Bulletin de la Société entomologique de France, **126** (4), 2021 : 509-512. ISSN 0037-928X
https://doi.org/10.32475/bsef_2204 eISSN 2540-2641

First record of *Telenomus kolbei* (Hymenoptera, Scelionidae) in France, parasitizing the eggs of *Nymphalis antiopa* and *Aglais io* (Lepidoptera, Nymphalidae)

Pieter Kan[1], Mark R. Shaw[2], Brigitte Kan-van Limburg Stirum[1], Sylvie Warot[3] & Géraldine Groussier[3]*

[1] Association FilmingVarWild, 295 chemin de la Croix, La Ferrage du Ray, F – 83830 Callas <filming.varwild@gmail.com>
[2] Honorary Research Associate, National Museums of Scotland, Edinburgh EH1 1JF, Royaume-Uni <markshaw1945@gmail.com>
[3] Institut Sophia Agrobiotech, INRAE, CNRS, Université Côte d'Azur, 400 route des Chappes, BP 167, F – 06903 Sophia Antipolis Cedex <sylvie.warot@inrae.fr> <geraldine.groussier@inrae.fr>
* Corresponding author

(Accepté le 3.XI.2021 ; publié le 7.XII.2021)

Abstract. – *Telenomus kolbei* is newly reported in France, parasitizing two new host species: *Aglais io* and *Nymphalis antiopa*. Oviposition by the parasitoid into eggs of both species was observed. Films were made, to which links are given. DNA sequences were obtained and deposited in Genbank.

Résumé. – **Premier signalement de *Telenomus kolbei* (Hymenoptera, Scelionidae) en France, parasitant les œufs de *Nymphalis antiopa* et *Aglais io* (Lepidoptera, Nymphalidae)**. *Telenomus kolbei* a récemment été signalé en France, parasitant deux espèces-hôtes inédites : *Aglais io* et *Nymphalis antiopa*. La ponte du parasitoïde a été observée dans les œufs des deux espèces. Des films ont été réalisés, auxquels des liens se réfèrent. Les séquences d'ADN ont été obtenues et déposées dans Genbank.

Keywords. – Sex ratio, protandry, egg parasitoid, DNA sequences

---

The scelionid *Telenomus kolbei* (Mayr, 1879) is a black egg-parasitoid of about 1.5-2 mm length. This solitary endoparasitoid parasitizes eggs of Lepidoptera but the only recorded host is *Euproctis pseudoconspersa* Strand, 1910 (Erebidae, Lymantriinae) in Japan (Ashiba, 1959). In Europe, *T. kolbei* is recorded from Austria, Ireland, Denmark, Sweden and the Ukraine (O'Connor & Notton, 2013), and it is also recorded from Greenland (Buhl, 1995). The relevant specimens were caught by Malaise trapping, so no associated host is known. There are no reports of *T. kolbei* from France. In the present paper, we detail the collecting and rearing of this species in the south of France (Var) from two nymphalid hosts. Barcode COI and ITS2DNA sequences were obtained and are referenced here.

Materials and methods

***Collecting method of* Telenomus kolbei**. – In the course of filming the life-history of *Aglais io* (Linnaeus, 1758), on 7.IV.2010 an adult *T. kolbei* was filmed parasitizing a one-day-old egg-mass of this butterfly on the underside of a leaf of *Urtica dioica* L., after which it was collected for determination. The host eggs were retained for long enough to rear *A. io* larvae, but not until parasitoids would have arisen. An egg-batch of *Nymphalis antiopa* (Linnaeus, 1758) was collected on a twig of *Salix alba* L. at Lac de Méaulx, Seillans, in 2011 and 2016, in the first instance following the observation of an egg parasitoid ovipositing into them when they were three days old, and in the second case when it was clear that some of the eggs were soon to produce a parasitoid. The twigs supporting the host eggs were kept indoors in a test tube (Ø 2 cm × 17 cm) closed with cotton wool, until *T. kolbei* adults emerged.

***Identification of* Telenomus kolbei**. – In 2014, Dr Norman F. Johnson (Ohio State University, USA) identified the egg-parasitoid on the egg-mass of *Aglais io* from 2010 as well as the

emerged egg-parasitoids from the egg-batch of *Nymphalis antiopa* from 2011, and the latter specimens were deposited in the C.A. Triplehorn Insect Collection, Columbus Ohio, and recorded in the database: https://www.gbif.org/fr/occurrence/1024643650 (on the website, the locality from *T. kolbei* ex *Nymphalis antiopa* is not correct and should be Seillans instead of Callas).

Dr Andrew Polaszek (Natural History Museum, London, UK) identified the parasitoids that emerged from the egg-batch of *Nymphalis antiopa* in 2016 as *T. kolbei* and they were deposited in the Natural History Museum (London, United-Kingdom) collection.

Documentation. – Films were taken with a Canon XL2 with a 20× zoom, XL 5.4-108 mm lens, supplemented when appropriate with the addition of a Canon 72 mm close-up 500D lens. For macro, a Canon EF 100mm 1:2:8 with an EF Canon XL adaptor was used. The footage is recorded on mini DV tapes of 60 minutes. Still pictures were taken from the footage. For still pictures of living specimens, a Lumix HD Panasonic DMC-TZ10 was used.

Molecular characterisation. – We carried out molecular characterisation because of the opportunity it offers to be used to identify future samples in synergy with morphological criteria.

Sylvie Warot and Géraldine Groussier obtained COI (barcode) and ITS2 sequences, and took pictures of 4 reared *T. kolbei* specimens from 2016.

Adults of *T. kolbei* 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'-GTCAACAAATCA-TAAAGATATTGG-3') and HCO 2198 (5'-TAAACTTCAGGGTGACCAAAAAATCA-3') (Folmer *et al*., 1994). A second PCR was performed with the primer pair ITS2 (Stouthamer *et al*., 1999). The COI-PCR and ITS2-PCR conditions were as follows: 95°C for 5 min, followed by 35 cycles of (i) 94°C for 30 s, (ii) 50°C for 1min, 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 Genebank® 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).

Results

Telenomus kolbei is recorded from France for the first time. Both *Aglais io* and *Nymphalis antiopa* are new host records. Genbank accession numbers for the CO1 and ITS2 sequences obtained are recorded in table I.

Observations and rearing of **Telenomus kolbei**. – On 6.IV.2010, an *Aglais io* was observed egg-laying on *Urtica dioica*, on the bank of a small stream in Callas (Var). The next day, a black egg-parasitoid was parasitizing these eggs and was collected after being filmed (fig. 1; https://www.youtube.com/watch?v=PwJlj06kmM4&ab_channel=FilmingVarWild). On 28.IV.2010, 22 days later, the *A. io* caterpillars hatched. Unfortunately, no *T. kolbei* specimens were reared from this egg mass because, at that time, no attention was paid to potentially parasitized eggs.

On 16.IV.2011, a *Nymphalis antiopa* was observed depositing about 150 pale-yellow coloured eggs around a terminal twig of *Salix alba*, in the form of a cylinder, at Lac de Méaulx, Seillans (Var). Three days later, on 19.IV.2011, the eggs had turned amber-yellow, and an egg parasitoid was present (fig. 2; https://www.youtube.com/watch?v=ZbcwRKOTZ0Y&ab_channel=FilmingVarWild). By 20.V.2011, 34 days

Table I. – Genbank accession numbers for the DNA sequences obtained for *Telenomus kolbei*.

Specimen voucher	CO1 Genbank accession number	ITS2 Genbank accession number
37422	MW629080	MW632098
37423	MW629081	MW632099
37425	MW629082	–

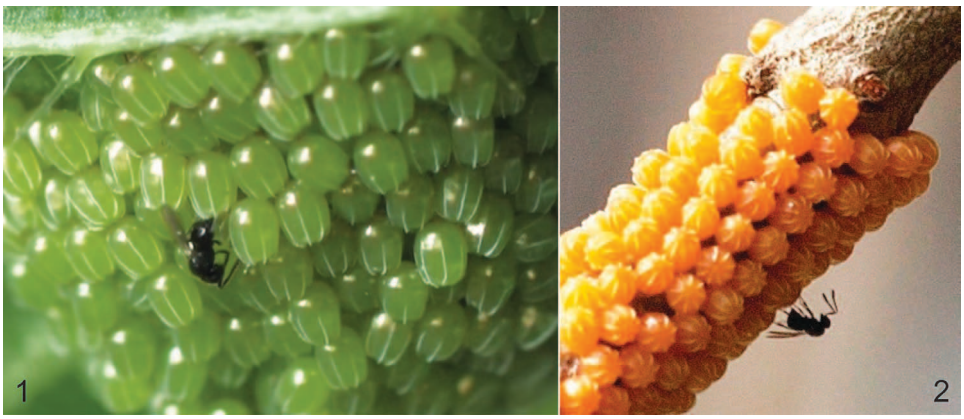

Fig. 1-2. – *Telenomus kolbei* (Mayr). – **1**, Ovipositing *Aglais io* eggs. – **2**, Ovipositing *Nymphalis antiopa* eggs.

Fig. 3. – *Telenomus kolbei* emerging from *Nymphalis antiopa* eggs.

after the egg-batch was laid, the eggs turned black and still no caterpillars had emerged. On 27.V.2011, the twig with the egg-package was collected. Around 44 days after the parasitoid wasp was observed, more than 150 black parasitoid wasps emerged between 2 and 5.VI.2011. In 2014, all these specimens were identified as males of *T. kolbei*.

On 5.IV.2016, a *N. antiopa* was observed depositing two cylindrical egg rings about 1 cm apart (considered as one egg-batch), on a twig of *Salix alba* at Lac de Méaulx, Seillans, at the same location as in 2011; one with about 140 eggs and the other with about 170 eggs. On 20.IV.2016, about 95 caterpillars had hatched in situ, leaving transparent white empty eggshells, while two-third of the eggs remained brownish. By 12.V.2016, after 37 days, all the unhatched eggs were black and the twig with eggs was collected. Over a period of nine days, between 20 and 28 May, 45 days after the eggs were deposited, about 206 *T. kolbei* emerged from the remaining eggs (fig. 3; table II). After emergence of the parasitoid, the host eggs remained black and the neat round exit hole differed in shape from the larger and more ragged hole through which the young larvae of *N. antiopa* hatches (fig. 3).

Table II. – 2016 emergence of *Telenomus kolbei*.

Date of emergence	Adults	♂	♀	Escaped
20.V.16	12	12	0	2
21.V.16	33	33	0	3
22.V.16	11	11	0	2
23.V.16	63	8	55	3
24.V.16	35	0	35	0
25.V.16	25	0	25	0
26.V.16	14	0	14	0
27.V.16	7	2	5	1
28.V.16	2	0	2	0
Total	**202**	**66**	**136**	**11**

Discussion and conclusion

Presuming that the species recorded from Japan from a lymantriine host (Ashiba, 1959) is indeed the same species, it is perhaps unlikely that this parasitoid is restricted to Nymphalinae hosts, but the extent to which it selects them *de facto* is also in need of further research. It is known that Scelionidae inject a substance that inhibits host embryonic development at the time they oviposit, just before their egg passes into the host egg (Strand *et al*., 1983). This suggests that a broad host range may be possible, since they are effectively idiobionts (*i.e.* rendering the host physiologically dead at the time of oviposition), though behavioural host-selection might be strong.

Since all the eggs were parasitized in the 2011 batch, from which only males emerged over a period of 3 to 4 days, it is likely that all of the host's eggs were parasitized by the same unfertilized female, or possibly by several sibling females that had emerged from an egg batch lacking males.

From the approximately 310 eggs in the 2016 *Nymphalis antiopa* batch, 95 caterpillars hatched after about 14 days (31%), and 213 *Telenomus kolbei* adults emerged between 44 and 47 days (69%), comprising 66 males and 136 females (11 more escaped) with a sex ratio male/female of about 1:2. It is not known if the eggs were parasitized by one or more females. It has been found that in some species of Scelionidae mated females parasitizing aggregated host eggs assign sex to their offspring in a sequential way, resulting in a balanced sex-ratio in which females predominate (Waage, 1982). While protandry (*i.e.* males hatching before females) is commonplace in insects, the reproductive strategy of Scelionidae outlined by Waage (1982), which facilitates sib-mating, suggests that protandry might not be expected if sequential sex-allocation is a general feature of that family. Because protandry clearly occurred in this case, a more out-breeding strategy seems indicated, not only because the males so overwhelmingly emerged before females, but also by the large number of males produced, far in excess of the sex-ratios expected for in-breeding systems. This conclusion does, however, depend on the presumption that no unmated females were involved in the parasitism of that particular egg-batch.

Because of the cylindrical single-layered way in which the eggs of *N. antiopa* are deposited they are, individually, easier to access by *T. kolbei*, compared to the egg mass of *Aglais io* in which the eggs are laid in a dense mass with the deeper eggs effectively concealed by those on top of them. In the future, it would be interesting to evaluate the extent of protection from parasitism the deeper eggs in *A. io* batches enjoy. It seems likely that *T. kolbei* specialises on gregarious egg batches, but additional work on its host range are required.

Acknowledgments. – Special thanks to Dr Norman F. Johnson and Dr Andrew Polaszek for identifying *Telenomus kolbei*. Many thanks to Peter Groenendijk for the picture of *T. kolbei* on the egg-batch of *Nymphalis antiopa.*

References

Ashiba M., 1959. – Some observation of egg parasite (*Telenomus kolbei*) of *Euproctis pseudoconspersa* under the low temperature breeding (III) [in Japanese]. *Medical Entomology and Zoology,* **10** (2) : 111. https://doi.org/10.7601/mez.10.111_2

Buhl P. N., 1995. – The proctotrupoid wasps of Greenland (Hymenoptera, Proctotrupoidea). *Entomologiske Meddelelser,* **63** : 7-10.

Drummond A. J., Ashton B. & Buxton S., 2010. – Geneious v5.5. http://www.geneious.com [accessed on 21.VI.2010]

Folmer O., Black M., Hoeh W., Lutz R. & Vrijenhoek R., 1994. – DNA primers for amplification of mitochondrial cytochrome c oxidase subunit I from diverse metazoan invertebrates. *Molecular Marine Biology and Biotechnology,* **3** : 294-299.

O'Connor J. P. & Notton D. G., 2013. – A review of the Irish scelionids (Hymenoptera: Platygastroidea, Platygastridae) including four species new to Ireland. *Bulletin of the Irish Biogeographical Society,* **37** : 20-44.

Strand M. R., Ratner S. & Vinson S. B., 1983. – Maternally induced host regulation by the egg parasitoid *Telenomus heliothidis. Physiological Entomology,* **8** : 469-475. https://doi.org/10.1111/j.1365-3032.1983.tb00381.x

Stouthamer R., Hu J., Van Kan F. J. P. M., Platner G. R. & Pinto J. D., 1999. – The utility of internally transcribed spacer 2 DNA sequences of the nuclear ribosomal gene for distinguishing sibling species of *Trichogramma*. *BioControl,* **43** : 421-440. https://doi.org/10.1023/A:1009937108715

Tamura K., Stecher G., Peterson D., Filipski A. & Kumar S., 2013. – MEGA6: Molecular Evolutionary Genetics Analysis version 6.0. *Molecular Biology and Evolution*, **30** : 2725-2729. https://doi.org/10.1093/molbev/mst197

Waage J. K., 1982. – Sib-mating and sex ratio strategies in scelionid wasps. *Ecological Entomology,* **7** : 103-112. https://doi.org/10.1111/j.1365-2311.1982.tb00648.x