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PENSOFT

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Biology of two European *Tenthredo* species (Hymenoptera, Tenthredinidae) feeding on *Gentiana*

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Abstract

Very few sawflies using Gentianaceae as larval host plants have been recorded. We identified larvae collected in Austria on *Gentiana asclepiadea* L. as *Tenthredo atra* Linnaeus, 1758 and *T. propinqua* Klug, 1817. If its current taxonomic circumscription as a single species is accepted, *T. atra* is a highly polyphagous species, whereas *T. propinqua* may be more specialised: *Gentiana asclepiadea* is its first recorded host. We sequenced plant DNA from the head of one *T. propinqua* larva, which confirmed that it had been feeding on this plant. This is the first recorded use of *G. asclepiadea* by sawfly larvae. Larvae are illustrated, and identification characters are described.

Key Words

Gentianaceae, host plants, larvae, sawflies, Symphyta, Tenthredinoidea

Introduction

The study of the immature stages of sawflies, including the identification of their larval hosts, has a long tradition in Europe, reaching back to the pioneering studies of Réaumur (1740). Despite many advances since then, we still know little or nothing about the biology of some taxa. Here, we fill one of these gaps by documenting the host plant association of two *Tenthredo* species with a member of the Gentianaceae, a plant family which has hitherto seldom been mentioned as a host of sawflies.

Recently, DNA sequencing has proved itself as a potent tool for the identification of sawfly larvae (e.g. Shinohara et al. 2017; Prous et al. 2019). In this study we used DNA sequences to identify one of the sawfly species. Compared to the traditional method of rearing an adult from a larva and determining the adult using morphological characters, sequencing can provide an identification result much more quickly, and the risk is avoided of all individuals dying before they reach maturity, in which case no identification will be obtained. We also demonstrate that DNA sequencing can be used to identify or confirm the host of a larva, using DNA extracted from the larva. This is especially useful for larvae that were collected, for example by sweeping, without any clear indication of what they were feeding on, and might also help to identify the hosts of species of Tenthredininae which do not feed on the plant species in which eggs are laid, as reported by Chevin (2009) for some *Macrophya* species.

Methods

Nearly fully grown larvae of two *Tenthredo* species were beaten from, or detected visually on *Gentiana asclepiadea* L. in the Gesäuse National Park, Styria, Austria, by E. Altenhofer and R. Netzberger in 2016 and 2019. Some larvae were kept by EA for rearing, and

others were preserved in 95% ethanol. Identification of *Tenthredo atra* Linnaeus, 1758 is based on the morphology of adults and larvae. *Tenthredo propinqua* Klug, 1817 was identified by genetic sequences obtained from a larva. Total DNA was extracted from the head of one *T. propinqua* larva (DEI-GISHym12639), and one mitochondrial (1087 bp of CO1) and two nuclear gene fragments (1654 bp of NaK and 2543 bp of POL2) were sequenced (methodology as in Prous et al. 2019). To test which host plant the larva had been feeding on, we used the same larval extract to amplify a plastid region between trnL 5′ exon and trnF using primers c and f (Taberlet et al. 1991). The region, which turned out to be 816 bp, contains two variable introns and the trnL 3′ exon and was sequenced with primers c, d, e, and f (Taberlet et al. 1991). The sequences have been deposited in the GenBank (NCBI) database (accession numbers MN856146–MN856149).

Material examined

The abbreviation SDEI refers to the insect collection of the Senckenberg Deutsches Entomologisches Institut (SDEI), Müncheberg, Germany.

Tenthredo atra Linnaeus, 1758

Austria: Styria: Gesäuse, Kroisalm, 47.60N 14.63E, 900 m, 26.08.2016, 3 females reared from larvae on *Gentiana asclepiadea* (emerged May 2017), specimens were overlooked after emergence, and are in very poor condition, i.e. fragmented, with diverse parts gummed on one card (DEI-GISHym12664), and 1 larva, leg. E. Altenhofer (SDEI). Gesäuse, E Admont, 47.58N 14.62E, 11.09.2019, 10 larvae on *Gentiana asclepiadea*, leg. E. Altenhofer (SDEI). The last of these larvae entered the ground to overwinter on 21.09.2019. Gesäuse, Hartelsgraben, 47.59N 14.73E, 23.08.2019, larva on *Gentiana asclepiadea*, photographic record by R. Netzberger (Fig. 1).

Tenthredo propinqua Klug, 1817

Larvae:

Austria: Styria: Gesäuse (E Admont), between Gstatterboden and Hochscheibenalm, 47.58N 14.62E, 600–1150 m, 15.09.2019, 10 larvae on *Gentiana asclepiadea*, leg. E. Altenhofer (2 larvae in SDEI [DEI-GISHym12639, 12640], others retained by EA for rearing).

Imagines:

Ukraine: 1 male (DEIGISHym20102), Jablunitsa, Berkut, 48.72N 24.37E, 840 m, 08.07.2004, leg. E. Heibo (SDEI). 1 male (DEIGISHym20103), Jablunitsa, Berkut, 48.72N 24.37E, 840 m, 06.07.2004, leg. E. Heibo (SDEI). 1 male (DEIGISHym20104), Jasinja, Tatariv, 48.37N 24.56E, 710 m, 03.07.2004, leg. E. Heibo (SDEI). 1 female (DEIGISHym20105), Jablunitsa, Berkut, 48.72N 24.37E, 840 m, 06.07.2004, leg. E. Heibo (SDEI).

Austria: 1 female (DEIGISHym17738), Carinthia, Eisenkappel 10km E, St Margarethen, 46.46N 14.66E, 28.06.1993, leg. L. Behne (SDEI).

Tenthredo scrophulariae Linnaeus, 1758

The larvae illustrated in Figs 6, 7 were photographed by Henri Savina in France, Ariège, Aulus-les-Bains, 42.80N 1.33E, respectively on 08.09.2007 and 30.09.2007. Host: *Scrophularia* sp.

Results

Tenthredo atra

Figs 1, 2

Notes. *Tenthredo atra* has already been associated by various authors with larval hosts in many higher plant taxa. Taeger et al. (1998), in a summary of these records, mentioned the families Brassicaceae, Caprifoliaceae, Lamiaceae, Plantaginaceae, and Solanaceae. There are also records of larvae of *T. atra* feeding on Asteraceae (Pschorn-Walcher and Altenhofer 2006), Betulaceae and Salicaceae (Loth 1913), Ranunculaceae (Conde 1934), Rosaceae (Kangas 1985), and Urticaceae (Pschorn-Walcher and Altenhofer 2000). It is not clear whether records from *Menyanthes trifoliata* (Menyanthaceae) and *Sedum telephium* (Crassulaceae), which are sometimes named as hosts of *T. atra* (e.g. Taeger et al. 1998), really refer to this species, or respectively to the closely related *T. moniliata* Klug, 1817 and *T. ignobilis* Klug, 1817. Taeger et al. (1998) mentioned some additional plant taxa on which oviposition by *T. atra* has been observed but which have not been proved to be hosts of the larvae. Our identification of the larvae from *Gentiana* as *T. atra* accepts the premise that the name refers to only one, highly polyphagous species. However, a wide morphological variability, most obvious in the colour pattern of *T. atra* imagines, might indicate that more than one species are currently grouped under this name.

The larvae from *Gentiana asclepiadea* (Figs 1, 2) are in general appearance not distinguishable from larvae of *T. atra* from other hosts, nor from the larvae of the related *T. moniliata* on *Menyanthes trifoliata* (Conde 1934; Liston personal observations). Lorenz and Kraus (1957) did not examine larvae of *T. atra*, and their description is based on those of Cameron (1882) and Carpentier (1888). Lorenz and Kraus (1957) did not mention the faint, oblique, darker dorso-lateral stripes shared by the larvae from *Gentiana*, the larva described by Cameron (1882; as *T. dispar* Klug, 1817 from *Succisa pratensis*), and larvae of *T. moniliata* examined by Liston. Note also that Carpentier's (1888) description, as *T. dispar*, is of larvae from *Menyanthes trifoliata*, and may therefore refer to *T. moniliata*, but he did not mention any body markings. On the other hand, the larva of *Tenthredo ignobilis*, another species in the complex with *T. atra*,

Figures 1, 2. *Tenthredo atra* larvae, nearly full-grown, on *Gentiana asclepiadea* (photographed respectively on 23.08.2019, 11.09.2019). Photos: R. Netzberger (**1**), E. Altenhofer (**2**).

possibly differs from *T. atra* in lacking the oblique body markings (Liston 2015: fig. 9).

Larvae of the later instars feed mainly on the leaves; they feed from the edge, leaving irregular holes. Inflorescences are also sometimes consumed, at least under rearing conditions (Fig. 2). The largest full-grown larvae are similar in size to those of *T. propinqua*, i.e. somewhat over 20 mm long.

Tenthredo propinqua

Figs 3–5

Notes. A mitochondrial CO1 sequence from one larva (DEI-GISHym12639) corresponded closely (maximum divergence 0.5%) with sequences from *T. propinqua* imagines (DEI-GISHym20102, DEI-GISHym20103, DEI-GISHym20104, DEI-GISHym20105, DEI-

Figures 3–7. Larvae of *Tenthredo propinqua* and *scrophulariae.* **3–5.** *Tenthredo propinqua*, from *Gentiana asclepiadea*. Arrows indicate some of the black dorso-median markings, which on the abdomen occupy the width of a single annulet; **3.** Preserved in ethanol (wax dissolved); **4.** Alive, with wax coating (photographed on 11.09.2019); **5.** Head, preserved in ethanol. **6, 7.** *Tenthredo scrophulariae* larvae, on *Scrophularia* species. Arrows indicate some of the black dorso-median markings, which on the abdomen extend across two annulets at least in part; **6.** Half-grown (photographed on 08.09.2007); **7.** Nearly full-grown (photographed on 30.09.2007). Photos: A. Liston (**3, 5**), E. Altenhofer (**4**), H. Savina (**6, 7**).

GISHym17738). Nuclear sequences (NaK and POL2) are available only for the specimen sequenced here (DEI-GISHym12639). The sequenced plastid trnL-trnF region (816 bp) from the larval DNA extract confirmed *Gentiana* as the host. The closest (99–100% similarity) according to NCBI BLAST (https://blast.ncbi.nlm.nih.gov/Blast.cgi) were four species of *Gentiana*, among them *G. asclepiadea*. A shorter *G. asclepiadea* sequence in GenBank (accession AB453085, 387 bp) was identical to our sequence, while a longer one (AJ580515) differed by three substitutions and one deletion over the length of 781 bp (because of apparently numerous sequencing errors at the 3′ end of AJ580515, 21 bp of that sequence were excluded from the comparison).

Gentiana asclepiadea is the first recorded host plant of the hitherto unknown larva of *T. propinqua*, which is a close relative of *T. scrophulariae* Linnaeus, 1758. These species have long been known to strongly resemble each other in the morphology of their imagines, but they are distinguishable using some colour characters (Enslin 1912). Their larvae are also closely similar in general appearance (see the description of a *T. scrophulariae* larva by Lorenz and Kraus 1957 and below). However, *T. scrophulariae* has a very different host plant spectrum, which consists mainly of *Scrophularia* and *Verbascum* species, but sometimes *Buddleja* species (Muche 1962), all of which belong to the Scrophulariaceae.

Differences in the pattern of black markings may enable *T. propinqua* larvae to be distinguished from *T. scrophulariae*, but a larger number of *T. propinqua* larvae should be checked, to confirm that the differences are consistent. In *T. propinqua*, each of the medio-dorsal black spots on the abdominal segments occupies only the width of single annulet (Figs 3, 4), whereas in *T. scrophulariae*, these spots occupy parts or the whole width of two annulets (Figs 6, 7). The position of the corresponding spots on the thorax is, however, similar in both species. At least in the later instars of *T. scrophulariae* larvae, the position of these markings is thought to be stable: compare Fig. 6 (half-grown) with Fig. 7 (nearly full-grown). After each moult, the integument of the larvae of both species temporarily lacks the covering of white wax and has a greenish ground colour. The head colour pattern of *T. propinqua* (Fig. 5) is the same as described by Lorenz and Kraus (1957) for *T. scrophulariae*. In practice, the identity of their host plant should be sufficient to distinguish larvae of these species.

The feeding habits of *Tenthredo propinqua* larvae are similar to those of *T. atra*, i.e. irregularly shaped parts of the leaf-blade are consumed from the edge. But, unlike for *T. atra*, we did not observe feeding on the inflorescences by *T. propinqua*. The largest full-grown larvae are 22–25mm long, which is about the same as given by Lorenz and Kraus (1957) for *T. scrophulariae*.

Discussion

As far as we are aware, neither *Gentiana* nor any other member of the Gentianaceae has previously been recorded as a larval host of a sawfly, except by Wang et al. (2015), who studied in China the effect of florivory by larvae on *Halenia elliptica* D. Don; they referred the larvae to as an undescribed species of Tenthredinidae. Otherwise, the only reported interaction between a sawfly species and a species of Gentianaceae involves visits to the inflorescences of *Frasera speciosa* Douglas ex Griseb. by the Nearctic *Tenthredo erythromera* Provancher, 1885 (Norment 1988).

Tenthredo propinqua is a rather rarely collected species (Ritzau 1998), whose known distribution comprises south-eastern Europe, Turkey, and the Transcaucasus (Lacourt 1999). Although the eastern part of the range of *Gentiana asclepiadea* is more or less congruent with that of *T. propinqua*, the sawfly has not yet been recorded further west than Berchtesgaden (Bavaria, Germany), although the plant is widespread in Switzerland and occurs as far west as northern Spain (Zajac and Pindel 2011). *Tenthredo propinqua* has been considered to be to some extent endangered or even locally extinct, at least in the Alps of eastern Bavaria on the north-western edge of its range (Ritzau 1998; Liston et al. 2012). In the future, we should be able to more effectively assess its distribution and conservation status by searching for its larvae.

Approximately 400 species of *Gentiana* occur worldwide in Eurasia, North Africa, the Americas, and eastern Australia, but South-East Asia is a hotspot of diversity of this genus, with 248 species known from China alone, whereas only 27–29 species occur in Europe (Ho and Pringle 1995; Mel'nyk et al. 2014). Because China also possesses a very rich fauna of *Tenthredo* species (Wei et al. 2006), it is possible that *Gentiana* is more widely used there as a host plant by these sawflies than it is in Europe.

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References

Cameron P (1882) A Monograph of the British Phytophagous Hymenoptera. (*Tenthredo*, *Sirex* and *Cynips*, Linné.). Volume 1. The Ray Society, London, 1–340. [21 pls] https://doi.org/10.5962/bhl.title.59527

Carpentier L (1888) Notes sur quelques larves de tenthrédides. Mémoires de la Societé Linnéenne du Nord de la France 7: 254–286.

Chevin H (2009) Biologie comparée des espèces francaises du genre *Macrophya* (Hymenoptera, Symphyta, Tenthredinidae). Bulletin des Naturalistes des Yvelines 36: 44–50.

Conde O (1934) Ostbaltische Tenthredinoidea, II. Teil. Korrespondenzblatt des Naturforscher-Vereins zu Riga 61: 168–198.

Enslin E (1912) Die Tenthredinoidea Mitteleuropas. Deutsche Entomologische Zeitschrift [1912](Beiheft 1): 1–98. https://doi.org/10.1002/mmnd.48019120209

Ho T, Pringle JS (1995) Gentianaceae: *Gentiana* Linnaeus. In: Wu Z, Raven PH (Eds) Flora of China, Volume 16 – Gentianaceae through Boraginaceae. Science Press and Missouri Botanical Garden Press, Beijing and St. Louis, 15–93.

Kangas JK (1985) Pälkäneen Sahapistiäisfauna 1953–1983. Pälkäne-Seuran Julkaisuja 5: 1–113.

Lacourt J (1999) Répertoire des Tenthredinidae ouest-paléarctiques (Hymenoptera, Symphyta). Mémoires de la Société Entomologique de France 1–432.

Liston AD (2015) New records and host plants of Symphyta (Hymenoptera) for Germany, Berlin and Brandenburg. Contributions to Entomology. Beiträge zur Entomologie 65(2): 383–391. https://www.contributions-to-entomology.org/article/view/1890/188

Liston AD, Jansen E, Blank SM, Kraus M, Taeger A (2012) Rote Liste und Gesamtartenliste der Pflanzenwespen (Hymenoptera: Symphyta) Deutschlands. Stand März 2011. In: Binot-Hafke M, Balzer S, Becker N, Gruttke H, Haupt H, Hofbauer N, Ludwig G, Strauch M (Eds) Rote Liste gefährdeter Tiere, Pflanzen und Pilze Deutsch-

lands. Wirbellose Tiere Teil 1. Naturschutz und Biologische Vielfalt, Bonn-Bad Godesberg 70 (3): 489–556.

Lorenz H, Kraus M (1957) Die Larvalsystematik der Blattwespen (Tenthredinoidea und Megalodontoidea). Abhandlungen zur Larvalsystematik der Insekten 1: 1–389.

Loth N (1913) Verzeichnis der im Gebiete des rheinischen Schiefergebirges und in einem Teile der niederrheinischen Tiefebene vorkommenden Tenthrediniden. Berliner entomologische Zeitschrift 58: 46–95.

Mel'nyk V, Drobyk NM, Twardovska MO, Kunakh V (2014) Karyology of European species of genus *Gentiana* L. In: Rybczynski JJ, Davey MR, Mikula A (Eds) The Gentianaceae- Volume 1: Characterization and Ecology. Springer, Berlin and Heidelberg, 219–230. https://doi.org/10.1007/978-3-642-54010-3_7

Muche WH (1962) Neue Wirtspflanze für *Allantus scrophulariae* L. Entomologische Nachrichten 6(2): 24.

Norment CJ (1988) The effect of nectar-thieving ants on the reproductive success of *Frasera speciosa* (Gentianaceae). American Midland Naturalist 120(2): 331–336. https://doi.org/10.2307/2426005

Prous M, Liston A, Kramp K, Savina H, Vårdal H, Taeger A (2019) The West Palaearctic genera of Nematinae (Hymenoptera, Tenthredinidae). ZooKeys 875: 63–127. https://doi.org/10.3897/zookeys.875.35748

Pschorn-Walcher H, Altenhofer E (2000) Langjährige Larvenaufsammlungen und Zuchten von Pflanzenwespen (Hymenoptera, Symphyta) in Mitteleuropa. Linzer biologische Beiträge 32(1): 273–327.

Pschorn-Walcher H, Altenhofer E (2006) Neuere Larvenaufsammlungen und Zuchten von mitteleuropäischen Pflanzenwespen (Hymenoptera, Symphyta). Linzer biologische Beiträge 38(2): 1609–1636.

Réaumur RAF de (1740) Troisieme Mémoire. Et le premier sur les Mouches à quatre aîles. Des fausses Chenilles, & des Mouches à scies, dans lesquelles elles se transforment. In: Réaumur RAF de 1740: Mémoires pour servir à l'Histoire des Insectes. Tome Cinquiéme. Suite de l'Histoire des Mouches à deux aîles, & l'Histoire de plusieurs Mouches à quatre aîles, sçavoir, des Mouches à scies, des Cigales, & des Abeilles. Imprimerie Royale, Paris, 87–144.

Ritzau C (1998) *Tenthredo propinqua* Klug, 1817 neu für Deutschland (Hymenoptera: Tenthredinidae). In: Taeger A, Blank SM (Eds) Pflanzenwespen Deutschlands (Hymenoptera, Symphyta). Kommentierte Bestandsaufnahme. Goecke & Evers, Keltern, 43–44.

Shinohara A, Hara H, Kramp K, Blank SM, Kameda Y (2017) Bird droppings on chestnut leaves or sawfly larvae: DNA barcodes verify the occurrence of the archaic *Megaxyela togashii* (Hymenoptera, Xyelidae) in Hokkaido, Japan. Zootaxa 4221(2): 220–232. https://doi.org/10.11646/zootaxa.4221.2.6

Taberlet P, Gielly L, Pautou G, Bouvet J (1991) Universal primers for amplification of three non-coding regions of chloroplast DNA. Plant Molecular Biology 17: 1105–1109. https://doi.org/10.1007/BF00037152

Taeger A, Altenhofer E, Blank SM, Jansen E, Kraus M, Pschorn-Walcher H, Ritzau C (1998) Kommentare zur Biologie, Verbreitung und Gefährdung der Pflanzenwespen Deutschlands (Hymenoptera, Symphyta). In: Taeger A, Blank SM (Eds) Pflanzenwespen Deutschlands (Hymenoptera, Symphyta). Kommentierte Bestandsaufnahme. Goecke & Evers, Keltern, 49–135.

Wang L, Meng L, Luo J (2015) Florivory Modulates the Seed Number-Seed Weight Relationship in *Halenia elliptica* (Gentianaceae). The Scientific World Journal 2015: 1–7. https://doi.org/10.1155/2015/610735

Wei M, Nie H, Taeger A (2006) Sawflies (Hymenoptera: Symphyta) of China - Checklist and Review of Research. In: Blank SM, Schmidt S, Taeger A (Eds) Recent Sawfly Research: Synthesis and Prospects. Goecke & Evers, Keltern, 505–574.

Zajac A, Pindel A (2011) Review of the Willow Gentian, *Gentiana asclepiadea* L. Biodiversity 12(3): 181–185. https://doi.org/10.1080/14888386.2011.628247