Morphology of a new blister beetle (Coleoptera, Meloidae) larval type challenges the evolutionary trends of phoresy-related characters in the genus Meloe

A. Di Giulio,1 M. Carosi,1 R. Khodaparast,2 M.A. Bologna1

1Department of Sciences, Roma Tre University, Rome, Italy; 2Young Researcher Club, Kazerun Branch, Islamic Azad University, Kazerun, Iran

Abstract

The discovery of some specimens of a new first instar larval type in blister beetles, collected in Iran on Anthophora bees, confirms the existence of repetitive and parallel trends in morphological specialization to phoresy in distinct lineages of Meloidae and in particular in the subfamily Meloinae. The new Iranian larva, herein described and illustrated, shows several characters and a peculiar phoretic strategy that closely parallel that of the Meloe subgenus Lampromeloe, with similar modifications of the fronto-clypeal setae into strong lanceolate spines used to pierce the intersegmental membranes of the bees. Both parallel and shared derived evolution of these characters seem possible.

Key words: Meloe, Lampromeloe, triungulin, phoresy, SEM, Iran.

Introduction

The Meloidae, or blister beetles, are a beetle family with about 120 genera and 3000 species, mostly tropical or subtropical and related to open habitats, almost cosmopolitan (absent only from New Zealand, Antarctica and most Polynesian islands; Bologna et al., 2013). Members of this family are morphologically very heterogeneous, but adults are well characterized by the head clearly separated from the pronotum by a neck and by bilobed claws. Adults are mostly diurnal and phytophagous, feeding on flowers and/or leaves of several plant families, while larvae are predaceous, feeding on the provisions and immature stages of various aculeate Hymenoptera (mostly Apoidea) or on Acridoidea egg pods.

Blister beetles are largely known in the biological literature because of their importance for applied science (biological control of grasshoppers, pharmacology, veterinary and agricultural problems) as well as their distinctive biology (hypermetamorphic development, parasitoid larval habits, defensive attributes, and diverse courtship behavior). The most recent extensive review of meloid systematics, bionomics and biogeography was published by Bologna (1991), but other syntheses were recently published by Bologna et al. (2010) and, specifically considering larval morphology and biology, by Bologna & Di Giulio (2011). The systematics of the family is well studied: two phylogenetic studies, based on morphology (adult and larva) and behavior (Bologna & Pinto, 2001), and on molecular evidence (Bologna et al., 2008), respectively, differ in considering four (Eleticinae; Nemognathinae; Tetraonycinae; Meloinae) or only three subfamilies (Eleticinae; Nemognathinae; Meloinae, including Tetraonycini). We adopted here the classification of Bologna et al. (2008).

The rich literature of the last century on larval morphology and biology of this family was reviewed by Bologna & Di Giulio (2011) who synthesized critically the information on the diversified radiation in both morphology and biology, proposing a new scenario which amended several previous hypotheses. In particular, based on the discovery of
primitive, non-parasitic larvae in the subfamily Eleticinae (Pinto et al., 1996; Bologna et al., 2001), hypermetabolobly must be considered a derived condition of Nemognathinae and Meloinae rather than a synapomorphy of the whole family. The evolution of parasitism and hypermetabolobly supported a great adaptive radiation in larval morphology and biology. At least in some meloid lineages such radiation seems connected to phoresy, a strategy typically related to dispersion of scarcely movable life stages or to specialized parasitic behaviors. The phoretic strategy of the meloid first instar larvae on bees represents a passive transport from flowers, where eggs are laid (some Nemognathinae) or where triungulins climb after their hatching from the ground (some Meloinae) to the host nest. It is now acknowledged that in Meloidea, phoresy evolved several times (Bologna & Pinto, 2001; Bologna et al., 2008), as a derived condition, in both Nemognathinae and Meloinae. A gradient of specialization to phoretic habits and specialized parasitic biology is clearly recognizable in triungulins of the nemognathine tribes (Di Giulio et al., 2010); Palaestrini, Horinii and Nemognathini, especially concerning the morphology of legs, mouthparts and abdominal spiracles. The subfamily Meloinae shows a similar, though independent, gradient of specialization to phoresy, with repeated and complex events of trophic and dispersal adaptations (Bologna & Pinto, 2001). Starting from a typical campodeiform larva actively searching the host bee nest, the adaptation to phoresy apparently evolved separately at least five times in this subfamily, as well as trophic specialization to feed on termite eggs (Di Giulio et al., 2003), but also on Masaridae and Vespidae nests (Bologna, 2003), or on Acridoidea egg batches in the tribes Mylabrini and Epicautini (for a synthesis see Bologna, 1991).

Focusing on the tribe Meloini, the Old World genus Meloe Linnaeus, 1758, represents a paradigmatic study case of the radiation in larval morphology. The genus includes ca. 155 species in 16 subgenera (Bologna, 1991; Bologna & Pinto, 1998; Di Giulio et al., 2002). All subgenera are represented in the Old World, the region harboring the vast majority of species (ca. 130). In the New World only the nominate subgenus, Treiodous Bugès, 1869, and, on the Aleutian Islands, Eurymeloe Reitter, 1911, occur.

The first instar larvae of 12 out of the 16 subgenera have been described: information on subgenera and references are reported in Table 1 (Di Giulio et al., 2002; 2013). The only subgenus for which larvae remain unknown include Alveomeloe Prisipinova, 1987, Chromie Reitter, 1911, Desertmeloe Kaszab, 1964, and Meloenellus Reitter, 1911, all from Central Asia.

Both the different degree of morphological specialization related to phoresy and the presence of progressive trends of complexity in morphological traits adapted to phoresy have been partially analyzed in the nominate subgenus and in Treiodous (Pinto & Bologna, 1993; Bologna & Pinto, 2001; Bologna & Di Giulio, 2011). Larval morphology and taxonomy of the subgenus Lampropeloe Reitter, 1911 have never been revised, even though the first instar larvae of two species have been described (namely M. cavanis Petagna, 1819 and M. variegatus Donovan, 1793; see Table 1 and Results). The main larval apomorphy of this subgenus is the modification of the anterior fronto-clypeal setae into nine strong lanceolate spines, four of which elongate, directed forward and inserted medially at the anterior apex of the head (Figure 1d-f). These spines form an anterior spear used to pierce the thin intersegmental abdominal membranes of the bees and act in combination with the modified trident-shaped claws and strongly curved mandibles to remain steadily attached to the host. As far as known, this is a unique phoretic strategy not only for Meloidea, that use only their modified mandibles to seize one or a few hairs of the host (Bologna, 1991; Bologna & Pinto, 2001), but also considering other phoretic insects.

The recent discovery of an unidentified Nemognathinae first instar larva from Iran, with phoretic adaptations very similar to those of Lampropeloe, provides the opportunity to explore deeply the conflicting

Table 1. Meloe subgenera with described larvae (left column), alphabetically ordered; Meloe species, divided per subgenera, with described larvae (middle column); main bibliographic references for larval taxonomy for each subgenus of Meloe (right column).

<table>
<thead>
<tr>
<th>Meloe subgenus</th>
<th>Meloe species</th>
<th>Main references</th>
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<tr>
<td>Afromeloe Schmidt, 1913</td>
<td>caffer Péringuey, 1886</td>
<td>Bologna &amp; Pinto (1998)</td>
</tr>
<tr>
<td>Coemeloe Reitter, 1911</td>
<td>taucius Rossi, 1792</td>
<td>Cros (1929); Di Giulio et al. (2013)</td>
</tr>
<tr>
<td>Eurymeloe Reitter, 1911</td>
<td>affinis Lucas, 1832; aperennis Bologna, 1889; apius Reitter, 1885; brevicollis Panzer, 1773; coronius Marseul, 1876; flavicollis Wollaston, 1854; ganglbaueri Apfelbeeck, 1905; glazunovi Plinianoki, 1910; mediterraneus C. Müller, 1925; niger Marsham, 1802; scabriusculus Brandt and Erichson, 1832</td>
<td>Bologna et al. (1989); Di Giulio et al. (2013)</td>
</tr>
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<td>Lampropeloe Reitter, 1911</td>
<td>caensis Petagna, 1819; variegatus Donovan, 1793</td>
<td>Cros (1927); present paper</td>
</tr>
<tr>
<td>Lusinomeloe Reitter, 1911</td>
<td>oliviervi Chevrolat, 1833</td>
<td>Bologna &amp; Pinto (1995)</td>
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<tr>
<td>Listromeloe Reitter, 1911</td>
<td>hungaricus Schrank, 1776</td>
<td>Cros (1930); Bologna &amp; Pinto (2001)</td>
</tr>
<tr>
<td>Meloe Linnaeus, 1758</td>
<td>americanus Leach, 1815; angusticolis Say, 1824; auricolus ? Marseul, 1877; campanicolis Pinto &amp; Selander, 1976; coarctatus Motschulsky, 1858; dianeilla Pinto &amp; Selander, 1970; daguesi Champion, 1891; or nebulosus Pinto &amp; Selander, 1970; hottenotus Péringuey, 1886; impressus Kirby, 1837; menokono Kono, 1936; niger Kirby, 1837; occultus Pinto &amp; Selander, 1970; proscarabaeus Linnaeus, 1757; rhodesianus Péringuey, 1904; strigulosus Mannerheim, 1852; violaceus Marsham, 1802</td>
<td>MacSwain (1956); Pinto &amp; Selander (1970); Kifune et al. (1973); Bologna &amp; Pinto (1998)</td>
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<td>cicatricosus Leach, 1815; ruifiventris Germar, 1832</td>
<td>Selander (1989); Lückmann &amp; Scharf (2004)</td>
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<tr>
<td>Mesomeloe Reitter, 1911</td>
<td>coelatus Reiche, 1857</td>
<td>Di Giulio et al. (2002)</td>
</tr>
<tr>
<td>Tapromeloe Reitter, 1911</td>
<td>erythrocnemis Pallas 1782</td>
<td>Bologna &amp; Pinto (1992)</td>
</tr>
<tr>
<td>Treiodous Bugès, 1869</td>
<td>afer Bland, 1864; autumnalis A.G. Olivier, 1797; barbarus LeConte, 1861; laeveis Leach, 1815</td>
<td>Pinto &amp; Selander (1970); Pinto &amp; Bologna (1995)</td>
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character evolution (phylogenetic traits and convergence related to phoresy) in Meloidae.

Aims of this paper are: i) to describe and illustrate a new Meloinae sp. (incertae sedis) first instar larval type and its peculiar phoretic strategy; ii) to comparatively analyze by SEM the morphological characters of the new meloine larva from Iran and those of the Meloe (Lampromeloe) larva; iii) to discuss the evolutionary implications of its placement in the genus Meloe, inside or outside the subgenus Lampromeloe, versus other possible alternative scenarios; iv) to discuss the general problem of convergent adaptation and trends of progressive derived condition in several morphological features of phoretic blister beetles, especially in the genus Meloe.

Figure 1. First instar larvae of Meloinae sp. (incertae sedis) and Meloe (Lampromeloe). Meloinae sp. (incertae sedis) (Iran): head capsule in dorsal (a), left lateral (g) and ventral (j) views; detail of clypeo-labral spines (d). M. (Lampromeloe) cavensis: head capsule in dorsal (b), left lateral (h) and ventral (k) views; detail of clypeo-labral spines (e). M. (Lampromeloe) variegatus: head capsule in dorsal (c), left lateral (i) and ventral (l) views; detail of clypeo-labral spines (f). Scale bars: a, j=100 µm; b, c, k=300 µm; d=40 µm; e, f, g=50 µm; h, i, l=200 µm.
Materials and Methods

The description of first instar larvae is based on the following material preserved in 70% ethanol and/or mounted on slides or on stubs. If not indicated otherwise, larvae and adults are kept at Roma Tre University, Rome, Italy, in the M.A. Bologna collection. Sampled localities are ordered by country, from north to south and from west to east.

Adults of Meloe (Lampromeloe) were reared at 24-25°C in a thermostatic chamber with photoperiod control (LD 12:12 h); females oviposited in a static chamber with photoperiod control (LD 12:12 h); females oviposited or mounted on slides or on stubs. If

Meloe (Lampromeloe) cavensis Petagna, 1819


The first instar larva of M. cavensis was described by Zanon (1919, 1922); Cros (1919, 1927, 1941) from Libya and Algeria. The specimens we examined from Italy and Palestine do not differ from these descriptions.

Meloe (Lampromeloe) variegatus Donovan, 1793


The first instar larva of M. variegatus was described by Brandt & Ratzenburg (1833), Assmus (1865), Cros (1929, 1941), Hachfeld (1931), Blair (1943), van Emden (1943), Krzyzanska (2000), Topolska et al. (2001), and Rückmann & Scharf (2004) from United Kingdom, Germany, Slovakia, Poland, and Algeria. Our specimens from Turkey and Algeria do not differ from these descriptions. We are able to confirm what Cros (1941) noted about the Maghrebian triungulins differing from the Euro-Anatolian ones because of a greater sclerotization of the body, and the head being wider on temples and less narrowed in front.

Meloinae sp. (incertae sedis)

- Slide M464 (2 triungulins), vial 609 (3 triungulins), stubs 148 and 149 (1+1 triungulins). Triungulins: Iran, Fars province, Shiraz, Zangench, 2128 m a.s.l., 25/31.iii.2012, leg. R. Khodaparast, on 59 females (100% parasitized) of the anthropid bee Anthophora (Pyganthophora) atroliaba Lepeletier, collecting honey on Matthiola Alton (Brassicaceae).

Morphological analysis was performed by an Olympus BX51 light microscope equipped with a Colorview II camera (SIS) and Cell*D image 285 analysis software (SIS) to examine and photograph the cleared specimens mounted on slides in Canada balsam, and a 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 & Pinto (2001). For some traits of larval chaetotaxy we followed notational conventions as suggested by Selander (1990).

Results

Description of Meloinae sp. (incertae sedis)

first instar larva of from Iran

Habitus and measures

Triungulin campodeiform (Figure 2a,d,g), distinctly tapered at the base of the abdomen, with maximum width on pronotum, characterized by large, oval head (Figure 1a,j), antennae posterolaterally directed (Figure 1a,i), long and stout legs (Figure 2a, d, g), fusiform abdomen (Figure 2a,g), elongate medial caudal setae (Figure 2a). Color pale yellow, weakly sclerotized.

Measures (in mm, based on two slide-mounted specimens, the first measure reported in the following always refers to the same specimen). Body length: 1.63-1.82; Head length: 0.25-0.26; head width: 0.34-0.37; Diameter of stemmata: 0.020-0.19; Epicranial stem length: 0.03; Length of antennomeres, f: 0.023-0.027, ii: 0.058-0.062, iii: 0.054-0.058; length of apical antennal seta: 0.19-0.20; Prothorax length: 0.23-0.25; prothorax width: 0.39-0.42; Mesothoracic spiracle maximum width: 0.036-0.045; abdominal spiracle I maximum width: 0.051-0.053; Length of abdomen: 0.9-1. Length of caudal setae: 0.31-0.36.

Head

Cephalic capsule (Figure 1a,g) transverse, 1.5 times wider than long, ovoid, widest behind stemmata; anterior margin broadly rounded. Stemmata latero-dorsal, small, slightly prominent, set at middle level of head. Transverse ridge at base of vertex present, partially covering the anterior margin of pronotum. Epicranial suture (Figure 1a) Y-shaped, with very short stem, dividing into arms at the level of transverse ridge, near base of head; arms basally V-shaped, clearly diverging from base, slightly sinuate at the level of stemmata, diverging towards the edge of frontoclypeus and ending just beyond the base of antennae. Frontoclypeus (Figure 1a,g) slightly concave between arms of epicranial suture. Frontoclypeal region (Figure 1a) with 19 setae: frontoclypeal row (FCR) with 5 pairs of setae and one impair seta in the middle: FCR1 short, normally trichoid, the others strong, spiniform, lanceolate, sharp at tip, antero-dorsally directed, forming a typical 9-spined anterior spear (Figure 1d); spines of the anterior spear alternating in size, long/short (4 long and 5 short, the impair short); sensory pit anterior to FCR1; frontal setae with very short stem, dividing into arms at the level of transverse ridge, near base of head; arms basally V-shaped, clearly diverging from base, slightly sinuate at the level of stemmata, diverging towards the edge of frontoclypeus and ending just beyond the base of antennae.

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dorsolateral pit; sensory appendix on segment II well developed, conical, lateroventral (outer side); segment III slender, slightly shorter than II, 2.5 times longer than I; III with a long apical seta (approximately 1.3 times longer than entire antenna), 3 long subapical setae, 2 lateral (1 on outer and 1 on inner side) and 1 dorsal in position, 1 minute seta near the base of apical seta, 1 lateral and 1 sub-basal pit. Mandibles (Figure 3g) elongate, directed forward, moderately curved, narrowed toward apex, with broad and prominent base; outer margin of mandible with 2 setae, 1 sensory pit between them and 1 pit dorsolaterally; inner and outer surfaces smooth, ventral surface mediately with 10-12 teeth that represent the apices of rounded notches. Maxillae (Figures 1j and 3g) with mala simple, lobiform, slightly protruding, with about 10 spiniform setae; cardo transverse, small, with 1 minute seta; maxillary palpi with joints as follows: I extremely short, with 1 ventral pit; II slightly longer than I, with 2 ventrolateral subequal setae, 1 on the external and 1 on the internal side; III about twice as long as segment I and II together, asymmetrical (lateral side longer than medial side), with 1 dorsolateral seta (inner side); apex of segment III obliquely truncate, with a large, sensory area composed of 1 apical, elongate, spiniform sensillum surrounded by several shorter papillae; outer side with 1 slender digitiform sensillum. Gulamentum (Figure 1j) weakly sclerotised with 2 very long anterior setae. Prementum (Figure 1j) and mentum fused, with 1 pair of basal setae lined with 1 pair of external sensory pits; 1 pair of ligular setae present dorsally between insertion of palpi and one pair ventrally; labial palpi well developed, with segment II longer than I, with 1 distinct cylindrical, bluntly tipped, apical sensillum inserted in a prominent base and surrounded by shorter subapical papillae. Labrum (Figure 1j) narrow, fusiform, transverse, with angulated sides, not visible dorsally and distinctly separated from frontoclypeus, with 18 setae: 10 setae and 2 pits along the anterior edge and 8 setae and 4 pits in middle of labrum.

**Thorax**

Segments transverse and subrectangular (Figures 2a and 4a): prothorax broader than head, meso- and metathorax about as wide as head; margins of pronotum almost straight and subparallel, meso- and metanotum campaniform, with margins slightly rounded. Ecdysial line (Figure 2a) well marked, complete on pro- and mesonotum, vestigial or absent on metanotum. Pronotum about 2 times wider than long; each half of pronotum with 13 small setae and 5 pits symmetrically placed along 3 transverse, subparallel rows; anterior row (AR) with 5 setae and 2 pits; medial row (MR) with 3 setae; posterior row (PR) with 5 setae and 2-3 pits; prosternum with 3 pairs of medial setae, the two anterior pairs similar in length, posterior pair longer than others. Mesonotum slightly shorter than pronotum; AR with 2 setae grouped with one pit mesodorsally, 2 setae laterally, anterior to the spiracle. MR with 5 setae (1 shorter near the spiracle); PR with 5 setae and 2 pits; 3 pairs of medial setae on mesosternum, the anterior pair extremely short. Setae of metathorax similar in number, position and relative dimensions to those of mesothorax. Legs elongate and stout (Figure 2a,d,g); coxa short and broad, with 4 elongate setae, transversally arranged, 4-5 minute basal setae and 1 pit; trochanter with 2 moderately long setae and 4-6 pits; femur not swollen,  

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![Figure 2. Comparative habitus (in dorsal, lateral and ventral view, from top to bottom) of first instar larvae of Meloinae sp. (incertae sedis) (Iran) (a, d, g), *Meloe (Lampromeloe) cavensis* (b, e, h), and *Meloe (Lampromeloe) variegatus* (c, f, i). Scale bar=500 µm.](image-url)
Figure 3. First instar larvae of Meloinae sp. (incertae sedis) and Meloe (Lampromeloe). Meloinae sp. (incertae sedis) (Iran): left ventral parietale of head capsule (a), with detail of microsculpture (d); mouthparts in ventral view (g); right antenna in ventral view (j). M. (Lampromeloe) cavensis: left ventral parietale of head capsule (b), with detail of microsculpture (e); mouthparts in ventral view (h); right antenna in ventral view (k). M. (Lampromeloe) variegatus: left ventral parietale of head capsule (c), with detail of microsculpture (f); mouthparts in ventral view (i); right antenna in ventral view (l). Scale bars: a, g, i, j=50 μm; b, c, h, k=100 μm; d, e, f=10 μm.
with 7 setae and 1 pit, the longest ventral femoral seta much shorter than femur; tibiae and claws slightly increasing in length from pro- to metathorax; tibiae tapered at apex and with 4 longitudinal rows of 5-6 moderately long setae; claw (Figure 4g) thin and elongate, curved basally inward, somewhat spatulate, acute at apex, flanked by 2 long and spini-
form setae, forming a slender trident.

**Abdomen**

Abdomen is fusiform with transverse, rectangular tergites (Figure 2a,g); maximum width at segment III-IV; sternites entire. Mediotergites with 3 transverse rows of setae; their arrangement on each half of tergites as follows: AR with 2 minute setae (4 on tergite I, 2 medial and 2 lateral) and 1 pit, MR (at the level of spiracles) with 2 small setae, PR with 8 setae and 1 pit, the 4 lateral (including 2 on laterotergites) setae distinctly elongate on abdominal segments II-VIII; tergite IX with 1 extremely elongate pair of setae on PR (medial caudal setae), which are shorter than the last four abdominal segments combined. AR of each sternite with 2 pairs of minute setae, MR absent and PR with 8 elongate setae, each slightly different in length. Sternite IX with 1 small seta on AR; 6 long setae on PR. Abdominal apex (segment X or pygopod) apically membranous, transversally divided in two parts: dorsal part semicircular with 6 extremely small setae transversally arranged, ventral part longitudinally divided in 2 lobes, moderately produced, with two pairs of setae along the posterior margin.

**Spiracles**

Marginal ring (peritreme) of mesothoracic and abdominal spiracles I distinctly protruding (Figure 4j). Mesothoracic spiracle elliptical, obliquely placed on laterotergite. Abdominal spiracle I (Figure 4j) dor-
sally placed on laterotergite, transversely elliptical; abdominal spiracles II-VIII small, round, subequal and slightly protruding, laterally positioned, placed on laterotergites, decreasing in size from II to VIII.

**Morphological comparison of the new larva with Meloe (Lampromeloe) first instar larvae**

The new Iranian first instar larva differs from the triungulins of all other Meloidea, except for those of the subgenus Lampromeloe, in having 9 modified frontoclypeal setae (5 short and 4 long, alternate, Figure 1d-f), lanceolate, strong and pointed, forming an anterior spear, com-
bined with the presence of mandibles ventrally denticulate, with teeth representing the apices of longitudinally serial, rounded indentations, and in the integument of parietalia having imbricate squamiform microsculpture (Figure 3d-f). The first instar larvae of M. cavensis and M. variegatus are very similar in most characters; however, triungulins of M. cavensis differ from those of M. variegatus in smaller size (length: ca. 3 mm; width: 0.4 mm; length of long caudal setae: 0.7 mm; Bologna, 1991), the presence of only two elongate and two short medial caudal setae, and a more triangular and pointed head.

The new larva from Iran differs from first instar larvae of both species of Lampromeloe (Meloe cavensis (Figures 1b,e,h,k; 2b,e,h; 3b,e,h,k and 4b,e,h,k) and M. variegatus, (Figures 1c,f,i,l; 2c,f,i; 3c,f,i,l; 4c,f,i,l)) by its smaller size (body length (BL) 1.73 mm vs BL of M. cavens-
is and M. variegatus: 4 mm); scarce sclerotization (es strong scleroti-
ization, especially of the head); subrectangular head (es triangular); frontal arms sinuate (es straight and parallel); antennomere II clavate with conical sensorium (es cylindrical, without sensorium); mandibles long and moderately curved, directed forward with smooth cutting edge (es short and abruptly curved, ventrally directed with microserrate cut-
ting edge); maxillary palpomere III wide and flattened (es cylindrical); thoracic sternum without (es with many elongate and spiniform) addi-
tional setae; femora only slightly swollen (es distinctly swollen); tibiae robust, regularly tapering to apex (es thin, cylindrical, stick-like); mesothoracic and abdominal sternite I large and distinctly protruding (es small and slightly protruding); claws very slender and slightly spatulated (es wide and distinctly spatulated).

### Table 2. Characteristics of some features related to phoresy in the triungulin of 12 Meloe subgenera.

<table>
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<th>Subgenus</th>
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<th>Labrum</th>
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<td>Regular</td>
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<td>Not swollen</td>
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<td>Meloegonius</td>
<td>Apical</td>
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<td>Not swollen</td>
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<td>Listromeloe</td>
<td>Apical</td>
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<td>Treiodus</td>
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<td>Meloe</td>
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<td>Lampromeloe</td>
<td>Apical</td>
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Figure 4. First instar larvae of Meloinae sp. (incertae sedis) and Meloe (Lampromeloe). Meloinae sp. (incertae sedis) (Iran): pro- and mesonotum in dorsal (a) and ventral (d) view; left mesothoracic claw, dorsal view (g); left half of abdominal segments I-III, dorsolateral view (i). *M. (Lampromeloe) caensis*: pro- and mesonotum in dorsal (b) and ventral (e) view; left mesothoracic claw, dorsal view (h); left half of abdominal segments I-III, dorsolateral view (k). *M. (Lampromeloe) variegatus*: pro- and mesonotum in dorsal (c) and ventral (f) view; left mesothoracic claw, dorsal view (i); left half of abdominal segments I-III, dorsolateral view (l). Scale bars: a, j, k, l=100 µm; b, c, i=400 µm; c=300 µm; d=200 µm; g=40 µm; h, i=50 µm.
Larval behavioral notes

The new Iranian triungulins have been collected in number (Materials and Methods) on Anthophora (Pyganthophora) atrotalba Lepeletier (Figure 5a) on flowers, attached by the modified frontal setae, to the intersegmental abdominal membranes of the bee (Figure 5b).

This phoretic strategy is exactly the same of that of first instar larvae of Lampromeloe (M. caensis and M. variegatus), a derived behavior, unique among blister beetles and phoretic insects in general. After piercing the host abdomen, some bee individuals are damaged and have neurologic problems (Bologna, 1991). Host bees of M. caensis and M. variegatus are Anthophora nigrocincta Lepeletier, A. pennata (Lepeletier), and A. femorata (Olivier) respectively. Rare cases of parastisism on honey bee (Apis mellifera Linnaeus) were also described in both species. Other Apoidea are reported as vectors but they are not confirmed hosts (Bologna, 1991).

Discussion and Conclusions

The body shape and most other characters of the new larva are typical of the subfamily Meloinae, and strongly differ from those of the other two subfamilies, Eleticinae and Nemognathinae (Bologna & Pinto, 2001; Bologna et al., 2008). For this reason, its assignment to a phoretic lineage of the Meloinae seems to be obvious. It has been demonstrated, however, that, in this subfamily, phoresy has evolved independently at least five times (Bologna et al., 2008), with convergent, phoresy-related adaptive characters. Twenty Meloinae genera are distributed in Iran, only 16 in Fars and in western Iran, while four are spread only in the extreme desert coastal area or in the north east semi-desert regions. The first instar larva of 13 out of 16 genera is well known, and the triungulins of only three Lyttini genera (Calydus Reitter, 1896; Eolydus Denier, 1913; Syrionalyta Kaszab, 1962) remain unknown.

Based on current knowledge (Bologna & Pinto, 2001), the dorsal position of the first abdominal spiracle on the tergite would refer the new larva to the tribe Meloini, genus Meloe. Although the occurrence of this character in other Meloinae (e.g. Cabalia, Bologna & Di Giulio, unpublished data) challenges its general validity, and its association with multiple phoretic adaptations (mouthparts, antennae, legs, claws, etc.) could also potentially characterize other Meloini genera, in our opinion the assignment of the Iranian larva to the genus Meloe is the most likely and parsimonious solution at the moment, waiting for more records from the same locality.

Within Meloe, we found that the new larva shares at least three strong similarities with the greatly specialized subgenus Lampropeloe: i) presence of nine homologous spiniform and directed forward frontoclypeal setae on medial anterior part of the head; ii) mandibles ventrally denticulate, with teeth representing the apices of longitudinally serial, rounded indentations; iii) parietalia with unique imbricate, squamiform microsculpture. Concerning the first, it is worth stressing that the presence of this anterior setal spear underlies an highly specialized phoretic strategy of piercing the abdominal intersegmental membranes of the bee, strengthening the action of legs and mandibles to remain more strongly attached to the host. This phoretic strategy, as well as the anterior spines, the indentations on the ventral side of the mandibles and the head pattern of microsculpture are found, until now, only in Lampromeloe. Although these derived characters seem to be potential synapomorphies of the new Iranian larva and Lampropeloe, several other characters of the new larva are in a plesiomorphic, or in a completely different derived condition, and would suggest to have followed a different evolutionary pathway from that of Lampropeloe, as well as from other Meloe subgenera.

Bologna & Pinto (2001) pointed out that phoresy of triungulins is a strategy evolved independently and repeatedly, at least 6 times in the Meloidae family in both Nemognathinae and Meloinae. In the first subfamily, the tribe Stenoderini is probably not phoretic, while the three remaining tribes (Palaestrini, Horini, Nemognathini) are phoretic, showing a progressive trend of morphological specialization (Di Giulio et al., 2010). Within Meloinae, larval phoresy evolved independently in some genera of Lyttini, Mylabrini and Meloini. In this last tribe, the genus Physomeloe Reitter, 1911, is not phoretic (Bologna & Aloisi, 1994; Bologna & Pinto, 2001; Bologna et al., 2008), while the genus Meloe

Figure 5. Anthophora (Pyganthophora) atrotalba Lepeletier, habitus in lateral view (a), parasitized by triungulins of Meloinae sp. (incertae sedis) and Meloe (Lampromeloe) sp., attached by the modified frontal setae (b, arrows) into the ventrolateral intersegmental abdominal membranes of the bee.
includes only phoretic species in 12 examined out of 16 subgenera.

As pointed out in the previous literature on larval morphology of *Meloe* (Di Giulio et al., 2002), the coexistence of characters in both pleisiomorphic and apomorphic conditions, most of them clearly related to phoresy, is of particular interest. This puzzle of character conditions is summarized in Table 2.

Compared to non-phoretic genera of Meloinae, pleisiomorphic/ancestral conditions include: body short and broad with abdomen being navicular; weak sclerotization; mandibles smooth; sensory appendix on antennomere II conical, positioned on apex; legs elongate with femora not swollen; claws conicofovalate with setae setiform; abdominal spiracle I oval and transverse; lateral pair of caudal setae short, medial pair elongate. Apomorphic conditions include: body elongate, subparallel-sided; great sclerotization; mandibles denticulate; sensory appendix on antennomere II apical but disk-like or on a ventro-apical platform and flattened; legs shortened with femora more or less swollen; claws and lateral setae more or less spathulate; abdominal spiracle I rounded; both pairs of caudal setae similar in length (short or elongate).

The presence of trends of parallel apomorphic conditions in different *Meloe* lineages supports the hypothesis of an evolutionary radiation related to phoresy in *Meloe*, rather than a unique progressive evolution. The ancestral condition is that of some subgenera (*Eurymeroe*, *Coelomeroe*, *Micromeloe*, *Mesomeloe*, *Lasiomeloe*), which have all characters pleisiomorphic; three (*Afrotemoe*, *Meloegonius*, *Taphromeloe*) have some of these characters moderately derived; others (*Listromeloe*, *Treiodous*, *Meloe*) have all characters derived, or both states (*Lampromeloe*). In the subgenus *Meloe*, Pinto & Bologna (1993) pointed out a modest progressive reduction of the antennal sensory appendix, but the derived state of this character is not present in the subgenus.

The new Iranian larva, is the first case of *Meloe* with a mosaic of both pleisiomorphic and apomorphic characters. The three characters discussed above (frontoclypeal spines, mandibles ventrally denticulated and squamiform microsculpture on parietalia), similar to those of *M. (Lampromeloe)*, represent clearly synapomorphic or independently apomorphic characters. However, comparing this larva with those known in other *Meloe* subgenera, and in other phoretic Meloini lineages, a peculiar *cocktail* of conflicting phoresy-related characters can be recognized: i) body short and broad with abdomen navicular, very small dimensions and integument weakly sclerotized (pleisiomorphic condition); ii) distinctive subrectangular head capsule with anterior margin broadly curved, and not triangular and pointed like in the derived head capsules of *triungulins of Lampromeloe* and other *Meloe* subgenera (Di Giulio et al., 2013); iii) mandibles long and wide, directed forward (derived). Similar mandibles seem to be unusual for the genus *Meloe*. The only *Meloe* subgenus known with mandibles similar in shape (but smooth and without ventral indentations), is the monotypic *Listromeloe* (*M. hungarus* Schrank, 1776), which lacks modified setae on fronto-clypeus and shows a distinct head (greatly rounded laterally), pronotum (greatly transverse and wider than mesonotum), and tarsusculus less modified (claw spatulate but lateral setae almost setiform) (Cros, 1930; Di Giulio et al., 2002). iv) Lhalial and maxillary apical palpomeres stout and flattened (pleisiomorphic); v) antennomere II with conical and well developed sensorium apically positioned (pleisiomorphic); vi) regular pattern of three main couples of setae on thoracic sterna (pleisiomorphic); vii) legs long and robust, with femora not swollen (pleisiomorphic); viii) tibiae robust regularly tapering to apex (pleisiomorphic); ix) claw and lateral setae only slightly spatulate (apomorphic); x) abdominal spiracle I large, suboval (pleisiomorphic); xi) medial caudal setae elongate (pleisiomorphic).

Given its tentative assignment to the genus *Meloe*, we can basically depict three different scenarios: i) this larva is actually a *Lampromeloe*; ii) it belongs to a different subgenus, sister to *Lampromeloe*; iii) it belongs to a different subgenus not related to *Lampromeloe*. In the following we explore these possibilities, discussing the related evolutionary implications.

*Meloe* (*Lampromeloe*) is a Palaeartic element, which includes with certainty two species: *M. cavensis* and the polytypic *M. variegatus*. Two other species were referred to this subgenus: *M. specularis* Gredler, 1877, from Gondokoro in southern tropical Sudan and *M. stellatus* Plaginsky, 1923, from Lorestan in western Iran. *M. specularis* is a mysterious species; we tried locating the type in the Gredler's collection without success (Natural History collection of Franciscans in Bolzano, Italy). It was possibly collected during a missionary expedition by father Combioni in Equatoria, but the occurrence of the genus *Meloe* in that part of central Africa is doubtful (Bologna & Pinto, 1998). We suspect that the specimen was collected in the subdesert area near Khartoum, in Sudan, where the catholic mission was established. According to its description, *M. specularis* is very similar to *M. cavensis*, which is distributed also in Egypt. *M. stellatus* was never studied after its description. We examined the photos of 14 syntypes of this species (Museum für Naturkunde, Berlin), which seems extremely similar to *M. cavensis*, but the synonymy cannot be defined without direct examination of types in the framework of a revision of the subgenus. In Iran 22 (and 2 uncertain) species of the genus *Meloe* have been recorded, belonging to 11 subgenera (Bologna, 2008; Fekrat & Modares Awal, 2012); representative larvae of all these subgenera are known. Adults of *Lampromeloe* are easily recognizable because of the entirely metallic (blue-green and cupreous) body coloration, male head with a longitudinal depression behind the eye, and elytra with flat areolae (Bologna, 1991). Both *M. variegatus* and *M. cavensis* are distributed in Iran, and *M. stellatus*, previously considered as possible synonym of *M. cavensis* by Bologna (1991, 2008), seems to be endemic to this country. We could infer that the new larva here described actually refers to *M. stellatus* or to a new unknown *Lampromeloe* species. However, there are no records of adult *Lampromeloe* specimens from the site where larvae have been collected by one of us (RK), neither during recent field researches, nor in historical entomological collections.

Alternatively the new triungulin from Iran could represent a sister taxon of the *Lampromeloe* lineage, distinct from the former by the characters listed in Results (second paragraph) and discussed above. In this case, we could hypothesize that this larva presumably shows the pleisiomorphic condition of the characters shown by the more specialized *Lampromeloe* larvae; in particular the modification of the spiniform frontal setae and the strategy of piercing the intersegmental membranes of the abdomen of the bees would have evolved in this lineage before the modification of other phoresy-related characters like those of the legs (swollen femora, slender tibiae and flattened, wide, trident shaped claws) or the reduction of the sensorium.

The less parsimonious hypothesis is that this larva belongs to another lineage of *Meloe* completely unrelated to *Lampromeloe*. In this scenario, the strikingly convergent characters of the anterior spear, the ventral mandibular indentations and the head microsculpture would have been acquired independently by the two lineages, which developed a similar phoretic strategy in parallel. In this case the polarization of the characters of the larva from Iran relative to a *Lampromeloe* larva would not be possible.

In synthesis, the present new finding strongly supports the previous hypothesis of a non-parasimnonous multiple evolution to phoresy in the genus, with several parallelisms. We hypothesize that the parasitic behavior on bees of most Meloidae, could have played an important role for the repeated evolution in several lineages of similar or less complex specializations to phoresy.

References

ASSMUS E., 1885 - Die Parasiten der Honigbiene und die durch dieselben bedingten Krankheiten diesese Insects. Nach eigenen


BOLOGNA M.A., PINTO J.D., 2001 - Phylogenetic studies of the Meloloeidae (Coleoptera), with emphasis on the evolution of phoresy. - Syst. Ent. 26: 33-72.


