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Additional information on the enigmatic *Lytta zubovi* (Coleoptera: Meloidae): description of the female and first instar larva with remarks on its phylogenetic relationships

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Abstract

Lytta zubovi is a poorly known sexually dimorphic species of blister beetles recently described from Crimea. The female, until now known only from a photograph, is described and the first instar larva obtained from eggs is described and figured using scanning electron microscopy (SEM). Larval morphology greatly supports the previous tentative placement in the genus *Lytta*.

Keywords: Sexual behaviour, larval morphology, sexual dimorphism, taxonomic placement

Introduction

An enigmatic new species of blister beetles, endemic to Crimea, was recently described by Bologna and Nikitsky (2010) as *Lytta zubovi* and tentatively referred to the Holarctic genus *Lytta* Fabricius, 1775. Only two males were examined and the female was only known from a photograph, in which it appeared brachyelytrous and possibly apterous.

The placement of this new species in the tribe Lyttini, genus *Lytta*, was based on the male genitalia and mesosternal structure. Some primitive characters (see Bologna & Nikitsky 2010 for details) are shared with the subgenus *Poreospasta*, an amphi-Pacific element from western North America and central Asia (Selander 1960), but the flattened head, pronotum and elytra, the transverse shape of the pronotum and the brachyelytrous condition of the female greatly distinguish this species from all other known *Lytta*.

The recent collection of two new specimens, including a female, adds to our knowledge of species distribution, ecology and morphology. Additionally, eggs and first instar larvae were obtained from the female. First instar larval morphology is of considerable importance in estimating phylogenetic relationships in Meloidae (Bologna & Pinto 2001), and is useful here in helping to determine the tribal and generic placement of *L. zubovi*.

The primary aims of this paper are to: (a) describe the female morphology; (b) describe the first instar larval morphology; (c) infer phylogenetic relationships of this species.

Materials and methods

One male and one female of *L. zubovi* were collected on 26 April 2012 (see Results) and both maintained in the laboratory. They were observed during copulation in the field; the female was held in a terrarium and, after oviposition, was killed in 95% alcohol. The mesothoracic muscle of this female was dissected and processed for DNA analysis. Afterwards the specimen was pinned for morphological study and deposited in the Marco A. Bologna collection, Department of Sciences, University Roma Tre. The male specimen is preserved at the Zoological Museum of Moscow, Lomonosov State University.

About 200 eggs were laid (27 April 2012) on leaves of *Senecio* positioned at the bottom of the rearing terrarium (Figure 1), and after 12 days

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Figure 1. Eggs of Lytta zubovi laid on leaves.

(9 May 2012), about 30 first instar larvae hatched and were fixed in 70% alcohol.

Adult morphological analysis was performed using an Olympus SZX12 stereomicroscope equipped with a drawing tube; larval morphology was studied with an Olympus BX51 light Colorview II camera (SIS) and Cell*D image 285 analysis software (SIS) for examining and photographing the cleared specimens mounted on slides in Canada balsam, and an FEI dualbeam FIB/ SEM Helios Nanolab for material mounted on stubs after critical point dehydration and gold sputtering. Terminology of larval structures follows MacSwain (1956), Lawrence (1991) and Bologna and Pinto (2001). For some traits of larval chaetotaxy, we followed notational conventions suggested by Selander (1990). Quantitative data presented in the larval description represent means based on three measured, randomly selected specimens.

Results

Faunistics

The first record of this species refers to eastern Crimea, Arabatskaya Strelka (near the Arabatskaya Fortress); the following two new specimens were collected by V. V. Savchuk at Peschanaya Ravine, 3.5 km northeast from Primorskyi settlement (45° 07'45.44"N-35°31'43.81"E), which is located ca. 12 km northeast of Feodosia.

Ethology

In our previous paper (Bologna & Nikitsky 2010), we described the linear phase of the sexual behaviour. This is confirmed by our new observation (Figure 2), and is similar to that of other genera of the subfamily Meloinae (see Bologna 1991 for a review). During the study of the new pair, a dorsal male posterior phase was observed (Figure 3) but not recorded in detail.



Figure 2. Linear phase of sexual behaviour of Lytta zubovi.



Figure 3. Dorsal phase of sexual behaviour of Lytta zubovi.

Adults are very agile and were observed to move rapidly both in the field and the laboratory. When disturbed, the beetles try to dig rapidly into the litter, which is close to the steppe type. Thanatosis (Figure 4) were also observed. Adults fed (Figure 5) on leaves of herbaceous plants of the genera *Senecio* (Asteraceae)



Figure 4. Thanatosis behaviour of Lytta zubovi.



Figure 5. Feeding activity of Lytta zubovi.

and *Salvia* (Lamiaceae) provided in the terrarium by the observers. No information is available on host plants in the field.

The female oviposited on the bottom of the rearing terrarium and on the plants positioned in the terrarium (Figure 1), as is typical in the laboratory. However, we assume that in the field eggs are laid in a hole excavated in the soil, as in other Meloinae. Courtship sporadically took place after oviposition, but no additional oviposition occurred.

Egg and first instar larva description

Egg. Length = 0.85-0.90 mm. Colour pale yellow, elongate, cylindrical, rounded at both ends, enlarged

antero-apically, maximum width 250 μ m. As in other meloid species (Bologna & Di Giulio 2002, 2003; Di Giulio et al. 2002), a micropylar area is present at the anterior pole of the egg, consisting of a crown of about 20 small, subcircularly arranged micropyles (diameter 30 μ m). Each micropyle consists of a sloping V-shaped chorionic flap overhanging a central cavity.

Habitus. Triungulin campodeiform (Figure 6a-c); body poorly sclerotized, thin and elongate, subparallel sided, with abdomen slightly fusiform. Body length 1.7 mm (from abdominal apex to labrum); head length 0.22 mm (from occipital foramen to clypeolabral suture), maximum width 0.25 mm; diameter of stemmata 16 µm; epicranial stem 53 µm; antennal length 75 µm, antennal seta length 125 µm; prothorax length 0.2 mm, maximum width 0.28 mm; abdominal length 1 mm, maximum width (segments III-IV) 0.27 mm, terminal setae length 0.28 mm; diameter of spiracles: mesothoracic 125 µm, abdominal I 120 µm, abdominal II-VIII respectively from 100 µm to 120 µm. Colour of membranous areas whitish, head, legs and sclerites light brown, distal half of mandibles dark brown. Terga of thorax and abdomen slightly sclerotized and entire; epipleura of thorax and abdomen completely fused with terga displacing spiracles in a dorsal position; sterna of thorax and abdomen membranous, with the exception of abdominal sternum IX and small areas around sternal setae. Cuticle reticulate with transverse polygonal meshes, more



Figure 6. Lytta zubovi, habitus of first instar larva. (a) Dorsal view; (b) left lateral view; (c) ventral view.



Figure 7. Lytta zubovi, head of first instar larva. (a) Dorsal view; (b) left lateral view; (c) ventral view; (d) frontal view.

evident around terga, discal areas of terga smooth. Tergal setae short.

Head. (Figure 7a-d). Rounded, slightly broader than long, greatest width at the level of the stemmata; sides subparallel, broadly curved in posterior half; basal elevation absent; anterior margin subtruncate, slightly rounded laterally. Epicranial suture Y-shaped, complete to antennal base; basal half of frontal arms parallel, widely diverging apically to antennal insertions. Stemmata small, circular, slightly convex, placed dorsolaterally. Frontoclypeal region with 16 setae; apex of frontoclypeus with one transverse row (frontoclypeal row, FCR) of three pairs of setae: FCR1 similar in length to FCR3, FCR2 longer than others; one additional pair of shorter setae present medially between FCR1; one sensory pit between FCR2 and FCR3. Four pairs of setae posterior to FCR along a curved line paralleling arms of epicranial suture (setae 1-4 from posterior to anterior); one sensory pit present between setae 1 and 2; setae 2-4 subequal, 1 shorter. Basal part of each epicranial plate dorsally with four minute setae and one posterolateral pit arranged in a longitudinal row (basal row, BR) parallel to basal stem of epicranial suture; nine pairs of setae and four pairs of sensory pits anterior to BR: one pair of setae close to epicranial stem with one pair of pits placed anteriorly; one pair of shorter setae anterior to BR; seven pairs of variously sized setae (including ocular seta) and two pits encircling stemmata. Ocular sensory pit slightly anterior to stemma; ocular seta anterior to stemma and to ocular pit. Posterior half of ventral surface of each epicranial plate with one medial sensory pit, anterior half with four setae and four sensory pits arranged as follows: two pairs of setae transversally lined at the level of the cardo and two pairs of setae and three pits (one lateral and two medial, near mandibular acetabulum) placed anteriorly; one large pit placed medially, close to base of maxillae. Labrum (Figures 7d, 8a–b) transverse, subrectangular with curved sides, bearing 11 pairs of setae of varying length and one pair of sensory pits.

Antennae (Figure 8c) short, directed anterolaterally; antennomere I short, ring-like with one dorsal sensory pit; II slightly shorter than I, distinctly asymmetrical, longer on anterior side, with three elongate setae (two dorsal and one ventral), one minute dorsal seta and one dorsal pit medial to others, near the sensory appendix; sensory appendix well developed, large, bulbous, hyaline and pointed at tip, positioned on outer side of antennomere II; III slender and elongate, cylindrical, slightly clavate, about as long as I and II together, with a long apical seta (less than three times as long as entire antenna), three elongate, subequal, subapical setae, two lateral (one on outer and one on inner sides) and one dorsal, curved towards apical one; one minute seta near base of apical seta. Mandibles (Figures 7d, 8a-b) robust,



Figure 8. *Lytta zubovi*, first instar larva. (a) Labrum and mouthparts in frontal view; (b) labrum and mouthparts in ventral view; (c) left antenna in dorsal view; (d) left hind tibiotarsus in dorsal view; (e) metanotum and first abdominal tergum; (f) apex of the abdomen in posterodorsal view.

conical-falcate, basal half broad and slightly sclerotized, apical half strongly sclerotized, narrowing and abruptly bending inward, ental margin medially keeled and with two edges: ventral edge smooth; dorsal edge with eight triangular, upcurved, toothlike emarginations; outer margin of mandible with two setae, one sensory pit between them and one pit mesodorsally. Maxillae with stipes about as long as wide and bearing two rows of setae: anterior row with two long setae and one medial pit; posterior row with two shorter setae and one pit between them; mala simple, lobiform, slightly protruding, with 7-8 spiniform setae, none of which is obviously stouter than others; cardo transverse, subrectangular, with one short lateral seta; maxillary palpomeres I and II (Figure 8a-b) short, I only slightly longer and wider; I with one ventral sensory pit; II with two subequal setae, one dorsal on outer side and one small ventral; III subrectangular, subparallel-sided, slightly narrower than II, dorso-ventrally flattened and spoon-like (slightly concave dorsally), 1.8 times the length of I and II together, with one basal and dorsolateral seta (inner side) and one ventrolateral pit (outer side); apex of palpomere III convex and membranous, with a sensorial area composed of about 30 conical and subequal sensilla, one medial, larger and cylindrical with ring-like base, and one very small close to medial; outer side of palpomere III with one slender digitiform sensillum. Gula without setae; submentum, mentum and prementum poorly sclerotized; submentum with two setae; mentum with two shorter setae and four pits; prementum with four setae, two short and basal and two longer and apical. Labial palpomeres (Figure 8a-b) narrow

and cylindrical, palpomere I slightly asymmetric (outer side longer) and one small ventral seta; palpomere II about twice as long as I, with a lateral pit and an apical circular slightly swollen sensorial area composed of 10 conical and subequal sensilla, one larger medially placed and cylindrical with large ring-like base, and one very small close to medial.

Thorax. Segments transverse, slightly broader than head; prothorax slightly wider than meso- and metathorax; margins of each thoracic segment rounded. Ecdysial line complete on pronotum, incomplete on mesonotum and absent on metanotum. Anterior margin of pronotum membranous; each half of pronotum with 12 short setae and five pits symmetrically placed along three transverse, subparallel rows; anterior row (AR) with four setae and four pits; medial row (MR) irregular, with four setae and one pit; posterior row (PR) with four setae and two pits; prosternum with three pairs of medial setae arranged longitudinally and one pair anterior. Mesonotum subrectangular, slightly narrower and shorter than pronotum, with AR composed of four pairs of setae; MR with two lateral setae and one pit; PR with four setae and two pits; three pairs of medial setae on mesosternum, anterior pair extremely short. Metanotum (Figure 8e) subequal to mesonotum, more rounded on sides; setae of metathorax similar in number, position and relative dimensions to those of mesothorax.

Legs. Slender, without lanceolate setae: coxa conical and elongate, with one small apical seta, four elongate medial setae, transversally arranged, gradually decreasing in length from base to apex, three minute basal setae and one pit; trochanter with four apical setae and five pits; femur not enlarged in the middle, slightly shorter than tibiotarsus, with six setae and one pit, longest ventral femoral seta much shorter than femur; fore femora slightly more robust and shorter than others; tibiotarsi and claws increasing in length from pro- to metathorax; tibiotarsi slightly tapered at apex and with five longitudinal rows of 5-7 spinelike, moderately long setae; claw (Figure 8d) conical-falcate, thin, acute and slightly curved at apex, with two setae of different length inserted at different levels near base (apical distinctly longer than basal).

Spiracles. (Figures 6a–b, 8e). Round, internally papillate, with a small opening. Peritreme round and flat, slightly elevated and with about 10 projections encircling opening. Mesothoracic spiracle anterolateral in position, abdominal spiracles medial in position, all spiracles distinctly dorsal and subequal in diameter.

Abdomen. Slightly fusiform, approximately 1.8 times as long as thorax; segments II-VI subequal,

maximum width at III; segment IX smallest and suboval; sternal abdominal area membranous, except for sternum IX. Tergites distinctly transverse, with rounded sides, with three transverse rows of setae on each half of tergum as follows: AR with three minute setae (four on tergum I, Figure 8e) and one pit; MR with three setae at level of spiracle, two medial and one lateral (long in segments II-VIII); PR with seven setae of various size and one pit. AR and MR of tergum IX with same setation as segments II-VIII, PR composed of five elongate setae, longer than those of segments II-VIII, and one pair of very long setae (caudal setae), about as long as the last two abdominal segments combined. AR and MR of sterna I-IX with one pair of minute medial setae each, PR with four pairs of setae (three pairs on segment I), two outer pairs (one pair on segment I) very long (except on segment IX). Pleurites completely fused with tergites, displacing spiracles dorsally. Segment X membranous (Figure 8f), divided by a transverse anal fold in two parts: dorsal part semicircular with six extremely small setae transversally arranged, ventral part (pygopod) longitudinally divided into two lobes, moderately produced.

Female morphology

The description of *L. zubovi* (Bologna & Nikitsky 2010) included only mention of the sexual dimorphism of this species, without a detailed account of female morphology.

The habitus of the female is represented in Figures 2 and 3. The female is similar to the male except for the following morphological details: (1) antennomere III visibly longer than IV; (2) median line of pronotum indistinct; (3) base of pronotum with very indistinct sinuosity medially; (4) scutellar setae brownish; (5) metasternum shorter; (6) elytra short, covering less than the anterior half of the abdomen, last four abdominal tergites not covered, terga distinctly rugose; (7) metathoracic wings absent; (8) foretarsi with ventral pad of longer and denser setae; (9) metatibiae slightly curved as mesotibiae; (10) posterior margin of penultimate sternite greatly curved and emarginated; (11) apical sternite with rounded posterior margin.

Discussion

The placement of the Crimean species in the tribe Lyttini, genus *Lytta*, is well supported by larval morphology; thus, any suspicion about a possible assignment to Meloini is resolved.

The larva definitely belongs to the tribe Lyttini based on the combination of the following morphological characters, considered diagnostic by MacSwain (1956): (1) epicranial suture complete to the antennal base; (2) very short antennomere II; (3) sensory appendix well developed on antennomere II; (4) terminal antennal seta more than twice as long as the entire antenna; (5) absence of modified seta at apex of mala; (6) maxillary palpomere III subparallel sided; (7) fusion between pleurites and tergites dorsally displacing the abdominal spiracles.

In particular, this larva has in common with other Lytta several characters such as: (1) head rounded, slightly wider than long; (2) weakly sclerotized gula; (3) boundary between clypeus and frons marked by a row of eight setae; (4) antennal sensory appendix bulbous, large, apically pointed; (5) tergites with posterior marginal row of 10 setae; (6) spiracle on abdominal segment I subequal to mesothoracic spiracle; (7) abdominal spiracles II-VIII subequal to I. However, it is worth noting the following characters that differ from the known Lytta larvae and that possibly support a distinct subgeneric assignment: (1) first antennal segment longer than the second (it is shorter than the second in Lytta); (2) antennal seta less than 3 times longer than the entire antenna (in Lytta 3-6 times longer than entire antenna); (3) ecdysial line complete on pronotum, incomplete on mesonotum and absent on metanotum (it is complete on all thoracic segments and on first abdominal tergite in Lytta, except L. vescicatoria, see MacSwain 1956); (4) abdominal sterna VIII and IX membranous, unsclerotized (sclerotized in Lytta).

Preliminary molecular data also support the placement of *L. zubovi* among Lyttini. In fact, in the framework of a molecular phylogenetic study of the tribe Lyttini (Pitzalis et al., in preparation), we compared the sequence of 16S mtDNA of *L. zubovi* with that of 65 other taxa (Bologna 1991; Bologna & Pinto 2001; Bologna et al. 2008). In all trees, *L. zubovi* is included in the clade of the genus *Lytta*, as the sister of *L.* (*Poreospasta*) auriculata Horn, 1870.

The form of sexual dimorphism in *L. zubovi* is characterized by brachyelytrous and apterous females, and holelytrous, fully winged males. Additionally, the female is considerably larger than the male. Although uncommon in the Lyttini, brachyelytry and complete absence of hind wings, believed to be independently evolved, do characterize a single other species of *Lytta*, one *Lydomorphus* Fairmaire, 1882, the genera *Berberomeloe* Bologna, 1989, *Trichomeloe* Reitter, 1911 and *Parameloe* Denier, 1933, as well as several additional genera in other lineages of Meloidae (see e.g. Bologna 1991; Bologna & Pinto 2002; Bologna & Nikitsky 2010 for a wider discussion). In the last three genera, however, both sexes share these reduced features. The only other meloid group with the same degree of sexual dimorphism in elytral and hind wing development as occurs in *L. zubovi* is in the North American genus *Cordylospasta*.

Due to its distinct adult morphology, we suspect that *L. zubovi* should eventually be referred to a new subgenus of *Lytta*. However, such action will require the study of a large number of species from all subgenera, particularly those from central and eastern Asia.

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