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# The egg endoparasitoids of *Macrolenes dentipes* (Olivier) (Coleoptera: Chrysomelidae), with description of a new species of *Aprostocetus* Westwood and notes on its host (Hymenoptera: Eulophidae)

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**Abstract**
A new species of *Aprostocetus* (*Chrysotetrastichus*) (Hymenoptera: Eulophidae), *A. macrolenei* Viggiani **sp. nov.**, has been obtained from the eggs of *Macrolenes dentipes* (Coleoptera: Chrysomelidae: Clytrinae) and described. It is confirmed that *Bloodiella andalusica* Nowicki is also an egg parasitoid of the mentioned host. Biological notes on both parasitoids are given. Phenological data on the chrysomelid host and diagnostic characters of its undescribed first-instar larva are provided.

**Key words**: *Bloodiella andalusica*, Clytrinae, Cryptocephalinae, leaf beetle, lentisk, *Aprostocetus macrolenei*, A. *oreophilus*.

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## Introduction

In May 2019 a severe infestation, caused by leaf beetles on several plants, was detected in Cassano allo Ionio, locality Silva (Cosenza province), Calabria, Italy. Adults and egg clusters were collected for identification and study. In June-beginning of July, several parasitoids emerged from the collected eggs. Some of them were identified and recorded as *Bloodiella andalusica* Nowicki (Chalcidoidea: Trichogrammatidae) (Viggiani & Filella 2019), however, the most abundant species was an *Aprostocetus* (Hymenoptera: Eulophidae) here described as a new species. Adults and egg clusters of chrysomelids were collected also in May-June 2020 in Cassano allo Ionio (CS), locality S. Venere, and the same parasitoids emerged. A total of 237 specimens of *Aprostocetus* and 90 of *B. andalusica* were collected in 2019-2020.

Egg endoparasitoids of chrysomelid beetles (Coleoptera: Chrysomelidae) are rather poorly known. Most of them are recorded as parasitoids of Chrysomelidae Galerucinae (*Galeruca* spp.) and only one species is associated with Chrysomelidae Cryptocephalinae (Domenichini 1964, 1966; Graham 1985, 1991; Noyes 2021). Biological information is known mostly about the parasitoids of the elm-leaf beetle *Xanthogaleruca luteola* (Müller) (Marchal 1905; Silvestri 1910).

## Material and methods

In the infested area in Cassano allo Ionio, adults of chrysomelids were collected on several plants (lentisk, olive tree, vine, strawberry tree). Some of them were used to make slides, mostly of the genitalia. Pieces of olive leaves with egg clusters of the dominant chrysomelid were collected since May 2019 and isolated in Petri dishes and vials. Egg clusters were sampled in the subsequent months of 2019 and the samplings were also extended to another area (Campania: Salerno province, S. Pietro di Castellabate). From the samples emerged several parasitoids in June-July of the same year. Some of them were slide-mounted in Canada balsam phenol and others on pin-cards. All the material was examined under a Leica stereoscope and a Zeiss Axiophot with phase contrast. Both instruments were also used to take pictures by a Canon Powershot S45 camera.

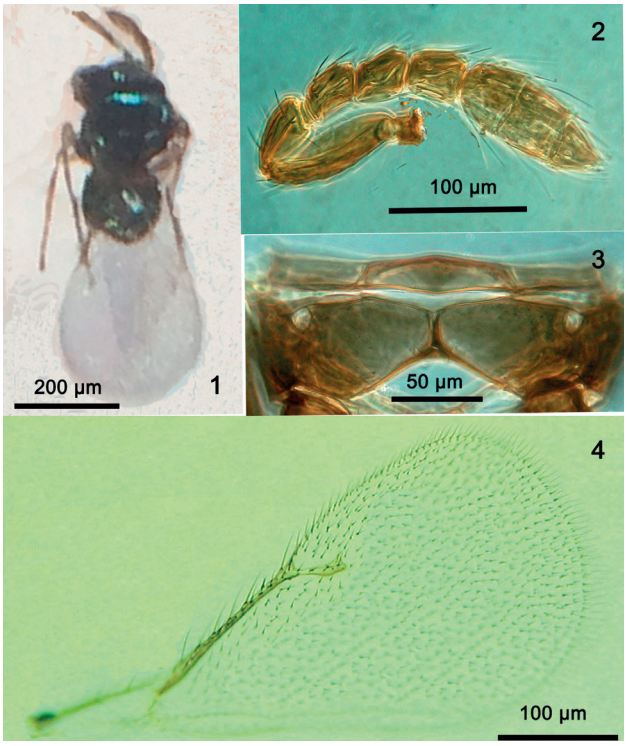
We used an integrative approach to characterize the collected specimens, starting from morphological identifications based on available keys (Graham 1987, 1991) and carried out a genetic characterization sequencing two genes. DNA was extracted from a couple of single specimens by a non-destructive Chelex and proteinase K method modified as in Gebiola et al. (2009). Two genes were sequenced: the mitochondrial cytochrome c oxidase sub-

unit I (COI) and the ribosomal gene, the expansion segment D2 of the 28S ribosomal subunit (28S-D2). The COI gene was amplified using LCO1490 paired with HCO2198 (Folmer et al. 1994). 28S-D2 was amplified with primers D2F and D2R (Campbell et al. 1993). PCR reactions and cycling conditions for COI were 1 min of initial denaturation at 94 °C, 40 cycles step at 94 °C for 30 sec, 48 °C for 1 min and 30 sec and 72 °C for 2 min, the amplification was completed by holding for 7 min at 72 °C and for 28S-D2 was set as described in (Gebiola et al. 2009).

PCR products were checked on a 1.2% agarose gel stained with GelRED® (Biotium, Fremont, CA, USA) and directly sequenced. Chromatograms were assembled using BioEdit 7.0 [28] and aligned manually.

COI sequences were virtually translated into amino acids to detect frame shift mutations and nonsense codons using EMBOSS Transeq (http://www.ebi.ac.uk/Tools/st/emboss_transeq/ (accessed March 16, 2020).

All sequences were deposited in GenBank with accession numbers MT218561 and MT218562, and lacking homologous sequences in GenBank database, our sequences were checked by Blast searches.




**Figs 1-4** – *Aprostocetus macrolenei* Viggiani, **sp. nov.** 1, female; 2, antenna; 3, metanotum, and propodeum; 4, fore wing.

## Results

The dominant leaf beetle species resulted to be *Macrolenes dentipes* (Olivier) (Chrysomelidae: Clytrinae); a few specimens of *Lachnaia italica* (Weise) and *Tituboea biguttata* (Olivier) were also collected.

During 2019-2020 several tens (237 specimens) of an eulophid tetrastichine were reared from the egg clusters of *Macrolenes dentipes*. The assignment of the reared specimens to the genus, according to the existing keys (Domenichini 1964; Graham1987, 1991) was rather difficult. The keys to several genera, subgenera, species groups and species are based frequently on characters of difficult evaluation or weak or contradictory. By the morphological approach, the species was assigned to *Aprostocetus* (subgenus *Chrysotetrastichus*), *oreophilus*-group, but the species exhibits different characters from the known species of the mentioned group (Graham 1987) and it is here described as new to Science.

From egg clusters of *M. dentipes* specimens of *Bloodiella andalusica* Nowicki (Hymenoptera: Trichogrammatidae) emerged.

### *Aprostocetus* (*Chrysotetrastichus*) *macrolenei* Viggiani sp. nov.

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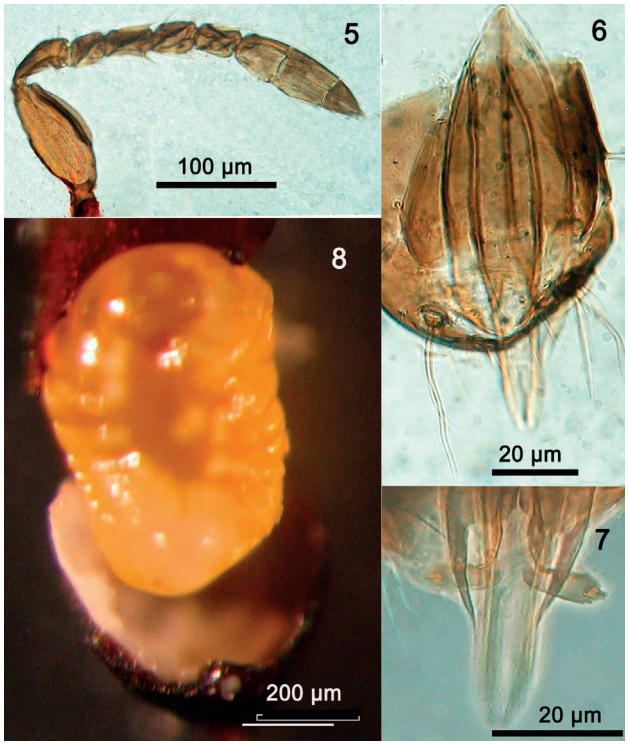
**Diagnosis**. Female. Antenna with funicle not stouter than pedicel, F1 slightly longer than wide, club ovate, about 2.5 as long as wide. Mid lobe of mesoscutum without median line, 2-4 adnotaular setae on each side. Fore wing 1.9-2.0× as long as wide, with speculum very small and rather short fringe. Gaster round-ovate, with ovipositor not extruded as long as the hind tibia. Male. With enlarged scape, 2× as long as wide; ventral plaque extended from about 1/3 of the basal margin to very near the distal end, on 2/3 of the segment.

**Description**. Female (Fig. 1). Body black, with green-blue metallic tints on the thorax; antennae brown; front legs with distal part of the femur, tibia, and tarsus yellowish to brownish; wings hyaline. Length: 0.6-0.7 mm. Head as wide as thorax, 2.3× as wide as long, POL1.8× of OOL; malar space 0.6 length of eye, sulcus nearly straight; mouth about 1.2×malar space; anterior margin of clypeus without teeth in the middle. Mandible 3-toothed; maxillary palp uni-segmented, 4× as long as wide, with a terminal seta about as long as its length; labial palp half length of the maxillary palp and with a terminal seta 1.5 as long as its length. Antenna (Fig.2) with scape 0.7× as long as the length of an eye, about 4× as long as wide, pedicel slightly longer than F1, 1 small discoidal anellus well evident funicle not stouter than pedicel, F1 slightly longer than wide (8:6) as F2 and F3, club ovate, about 2.5× as long as wide, 4.6-5.0 as wide as funicle, club segments subequal, with C2× slightly longer than C1 and C3; terminal spine very short. Linear sensilla on funicular and club segments with their distal part markedly prominent (about 0.8× their base), like a strong seta, their number is 2-3 on one surface of each segment; setae




**Figs 5-8 –** *Aprostocetus macrolenei* Viggiani, **sp. nov.** 5, male antenna; 6, genitalia; 7, particular of genitalia; 8, last instar larva of *A. macrolenei*.

of different lengths on each segment, the longest from the base of a segment to the base of the following. Thorax about as long as gaster. Mid lobe of mesoscutum without median line, with a very superficial reticulation composed of elongate areoles; 2-4 adnotaular setae on each side; scutellum 1.2× as wide as long, with well-marked submedian lines, sublateral lines at a distance of 0.8× to each other and 2 pairs of setae. Dorsellum (Fig. 3) shorter than propodeum in the middle (5:7), the latter with small spiracles 0.25-0.3× of the corresponding propodeum length, near its anterior margin, 1 paraspiracular seta on callus, a median carina and without reticulation. Fore wing (Fig.4) rather large, 1.9-2,0× as long as wide, extended beyond the end of gaster as the length of the thorax and gaster combined, costal cell slightly shorter or as long as the marginal vein, submarginal vein normally with 2 dorsal setae, marginal vein 2.7-3.0× the length of stigmal vein, its front edge with 5-8 setae, not longer than longest discal fringe, speculum very small, closed below, discal ciliation rather dense and short marginal fringe 1/7-1/8 of discal width. Hind wing acute, with ciliation regularly extended from below the base level of the marginal vein to the distal end of the blade; longest marginal setae of the disc about as half of the discal width. Legs rather slender, basitarsomeres slightly shorter than subsequent segments (5:7) or of the same length, middle spur slightly shorter than corresponding basitarsomere. Gaster round-ovate, as long as the thorax, 1.2× as long as wide, with short, transverse petiole, cercal setae with the longest kinked; tip of hypopygium at about base of the ovipositor; ovipositor not extruded, as long as the hind tibia, with 3rd valvulae short, 1/4 of the ovipositor length.

Male. Color and general characters as female. Antenna (Fig.5) longer, with enlarged scape, 2× as long as wide, with ventral, sensorial plaque extended from about 1/3 of the basal margin to very near the distal end, on 2/3 of the segment, pedicel 2× the length of F1, the latter about as long as wide, F2-F4 slightly longer; club 2.5× as long as wide; sensilla and setae as in the female. Gaster oblong-ovate. Genitalia (Fig. 6) small, 0.15 mm in length, with phallobase ovate, basally pointed, with a ventral ridge, parameres (Fig. 7) with a seta at about 1/3 of their base and another longer at the distal end, volsella with one digital spine, aedeagus longer than phallobase (55:50), with long apodemes, 2.7× as long as the aedeagal body.

**Taxonomic remarks**. The specimens studied in this paper exhibit characters of *Oomyzus* Rondani, *Aprostocetus* Westwood and *Baryscapus* Foerster. The genus *Oomyzus*, as defined by Graham (1991) is hardly distinguishable from *Baryscapus* Foerster and rather heterogeneous. Their distinction is mainly based on the "malar sulcus straight or virtually so" in *Oomyzus* ("malar sulcus present and distinctly, often rather strongly curved" in *Baryscapus*) and "only one dorsal seta on SM (2 only in rare cases)" in *Oomyzus* ("2 or more dorsal setae on SM, except some species in *Baryscapus*"). The wide genus *Aprostocetus*, including several subgenera, is characterized by having "malar sulcus present, usually straight or weakly curved, rarely strongly curved, usually simple, but occasionally foveate below the eye". The variability of the mentioned characters may explain the uncertainty in the genus identification.

Egg endoparasitoids of Coleoptera Chrysomelidae have been described in *Oomyzus* and *Aprostocetus* (previously in *Tetrastichus*) genera, hence the species included in these two genera were taken into consideration. In the key provided by Domenichini (1964) the species fits with *Tetrastichus oreophilus* Förster, 1861, recorded for the first time as an egg parasitoid of *Cryptocephalus pini* (L.) (Coleoptera: Chrysomelidae, Cryptocephalinae), on which the same author based the *oreophilus* species-group (Domenichini 1966). Graham (1987) transferred this species under *Aprostocetus* (subgenus *Chrysotetrastichus*), in the *oreophilus*-group. In Graham's key to the species included in *Aprostocetus* (*Chrysotetrastichus*) the eulophid under study runs to couplet 9, concerning the uncertain species *A. masculinus* Graham, 1987, and *A. setulosus* Graham, 1959, and based mostly on male characters. From the first species *A. macrolenei* sp. nov. differs mostly for the sensorial plaque on the scape (about as long the ventral margin in *masculinus*, 3/4 of the same in *macrolenei*) and from the length of F1 in the male antenna (1.6-1.8× as long as wide in *setulosus*, slightly longer than wide in *macrolenei*). From *A. oreophilus* the new species differs

for having the funicle of the female antenna not stouter than the pedicel, the marginal vein of the fore wing 2.7-3.0×the length of the stigmal vein (in *oreophilus* funicle stouter than the pedicel and marginal vein 2.0 - 2.5× of the stigmal vein) and the ventral plaque of the scape in the male antenna longer (in *macrolenei* 0.66-0.75× the length of scape, in *oreophilus* 0.42-0.55×).

The molecular results showed that the species is close to *Aprostocetus ceroplastae* (Girault, 1916) and *A. dendroctoni* Yang, 1996; 99.6% similarity for 28S and to *A. ceroplastae* 91.84% of similarity for COI, but both mentioned species are morphologically and biologically very distinct.

At present three species of egg parasitoids of Coleoptera Chrysomelidae Galerucinae are included in *Oomyzus*, *gallerucae*-group. After the description of this group by Domenichini (1966) and Graham (1985), the latter author (Graham 1991) writes that *gallerucae* is a "natural group, which however, is difficult to define". It seems reasonable, after the description of the new species *A. macrolenei*, to evaluate the elimination of the mentioned group-name and transfer the included species in *Aprostocetus,* although this formal act will be considered in a separate paper.

**Etymology**. The species name is referred to the host.

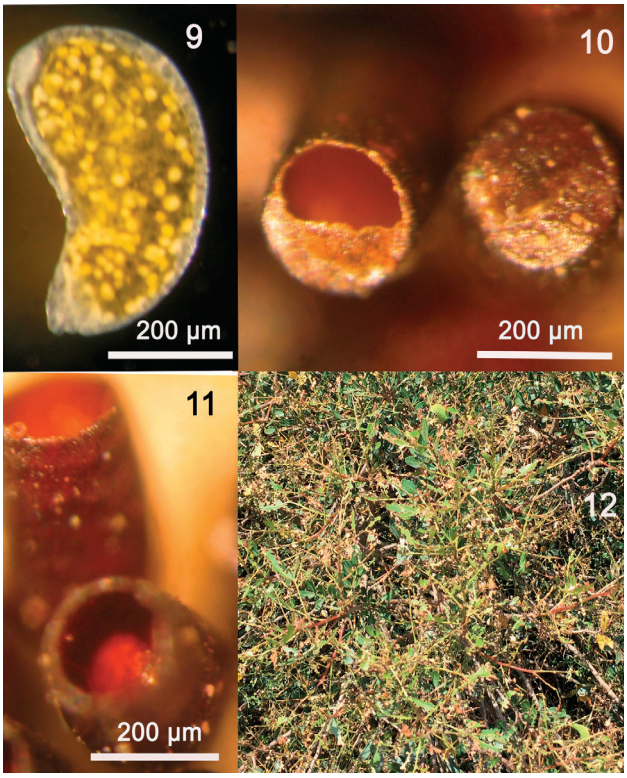
**Material examined**. Holotype ♀ (on slide), **Italy**: Calabria (Cosenza province), Cassano allo Ionio, locality Silva, 21-30.vi.2019, 39°47'09.97"N, 16°22'01.80", from egg of *Macrolenes dentipes* on an olive tree leaf, F. Filella lgt. Paratypes. 4♀ and 2♂ (on slide), same data of holotype. Additional material. 2♀ (on pin-card), Follonica, 27.vi.1984, from eggs of *M. dentipes* on an olive tree leaf, R. Tiberi lgt. Several tens of additional specimens (on pin-card) reared from eggs of *M. dentipes* on olive tree leaves collected in Cassano allo Ionio, 10.v.-26.vi.2020.

Holotype and paratypes will be deposited in the entomological collection of the Dipartimento di Agraria dell'Università degli Studi "Federico II", Portici, Napoli, Italy. Two paratypes will be deposited in the Natural History Museum, London.

*Last instar larva*. Body chalcidoid-type (Figs 8-9), yellowish, without setae or other cuticular structures; head small, subtriangular mandibles and unmarked endoskeleton; no trace of tracheal system; length about 0.5 mm.

## Life cycle

The first annual emergence of *A. macrolenei* sp. nov. occurs in April-May when the host oviposits. The parasitoid emerges from the host eggs (Fig. 10, left) of the preceding year, where its last instar larva undergoes a long period of apparent inactivity, from spring to spring. A probable diapause and not a simple quiescence is involved. The exit




**Figs 9-12 –** 9, last instar larva of *Aprostocetus macrolenei* Viggiani, **sp. nov.** mounted on slide; 10, egg of *Macrolenes dentipes* with exit hole of *Aprostocetus macrolenei* (left) and an unhatched egg (right); 11, hatched eggs of *Macrolenes dentipes*; 12, severe damage of *M. dentipes* infestation on lentisk.

hole of the parasitoid appears of various shapes, but not as a regular circle extended to the complete distal cap, as in the hatched eggs (Fig. 11). The ratio males/females was 2.64 (n = 50). *A. macrolenei* sp. nov. is a solitary parasitoid. The rate of parasitization recorded was variable from some eggs/cluster to all eggs/cluster. The parasitoid oviposits in the fresh host eggs of the year. Probably the parasitoid, as in other species, is near the ovipositing host female while it lays its eggs (Masutti 1960). The larval and pupal development lasts about one month. The new parasitoid emergence was mostly observed in the second half of June. In some clusters, unhatched eggs were observed and in each of those eggs, a mature larva of the parasitoid was found.

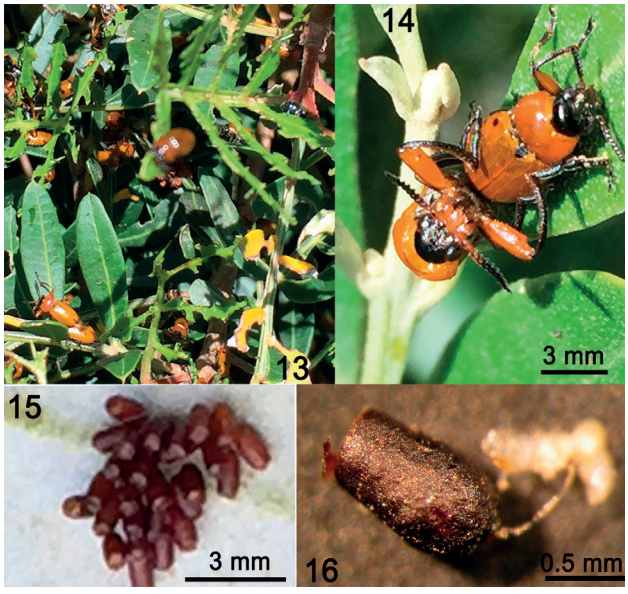
The life cycle of *A. macrolenei* sp. nov. appears markedly different from that of the allied species *A. oreophilus*. In fact, according to Masutti (1960), the latter species (named *Tetrastichus* = *Aprostocetus*) emerges in late summer, when the host, *Cryptocephalus pini*, starts progressively to oviposit until November. From the end of October in the eggs oviposited in the preceding year, mature larvae, pupae, exuviae of pupae, and emerging adults of the parasitoid were found. After oviposition in the host eggs of the year, probably before they fall on the soil, the parasitoid remains inactive until the autumn of the next year.

### *Bloodiella andalusica* Nowicki, 1935

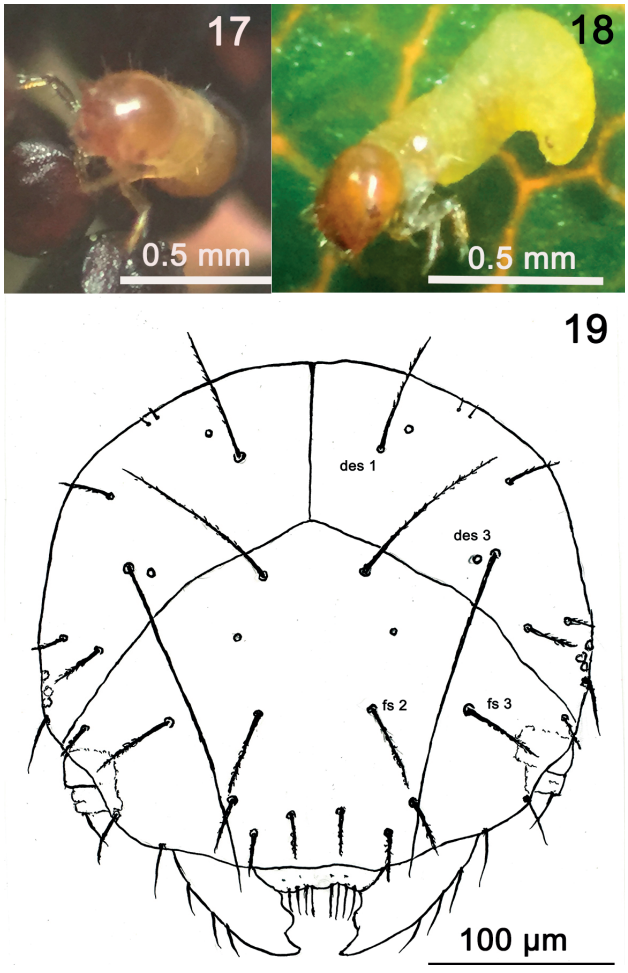
In a previous paper (Viggiani & Filella 2019), the eggs of *M. dentipes* were hypothesized to be the host of *B. andalusica*. The observations carried out in 2020 demonstrate that *B. andalusica* is a solitary egg endoparasitoid of *M. dentipes*. In May 2020 adults emerged from overwintering egg clusters of the chrysomelid host, they oviposited in fresh host eggs and a new adult emergence occurred in June, when 90 specimens of *B. andalusica* were reared. The trichogrammatid shows the same phenology of the eulophid *A. macrolenei* described above.

### Notes on *Macrolenes dentipes*

The genus *Macrolenes* Chevrolat, 1836, includes the species *Macrolenes dentipes* (Olivier, 1808) and *Macrolenes bellieri* (Reiche, 1860); the latter recorded only in Sicily. *M. dentipes* is widely distributed in the Mediterranean region (Regalin & Medvedev 2010) and rather easily recognized for the male front legs having long and dentate femora (Fig. 14). The adults are observed on several plants [*Pistacia*, *Rhus* (Anacardiaceae), *Quercus* (Fagaceae), *Fraxinus* (Oleaceae), *Paliurus* and *Ziziphus* (Rhamnaceae)] from May to August, but they are preferably active leaf-eater on *Pistacia lentiscus* L. (Agoiz-Bustamante et al. 2019). Adults of *M. dentipes* are phytophagous, while larval stages are phyto-zoo-saprophagous (Schöller 1998). The females oviposit eggs in clusters attached to the leaf surface by long strands. The first instar larva is undescribed. Larval development occurs on the soil. The third larva, described by Medvedev & Schöller (2002) on specimens collected under a stone covering an ant's nest under a *Quercus ilex* tree, is protected by a simple case, light grey to brownish, with the fine line-like structure on the surface. *M. dentipes* is one of the ant-nest beetles (Agrain et al. 2015), but according to Schöller & Witte (2007), the larvae of the most advanced genera of Clytrinae (*Labidostomis* and *Macrolenes*) have lost secondarily the ability to penetrate into the ant nests and remain at the entrance of the host's nest, namely, a species of *Tapinoma*: a new, protected and food-rich niche of a dolichoderine.




**Figs 13-16 –** 13, adults of *Macrolenes dentipes* infesting lentisk; 14, *M. dentipes* in copulation; 15, egg cluster; 16, egg with attached strand.




**Figs 17-19 –** 17, *Macrolenes dentipes*, hatching first instar larva; 18, first instar larva; 19, head of the first instar larva, frontal view.

In our study area a massive population of *M. dentipes* was recorded in May 2019. Adults were observed on several plants (*Arbutus firstly unedo*, *Olea europaea, Pistacia lentiscus*, *Vitis vinifera*), but they produced severe damages mostly on lentisk (Figs 12-13). Mating (Fig. 14) and egg clusters (Fig. 15) were observed mostly on olive tree leaves. Eggs (Fig. 16) are subcylindrical, basally pointed and distally trunked, 0.7-0.9 mm in length and 0.4 mm in width. The diameter of the egg distal end is about as that of the head of the first emerging larva of the leaf beetle (0.3 mm). The chorion of the egg is coated with brown material.

The number of eggs/cluster varied from 4 to 38 (average: 18; SD ± 10.6, n = 23). The eggs hatched at the beginning of June (Fig. 17); after hatching, they showed the distal cap completely detached (Fig. 11). The first instar larva (Fig. 18), 0.8-0.9 mm in length, is typically J-shaped, with the last abdominal segments directed ventrally. The head chaetotaxy (Fig. 19) offers diagnostic characters for the identification of the species; in particular: the single seta, on each side of the epicranial suture, not simple but at least slightly spiny as the other dorsal epicranial setae, except the very long 3rd seta; all frontal setae moderately or densely spiny, with the 3rd pair not longer than the second one.In the key for the identification of the clytrine genera by Wasowska (2007), based on the first instar larva, the larva of *M. dentipes* runs near *Lobidostomis*, having one seta on each side of epicranial suture, on top of the head, but simple in the latter species, not spiny.

## Concluding remarks

The biology of the known parasitoids linked to the eggs of Chrysomelidae Clytrinae and Cryptocephalinae appears very interesting. The biological information concerning *Aprostocetus oreophilus*, an egg parasitoid of *Cryptocephalus pini*, given by Masutti (1960), showed long inactivity of the eulophid into the host eggs, from fall to fall, timed with the single annual host period of oviposition. The author did not specify the instar or instars, but probably young stages are involved in this behavior. The studied case of *A. macrolenei* shows another strict host-parasitoid adaptation mediated by the last instar larva of the latter, which remains inactive in the host egg for about a year, from spring to spring. This apparent inactivity, interrupted in April-May, is probably maintained by diapause and not by quiescence. In fact, the only change in temperature is not effective. The occurrence of this phenomenon in egg parasitoids of both Clytrinae and Cryptocephalinae is another indication of the affinity between the species belonging to the two groups mentioned.

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