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A NEW SPECIES OF DACNUSINI FROM MONTECRISTO ISLAND, WITH DESCRIPTION OF THE PREIMAGINAL PHASES AND VENOM APPARATUS OF *ANTRUSA CURTITEMPUS* (HYMENOPTERA, BRACONIDAE, ALYSIINAE)

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ABSTRACT

Antrusa montecristiensis, a new species of Dacnusiini from Montecristo island (Italy), is described, illustrated, and compared with allied species. Specific keys of the genus *Antrusa* for the West Palaearctic region are provided. The preimaginal phases and the venom apparatus of *A. curtitempus* Fischer, Tormos, Docavo & Pardo, are described, illustrated, and compared with species of allied genera. The larva stages are similar to those of *Dacnusa*; the immature larvae differ in the number and distribution of the setae of the abdominal and thoracic segments, and the mature larva in the type of the tegumental differentiations of the thorax and abdomen as well as in the number and size of the sensilla of the maxillary palpi. The venom apparatus of this species is very similar to that of *Antrusa flavicoxa* (Thomson), differing from it in length of the reservoir and the number of gland filaments.

Key Words: Hymenoptera, Braconidae, new species, immature stages, venom apparatus, *Antrusa*, Spain

RESUMEN

Se describe, ilustra, y compara con las especies más próximas, una nueva especie de Dacnusiini de la isla de Montecristo (Italia): *Antrusa montecristiensis*. Se proporcionan claves dicotómicas para la separación de las especies del género *Antrusa* presentes en el oeste paleártico. Adicionalmente, las fases preimaginales y el aparato del veneno de *A. curtitempus* Fischer, Tormos, Docavo & Pardo, se describen, ilustran, y comparan con las especies de los géneros más afines. Los estados larvarios de esta especie son similares a los de *Dacnusa*, pudiéndose separar las larvas inmaduras a partir del número y distribución de las setas de los segmentos torácicos y abdominales, mientras las larvas maduras pueden caracterizarse mediante el tipo de diferenciaciones tegumentarias presentes en el tórax y abdomen, así como a partir del número y tamaño de los palpos maxilares. El aparato del veneno de *Antrusa montecristiensis* difiere, del de *Antrusa flavicoxa* (Thomson), en el número de filamentos glandulares y longitud del reservorio.

Translation by the authors.

The taxonomic rehabilitation of the genus *Antrusa* Nixon was proposed by Fischer et al. (2004). *Antrusa* can be characterized and delimited from *Exotela* Förster, *Dacnusa* Haliday and *Chorebus* Haliday by a combination of the following character states: (a) mandibles three-dentate, (b) vein nr antefurcal, (c) T1 with medial longitudinal carina, and (d) no sexual dimorphism of the pterostigma. The latter character is significant for separation from *Dacnusa*, but cannot be applied easily without having both sexes available. The longitudinal carina of T1 may be helpful. The three-dentate mandibles separate *Antrusa* from *Chorebus*, and the antefurcal nr separates it from *Exotela* in the restricted sense. We discovered a new species of this genus, *Antrusa montecristiensis* **sp. n.**, described below, in Italy (Tuscan Archipelago: isola di Montecristo). Regarding the preimaginal stages of the species of this genus, it should be

noted that the structures that allow characterization of the final larval instar have not been described (although see Čapek 1970). Detailed studies addressing the variation in gland and reservoir morphology of the venom apparatus in species of Dacnusiini have been conducted by Quicke et al. (1997) and Tormos et al. (2003). In this article, the preimaginal phases and venom apparatus of *Antrusa curtitempus* Fischer, Tormos, Docavo & Pardo, 2004 are described.

MATERIAL AND METHODS

Adults of *Antrusa montecristiensis* **sp. n.** were obtained in Mar 2000 with use of malaise trap placed in the Tuscan Archipelago, isola di Montecristo. To study the preimaginal phases of *A. curtitempus*, at beginning of Aug 1992, we collected larvae of *Chromatomyia horticola* (Goureau) (Diptera, Agromyzidae) mining leaves

of *C. arietinum* Linnaeus and took them to the laboratory. We had obtained adults of this parasitoid from parasitized host pupae at Ayora (Valencia, Spain) 2 years previously. To collect the larvae we picked leaves from plants infested with the agromyzids and placed them in containers of suitable dimensions whose openings were covered with gauze held in place with a rubber band. These receptacles were kept under environmental conditions of temperature, relative humidity (RH), and photoperiod. The larval and pupal hosts were dissected periodically. The dissections allowed us to study the egg, 2 immature instars, mature larva, and the pupa of this species. All dissections were performed in 0.9% saline. For microscopic preparation of the preimaginal phases, the methods of Tormos et al. (2003, 2004) were employed.

The venom apparatus was prepared and drawn according to the method described by Quicke et al. (1992, 1997) (clorazol black method) for dry museum specimens. The venom apparatus was treated with a sodium hydroxide solution, after which the soft tissue was removed. It was then possible to observe the characteristics of the remaining chitinous gland intima, which are not apparent from examination of an intact gland and reservoir.

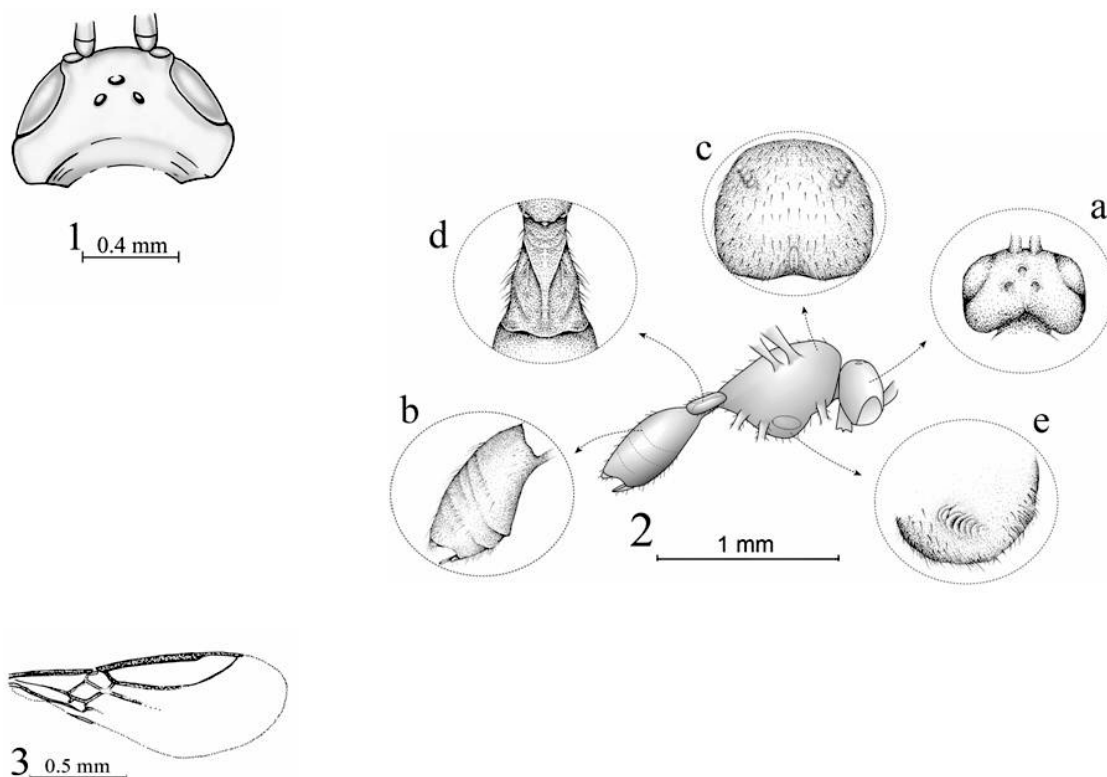
The material examined (adults, immature stages, and venom apparatus) is deposited at the "Torres Sala" Entomological Foundation (Valencia, Spain).

The terms for body morphology and wing venation, together with the criteria for collecting biometric data of adults, follow Fischer (1973, 2002) with 2 modifications: (1) mesosoma vs. thorax, and (2) setae vs. hairs. All the material examined is deposited at the Museo del Medio Ambiente (Valencia, Spain). The following abbreviations have been used in the descriptions: a2 = lower vein of B (brachius); B = brachial cell; cq1 = first cubital cross-vein; cu2 = 2nd abscissa of cu (= cubital vein); cu2' = second abscissa of cubital vein of hind wing; cu1b = lower cubital-anal cross vein (3rd discoideal segment); d = discoidal vein; F, Fl, F2, etc. = flagellomere (s), flagellomere 1, 2, etc.; Fm, Fp = middle flagellomere (s), penultimate flagellomere; M = medial cell of hind wings; np = parallel vein; r' = radiellus (radial vein of hind wing); nr = recurrent vein; nr' = recurrent vein of hind wing; nv = nervulus; R = radial cell; r, rl, r2 = radial vein, first, second abscissa of radius; st = pterostigma; SM' = submedial cell of hind wings; T, T1, T2, T3, T2 + 3 = tergite (s), first, second, third tergite, first + second tergite. The terminology used in the description of the different structures of the immature stages is that used by Tormos et al. (2003, 2004). The terminology used for characteristics of the gland and reservoir parts of the venom apparatus follows Tormos et al. (2003).

RESULTS AND CONCLUSIONS

Antrusa montecristiensis sp. n. (Figs. 2-3)

Female—Head (Fig. 1 and 2a): Head 1.6-times as wide as long, 1.7 times as wide as face, 1.35 times as wide as mesoscutum; eyes as long as temples, protruding, behind eyes as wide as at eyes; toruli in normal position, occiput bayed inwards; upper side, occiput and in the ocellar area smooth; distance between ocelli greater than ocellus width, distance between an ocellus and eye as long as width of ocellar area. Face 1.6 times as wide as high, slightly and evenly convex, middle elevation almost missing, with fairly evenly distributed, scattered setae, seta points discernable, edges of eyes only slightly converging below, nearly parallel sided. Clypeus slightly convex, 2.7 times as wide as high, with few outstanding setae. Tentorial pits round, their diameter as great as the distance from eyes. Labrum triangular, protruding, with inconspicuous setae. Mandible slightly longer than wide, lower edge straight, upper edge slightly directed upwards, with 3 teeth almost equally developed, tooth 1 rounded, tooth 2 pointed and slightly protruding, tooth 3 rounded, an incision between tooth 2 and 3, outer surface shiny-to-uneven and a few scattered setae. Antennae 30 antennomeres, longer than body, the basal flagellomeres about 3 times as long as wide, the following slightly shorter, Fp about 2 times as long as wide; the setae as long as the segment width; in lateral view 3 placodes visible. Mesosoma: 1.3 times as long as high, upper side convex. Mesoscutum (Fig. 2c) about 1.4 times wider than long, notauli developed almost to the centre and crenulate, lateral and central lobes and declivity setose, dorsal slit well differentiated. Prescutellar furrow rectangular, with 3 longitudinal ridges. Scutellum triangular. Postaxillae and metascutum glabrous. Propodeum reticulate, shiny, with short and scattered pubescence, with pentagonal area, a longitudinal carina inside, with basal carina and costulae. Furrows of sides of pronotum crenulate below. Prescutellar furrow broad, irregularly striated, tapering anteriorly and reaching edge, not reaching middle coxa, prepectal furrow narrow, passing into the crenulate anterior mesopleural furrow, posterior mesopleural furrow simple, epicoxal area of middle coxa with a few scattered setae only, sternaulus short (Fig. 2e), fairly deep and crenulated, not extending to the posterior margin. Metapleuron with long scattered setae distributed regularly, delimited from propodeum by an irregular lamella. Hind femur 5 times as long as wide, hind tarsus scarcely shorter than hind tibia. Wings (Fig. 3): st parallel-sided, reaching beyond middle of R, r arising from base of st by a distance as long as r1, the latter slightly longer than the width of st when



Figs. 1-3. Fig. 1. *Antrusa curtitempus* Fischer, Tormos, Docavo & Pardo (female): Head in dorsal view; Fig. 2. *Antrusa montecristiensis* **sp. n.** (female); Fig. 2a. Head in dorsal view; Fig. 2b. T2+3; Fig. 2c. Mesoscutum; Fig. 2d. T1; Fig. 2e. Sternaulus; Fig. 3. Anterior right wing

infolded, distal half of r2 nearly evenly bent, R not reaching tip of wing, cu2 developed by a distance greater than cql long, nr clearly antefurcal, d slightly longer than nr, nv postfurcal, B about twice as long as wide, closed by vein cu1b, np arising from middle of B; r' and cu2' indicated only as folds, nr' absent. Metasoma: T1 (Fig. 2d) as long as wide, apically 1.5-times as wide as basally, longitudinal median keel rather weak, the remainder practically smooth, with a few setae on its sides. T2 smooth (Fig. 2b). Setae of T2 + 3 (Fig. 2b) not distributed over the entire surface; a broad, bare area between T2 and T3 (Fig. 2b). Ovipositor sheath as long as hind basitarsus, reaching slightly beyond tip of metasoma. Color:

Black. Dark brown on metasoma, except T1. Yellow on anellus, mouth parts, wing venation, T1, and legs. Body length: 1.4 mm. Male—Like female except antennae with 32 antennomeres.

Host—Unknown.

Material Examined: Holotype: ♀, ITALY: Tuscan Archipelago: Montecristo island, 15–30.III.2000 (obtained through malaise trap). Paratype: ditto, 1 ♂, 1 ♀.

Etymology: The specific name of this species refers to Montecristo island, where it was captured.

Taxonomic Position: The new species can be distinguished with the following keys which are indicated continuously.

KEYS OF THE WEST PALAEARCTIC SPECIES OF *ANTRUSA* NIXON

1. Head (Fig. 1) behind eyes strongly narrowed; temples about half as long as eyes. Body length: 1.5 mm. Spain
..... *A. curtitempus* Fischer, Tormos, Docavo & Pardo, 2004
- Head (Fig. 2a) behind eyes as wide as at eyes or wider; temples about as long as eyes 2
2. Head behind eyes widened. T2 weakly sculptured; T2 + 3 setose all over. Antennae 29-32 segmented; scape and pedicel yellow. Body length: 2.5 mm. England, Germany, Central Russia *A. vaenia* Nixon, 1954
- Head behind eyes not or only slightly wider than at eyes (Fig. 2a). Setae of T2 + 3 not distributed over the entire surface; a broad, bare area between T2 and T3 (Fig. 2b). T1 sometimes longitudinally striated 3

3. r2 nearly evenly bent (Fig. 3). 4
- r2 distally bisinuate. 6
4. Mesoscutum setose only anteriorly, the rest predominantly bare. T1 longitudinally striated, weak points between the striae, shiny. T2 bare, smooth. Hind femora 5.5-times as long as wide in apical third. T1 brownish. T2+3 dark brown. Antennae 31-segmented. Body length: 2.2 mm. Central Russia *A. chrysotegula* (Tobias, 1986)
- Mesoscutum nearly entirely covered with short, fine setae (Fig. 2c). Antennae 30-32 segmented. 5
5. Hind femora 4.5 times as long as wide. T1 rugose-punctate, matte. T2 weakly longitudinally rugose at base. T1 black, T2 dark brown, rest of metasoma yellow. Body length: 2.3 mm. Moldavia *A. chrysogastra* (Tobias, 1986)
- Hind femora 5 times as long as wide. T1 with the longitudinal median keel rather weak, laterally practically smooth (Fig. 2d). T2 smooth (Fig. 2b). T1 yellow, rest of metasoma dark brown. Body length: 1.4 mm. Italy *A. montecristiensis* **sp. n.**
6. Head behind eyes widened. Antennae 23-36-segmented. T1 narrow, folds stronger. T2 smooth. Body length: 2.2-2.3 mm. Western Europe; North-West and Central Russia; Azerbaijan *A. melanocera* (Thomson, 1895)
- Head behind eyes not widened. Antennae 28-34-segmented. T1 somewhat wider, the folds rather weak. T2 sometimes weakly sculptured. Body length: 2.0-2.4 mm. Western Europe; North-, Central and South-West Russia; Azerbaijan; Siberia (Irkutsk) *A. flavicoxa* (Thomson, 1895)

IMMATURE STAGES (FIGS. 4-7)

The egg and first instar were found in host larvae at different developmental stages. The first instar remained within the trophamnion until the host formed its puparium. The trophamnion layer consist of polygonal cells. Second and third instars were only found in hosts that had pupated. Second and third instars and pupa were found in host puparia. A specimen of each stage was studied for description.

Egg. The egg (Fig. 4) is ovoid, slightly pointed at one end, and hymenopteriform in shape. Shortly after being laid, the egg varies between 130-160 μm (X (mean) \pm SD = 141 ± 12.02 , $n = 4$) in length and between 50-71 μm ($X \pm$ SD = 57.4 ± 8 , $n = 4$) in width. The developing eggs, during segmentation, increase in size (430-517 μm , $n = 2$), and become more spherical, tending towards an oblong shape at the end of their development.

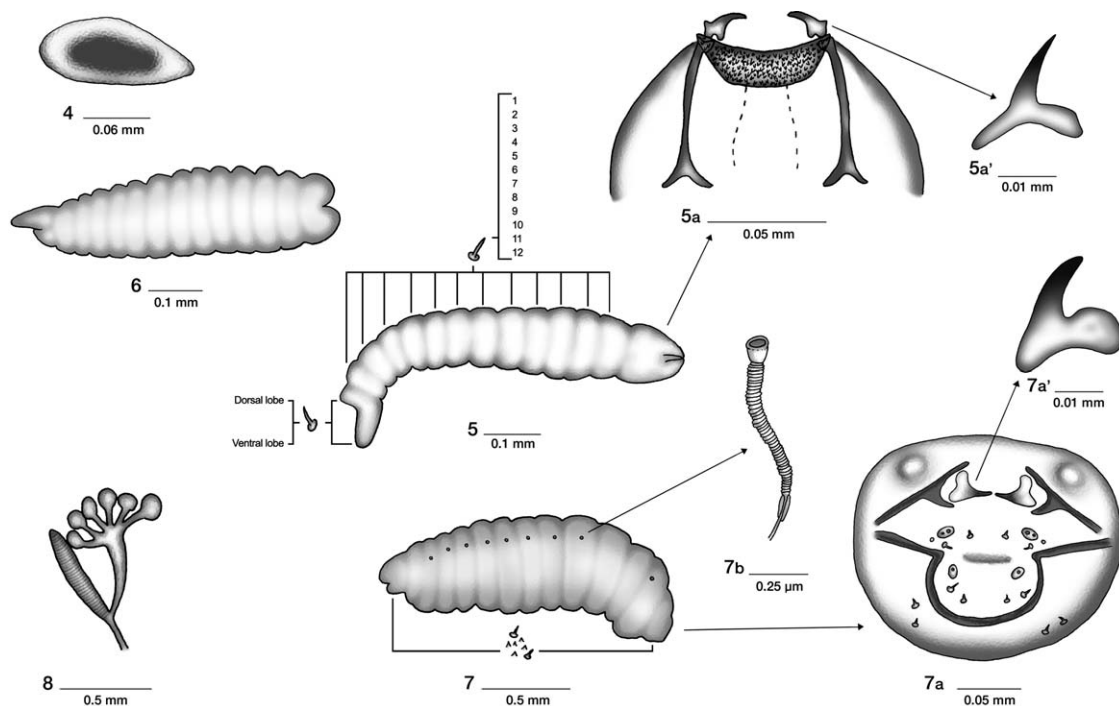
Larva. First instar *Body* (Fig. 5): Length and width (at the level of the mesothoracic segment): 0.8×0.25 mm, with head well defined and 13 body segments, caudate, vermiform. Last abdominal segment with a well-differentiated ventral lobe in the form of a tail ($l = 90$ -85 μm), with 28 setae ($l = 60$ μm) distributed in a fan around the anus. Segments 1-12 with a row of setae ($l = 45$ μm) on their posterodorsal part, the numbers corresponding to 10 (mesothorax), 16 (metathorax), and between 20 and 40 (abdominal segments). *Cranium* (Fig. 5a) ($w = 140$ μm) with pleurostomal processes and the hypostoma heavily sclerotized, and with an area of small sclerotized spicules positioned antero-ventrally. *Mouthparts*: Mandibles (Fig. 5a') well defined, with an oblong molar lobe and one blade ($l = 15$ μm) sharp, curved, and well sclerotized.

Second instar. *Body* (Fig. 6): Length = 0.7 mm; w (at the level of the mesothoracic segment) = 0.20 mm, cylindrical, long with respect to mesothoracic width, with a reduced tail. Integument bare. Without prominent cephalic sclerites or mouthparts.

Third instar. *Body* (Fig. 7): Typical hymenopteriform ($l = 1.70$, $w = 0.60$ mm), more grub-like, with the tail further reduced in length, yellowish. Integument with small setae ($l = 3$ μm) and microtrichiae covering the thoracic and abdominal segments, except the intersegmental zones and around the spiracles and anus. Nine pairs of spiracles (Fig. 7b) (diameter = 10 μm), with the atrium and closing apparatus well differentiated, 1 pair on the prothorax and another on the anterior edge of each of the first 8 abdominal segments. *Cranium* (Fig. 7a): Width (maximum) = 0.20 mm, height (taken from the base of the mandibles) = 0.10 mm, reduced, weakly sclerotized, with setae ($l = 3$ μm); orbital antennal circular ($d = 85$ μm), weakly protuberant; pleurostoma, superior and inferior mandible processes, hypostoma and stipital sclerite well differentiated and sclerotized; the latter joined to the labial sclerite, which is weakly sclerotized. *Mouthparts*. Mandibles (Fig. 7a'): Length of blade = 20 μm with broad base and relatively long blade, curved, unarmed (smooth) unidentate, sclerotized; maxillary and labial palpi oval, slightly protuberant, with one sensilla [$d = 3$ μm] in the case of the labial palpi, and with 1 sensilla [$d = 3$ μm] and the other minute [$d = 2$ μm] in the case of the maxillary palpi; salivary orifice well defined ($l = 20$ μm).

Pupa. Exarate. Without cocoon.

The preimaginal phases of *A. curtitempus* are similar to those described for *Dacnusa*. The differences mainly lie in the number and distribution of



Figs. 4-7. Fig. 4. Egg; Fig. 5. First instar (lateral view) showing the distribution of the setae of the body and lobes of the last abdominal segment; Fig. 5a. Cranium; Fig. 5a'. Mouthparts; Fig. 6. Second instar (lateral view); Fig. 7. Third instar (lateral view) showing the distribution of the tegumental differentiations of the body; Fig. 7a. Cranium; Fig. 7a' Mandible; Fig. 7b. Second spiracle; Fig. 8. Venom apparatus with detail of reservoir, secondary venom duct, venom gland and gland filaments (sacks).

the setae of the abdominal and thoracic segments of the first instar larva and in the type of the tegumental differentiations of the thorax and abdomen, as well as in the number and size of the sensilla of the maxillary palpi of the mature larva. Unlike what was reported by Čapek (1970) for *Antrusa melanocera* (Thomson 1895), the last instar of *A. curtitempus* has a well differentiated and sclerotized stipital sclerite.

VENOM APPARATUS (FIG. 8)

This species has a venom apparatus with the character states specified by Quicke et al. (1997) for *Antrusa flavicoxa* (Thomson 1895). Thus, *A. curtitempus* has (a) an undivided reservoir, (b) a reservoir neck region without narrowing, (c) a secondary venom duct absent, (d) an extensively branched venom gland (in this case with 6 sacks, in *A. flavicoxa* with 7), (e) a venom gland inserted at the extreme posterior end of the reservoir, and (f) a secondary venom duct that is not narrow. The morphological differences with the venom apparatus of *A. flavicoxa* are (a) the reservoir in *A. curtitempus* is between 4-6 times longer than maximally wide and in *A. flavicoxa* is 8 times longer than maximally wide, and (b) the venom gland in

A. curtitempus has 6 sacks and in *A. flavicoxa* the venom gland has 7 sacks.

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