

Taxonomy, larval morphology and cytogenetics of *Lihelophorus*, the Tibetan endemic subgenus of *Helophorus* (Coleoptera: Hydrophiloidea)

Robert B. ANGUS^{1,7)}, Fenglong JIA^{2,7)}, Zhen-ning CHEN^{3,7)}, Ying ZHANG⁴⁾,
Dominik VONDRÁČEK^{5,6)} & Martin FIKÁČEK^{5,6,7)}

¹⁾ Division of Life Sciences (Insects), Natural History Museum, Cromwell Road, London SW7 5BD, UK;
e-mail: r.angus@royalholloway.ac.uk

²⁾ Museum of Biology, Sun Yat-sen University, Guangzhou, China; e-mail: lssjfl@mail.sysu.edu.cn

³⁾ Biology and Geography School, Qinghai Normal University, Wusi West Road 38#, 810000, Xining, Qinghai
Province, China; e-mail: 149470880@qq.com

⁴⁾ Computer Network Information Center, Chinese Academy of Sciences, Beijing, 100190, China;
e-mail: 344280438@qq.com

⁵⁾ Charles University in Prague, Faculty of Science, Department of Zoology, Viničná 7, CZ-128 43 Prague 2,
Czech Republic; e-mail: dominik.vondracek@gmail.com

⁶⁾ Department of Entomology, National Museum, Cirkusová 1740, CZ-193 00 Praha 9 – Horní Počernice,
Czech Republic; e-mail: mfikacek@gmail.com

⁷⁾ corresponding authors

Abstract. The taxonomy, larval morphology and cytogenetics of *Lihelophorus* Zaitzev, 1908, a subgenus of *Helophorus* Fabricius, 1775 endemic to the Tibetan Plateau, are examined based on recently collected material and the inspection of type and historical specimens. Three species are recognized: *Helophorus* (*Lihe-**lophorus*) *lamicola* Zaitzev, 1908 (type species), *H. (L.) ser* Zaitzev, 1908 and *H. (L.) yangae* sp. nov.; all of them are (re)described and their diagnostic characters are discussed and illustrated. First instar larvae of two species, *H. lamicola* and *H. yangae*, both obtained from egg cases laid by field-collected adults and confirmed as conspecific with them by means of *cox1* DNA sequences, are described and compared in detail with the larva of *H. (Helophorus) liguricus* Angus, 1970. Larvae of both species differ from all other *Helophorus* larvae by strongly asymmetrical nasale and extremely long legs, larvae of *H. lamicola* are unique among *Helophorus* in the presence of abdominal tracheal gills. Mitotic karyotypes from mid-gut cells and meiotic karyotypes from testes are described for all three species, revealing the karyotype $2n = 20 + X_{Y_p}$ for all of them. All these data (larval morphology, DNA sequences and karyotypes) are in agreement with the presence of three species of *Lihelophorus* revealed by adult morphology, and also support the monophyly of the

subgenus. Known data on biology and distribution of *Lihelophorus* are summarized, including the highest known altitudinal record for an aquatic beetle (5350 m a.s.l.). The unusual larval morphology of *Lihelophorus* is discussed in detail and hypothesized as adaptation for high altitude cold changeable environment. The systematic position of *Lihelophorus* and the composition of the *Helophorus* fauna of the Tibetan Plateau are briefly discussed.

Key words. Coleoptera, Helophoridae, new species, immature stages, egg case, biology, tracheal gills, high altitude, Tibetan Plateau, China, Palaearctic Region

Introduction

When studying the aquatic beetles collected on the Tibetan Plateau by the Swedish traveller Sven Hedin on his second expedition to Tibet (1899–1902), and by the Russian traveller Pyotr K. Kozlov on his first Mongol-Tibetan expedition (1899–1901), ZAITZEV (1908) found short series of two unusual species of *Helophorus* Fabricius, 1775, *H. lamicola* Zaitzev, 1908 and *H. ser* Zaitzev, 1908 which he placed in a separate newly described subgenus *Lihelophorus* Zaitzev, 1908. Due to a general scarcity of material from the Tibetan Plateau, only a few additional specimens became available within 100 years after his paper was published. Some specimens collected by the Austrian zoologist Erich J. G. Zugmayer during his expedition to Central Asia in 1906 were reported by KNISCH (1910). A few additional specimens from Ladakh and the western part of the Tibetan Plateau were reported by ORCHYMONT (1943), partly collected by the American expedition to northern India in 1932, and partly found in Indian museums. No more specimens have been reported more recently, and part of the specimens examined by the above authors was moreover not found in the collections subsequently. A part of original type series was examined recently by ANGUS (1995), who redescribed both species, designated their lectotypes and illustrated their genitalia. He also noticed that the genitalia of type specimens partly disagree with those illustrated for the Ladakh and West-Tibetan specimens by ORCHYMONT (1943). Nevertheless, a proper revision of *Lihelophorus* was never done, as the very limited number of specimens (fewer than 10) available to study did not allow either the problems mentioned by ANGUS (1995) to be addressed, or the distribution of the species and their biology to be understood.

When studying the morphology of modern *Helophorus* in order to understand the taxonomic placement of the Mesozoic fossils, FIKÁČEK et al. (2012) accidentally found a short series of *Lihelophorus* collected by Czech entomologists in 2005 in the Chinese provinces Gansu and Qinghai. Thanks to that discovery, *Lihelophorus* was included in the phylogenetic analysis and revealed as the earliest diverging clade of modern *Helophorus*. This result was rather unexpected, as *Lihelophorus* was until that moment considered an aberrant relative of the species grouped in the nominotypical subgenus (*Helophorus* s. str.). The position of *Lihelophorus* as a sister group to all remaining modern *Helophorus* provoked questions concerning the biology, larval morphology and karyotypes, and emphasised the need to accumulate fresh

material. In 2013, R. B. Angus and F.-L. Jia organized a joint collecting trip to eastern Qinghai to collect fresh specimens. Despite being very short and facing severe weather including snowfall (see ANGUS 2013), the collecting trip was successful and produced not only freshly collected DNA-grade material of three different *Lihelophorus* species, but also their karyotypes and larvae of two of them. Additional collecting efforts of F.-L. Jia and J. Schmidt, and inspection of Chinese and European museums also resulted in a rather rich material counting in total slightly more than 200 specimens, which allows us to revise *Lihelophorus* in detail.

Because of the large amount of data obtained when examining the material accumulated and when re-analysing the phylogenetic position of *Lihelophorus* using molecular data, we have divided the results into two parts. In this paper, we are summarizing all facts concerning the taxonomy, adult and larval morphology, cytogenetics and distribution of the *Lihelophorus* species. In the second contribution (FIKÁČEK et al., in prep.), we are providing the results concerning the phylogenetic position of *Lihelophorus* and its consequences for the phylogeny of the Helophoridae and for interpretation of the Mesozoic fossil record of the family.

Material and methods

Adult morphology and taxonomy. Some of the examined specimens were dissected and the genitalia were placed in water-soluble dimethyl hydantoin formaldehyde (DMHF) resin. Habitus photographs were taken using Canon D-550 digital camera with Canon MP-E65mm f/2.8 1–5× macrolens; aedeagus photographs were taken using Zeiss Axioskop microscope with Canon EOS 550D digital camera. In all cases, multiple photographs taken were combined with Helicon Focus image stacking software. The external morphology of uncoated (in *H. lamicola* and *H. yangae*) and gold-coated (in *H. ser*) specimens was examined using the Hitachi S-3700N electron microscope at the Department of Palaeontology, National Museum in Prague, using high vacuum for gold-coated specimens, and low vacuum for uncoated ones. Detailed morphology was examined on the completely dissected specimen of *H. ser* mounted as a permanent slide using the Euparal resin. Mouthparts of *H. yangae* were dissected in the molecular voucher MF663 and mounted in a drop of Euparal resin on a slide attached to the mounted specimen; mouthparts of *H. lamicola* were studied in the card-mounted dissected mouthparts of one of the paralectotypes (these were already dissected when the specimen was received). Morphological terminology follows ANGUS (1992) with some updates concerning the morphology of the aedeagus ('basal piece' = phallobase, 'tube' = median lobe) and elytral morphology ('intercalary stria' = scutellary stria, 'elytral interstices' = elytral intervals). Sub-generic classification follows HANSEN (1999) with updates by FIKÁČEK et al. (2012).

Larval morphology and taxonomy. Two egg cases were obtained from adults kept alive in rearing boxes (aquaria), one from the locality of Zuimatan, the other from Yematan. When discovered, the egg case was transferred to a Petri dish with a piece of wet filter paper, to allow the hatching of the larvae. Five larvae hatched from each egg case, differing on the first view by the presence/absence of finger-like projections (tracheal gills) on side of abdominal segments (Figs 9b–c). Since the adults laying the egg cases were not known, we were originally not sure about the association of both egg cases and both larval forms with the adults:

all three *Lihelophorus* species co-occur in Zuimatan and were present in the rearing box from which the egg case was obtained, and specimens of *H. yangae* sp. nov. and *H. (Helophorus) hammondi* Angus, 1970 were present in the rearing box with the egg case from Yematan. We hence fixed a few adults and one larva of each morphotype in 96% alcohol and these were later used for DNA isolation and testing the larval-adult association using *cox1* sequences. The DNA-grade specimens after DNA isolation (and hence cleaned from internal tissues by proteinase K) were subsequently mounted in temporary glycerine slides allowing re-mounting, and used for the main part of the larval descriptions. Remaining larvae of both morphotypes were mounted in dimethyl hydantoin formaldehyde (DMHF) resin which caused their partial deformation; for these reasons, they were only used for double-checking some morphological characters, principally those of abdominal morphology. A side effect of mounting the larvae in DMHF was the retention of air in the tracheal system, which allowed us to study it in detail (see Figs 8g–j). The comparison with *H. (Helophorus) liguricus* was based on the first instar larvae of that species reared from identified adults collected in the Czech Republic (Sedlec, Slanisko u Nesytu National Reserve, 16.iii.2003, M. Fikáček lgt.) and mounted as permanent slide in Hoyer's medium.

Observations were carried out using an Olympus BX41 compound light microscope. Illustrations were made with the aid of a drawing tube attached to the microscope. Photographs were taken using the Canon EOS 550D digital camera attached to the microscope. The morphological terminology of larva generally follows ARCHANGELSKY (1997) and MINOSHIMA & HAYASHI (2011). The only exception is the terminology of maxillary parts, in which we follow the terminology by BEUTEL (1994, 1999): maxillary palpus is considered having 3 palpomeres, the third maxillary 'segment' is called palpifer (and considered as distal divided portion of stipes bearing the galea). For the chaetotaxy of the larval head we refer to FIKÁČEK et al. (2008) and BYTTEBIER & TORRES (2009). The following abbreviations are used: **AN** – antenna; **FR** – frontale; **gAN** – group of antennal sensilla; **gAPP** – group of sensilla on galea (= inner appendage of maxilla); **gFR** – group of sensilla on frontale; **gLA** – group of sensilla on labium; **gMX** – group of sensilla on maxilla; **LA** – labium; **MN** – mandible; **MX** – maxilla; **PA** – parietale; **SE** – sensorium. Additional sensilla are marked by a black square (■) in Figs 13–14.

Cytogenetics. Chromosome preparations were made from mid-gut and testis of adult beetles using the methods outlined by DUTTON & ANGUS (2007). The treatments with colchicine solution and hypotonic KCl were both for 12.5 min, and fixation was for 1 hour in 4 : 1 ethanol-acetic acid solution. Preparations were made and Giemsa-stained in mid-June 2013 in Qinghai Normal University. Photography and C-banding were done in the Natural History Museum, London in the second half of July that year. Ba(OH)₂ treatment was for 2 and 3 minutes (at about 24°C). The 3-minute treatment gave better results. The numbers of karyotypes obtained are too low for statistical treatment of measurements, but two parameters are used as a rough guide: Relative Chromosome Length (RCL) is the length of each chromosome expressed as a percentage of the total haploid autosome length of karyotype while Centromere Index (CI) is the length of the short arm of a chromosome expressed as a percentage of the total length of the chromosome. This gives a measure of the position of the centromere in the chromosome. Centromere positions are frequently given in terms of four chromosome categories, of which

Table 1. List of sequenced specimens with the GenBank accession numbers of the *cox1* sequences.

Code	Species (stage)	Locality data	Accession number
MF658	<i>H. ser</i> (adult)	China: Qinghai, Golo, Huamuxia, Zuimatan (35°19'52"N 99°03'57"E)	KU991588
MF659	<i>H. ser</i> (adult)	same as MF658	KU991589
MF660	<i>H. lamicola</i> (larva with abdominal appendages)	same as MF658	KU991590
MF661	<i>H. lamicola</i> (adult)	same as MF658	KU991591
MF662	<i>H. lamicola</i> (adult)	same as MF658	KU991592
MF663	<i>H. yangae</i> (adult)	China: Qinghai, Golo, Maduo, roadside pool near Yematan (34°40'47"N 99°03'57"E)	KU991593
MF664	<i>H. yangae</i> (adult)	same as MF663	KU991594
MF666	<i>H. yangae</i> (larva without abdominal appendages)	same as MF663	KU991595
MF1247	<i>H. lamicola</i> (adult)	China: Qinghai, road to Tuotuohe (35°21'53"N 93°26'28"E)	KU991587
MF1248	<i>H. ser</i> (adult)	China: Tibet, Pumo Tso to Monda La (28°29'N 90°33'E)	KU991596

two are relevant here – chromosomes with CI from 50–46 are classed as metacentric, and those with a CI from 45–26 are classed as submetacentric.

Successful chromosome preparations were obtained from the following specimens collected by R. B. Angus, F. L. Jia and Y. Zhang during the joint trip to Qinghai in 2013 (see Material examined for detailed locality data): *H. lamicola* – 1 male: Zuimatan; *H. ser* – 1 male, Zuimatan; *H. yangae* – 1 male, Zuimatan; 1 male, Yematan; 1 male, Maduo river flats; 1 female, Gangca.

DNA sequencing. We used DNA sequences to test for the presence of three separate clades (species) in our material as it was expected based on the morphology, and to confirm the identity of reared larvae. DNA was isolated from several adult specimens and one larva of each morphotype. A 721-bp long fragment of the 3' end of cytochrome oxidase I (*cox1*) was amplified for all these specimens, using the *stev_jerryF* (5'-CAACATYTATTYTGATTYTTTGG-3') and *stev_patR* (5'-GCACTAWTCTGCCATATTAGA-3') primers and the PCR conditions as specified by FIKÁČEK et al. (2013). The sequences were submitted to GenBank under the accession numbers listed in Table 1. We combined the new sequences with those of other *Helophorus* available on GenBank, and used 5 representatives of other hydrophiloid families to root the tree. Sequences were aligned using the MUSCLE alignment as implemented in Geneious. Models of sequence evolution for each codon position were compared using PartitionFinder (LANFAR et al. 2012). The phylogenetic tree was constructed under Bayesian inference using MrBayes v. 3.1.2 (HUELSENBECK & RONQUIST 2001) using four chains of 5,000,000 generations and sampling the chain every 1000 generations with the first 25% of trees eliminated as burn-in. Genetic distances of within and among *Lihelophorus* species were estimated using maximum composite likelihood model as implemented in MEGA7.0 software (KUMAR et al. 2016).

Specimen depositories. Examined specimens are deposited in the following collections:

BMNH Natural History Museum, London, UK (M. Barclay, C. Taylor);
IRSNB Institute Royal des Sciences Naturelles, Brussels, Belgium (P. Limbourg);
NHMW Naturhistorisches Museum, Wien, Austria (M. A. Jäch);
NHRS Naturhistoriska Riksmuseet, Stockholm, Sweden (J. Bergsten);
NMPC National Museum, Prague, Czech Republic (M. Fikáček, J. Hájek);
SYSU Entomological collection of Sun Yat-sen University, Guangzhou, China (F.-L. Jia);
ZIN Zoological Institute, Russian Academy of Sciences, St. Petersburg, Russia (M. Volkovitsh).

Taxonomy and adult morphology

Subgenus *Lihelophorus* Zaitzev, 1908

Type species. *Helophorus* (L.) *lamicola* Zaitzev, 1908 (designated by HANSEN 1991: 285).

Diagnosis of adults. Body moderately large to large (4.4–6.1 mm); antenna with 9 antennomeres; maxillary palpus 4 asymmetrical (Figs 4–5); labial palpus ca. as long as cardo (Fig. 1a–b); labial palpomere 3 slightly asymmetrical, with fringe of long setae (Figs 1b, g); gular sutures widely separate (Fig. 1a); clypeus and frons without well-defined granules (Figs 4–5); pronotum with 5 longitudinal grooves (largely reduced and weakly distinct only posteriorly in *H. lamicola*), without granules (Figs 2a,c,e, 4–5); scutellary stria of elytron present (Figs 2b,d,f); elytron without elevated alternate intervals, tubercles or erect setae (Fig. 3); elytral interval 10 flat, i.e. elytral flanks absent (Figs 2j–k); epipleuron with wide inner pubescent portion (Fig. 2j); whole metaventrite and abdominal ventrites with dense pubescence; meso- and metatarsomere 2 longer than metatarsomere 3 (Figs 1h–j); tibiae and tarsi of middle and posterior legs with row of long swimming-hairs (Figs 1h–k); posterior margin of last abdominal ventrite with minute irregular teeth (Fig. 2i).

By body size and general habitus, the presence of distinct scutellary stria and asymmetrical ultimate maxillary palpomere, the *Lihelophorus* species resemble representatives of subgenera *Helophorus* s. str. and *Gephelophorus* Sharp, 1915. They differ from them by (1) presence of series of swimming-hairs on meso- and metatibiae and (2) flat last elytral interval. The well-developed series of swimming-hairs on meso- and metatibiae are only present in the species of the subgenus *Rhopalohelophorus* Kuwert, 1886, which can be however easily distinguished from *Lihelophorus* by the absence of scutellary stria on the elytron.

Distribution. Endemic to the Tibetan Plateau (Fig. 7a)

Analysis of molecular data. The division of the molecular dataset into three partitions was selected as the best partition scheme, with different substitution model selected for each codon position: GTR+G for the first, F81+I for the second and HKY+G for the third codon position. The analysis revealed strongly supported clades for the subgenus *Lihelophorus* as well as for its three species recognized by means of morphology (Fig. 9a). Both larval morphotypes reared from egg cases laid by field-collected adults were reliably assigned to adults from the same locality: the larva with abdominal appendages (MF660) to *H. lamicola* and the larva without the appendages (MF666) to *H. yangae* sp. nov. Intraspecific variation of *cox1* sequences ranged between 0.0–2.9 % (mean 1.9 %) in *H. lamicola*, 0.8–6.3 % (mean 4.4 %) in *H. ser* and 0.0–0.1 % (mean 0.1 %) in *H. yangae* sp. nov. Interspecific distances range from 10.4–13.4 %.

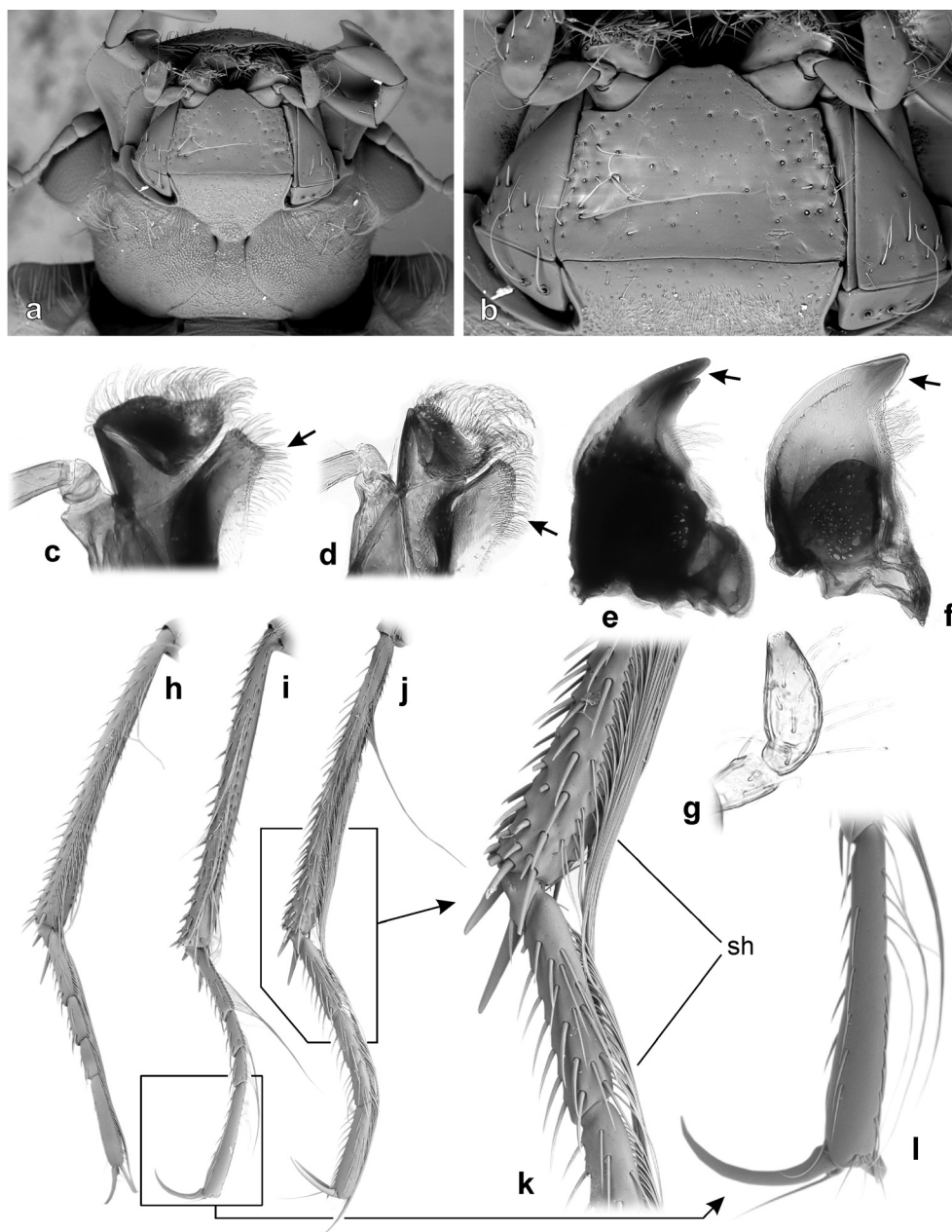


Fig. 1. Adult morphology of *Helophorus* (*Lihelophorus*). a–b – *H. yangae* sp. nov. (a – ventral view of the head; b – detail of mentum and labial palps); c–d – maxillary galea and lacinia (c – *H. yangae* sp. nov.; d – *H. ser* Zaitzev, 1908); e–f – mandible (e – *H. yangae* sp. nov.; f – *H. ser*); g – labial palpus of *H. ser*; h–l – hind legs in dorsal view (h – *H. yangae* sp. nov.; i – *H. ser*; j – *H. lamicola* Zaitzev, 1908; k – detail of apex of tibia and base of tarsus of *H. lamicola*; l – detail of tarsomere 5 and claw of *H. ser*). Abbreviations: sh – swimming hairs. Not to scale.

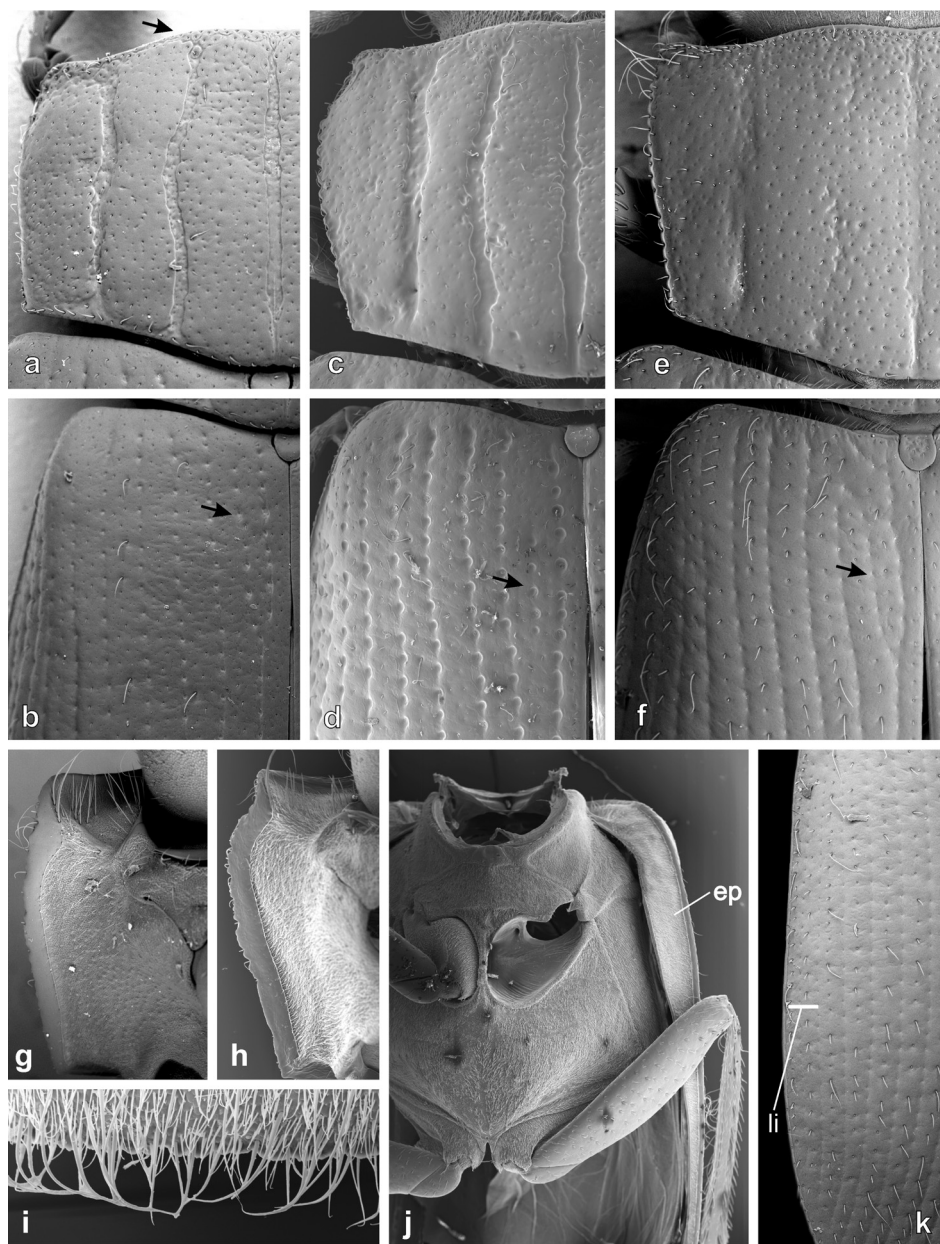


Fig. 2. Adult morphology of *Helophorus* (*Lihelophorus*). a–f – details of left half of pronotum (a,c,e) and base of left elytron (b, d, f): a–b – *H. yangae* sp. nov.; c–d – *H. ser* Zaitzev, 1908; e–f – *H. lamicola* Zaitzev, 1908; arrow in (a) shows remnants of pronotal granulations, arrows in (b, d, f) show scutellary stria. g–h – prothoracic hypomerion and ‘suprapleural area’ (g – *H. yangae* sp. nov.; h – *H. ser*). i – detail of posterior margin of abdominal ventrite 5 of *H. ser*, showing the irregular denticulation. j – ventral view of the thorax of *H. ser*. k – dorsolateral portion of left elytron of *H. lamicola*. Abbreviations: ep – epipleuron; li – last elytral interval. Not to scale.

***Helophorus (Lihelophorus) lamicola* Zaitzev, 1908**

(Figs 1j–k; 2e–f, k; 3a–c; 4a–c; 6f–i)

Helophorus (Lihelophorus) lamicola Zaitzev, 1908: 421.*Helophorus (L.) lamicola*: ANGUS (1995): 189 (redescription, lectotype designation).**Type locality.** China, Qinghai Province, Zhaling Hu (Lake) (= Djarin Nor), 34.911°N 97.273°E.

Type material examined (3 spec.). LECTOTYPE (designated by ANGUS 1995: 189): 1 ♂ (ZIN): 'LECTO- / TYPE [rounded label with violet margin] // Дол. озер верхн. Хуан-хе: [= Dol. ozer verkhn. Khuan-khe] / оз. Джарин-нор. [= oz. Dzharin-nor.] / Кон. [= Kon.] VI. 1900. / Эксп. Козлова. [= Eksp. Kozlova.] // LECTOTYPE March 1970 / *Helophorus lamicola* / Zaitz. / R. B. Angus det. // coll. Zaitzev'. PARALECTOTYPES: 1 damaged specimen with dissected mouthparts, 1 ♀ (ZIN): 'PARA- / LECTO- / TYPE [rounded label with blue margin] // Дол. озер верхн. Хуан-хе: / оз. Джарин-нор. / Кон. VI. 1900. / Эксп. Козлова. // March 1970 / Paralectotype. / *H. lamicola* Zaitzev / R. B. Angus det. // coll. Zaitzev'; 1 ♂ (NHRS): 'Vatten djur [= water animal] / 25 Juni [19]01 / L. XXIII [= camp XXIII] / Hedin // Hel. (*Lihelophorus*) / *lamicola* m. / III. 08 Zaitzev det. / 8248 // NHRS-JKLB / 000021180 // 3817 / E91". Based on ORCHYMONT (1943) who studied Sven Hedin's journal, camp 23 was situated south of Arka-tagh at altitude of 5073 m a.s.l., at approximate GPS coordinates 36°16'N, 88°21'E.

Additional material examined (9 spec.). CHINA: QINGHAI: 1 ♀ (BMNH): same label data as the lectotype, but from Semenov collection and without type labels; 3 ♂♂ 1 ♀ (BMNH): Golo, Huamuxia, Zuimatan, roadside pool, 35°19'52"N, 99°03'57"E, 4141 m, 7.vi.2013, R. B. Angus, F. L. Jia & Y. Zhang lgt.; 1 ♂ (SYSU): same label data; 2 spec. (NMPC): same label data [DNA vouchers MF661 and MF662]; 1 ♀ (NMPC): road to Tuotuohe, 4500 m, 35°21'53"N 93°26'28"E, 22.vii.2015, J. Schmidt lgt. [DNA voucher MF1247].

Differential diagnosis. Differs from other *Lihelophorus* by narrow pronotum (maximum width clearly less than length of mesotarsus + claw) with rather straight sides and almost entirely effaced pronotal grooves (Figs 2e, 3a–c, 4a–c). In contrast to other *Lihelophorus*, elytral series are often not impressed basally, punctures of elytral series are small and interval punctures very small; because of that, the scutellary stria is sometimes rather indistinct. Meso- and metatarsal claws are long (as in *H. ser*, but unlike *H. yangae* sp. nov.). The aedeagus is very similar to that of *H. ser* (based on pointed parameral apices) but the median lobe is wider and the apical portion shorter (Figs 6f–i). For detailed comparison see Table 2.

Redescription. Body length: 5.1–5.5 mm in males, 5.2–6.1 mm in females.

Head. Dull brownish bronze with green reflections, closely and irregularly punctate, the punctures not round, giving a generally rough effect. Y-groove shallow, its stem rather indistinct, wider than the lateral arms, widened anteriorly. Maxillary palpi pale yellow, elongate, apical segment asymmetrical. Antennae yellow, 9-segmented. Maxillary galea in shape of simple pubescent lobe, without strong spines (as in Fig. 1d). Mandible rather short, with simple blunt apex (as in Fig. 1f).

Pronotum. Yellow, a little wider than the head, moderately and evenly arched, widest before the middle, sides weakly curved to base. Anterior angles sharper and more pronounced than is usual in *Helophorus*. Grooves scarcely traceable, mid groove straight, narrow; submedian grooves angled outwards medially, petering out anteriorly but sinuate a quarter of the way from each end; submarginal grooves with their basal pits distinct, traceable forward from these, petering out at middle of pronotum. Narrow raised lateral margin distinct. Intervals without granules but with large punctures and smaller ones between them.

Elytra. Yellow, as pronotum, without apparent darker marks, slightly dull. Striae weak and shallow, scutellary stria present but often rather indistinct due to small size of serial punctures. Interval punctures only slightly smaller than the striae, with fine erect hairs. All intervals flat, including the 10th.

Legs. Long, with long swimming-hairs on meso- and metatibiae and meso- and metatarsi; metatarsomere 5 ca. as long as metatarsomeres 3–4 combined; metatarsal claw ca. $0.65\times$ as long as ultimate metatarsomere.

Aedeagus. Parameres pointed, very weakly to strongly sinuate on outer margin. Phallobase ca. as long as or slightly shorter than parameres. Median lobe wide, its distal portion short (in shape of more or less isosceles triangle); base of median lobe (between the struts) rather straight but with median backwardly directed spur.

Variation. The species varies considerably in size and body proportions, i.e. both smaller narrower (Fig. 3b) and larger wide (Fig. 3c) are known. The shape of the sides of the pronotum varies slightly from weakly arcuate (Fig. 4a) to indistinctly sinuate (Figs 4c). The aedeagus varies considerably in the shape of the apical portions of parameres; they may be narrow with lateral margin indistinctly sinuate (Figs 6f–g) or apically widely expanded with lateral margin strongly sinuate (Figs 6h–i); the proportion of the phallobase to parameres varies slightly, but no specimens with extremely long phallobase were found; the width of the apical portion of the median lobe varies from very wide (Figs 6g–h) to rather narrow (Figs 6f, i). The apparent paler coloration of the aedeagus of the lectotype (Fig. 6f) in comparison to other specimens examined (Figs 6g–i) is very likely caused by the fact that the lectotype is slightly teneral.

Distribution (Fig. 7a). Confined to the northern part of the Tibetan Plateau, from Zuimatan in the east, via Lake Zhaling Hu (type locality) to Sven Hedin's camp 23, situated south of the Arka-tagh mountains. The altitudes of the localities range from about 4140 to 5070 m a.s.l.

Note. The record of *H. lamicola* from Apo Tso by ORCHYMONT (1943) was based on a misidentified specimen of *H. ser* (see under that species for details).

Helophorus (Lihelophorus) ser Zaitzev, 1908

(Figs 1d, f–g, i, l; 2c–d, h–j; 3d–g; 4d–h; 6a–e)

Helophorus (Lihelophorus) ser Zaitzev, 1908: 422.

Helophorus (L.) ser: ANGUS (1995): 190 (redescription, lectotype designation); FIKÁČEK et al. (2012): 437 (phylogenetic analysis).

Type locality. China, Qinghai Province, valley of Alag Hu lake, 35.573°N, 97.120°E.

Type material examined (1 spec.). LECTOTYPE (designated by ANGUS 1995): 1 ♂ (ZIN): 'LECTO-/TYPE [rounded label with violet margin] // Ю. скл. хр. Бурхан-Будда: [= Yu. skl. khr. Burkhan-Budda:] / дол. оз. Алык-Нор. [dol. oz. Alyk-Nor] 30.V.1900. / Эксп. Козлова. [= Eksp. Kozlova.] // Hel. (Lihelophorus) / ser sp. n. / iv.08 Zaitzev det. // LECTOTYPE March 1970 / Helophorus ser Zaitz. / R. B. Angus det. // колл. Семенова-Т. III. [koll. Semenova-T. Sh.]'

Additional material examined (95 spec.). CHINA: XIZANG: 1 ♂ (IRSNB): Apo Zo, Westtibet, Aug. 1906, Zugmayer (identified as *H. lamicola* by Orchymont and Knisch); 1 unsexed spec. (BMNH): 'S. Tibet, vall. SE of Dongo La Pass [= valley SE of Dong La pass], 28°46'N, 87°57'E, 4900–5300 m', 18.vi.2011, J. Schmidt lgt.; 16 ♂♂, 16 ♀♀ (BMNH), 1 ♂ 1 unsexed spec. (NMPC): 'S. Tibet E Nyainqentanglha Feng [Mt.], E slope Lha Tsu Vall [Lha Tsu valley], 5000–5350 m, 30°22'47"N 90°43'22"E', 20.vii.2010, J. Schmidt lgt.; 1 ♀ (NMPC): Pumo Tso to Monda La, 5000–5120 m, 28.vi.2015, 28°29'N 90°33'E, J. Schmidt lgt. [DNA voucher MF1248]. GANSU: 9 spec. (NMPC): Xiahe (= Labrang) env., 35°11.5'N 102°30.6'E, 2940 m, 19–22.vi.2005, J. Hájek, D. Král & J. Růžicka lgt. QINGHAI: 4 spec. (NMPC): Gangca Dasi (lamasery), 37°32.4–33.0'N 100°05.3–06.0'E, 3505–3840 m, 11–12.vi.2005, J. Hájek, D. Král & J. Růžicka lgt.; 1 ♂ 5 spec. (NMPC): Haibu env., 3190–3270 m, 36°48.4–49.8'N 100°45.4–49.7'E, 13–15.vii.2005, J. Hájek, D. Král & J. Růžicka lgt.; 1 ♂ 3 spec. (NMPC): Hairag, 3240 m, 37°10.1'N 100°24.7'E, 3–5.vii.2005, J. Hájek, D. Král & J. Růžicka lgt.; 1 ♂ (BMNH): Golo, Maduo, roadside pools on river flats 20 km E of Maduo, 34°51'17"N, 98°17'18"E, 4290 m, 8.vi.2013, R. B. Angus, F. L. Jia & Y. Zhang lgt.; 2 ♂♂ 6 ♀♀ (BMNH): Golo, Huamuxia, Zuimatan, roadside pool, 35°19'52"N, 99°03'57"E, 4141 m, 7.vi.2013, R. B. Angus, F. L. Jia & Y. Zhang lgt.; 3 ♂♂ 2 ♀♀,

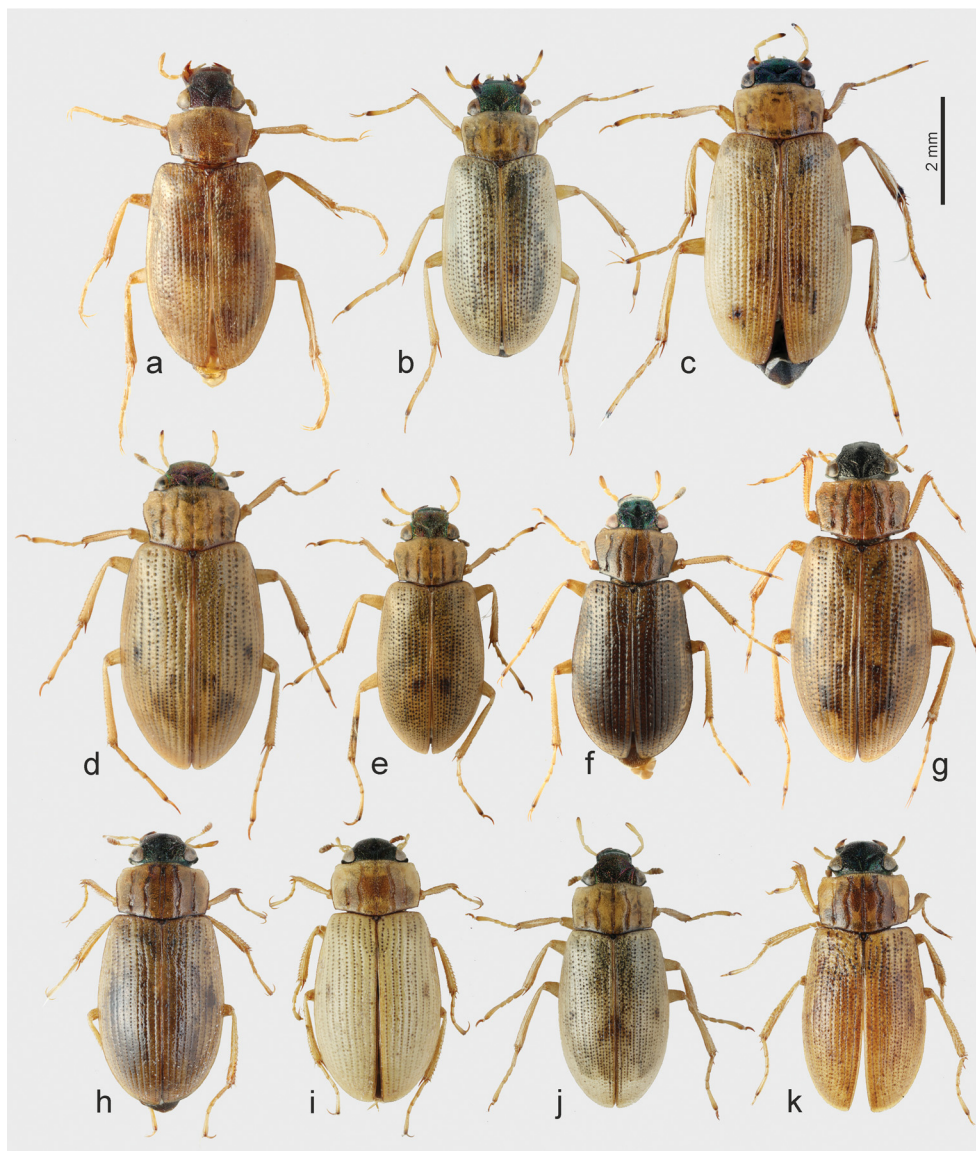


Fig. 3. Habitus of the species of *Helophorus* (*Lihelophorus*). a–c – *H. lamicola* Zaitzev, 1908 (a – Qinghai: Zhaling Hu (lectotype); b–c – Qinghai: Golo). d–g – *H. ser* Zaitzev, 1908 (d–e – Qinghai: Golo; f – Xizang, Nyainqentanglha Feng; g – Gansu: Xiahe env.). h–k – *H. yangae* sp. nov. (h – Xizang: 90 km W of Amdo; i – Xizang: 25 km W of Amdo; j – Qinghai: Golo; k – India: Kar Tso (Orchymont coll.)). All specimens to scale.



Fig. 4. Details of the head and pronotum of the *Helophorus* (*Lihelophorus*) specimens. a–c – *H. lamicola* Zaitzev, 1908 (a – Qinghai: Zhaling Hu (lectotype); b–c – Qinghai: Golo). d–h – *H. ser* Zaitzev, 1908 (d – Qinghai: valley of Alag Hu lake (lectotype); e–f – Qinghai: Golo; g – Xizang, Nyainqentanglha Feng; h – Gansu: Xiahe env.). Not to scale.

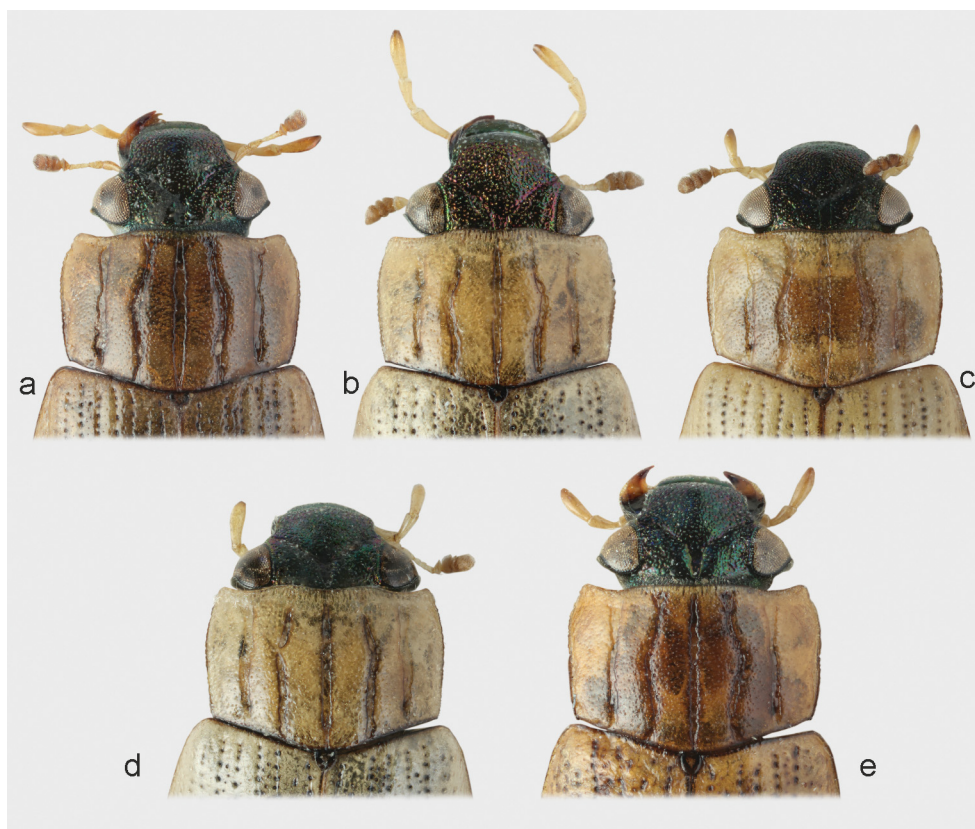


Fig. 5. Details of the head and pronotum of the *Helophorus (Lihelophorus) yangae* sp. nov. (a – Xizang: 90 km W of Amdo; b,d – Qinghai: Golo; c – Xizang: 25 km W of Amdo; e – India: Kar Tso (Orchymont coll.). Not to scale.

6 unsexed spec. (SYSU): same label data; 2 spec. (NMPC): same label data [DNA vouchers MF658 and MF659]; 1 ♂, 2 unsexed spec. (SYSU): Kekexili [= Kekesili], Kangzhagri, 5130 m, 15.viii.1990, Xuezhong Zhang lgt.; 2 ♂♂ 1 ♀ (SYSU): Kekexili, Xinqingfeng [= Syn Qing Feng, or Buka Daban Feng] Mt. hot spring, 4900 m, 2.viii.1990, Xuezhong Zhang lgt.; 1 ♂, 2 unsexed spec. (SYSU): Kekexili, Malanshan, 4950 m, 1.viii.1990, Xuezhong Zhang lgt.; 1 ♂ (SYSU): Kekexili, Kusaihu lake, 4600 m, 10.viii.1990, Xuezhong Zhang lgt.; 1 ♂ 1 ♀ (SYSU): Golo, Maduo, roadside pools near Yematan, 34°40'47"N 99°03'57"E, 4240 m a.s.l., 8.vi.2013, R. B. Angus, F. L. Jia & Y. Zhang lgt. **INDIA: JAMMU AND KASHMIR:** 1 ♀ (IRSNB): Indian Tibet, L73 Chushol, pond below village, 10.viii.1932 (identified as *H. ser* by Orchymont).

Differential diagnosis. Characterized by moderately wide pronotum with distinctly sinuate sides abruptly narrowed just before anterior corners, with five longitudinal grooves distinctly impressed (Figs 2c–d, 3a–g, 4d–h); in this it is more similar to *H. yangae* sp. nov. (which differs by aedeagus with rounded parameral apices) than to *H. lamicola*. Elytral series, including the scutellary stria, are always very distinct (in contrast to *H. lamicola*). Meso- and metatarsal claws are long (as in *H. lamicola*, but unlike *H. yangae* sp. nov.). Metatarsomere 5 is usually longer (often much longer) than metatarsomeres 3–4 combined. The aedeagus is very similar

to *H. lamicola* (based on pointed parameral apices) but the median lobe is narrower and its apical portion longer (Figs 6a–e). For detailed comparison see Table 2.

Redescription. Body length: 4.4–5.7 mm in males, 4.9–6.1 mm in females.

Head. Dull bronze with green to purple reflections to completely black, closely and irregularly punctate, giving a rough effect in some portions, punctation consisting of two sizes of punctures. Y-groove shallow to moderately deep, with rather distinct stem wider than lateral arms, widened anteriorly. Maxillary palpi pale yellow, elongate, apical segment asymmetrical. Antennae yellow, 9-segmented. Maxillary galea in shape of simple pubescent lobe, without strong spines (Fig. 1d). Mandible rather short, with simple blunt apex (Fig. 1f).

Pronotum. Yellow, wider than the head, moderately and evenly arched. Widest closely before anterior corners; sides strongly arcuate, abruptly narrowed anteriorly, straight to indistinctly sinuate posteriorly. Grooves distinct, usually deeply and sharply impressed; mid groove straight, narrow; submedian grooves slightly sinuate; submarginal grooves with their basal pits distinct, bent mesally in anterior fourth. Narrow raised lateral margin distinct. Intervals without granules but with large punctures and smaller ones between them.

Elytra. Yellow as pronotum, with or without apparent darker sutural Λ -mark and sublateral spots. Striae distinct, consisting of rather large punctures, scutellary stria always distinct. Interval punctures much smaller than the striae, with fine erect hairs. All intervals flat, including the 10th.

Legs. Long, with long swimming-hairs on meso- and metatibiae and meso- and metatarsi; metatarsomere 5 distinctly longer than metatarsomeres 3–4 combined; metatarsal claw ca. 0.5× as long as ultimate metatarsomere.

Aedeagus. Parameres pointed, very weakly to strongly sinuate on outer margin. Phallobase ca. as long as or slightly shorter than parameres. Median lobe narrow, its distal portion long (in shape of more or less equilateral triangle); base of median lobe (between the struts) arcuate, with median backwardly directed spur.

Variation. The species varies considerably in size and body proportions; both smaller narrower (Fig. 3e) and larger wide (Figs 3d, g) specimens are known. Coloration also varies slightly, especially as it concerns the colouration of the head (Figs 4d–h) and the absence / presence of the dark patterns on elytra; elytra of the specimens from high altitudes of southern Xizang (collected by J. Schmidt) are often slightly darker than in other examined specimens (Fig. 3f). The pronotum varies slightly in the shape of the sides as well as in the depth and shape of the pronotal grooves (Figs 4d–h), with rare specimens with partly reduced shallow grooves (Fig. 4e) which may resemble *H. lamicola*. The aedeagus varies considerably in the shape of the apical portions of parameres: the variation is the same as in *H. lamicola*, see there for details. In addition to that, we observed the considerable variation in the length of the aedeagus (compare Figs 6a–c and 6d–e) which was not found in *H. lamicola*. Examined specimens with shorter aedeagus were mostly from southern Xizang (specimens collected by J. Schmidt, Figs 6b–c), but the lectotype from central Qinghai also has this kind of the aedeagus; on the other hand, most specimens examined from Qinghai and Gansu have longer aedeagus (Figs 6d–e). The apparent paler coloration of the aedeagus of the lectotype and specimens collected by J. Schmidt (Figs 6a–c) may be an artefact of long-term storage of the specimens in fixation fluid.

Distribution (Fig. 7a). Very widely distributed on the Tibetan Plateau, from Indian province Jammu and Kashmir to southern Gansu in China. It occurs at altitudes ranging from 2940 m (Gansu: Labrang) to ca. 5350 m (Xizang: Nyainqentanglha Feng). As pointed out by Joachim Schmidt (pers. comm. to R. B. Angus) this makes it one of the highest-living of all Coleoptera. **Note.** The record of *H. ser* from Indian Tibet by ORCHYMONT (1943) was based on a misidentified specimen of *H. yangae* (see under that species for details).

***Helophorus (Lihelophorus) yangae* Angus, Fikáček & Jia, sp. nov.**

(Figs 1a–c, e, h; 2a–b, g; 3h–k; 5a–e; 6j–n)

Type locality. China, Qinghai Province. Zuimatan, Roadside pool, 35.333°N 99.007°E.

Type material (143 spec.): HOLOTYPE: ♂ (SYSU): 'CHINA QINGHAI Golo, Huamuxia, Zuimatan, Roadside pool, 35°19'52"N 99°03'57"E, 4141 m, 7.vi.2013, R.B. Angus, F.L. Jia & Y. Zhang lgt.' PARATYPES: **CHINA: QINGHAI:** 2 ♂♂ 2 ♀♀ (BMNH): same data as the holotype [1 ♂: chromosome prep. 3, 12.vi.2013, R.B. Angus; 1 ♂: chromosome prep. 1, 13.vi.2013, R.B. Angus]; 2 ♂♂ 1 ♀ (SYSU): same data as the holotype; 1 ♂ 2 ♀♀ (BMNH): Golo, Maduo, roadside pools near Yematan, 34°40'47"N 99°03'57"E, 4240 m, 8.vi.2013, R. B. Angus, F. L. Jia & Y. Zhang lgt. [♂: chromosome prep. 2, 10.vi.2013, R. B. Angus]; 6 ♂♂ 9 ♀♀ (SYSU): same label data; 2 ♀♀ (NMPC): same label data [DNA vouchers MF662 and MF664]; 3 ♂♂ 2 ♀♀ (SYSU): Golo, Maduo, Heihexiang, roadside pools near Xingxinghai lake 34°40'47"N 99°03'57"E, 4240 m a.s.l., 8.vi.2013, R. B. Angus, F. L. Jia & Y. Zhang lgt. 1 female (BMNH): Golo, Maduo, roadside pools on river flats 20 km E of Maduo. 34°51'17"N, 98°17'18"E, 4290 m, 8.vi.2013, R. B. Angus, F. L. Jia & Y. Zhang lgt.; 1 ♀ (BMNH): N. Qinghai Hu, Gangca, roadside pool. 37°18'N, 100°11'E, 3370 m, 5.vi.2013, F. L. Jia & Y. Zhang lgt. [chromosome prep. 2, 12.vi.2013, R. B. Angus]. **XIZANG:** 3 ♂♂ 4 unsexed spec. (SYSU): suburb of Rikaze, 3826 m, 20–23.vii.1986, Liang Ge-Qiu lgt.; 1 unsexed spec. (SYSU): Jiangzi, 27–28.vii.1986, Liang Ge-Qiu lgt.; 3 ♂♂ 3 ♀♀, 19 unsexed spec. (SYSU): Dangxiong, Yangbajing town, 4320 m a.s.l., 6.viii.2013, 30°5'43.18"N, 90°32'21.33"E, Jia Yue & Jia Fenglong lgt.; 2 ♂♂ 3 ♀♀ 8 unsexed spec. (SYSU): Nanshan, Zhanang County, Sangye Fery, 29°19'23.12"N, 91°30'33.79"E, 3658 m a.s.l., 2.viii.2013, Jia Yue & Jia Fenglong lgt.; 2 ♂♂ 2 ♀♀ 6 unsexed spec. (SYSU): Nanshan, Zhanang County, near Sangye temple, 29°19'25.49"N, 91°30'13.42"E, 3575 m a.s.l., 6.viii.2013, Jia Yue & Jia Fenglong lgt.; 2 ♂♂ 2 ♀♀ 9 unsexed spec. (SYSU): Shannan, Zhanang County, Sangye town, 29°19'23.12"N, 91°30'33.79"E, 3620 m a.s.l. Jia Yue & Jia Fenglong lgt.; 1 ♂ (SYSU): Shannan, Langkazi, 28°58'4.91"N, 90°23'52.72"E, 5030 m a.s.l., 5.viii.2013, Jia Yue & Jia Fenglong lgt.; 2 ♂♂ 2 ♀♀, 7 unsexed spec. (SYSU): near Yangzhuoyongcuo lake, 28°54'57.55"N, 90°43'29.18"E, 4447 m a.s.l., 3.viii.2013, Jia Fenglong & Jia Yue lgt.; 1 ♂ (SYSU): Gongbujiangda, 29°53'7.01"N, 93°14'45.88"E, 4155 m a.s.l., 2.viii.2013, Jia Fenglong & Jia Yue lgt.; 4 ♂♂ 2 ♀♀ 15 unsexed spec. (SYSU): Rikaze, Jiamudui village, 29°9'11.57"N, 89°0'40.67"E, 3872 m a.s.l., 2.viii.2013, Jia Yue lgt.; 1 ♂ 3 ♀♀ (NHMW): 'CHINA: Tibet 9.VIII. / 90 km W Amdo / Cigetang 4400m / leg. X. Guo 1998'; 1 ♀ (NHMW): 'CHINA: Tibet 14.VIII. / 25 km W Amdo, 4400m / Couana Lake – brook / leg. X. Guo 1998'. **INDIA: JAMMU AND KASHMIR:** 1 ♂ (IRSNB): 'male symbol // Indian Tibet / Tzo-Kar [= Kar Tso] Sta- / ktsak-Puk-tso [= Startsapuk Tso] / pool in swamp / 4-IX, 1932 // A. d'Orchymont det. / *H. (Lihelophorus)* / ser Zaitzev'.

Differential diagnosis. Distinguished from the other two *Lihelophorus* species (*H. lamicola* and *H. ser*) by the rather wide evenly arched pronotum (width approximately equal to length of mesotarsus + claw) with rounded sides and narrow marginal grooves, rather short metatarsal claws and by the bluntly rounded apices of the parameres. For detailed comparison see Table 2.

Description. Body length: 4.6–5.1 mm in males, 5.0–6.1 mm in females.

Head. Greenish bronze to maroon bronze, surface with flattened granulation, the granules with large median pits and the grooves between the granules sometimes interrupted resulting in partial coalescence of the granules. Y-groove with the stem at least twice as wide as the arms, widened anteriorly, its floor rugulose. Maxillary palpi yellow, elongate, the apical segment asymmetrical, darkened towards the tip. Antennae with 9 antennomeres, yellow, the clubs a

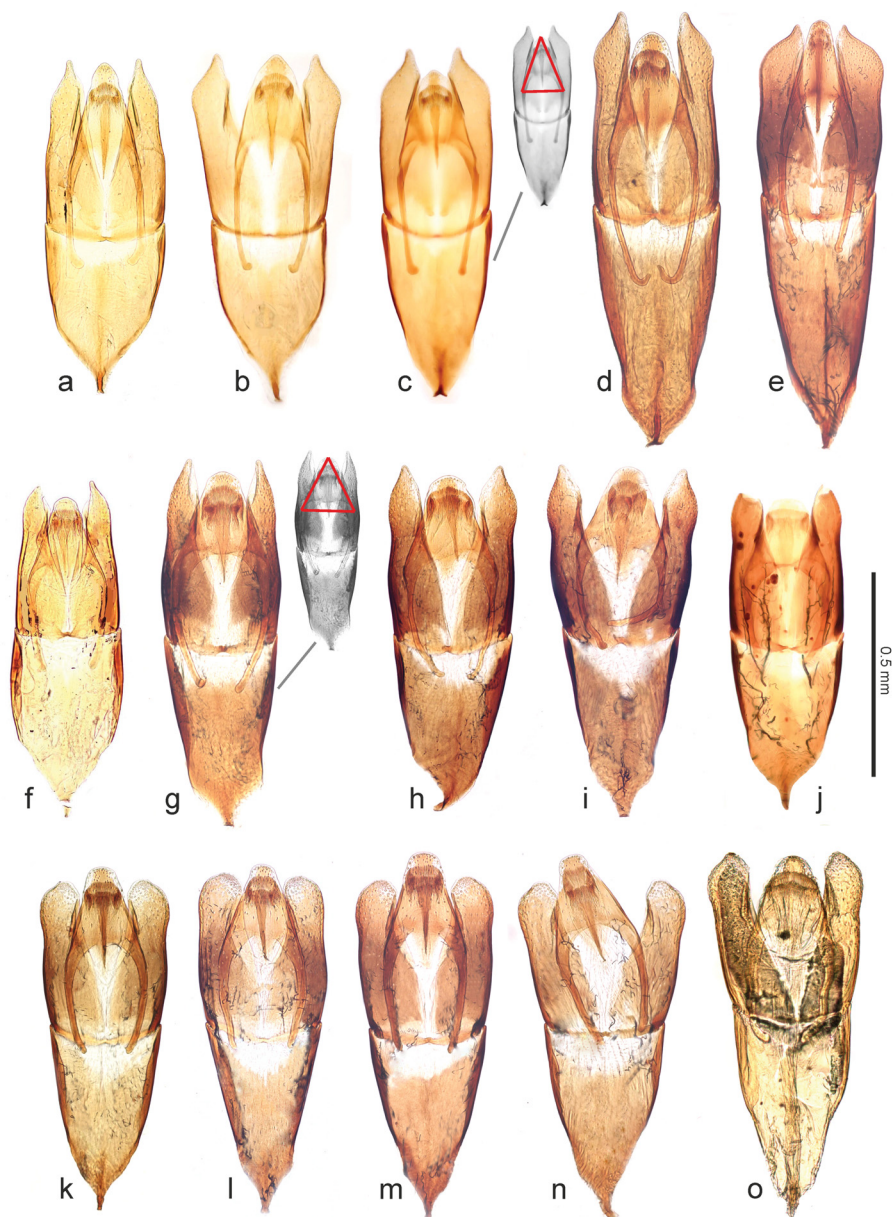


Fig. 6. Aedeagophores of *Helophorus* (*Lihelophorus*) species. a–e – *H. (L.) ser* Zaitzev, 1908 (a – lectotype, Qinghai: valley of Alag Hu lake; b–c – Xizang: valley SE of Dong La pass; d – Qinghai: Golo, 20 km E of Maduo; e – Qinghai: Zuimatan). f–j – *H. (L.) lamicola* Zaitzev, 1908 (f – lectotype, Qinghai: Djarin Nor; g–i – Qinghai: Zuimatan; j – paralectotype, Xizang: S of Arka-tagh, 5073 m a.s.l., lgt. S. Hedin). k–o – *H. (L.) yangae* sp. nov. (k – holotype, Qinghai: Zuimatan; l–m – paratypes, same locality; n – Qinghai: Golo, Yematan; o – Xizang: Gongbujiangda).

Table 2. Comparison of adult morphology of *Lihelophorus* species.

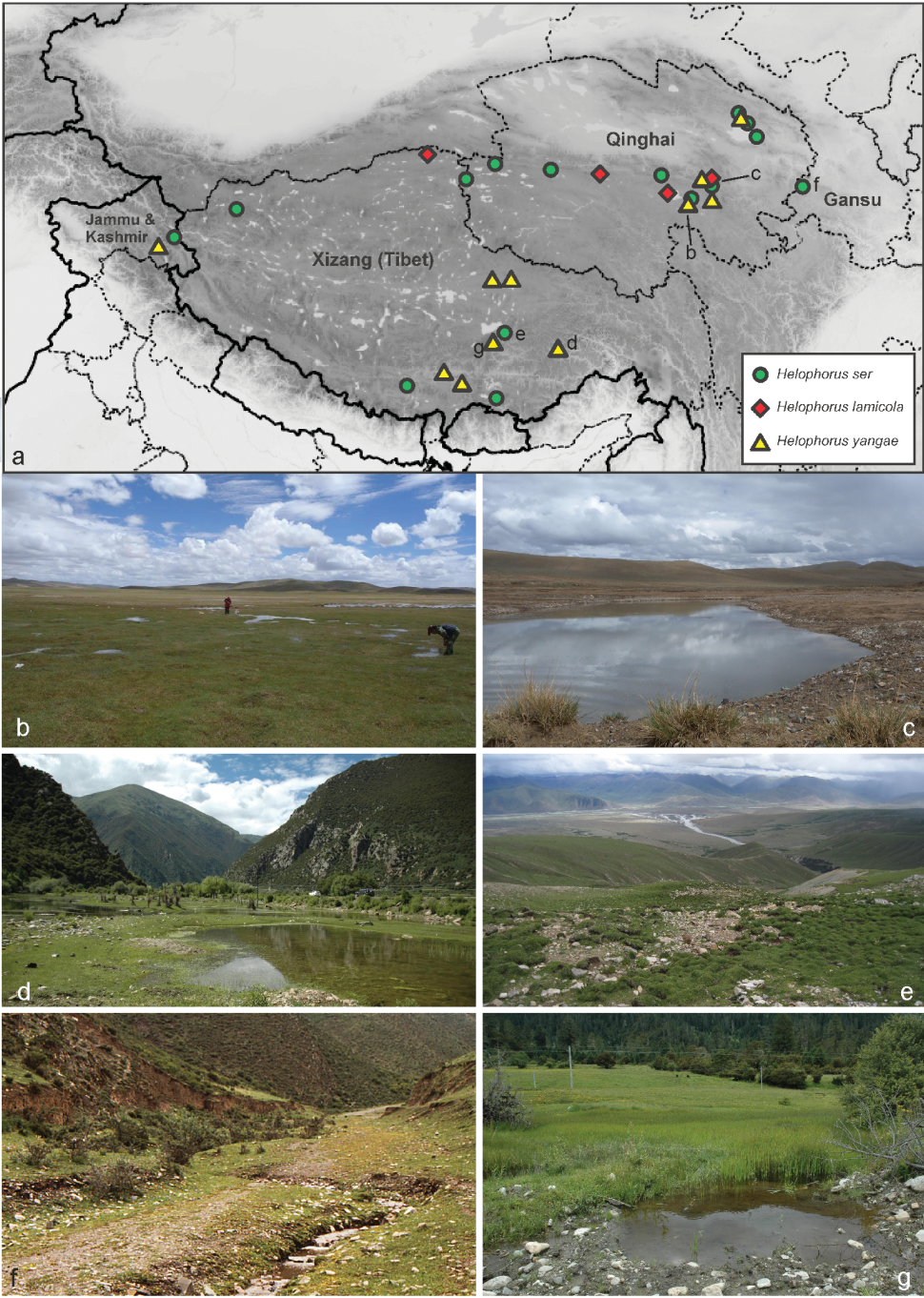
Character	<i>H. lamicola</i>	<i>H. ser</i>	<i>H. yangae</i> sp. nov.
Mandibular apex	simple (as in Fig. 1f)	simple (Fig. 1f)	bifid (Fig. 1e; rare specimens with simple apex exist)
Lacinia	simple, with trichoid setae (as in Fig. 1d)	simple, with trichoid setae (Fig. 1d)	bilobate, with stout setae distally (Fig. 1c)
Pronotum shape	flat	weakly arched	highly and evenly arched
Pronotal grooves	largely reduced (Figs 2e, 4a–c)	well developed (Figs 2c, 4d–h)	well developed (Figs 2a, 5)
Lateral margins of pronotum	very weakly arcuate (Figs 4a–c)	strongly arcuate, strongly narrowed anteriorly (Figs 4d–h)	evenly rounded (Fig. 5)
Basal pronotal margin	narrower than elytral bases combined (Figs 3a–c)	narrower than elytral bases combined (Figs 3e–g)	ca. as wide as elytral bases combined (Figs 3h–k)
Strial punctures of elytra	minute, scarcely impressed (Fig. 2b)	coarse, well impressed (Fig. 2d)	minute, scarcely impressed (Fig. 2f)
Interval punctures	ca. as large as strial punctures	much smaller than strial punctures	ca. large as strial punctures
Punctures of scutellary stria	7–9, often become indistinct	4–7, coarse and always distinct	6–8, always distinct
Legs including claws	conspicuously long	conspicuously long	less obviously long
Metasomere 5	ca. as long as metatarsomeres 3–4 combined (sometimes longer)	much longer than metatarsomeres 3–4 combined (sometimes as long as)	slightly longer or as long as metatarsomeres 3–4 combined
Metatarsomere / tarsal claw	1.5	1.7	1.9
Apex of parameres	pointed	pointed	blunt
Apical portion of median lobe	equilateral triangle	isosceles triangle	isosceles to almost equilateral triangle
Phallobase	short	short to very long	short

little darker. Maxillary galea angulate apically, with several stout setae (Fig. 1c). Mandible wide, apex bifid (Fig. 1e).

Pronotum. Yellow, grooves sometimes slightly darker. Moderately and very evenly arched, widest medially, the sides evenly curved. Grooves narrow and shallow, sometimes weaker over anterior fifth of the pronotum; submedian grooves weakly angled outwards medially, occasionally interrupted in the region of the angle; marginal grooves rather indistinct, not obviously widened medially. Intervals punctate, sometimes very finely so; surface between the punctures either smooth or rugulose.

Elytra. Yellow to dirty yellow with vague darker mottling, the brownish sutural Δ -mark absent or distinct. Striae present as rows of punctures, very weakly impressed below the level of the interstices. All interstices, including the outermost one, completely flat. Scutellary stria with 5–8 punctures. Elytra widest just behind middle, tapering to blunt apex, the sides gently rounded.

Legs. Yellow with apical tarsal segment dark brown apically, and claws brown. Legs rather long, with long swimming-hairs on meso- and metatibiae and meso- and metatarsi;



metatarsomere 5 distinctly ca. as long as metatarsomeres 3–4 combined; metatarsal claw ca. $0.4\times$ as long as ultimate metatarsomere.

Aedeagus. Parameres bluntly rounded apically, weakly sinuate on outer margin. Phallobase ca. as long as parameres. Median lobe wide, its distal portion short (in shape of more less isosceles triangle); base of median lobe (between the struts) straight, with median backwardly directed spur.

Variation. The species varies slightly in size and body proportions, both smaller narrower (Fig. 3j) and larger wider (Fig. 3i) are known. Coloration varies slightly as well, both in coloration of the head (black or with weak greenish to purple sheen) and the elytra (from yellowish to brown, sutural Δ -mark present or absent). Pronotum varies slightly in the extent of the impression of the grooves as well as in their shape, in rare specimens the submedian grooves may be even Y-shaped anteriorly (Fig. 5d). Aedeagus varies slightly in the proportions of the median lobe, but otherwise its morphology is very constant in all specimens examined.

Etymology. This species is named after Dr Xiaomei Yang, wife of Fenglong Jia.

Distribution (Fig. 7a). Widely distributed on the Tibetan Plateau, at altitudes ranging from 3300 m (Gangca) to ca. 5000 m (Langkazi).

Immature stages

Helophorus (Lihelophorus) yangae sp. nov.

(Figs 8c–d, f, i–j; 9b; 10; 12c–d; 13b, f, g–h; 14e–f)

Larval material examined (5 larvae). 1 first instar larva (NMPC, DNA voucher MF666): **CHINA: QINGHAI:** Golo, Maduo, roadside pools near Yematan, $34^{\circ}40'47''$ N $99^{\circ}03'57''$ E, 4240 m a.s.l., 8.vi.2013, R. B. Angus, F. L. Jia & Y. Zhang lgt.; 4 first instar larvae (BMNH): same label data. All larvae were reared from the single egg case (deposited in BMNH) laid by the field-collected female, association with adults from the same locality was confirmed by the *cox1* sequence data.

Egg case (Fig. 8f). Consisting of an egg-bag surmounted by a thin trailing mast which takes the form of a long narrow tube, thus corresponding to Type 4 egg case of ANGUS (1992); it is similar to that of *H. lamicola*, but with a shorter mast which may represent individual variation.

Larva. General morphology. Body (Fig. 9b) elongate, slender, almost parallel-sided. Body length (without urogomphi) ca. 3.0 mm. Head (Figs 8c–d) subquadrate in shape, ca. $1.15\times$ wider than long, prognathous, with occipital foramen directing posteriorly.

Head width 0.43–0.47 mm ($n = 5$). Parietale with numerous cuticular spines dorsally, laterally and ventrally. Frontal lines V-shaped, arising posteriorly of antennal socket and reaching posterior margin of head capsule, nearly straight; coronal line absent. Antennal sockets situated on the laterodorsal portion of head. Frontoclypeus (Fig. 12c) with small asymmetrical

Fig. 7. Distribution and habitats of *Lihelophorus* species. a – known distribution of *Lihelophorus* species based on examined specimens. Examples of habitats (see the letters in Fig. 7a for geographic position of each locality): b – Qinghai: Golo, Maduo, roadside pools on river flats, 4290 m (habitat of *H. ser* and *H. yangae* sp. nov.); c – Qinghai: Golo, Huamuxia, Zuimatan, roadside pool, 4141 m (habitat of *H. lamicola*, *H. ser* and *H. yangae* sp. nov.); d – Xizang: Gongbujiangda, 4155 m (habitat of *H. yangae* sp. nov.); e – Xizang: E Nyainqentanglha Feng, eastern slope of Lha Tsu valley, 5000–5350 m (habitat of *H. ser*); f – Gansu: Xiahe env., 2940 m (habitat of *H. ser*); g – Xizang: Dangxiong, Yangbajing town, 4320 m (habitat of *H. yangae* sp. nov.).

nasale bearing single median tooth, and large symmetrical epistomal lobes overlapping nasale, well-sclerotized including laterally. Six small stemmata situated on anterolateral portion of parietale, widely separated from each other. Gular suture absent, posterior tentorial pits distinct, situated anteromesally. Cervical sclerites absent. Antenna (Fig. 13f) 3-segmented, long and rather stout. Scape ca. as long as pedicel, pedicel slightly widened distally, flagellum slightly shorter than scape.

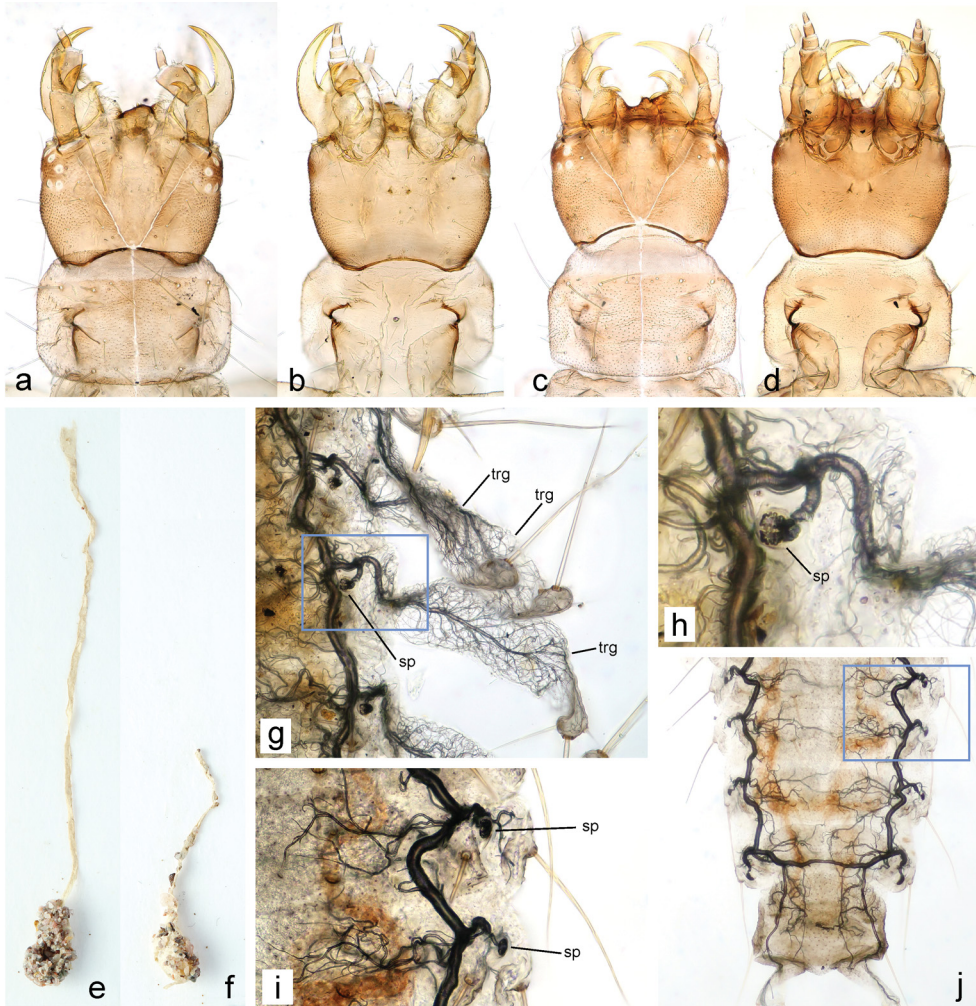


Fig. 8. Immature stages of *Lihelophorus*. a–d – head and prothorax of first instar larvae: a–b – *H. (Lihelophorus) lamicola* Zaitzev, 1908 (a – dorsal; b – ventral); c–d – *H. (Lihelophorus) yangae* sp. nov. (c – dorsal; d – ventral). e–f – egg cases: e – *H. (Lihelophorus) lamicola* Zaitzev, 1908; f – *H. (Lihelophorus) yangae* sp. nov.; g–j – abdomen with spiracles containing air bubbles: g–h – *H. (Lihelophorus) lamicola* Zaitzev, 1908 (g – tracheal gills and its tracheal system; h – detail of tracheal branching around abdominal spiracle); i–j – *H. (Lihelophorus) yangae* sp. nov. (i – detail of tracheal system around abdominal spiracles; j – tracheal system of abdomen). Abbreviations: sp – spiracle; trg – tracheal gill.

Mandibles (Fig. 13b) symmetrical, with falcate apical portion. Retinaculum with two teeth; distal tooth large, bearing dense tuft of cuticular spines; basal tooth small, lacking cuticular projections. Basal inner face not projecting, with a basal field of fine spine-like cuticular projections.

Maxilla (Figs 14e–f) 6-segmented (including cardo), slightly longer than antenna. Cardo rather small, situated laterally, and associated with two additional sclerites lying between cardo and labium. Stipes ca. as long as palpifer, subcylindrical; palpifer completely sclerotized, mesally with a long well-sclerotized finger-like galea which has an additional ring-like sclerite basally. Maxillary palpus with 3 segments, ca. as long as palpifer; palpomere 1 ca. half as long as palpomere 2 and 3 each.

Labium (Figs 13g–h). Submentum large, trapezoid, fused with parietale, submental suture indistinct. Mentum short, ca. as long as wide, with cuticular spines on dorsal surface. Prementum V-shaped, ligula absent. Labial palpus 2-segmented, with basal palpomere ca. 2× shorter than distal palpomere.

Thorax. Prothorax ca. as wide as head capsule. Proscutum, meso- and metanotum (Fig. 15f) each formed by a large plate subdivided mesally by a sagittal line. Ventral portion of prothorax (Fig. 8d) with a pair of small widely isolated presternal sclerites, each articulating posterolaterally with a precoxal sclerite which connects presternum with coxal articulation; remaining parts largely membranous. Mesothorax with two pleural sclerites: the anterior one bulge-like bearing spiracle, the posterior rounded and flat. Legs long, clearly visible in dorsal view, all pairs similar in shape. Leg 5-segmented (Figs 15b–c); all pairs of coxae well separated from each other, procoxae slightly closer to each other than meso- and metacoxae; trochanter ca. half as long as femur, rather firmly joined with femur; tibiotarsus

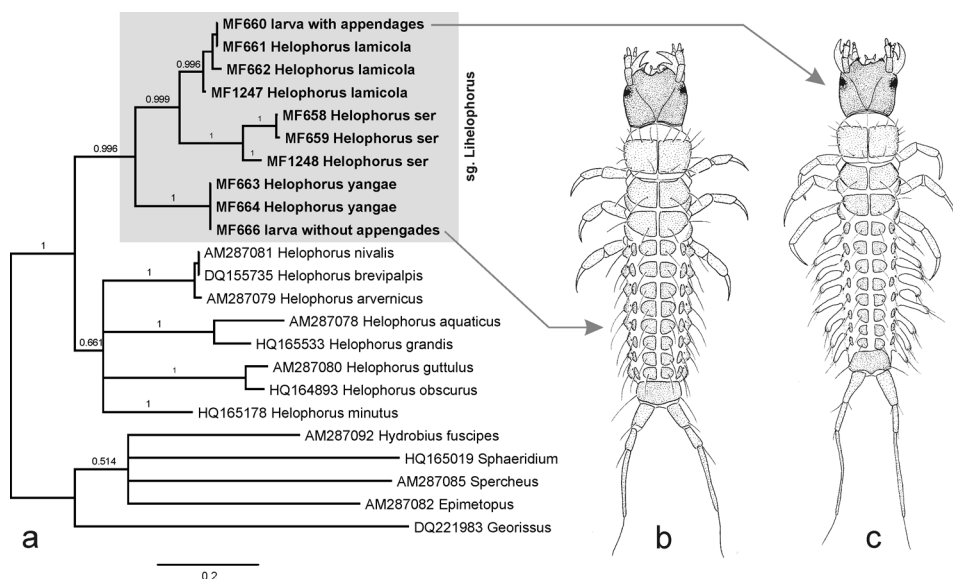


Fig. 9. Association of reared larvae and field-collected adults of *Helophorus* (*Lihelophorus*) based on *coxI* sequences. a – Bayesian inference of newly obtained and available GenBank sequences; b–c – habitus of reared larvae (b – *H. yangae* sp. nov.; c – *H. lamicola* Zaitzev, 1908).

cylindrical, ca. as long as trochanter and femur combined. Claw ca. as long as tibiotarsus, slightly bent ventrad.

Abdomen (Figs 9b, 15e–f, g) with 9 well developed segments, not subdivided into folds. Segments 1–8 each with a pair of large central ('dorsal sclerites' sensu ANGUS 1992) and smaller lateral sclerites ('dorsolateral sclerites' sensu ANGUS 1992); dorsally, one small sclerite below spiracle ('lateral sclerite' sensu ANGUS 1992), and transverse series of three small sclerites ventrally; spiracle is situated in membranose area. Segment 9 with large entire sclerite dorsally; bearing a pair of long three-segmented urogomphi (Fig. 15j). Spiracular atrium not developed.

Primary chaetotaxy. Head. Frontale with 44 sensilla (Figs 10b, 12c). Central part with three pairs of sensilla diverging posteriad, small seta FR1 situated at midlength near frontal line, pore FR2 and small seta FR3 close to each other and situated more anteromesally. Lateral portions posterior to antennal socket each with two setae and one pore: short seta FR5 situated close to frontal line, long seta FR6 anteromesally of the latter, and pore FR4 even more mesally. Inner part of antennal socket with a small seta FR7, central portion of frontale between antennae with two pairs of small setae (FR8–9) converging anteriad. Nasale with a pair of short stout setae mesally at sides of median tooth and a sensillum situated on based on epistomal lobe on each side (gFR1), pores FR15 absent. Each epistomal lobe with a group of eight setae (gFR2) situated on anterior margin, all setae lanceolate, with pilose inner margin; long seta FR12, shorter seta FR10 and a pore FR14 situated on epistomal lobe anteromesally of antennal socket, pores FR11 and FR13 seta FR9 and anterior margin of head. Parietale with 29 sensilla each (Fig. 10). Dorsal face with posterior portion bearing a row of four short setae and one pore (PA1–5) sublaterally, and a pore (PA6) and a short seta (PA7) mesally. Dorsomesal portion with one moderately long seta (PA12) and one very long seta (PA8), ocular area with one pore (PA10) and one seta (PA11) dorsally, and one short seta (PA9) between stemmata of anterior row, and one seta (PA16) ventrally. Lateral portion with a pore (PA30) posteriorly, three long setae (PA13–14 and PA18) situated in posterior half and two pores (PA15 and PA17) situated more anteriorly. Anterior margin laterally with a pore (PA19) dorsally, long seta (PA20) and short seta (PA21) laterally, and three pores (PA23–25) ventrally; sensilla PA22 either absent, or present as a pore anteriorly of PA21. Ventral portion with four sensilla: short seta PA26 anteriorly, pore PA27 and long seta PA28 at midlength, and pore PA29 posteriorly.

Antenna (Fig. 13f). Scape with two pores (AN1–2) situated dorsally and three pores (AN3–5) situated along distal margin on lateral, mesal and ventral faces; pedicel with one pore (AN6) dorsally in distal half of sclerotized area, two short setae (AN10–11) situated mesally and three short setae (AN7–9) situated laterally below antennal sensorium (SE1), AN7 large and stout, similar to SE1 in shape. Sclerotized portion of flagellum lacking sensilla, apical group of sensilla (gAN) bearing several moderately long and short setae.

Mandible with 9 sensilla (Fig. 13b). A short long seta (MN1) situated in basal half of outer face, two pores (MN2–3) on dorsal surface ca. at midlength, a pore (MN4), a short seta (MN5) and a small pore (MN6) subapically on inner face.

Maxilla (Figs 14e–f) with one short seta (MX1) situated ventrally on cardo. Stipes with five moderately long setae on inner surface: MX7 situated subbasally, MX8–11 in distal third. Ventral surface with two pores (MX3 and MX4) and one very long (MX5) and one short seta

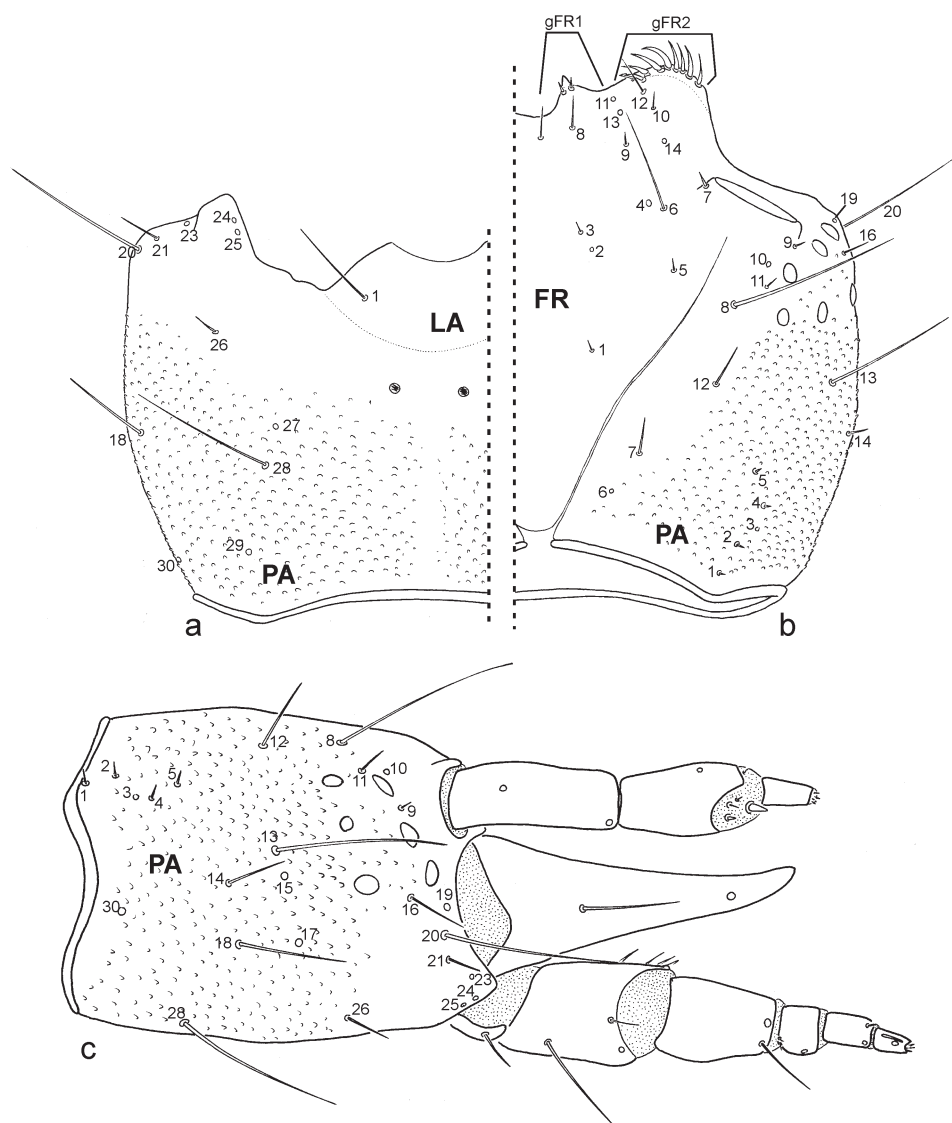


Fig. 10. Head capsule of the first instar larva of *Helophorus (Lihelophorus) yangae* sp. nov. a – ventral view; b – dorsal view; c – lateral view.

(MX6). Palpifer with a long seta (MX16) mesally close to base, two short setae (MX13–14) and one pore (MX12) situated along distal margin; situated distally on lateral surface, MX14 ventrally of MX16; distal margin ventrally with a seta (MX13) and a pore (MX12). Base of inner appendage with one pore ventrally (MX15) and one dorsally (MX17). Inner appendage (gAPP) with one moderately long seta and several very short setae apically. Palpomere 2 with

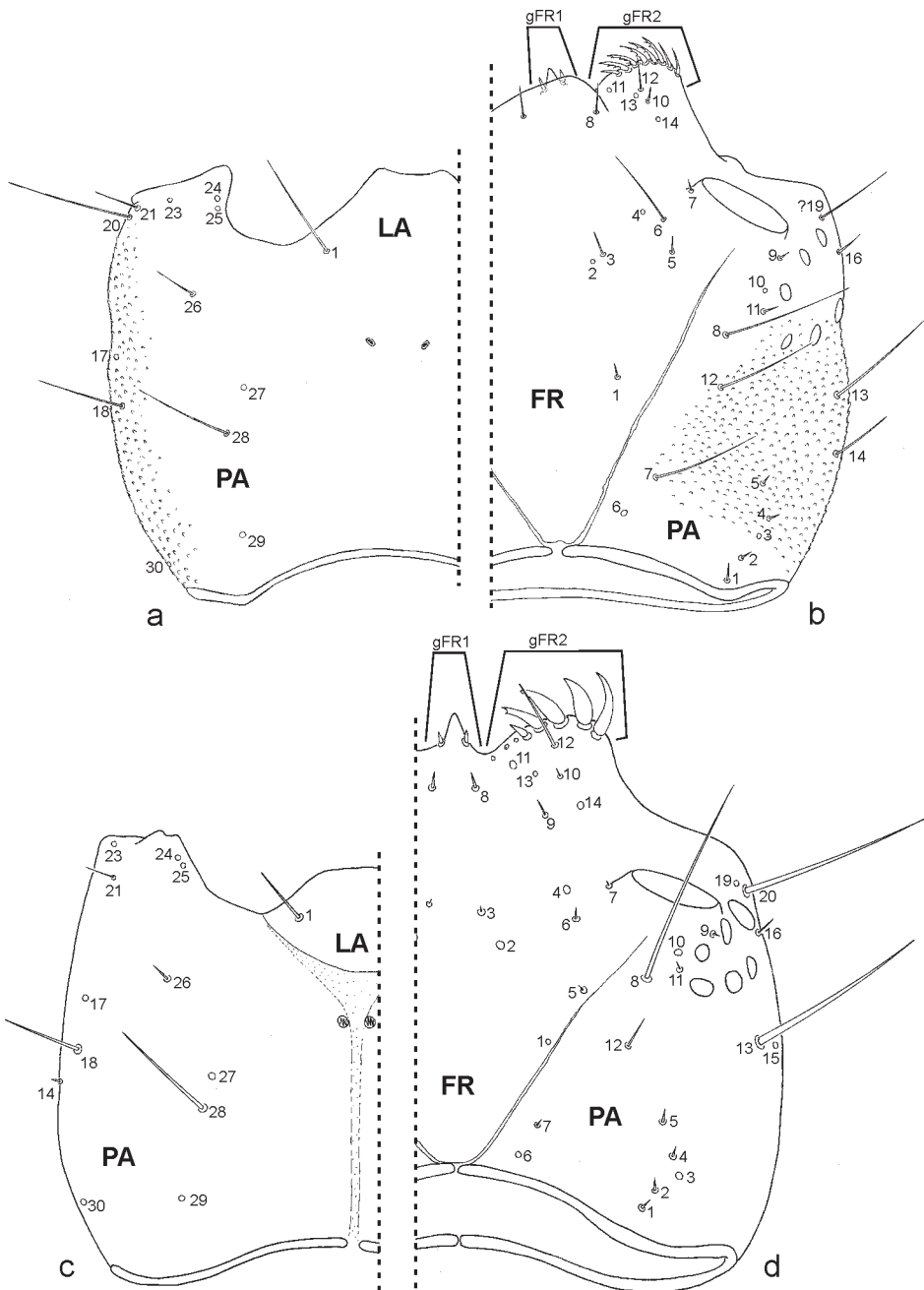


Fig. 11. Head capsules of first instar larvae of *Lihelophorus* and *Helophorus* s.str. a-b – *H. (Lihelophorus) lamicola* Zaitzev, 1908 (a – ventral; b – dorsal). c-d – *H. (H.) liguricus* Angus, 1970 (c – ventral; d – dorsal).

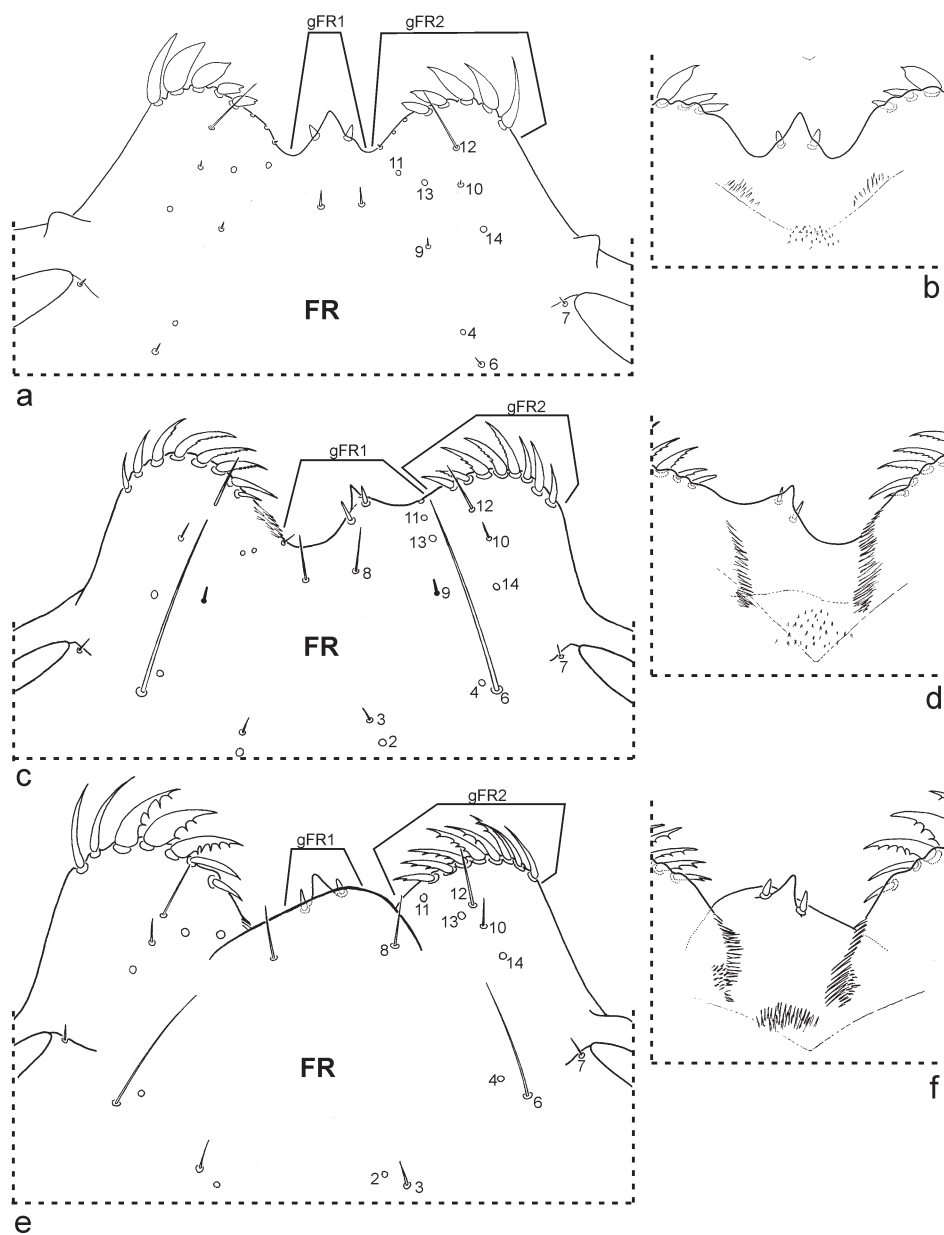


Fig. 12. Labroclypeus and epistome of larvae of *Lihelophorus* and *Helophorus* s.str. a–b – *H. (H.) liguricus* Angus, 1970 (a – dorsal view; b – ventral view); c–d – *H. (Lihelophorus) yangae* sp. nov. (c – dorsal; d – ventral); e–f – *H. (Lihelophorus) lamicola* Zaitzev, 1908 (e – dorsal; f – ventral).

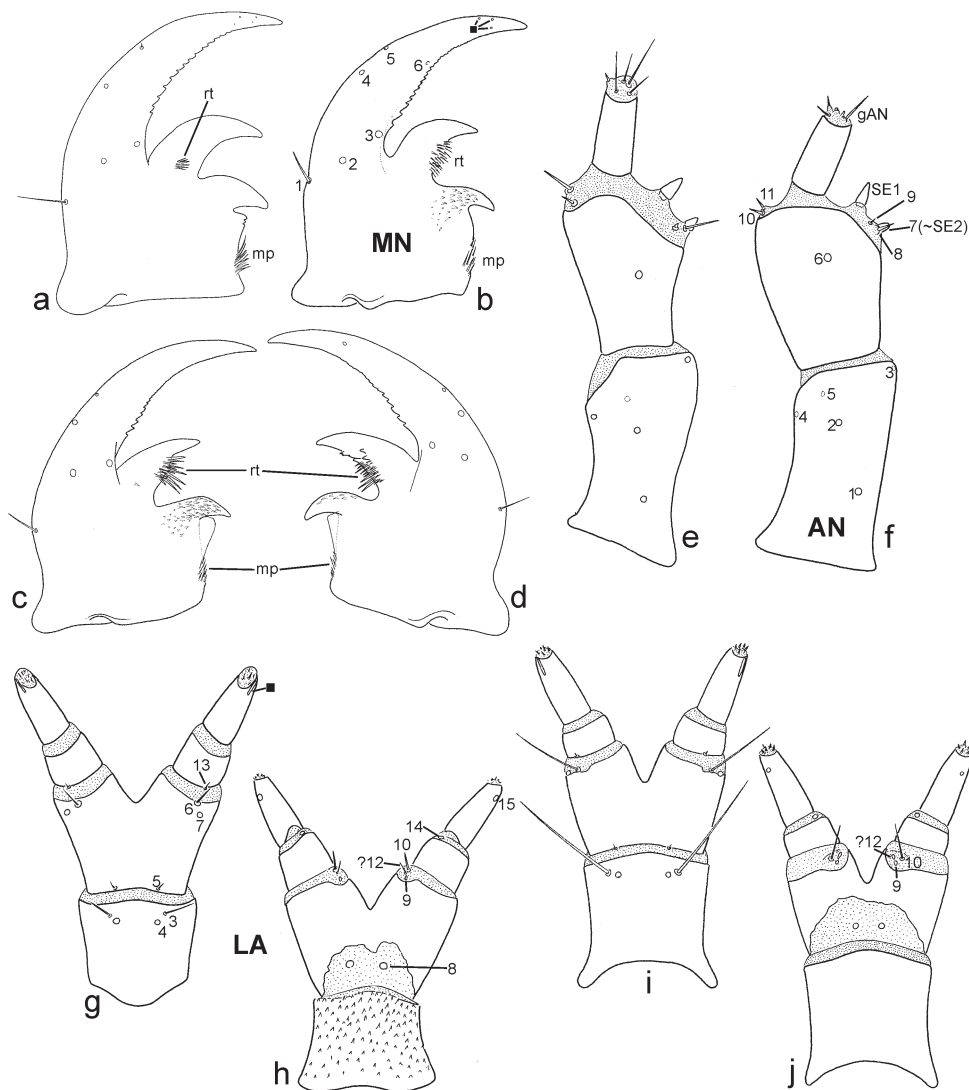


Fig. 13. Head appendages of larvae of *Lihelophorus* and *Helophorus* s.str. a–d – mandibles in dorsal view: a – *H. (H.) liguricus* Angus, 1970 (left mandible); b – *H. (Lihelophorus) yangae* sp. nov. (left mandible); c–d – *H. (Lihelophorus) lamicola* Zaitzev, 1908 (c – left mandible; d – right mandible). e–f – left antenna in dorsal view: e – *H. (H.) liguricus* Angus, 1970; f – *H. (Lihelophorus) yangae* sp. nov. g–j – labium: g–h – *H. (Lihelophorus) yangae* sp. nov. (g – ventral; h – dorsal); i–j – *H. (Lihelophorus) lamicola* Zaitzev, 1908 (i – ventral; j – dorsal). Abbreviations: mp – mandibular penicillus; rt – retinacular tuft.

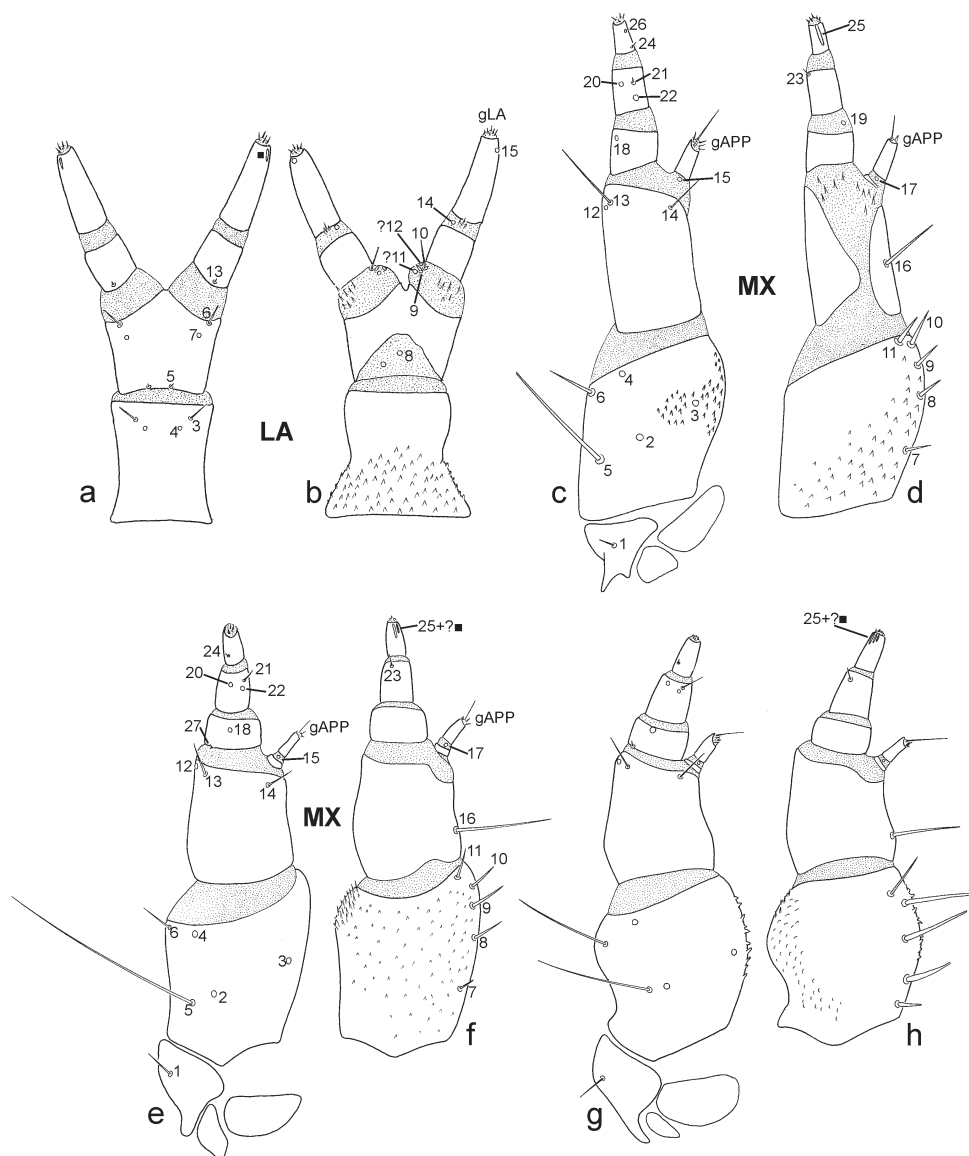


Fig. 14. Head appendages of larvae of *Lihelophorus* and *Helophorus* s.str. a–b – labium of *H. (H.) liguricus* Angus, 1970 (a – ventral; b – dorsal). c–h – maxilla: a–d – *H. (H.) liguricus* (c – ventral; d – dorsal); e–f – *H. (Lihelophorus) yangae* sp. nov. (e – ventral; f – dorsal); g–h – *H. (Lihelophorus) lamicola* Zaitzev, 1908 (g – ventral; h – dorsal).

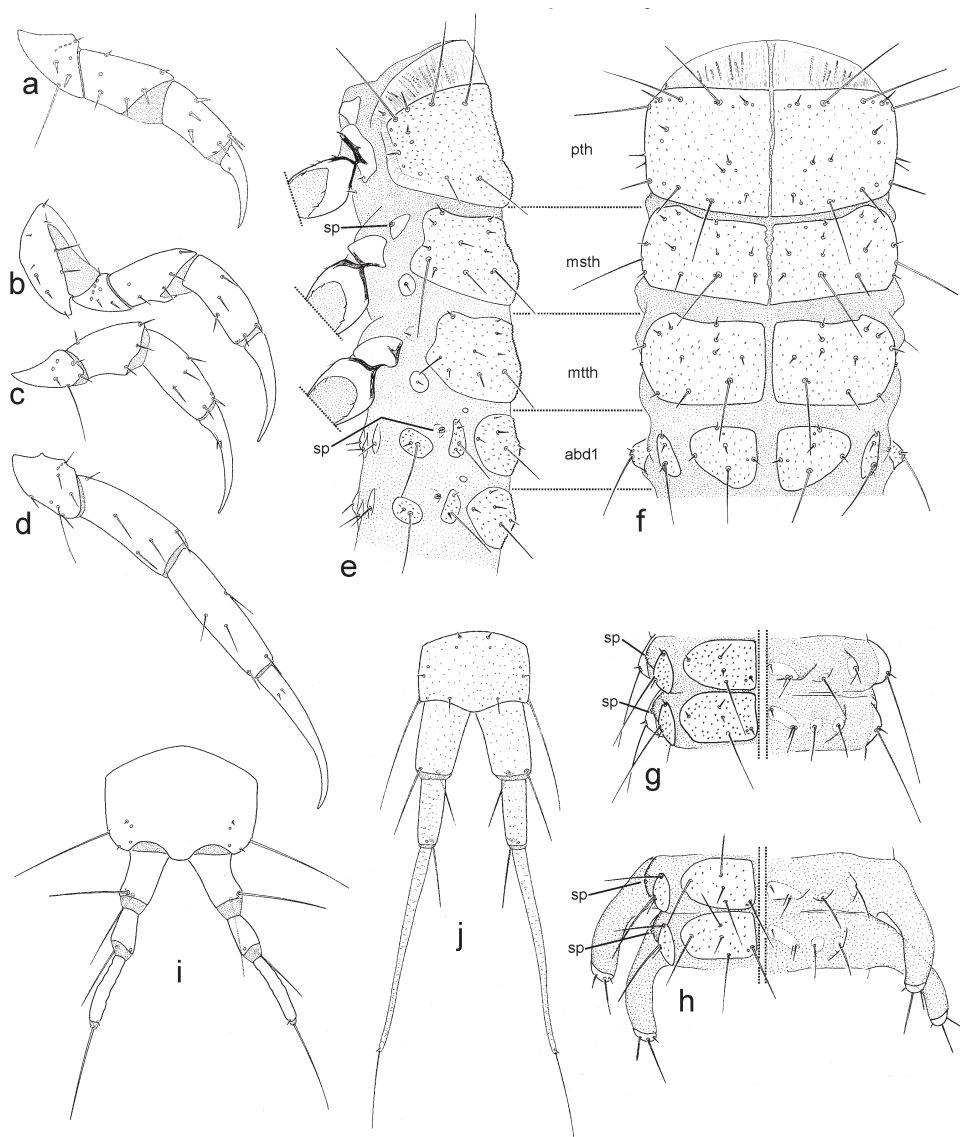


Fig. 15. Thorax and abdomen of *Lihelophorus* and *Helophorus* s.str. a–d – metathoracic legs (a – *H. (H.) liguricus* Angus, 1970, frontal view; b–c – *H. (Lihelophorus) yangae* sp. nov. (b – frontal; c – posterior); d – *H. (Lihelophorus) lamicola* Zaitzev, 1908). e–f – thorax and first abdominal segments of *H. (Lihelophorus) yangae* sp. nov. (e – lateral view; f – dorsal view). g–h – abdominal segments (dorsal and ventral views): g – *H. (Lihelophorus) yangae* sp. nov.; h – *H. (Lihelophorus) lamicola* Zaitzev, 1908. i–j – abdominal segment 9 and urogomphi: i – *H. (H.) liguricus* Angus, 1970; j – *H. (Lihelophorus) yangae* sp. nov. Abbreviations: abd1 – abdominal segment 1; msth – mesothorax; pth – prothorax; sp – spiracle.

one tiny seta (MX27) basally and one pore (MX18) at midlength. Palpomere 3 with a series of sensilla on distal margin (MX20–23). Palpomere 4 with a short basal seta (MX24) and two digitiform sensilla situated subapically. Apical membranous area with short sensilla (gMX).

Labium (Figs 10a, 13g–h). Submentum with one pair of long setae (LA1), LA2 absent. Mentum with a pair of pores (LA4) and pair of short setae distally on ventral surface. Prementum ventrally with a pair of minute setae (LA5) basally, and a pair of pores (LA7) and a pair of short setae (LA6) distally below articulation of labial palp; dorsal surface with one pair of basal pores (LA8) basally in membranous area, anteromedian portion between palpal bases with two pores (LA9, LA?12) and a pair of setae (LA10). Palpomere 1 with one tiny seta (LA13) basally and a pore (LA14) on intersegmental membrane, palpomere 2 with a subapical pore (LA15) and additional digitiform sensilla. Apical membranous field with multiple short setae.

Thorax (Figs 8c–d, 15e–f). Proscutum with 15 setae and 7 pores on each half situated in three transverse rows; all setae trichoid, pores usually situated close to seta articulation. Ventral portion with two long setae on anterior part of each precoxal sclerite, and a pair of setae situated between procoxa in a membranous area. Lateral portion with a one minute seta behind procoxal articulation. Meso- and metanotum with sensilla arranged in 4 longitudinal rows; each half of mesonotum with 13 setae and 3 pores (2 of them situated next top setal articulation), each half of metanotum with 11 setae and one pore situated next to articulation of posteromesal long seta. Ventral portion of meso- and metathorax with one small sclerite mesally, each with one pair of short setae.

Metathoracic leg (Figs 15b–c). Coxa with long setae on a ridge anteriorly above trochanter articulation, and a row of short setae more proximally of articulation. Trochanter with a series of elongated pores ca. at midlength on both anterior and posterior surface, two short setae and one pore on anterior face, three setae posteriorly, and one long seta on posterior face. Femur with 8 short setae and one pore; 4 setae and one pore on frontal surface, four setae on posterior surface. Tibiotarsus with an oblique row of three moderately long setae on anterior and posterior face, distal portion with four trichoid setae around claw articulation. Claw bisetose; setae minute, situated in proximal third of claw length.

Abdomen (Figs 8i–j, 15e–g, 15j). Each abdominal segment with four longitudinal rows of sensilla serially homologous with those of thoracic segments; first three mesal rows situated on dorsal sclerite, lateralmost series situated on dorsolateral sclerite. Lateral sclerite with one long and one short seta. Medioventral sclerite with three short setae on each side, lateroventral sclerites each with one short seta. Tergite of segment 9 with 7 sensilla on each side, posterolateral seta very long. Urogomphus with two sensilla (one dorsal, one ventral) on segment 1 and 2 each, segment with with long terminal seta only.

Helophorus (Lihelophorus) lamicola

Larval material examined (5 larvae). 1 first instar larva (NMPC, DNA voucher MF660): **CHINA: QINGHAI:** Golo, Huamuxia, Zuimatan, roadside pool, 35°19'52"N, 99°03'57"E, 4141 m, 7.vi.2013, R. B. Angus, F. L. Jia & Y. Zhang lgt.; 4 first instar larvae (BMNH): same label data. All larvae were reared from the single egg case (deposited in BMNH) laid by a field-collected female, association with adults from the same locality was confirmed by the *coxI* sequence data.

Egg case (Fig. 8e). Consisting of an egg-bag surmounted by a thin trailing mast which takes the form of a long narrow tube, thus corresponding to Type 4 egg case of ANGUS (1992). The egg case was placed in the sand at the water's edge in the aquarium housing the beetles, and has sand grains adhering to the egg-bag. A similar egg case was observed in the ground at the water's edge in the Zuimatan locality where the beetles were collected

Larva. General morphology (only characters different from *H. yangae* are mentioned). Body (Fig. 9c) elongate, slender, almost parallel-sided. Body length ca. 2.9 mm. **Head** width 0.43–0.46 mm (n = 5). Parietale (Figs 12a–b) with numerous cuticular spines dorsally and laterally, ventral face smooth. Frontoclypeus (Fig. 12e) with large bulged asymmetrical nasale bearing single median tooth, and large slightly asymmetrical epistomal lobes overlapping nasale. Gular suture absent, posterior tentorial pits minute, situated anteromesally (Figs 8a–b). **Mandibles** (Figs 13c–d) slightly asymmetrical, with falcate apical portion. Retinaculum with two teeth; distal tooth large, bearing dense tuft of long cuticular spines; basal tooth small, with numerous cuticular spines in left mandible and few projections in right one. Basal inner face projecting into a small lobe, with a basal field of fine spine-like cuticular projections. **Maxilla** (Figs 14e–f). Stipes ca. as long as palpifer, sinuate laterally, mesal face with a series of irregular cuticular spines, lateral face with small blunt cuticular spines; palpifer slightly longer than stipes. **Labium** (Figs 13i–j). Submentum completely fused with parietale, submental suture absent. Mentum without cuticular spines on dorsal surface. **Thorax**. Legs (Fig. 15d) very long, 5-segmented; trochanter ca. half as long as femur; tibiotarsus cylindrical, slightly longer than trochanter and femur combined. Claw ca. as long as tibiotarsus, slightly bent ventrad. **Abdomen** (8g–h, 15h). Segments 1–8 each with a pair of long finger-like projections laterally below spiracles, bearing lateral sclerite on apex; each projection with trachea connected to tracheal branch connecting main tracheal trunk with spiracle lying above base of the particular projection.

Chaetotaxy. Head. Frontal pores FR2 closer to each other than setae FR3; setae FR8 widely separated from each other; parietal setae PA7, PA12, and PA14 much longer than in *L. yangae*. Labial setae LA3 very long. Maxillary setae MX5 and MX6 of the same length, both rather long; setae MX8–11 very long, situated along the whole length of stipes; apical maxillary palpomere with multiple additional digitiform sensilla. **Abdomen** with lateral abdominal sclerite (situated on the top of tracheal gill) with three setae.

Cytogenetics

Mitotic chromosomes, arranged as karyotypes, are shown in Fig. 16, first metaphase of meiosis in Fig. 17 and second metaphase of meiosis in Fig. 18. All three species have the karyotype $2n = 20 + Xy_p$ and there are some small but clear differences between the karyotypes of the three species.

Helophorus lamicola. Mitotic chromosomes, arranged as karyotypes, are shown in Figs 16a–b. The autosomes are metacentric to submetacentric, with autosome pairs 2, 4–6, and the X chromosome submetacentric. Autosome pair 5 and the X chromosome are almost subacrocentric. The RCLs of the autosomes range between ca. 14–7.5, with a fairly even

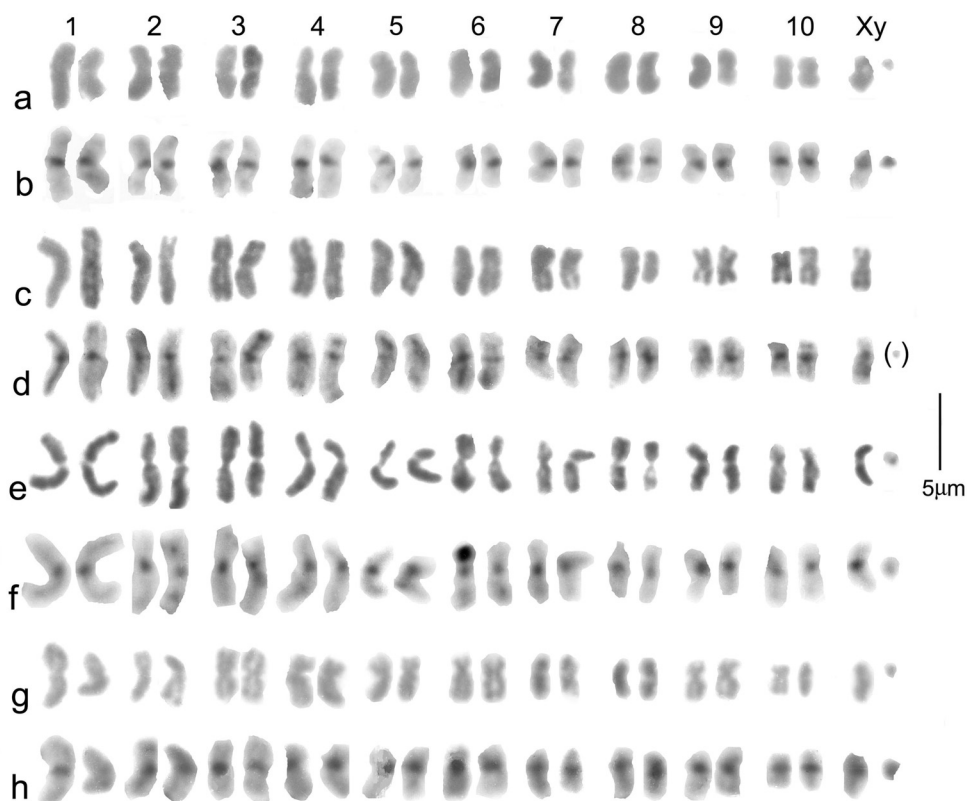


Fig. 16. Mitotic chromosomes from mid-gut cells of males of *Helophorus* (*Lihelophorus*) species from Qinghai: Zuimatan, arranged as karyotypes. a–b – *H. lamicola* Zaitzev, 1908 (a – plain (Giemsa stained); b – same nucleus, C-banded). c–d – *H. ser* Zaitzev, 1908 (c – plain; d – same nucleus, C-banded). e–h – *H. yangae* sp. nov. (e – plain; f – same nucleus, C-banded; g – plain; h – same nucleus, C-banded). The y chromosome is missing from the preparation shown in c and d and in d the y chromosome from a different C-banded preparation with a similar degree of chromosomal condensation has been inserted.

decrease along the karyotype. The X chromosome (RCL about 8.2) is about the same size as autosome pairs 8 and 9. The y chromosome is dot-like. C-banding (Fig. 16b) shows distinct centromeric C-bands on all the autosomes and the X chromosome, with the y at least partly heterochromatic. Metaphase I of meiosis (Fig. 17a) shows the Xy_p bivalent very clearly, and all the autosomal bivalents are rod-like, each with one terminalised chiasma. Metaphase II (Fig. 18a–b) shows the shape of the chromosomes clearly, especially the small X chromosome with one very short arm.

Helophorus ser. Mitotic chromosomes, arranged as karyotypes, are shown in Figs 16c–d. The autosomes are mainly submetacentric, only pairs 1 and 10 appearing metacentric. None of the chromosomes approaches the subacrocentric category. The RCLs of the autosomes range

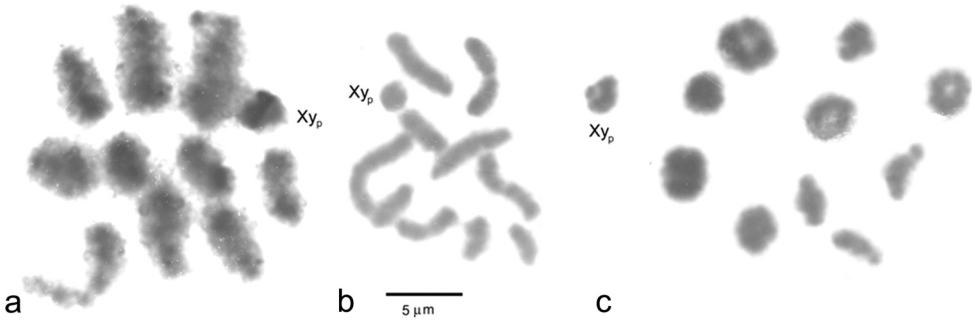


Fig. 17. Meiosis metaphase I from testes of *Helophorus* (*Lihelophorus*) species from Qinghai: Zuimatan. a – *H. lamicola* Zaitzev, 1908; b – *H. ser* Zaitzev, 1908; c – *H. yangae* sp. nov.

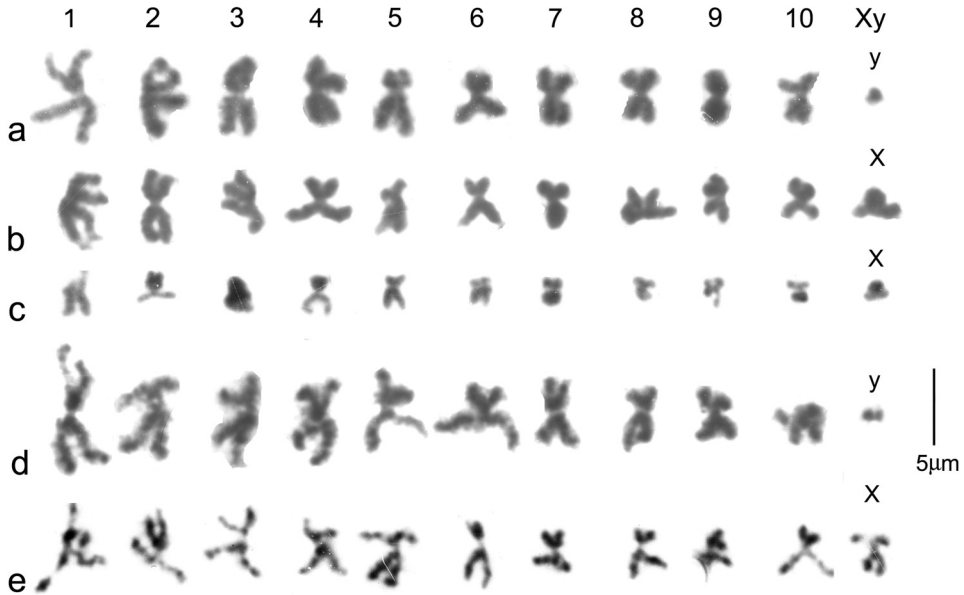


Fig. 18. Meiosis metaphase II from testes of *Helophorus* (*Lihelophorus*) species from Qinghai: Zuimatan. a–b – *H. lamicola* Zaitzev, 1908 (a – with y chromosome; b – with X chromosome). c – *H. ser* Zaitzev, 1908, with X chromosome. d–e – *H. yangae* sp. nov. (d – with y chromosome; e – with X chromosome).

between ca. 14.5–7 with a fairly even decrease along the karyotype. The X chromosome (RCL about 8.0) is about the same size as autosome pair 7 and submetacentric (CI about 40), but not nearly subacrocentric as in *H. lamicola*. The y chromosome is dot-like. C-banding (Fig. 16d) shows distinct centromeric C-bands on all the autosomes and the X chromosome, but these bands are smaller than in *H. lamicola*. The y chromosome does not show any obvious heterochromatin. Metaphase I of meiosis (Fig. 17b) shows the autosomes as rod-bivalents

and the sex bivalent as Xy_p . Only one X-bearing metaphase II was obtained (Fig. 18c). The X chromosome appears small, similarly shaped to that of *H. lamicola*. Its small size apparently reflects its greater degree of condensation than some of the autosomes.

***Helophorus yangae*.** Mitotic chromosomes, arranged as karyotypes, are shown in Figs 16e–h. Autosome pairs 1–3 are metacentric with the rest submetacentric. As in *H. ser*, none approaches the subacrocentric category. The RCLs of the autosomes range between ca. 13–8, with a fairly even rate of decrease along the karyotype. The X chromosome is about the same size as autosome pair 9 and is metacentric. The y chromosome is dot-like. C-banding (Figs 16f–h) shows distinct centromeric C-bands on all the autosomes and the X chromosome with a particularly heavy C-band. The y chromosome has a small heterochromatic area. Metaphase I of meiosis (Fig. 17c) shows that seven of the autosome pairs form ring-bivalents, each with two terminalised chiasmata, and the Xy_p configuration of the sex bivalent is very clear. Metaphase II (Figs 18d–e) shows the shape of the chromosomes and in particular the small X chromosome with a rather long centromere region probably reflecting its larger C-band.

Distribution and biology

Lihelophorus species are all endemic to the Tibetan Plateau, and were recorded in altitudes between 2940 and 5350 m a.s.l. The latter highest record is based on a long series of *H. ser* found in an almost dried-up streambed in the eastern slope of the Lha Tsu valley (Fig. 7e), and is the highest record of an aquatic beetle ever recorded (J. Schmidt, pers. comm.). Two of the *Lihelophorus* species (*H. ser* and *H. yangae* sp. nov.) are widespread, with the range covering the complete Tibetan Plateau, and both of them are recorded also from lower altitudes below 4000 m a.s.l. In contrast, *H. lamicola* seems to be restricted to the northern part of the Plateau, and all recorded specimens were found in higher altitudes only (above 4100 m a.s.l.).

All three species seem to share very similar habitat requirements based on our experience, which is also illustrated by their syntopic occurrence in the roadside pool at Zuimatan in Qinghai (Fig. 7c). Most specimens were collected from various types of exposed shallow pools in non-forested areas, both with and without submerged vegetation (Figs 7b–d, g). Adults of all three species are good swimmers thanks to the tibial and tarsal swimming-hairs of meso- and metathoracic legs. There are only two records of *Lihelophorus* from streams (in both cases concerning *H. ser*): a short series was collected in Gansu: Xiahe in side pools at a small stream, and a long series was collected in an almost dried-up stream in Lha Tsu valley. Based on these records, it seems that *Lihelophorus* species occasionally inhabit side pools of the streams with no or limited current, but their adaptation to swimming clearly indicates that they are not inhabiting fast-flowing waters.

After hatching from the egg case, larvae of *H. yangae* sp. nov. were observed to crawl on the moist (non-submerged) substrate, as it is usual for all other *Helophorus* larvae. The larva also corresponds with those of other *Helophorus* in the presence of open abdominal spiracles and absence of any special adaptations for aquatic life style. We hence conclude that larvae of *H. yangae* are terrestrial, occurring among vegetation and on soil near water, as it is the case for all *Helophorus* larvae which biology is known (ANGUS 1992).

In contrast, the larva of *H. lamicola* seems to be more aquatic, as indicated by the presence of the lateral projections of the abdomen. These projections are tracheal gills, as may be concluded from their extensively dense tracheation (Fig. 8g). The fact that an egg case similar to that from which larvae of *H. lamicola* were obtained was observed attached to fine thread-like vegetation some distance from the shore at Zuimatan would also accord with the aquatic life style of the larva of that species.

Discussion

Larval morphology of *Lihelophorus*

The knowledge of larval morphology of *Helophorus* is fairly complete especially for the western Palaearctic species due to the studies by ANGUS (1973, 1992), and larvae are known for 6 of 9 currently recognized subgenera. All of them are rather uniform in morphology, and only few characters may be used to distinguish the subgenera: presence/absence of the retinacular tuft on the mandible, presence/absence of ventral cuticular projections and lateral teeth of the nasale, number of supernumerary sclerites on abdominal segments and the presence/absence of tubercles around abdominal tergites (ANGUS 1992). At species level, diagnostic characters usually comprise slight differences in proportions of head appendages, legs and urogomphi, detailed morphology of the mandibular base and nasale, size of mesothoracic spiracles, the extent of sclerotization of abdominal tergites and arrangement of cuticular asperities on the abdomen (ANGUS 1992). Larvae of only few species are morphologically so unusual that they can be easily distinguished at a glance. For example, the larva of *H. pumilio* Erichson, 1837 differs from all other *Helophorus* in the morphology of the mandible (strongly falcate apical portion with large teeth on inner face and small retinacular teeth; DROST 1989, ANGUS 1992). This difference may be the consequence of food preferences, as larvae of *H. pumilio* were recorded to feed on nematodes, which was never observed for other *Helophorus* species (ANGUS 1992, DROST 1989).

The larvae of *Lihelophorus* described here bear all synapomorphies of *Helophorus*, but differ from all other *Helophorus* larvae in many aspects. The most apparent differences are the asymmetrical nasale and the extremely long legs and urogomphi, but few others may be listed: basal retinacular tooth of the mandible bears cuticular spines ventrally, retinacular tuft bears very many long projections, epistomal lobes bear 7–8 setae, and the surface of parietale as well as thoracic and abdominal tergites bear numerous cuticular denticles. In addition to these characters, which are shared by both *H. yangae* sp. nov. and *H. lamicola*, additional unusual features (unique within *Helophorus*) may be found in the larva of *H. lamicola*: nasal region is bulging resulting in widely separated setae FR8, maxillary stipes bears very long mesal setae (MX8–11) and equally long setae MX5–6, and abdominal segments bear tracheal gills. Analogically to the case of *H. pumilio* mentioned above, many of these unique characters may be potentially considered as adaptation for a specialized life style (despite the absence of direct observations): modification in the morphology of mandibles, nasale and the setation of epistomal lobes likely has a connection with feeding habits, the presence of tracheal gills seems to be an adaptation for aquatic life style. Interestingly, a gradual transition may be observed in these characters when the usual

Table 3. Comparison of *Lihelophorus* larvae and larvae of other *Helophorus* species.

Character	<i>H. liguricus</i> /other spp.	<i>H. yangae</i> sp. nov.	<i>H. lamicola</i>
Nasale: shape	symmetrical	asymmetrical	asymmetrical
Nasale ventrally	without / with teeth ¹	without teeth ¹	without teeth ¹
Epistomal lobes	with 5 setae / 5–6 setae ²	with 8 setae	with 7–8 setae
Setae FR8	close to each other	close to each other	widely separated
Frontal seta FR6	minute / long ³	long	long
Parietale	smooth	with cuticular denticles	with cuticular denticles
Parietale setae PA8, PA13 and PA20	long and stout	long but fine	long but fine
Pariental seta PA7	minute / long ⁴	long	long
Mandibular retinacular tuft	present: minute / absent ⁵	present: large	present: large
Ventral cuticular spines on basal retinacular tooth	absent	present	present
Sclerotized portion of prementum	subconical / shortly V-shaped ⁶	V-shaped	V-shaped
Stipes	much shorter / as long as palpifer ⁷	ca. as long as palpifer	ca. as long as palpifer
Stipes dorsally	incompletely / completely sclerotized ⁸	completely sclerotized	completely sclerotized
Setae of outer face of maxillary stipes	MX5 much longer than MX6	MX5 much longer than MX6	MX5 and MX6 of the same length
Setae of inner face of stipes (MX8–11)	moderately long / short, situated distally ⁹	moderately long, situated distally	very long, along whole inner face
Legs	very short to short	moderately long	very long
Tracheal gills on abdomen	absent	absent	present
Urogomphi	short, segments widened distally	long, segments tapering distally	long, segments tapering distally

¹ many species of *Helophorus* bear ventral cuticular teeth on nasale, incl. species of *Helophorus* s.str., *Eutrichelophorus*, and a part of *Rhopalohelophorus*; spines are absent in examined first instars of *H. (s.str.) liguricus*, despite higher instars of the subgenus usually bears distinct teeth, indicating possible between-instar variation in this character;

² five setae in most *Helophorus*, 6 only in some *Empleurus* species;

³ long in all examined *Helophorus* except *H. liguricus* Angus, 1970;

⁴ short in most examined species, long in *H. tuberculatus* Gyllenhal, 1808 and *H. nanus* Sturm, 1836;

⁵ present in all known larvae of *Helophorus* s.str. and *Rhopalohelophorus*, absent from all other subgenera with known larvae (ANGUS 1992, FIKÁČEK et al. 2012);

⁶ intermediate between states illustrated in this paper for *H. liguricus* and *Lihelophorus* (i.e. V-shaped with short distal portion) in third instars of *H. rufipes* (Bosc, 1791), *H. alternans* Gené, 1836, *H. maritimus* Rey, 1885 and *H. grandis* Illiger, 1798;

⁷ rather variable within *Helophorus*, but in most species with stipes and palpifer subequal in length maxilla is much longer than in *Lihelophorus*, resulting in much longer stipes and palpifer; in most examined *Rhopalohelophorus* larvae, stipes and palpifer are subequal in length and similarly short as in *Lihelophorus*;

⁸ seems to be rather variable within *Helophorus* and partly maybe even between instars, and moreover it is rather difficult to examine in many slide-mounted larvae;

⁹ the morphology of MX8–11 is rather variable within *Helophorus*, but they are never as long as in *H. lamicola*; in all examined specimens of *Rhopalohelophorus* these setae are very short.

Helophorus larva (here represented by *H. liguricus*) is compared with those of *H. yangae* and *H. lamicola*: nasale (Fig. 12) is simple and symmetrical with few epipharyngeal minute spines in *H. liguricus*, it becomes simply asymmetrical and with enlarged lateral epipharyngeal spines in *H. yangae*, and bulging and extremely asymmetrical with all epipharyngeal spines enlarged in *H. lamicola*. Similar transition is observed in the length of legs (short in *H. liguricus*, moderately long in *H. yangae*, very long in *H. lamicola*; Figs 15a–d) and in fact also in the development of the tracheal gills (*H. yangae* bears all unique characters of *Lihelophorus* but still lacks the gills, *H. lamicola* bears these characters plus the gills). These transformation series actually agree with the phylogenetic positions of both species revealed by *cox1* sequences (Fig. 9a).

Modified head morphology and tracheal gills as high altitude adaptations?

The presence of the tracheal gills in larvae of *H. lamicola* was the biggest surprise in the course of our study, as tracheal gills are not only absent from all known *Helophorus* larvae, but also from most larval Hydrophiloidea, with three known exceptions: *Berosus* (tribe Berosini) and *Laccobius* (*Yateberosus*) (tribe Laccobiini) in the Hydrophilidae, and larvae of some *Epimetopus* in the Epimetopidae (MINOSHIMA & HAYASHI 2015; FIKÁČEK et al. 2011; FIKÁČEK & MINOSHIMA, in press). In all cases, the tracheal system is modified in these taxa, with abdominal spiracles reduced in size (and likely closed) and, in members of the Hydrophilidae, with the spiracular atrium largely to totally reduced. In all three cases, the closed spiracular system and abdominal tracheal gills evolved in clades in which the head morphology is highly modified, allowing the food to be processed underwater (in other groups prey has to be held above water to be processed extraorally). FIKÁČEK & MINOSHIMA (in press) considered the modification of the head of these clades as preadaptation for benthic (i.e. permanently submerged) life style, which was subsequently followed by modification of tracheal system in those taxa which became really benthic.

The situation found in *Lihelophorus* may be analogous: head morphology is highly modified in both *H. yangae* and *H. lamicola* compared to other *Helophorus* larvae, and this modification clearly predated the modification of the tracheal system which is found only in *H. lamicola*. In difference, larvae of *Lihelophorus* are not benthic, as they have open abdominal spiracles. However, usual *Helophorus* larvae are terrestrial and are not adapted for processing the food in the water, where they would face the same problem that larvae of Hydrophilidae do (i.e. dissolution of digestive fluids while processing the prey extraorally). Head modifications observed in *Lihelophorus* may possibly be adaptations allowing processing of the aquatic prey, which would also be supported by the presence of tracheal gills (i.e. other adaptation for aquatic life style) in *H. lamicola*. The presence of open spiracles in both *Lihelophorus* species would however indicate that they still remain to some extent terrestrial. Possibly, head morphology of *Lihelophorus* may actually be adapted to process the prey both emerged and underwater, i.e. to diversify prey sources. This may be an advantageous strategy in the high altitude environment with short breeding season and highly variable environment (e.g., changeable weather).

The combination of tracheal gills and open abdominal spiracles found in *H. lamicola* is rather perplexing, as both modes of breathing were so far considered as mutually incompatible:

the use of tracheal gills is possible only when the larva is submerged, and the presence of open spiracles was supposed impossible in submerged insects due to the risk of flooding the tracheal system. Moreover, the branchial trachea (i.e. that of the tracheal gill) is connected directly to the spiracular trachea closely before its connection to the spiracle in *H. lamicola* (Fig. 8h), and both the spiracle and tracheal gill hence seems to form a single functional unit. Additional experiments would be necessary to find out whether this close connection of the spiracular and branchial tracheae may help to keep the tracheal system unflooded in the submerged larva (e.g., by keeping the gas content and pressure at the spiracle high enough to prevent the water from entering the tracheal system through the spiracle). Another question is the function of tracheal gills of *H. lamicola*, given the fact that the closely related *H. yangae* likely lives in a very similar way and easily survives without them. Possibly, tracheal gills may provide an advantage to larvae trapped submerged by the ice suddenly formed over the water surface (as it actually happened during our expedition, see ANGUS 2013) and hence solely dependent on oxygen dissolved in water. VERBERK et al. (2011) demonstrated that at high altitudes aquatic animals face a combined effect of low partial pressure of oxygen (due to the altitude) and low diffusivity of oxygen in water (due to low temperatures) which decreases strongly the oxygen supply available to them. Tracheal gills make the intake of oxygen more effective under these conditions, and may be hence hypothesized as adaptation for (semi)aquatic lifestyle at high altitudes in cold environment with unstable weather as well.

Systematic position of *Lihelophorus*

The subgenus *Lihelophorus* was originally considered as closely related to *Helophorus* s.str. by ANGUS (1995), given the large body size, presence of the scutellary stria and the asymmetrical apical maxillary palpomeres. FIKÁČEK et al. (2012) included *Lihelophorus* in the phylogenetic analysis of *Helophorus* based on morphological characters, and concluded that it forms a sister clade to all remaining extant *Helophorus* species, a position revealed mainly due to the presence of many plesiomorphic characters in *H. ser* (i.e. the only species of *Lihelophorus* included in the analysis). The discovery of the morphologically very unusual larvae (see above) originally supported the separate position of *Lihelophorus* as well.

On the other hand, examination of all three species of *Lihelophorus* and multiple specimens of *H. ser* showed that some characters considered as plesiomorphies by FIKÁČEK et al. (2012) have to be reevaluated. For example, the simple adult galea without large spines (characters 43:0 and 44:0, Fig. 1d) are only present in *H. ser* and *H. lamicola*, but not in *H. yangae*, in which lobate galea resembling that of remaining *Helophorus* is present (Fig. 1c). Taking the basal position of *H. yangae* in *Lihelophorus* into account (Fig. 9a), this indicates that the simple galea of *H. ser* observed by FIKÁČEK et al. (2012) is not a plesiomorphic condition, but a reversal. Similar is the case of the shape of apical labial palpomere (character 49) which is clearly asymmetrical in *H. yangae* (Fig. 1b). Moreover, the fringe of setae is clearly present on the outer face of the apical labial palpomere of all *Lihelophorus* species (Figs 1b,g) but was previously overlooked (coded as 50:0 by FIKÁČEK et al. 2012). All these findings indicate that the phylogenetic position of *Lihelophorus* may actually be more inside of *Helophorus* than originally expected.

When larval unique autapomorphies are not considered, a few other characters may be compared between *Lihelophorus* and other *Helophorus* species. The presence of retinacular tuft on the mandible is shared with the subgenera *Helophorus* s.str. and *Rhopalohelophorus* only. *Rhopalohelophorus* larvae also resemble those of *Lihelophorus* in the rather short maxillary stipes and palpifer, both ca. subequal in length, and by the absence of supranumerary sclerites on abdominal segments. Cytogenetic data are also similar for *Lihelophorus* and *Rhopalohelophorus* (both with karyotypes of 22 chromosomes), and differ from those of *Helophorus* s.str. (18 chromosomes).

These results are in agreement with an ongoing phylogenetic analysis of *Helophorus* (FIKÁČEK et al., in prep.) which supports *Lihelophorus* as a monophyletic clade situated deeply within *Helophorus* and standing close to *Rhopalohelophorus*.

Table 4: List of the *Helophorus* species occurring in Tibet (distribution data adopted from ANGUS 1995, HEBAUER 1999, ANGUS et al. 2014, FIKÁČEK et al. 2015, and this paper). The column ‘margins’ includes marginal parts of the Tibetan Plateau in Nepal, Bhutan, northern Sichuan and western Gansu.

Species	Ladakh	Xizang	Qinghai	margins	Distribution outside Tibet
sg. <i>Orphelophorus</i> Orchymont, 1927					
<i>H. obscurellus</i> Poppius, 1907			•		N Europe, Central Asia, E Siberia
sg. <i>Transithelophorus</i> Angus, 1970					
<i>H. crinitus</i> Ganglbauer, 1901			•		Siberia, Far East, N China, Mongolia
sg. <i>Kyphohelophorus</i> Kuwert, 1886					
<i>H. tuberculatus</i> Gyllenhal, 1808				•	Europe, Siberia, Far East, North America
sg. <i>Helophorus</i> s.str.					
<i>H. hammondi</i> Angus, 1970	•		•		E Siberia, Mongolia, relicts in W Russia
<i>H. kozlovi</i> Zaitzev, 1908			•		Uzbekistan (Tian Shan)
<i>H. jaechi</i> Angus, 1995			•		China: Sichuan, Fujian
sg. <i>Lihelophorus</i> Zaitzev, 1908					
<i>H. lamicola</i> Zaitzev, 1908		•	•		endemic to Tibet
<i>H. ser</i> Zaitzev, 1908	•	•	•	•	endemic to Tibet
<i>H. yangae</i> sp. nov.	•	•	•		endemic to Tibet
sg. <i>Rhopalohelophorus</i> Kuwert, 1886					
<i>H. angusi</i> Hebauer, 1999				•	only known in Nepal
<i>H. aquila</i> Angus, Jia & Chen, 2014			•		endemic to Tibet
<i>H. aspericollis</i> Angus, 1973			•		Kazakhstan, Siberia, Far East, Mongolia
<i>H. carsoni</i> Angus, 1970			•	•	Mongolia, N China, S Siberia, Altai
<i>H. frater</i> Orchymont, 1926	•	•	•	•	E Turkey, Iran, S. Siberia
<i>H. imaensis</i> Orchymont, 1926	•	•	•		endemic to Tibet
<i>H. kerimi</i> Ganglbauer, 1901			•		Central Asia to Far East, incl. N China
<i>H. mongoliensis</i> Angus, 1970			•		Kazakhstan, Mongolia
<i>H. montanus</i> Orchymont, 1926		•	•	•	Sichuan, Afghanistan, Central Asia, Kyrgyzstan
<i>H. praeanus</i> Lomnicki, 1894			•		Siberia, Far East, Mongolia

Helophorus of the Tibetan Plateau

The subgenus *Lihelophorus*, treated in detail in this paper, is the only larger clade of *Helophorus* endemic to the Tibetan Plateau. Apart from it, only two species, both belonging to the subgenus *Rhopalohelophorus*, are endemic for the region: *H. imaensis* Orchymont, 1926 and the recently described *H. aquila* Angus, Jia & Chen, 2014. In addition, *H. kozlovi* Zaitzev, 1908 occurs exclusively in the Tibetan Plateau and the adjacent Tian Shan Mts., and *H. angusi* Hebauer, 1999 is so far known only from Nepalese Himalaya on southern margin of the Tibetan Plateau (ANGUS 1970, 1995; ANGUS et al. 2014; HEBAUER 1999). Although the phylogenetic relationships have not been tested within *Helophorus* so far, all these species seem to be related to Central Asian species: *H. imaensis* appears to be closely related to the Mongolian *H. kaszabianus* Angus, 1970, *H. aquila* is a largely parthenogenetic species standing probably close to *H. frater* Orchymont, 1926, *H. kozlovi* shows similarities with the species of the Eastern Palaearctic *H. bergrothi* species group, and *H. angusi* seems most similar to *H. altaicus* Ganglbauer, 1901. Apart from these endemics, the Tibetan *Helophorus* fauna includes widespread Eastern Palaearctic or Central Asian species (see Table 4).

Acknowledgements

We are indebted to the curators of the museum collections listed above for the access to the specimens of *Lihelophorus*, to Dr. Joachim Schmidt (Rostock, Germany) for the gift of important material, for valuable information on distribution, especially of *H. ser* and *H. lamicola* and the photograph of the locality, and to Jiří Hájek (NMPC) for providing the biology data and locality photos for specimens collected by him. We are indebted to Yūsuke Minoshima (Kitakyushu Museum of Natural History and Human History, Japan) and an anonymous reviewer for their comments and corrections of the manuscript. The Natural History Museum (London, UK) and Royal Holloway University of London provided research facilities for R. B. Angus. The study was supported by grant SVV 260 313/2016 to D. Vondráček and by the Ministry of Culture of the Czech Republic (DKRVO 2016/14, National Museum, 00023272) to M. Fikáček, and by the National Natural Science Foundation of China (grant no. 31272266) to F.-L. Jia.

References

- ANGUS R. B. 1970: Revisional studies on East Palearctic and some Nearctic species of *Helophorus* F. (Coleoptera: Hydrophilidae). Ergebnisse der zoologischen Forschung von Dr. Z. Kaszab in der Mongolei (No. 226). *Acta Zoologica Academiae Scientiarum Hungaricae* **16**: 249–290.
- ANGUS R. B. 1973: The habitats, life histories and immature stages of *Helophorus* F. (Coleoptera: Hydrophilidae). *Transactions of the Royal Entomological Society* **125**: 1–26.
- ANGUS R. B. 1992: Insecta Coleoptera Hydrophilidae Helophorinae. In: SCHWOERBEN J. & ZWICK P. (eds) *Süßwasserfauna von Mitteleuropa 20/10-2*. Gustav Fischer Verlag, Stuttgart – Jena – New York, 144 pp.
- ANGUS R. B. 1995: Helophoridae: The *Helophorus* species of China, with notes on the species from neighbouring areas (Coleoptera). Pp 185–206. In: JÄCH M. A. & JI L. (eds): *Water Beetles of China. Volume 1*. Zoologisch-Botanische Gesellschaft & Wiener Coleopterologenverein, Wien, 410 pp.
- ANGUS R. B. 2013: On the roof of the world – wanderings of an old foreigner. *Latissimus* **34**: 5–12.
- ANGUS R. B., JIA F.-L. & CHEN Z.-N. 2014: A review of the *Helophorus frater-praenanus* group of species, with description of a new species and additional faunal records of *Helophorus* Fabricius from China and Bhutan (Coleoptera: Helophoridae). *Koleopterologische Rundschau* **84**: 209–219.

- ARCHANGELSKY M. 1997: Studies on the biology, ecology, and systematics of the immature stages of New World Hydrophiloidea (Coleoptera: Staphyliniformia). *Bulletin of the Ohio Biological Survey, New Series* **12**: 1–207.
- BEUTEL R. G. 1994: Phylogenetic analysis of Hydrophiloidea based on characters of the head of adults and larvae (Coleoptera: Staphyliniformia). *Koleopterologische Rundschau* **64**: 103–131.
- BEUTEL R. G. 1999: Morphology and evolution of the larval head of Hydrophiloidea and Histeroidea (Coleoptera: Staphyliniformia). *Tijdschrift voor Entomologie* **142**: 9–30.
- BYTTEBIER B. & TORRES P. L. M. 2009: Description of the preimaginal stages of *Enochrus* (*Hugoscottia*) *variegatus* (Steinheil, 1869) and *E. (Methydrus) vulgaris* (Steinheil, 1869) (Coleoptera: Hydrophilidae), with emphasis on larval morphometry and chaetotaxy. *Zootaxa* **2139**: 1–22.
- DROST M. B. P. 1989: *Helophorus croaticus* and *H. pumilio* (Coleoptera: Hydrophilidae) in the Netherlands and the diagnoses of their larvae. *Entomologische Berichten* (Amsterdam) **49**: 1–7.
- DUTTON L. A. & ANGUS R. B. 2007: A karyosystematic investigation of a group of sibling species related to *Stictotarsus griseostriatus* (De Geer) (Coleoptera: Dytiscidae). *Comparative Cytogenetics* **1**: 3–16.
- FIKÁČEK M., ANGUS R. B., GENTILI E., JIA F.-L., MINOSHIMA Y. N., PROKIN A., PRZEWOŹNY M. & RYNDEVICH S. K. 2015: Family Helophoridae Leach, 1815. Pp. 25–33. In: LÖBL I. & LÖBL D. (eds) *Catalogue of Palaearctic Coleoptera. Hydrophiloidea – Staphylinoidea. Volume 2/1. Revised and updated edition*. Brill, Leiden – Boston, 900 pp.
- FIKÁČEK M., ARCHANGELSKY M. & TORRES P. L. M. 2008: Primary chaetotaxy of the larval head capsule and head appendages of the Hydrophilidae (Coleoptera) based on larva of *Hydrobius fuscipes* (Linnaeus, 1758). *Zootaxa* **1874**: 16–34.
- FIKÁČEK M., BARCLAY M. V. L. & PERKINS P. D. 2011: Two new species of the *Epimetopus mendeli* species group and notes on its adult and larval morphology (Coleoptera: Hydrophiloidea: Epimetopidae). *Acta Entomologica Musei Nationalis Pragae* **51**: 477–504.
- FIKÁČEK M. & MINOSHIMA Y. (in press): Larval morphology of *Laccobius*, subgenus *Yateberosus* Satô and notes on parallel evolution of ‘Berosus-like’ larval morphology in the Hydrophiloidea (Coleoptera). In: JÄCH M. A. & BALKE M. (eds.): *Water Beetles of New Caledonia*, volume 2. *Monographs of Coleoptera* **4**: in press.
- FIKÁČEK M., PROKIN A., ANGUS R. B., PONOMARENKO A., YUE Y., REN D. & PROKOP J. 2012: Phylogeny and the fossil record of the Helophoridae reveal Jurassic origin of extant hydrophiloid lineages (Coleoptera: Polyphaga). *Systematic Entomology* **37**: 420–447.
- HANSEN M. 1991: The hydrophiloid beetles. Phylogeny, classification, and a revision of the genera (Coleoptera: Hydrophiloidea). *Biologiske Skrifter* **40**: 1–367.
- HANSEN M. 1999: *World catalogue of insects. Volume 2. Hydrophiloidea (s.str.) (Coleoptera)*. Apollo Books, Stenstrup, 416 pp.
- HEBAUER F. 1999: *Helophorus angusi*, a new species from Nepal (Coleoptera: Hydrophiloidea). *Acta Coleopterologica* **15**(2): 3–4.
- HUELSENBECK J. P. & RONQUIST F. 2001: MRBAYES: Bayesian inference of phylogenetic trees. *Bioinformatics* **17**: 754–755.
- KNISCH A. 1910: Über einige von Dr. Erich Zugmayer in Tibet und Turkestan gesammelte Hydrophiliden. *Zoologische Jahrbücher, Abteilung für Systematik, Geographie und Biologie der Tiere* **29**: 4515–454.
- KUMAR S., STECHER G. & TAMURA K. 2016: MEGA7: Molecular Evolutionary Genetics Analysis version 7.0 for bigger datasets. *Molecular Biology and Evolution* (accepted).
- LANFEAR R., CALCOTT B., HO S. Y. W. & GUINDON S. 2012: PartitionFinder: Combined selection of partitioning schemes and substitution models for phylogenetic analyses. *Molecular Biology and Evolution* **29**: 1695–1701.
- MINOSHIMA Y. & HAYASHI M. 2011: Larval morphology of the Japanese species of the tribes Acidocerini, Hydrobiusini and Hydrophilini (Coleoptera: Hydrophilidae). *Acta Entomologica Musei Nationalis Pragae* **51** (supplementum): 1–118.
- MINOSHIMA Y. & HAYASHI M. 2015: Description of the larval stages of the berosine genera *Berosus* and *Regimbartia* based on the Japanese species *B. japonicus* and *R. attenuata* (Coleoptera: Hydrophilidae). *Acta Entomologica Musei Nationalis Pragae* **55**: 47–83.
- ORCHYMONT A. d’ 1943: Les Palpicornia du Tibet (Coleoptera). *Bulletin du Musée Royal d’Histoire Naturelle de Belgique* **19**(57): 1–16.
- VERBERK W. C., BILTON D. T., CALOSI P. & SPICER J. I. 2011: Oxygen supply in aquatic ectotherms: partial pressure and solubility together explain biodiversity and size patterns. *Ecology* **92**: 1565–1572.
- ZAITZEV F. A. 1908: Beitrag zur Kenntniss der Waserkäfer von Chinesisch-Centralasien. *Annuaire du Musée Zoologique de l’Académie Impériale des Sciences de St. Pétersbourg* **13**: 417–426.