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Description of Adults, Preimaginal Phases, and the Venom Apparatus of a New Species of *Aspilota* Förster from Spain (Hymenoptera: Braconidae)

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Maximilian Fischer, Josep Tormos, Xavier Pardo, and Josep Daniel Asís (2008) Description of adults, preimaginal phases, and the venom apparatus of a new species of *Aspilota* Förster from Spain (Hymenoptera: Braconidae). *Zoological Studies* **47**(3): 247-257. The adults, egg, preimaginal stages, and venom apparatus of *Aspilota propeminimam* sp. nov. are described and compared with those of allied species. The new species is an endoparasitoid and was reared from sarcophagid flies, *Sarcophaga carnaria* (Linnaeus), that had infested a rat carcass in Spain. Morphological structures of potential phylogenetic value are discussed, and a key for the discrimination of the imagines is provided. Additionally, the different phases and preimaginal stages of the Alysiinae are described and characterized; the following characters that define the mature larvae of *Aspilota* Förster and some of its closest genera (e.g., *Dinotrema* Förster and *Eudinostigma* Tobias) are provided: (a) tegument smooth, (b) epistoma and epistomal arch complete, (c) mandibles straight and thorn-shaped, (d) mandibular blade without teeth, (e) labial sclerite completely reduced, (f) only mandibles and palpi present and clearly differentiated, (g) all cephalic structures non-sclerotized, except for epistoma, hypostoma, and mandibles, and (h) spiracles with differentiated atrium and closing apparatus; closing apparatus not close to atrium.
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Key words: Hymenoptera, Preimaginal phases, *Aspilota*, Venom apparatus, New species.

Alysiinae is a species-rich subfamily of the Braconidae that has over 1000 described species placed in 2 tribes: the Alysiini and Dacnusini (Shenefelt 1974, Yu et al. 2005). Its monophyly is based on the possession of exodont mandibles and the koinobiont endoparasitism of cyclorrhaphous Diptera (Docavo et al. 2007).

Within the Alysiini, the *Aspilota* Förster, 1862 complex (= *Aspilota* sensu lato), is characterized by the following derived character states: a) a tendency to have a linear pterostigma, and b) a venom reservoir with a long neck and parallel sides (i.e., a fairly simple poison gland). Within this complex, one of the groups of the Braconidae with a large number of species, *Aspilota*, is characterized by the autapomorphy "tentorial pits reaching the edge of the eye". A revision and discussion of the relationships among the genera of this complex are provided by van Achterberg (1988) and Wharton (2002).

Most of the systematics and taxonomy of braconid wasps are based on the external morphology of adults, and relatively little attention has been paid to interspecific differences in larval or internal characters, even though these could be useful.

Hagen (1964), Finlayson and Hagen (1979), Finlayson (1987), Pardo et al. (2000 2001), Tormos et al. (2003), and Fischer et al. (2006) have provided most of the information on the functional

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morphology and evolutionary connotations of the different phases and preimaginal stages of the Braconidae, particularly the Alysiinae of the tribe Dacnusini. For the Alysiini, the other tribe of the Alysiinae, the main contribution is a description of exuviae of mature larvae given by Čapek (1970 1973), together with recompilations of Clausen (1962) and Finlayson (1987). With regard to *Aspilota*, no cast skin of the last larval instar has yet been described, because the exuviae studied by Čapek (1970) and the descriptions compiled by Finlayson (1987) belong to species included in *Dinotrema* Förster, 1862. Additionally, the identity of many of the species previously studied (e.g., by Evans 1933) and their generic placement are controversial (Fischer et al. 2006).

The reproductive apparatus of the Hymenoptera and its associated venom apparatus may provide phylogenetic information and/or be useful for morphologically discriminating similar species (Edson and Vinson 1979, Quicke and van Achterberg 1990, Whitfield 1992, Fischer et al. 2006). A detailed study of variations in the gland and morphology of the reservoir of the venom apparatus of Alysiini was conducted by Quicke et al. (1997). Nevertheless, the venom apparatus has been described for only 2 species of *Aspilota*.

In this paper, the imagines, immature stages, and venom apparatus of a new species of *Aspilota*, *A. propeminimam*, are described and compared with those of allied species. A key for discriminating adults of this genus is provided. The species was reared from sarcophagid flies, *Sarcophaga carnaria* (Linnaeus, 1758), feeding on a carcass. Additionally, different phases and preimaginal stages of the Alysiinae are described and characterized.

MATERIALS AND METHODS

Adults and exuviae of the final larval instar of *A. propeminimam* sp. nov. were obtained at the beginning of July 1988 from puparia of *S. carnaria* whose larvae were feeding on a carcass of *Rattus* sp. at Calvarrasa de Abajo, Salamanca, Spain. Additionally, adults were obtained in July and Sept. 1988 by net sweeping over *Ligustrum vulgare* L. (Oleaceae). To collect hosts and obtain parasitoids and their exuviae, remains of rats infested with sarcophagid larvae were collected and placed in plastic bottles of suitable dimensions; the openings were covered with gauze held in place with a rubber band. These receptacles were kept under favorable environmental conditions of a temperature of 21-23°C, a relative humidity (RH) of 60-80%, and a photoperiod 16L: 8D. We used the methodology proposed by Tormos et al. (2003) for opening the puparium and preparing the cast skins.

At the end of July and beginning of Aug. 1988 in order to study the egg and different larval instars of *A. propeminimam*, we collected host larvae feeding on the same substrate from which we had obtained parasitized pupae and from which adults of the parasitoid emerged, and took them to the laboratory. The larvae were placed in containers and kept in a chamber (hot-cold) under environmental conditions described above; hosts were dissected periodically. The dissections allowed us to study the egg, 2 immature larval instars, mature larvae, and the pupa of a species of Alysiini that was probably the species described here, as it was the only parasitoid from which adults were obtained. Additionally, the morphological characters of the different larval instars were in full agreement with those of previously described *Aspilota* species. All dissections were performed in 0.9% saline. For microscopic preparation of the larval instars, methods of Tormos et al. (2003 2004) were used.

The venom apparatus was prepared according to the clorazol black method described by Quicke et al. (1992 1997) and used by Tormos et al. (2003) and Fischer et al. (2006) for dry museum specimens. The venom apparatus was treated with a hydroxide solution to remove the soft tissue and allow study of the characteristics of the chitinous gland intima; these structures are not apparent from examination of an intact gland and reservoir.

The morphological terms for the adults (body and wing venation), together with the methodology used to obtain their biometric data, follow Fischer (1973). The terminology used in the description of the immature stages followed that of Tormos et al. (2003). The characteristics and terminology used to describe the gland and reservoir parts of the venom apparatus follow Tormos et al. (2003) and Fischer et al. (2006). The holotype is deposited at the Fundación Entomológica “Torres-Sala” (Valencia, Spain); the paratypes are deposited at the Naturhistorisches Museum Wien (Wien, Austria) (2 ♀♀ on *L. vulgare* L.), Zoological Institute of the Russian Academy of Sciences (St. Petersburg, Russia) (1 ♀ on *L. vulgare* L.), and the Fundación Entomológica “Torres-Sala” (1 ♀ on *L. vulgare* L.; 1 ♀, 1 ♂ from puparia of *S. carnaria*

(Linnaeus)). The following abbreviations are used: (1) antennae: F, Fl, F2, Fm, Fp = flagellomere(s), flagellomere 1, 2, etc., middle flagellomere(s), penultimate flagellomere; (2) metasoma: T, T1, T2 = tergum(a), 1st tergum, 2nd tergum; (3) wings: forewing: st, pterostigma; r, r1+r2, r3, = radial vein, combined 1st and 2nd abscissa of r, 3rd abscissa of radius (r); cc1 and cc2, 1st and 2nd transverse-cubital veins; cu2, 2nd abscissa of cu (= cubital vein); d, discoidal vein; nr, recurrent vein (nervus recurrens); np, parallel vein (nervus parallelus); nv, nervulus; R, radial cell; Cu2, 2nd cubital cell; B, brachial cell; hindwing: cu2’, 2nd abscissa of cubital vein; M’ and SM, medial and submedial cells; nr’, radiellus (recurrent vein); r’, radial vein; (4) biometric data: d, diameter; l, length; and w, width.

RESULTS

Adults

Genus *Aspilota* Förster, 1862

Type species: *Alysia ruficornis* Nees von Esenbeck, 1834.

Diagnosis: Mandibles small, simple, often with tooth 1 diminished relative to tooth 3, external surface almost smooth, keels diagonal and ventral, often poorly developed or absent. Anterior tentorial pits broadened, generally extending to ventral edge of eye. Notauli short, generally not extending beyond decliving of mesoscutum. Forewing st reduced, linear, metacarpus not distinguished; 1st transverse-cubital vein shorter than r2; n-r clearly postfurcal; B closed.

Hosts: Anthomyiidae, Phoridae, Platypezidae, and Sarcophagidae.

Aspilota propeminimam sp. nov.

(Figs. 1, 2)

Holotype (M.A.G. Vacas leg.): ♀, SPAIN, Salamanca, Calvarrasa de Abajo, 15 July 1988, on *Ligustrum vulgare* L.

Paratypes (M.A.G. Vacas leg.): 4 ♀♀, SPAIN, Salamanca, Villamayor, 20 Sept. 1988, on *L. vulgare* L.; 1 ♀, same as previous, July 1988, from puparium of *S. carnaria* (Linnaeus) on a rat carcass (pedotrophic material), 1 ♂, same as previous.

Host: *Sarcophaga carnaria* on the carcass of *Rattus* sp. Most of the biological information available about species of Alysiini refers only to the habitats where they were collected and lists of parasitoids cited in the study of some hosts responsible for the pests. Thus, little is known about the hosts of species of the *Aspilota* group (particularly species of *Aspilota*), but the hosts appear to be larvae of the Phoridae that occur in decaying organic matter (Wharton 1984, van Achterberg 1988, Tormos and Gayubo 1989). Nevertheless, the new species described herein was obtained from saprophagous sarcophagid flies.

Etymology: *prope* (Lat prep. accusative) = near, i.e., near the species *Aspilota minima* (Thomson, 1895).

Taxonomic position: The new species is ascribed to the *lobidens*-group because of the following character states: a) mesoscutum without a dorsal fovea; b) T1 twice as long as wide; and c) tooth 1 of the mandibles normally developed. The species runs in the key by Fischer (1976) at couplet 12 to *Aspilota minima*. The 2 species are morphologically very close to each other. They can be distinguished as follows:

1. r2 at most twice as long as cc1; r3 (Fig. 3b’) 3.0x as long as r2. Tooth 1 of mandible a little shorter than tooth 3. Propodeum with fine, reticulated sculpturing, and smooth spots on either side near front edge. Posterior mesopleural furrow smooth. Head (Fig. 3c’) between temples slightly widened. Antennae (Fig. 3a’) 13-segmented. 1.5 mm. Sweden.... *A. minima* (Thomson)

\- r2 nearly 3 times as long as cc1; r3 (Fig. 2) 2.5x as long as r2. Tooth 1 of mandible as long as tooth 3. Propodeum with basal carina, pentagonal area, and costulae almost smooth and shiny. Posterior mesopleural furrow crenulate. Head (Fig. 1) not widened between temples. Antennae (Figs. 1, 2) 14-16 segmented. 1.2 mm. Spain.................................*A. propeminimam* sp. nov.

Female

Body length: 1.2 mm.

Head: 1.9x as wide as long; 1.8x as wide as mesoscutum; 1.4x as wide as face; between eyes as wide as between temples, eyes 1.2x as long as temples; eyes and temples rounded in a common curve; distance between toruli as great as their diameter; distance of toruli from eyes not much greater; occiput slightly excavated. Distance between ocelli slightly greater than their diameter; distance of ocelli from eyes greater than ocellar area width; epicranial suture visible; only a few setae near occiput and ocellar area. Face 1.33x as wide as high, evenly arched; eye margins parallel; middle elevation absent, with some moderately long setae mesally and close to eye margins. Clypeus 3x as wide as high, slightly arched; lower edge slightly curved. Paraclypeal area extending as far as eye margin, twice as wide as high and slightly widened towards eyes. Mandible 1.8x as long as wide; lower edge straight; upper edge curved slightly inwards in middle, at apex as wide as base; teeth 1 and 3 more or less rounded and of equal width; tooth 2 pointed and slightly projecting; with clear incisions between teeth. Maxillary palpi as long as height of head; labial palpi short, segments 3 and 4 scarcely longer than broad. Antennae (Figs. 1, 2) approximately as long as body, 14 or 15 segmented; F1 3.0x as long as wide and slightly narrower than following flagellomeres; F2 and F3 gradually wider; from F4 onwards, equally wide; F1, F2, and F3 of equal length; F6 twice and Fp 1.8x as long as wide; F clearly separated from each other; if present at all, 2 sensilla visible in lateral view; some setae longer than width of F.

Mesosoma: 1.33x as long as high, upper side arched. Mesoscutum 1.4x as wide as long, rounded in front of tegulae; dorsal fovea absent; notauli (Fig. 1a) present on declivity, absent from disc, each imaginary course indicated by a row of setae joining marginal furrows, latter mainly smooth. Prescutellar furrow divided, lateral areas as long as wide; smooth. Rest of mesosoma smooth. Furrow between metascutum and propodeum narrowly crenulate. Propodeum (Fig. 1b) with basal carina, pentagonal areola and costulae, areas almost smooth and shiny. Spiracles small. Lower furrow of 1 side of pronotum with some poorly recognizable scores. Sternaulus (Fig. 2a) crenulate, shortened on either side; posterior mesopleural furrow crenulate. Metapleuron with smooth furrows. Hind femora 5x as long as broad.

Wings (Figs. 1, 2): r arising by length of r1 behind base of st; r1 longer than width of st; angle between r1 and r2 obtuse; r2 2.8x as long as cc1; r3 straight, 2.5x as long as r2; R reaching tip of wing; Cu2 slightly narrowed toward apex of wing; nr highly postfurcal; d 1.8x as long as nr; nv postfurcal by its own length. B closed; distal side angled at middle; np almost absent; r’ and cu2’ at most indicated as folds; SM’ 1/2 as long as M’.

Metasoma: T1 (Fig. 1c) twice as long as wide apically, slightly wider behind than in front, parallel-sided behind spiracles; slightly narrowed in front of spiracles, evenly cross-arched, striate; dorsal carinae converging and disappearing in striation; basal excavation large, as wide as long, slightly widened towards base at excavation. T6-T8 folded above. Ovipositor sheaths (Fig. 2) only slightly curved upward, almost straight, as long as T1 (Fig. 1c).

Color: black, except the following features yellow: base of antenna as far as F1, mouthparts, legs, tegulae, wing venation, and T1. T2-T8 gradually darkening. Wing membrane hyaline.

Male. Like female except antennae 16-segmented.

Immature Stages
(Figs. 3-7)

The egg and 1st larval instar were found in host larvae at different developmental stages. Second and 3rd larval instars and pupae were found in host puparia. A specimen of each stage was studied for the description.

Egg (Fig. 3). Apparently free in the hemocele, hydropic. Cylindrical chorion smooth and translucent; 300 μm long x 35 μm wide.

Larvae

First instar (Fig. 4). Body: length and maximum width 370 and 116 μm, respectively; hymenopteriform, transparent, slightly broader in anterior region and very slightly curved to ventral side, with well-defined head and distinct segmentation. Last abdominal segment slightly modified into a short, blunt, rounded organ in shape of a tail. Tegument, with exception of head and last abdominal segment, densely microspinulose (spinulae length, 1-2 μm) except for constrictions between segments and areas where spiracles would be located. Anal segment very slightly notched transversely. Cranium: with the following differentiated, although only very slightly, sclerotized sclerites (Fig. 4a): epistoma, pleurostoma, anterior and posterior pleurostomal processes, and hypostoma. Antennae papilliform (Fig. 4a). Mouthparts: mandibles well defined, slender, with sharp blade slightly curved and well sclerotized; maxillary and labial palpi poorly defined.

Second instar (Fig. 5). Body: length and maximum width 400 and 125 μm, respectively; tegument very similar to that of 1st instar. Only appreciable differences lie in the more-reduced dimensions of head, absence of a tail, and scattered microspinulae of tegument. Cranium: without cephalic sclerites (Fig. 5a). Mouthparts: mandibles present but almost imperceptible, 14 μm long and 10 μm wide.

Third instar (Fig. 6). Body: length and

maximum width 530 and 200 μm, respectively; hymenopteriform, slightly broader anteriorly, with head (reduced as in 2nd instar) and segments well defined. Color: yellowish-white, weakly sclerotized. Pleural lobes not developed. Tegument glabrous (bare, smooth). Anal segment slightly notched transversely. Nine pairs of spiracles (Fig. 6b) (d = 8 μm), 1 pair on posterior edge of 1st thoracic segment and 1 pair on 1st 8 abdominal segments; atrium and closing apparatus (l = 10 μm; w = 5 μm) well differentiated; atrium almost round, with peritreme, separated from closing apparatus by a section of funnel-shaped trachea with approximately 23 chambers. Cranium and mouthparts (Fig. 6a): see description of exuviae. Head of fresh mature larva also shows hypostoma.

Exuviae of final instar larva (cast skin). Only 1 exuviae available for study. Tegument very weakly sclerotized, except spiracles, glabrous and smooth. Spiracles with differentiated atrium (d = 8 μm) and closing apparatus; closing apparatus not close to atrium. Cranial structures (Fig. 7): complete epistomal arch. Mouthparts: mandibles (l = 64 μm) highly sclerotized, with relatively broad base and long blade, thorn shaped, blade without teeth, smooth, pointed, with overlapping apex. Maxillary (d = 4 μm) and labial (d = 3 μm) palpi slightly protuberant, with 1 sensillum; salivary orifice defined.

Pupa. Exarate. In single pupa examined, presence of a cocoon not observed.

DISCUSSION

The morphology and size of the egg of *A. propeminimam* are similar to those reported for other species of the Alysiinae: *Dacnusa areolaris* (Nees von Esenbeck, 1811) (Haviland 1922), *Dinotrema nervosum* (Haliday, 1933) (Evans 1933), *Dac*. *dryas* (Nixon, 1948) (Guppy and Meloche 1987), *Dac*. *sibirica* Telenga, 1935 (Croft and Copland 1994), and *Chorebus pseudoasphodeli* Tormos, Pardo, Jiménez, Asís and Gayubo, 2003 (Tormos et al. 2003). An embryonic membrane (trophamnion) surrounds the embryo, which is in a curled position within it. The function of this membrane is probably trophic (Gauld and Bolton 1988, Croft and Copland 1994, Tormos et al. 2003); it is very morphologically similar to that found in *Dac*. *areolaris*, *Dac*. *dryas*, *Dac*. *sibirica*, and *C*. *pseudoasphodeli*.

The knowledge acquired to date has allowed the following description of the alysiine egg to be made: oval, translucent, slightly viscous, and with a smooth, transparent chorion. The size of the egg, hundreds of microns in length and dozens in width, increases during segmentation, as it becomes more spherical. Towards the end of its development, the egg tends to have an oblong shape.

According to the classifications of Clausen (1962) and Hagen (1964), the 1st larval instar of Alysiinae can be considered caudate-mandibulate. In *A*. *propeminimam*, the trophamnion remains practically intact until the 1st molt of this stage, as occurs in other braconids (Polaszek 1986), and among alysiines, in *Dac*. *areolaris* (Haviland 1922) –although Hopkins (1977) did not find a trophamnion around the 1st instar of this species-, *C*. *posticus* (Haliday, 1839) (= "*C*. *gracilis*" (Nees von Esenbeck, 1834)) (Wright et al. 1946), *Dac*. *dryas* (Guppy and Meloche 1987), *Dac*. *sibirica* (Croft and Copland 1994), or *C*. *pseudoasphodeli* (Tormos et al. 2003). According to Salt (1968), the presence of this embryonic layer in the 1st larval instar may serve as a protective barrier against the host immune defense system. In this species, the larva is more similar to the 1st instar of *Dac*. *areolaris* (Haviland 1922, Hopkins 1977), *Dac*. *dryas* (Guppy and Meloche 1987), *Dac*. *sibirica* (Croft and Copland 1994, van der Linden and van Achterberg 1989), *C*. *pseudoasphodeli* (Tormos et al. 2003), and *Dac*. *cicerina* Tormos, Pardo, Asís and Gayubo (Tormos et al. unpubl. data) than to *C*. *posticus* (Wright et al. 1946) or *Din*. *nervosum* (Evans 1933). Other species of the Alysiini such as *Grandia cynaraphila* (Ricchello, 1928) (Ricchello 1928, Clausen 1962), Dapsilarthra gahani (de la Baume-Pluvinel 1915) (de la Baume-Pluvinel 1915, Clausen 1962), and *Dap*. *apii* (Curtis, 1826) (Keilin and Tate 1943) show a 1st "atypical instar" as detailed below. Like the larva of the species mentioned above, it shows tegumental differentiations on a large number of body segments and is of the caudate type. In this respect, it should be indicated that although the 1st larval instar of braconids is extremely variable in form (Clausen 1962, Hagen 1964, Finlayson 1987), the caudate type is relatively common among endoparasitic braconids (Gauld and Bolton 1988), and has been described for 3 species of *Dacnusa* Haliday, 1833 and *C*. *pseudoasphodeli*. As for species of the Alysiinae with a 1st larval instar that could be called "atypical", mention should be made of 3 species of the Alysiini: *G*. *cynaraphila* (= *Dacnusa navicularis cynaraphila*) (Clausen 1962), *Dap*. *gahani* (= *Adelura gahani*) (de la

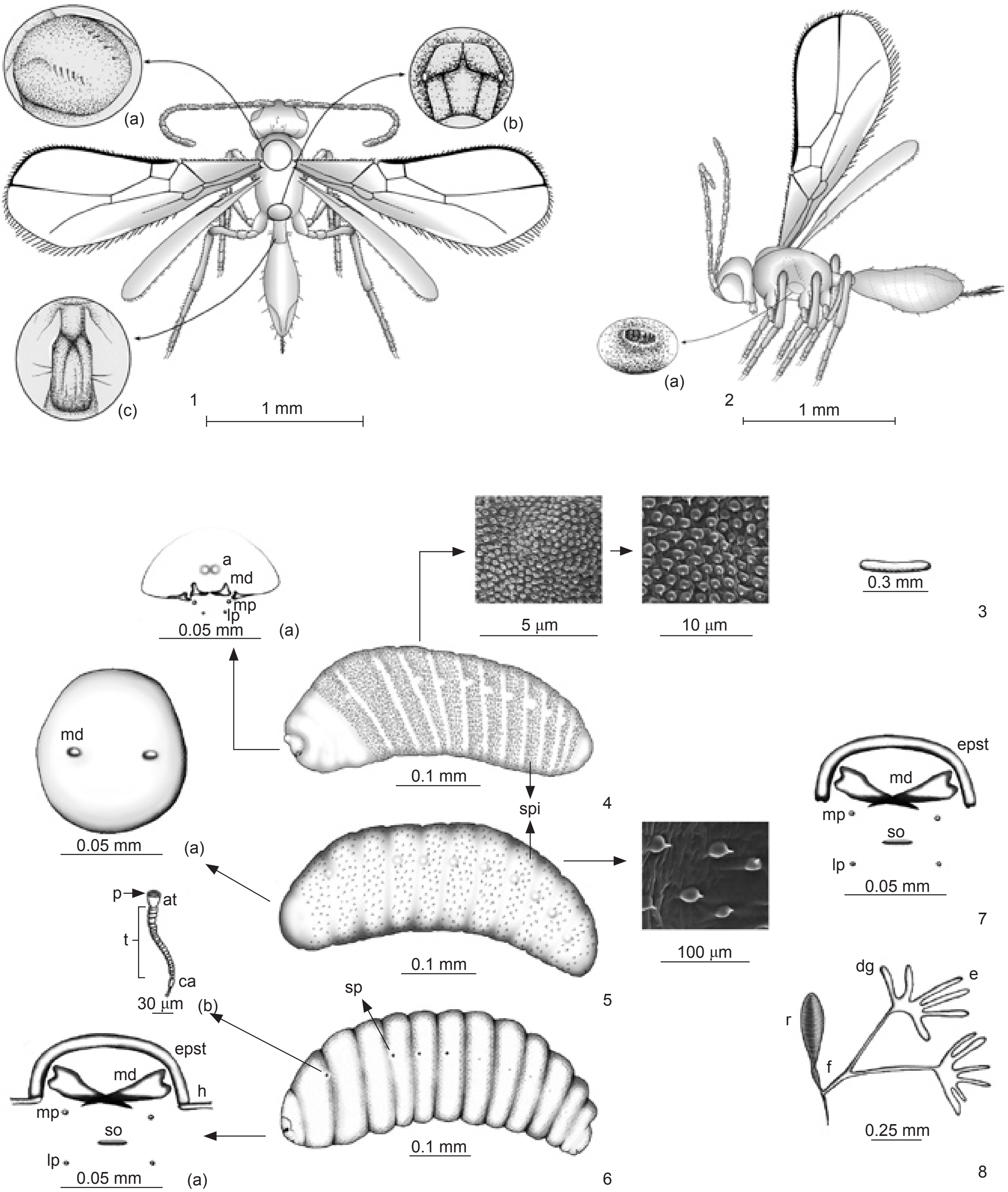
Baume-Pluvinel 1915) and *Dap*. *apii* (Keilin and Tate 1943). Of interest is the presence of paired ventral processes in the 1st case and of “paired ventral pseudo-processes” in the 2nd and 3rd cases, surmounted in all 3 cases by tegumental differentiations of the “setae” type. These larvae, which can be included in the polypodeiform type (Clausen 1962), are also typically caudate, except for their paired processes/pseudo-processes. From the point of view of functional morphology, it is possible that the mandibles of this 1st instar might serve to break the chorion, as indicated by Guppy and Meloche (1987) and Tormos et al. (2003, unpubl. data) referring to *Dac*. *dryas*, *C*. *pseudoasphodeli*, and *Dac*. *cicerina*. It is also possible that they could contribute to the avoidance of super- or multiparasitoidism, as mentioned by Hagen (1964) and Tormos et al. (2003), referring to caudate-mandibulate braconid larvae. The functions of the tail and tegumental differentiations are highly controversial (Ullyett 1944, Hagen 1964, Tormos et al. 2003). The most significant adaptations developed by 1st-stage larvae of the Alysiinae in order to exit the egg and detach from the trophamnion, and to feed and reach adequate development, are as follows: (1) those related to support of the mandibles: the appendices are necessary for exiting the egg, detaching from the tophamnion, and avoiding super- or multiparasitism by causing the death of their competitors. In this respect, note should be taken that in the final instar, the mandibles have a more-triangular shape, in contrast with the sickle-shaped type shown by the 1st instar: a cranium with differentiated and sclerotized sclerites. (2) Those related to the capacity for sensory exploration and mobility: a) maxillary and labial palpi present; b) body covered with tegumental differentiations (setae, spines, spinules, microtrichia, etc.); and c) last abdominal segment well developed. In this regard it should be emphasized that although the function of the last of the structures mentioned and that of the tegumental differentiations are controversial, as previously indicated, the larva is able to contract causing the body segments and the tail to markedly arc, and the tegumental differentiations can even help with this. Both types of structure may therefore contribute to movement and displacement. It is not easy to infer phylogenetic relationships from the study of immature larvae, because many of the characters are adaptive. If more data about the 1st stage were available, it would be possible to determine in which taxon or taxa of the Alysiinae hypermetamorphosis is more or less pronounced, and, hence, the larvae are “more or less specialized”. This would afford a better understanding of postembryonic development, the preimaginal phases, and the biology of this subfamily.

Based on the information provided here and on the above contributions, it is possible to produce the following description of the 1st larval instar of the Alysiinae: body with well-defined head and 13 segments; caudate, vermiform, transparent, and weakly curved. The last 2 abdominal segments are often almost fused; the last slightly modified into a short, rounded organ in the form of a tail, sometimes with microtrichia grouped around where the anus would be found. Segments 2-12, with the exception of the prothoracic and the last abdominal ones, have tegumental differentiations. Cranium slightly flattened, with the following sclerites differentiated: epistoma, pleurostoma, anterior, and posterior pleurostomal processes and hypostoma. Mouthparts: mandibles well developed, with an oblong molar lobe and a weakly curved and pointed well-sclerotized blade. Maxillary and labial palpi with varying differentiation.

The 2nd larval instar resembles the mature larva, as happens in many parasitic hymenopterans. This larva has lost its tail (or it is very reduced) and tegumental differentiations and has very reduced or almost-absent mandibles. The character state “presence of mandibles” brings the 2nd instar of *A*. *propeminimam* close to *Din*. *nervosum* (Evans 1933), the only 1st instar of the Alysiini described in detail. Likewise, the same character state separates this 2nd instar from those already described for *Dacnusa* (Haviland 1922, Guppy and Meloche 1987, Croft and Copland 1994), while it places it close to the 2nd instar of *C*. *pseudoasphodeli* (Tormos et al. 2003) and possibly to the “3rd instar” of “*C*. *gracilis*” (Wright et al. 1946). According to Wright et al. (1946), the 3rd instar of this species, although without mandibles, does have a pair of oral papillae. Perhaps these structures correspond to extremely reduced and depigmented mandibles, as occurs in *C*. *pseudoasphodeli*.

With the information available at present, the following description of the 2nd larval instar of the Alysiinae can be offered: body generally cylindrical and slightly spindle-shaped at the ends, with differentiated head and 13 segments. Tegument without tegumental differentiations. Cranium without head sclerites. Mouthparts: mandibles generally present, although almost imperceptible.

Regarding the mature larva, it should be

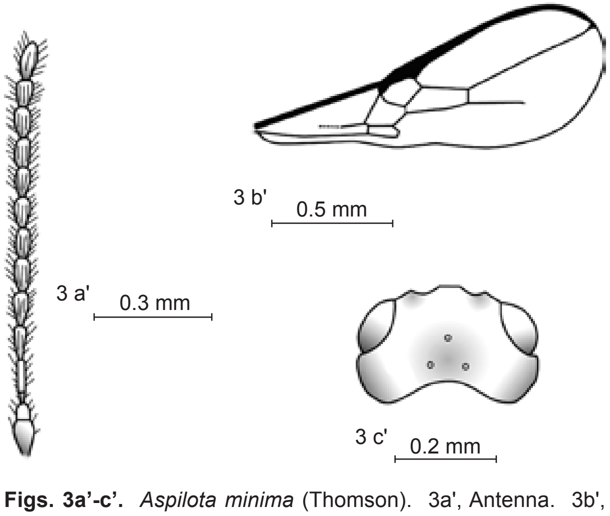


Figs. 1-8. *Aspilota propeminimam* sp. nov. **1.** Body in dorsal view with details of notauli (a), propodeum (b), and T1 (1st tergum of metasoma) (c). **2.** Body in lateral view with details of sternaulus (a). **3.** Egg. **4.** First instar larva (lateral view) with details of the cranium and mouthparts (a). **5.** Second instar larva (lateral view) with details of the cranium (a). **6.** Third instar larva (lateral view) with details of the cranium and mouthparts (a) , and 2nd spiracle (b). **7.** Exuviae of final larval instar: cephalic structures. **8.** Venom apparatus with details of reservoir, secondary venom duct, venom gland, and gland filament (sack). a, antenna; at, atrium; epst, epistomal arch; ca, closing apparatus; e, gland filament; h, hypostoma; md, mandible; mp, maxillary palpi; lp, labial palpi; p, peritreme; r, reservoir; so, salivary orifice; f, secondary venom duct; spi, spinulae; sp, spiracle; t, trachea; dg, venom gland.

noted that the final larval instar of the Hymenoptera Parasitica has been studied by several authors: notably, the classic works of Clausen (1962) and Hagen (1964), together with keys for the taxonomic separation of mature larvae elaborated by Beirne (1941), Short (1952 1959 1970 1976 1978), Finlayson (1967 1975), Čapek (1970), and Finlayson and Hagen (1979). Within this broad set, the Braconidae, and in particular the Alysiinae, has scarcely been studied. In *Chorebus*, the broadest genus of the Dacnusini, the final larval instar of only 9 species is known, although only detailed descriptions of head structures, spiracles (usually the prothoracic one) and tegument have been reported for *C. denticurvatus* Pardo, Tormos and Verdú, 2001, *C. pseudoasphodeli*, and *C. pseudoasramenes* Tormos, Pardo, Jiménez, Asís and Gayubo, 2003. In *Dacnusa*, one of the most common genera in the Spanish fauna, the final larval instar of 7 species has been described, but only 4, *Dac. sibirica*, *Dac. dryas*, *Dac. rodriguezi* Docavo and Tormos, 1997, and *Dac. cicerina* (unpubl. data), have been described in depth. According to the descriptions made to date, *Chorebus* and *Dacnusa* appear to be fairly homogeneous in terms of their larval morphology.

In the Alysiini, unlike the Dacnusini, the identity of many of the species studied previously, and their generic cutoff, is extremely controversial (Fischer et al. 2006). For example, *A. signifrons* Viereck, 1906 and *A. nervosa* Haliday, 1833, whose exuviae of the last larval instar were studied by Čapek (1970) (the exuviae of *A. nervosa* were also studied by Evans (1933)), are now placed in *Dinotrema*. *Aspilota vesparum* Stelfox, 1943, whose exuviae of the last larval instar were described by Short (1952) and Finlayson (1979), is currently considered to be a synonym of *Din. nervosum*. Despite this, it may be stated that the final larval instar of the Alysiini, together with that of the Alysiinae overall, shows great variations in the differentiation of morphological characters (Short 1952 1979, Čapek 1970 1973). Thus, although there are morphologically heterogeneous genera such as *Dapsilarthra* Förster, 1862 (Short 1979), it is possible to observe a series from the almost-complete set of head sclerites in *Alysia* Latreille, 1804 and *Phaenocarpa* Förster, 1862 (Short 1952 1979) to the condition in *Aspilota*, *Dinotrema*, and *Coelinius* Nees von Esenbeck, 1818, where almost the only sclerotized structures are the epistoma and mandibles (Short 1952, 1979, Finlayson 1987, Fischer et al. 2006). The mature larva and its exuviae of the species of *Aspilota* described here have the chief larval characters of the final instar larva of the Alysiini, with well-defined (a) simple, smooth mandibles, and (b) reduced labial sclerite (Čapek 1970). They also show most of the characteristics of the phylogenetically closest genera (van Achterberg 1988, Wharton 2002) of which the cast skin of the final larval instar has been described: *Dinotrema* and *Eudinostigma* Tobias, 1986: (a) tegument smooth (Short 1952), (b) epistoma and epistomal arch complete (Čapek 1970 1973), (c) mandibles straight, thorn-shaped (Čapek 1970 1973), (d) mandibular blade without teeth (Finlayson 1987), (e) labial sclerite completely reduced (Čapek 1970, Finlayson 1987), (f) of the mouthparts and their differentiations, only mandibles and palpi present (Čapek 1973), (g) all cephalic structures non-sclerotized except epistoma, hypostoma, and mandibles (Čapek 1970), and (h) spiracles with differentiated atrium and closing apparatus (Čapek 1973), and with closing apparatus not close to atrium (Short 1952). Differences from the previously described species of the genera *Dinotrema* (= *Synaldis*) and *Eudinostigma* (Čapek 1970, Finlayson 1987) lie in the number and arrangement of the sensory structures.

With the available information, and despite the manifest variability shown by different structures (e.g., the cephalic structures), the following description of the mature larva of the Alysiinae can be proposed. Body: Hymenopteriform, with head, 3 thoracic and 8 abdominal segments well defined: usually whitish-yellow. Tegument weakly sclerotized, with the exception of spiracles and, on some occasions, with tegumental differentiations



Figs. 3a'-c'. *Aspilota minima* (Thomson). 3a', Antenna. 3b', right anterior wing. 3c', head in dorsal view.

(spinules, microtichiae, papillae, setae, or sensilla) with varying morphology, number, and arrangement. Spiracles located on prothorax and on 1st 8 abdominal segments, with atrium not very well developed. Cranium weakly sclerotized, with different sensory structures: antennae sometimes patent, weakly protuberant, with an almost-circular antennal orbit, sometimes without differentiated sensilla; head structures (sclerotized areas of outer head skeleton) differentiated to a greater or lesser extent, although those present generally well differentiated and sclerotized. Mouthparts: mandibles with broad base and relatively long, curved, thin, glabrous, smooth, unidentate, sclerotized blade; maxillae and labium reduced, poorly defined or absent; maxillary and labial palpi circular or oval, slightly protruding, with a varying number of sensilla, which may vary in size, at apex; salivary orifice defined. Although it is still not possible to find an autapomorphy or group of derived states that defines the final larval instar of Alysiinae overall, it is possible to adduce that a character state common to all known larvae of Alysiini, except those of *Alysia*, lies in the absence of a defined labial sclerite. *Dacnusini*, except for *Synelix* Förster, 1862, has a well-differentiated hypostoma (Tormos et al. 2003).

Finally, with respect to the number of larval instars shown by species of the Alysiinae, it should be noted that although some authors have indicated the presence of a larval phase with more than 3 instars (Caudri 1941, Wright et al. 1946), in our studies to date, we have only managed to differentiate 2 immature larvae and 1 final instar (Fischer et al. 2006, Tormos et al. 2003, unpubl. data). Additionally, many authors (e.g., Evans 1933, Keilin and Tate 1943, Salked 1959) described 3 larval instars for the Alysiinae, and more recently, Croft and Copland (1994) also described a larval phase with 3 instars for *Dac. sibirica*. Moreover, it should be stressed that Rocha et al. (2004) indicated that *Fopius arisanus* (Sonan, 1932) (a species belonging to the Opiinae, a sister group to the Alysiinae) has 3 larval instars, in contrast to the 4 instars previously reported by Ibrahim et al. (1992).

Venom apparatus
(Fig. 8)

This species has a venom apparatus with the character states specified by Quicke et al. (1997) for *Aspilota*: (a) reservoir undivided; (b) reservoir neck region with narrowing; (c) reservoir less than 4x longer than maximally wide; (d) secondary venom duct without annular/spiral sculpturing; (e) venom gland with numerous sacs at end of a pair of long ducts; (f) a venom gland inserted at extreme posterior end of reservoir; and (g) secondary venom duct not narrow, more or less gradually developing into gland filaments. Morphological differences from the 2 species of *Aspilota* for which the venom apparatus has been described (the venom apparatus of another 3 *Aspilota* spp. is known, although with no specific determination) (Edson and Vinson 1979, Quicke et al. 1997) lie in the morphology of the secondary venom duct and the number of gland filaments (apical subdivisions of the venom gland).

From the data available on the preimaginal stages and morphology of the venom apparatus of Alysiini, it is clear that further descriptions are necessary, both of the larvae and the venom apparatus, before phylogenetic relationships can be inferred from their morphological features.

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