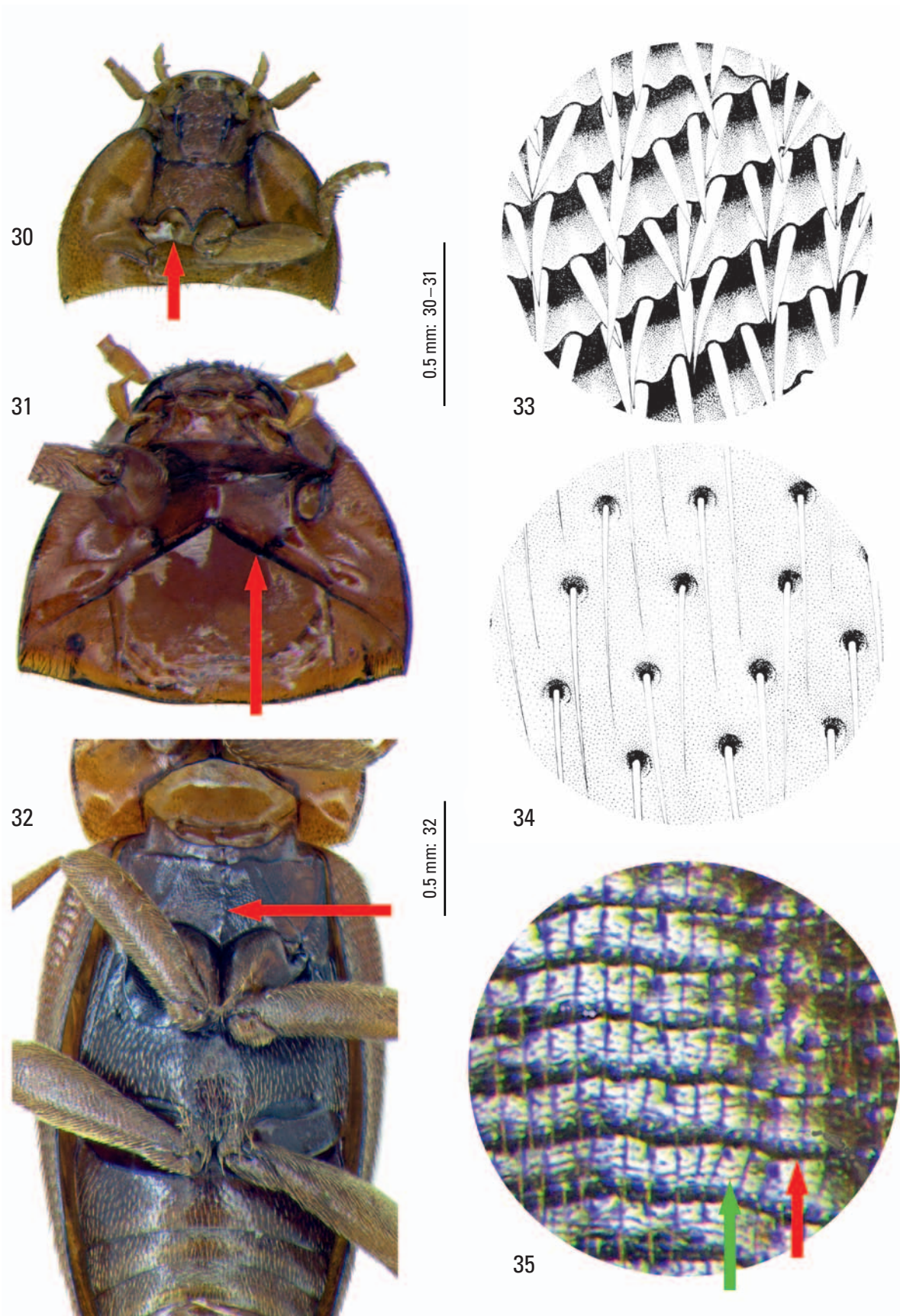
Figs. 8–18. *Sciaphyes shestakovi* sp.n. holotype male, aedeagus (8) dorsal and (9) lateral view; (12) detail of the apex of the paramere; abdominal segment IX in (15) dorsal and (16) lateral view. *Sciaphyes sibiricus* males, aedeagus of specimens from (10) Anisimovka and (11) Gamova peninsula; detail of the apex of the paramere of the specimen from Anisimovka in (13) dorsal and (14) lateral view; abdominal segment IX in (17) dorsal and (18) lateral view. Arrows mark the ventral lamella of the tegmen (character 25(0)).



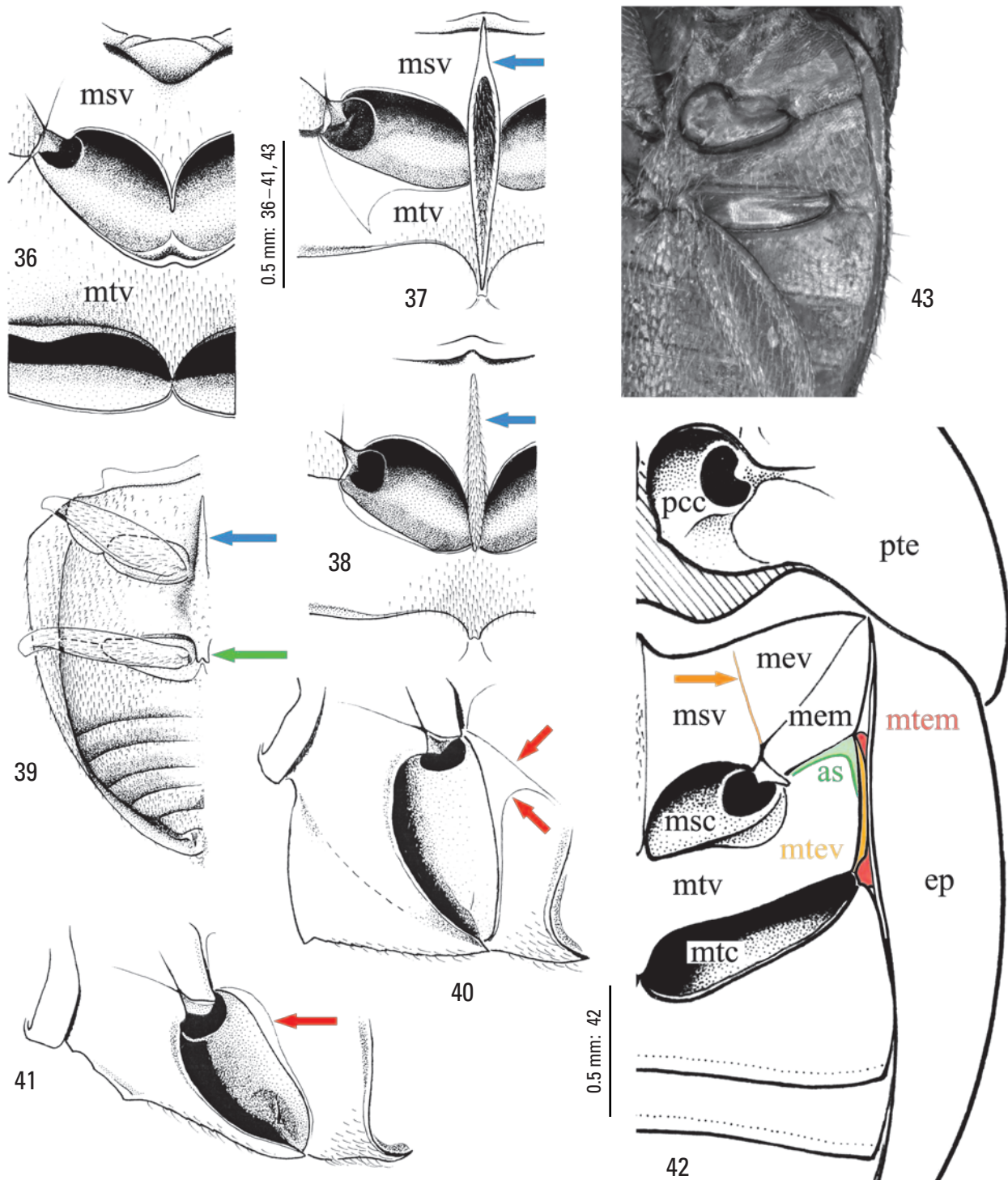
Figs. 19–29. *Sciaphyes sibiricus*, habitus in (19) dorsal and (20) ventral view, arrows: mesoventral, metaventral and abdominal carinae (characters 3(0), 10(1)); (21) antenna; (22) head and pronotum, ventro-lateral view, arrow: fronto-clypeal suture (character 1(0)); (23) thoracic and abdominal ventromedian carina, ventro-lateral view (character 10(1)); (24) metaventral carina (character 6(0)). (25) Habitus of *Aranzadiella leizaolai* Español, 1972 without parasutural striae (character 16(0)). (26) Habitus of *Ptomaphagus tenuicornis* (Rosenhauer, 1856), with parasutural striae (red arrow) (character 16(1)). Head of (27) *Ptomaphagus tenuicornis* (anterior view), without fronto-clypeal suture (character 1(1)), orange arrow: eye; (28) *Speonemadus angusticollis* (Kraatz, 1870) (anterior view), green arrow: fronto-clypeal suture (character 1(0)); orange arrow: eye; (29) *Speocharidius breuili* Jeannel, 1919 (dorsal view), with fronto-clypeal suture (green arrow) (character 1(0)), without eyes.

and no apparent sclerotized structures in the endophallus. There are apparent differences in the morphology of the aedeagus among the specimens of *S. sibiricus* from the two localities where they were found, Anisimovka (Fig. 10) and the Gamova peninsula (Fig. 11)

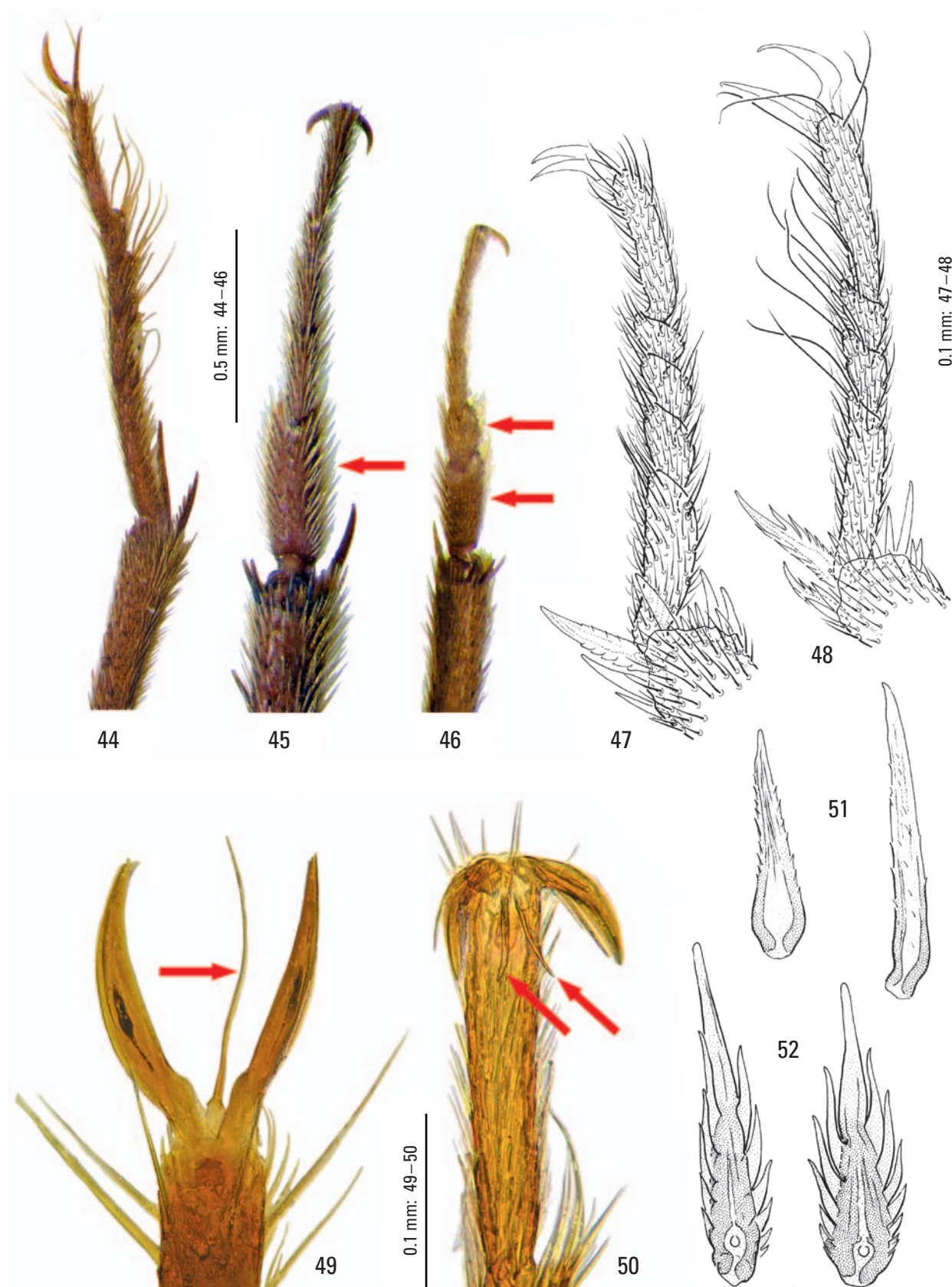
(see Fig. 6 for the geographic situation of the localities). More material would be necessary to establish if this variation is constant and geographically structured.



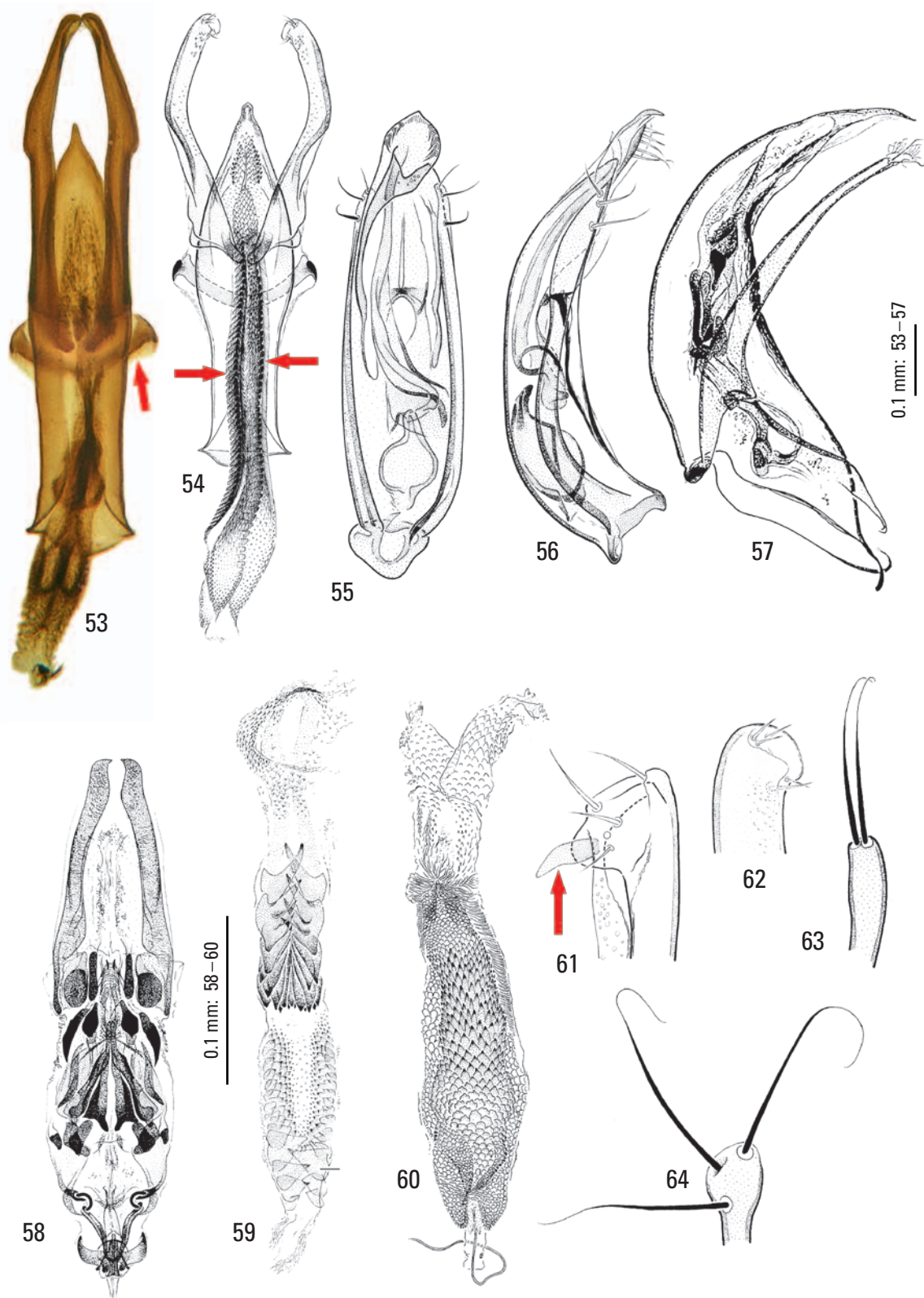
Figs. 30–35. Ventral view of the prothorax of (30) *Leptinus testaceus* Müller, 1817, with open procoxal cavities (arrow) (character 2(0)); (31) *Ptomaphagus troglodytes* Blas & Vives, 1983, with closed procoxal cavities (arrow) (character 2(1)). (32) Ventral surface of *Speonemadus clathratus* (Perris, 1864), with mesoventral carina (arrow) and confluent mesocoxal cavities (character 3(1)). Elytral disc of (33) *Ptomaphagus subvillosus* (Goeze, 1777), with transverse striae (character 17(0)); (34) *Quaestus* (*Asturianaella*) *incognitus* Salgado & Fresneda, 2004, with irregular punctation (character 17(2)); (35) *Speonemadus clathratus*, with two series of transverse striae (marked with arrows) forming a microreticulation (character 17(1)).



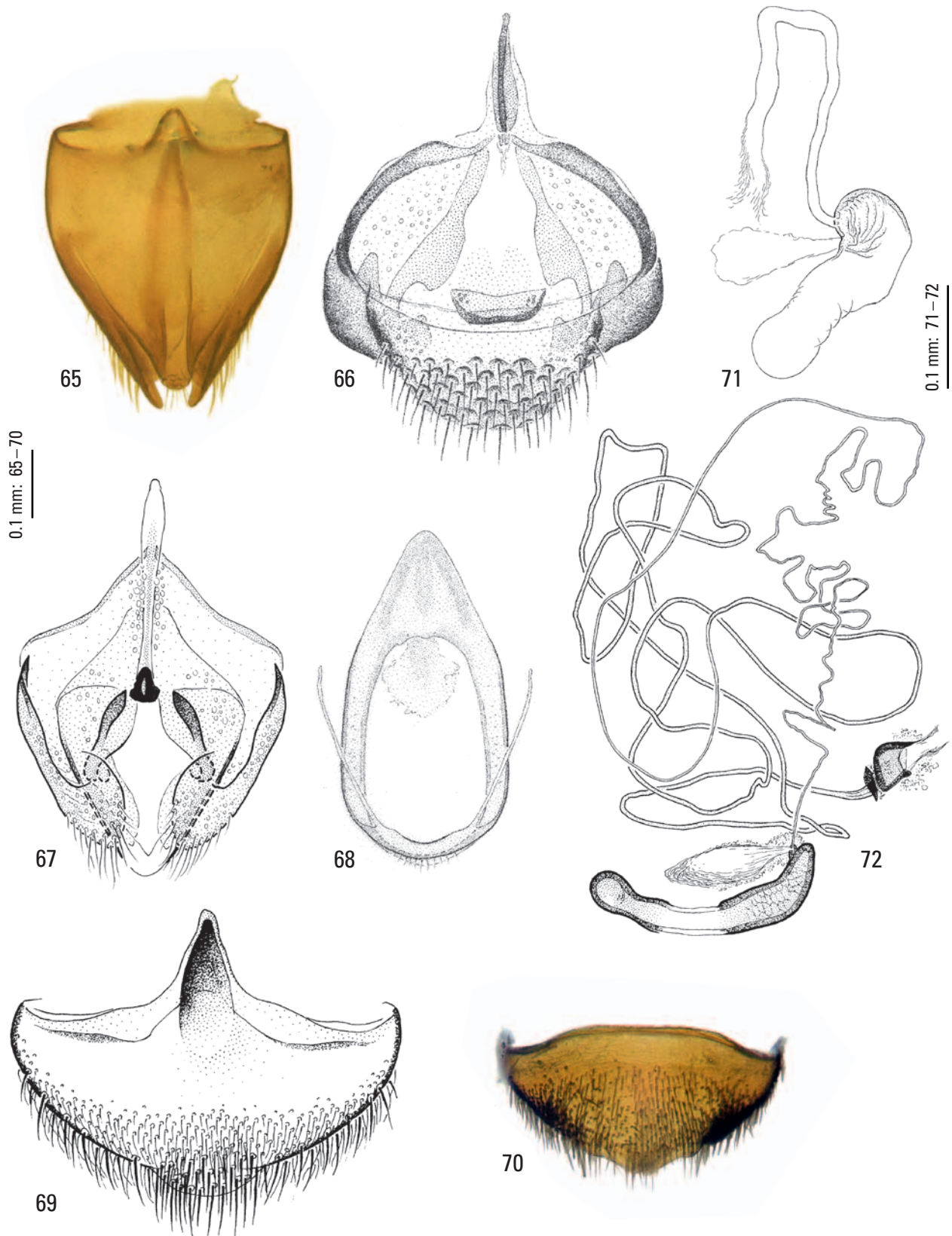
Figs. 36–43. Meso- and metaventrite of (36) *Catops fuliginosus* Erichson, 1837, without mesoventral carina and mesocoxal cavities confluent (character 3(2)); (37) *Quaestus (Quaesticulus) noltei* (Coiffait, 1965), with mesocoxal cavities separated by a mesoventral carina (blue arrow) which overlies the metaventrite (character 3(0)); (38) *Stygiophyes puncticollis* (Jeannel, 1910), with mesocoxal cavities separated by a mesoventral carina (blue arrow) which does not extend over the metaventrite (character 3(1)). (39) Ventral surface of *Paranillochlamys catalonica* (Jeannel, 1913), with mesoventral carina (blue arrow) and metacoxae separated by a metaventral apophysis (green arrow) (character 6(0)). Ventral side of metathorax of (40) *Speonomidius crotchi* (Sharp, 1873), with two metaventral sutures (red arrows) (character 7(1)); (41) *Troglocharinus (Troglocharinus) ferreri* (Reitter, 1908), with metaventral sutures reduced (red arrow) (character 7(0)). (42) Ventral surface of *Trapezodirus escollae* (Fresneda & Hernando, 1994) with mesoventrite-mesoepiventrite suture (orange arrow) (character 4(1)). (43) Ventral surface of *Leptinus testaceus*, with suture separating mesoepisternum and mesoepimeron absent (character 7(0)), metaepisternum and metaepimeron not externally visible (character 10(0)) and anapleural suture absent (character 11(0)). Abbreviations: as, anapleural suture; ep, epipleura; mev, mesoepiventrite (mesothoracic epiventrite); mem, mesoepimere (mesothoracic epimere); msc, mesocoxal cavity; msv, mesoventrite; mtc, metacoxal cavity; mtem, metaepimere (metathoracic epimere); mtev, metaepiventrite (metathoracic epiventrite); mtv, metaventrite; pcc, procoxal cavity; pte, prothoracic epimere.



Figs. 44–52. Male mesotarsi of (44) *Trapezodirus escollae*, without any dilated tarsomere (character 12(0)); (45) *Catops punctatulus* Fresneda, Lencina & Salgado, 2006, with the first mesotarsomeres dilated (red arrow) (character 12(1)); (46) *Speonemadus clathratus*, with two mesotarsomeres dilated (red arrows) (character 12(0)). Female protarsi of (47) *Catops fuliginosus*, with five tarsomeres (character 11(0)); (48) *Trapezodirus gimenezi* (Fresneda, Hernando & Lagar, 1998), with four tarsomeres (character 11(1)). Pretarsal empodium of (49) *Trapezodirus escollae*, with one seta (red arrow) (character 14(1)); (50) *Ptomaphagus troglodytes*, with two setae (red arrows) (character 14(0)). Internal meso- and metatibial spurs of (51) *Catops fuliginosus*, non pectinate (character 13(0)); (52) *Stygiophyes akarsticus* (Escollà, 1980), pectinate (character 13(1)).



Figs. 53–64. Aedeagus of (53) *Anemadus graecus* (Kraatz, 1870) in dorsal view, with a reduced lamina of the tegmen (arrow) (character 25(1)) and well developed basal lamina (character 21(1)); (54) *Speonemadus vandaltitiae* (Heyden, 1870) in dorsal view, with the internal sac bearing two spiny bands (arrows) (character 26(1)) and well developed basal lamina (character 21(1)); *Ptomaphagus tenuicornis* in (55) dorsal (character 20(1)) and (56) lateral view (character 19(1)), with basal lamina absent (character 21(2)); (57) *Pallaesiella pallaesana* (Jeannel, 1911) in lateral view, with reduced basal lamina and distal pore (character 21(0)). Internal sac of the aedeagus of (58) *Pallaesiella pallaesana*; (59) *Espanoliella jeanneli* (Bolívar, 1917); (60) *Catops fuliginosus*. Apex of the lateral stylus of the aedeagus (paramere) of (61) *Speonemadus maroccanus* (Jeannel, 1936), with one stout spine-like seta (arrow) (character 23(2)); (62) *Speonemadus vandaltitiae*, with five simple setae (character 23(1)); (63) *Catops fuliginosus*, with two slender simple setae (23(1)); (64) *Quaestus (Quaesticulus) nadali* Salgado, 1978, with three slender simple setae (character 23(1)).



Figs. 65–72. Male IXth abdominal segment of (65) *Anemadus graecus*, fully developed and forming a hood-like receptacle for the aedeagus (character 18(1)); (66) *Catops fuliginosus*, open and partially reduced (character 18(0)); (67) *Ptomaphagus sericatus medius* (Rey, 1889), open and strongly reduced, with two short, robust lateral processes (character 18(0)); (68) *Josettekia angelinae* Bellés & Déliot, 1983, reduced to a simple ring, with two long and narrow lateral processes (character 18(0)). Female 8th abdominal segment of (69) *Catops fuliginosus*, with spiculum ventrale (character 28(0)); (70) *Ptomaphagus troglodytes*, without spiculum ventrale (character 28(1)). Spermathecal complex of (71) *Catops fuliginosus*, “atypical spermatheca” of PERREAU (1989), fully membranous (character 27(0)); (72) *Troglocharinus (Troglocharinus) ferreri*, “spermatheca sclerotised type 1” of PERREAU (1989) (character 27(1)).

Table 2. Character matrix.

Character	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	
<i>Sciaphyes sibiricus</i>	0	1	0	1	0	0	1	1	1	1	0	2	0	0	0	0	1	1	0	0	1	0	1	1	0	1	0	0	
<i>Sciaphyes shestakovi</i>	0	1	0	1	0	0	1	1	1	1	1	0	2	0	0	0	0	1	1	0	0	1	0	0	1	0	?	0	0
<i>Micronemadus pusillimus</i>	1	1	1	1	1	1	1	1	1	0	0	2	0	0	0	1	1	1	0	0	1	0	2	1	1	1	0	0	
<i>Nemadiolus kuscheli</i>	1	1	1	1	1	1	0	1	1	0	0	2	0	0	0	1	1	1	0	0	1	0	0	1	0	1	0	0	
<i>Speonemadus clathratus</i>	0	1	1	1	1	1	0	1	1	0	0	0	0	0	0	1	1	1	0	0	1	0	1	1	1	1	0	0	
<i>Anemadus graecus</i>	0	1	1	1	1	1	0	1	1	0	0	0	0	0	0	1	1	1	0	0	1	0	0	1	1	1	0	0	
<i>Catops punctatulus</i>	0	1	2	1	1	1	0	1	1	0	0	1	0	0	0	1	2	0	1	0	0	1	0	0	0	0	0	0	
<i>Choleva grupoi</i>	0	1	2	1	1	1	0	1	1	0	0	1	1	0	0	1	2	0	1	0	0	0	0	0	0	0	1	0	
<i>Ptomaphagus tenuicornis</i>	1	1	0	1	1	1	1	1	1	0	0	2	0	0	1	1	0	0	1	1	2	1	0	2	?	0	1	1	
<i>Ptomaphagus troglodytes</i>	1	1	0	1	1	1	1	1	1	0	0	2	0	0	1	1	0	0	1	1	2	1	0	2	?	0	1	1	
<i>Trapezodirus escollae</i>	0	1	0	1	1	0	0	1	1	0	1	2	1	1	0	0	0	0	1	0	0	1	0	0	0	0	1	0	
<i>Spelaeochlamys ehlersi</i>	0	1	0	0	1	0	1	1	1	0	1	2	1	1	0	0	2	0	1	0	0	1	0	0	0	0	0	1	0
<i>Bathyscia montana</i>	0	1	0	1	1	0	1	1	1	0	1	2	1	1	0	0	2	0	1	0	0	1	0	0	0	0	2	1	0
<i>Leptinus testaceus</i>	0	0	0	0	0	0	0	0	0	0	0	2	0	2	0	0	2	0	0	0	0	0	0	0	0	0	0	0	0
<i>Agathidium</i> sp.	0	1	0	0	1	0	0	0	0	0	0	1	0	0	0	0	2	1	1	0	0	1	0	0	0	2	0	0	

4. Phylogenetic analyses

4.1. List of morphological characters for *Sciaphyes* phylogenetic analysis

In the following, multistate characters are marked with asterisks. The character matrix is shown in Table 2.

- Suture between the frons and the clypeus: visible externally (at least in lateral parts) (0) (Figs. 22, 28, 29: green arrows); not visible externally (1) (Fig. 27).
- Posterior part of the procoxal cavities: open (0) (Fig. 30: red arrow); closed (1) (Fig. 31: red arrow).
- * Length of the mesoventral carina: long, extending over the entire mesoventrite (0) (Fig. 37: blue arrow); short, extending over the anterior part of the mesoventrite (1) (Figs. 32: red arrow, 38: blue arrow); absent (2) (Fig. 36).
- Suture between mesoventrite and mesoepiventrite: absent (0); present (1) (Fig. 42: orange arrow).
- Suture between mesoepimere and mesoepiventrite: not visible externally (0) (Fig. 43); visible externally (1) (Fig. 42, between mem and msv + mev).
- Mesothoracic apophysis separating metaventral coxal cavities: present (0) (Fig. 39: green arrow); absent (coxal cavities contiguous) (1) (Figs. 32, 36).
- Metaventral sutures: absent (0) (Fig. 41: red arrow); present (1) (Fig. 40: red arrows).
- Metepisternum and metepimeron: not exposed, covered by elytral epipleura (0) (Fig. 43); at least partly exposed, not covered by elytral epipleura (1) (Fig. 42).
- Anapleural suture of the metaventrite: absent (0) (Fig. 43); present (1) (Fig. 42).
- Longitudinal median carina on abdominal sternite III (= 1st visible ventrite): absent (0) (Fig. 32); present (1) (Figs. 20, 23).
- Number of protarsomeres in female: five (0) (Fig. 47); four (1) (Fig. 48).
- * Dilated mesotarsomeres in male: none (0) (Fig. 44); 1st (1) (Fig. 45: red arrow); 1st and 2nd (2) (Fig. 46: red arrows).
- Structure of the internal (ventral) tibial spurs: simple (non pectinate or with a shallow serration) (0) (Fig. 51); complex (pectinate or polytoothed) (1) (Fig. 52).
- * Number of articulated setae (in some species shaped as small lobes) between the claws on the pretarsal empodium: none (0); one (1) (Fig. 49: red arrow); two (in some species one is greatly reduced) (2) (Fig. 50: red arrows).
- Type of dorsal sculpture of the pronotum: punctation (0) (Fig. 34); transverse striation (1) (Fig. 33).
- Elytral parasutural stria: absent (0) (Fig. 25), present (1) (Fig. 26: red arrow).
- * Type of dorsal sculpture of the elytra: transverse striae, maybe effaced towards the apex, with smooth surface between them (0) (Fig. 33); transverse striae with microreticulated surface, at least towards apex (1) (Fig. 35: arrows); irregular punctation (2) (Fig. 34).
- Male abdominal segment IX: reduced (0) (Figs. 66, 67, 68); fully developed, forming a complete hood (1) (Fig. 65).
- Shape of the median lobe of the aedeagus in lateral view: weakly curved, with a straight base (0) (Fig. 9); strongly curved all along its length (1) (Figs. 56, 57).
- Shape of the aedeagus in dorsal view: symmetric (0) (Figs. 53, 54); asymmetric (1) (Fig. 55).

- 21.* Size of the basal lamina of aedeagus: poorly developed, with a distal pore (0) (Fig. 57); well developed, with base extended forming a tube, with a basal pore (1) (Figs. 8, 10, 11, 53, 54); absent (2) (Figs. 55, 56).
22. Shape of the paramere (= lateral stylus) of the aedeagus: robust (0) (Figs. 8, 10, 11, 53, 54); slender (1) (Figs. 55, 56, 57).
- 23.* Setae of the apex of the parameres: absent (0); slender (1) (Figs. 62–64); robust, spine-like (2) (Fig. 61: red arrow).
- 24.* Location of the tegmen along the aedeagus: at base (0) (Figs. 55, 56); in basal third (1) (Figs. 8, 10, 11, 57); in middle (2) (Figs. 53, 54).
25. Ventral lamella of tegmen: large (0) (Figs. 8, 10, 11: red arrows); small, poorly developed (1) (Fig. 53: red arrow). Terminal taxa with character state (2) in character 24 were scored as inapplicable.
- 26.* Structures of the internal sac of median lobe of aedeagus: absent (0) (Fig. 8); forming two bands of spines (1) (Figs. 10, 11, 54: red arrows); with other structures (2) (Figs. 58–60).
27. Sclerotisation of the spermatheca: absent (i.e. fully membranous) (0) (Fig. 71); present (1) (Fig. 72).
28. Spiculum ventrale in the 8th female sternite: present (0) (Fig. 69); absent (1) (Fig. 70).

4.2. Morphology-based relationships of *Sciaphyes*

The analyses of the matrix containing 28 characters with equal character weight (all of them but character 2 informative, Table 2) resulted in 17 most parsimonious trees 56 steps long. The strict consensus of these trees (Fig. 73) recovered Cholevinae and all its tribes, including Sciaphyini, as monophyletic, and the latter as sister to Anemadini. The relationships of the Sciaphyini + Anemadini clade with Ptomaphagini and Cholevini were unresolved. The search stabilized after two rounds of re-weighting, with 16 characters weighted lower than 1, i.e. all except those with unambiguous character state changes (marked with red boxes for the binary characters in Fig. 73: 8–11, 15, 20, 25 and 28; plus some unambiguous changes in multistate characters 3, 14, 21, 24). The topology of the strict consensus of the resulting 5 most parsimonious trees was identical to that described before and with slightly higher node support values (Fig. 73).

The monophyly of Sciaphyini + Anemadini was recovered with high support in all analyses. Unambiguous synapomorphies supporting this relationship

were character states 17(1), 21(1) and 24(1), i.e. dorsal sculpture of the elytra with transverse striae and smooth interspaces (Fig. 33), basal lamina of aedeagus reduced (Figs. 55, 57) and tegmen inserted in the basal third of aedeagus (Figs. 8, 10, 57) (see Table 2). Other character states supporting this node, although with some degree of homoplasy, were 18(1) (shared with *Agathidium*), 16(0) (shared with *Leptinus*), and 21(1) (shared with *Choleva grupoi*) (Fig. 73, Table 2).

4.3. Molecular phylogenetic analysis of Cholevinae

The two runs with MrBayes converged after 5.3×10^6 generations to a standard deviation of the split frequencies of ca. 0.01, with enough effective sample size (as measured in Tracer v1.5, DRUMMOND & RAMBAUT 2007). This value was taken as the “burnin” fraction, and a majority rule consensus tree was built with the remaining 7,400 trees combined from the two runs.

The trees obtained with Bayesian Probability and Maximum Likelihood were largely congruent (Fig. 74), with most nodes having relatively high support. The monophyly of Leiodidae and Cholevinae was strongly supported (Bayesian posterior probability, Bpp=1 and ML bootstrap, MLb=100), but the sister relationship between Leiodidae (including *Eucatops*) and Platypsyllinae, although present with the two methods used, was not supported (Bpp<0.5; MLb<50%, Fig. 74). *Eucatops* was consistently recovered as sister to *Agathidium* and not related to Cholevinae, as previously assumed (e.g. NEWTON 1998), although the incomplete sampling of other Leiodidae subfamilies is inadequate to make any conclusion about the deep Leiodidae phylogeny.

The subfamily Cholevinae was split in two large clades, a strongly supported Leptodirini (including *Platycholeus* as a sister to the rest) and another including all other sampled tribes, the latter with good support with Bayesian methods (Bpp=0.95) but low support with ML (MLb=54, Fig. 74). This non-Leptodirini clade included both *Fusi* and *Sciaphyes*, which were never recovered in direct relationship with each other or with Leptodirini.

All recognised tribes within Cholevinae were recovered as monophyletic, however Cholevini was poorly supported and contained a trichotomy of (1) *Fusi*, (2) *Catops* + *Sciodreporides*, and (3) *Choleva* + *Nargus*. The sister relationship between *Sciaphyes* and Anemadini was recovered with ML only and with low support (Fig. 74).

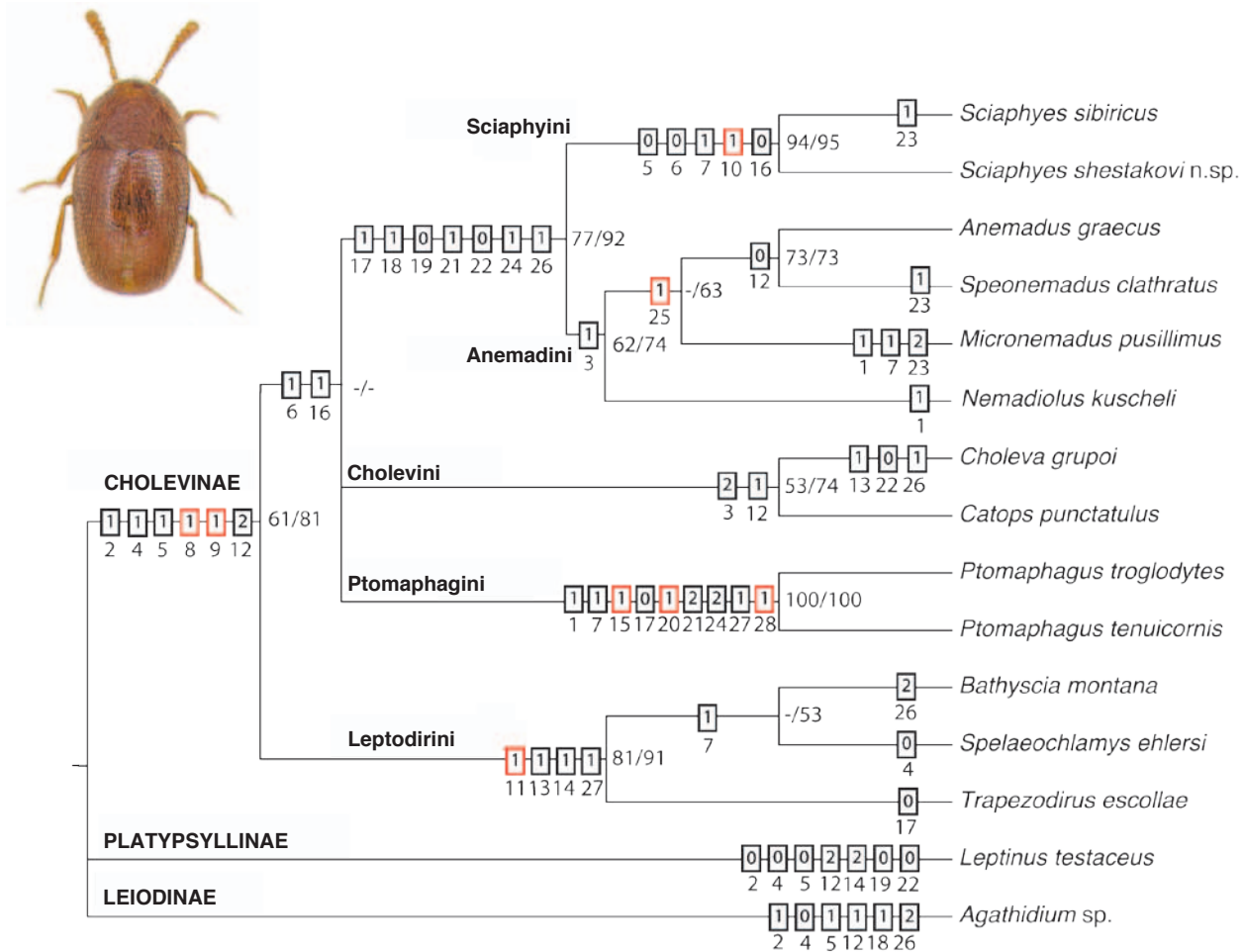


Fig. 73. Summary cladogram obtained with the matrix of morphological data. Numbers at nodes are bootstrap support values: characters unweighted / characters reweighted. Dashes in the same position indicate bootstrap support below 50%. Character state transformations are shown in the branches (unequivocal changes; see Table 2 for the character matrix). In red: unambiguous synapomorphies. Habitus: *Sciaphyes shestakovi* sp.n.

Fig. 74. → Phylogram of the best of 100 replicas obtained with RAxML for the phylogeny of Cholevinae. Numbers at nodes are ML bootstrap support values / Bayesian posterior probabilities. “-”, compatible node (i.e. present with support lower than 50% or 0.5 respectively); “x” incompatible node (i.e. not recovered). Habitus: *Sciaphyes shestakovi* sp.n.

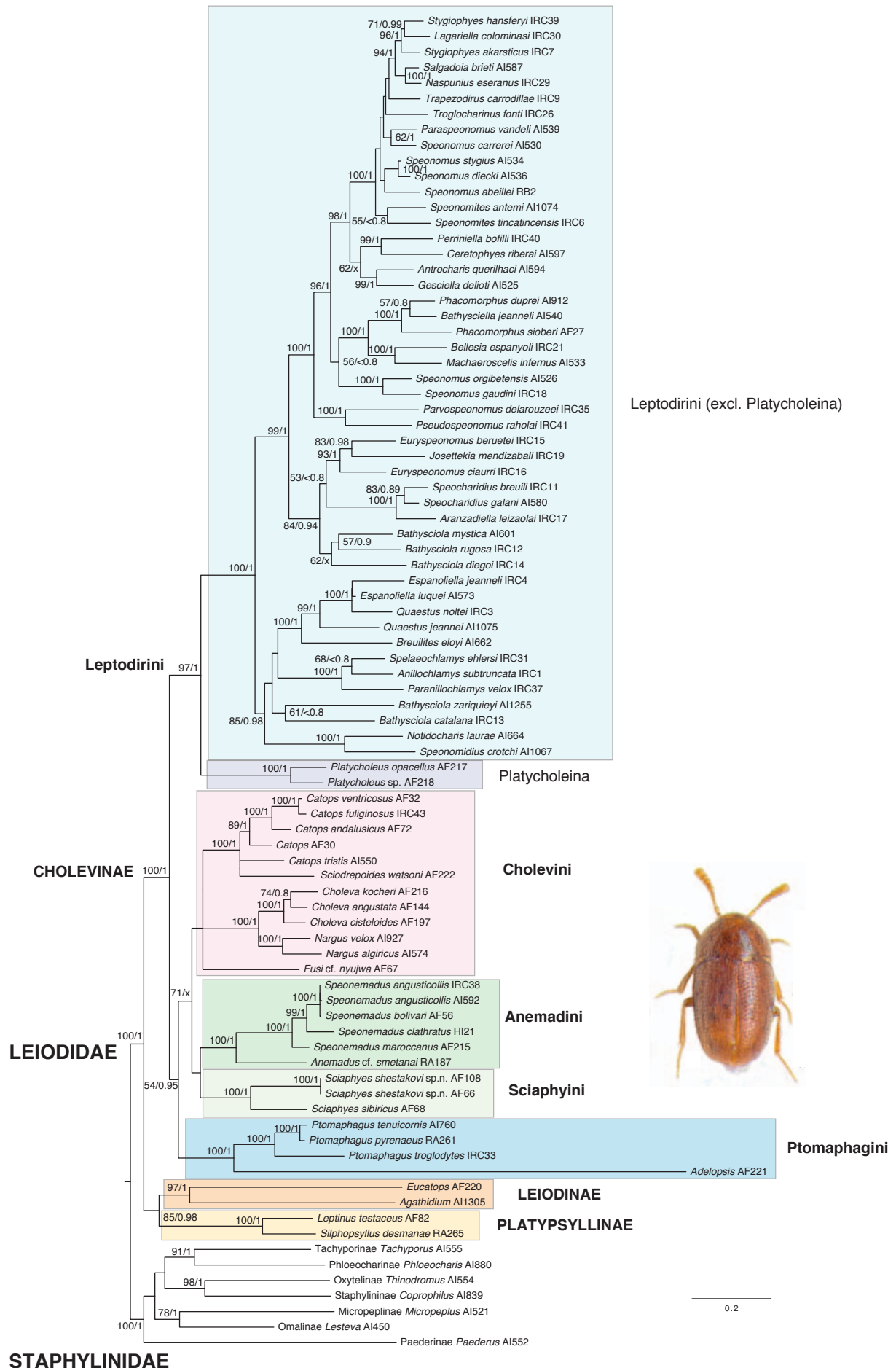
Within Leptodirini, the two species of *Platycholeus* were sister to the rest of the tribe, with strong support (Fig. 74). The relationships within Leptodirini were largely congruent with those obtained previously with the same dataset (RIBERA et al. 2010).

To conclude, among the three non-Mediterranean genera traditionally linked with Leptodirini, *Platycholeus* was found to be sister to the rest of this tribe. *Sciaphyes* seems to occupy an isolated and somewhat uncertain position warranting its own tribe (PERREAU 2000) and not directly related to either Leptodirini or Ptomaphagini, as previously suggested (HOSHINA & PERREAU 2008). Finally, *Fusi* was found to cluster with Cholevini, and not with Leptodirini as it has been previously assumed (PERKOVSKY 1989; PERREAU 2000).

5. Discussion

5.1. *Platycholeus*

The sister relationship of the North American *Platycholeus* (the sole genus of Platycholeina) and the monophyletic rest of the tribe comprising all western Palaearctic Leptodirini is in agreement with the previous hypotheses (e.g. JEANNEL 1924; NEWTON 1998). The opening of the North Atlantic Ocean started in the Late Cretaceous, but North America and Europe maintained some continuous land connections until the Eocene. The Thulean land bridge connected



North America, Greenland and the Iberian peninsula through the British Isles during the early Eocene (55 Mya to 50 Mya; MCKENNA 1983; SANMARTÍN et al. 2001). The origin of the tribe Leptodirini was estimated to be at ca. 44 Mya in RIBERA et al. (2010) based on a calibration using the split of the Corso-Sardinian plate at 33 Mya. This agrees well with a scenario in which stem Leptodirini were distributed during the Palaeocene throughout the united landmass of what is presently Western Europe and North America, with a vicariant separation between the two lineages in Early Eocene. The fate of these two vicariant lineages could not have been more divergent: while the Nearctic branch (Platycholeina) currently includes three species restricted to the forests of western North America, the western Palaearctic branch (the rest of Leptodirini) includes more than 900 species in one of the most diverse animal subterranean radiations.

5.2. *Sciaphyes*

Even though the genus *Sciaphyes* had long been considered to be related to Leptodirini, PERREAU (2000) and HOSHINA & PERREAU (2008) recognised that the absence of eyes and the body depigmentation were convergent consequences of the subterranean way of life and that parallel invasion to the subterranean strata has occurred multiple times within Cholevinae and in other families of Coleoptera (see e.g. DECU & JUBERTHIE 1998). PERREAU (2000) and HOSHINA & PERREAU (2008) pointed also to some characters separating *Sciaphyes* from Leptodirini, such as the five-segmented female protarsi (four-segmented in all Leptodirini); two metaventral sutures (though actually present also in some Leptodirini, see FRESNEDA et al. 2007: character 12, and in Ptomaphagini, see PERREAU 2000: figs. 24, 25); the presence of a median carina on the first visible abdominal ventrite (not known in any other Cholevinae), which continues the median carinae of the thoracic mesoventrite (present also in Leptodirini and Ptomaphagini) and metaventrite (present also in the males of the Leptodirini *Speocharidius breuili* Jeannel, 1919).

According to PERREAU (2000), in male *Sciaphyes* the sternite of the abdominal segment IX (= spiculum gastrale) is completely fused with the lateral parts of segment IX. The males of the three known species, however, have segment IX almost fully developed, with only the tergite base somewhat reduced in size and forming a hood in which the apex of the aedeagus is placed when inside the body in resting position (see the structure in *S. sibiricus*, Figs. 17, 18, *S. shestakovi* sp.n., Figs. 15, 16, and *S. kawaharai*, HOSHINA

& PERREAU 2008: figs. 16–17). In male Anemadini, abdominal segment IX is represented by a triangular structure with a pubescent apex, two pubescent pleurites and a sternite (= spiculum gastrale) reduced to a narrow longitudinal band (Fig. 65). In other Cholevinae (Cholevini, Fig. 66, and Ptomaphagini, Fig. 67), the male genital segment is reduced to varied extents, and the articulation of the spiculum gastrale is differently shaped. In Leptodirini the entire abdominal segment IX is reduced to a simple ring (Fig. 68). Based on the structure of the abdominal segment IX it seems thus difficult to establish phylogenetic relationships among the tribes of Cholevinae.

Sciaphyes shares some characters with other non-Leptodirini Cholevinae. The aedeagus (Figs. 8, 10) clearly resembles that of Anemadina (Figs. 53, 54), with an almost straight median lobe and a well developed, long basal lamina, with the margins recurved almost to the degree of forming a tube. The parameres are longer than the median lobe, well developed and of similar width all along their length; they are inserted in the dorsal part of the aedeagus and have five apical setae in *Anemadus* as well as in some species of *Speonemadus* (Fig. 62) and in *S. shestakovi* sp.n. (Fig. 12). Other species of *Speonemadus* (like *S. maroccanus* (Jeannel, 1936), Fig. 61) have parameres with four setae plus a spine; *Sciaphyes sibiricus* has five setae plus two modified spines (Figs. 13, 14), while *S. kawaharai* has three small setae. The ventral lamina of the tegmen is well developed in *Sciaphyes* (Figs. 8, 10, 11) but reduced to a narrow sclerotised band in Anemadina (Figs. 53, 54). Other subtribes of Anemadini such as Eunemadina and Nemadina do not have the same type of reduced ventral lamina of the tegmen. The *Sciaphyes* aedeagal internal sac with two longitudinal bands is more similar to Eunemadina and Nemadina than to Anemadina (Fig. 54).

Due to the clear non-Leptodirini affinities of some characters of *Sciaphyes*, PERREAU (2000) and HOSHINA & PERREAU (2008) hypothesized that the genus has an “intermediate” position between Leptodirini and Ptomaphagini, with a tribe of its own, Sciaphyini (PERREAU 2000). The three tribes share the presence of the two metaventral sutures, mesoventral carinae and, as a consequence, the non-confluent metacoxae. The general aedeagus structure of Sciaphyini (Figs. 8, 9, 10, 11) is very different from that in Ptomaphagini (Figs. 55, 56), and the same is true for the reticulation of the pronotum (with transverse striae in Ptomaphagini) and the structure of the male genital segment IX (see above).

Our results corroborate the convenience of erecting a tribe Sciaphyini to accommodate the genus *Sciaphyes*, but contrary to the earlier hypotheses (JEANNEL 1924; PERREAU 2000) we found it to be sister to Anemadini and, therefore, not closely relat-

ed to either Leptodirini or Ptomaphagini. This Sciaphyini-Anemadini relationship is not well supported with the molecular data, but strongly supported with the morphological data, also when the 28 characters listed in section 4.1. were recoded to 36 binary characters (data not shown).

5.3. *Fusi*

The original inclusion of *Fusi* among the Leptodirini by PERKOVSKY (1989) was apparently based on the peculiar general appearance of this subterranean beetle and other characters commonly associated with such a way of life. We could not study the male genital characters for this monotypic genus. However, the pentamerous female protarsi, together with the absence of other synapomorphies of Leptodirini (Fig. 73), suggest that a close relationship with Leptodirini is highly unlikely. Our molecular data clearly place *Fusi* in the Cholevini + Anemadini + Sciaphyini + Ptomaphagini clade, and with low support in the tribe Cholevini.

5.4. The geographic limits of Leptodirini

With the exclusion of the eastern Palaearctic *Fusi* and *Sciaphyes*, and the corroboration of *Platycholeus* as sister to the Palaearctic Leptodirini, the phylogenetic and geographic limits of the tribe become coherently defined. Two other non-Mediterranean questionable genera earlier attributed to Leptodirini were shown to be either members of another tribe (*Perkovskius* Lafer, 1989, from the Russian Far East is a member of Catopocerinae; PERREAU & RŮŽIČKA 2007) or were based on a mislabelled specimen (*Neotropospeonella* Pace, 1987, allegedly from Venezuela, is the European *Oryotus ravasinii* Müller, 1922; PERREAU 2003). There are, however, three further poorly known non-Mediterranean genera currently included in Leptodirini, all of them from Palaearctic East Asia: *Proleptodirina*, *Sinobathyscia* and *Coreobathyscia* (see Introduction). Our working hypothesis is that the subtribe Leptodirini (excl. *Platycholeina*) comprises mostly subterranean species geographically restricted to the north Mediterranean basin, with the eastern-most species reaching the Caucasus and north Iran, while all eastern Palaearctic taxa will be eventually shown to be not included in Leptodirini as here defined. Such a hypothesis is consistent with the biogeographic pattern suggested for the blind Raymondionymidae weevils

(GREBENNIKOV 2010) and apparently other predominantly Mediterranean radiations of blind subterranean beetles, with the exception of the Leptotyphlinae rove-beetles. These apparently form a monophyletic and exceptionally ancient clade with extant species widely scattered throughout the main zoogeographical regions (GREBENNIKOV & NEWTON 2009).

5.5. How many Leiodidae lineages lost adult eyes?

Reduction of compound eyes (= microphthalmmy) or even complete lack of eyes (= anophthalmmy) is a phenomenon commonly observed in Coleoptera, particularly in groups inhabiting leaf litter, soil or caves. The beetle family Leiodidae appears to be particularly prone to eye reduction, which is even more striking considering that with ca. 3,500 species this is not a very large family (NEWTON 1998; PERREAU 2000). As presently known, complete loss of adult eyes took place independently not less than eleven times in the following Leiodidae lineages:

- (1) all members of the subfamily Catopocerinae (*Catopocerus* and *Glaciavicola* in leaf litter and caves across most of the formerly un-glaciated Nearctic, *Perkovskius* Lafer, 1989 from the Russian Far East, and an undescribed genus from South America, A. Newton pers. comm.);
- (2) most members of the tribe Leptodirini (excl. *Platycholeus*) (Cholevinae: Leptodirini; see above for distribution and biology), except the genus *Notidocharis* Jeannel, 1956, the monotypic *Adelopsella* Jeannel, 1924 (with about ten pigmented eye facets; PECK 1973; NEWTON 1998), and some species in various genera in northern Turkey, the Caucasus and Iran (ZOIA & RAMPINI 1994); the eyeless *Coreobathyscia solivaga* Szymczakowski, 1975, described from a South Korean cave and known only from the type specimens, remains dubiously associated with Leptodirini (see above);
- (3) at least one species of the genus *Anemadus* Reitter, 1884 (*A. kabaki* Perreau, 2009 from China: Sichuan; Cholevinae: Anemadini), while some of its congeners are microphthalmmic (PERREAU 2009);
- (4) the monotypic *Fusi* from the Russian Far East (see above for more information on this genus);
- (5) all species of *Sciaphyes* (Cholevinae: Sciaphyini; see above for more information on this genus);

- (6) all species of mammal-associated Holarctic Platypyllinae, except *Leptinillus validus* Horn, 1872 with an unafaceted eye remnant (A. Newton pers. comm.);
- (7) both known species of *Zelodes* Leschen, 2000 from New Zealand (Leiodinae: Pseudoliadini) collected from litter or rotten potatoes (LESCHEN 2000);
- (8) all three known species of *Typhlocolenis* Hoshina, 2008 from caves in Japan (Leiodinae: Pseudoliadini; HOSHINA 2008);
- (9) various, and some possibly not closely interrelated, members of Scotocryptini (NEWTON 1998), including those associated with bee nests such as *Parabystus* Portevin, 1907 and *Scotocryptus* Girard, 1874 (PECK 2003); *Cyrtusiola anophthalma* Švec, 2004 from Madagascar, in spite of the suggestive name, has a single eye facet mentioned in the description;
- (10) *Agathidium (Agathidium) anophthalmicum* Angelini & De Marzo, 1984 from Darjeeling in India (Leiodinae: Agathidiini) (ANGELINI & DE MARZO 1984);
- (11) *Ptomaphagus troglodytes* Blas & Vives, 1983 from caves in southern Spain; although apparently none among the numerous cave- and soil-inhabiting *Ptomaphagus* Hellwig, 1795 species is truly anophthalmic in North America (PECK 1973), some cave species with an unafaceted eye spot were thought to be certainly blind with the optic nerves being lost (PECK 1973); this is currently being reinvestigated (S. Peck pers. comm. 2011).

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supported VG's sampling activities. Evgeny E. Perkovsky and Valery A. Korneyev (Schmalhausen Institute of Zoology, Kiev, Ukraine) prepared and sent us images of the *Fusi nyujwa* holotype. J.M. Salgado and Alexandra Cieslak (Institute of Evolutionary Biology, Barcelona, Spain) provided comments and variously supported our work, while Arnaud Faillie (Institute of Evolutionary Biology, Barcelona, Spain) helped with the lab work. Joyce Cook and Stewart B. Peck (Ottawa, Canada) critically reviewed the manuscript prior to submission, while Stewart and his family variously and consistently stimulated VG's beetle work. All collectors mentioned in Appendix 2 spent their time and efforts in catching beetles included in our analyses. The Fauna Ibérica project (MNCNM) permitted reproducing some of the figures. Part of the work resulting in the present paper was funded through projects CGL2006-11403 and CGL2007-61943 to A. Cieslak.

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Appendix 1. List of primers used

Gene	Name	Sense	Sequence	Reference
cox1	Jerry (M202)	F	CAACATTTATTTTGATTTTTGG	SIMON et al. (1994)
	Pat (M70)	R	TCCA(A)TGCACTAATCTGCCATATTA	SIMON et al. (1994)
	Chy	F	T(A/T)GTAGCCCA(T/C)TTTCATTA(T/C)GT	RIBERA et al. (2010)
	Tom	R	AC(A/G)TAATGAAA(A/G)TGGGCTAC(T/A)A	RIBERA et al. (2010)
	Tom-2	R	A(A/G)GGGAATCATTGAATAAA(A/T)CC	RIBERA et al. (2010)
cyb	CB3	F	GAGGAGCAACTGTAATTAATA	BARRACLOUGH et al. (1999)
	CB4	R	AAAAGAAA(A/G)TATCATTGAGGTTGAAT	BARRACLOUGH et al. (1999)
rrnL-nad1	16saR (M14)	F	CGCCTGTTTA(A/T)CAAAAACAT	SIMON et al. (1994)
	16Sa	R	ATGTTTTTGTAAACAGGCG	SIMON et al. (1994)
	16Sb	R	CCGGTCTGAACTCAGATCATGT	SIMON et al. (1994)
	16SAIf1	R	GCATCACAAAAAGGCTGAGG	VOGLER et al. (1993)
	ND1A (M223)	R	GGTCCCTTACGAATTTGAATATATCCT	SIMON et al. (1994)
	16Sbi	F	ACATGATCTGAGTTCAAACCGG	SIMON et al. (1994)
	FawND1	R	TAGAATTAGAAGATCAACCAGC	SIMON et al. (1994)
SSU	5'	F	GACAACCTGGTTGATCCTGCCAGT	SHULL et al. (2001)
	b5.0	R	TAACCGCAACAACCTTAAT	SHULL et al. (2001)
LSU	Ka	F	ACACGGACCAAGGAGTCTAGCATG	RIBERA et al. (2010)
	Kb	R	CGTCCTGCTGTCTTAAGTTAC	RIBERA et al. (2010)

Appendix 2. Material used for morphological study

Checklist of the material used for the morphological study, with collecting data and type of environment in which the species is usually found (according to published information and unpublished records). Abbreviations: MSP, deep subterranean medium; MSS, shallow subterranean medium; AE, endogean, humicolous or muscicolous in forest; F, forest litter. All listed taxa are from Leiodidae. Classification follows LAWRENCE & NEWTON (1995) and PERREAU (2004) down to tribe level.

Cholevinae, Anemadini – Anemadina: *Anemadus graecus* (Kraatz, 1870): Greece, Drama, Oros Falakro-Volax, Gr. S. Theodora, 27.vi.1987, Etonti leg. (AE & MSP); *Speonemadus clathratus* (Perris, 1864): Spain, Jaén, Linarejos, 6.xii.2002, Baena leg. (AE). – **Eunemadina:** *Nemadiolus (Subnemadiolus) kuscheli* Jeannel, 1962: Chile, Cautín Pr. 9 km S, Pucon P. Nat. Volcán Villarrica, 900 m, 15.xii.1984/10.ii.1985, S. & J. Peck leg. (AE). – **Nemadina:** *Micronemadus pusillimus* (Kraatz, 1877): Nepal, Kathmandu valley, Dike Paku Gupha, Balaju cave, 6.vii.1983, Perreau leg. (AE).

Cholevinae, Cholevini – Catopina: *Catops punctatulus* Fresneda, Lencina & Salgado, 2006: Spain, Ciudad Real, Navas de Estena, El Boquerón, 4.xi.2002, Lencina leg. (AE). – **Cholevina:** *Choleva (Cholevopsis) grupoi* Salgado, Blas & Fresneda, 2004: Spain, Cantabria, Rucandio-La Cavada, Cueva del Rión, 26.x.2003, Salgado leg. (AE).

Cholevinae, Leptodirini – Bathysciina: *Bathyscia montana montana* Schiödte, 1848: Slovenia, Javornik (ancient material without other collecting data) (AE & MSP). – **Pholeuina:** *Spelaeochlamys ehlersi ehlersi* Dieck, 1870: Spain, Alicante, Cocentaina, Cova de les Meravelles, 30.iii.2002, Escoll & Fresneda leg. (MSP); *Trapezodirus escollae* (Fresneda & Hernando, 1994): Spain, Huesca, Seira, 4.i.1992/4.iv.1992, Escoll & Fresneda leg. (MSS).

Cholevinae, Ptomaphagini: *Ptomaphagus (Ptomaphagus) tenuicornis tenuicornis* (Rosenhauer, 1856): Spain, Lleida, El Pont de Suert, Selva de Malpàs, 5.iii.1986/3.vii.1986, Fresneda leg. (AE & MSS); *Ptomaphagus (Ptomaphagus) troglodytes* Blas & Vives, 1983: Spain, Granada, Gualchos, Cueva de las Campanas, 27.iii.2002, Tinaut, Baena, Escoll & Fresneda leg. (MSP).

Cholevinae, Sciaphyini: *Sciaphyes sibiricus* (Reitter, 1887): see main text (AE); *Sciaphyes shestakovi* sp.n.: see main text (AE).

Platyptyllinae: *Leptinus testaceus* Müller, 1817: Spain, Navarra, Villanueva de Aezkoa, Cueva de Artekia, 9.vii.2000, Fresneda leg. (F & MSP).

Leiodinae, Agathidiini: *Agathidium* sp.: Spain, Córdoba, Cabra, Via Verde, 5.xi.2006, A. Castro leg.

Additional material used for the figures:

Cholevinae, Anemadini – Anemadina: *Speonemadus angusticollis* (Kraatz, 1870): Spain, Córdoba, Priego de Córdoba, Cueva de los Mármoles, 28.iii.2002, Fresneda, Escoll & Baena leg. (AE & MSP); *Speonemadus maroccanus* (Jeannel,

1936): Spain, Cádiz, Grazalema, 7.v.1983, Baena leg. (AE); *Speonemadus vandalitiae* (Heyden, 1870): Spain, Madrid, Rivas-Vaciamadrid, 22.v.1999, López-Colón leg. (AE & MSP).

Cholevinae, Cholevini – Catopina: *Catops fuliginosus* Erichson, 1837: Spain, Lleida, Sant Miquel de la Vall, Avenc de Sant Gervàs, 28.v.1986, Fresneda leg. (AE & MSP).

Cholevinae, Leptodirini – Pholeuina: *Aranzadiella leizao-lai* Español, 1972: Spain, Guipuzcoa, Mendaro, Cueva del Viento, 16.vii.1998, Fresneda leg. (MSP); *Espanoliella jeanneli* (Bolívar, 1917): Spain, Cantabria, Sámano, Cueva de la Lastrilla, 12.vii.1998, Fresneda leg. (MSP); *Josettekia angelinae* Bellés & Déliot, 1983: Spain, Navarra, All-Larraun, Cueva de Akelar, 25.xii.2002, Bourdeau leg. (MSP); *Pallaresiella pallaresana* (Jeannel, 1911): Spain, Lleida, Sort, Cova Saverneda (= Cova del Drac), 31.x.1992, Fresneda & Escoll leg. (MSP); *Paranillochlamys catalonica* (Jeannel, 1913): Spain, Tarragona, Torre de l'Espanyol, Mina dels Horts n° 2, 29.v.1999, Fadrique leg. (MSP); *Quaestus (Asturianella) incognitus* Salgado & Fresneda, 2004: Spain, Asturias, Caso-Caleao, Cueva de Julió, 31.vii.2002, Salgado leg. (MSP); *Quaestus (Quaesticulus) nadali* Salgado, 1978: Spain, Cantabria, Arredondo, Bustablado, Cueva de la Cañuela, 13.x.1997, Salgado leg. (MSP); *Quaestus (Quaesticulus) noltei* (Coiffait, 1965): Spain, Guipúzcoa, Mendaro, Cueva del Viento, 9.iv.1993, Fresneda leg. (MSP); *Speocharidius (Speocharidius) breuili* Jeannel, 1919: Spain, Guipúzcoa, Albiztur, Cueva de Mendikute, 15.vii.1998, Fresneda leg. (MSP); *Speonomidius crotchi crotchi* (Sharp, 1873): Spain, Navarra, Zegama, Cueva de Orobe, 16.vii.1998, Fresneda leg. (MSP); *Stygiophyes akarsticus* (Escoll, 1980): Spain, Lleida, Forat del Tincatinc, Altrón, 9.vii.1998/14.vii.1999, Fresneda leg. (MSP); *Stygiophyes puncticollis* (Jeannel, 1910): Spain, Lleida, Llimiana, Forat de l'Or, 5.viii.1992/31.x.1992, Fresneda & Escoll leg. (MSP); *Trapezodirus gimenezzi* (Fresneda, Hernando & Lagar, 1998): Spain, Huesca, Egea, Forau de las Grallas del Turbón, 20.viii.1992, Fresneda leg. (MSP); *Troglocharinus (Troglocharinus) ferreri ferreri* (Reitter, 1908): Spain, Barcelona, Begues, Avenc del Marge del Moro, 4.iii.1982, Fresneda leg. (MSP).

Cholevinae, Ptomaphagini – Ptomaphagina: *Ptomaphagus (Ptomaphagus) sericatus medius* (Rey, 1889): Spain, Barcelona, Centelles, 24.ix.1914 (ancient material without other collecting data) (AE & F & MSP); *Ptomaphagus (Ptomaphagus) subvillosus* (Goeze, 1777): Spain, Barcelona, Begues, Avenc de la Parrilla, 7.iii.1965, Auroux leg. (AE & F & MSP).

Electronic Supplement Files

at <http://www.arthropod-systematics.de/> (“Contents”)

File: FresnedaEtAl-LeiodidaeLeptodirini-ASP2011-1: Checklist of the material used in the molecular study, with collecting data and accession numbers (classification follows LAWRENCE & NEWTON 1995 and PERREAU 2004 down to tribe level).