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First rearing records of *Bloodiella andalusica* Nowicki, 1935 (Hymenoptera: Chalcidoidea, Trichogrammatidae) in France, from the eggs of *Labidostomis taxicornis* and *Macrolenes dentipes* (Coleoptera: Chrysomelidae, Clytrini)

ANDREW POLASZEK

Dept of Life Sciences, Natural History Museum, London SW7 5BD, U.K.
A.Polaszek@nhm.ac.uk

PIETER KAN

Filming VarWild, 295, Chemin de la Croix, La Ferrage du Ray, 83830 Callas, France
pieterkan83@gmail.com

BRIGITTE KAN-VAN LIMBURG STIRUM

Filming VarWild, 295, Chemin de la Croix, La Ferrage du Ray, 83830 Callas, France

SYLVIE WAROT

Institut Sophia Agrobiotech, INRA, CNRS, Université Côte d'Azur, 400 route des Chappes, BP 167, F-06903 Sophia Antipolis Cedex, France
sylvie.warot@inra.fr

GÉRALDINE GROUSSIER

Institut Sophia Agrobiotech, INRA, CNRS, Université Côte d'Azur, 400 route des Chappes, BP 167, F-06903 Sophia Antipolis Cedex, France
geraldine.groussier@inra.fr

Abstract

Bloodiella andalusica is a solitary egg-parasitoid with a body length of 0.5–0.7 mm, recorded from mainland France for the first time. Specimens were reared in Var, in the Provence-Alpes-Côte d'Azur region of South-eastern France, from egg clusters of two leaf beetle species: *Labidostomis taxicornis* (Fabricius, 1792) and *Macrolenes dentipes* (Olivier, 1808). This is the first record of *B. andalusica* from *L. taxicornis*, *M. dentipes* having been recorded as a host previously in Italy. DNA barcode sequences of *B. andalusica* were obtained and deposited in Genbank. Our observations on this species are summarised and illustrated.

Keywords: Barcode DNA sequence, egg parasitoid, ooparasitoid, Genbank, new host record, new distribution record

Introduction

Bloodiella Nowicki is a small genus of trichogrammatid ooparasitoids with just three currently valid species. *B. andalusica* Nowicki is recorded previously from France (Corsica), Hungary, Italy, Poland, Spain, Turkey and Yugoslavia (Bouček, 1977; Erdös, 1956; Nowicky, 1935; Öncüer, 1991; Viggiani & Filella, 2019). *B. carbonelli* De Santis is known only from Uruguay (De Santis, 1970) and *B. gynandrophthalmae* (Risbec) from Senegal (Risbec, 1951).

Previously recorded hosts are the chrysomelid leaf beetles *Macrolenes dentipes* (Italy: Viggiani & Filella, 2019; Viggiani, Filella & Bernardo, 2021) for *B. andalusica*, and *Smaragdina immaculata* (Lacordaire) (as *Gynandrophthalma weisei* Jacoby) for *B. gynandrophthalmae* (Senegal: Risbec, 1951).

Between 2014 and 2019 the project; '*Trichogramma* for plant protection' (TriPTIC), was carried out by the French National Research Institute for Agriculture, Food and Enviroment (INRAE). One of the objectives of the project was to gain more knowledge of the biodiversity of egg parasitoids in natural hosts. Eggs collected during surveys included those of *Labidostomis taxicornis* in 2016 and 2020, and *M. dentipes* in 2019 (both Chrysomelidae: Clytrini), and details of rearing *B. andalusica* from them are given here.

Materials and Methods

Collecting

In collaboration with the TriPTIC project, which started in 2014, PK and BK looked for eggs, and observed oviposition behaviour, of insect species in their natural environment. Eggs of different butterfly species were initially monitored and collected, later eggs of bugs, beetles and lacewings were collected and reared.

In May and June 2020, females of species from four genera were collected from the two localities and placed separately in glass containers to observe the oviposition and eggs in order to compare them with the two egg clusters from which *B. andalusica* emerged. The collected species were four female and four male *Labidostomis taxicornis*, one female *Macrolenes dentipes*, two female *Lachnaia italica* (Chevrolat) and three female *Clytra atraphaxidis* (Pallas).

Data logging and preservation

When oviposition was observed, the location was tagged with red tape and information recorded about the species, host plant, dates and site location. When a species could not be identified in the field the ovipositing female was collected for identification. After about 3–5 days the eggs were collected and put in small glass test tubes (10 mm Ø × 65 mm), provided with a label with the data, and closed with cotton wool. When parasitoids emerged from the collected eggs they were placed in 70% ethanol with a label with a code number and kept in a fridge. Specimens were initially sent to GG (INRAE) for identification. The empty eggs were preserved dry, in separate tubes with labels provided with the same code, serial number and data as the egg parasitoid which emerged from it.

Imaging

Pictures of the various egg clusters were taken by PK (Figs 1–4) with a LUMIX Panasonic DMC-TZ10 HD AVCHD Lite. The habitus image of *Bloodiella andalusica* (Fig. 5) was made by AP using a Canon DSLR with 10× Mitutoyo objective, processed with Helicon Focus stacking software; male genitalia (Fig. 6) and the antennae of a male and female (Figs 7 & 8) were made by AP using a Leitz Dialux 20EB compound microscope using Nomarski Differential Interference Contrast (DIC) illumination, photographed with MicroPublisher 5.0 RTV camera and scanned sections stacked and combined using Synoptics AutoMontage® software. All final image editing was with Adobe Photoshop CC®. Figs 4–8 are of specimens reared in 2016.

Parasitoid identification (morphological)

43 parasitoids that emerged in 2016, and 6 parasitoids (out of 30) that emerged in 2020, from the eggs of *Labidostomis taxicornis*, as well as 14 parasitoids that emerged in 2019 from the eggs of *Macrolenes dentipes* were sent to the Natural

History Museum (NHMUK) in London for examination by AP. Genomic DNA extraction was undertaken using the protocol in Polaszek *et al.* (2014) and Cruaud *et al.* (2019), which leaves the sclerotized parts of the specimen intact. Specimens were then critical point dried and card-mounted, with selected individuals then dissected and mounted in Canada balsam on microscope slides and examined at magnifications up to 400× using Nomarski Differential Interference Contrast (DIC) illumination. All specimens, as well as the host egg remains, have been deposited in the NHMUK collection. About 20 specimens reared from the egg cluster of *Labidostomis taxicornis* in 2020 are deposited in the collection of Institut Sophia Agrobiotech (INRAE).

Parasitoid identification (molecular)

Adults of *B. andalusica* were kept in 70% ethanol and brought back to Biological Resource Center 'Egg Parasitoids Collection' (BRC EP-Coll.), https://www6.inrae.fr/crb-eggparasitoids-coll_eng/Presentation for molecular characterisation. Non-destructive DNA extraction was performed for each sample by using a Quick Extract™ DNA Extraction Solution kit from Lucigem® (following the manufacturer's protocol, QE09050). A portion of the mitochondrial gene Cytochrome oxydase I (COI) was amplified using the primer pair: LCO 1490 (5'-GTCAACAAATCATAAAGATATTGG-3') and HCO 2198 (5'-TAAACTTCAGGGTGACCAAAAAATCA-3') (Folmer *et al.*, 1994). The COI-PCR conditions were as follows: 95°C for 5 min, followed by 35 cycles of (i) 94°C for 30s, (ii) 50°C for 1 min, and 72°C for 1 min with a final extension at 72°C for 10 min. PCR products were sequenced by Genewiz (Leipzig, Germany). Sequences were compared to already existing sequences in the international database Genbank® and the internal database of BCR EP-Coll. Molecular analyses were carried out with the use of Geneious software version R10 (Drummond *et al.*, 2010) and MEGA software version 7.0.25 (Tamura *et al.*, 2013). Specimens are deposited in the INRAE collection.

Results

Parasitoid emergence

On 1.viii.2016 an egg cluster of about 50 eggs, on a *Ptychotis saxifraga* stem, about 30 cm high (Fig. 1), was collected at an old quarry in Bargemon (Var). 46 trichogrammatids emerged between 4–9.viii.2016.

On 26.vi.2019 an egg cluster of 15 eggs of a different species was collected from a *Prunus dulcis* leaf at a height of 1.55 m (Fig. 2), in the garden at La Ferrage du Ray in Callas (Var). 14 trichogrammatids emerged on 4.vii.2019.

On 2.vi.2020 a female *L. taxicornis* was observed and photographed during oviposition, about thirty eggs were laid, 25 cm high on a *Knautia arvensis* (L.) leaf, placed on a forest trail at Taradeau (Var) (Fig. 3). When the female leaf beetle stopped egg-laying, she was captured for identification. On 9.vi.2020, the leaf with the egg cluster was collected from which on 29.vi.2020, thirty trichogrammatids emerged.

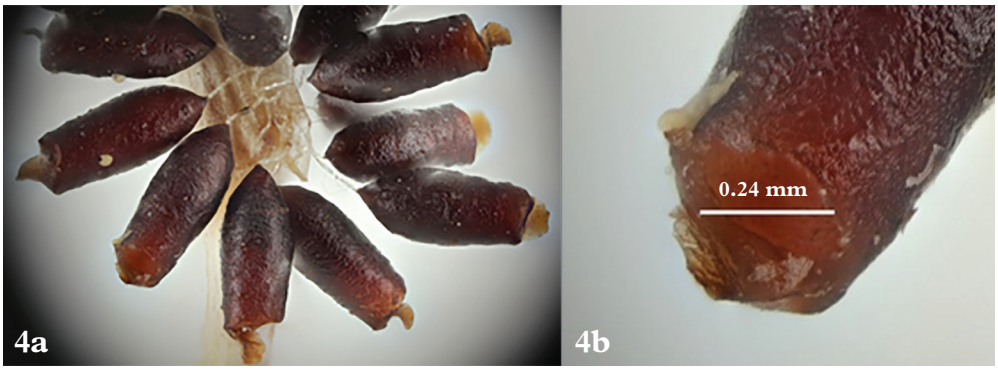
Labidostomis taxicornis

From the *L. taxicornis* egg cluster collected at Bargemonon on a *Ptychotis saxifraga* stem, with about 50 eggs, 46 *B. andalusica* emerged between 4 and 9.viii.2016. Emergences were as follows: 4.viii: 3 (escaped); 6.viii: 1; 7.viii: 4;

Fig. 1. Egg cluster of *Labidostomis taxicornis* (1.viii.2016).

Fig. 2. Egg cluster of *Macrolenes dentipes* (26.vi.2019).

Fig. 3. Oviposition of *L. taxicornis* (2.vi.2020).



Figs 4a, 4b. *B. andalusica* exit hole of 0.24 mm in *L. taxicornis* eggs.

Fig. 5. *Bloodiella andalusica* ♀.

8.viii: 6; 9.viii: 32. Of the 43 specimens AP identified, 10 were males 29 females (4 were lost during DNA extraction/mounting). The *L. taxicornis* host eggs are 1.3 mm long, with an exit hole of 0.24 mm (Fig. 5). From the *L. taxicornis* egg cluster collected at Taradeau, with 30 eggs, laid on 2.vi.2020 (Fig. 3), collected on 9.vi.2020, from which 30 female *B. andalusica* emerged on 29.vi.2020.

Macrolenes dentipes

From the *M. dentipes* egg cluster, with 15 eggs, collected at Callas on 4.vii.2019 – 14 *B. andalusica* emerged; one escaped. Of the 13 specimens, 2 were used for DNA sequencing (done by GG at INRAE), 11 females were sent to AP and identified as *B. andalusica*. The *M. dentipes* eggs are 1.0 mm; with an exit hole of 0.25 mm.

Host identification

Although the first two collected egg clusters were quite different, and obviously from two different species, most probably belonging to leaf beetles of the Clytrini, it was not clear to which species they belonged. In France there are ten genera in the tribe Clytini. Only the species of four genera are large enough to produce these egg clusters, namely: *Macrolenes*, *Labidostomis*, *Lachnaia* and *Clytra*. Unfortunately, very little is published on the biology of the species in these genera.

The eggs of *L. italica* and *C. atraphaxidis* were completely different from the two collected egg clusters and the species from these genera were thus excluded.

The egg cluster from *M. dentipes*, which has no congeners occurring in Var, looked unmistakably like the egg cluster found in Callas in 2019 (Fig. 2), and we therefore assume that this egg cluster is from *M. dentipes*, which corresponds with the findings of Viggiani & Filella (2019).

The egg cluster from *L. taxicornis* looked exactly like the egg cluster collected in Bargemon in 2016 (Fig. 1). However, in France there are about nine (very similar) species in the genus *Labidostomis*, of which at least three could occur in Var. Since *L. taxicornis* is the most common species of which the eggs are identical with the egg cluster collected in Bargemon in 2016, we assumed that this is the species whose eggs were parasitized by *B. andalusica*. Mating males and females producing the reference egg clusters were sent for identification to Dr Michael Geiser (Natural History Museum, London) who confirmed their identification as as *Labidostomis taxicornis*.

In each of the three collected chrysomelid egg clusters, all the eggs were parasitized by *B. andalusica*. Although *B. andalusica* was found in an area where intensive research was done on egg parasitoids of different insect species, from which multiple different trichogrammatids were reared (Kan *et al*, in preparation), mostly from eggs of different butterfly species, *B. andalusica* was only reared from eggs of *Macrolenes dentipes* and *Labidostomis taxicornis* and seems to be a specialist of the eggs of leaf beetles.

Parasitoid identification (morphological)

Using keys to trichogrammatid genera by Doutt & Viggiani (1968) and Pinto (2006) the distinctive genus *Bloodiella* was identified. Although *B. andalusica* was the obvious candidate, no specimens were present in the NHMUK collections. Reference to Nowicki's detailed and well-illustrated description of *B. andalusica* confirmed the identification, further confirmation became available with the publication by Viggiani & Filella (2019) which contains many figures and a re-description. Male and female specimens can be differentiated from each other mainly by the presence of the ovipositor in the latter, and aedeagus in the former (Fig. 6), and minor differences in antennal structure (Figs 7 & 8). In some slide preparations (e.g. Fig. 7) the presence of a second, minute funicle segment is indicated. Pinto (2006) distinguished *Burksiella* from *Bloodiella* based on the presence of a second, sometimes minute, funicle segment in *Burksiella*. Our observation suggests the relationship between these genera needs to be re-examined.

Parasitoid identification (molecular)

A CO1 sequence of 559 bp was obtained. No close matches were found in Genbank, the nearest was a *Perilampus* sp. at 85% similarity (very distantly

Fig. 6. *B. andalusica* ♂ genitalia: aedeagus.

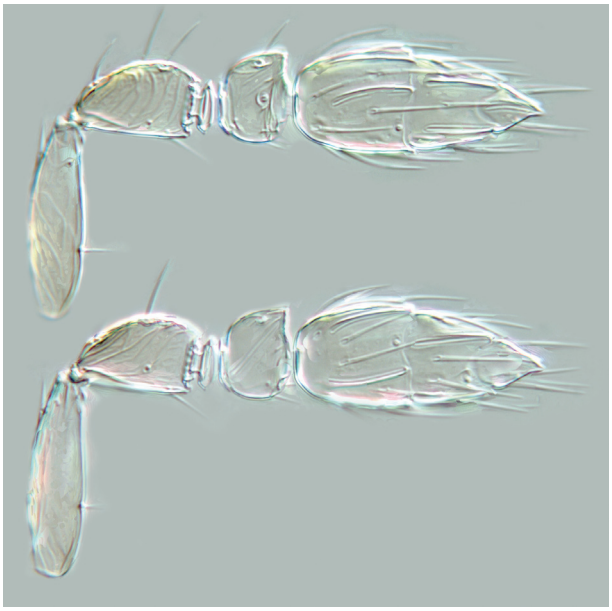


Fig. 7. *B. andalusica* ♂ antennae.

Fig. 8. *B. andalusica* ♀ antennae.

related, and in a different chalcidoid family). No matches were found in the BOLD database. The accession number in Genbank is MZ539944.

Biology of the two chrysomelid host species

Labidostomis taxicornis is recorded in Europe from Croatia, France, Italy, Malta, Portugal and Spain, and in North Africa from Algeria, Libya, Morocco and Tunisia (Regalin & Medvedev 2010). As little is published about the biology of this species, we will mention some of our own observations and rearing results. This leaf beetle is a very common in Var, and adults can be found from May to August. In spring males and females were observed flying around small oak trees, where they feed on the leaves of deciduous (*Quercus robur* (L.)) and evergreen (*Quercus ilex* (L.)) oak trees and mating occurs while feeding (PK, pers. observ.). The collected and reared egg clusters (Figs 1–4) have between 25 and 50 eggs of about 1.3 mm in diameter. The collected egg clusters were oviposited on the stems or leaves of herbaceous plants between 5 and 30 cm high.

Macrolenes dentipes is a mostly a Mediterranean species (Regalin & Medvedev, 2010) that has been recorded in Europe from Albania, Austria, Bosnia-Herzegovina, Bulgaria, Croatia, Slovenia, Spain, France, Greece, Italy, Macedonia and Montenegro; in Africa, from Algeria, Morocco and Tunisia; and in Asia from Iran, Israel, Lebanon, Syria and Turkey (Debreuil, 2010; Regalin & Medvedev, 2010). *M. dentipes* is less common than *L. taxicornis*. Adults have been observed on several plants: *Quercus* (Fagaceae), *Olea europaea* (Oleaceae), *Paliurus* and *Ziziphus* (Rhamnaceae) from May to August, but they prefer feeding on leaves of *Pistacia lentiscus* L. (Agoiz-Bustamante, Recalde Irurzun & Prieto Piloña, 2019). Adults of *M. dentipes* are phytophagous, while larval stages are phyto-zoo-saprophagous in ant nests (Schöller 1998). Viggiani, Filella & Bernado (2021) described the first instar larvae for the first time. Females lay eggs in clusters attached to the leaf surface or twigs by strands. Larval development occurs in the soil.

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