

# Taxonomy of the *Phyllocnistis saligna* (Zeller, 1839) complex (Lepidoptera, Gracillariidae) in North and Central Europe, with the description of a new species

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The *Phyllocnistis* Zeller, 1848 species of North and Central Europe feeding on *Salix* L. are reviewed. *Phyllocnistis triandricola* sp. n. is described new to science. DNA barcoding and discovery of a new character in the female genitalia resulted in robust species delimitation within the group. Remarks on the biology of the species are given.

Key words: Lepidoptera, Gracillariidae, *Phyllocnistis*, *Phyllocnistis saligna*, new species, cryptic diversity, DNA barcoding, leaf miner, moths, *Salix*, stem miner.

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## Introduction

In July 2019 a specimen of the leaf miner genus *Phyllocnistis* Zeller, 1848 was collected at Lillehammer, South Norway, in a trap with pheromones designed to attract the clearwing

moth *Synanthedon polaris* (Staudinger, 1877). The specimen was fairly worn, but was identified as *Phyllocnistis saligna* (Zeller, 1839) as the characteristic longitudinal fascia in the forewing was present (Bengtsson & Johansson 2011). Subsequently legs from the specimen were sent to

the DNA barcoding centre at Guelph in Canada for sequencing. The unexpected result from this suggested that the specimen was not conspecific with *Ph. saligna*. During 2020–2022, numerous additional specimens were collected at different localities in South Norway. All specimens were obtained on or close to bushes of *Salix triandra* L. A search for *Phyllocnistis* mines on this *Salix* L. species was successful, and additional specimens of the supposedly new species were reared.

Until recently the only *Phyllocnistis* species known to feed on *Salix* in Central and North Europe was *Ph. saligna* (Zeller, 1839). Over the last few years, this situation has changed. Three species have been added to the fauna of this area. *Phyllocnistis ramulicola* Langmaid & Corley, 2007 was described from Portugal and England (Langmaid & Corley 2007); Huemer (2013) recorded the otherwise Mediterranean *Ph. valentinensis* (M. Hering, 1936) new to Austria and Central Europe, and Pastorális *et al.* (2018) recorded *Ph. asiatica* Martynova, 1955 in Slovakia. These species have, since they were first recorded, also been identified in additional countries, and they appear now to be widespread. In the present study, we describe one more *Salix*-feeding *Phyllocnistis* species from Europe: *Ph. triandricola* sp. n.

The two European species feeding on *Salix*, which are closely related to the Norwegian taxon are *Phyllocnistis saligna* and *Ph. asiatica* Martynova, 1955. These species have the characteristic longitudinal forewing fascia and in their larval stage combine leaf- and stem-mining. *Phyllocnistis ramulicola* has the longitudinal forewing fascia, but only mines the stem of the food-plant and uses a leaf for pupation only. *Phyllocnistis valentinensis* is also a *Salix*-feeder, but only mines the leaves. The summer generation of *Ph. valentinensis* has the basal part of the forewing white, whereas the late, hibernating generation has dark grey suffusion in the basal part of the forewing. This suffusion may form an ill-defined longitudinal fascia (Laštůvka *et al.* 2018). This group also contains *Ph. canariensis* M. Hering, 1927 known from the Canary Islands and Madeira (Aguiar & Karsholt 2006). In the present work we discuss in particular the four

species *Ph. saligna*, *Ph. asiatica*, *Ph. ramulicola* and *Ph. triandricola* sp. n. (described below).

DNA sequences of the European species are available on the Barcode of Life Data System (BOLD) (Ratnasingham & Hebert 2007). The DNA barcode is a ca 650 bp long fragment of the mitochondrial cytochrome c oxidase subunit 1 (CO1) used as a standard marker to identify animal specimens into species. Sequences of *Ph. saligna*, *Ph. asiatica*, *Ph. ramulicola* and *Ph. triandricola* sp. n. form four well-defined clusters, and each cluster has received a separate Barcode Index Number (BIN-code): *Ph. triandricola* sp. n. (BIN: BOLD:ABW1114), *Ph. saligna* (BIN: BOLD:AAQ1589), *Ph. asiatica* (BIN: BOLD:AAL5482) and *Ph. ramulicola* (BIN: BOLD:AAL5481). In the BOLD database there are additional sequences from Austria and the Czech Republic in the same cluster as the Norwegian samples and which share the same BIN. In addition, a series of barcoded specimens from Japan form a separate cluster on BOLD and has received the BIN: BOLD:AAZ7400. It is probable that this represents another separate species within the complex. Dealing with this taxon, however, is outside the scope of the present work.

Independent of observations initially made in Norway, as described above, the possible presence of a cryptic species in *Ph. saligna* was reported in connection with a large-scale data release of European Gracillariidae barcode data (Lopez-Vaamonde *et al.* 2021). This team even examined the possible presence of a cryptic species in *Ph. saligna* and some other species with deep intraspecific barcode splits using ddRAD sequencing as a tool. While they refrained from making any taxonomic conclusions, the generated massive genomic data with 31,803 SNPs supported the view that two separate species are involved in *Ph. saligna*. In this comparison, no specimens of *Ph. asiatica* were included, but six specimens of true *Ph. saligna* from both North and Central Europe and three specimens of *Ph. triandricola* sp. n. from Central Europe were included.

In the genus *Phyllocnistis* differences in the genitalia between species are minor, and we have not found distinguishing characters in

the male genitalia. However, there is a reliable character in the female genitalia, which has enabled us to identify the members of the complex morphologically. This character confirms that the clusters discovered through DNA barcoding represent distinct species.

## Material and methods

The DNA barcode of the specimen captured in 2019 indicated that this potentially represented a new species. The specimen had been captured on the bank of the river Gausa and a systematic search for additional material began by the first author in the autumn of 2019 and continued in 2020 in the riverine forest along the rivers Gausa and Lågen, which merge just north of Lillehammer. The seasonal flooding from May until the end of June interrupted the search. On 6 July some specimens of *Phyllocnistis* were captured on *Salix triandra*, and the following days some twenty more were obtained along one kilometre of the bank and estuary of Gausa where this meets the river Lågen. The species was abundant on *S. triandra*, and a number of adults, mines and pupae were found. This prompted an extensive search along the rivers of Lågen and Glomma during the remaining part of the 2020 season, from the estuary of the Glomma at the south east coast of the country up to Dombås in the foothills of the Dovre Mountains. Leaves containing pupae were collected from bushes of *S. triandra* and kept singly in glass vials for breeding. This resulted in imagines from Elverum and mines and pupal exuviae from six more localities, all on *S. triandra* growing on riverbanks. The localities at Lillehammer were visited several times from the beginning of July until early October, and the biology was partly documented. Seven pupae were collected from which four adults and three parasitoids emerged. The parasitoids were sent to the Natural History Museum, University of Oslo (NHMO) for identification. Altogether, some 50 specimens were collected during 2020. Continued search during 2021 and 2022 resulted in records from additional localities along the rivers Glomma and Lågen. In addition to the Norwegian material,

bred specimens of the complex from Sweden, Denmark, the United Kingdom, and Austria were also examined.

Dissections of the genitalia were made according to standard procedures (Robinson 1976) and the genitalia mounted on slides. Dissections of barcoded females were given priority because the female genitalia offered a useful character to separate the taxa.

The terminology of the forewing pattern elements is in accordance with Cerdeña *et al.* (2020).

We examined DNA barcodes for all specimens of *Phyllocnistis* accessible for us in the BOLD database. Additionally, using BOLD tools, we confirmed that the DNA barcode sequences of *Ph. triandricola* sp. n. do not match with those of any other species deposited in BOLD accessible for us. The data of *Phyllocnistis* accessible to us includes 664 specimens with sequences of over 300 bp. They represent 23 named species, but as many as 34 BINs, demonstrating that there are probably many identified and/or undescribed species represented in this dataset. We used this dataset as a baseline to compare the genetic distances between the species treated here. As many of these sequences remain unpublished, and as many represent different species groups only distantly related to the *Salix* feeding species that together with *Populus* feeding species probably form a monophyletic clade, we established a BOLD dataset, public BOLD dataset, DS-PHYLLOCN ([dx.doi.org/10.5883/DS-PHYLLOCN](https://dx.doi.org/10.5883/DS-PHYLLOCN)), for the 478 records with a sequence length of over 500 bp and that are of relevance for our study (feeders of Salicaceae plants). In addition, one public record of *Ph. citrella* Stainton, 1856 was included for outgroup purposes only. Besides our own data, this dataset includes a large number of previously published records of European *Phyllocnistis* (Lopez-Vaamonde *et al.* 2021) supplemented with some public records of North American specimens of related species. We used BOLD analytical tools to calculate genetic divergences between species. We also constructed a Maximum Likelihood tree Mega v. 10.0.5 (Kumar *et al.* 2016) to visualize the genetic divergences and estimate the phylogenetic relationships, however, fully acknowledging

that this sampling represents only a preliminary hypothesis for the latter.

Abbreviations. NHMO = Natural History Museum, University of Oslo (Norway); NHMUK = Natural History Museum, London (United Kingdom); RVO = Reidar Voith; ZMUC = Zoological Museum, Natural History Museum of Denmark; and ZMUO = Zoological Museum, University of Oulu (Finland).

## Nomenclature

### Identity of *Phyllocnistis saligna* (Zeller, 1839)

In his description Zeller (1839) stated: «Bei Berlin, Gl[ogau] und Fr[ankfurt/Oder] im Frühjahr und Herbst um Weiden und Pappeln, deren Blätter die Raupe miniert, an warmen, stillen Abenden äusserst gemein». It is obvious that Zeller in this case did not recognize that the species on «Pappeln» (*Populus* L.) was not the same as the one(s) on «Weiden» (*Salix* L.). None of the *Populus* feeders have the longitudinal fascia in the forewing. In NHMUK two syntypes are present. One syntype is without collecting data, the other (Figure 1) (with one forewing missing) is labelled «Gross Glogau/ SILESIA/ J. 1839?/, Zeller Coll» (Figure 2). The labels on this syntype were added after the Zeller collection had been transferred to London (Heckford pers. comm.). On the website Global Taxonomic Database of Gracillariidae (De Prins & De Prins 2006–2022) the syntype from Glogau is referred to as the holotype. This is incorrect because Zeller in his description did not select a holotype. All type specimens are syntypes. The syntype labelled «Glogau» is a potential lectotype. It has only one forewing, but this wing is in good condition. In our opinion the forewing pattern of this *saligna* syntype (Figure 1) is most similar to that of *P. ramulicola* (Figure 5). The syntype labelled «*Phyllocnistis saligna* var. b» (Figure 3) is not conspecific with the other syntype. It has a label (Figure 4) referring to a publication in 1848 (Zeller 1848). A specimen mentioned in the 1848 paper could still be a syntype if it was collected before 1839. It lacks data, but can be considered as a syntype and a candidate

for lectotype designation. Although it cannot be proved for certain, we consider it most likely that this syntype is identical with the cluster in BOLD with BIN: BOLD:AAQ1589. If the identity of the name *saligna* is fixed to this syntype (and the species with BIN: BOLD:AAQ1589) it will serve stability and the name *Ph. ramulicola* will not be threatened. Also, the usual interpretation of the name *Ph. asiatica* (BIN: BOLD:AAL5482) will not be threatened. Because Zeller described *saligna* from an unspecified number of specimens there is still a chance that additional syntypes will turn up. In this situation we prefer not to select a lectotype, but simply continue current usage of the names involved. This means that we propose to apply the name *saligna* for the species with BIN: BOLD:AAQ1589.

There exist a few synonyms of *Phyllocnistis saligna* in the literature (De Prins & De Prins 2006–2020). These names could potentially represent older names for taxa in the complex and are discussed below.

*Opostega salignatella* Bruand, 1851 and [*Phyllocnistis*] *salignella* Herrich-Schäffer, 1855 are unjustified emendations of Zeller's name, *saligna* (De Prins & De Prins 2006–2020).

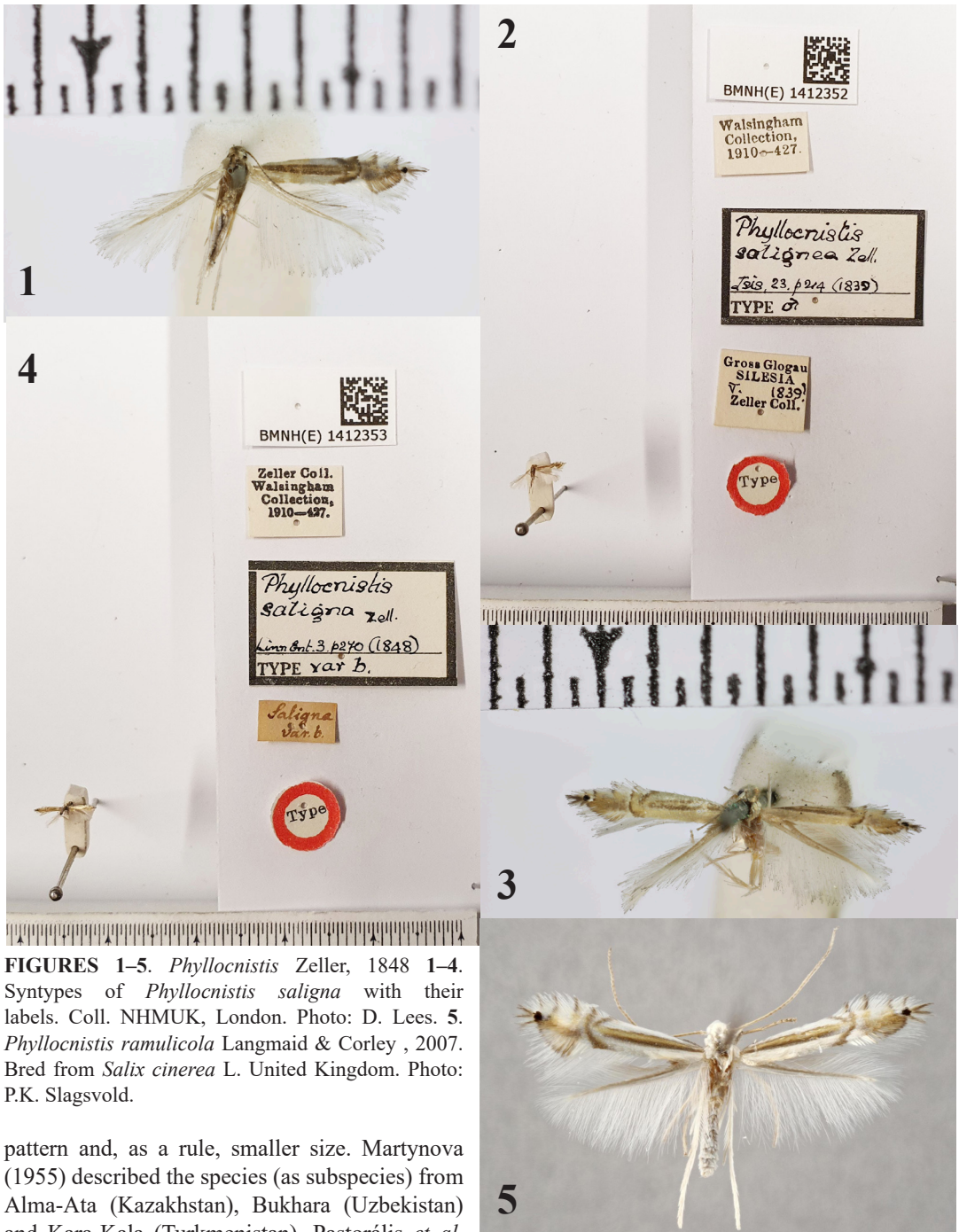
[*Tinea*] *cerasifoliella* Hübner, 1796 was stated by Stainton (1849) to differ from *Ph. saligna* by the lack «of the double brown line proceeding from the base of the wings». Thus, it cannot be conspecific with any species of the *Phyllocnistis saligna* complex which all have a longitudinal fascia from the base of the wing.

[*Opostega*] *lugdunensella* Bruand, 1858 was stated by the author to differ from *Ph. saligna* in having much smaller eye caps («des cuillerons, mais tres petits») (Bruand 1858). All species in the *Phyllocnistis saligna* complex have eye caps of similar size, and this makes it unlikely that Bruand was describing specimens of the complex.

### Identity of *Phyllocnistis asiatica* Martynova, 1955

Martynova (1955) described *Phyllocnistis asiatica* as a subspecies of *Ph. saligna* and stated it to differ from Western European specimens in a much lighter colour, the absence of brown tones and the replacement of them by a yellow, more subtle





**FIGURES 1–5.** *Phyllocnistis* Zeller, 1848 1–4. Syntypes of *Phyllocnistis saligna* with their labels. Coll. NHMUK, London. Photo: D. Lees. 5. *Phyllocnistis ramulicola* Langmaid & Corley, 2007. Bred from *Salix cinerea* L. United Kingdom. Photo: P.K. Slagsvold.

pattern and, as a rule, smaller size. Martynova (1955) described the species (as subspecies) from Alma-Ata (Kazakhstan), Bukhara (Uzbekistan) and Kara-Kala (Turkmenistan). Pastorális *et al.* (2018) recorded the species new to Europe from Slovakia. The material was bred from mines on *Salix alba* L. The illustrations in Pastorális *et al.* (2018) and Laštůvka *et al.* (2018) agree with

the description and figure given by Martynova (1955), and we follow their interpretation of the name *asiatica*. This interpretation agrees with the taxon with BIN: BOLD:AAL5482.

***Phyllocnistis triandricola* Voith, Aarvik & Berggren sp. n.** Figures 6–8, 12–15

**Type material.** *Holotype* ♀, NORWAY: Innlandet, Lillehammer: Graverberget, 61.1579°N 10.3888°E, 11.VII.2020, leg. R. Voith, BOLD sample ID: NLON807-20, coll. NHMO. *Paratypes*, 3♀♀, same data as holotype, genitalia slide RVO 3360; 4♂♂, 9♀♀, same locality as holotype, 20.VII.2020, genitalia slides RVO ♀ 3354, 3359, 2♀♀ ex pupa *Salix triandra*, 4♂♂, 6♀♀ coll. NHMO, 2♀♀ coll. RVO, 1♀ coll. ZMUC; 9♂♂, 7♀♀, NORWAY, Innlandet, Lillehammer: Bronsøya, 61.1571°N 10.3978°E, 6–9.VII.2020, BOLD sample ID: NLON808-20, genitalia slides ♂ RVO 3229, 3232, 3233, 3234, 3235, genitalia slides RVO ♀ 3230, 3231, 8♂♂, 7♀♀ coll. NHMO, 1♂ coll. RVO; 3♀♀, 12.VIII.2020, 2♀♀ coll. NHMO, 1♀ coll. RVO; 1♀, 29.VIII.2020, genitalia slide RVO 3358 coll. NHMO, 1♂ coll. ZMUC; 4♀♀, 18.V.2021, genitalia slides RVO 3701, 3702, 3703, 3704; 1♀, 17.VIII.2021, genitalia slide RVO 3671; 2♂♂, 3♀♀, ex pupa *Salix triandra*, 28.VI.2022, hatched 1–6.VII.2022; 1♀, NORWAY, Innlandet, Lillehammer: Søre Jørstad, 61.1580°N 10.3867°E, 14.VII.2019, in pheromone trap, BOLD sample ID: NLON264-19, coll. RVO; 4♂♂, 7♀♀, NORWAY: Innlandet, Elverum: Prestøya, 60.8696°N 11.5519°E, 21.VII.2020, genitalia slides ♂ RVO 3237, 3356, genitalia slides RVO ♀ 3353, 3355, 3357, coll. NHMO); 1♂, 2♀♀, same locality, 23.VIII.2020, 2♀♀ ex pupa *Salix triandra*, 1♂, 2♀♀ coll. NHMO, 2♀♀ coll. RVO; 1♂, NORWAY, Innlandet, Hamar: Åkersvika NR, Vikingskipet, 60.7915°N 11.0989°E, 19.V.2022, genitalia slide RVO 3700, coll. RVO; 2♀♀, NORWAY, Innlandet, Alvdal: Holmen, 62.0925983°N E10.6467847°E, 30.V.2022 coll. RVO; 4♀♀, NORWAY, Innlandet, Stor Elvdal: Koppang, Søndre Bakken, 61.5496118°N 11.0453668°E, 30.V.2022, genitalia slides RVO 3807, 3809 coll. RVO. All paratypes from Innlandet collected by R. Voith.

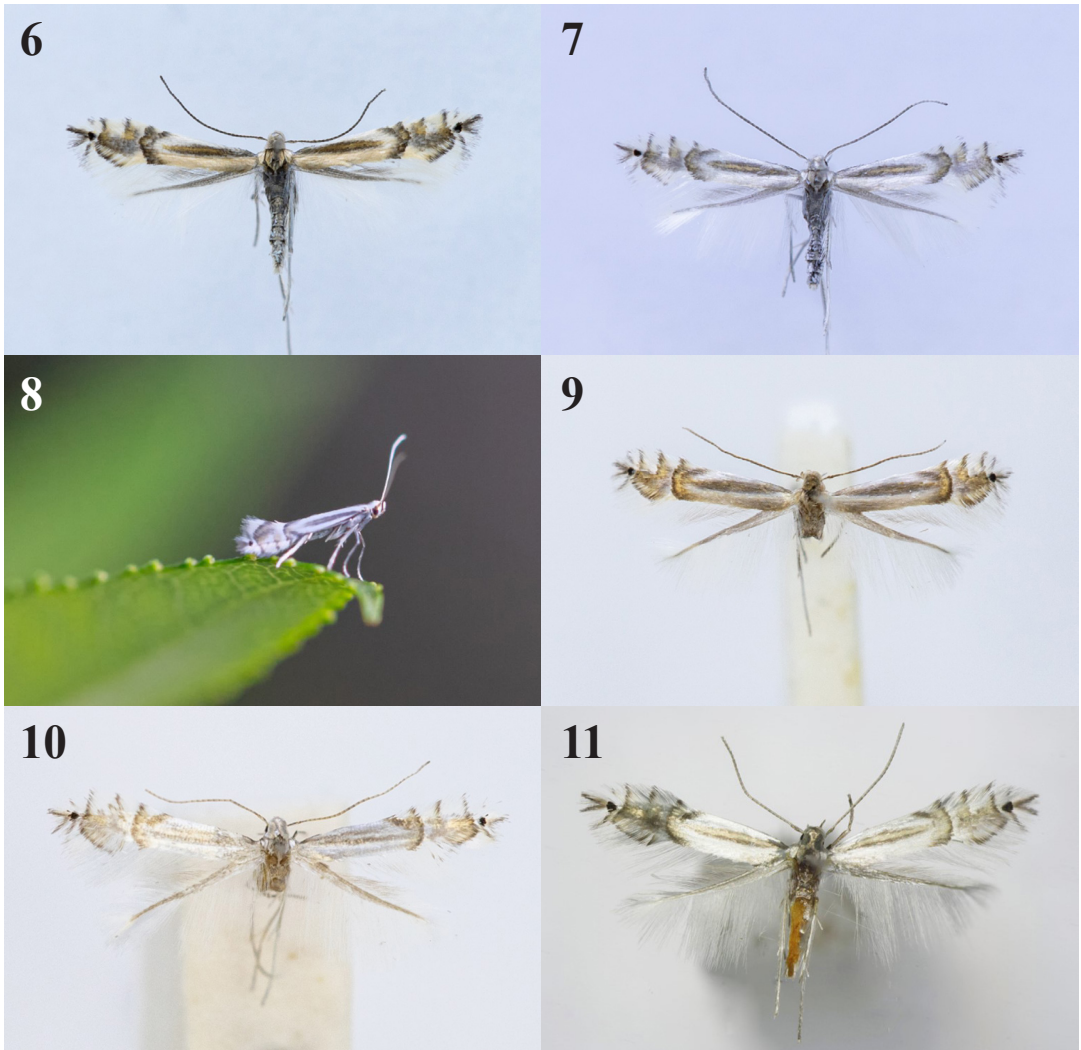
**Additional material:** 1♂, NORWAY: Viken, Lillestrøm: Sørums, Nordre Hammaren 59.9904434°N 11.2091675°E, 11.VI.2022, ex pupa *Salix triandra*, leg. P.K. Slagsvold; 1♀, same locality, 20.VI.2022, ex pupa *Salix triandra*, leg.

L. Aarvik (both NHMO); 1♀, AUSTRIA, Kärnten, Tiebelmündung, 2.VII.2018, KLM Lep 14420, BOLD sample ID: LEASV209-19, genitalia slide RVO 3743, leg. & coll. C. Wieser

**Diagnosis.** The most obvious differences between *Ph. triandricola* sp. n. (Figures 6–8) and other members of the complex are the length of the longitudinal fascia and the curvature of the transverse fasciae 1 and 2. The longitudinal fascia in *Ph. triandricola* sp. n. is well defined and comparatively shorter than in the other species, usually just reaching the middle of the wing and usually not to the transverse fascia 1. If it reaches the transverse fascia 1, this is only with a few dark scales. In *Ph. saligna*, *Ph. ramulicola* and *Ph. asiatica* the longitudinal fascia is longer, reaching nearly to two-thirds of the wing, and it will usually reach the transverse fascia 1. In *Ph. triandricola* sp. n. transverse fasciae 1 and 2 normally merge, forming a broad angulate band. In *Ph. saligna* and *Ph. asiatica* fascia 1 is reduced in the dorsal half and fascia 2 is slightly curved. In *Ph. ramulicola* fascia 1 is represented by a short oblique streak from the costa and fascia 2 forms an angled band, at 90–130 degrees, extending a short distance along the dorsum. The latter species is smaller than *Ph. triandricola* sp. n., wingspan 5.7–7.0 mm., whereas *Ph. triandricola* sp. n. measures 7.5–8.0 mm. *Ph. asiatica* has a defined seasonal dimorphism whereas this is not the case with *Ph. triandricola* sp. n., where no seasonal dimorphism has been noted. Adults of *Ph. saligna* and *Ph. ramulicola* are illustrated by Laštůvka & Laštůvka (2014), and adults of *Ph. saligna*, *Ph. ramulicola* and *Ph. asiatica* are illustrated by Laštůvka *et al.* (2018).

In the male genitalia (Figures 12–13) no significant character separating *Ph. triandricola* sp. n. from the other species of the complex has been found.

In the female genitalia (Figs 14–15) *Ph. ramulicola* differs from *Ph. triandricola* sp. n., as well as *Ph. saligna* and *Ph. asiatica*, by having two signa of unequal size. In the latter three species the two signa are of equal size. According to the photograph in Gomboc & Kirichenko (2022) the female of *Ph. valentinensis* also has signa of unequal size. In the ovipositor there are



**FIGURES 6–11.** Adults of *Phyllocnistis* Zeller, 1848. **6.** *Phyllocnistis triandricola* sp. n. Holotype. **7.** *Ph. triandricola* sp. n. Paratype. **8.** *Ph. triandricola* sp. n. Live specimen on leaf of *Salix triandra* L. **9.** *Ph. saligna* (Zeller, 1837) from Kista, Sweden. **10.** *Ph. asiatica* Martynova, 1955 from Lund, Sweden. **11.** *Ph. asiatica* from Wicken Fen, United Kingdom. Photos figures 6–10: R. Voith. Photo figure 11: R.J. Heckford.

two parallel sclerites in *Ph. triandricola* sp. n., *Ph. saligna* and *Ph. asiatica* (we have not checked for this character in other species). The sclerites are of different size and shape in the three species. In *Ph. triandricola* sp. n. (Figures 14–15) the sclerites are flat and become less sclerotized towards the broader dorsal end. In *Ph. saligna* (Fig. 16) and *Ph. asiatica* (Figure 17) the sclerites are more three dimensional and curved. The length of the sclerites in *Ph. triandricola* sp. n. is 81–103  $\mu\text{m}$

( $n = 12$ ); in *Ph. asiatica* they are 98–122  $\mu\text{m}$  ( $n = 4$ ) and in *Ph. saligna* (58–70  $\mu\text{m}$ ,  $n = 5$ ). In *Ph. saligna* the sclerites are banana-shaped, slender and pointed in both ends. In *Ph. asiatica* the sclerites are wider and usually banana-shaped, but they may also be more irregular, approaching the shape of an ‘s’. The sclerites in both these species are three dimensional and may change shape from pressure from the cover glass during preparation.

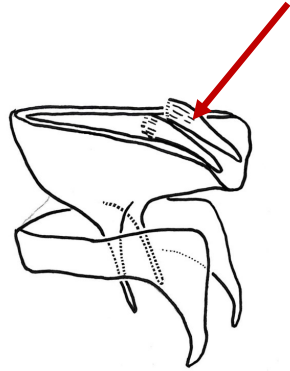


12



500  $\mu$ m

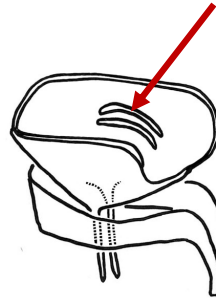
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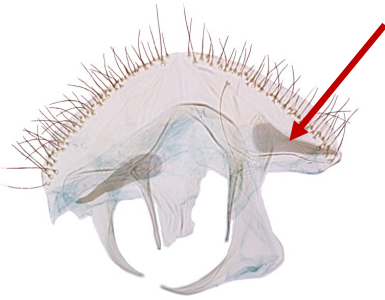
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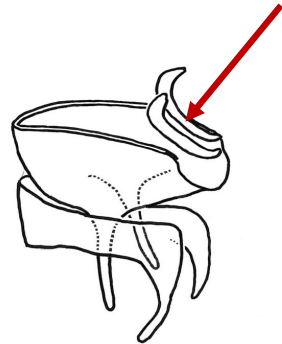


14



300  $\mu$ m

17

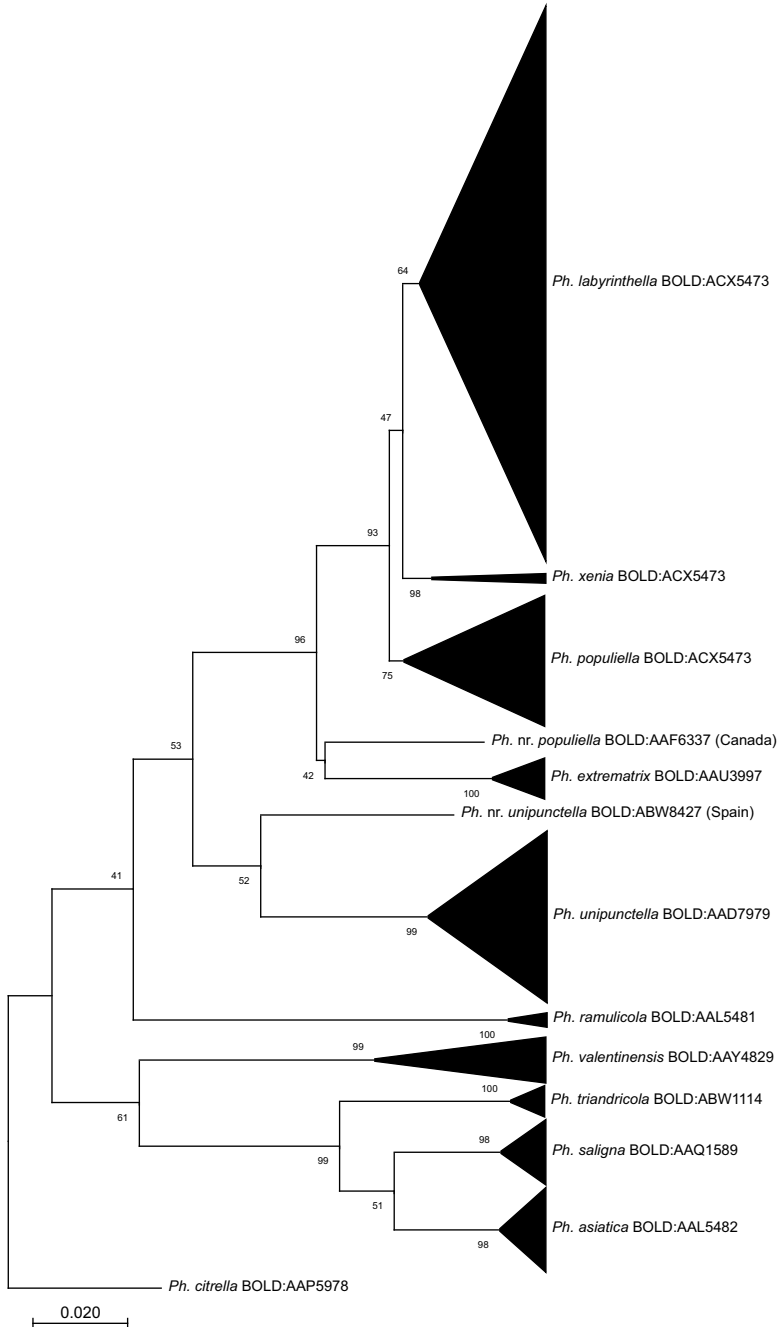


**FIGURES 12–17.** Genitalia of *Phyllocnistis* Zeller, 1848 species. **12, 13.** Male *Ph. triandricola* sp. n. **13.** Phallus. **14.** Female *Ph. triandricola* sp. n., ventral view of opened ovipositor. **15.** Female *Ph. triandricola* sp. n., ovipositor lateral view. **16.** Female *Ph. saligna* (Zeller, 1837), ovipositor lateral view. **17.** Female *Ph. asiatica* Martynova, 1955, ovipositor lateral view. Red arrows indicate the paired sclerites of the ovipositor.

**Molecular diagnosis.** *Phyllocnistis triandricola* sp. n., with BIN: BOLD:ABW1114, is separate from its nearest neighbour *Ph. asiatica*, with BIN: BOLD:AAL5482, by a p-distance of 5.84%. *Ph. saligna* is almost equally close to

*Ph. triandricola* sp. n. Intraspecific variability in *Ph. triandricola* is 0.33%. In the Maximum Likelihood tree (Figure 18), *Ph. triandricola* forms a well-supported (BS=99) cluster and forms a sister group to *Ph. saligna* + *Ph. asiatica*.





**FIGURE 18.** A Maximum Likelihood tree of 478 specimens and 13 species or putative species of *Phyllocnistis* Zeller, 1848, of which 12 represents the clade associated with Salicaceae plants. The tree was rooted on *Ph. citrella* Stainton, 1856. The node confidence was estimated based on 500 bootstrap pseudoreplicates. In general, BS support values for deeper nodes are relatively low, highlighting that phylogenetic relationships between species should be considered as tentative. Species form highly supported clusters except for the *Ph. labyrinthella*/*populiella*/*xenia* complex, which all belong to the same BIN and show rather minor genetic divergences.

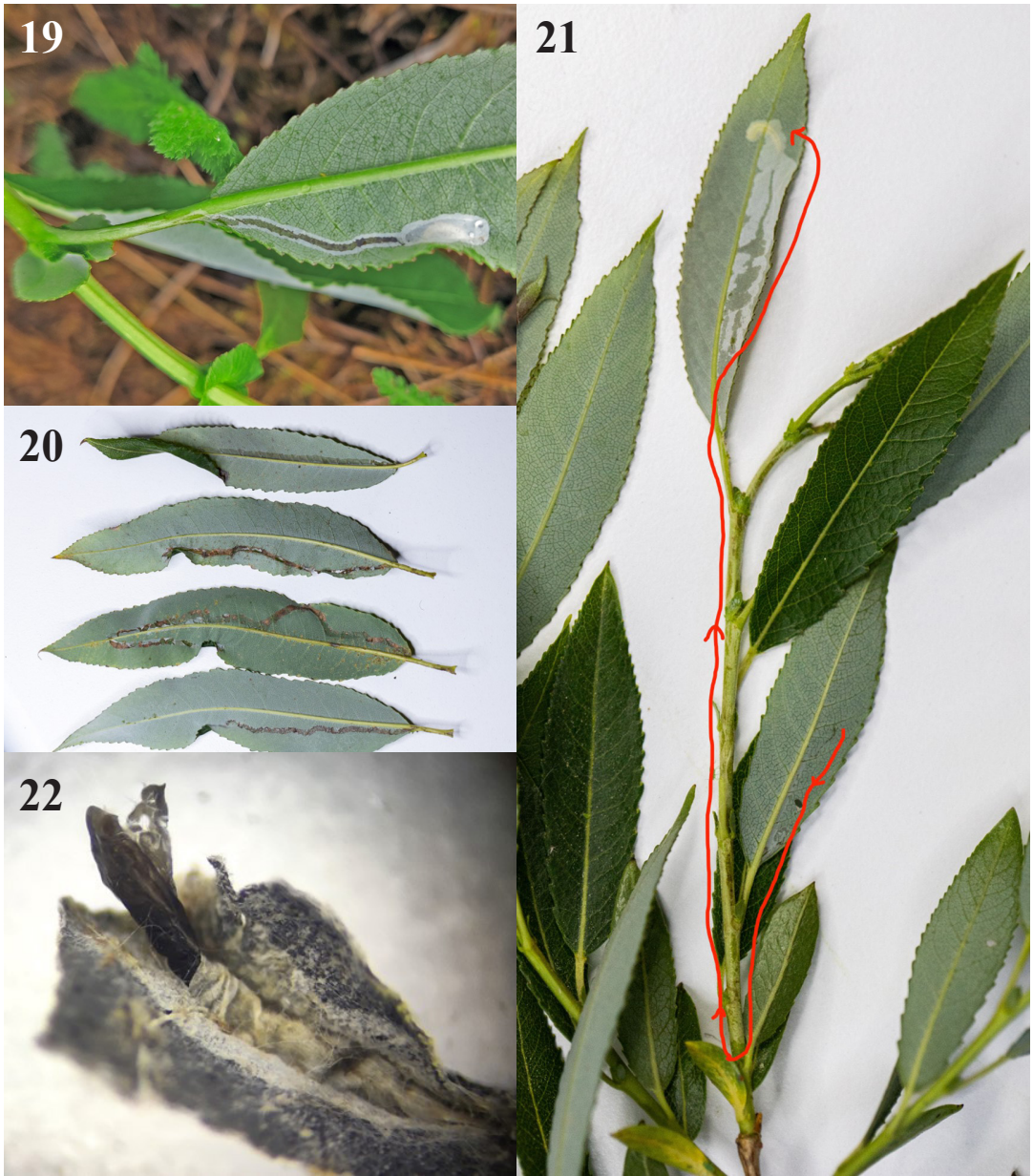
**Description.** Wingspan 7.5–8.0 mm. Head, thorax and tegulae cream-white, shiny; face and palp whitish, palp length twice the diameter of eye; scape of antenna covered with silvery, shining scales, antenna dark fuscous, greyish white on underside, 60–70 % length of forewing. Forewing cream-white, glossy, with ochreous hue particularly dorsad to longitudinal fascia; base of costa narrowly edged blackish; dorsum basally with small, oblique, brownish patch; a broad brown longitudinal fascia edged fuscous reaches to about middle of wing, not touching transverse fascia 1, ground colour visible between apex of longitudinal fascia and transverse fascia 1; transverse fasciae 1 and 2 merge, forming a broad angulate band partly ochreous within, narrower and more distinct dorsally; transverse fasciae 3 and 4 form two coastal strigulae reaching to middle of wing, inwardly edged ochreous; three fuscous apical strigulae emerge from black dot; transverse fascia 4 continued dorsally as an oblique, fuscous cilia line inwardly edged ochreous; cilia – apart from strigulae – white. The upper side forewing pattern is reflected on the underside. Hindwing grey, shining; cilia grey at costa, apically and dorsally white.

Male genitalia (Figures 12–13). Valva long and slender, medially narrower, terminally spoon-shaped, with triangular process, basally with two finger-like processes, lower edge of lower «finger» with membranous extension; phallus tubular, gradually narrowing, cornutus long and slender. Valvae three-dimensional, and their shape on the genitalia slides varies according to the position; also, position and visibility of the triangular process vary according to the position in which the genitalia are fixed.

Female genitalia (Figures 14–15). Margin of papillae anales with row of strong setae, apophyses posteriores short, strong, eighth segment short; apophyses anteriores very broad, horn-shaped; within ovipositor two slender sclerites, widened at dorsal end, slightly resembling a spatula, relatively weakly sclerotised and gradually membranous towards dorsum; ductus bursae membranous, narrow; corpus bursae with numerous denticles and two large signa of equal size, signa banana-shaped with one strong spine in middle.

**Variation.** The ground colour of the forewing varies from creamy ochreous white to shining silky white. Head, thorax and tegulae vary in the same way as the forewing, but may contrast with the ground colour of the forewing. The longitudinal fascia may be almost unicolorous blackish brown or ochreous with blackish brown margins. It may nearly reach the transverse fascia, but in such cases only with single dark scales. The transverse fascia 1 might occasionally extend a little basad along dorsum, resembling the fascia of *Ph. ramulicola*. In a few cases the transverse fasciae 1 and 2 are separate, making the forewing pattern resemble that of *Ph. saligna* and *Ph. asiatica*.

**Biology.** According to information in the BOLD database (accessed 28.10.2022) bred from *Salix babylonica* var. *matsudana* Koidz and *S. euxina* V.I. Belyaeva in the Czech Republic. In Norway the only observed host-plant is *Salix triandra* (Figures 19–21). Oviposition takes place on the underside of the leaf. The newly hatched larva enters the leaf and starts to form a tiny mine, always on the underside. The mine is extended, directly or with some sinuosity, to the main vein of the leaf which it then follows to the petiole and hence into the stem. The mine continues in the stem usually downwards for 5–10 cm, normally either to the first or second petiole before it turns 180 degrees and continues upwards for some 10–20 cm, either to the third or fourth petiole. Then the larva will enter a new leaf via the petiole where it makes a fairly straight mine in the middle up to two thirds or three quarters of the leaf's length. From there it will either mine straight to the margin to pupate, or cross over to the opposite side of the leaf where it makes a fold at the margin in which it forms a chamber under the epidermis and spins a loose white cocoon where the pupation takes place. The cigar-shaped fold with the cocoon is on the underside of the leaf. The fresh mine is more or less of the same colour as the stem (Figure 21) but turns whitish after some days and is then easy to see. After some weeks the mine within the leaf will turn darker as the frass in the mine decomposes and it becomes quite conspicuous. Due to their snail-track appearance the mines are obvious on the underside (Figure 20). The larvae of *Ph. saligna* and *Ph. asiatica* mine more in the



**FIGURES 19–21.** Mines of *Phyllocnistis triandricola* sp. n. in leaves and stem of *Salix triandra* L. The mine and direction of the larva indicated with a red line. **FIGURE 22.** Exuvia pupae of *Phyllocnistis triandricola* sp. n.. Photos: Figures 19, 22: P.K. Slagsvold; figures 20, 21: R. Voith.

leaves than does *Ph. triandricola* sp. n., which is predominantly a stem-miner.

The young larva is almost translucent, maggot-like with a broad head capsule and with a greenish hue. Fully grown it becomes more yellowish.

The pupa is dark brown – black and with

a conspicuous process on the head for cutting through the cocoon. The duration of the pupal stage is not known, but moths have emerged between one and eight days after the pupae were collected. Cocoons with pupae were found – not more than one per leaf – in July and the beginning



of August. Mines and exuviae have been found as late as the beginning of October. Hibernating adults occur from mid-May, and the imagines of the first generation appear from approximately 20 June, depending on the weather. Then there will be a continuous flight in two overlapping generations until the end of August. The moths gradually disappear in late August and are completely absent by mid-September. The species hibernates as an adult. If their hibernating habitat is not covered with snow, they will have to be able to withstand winter temperatures dropping regularly down to  $-30^{\circ}\text{C}$  degrees and occasionally even  $-40^{\circ}\text{C}$ .

**Ecology.** In Norway the species is local and occurs exclusively where *Salix triandra* grows (Figures 23–24). A few specimens can occasionally be found on nearby trees and bushes, but almost all were observed on 2–5 m high bushes of *S. triandra* growing on riverbanks. The flight time indicates that the moth completes its first generation during the flood season when its food-plant is, at least partly, submerged in water. The type locality is a riverbank which is flooded annually in early summer, normally with about

one metre of water; in extreme cases with up to two metres of water and strong currents. It seems possible that larvae are mining under water during the flood.

**Distribution.** In Norway present along the rivers Glomma and Lågen (Figure 25), most northerly in the Sel and Alvdal municipalities in the foothills of the Dovre Mountains. These two rivers run in a southerly direction and meet just north of  $60^{\circ}\text{N}$ . The total distance with localities along the two rivers is approximately 450 km, and the moth has up to 2022 been found in 17 localities. It has been searched for along other rivers in South Norway, but this has not been successful. *Phyllocnistis saligna* was recorded new to Norway by Aarvik *et al.* (2013). The record was based on a male specimen collected at Elverum in 1980 and which had remained misidentified as *Ph. labyrinthella* (Bjerkander, 1790) for many years. This specimen belongs to *Ph. triandricola* sp. n. Otherwise known from the Czech Republic (data in BOLD) and Austria. Probably confused with its relatives and more widespread than current data indicates.



**FIGURE 23.** Bronsøya in Lillehammer. Type locality of *Phyllocnistis triandricola* sp. n. Photo: R. Voith.





**FIGURE 24.** Prestøya in Elverum. Locality for *Phyllocnistis triandricola* sp. n. Photo: R. Voith.

**Etymology.** The species name, *triandricola*, is from *Salix triandra*, the scientific name for ‘almond willow’, and the feminine form of *incolus*, inhabitant. *Phyllocnistis triandricola*, the *Phyllocnistis* living on *Salix triandra*.

#### Remarks on *Phyllocnistis saligna*

**Examined material.** 1♀, SWEDEN, Uppland, Alvik, 11.X.2011, genitalia slide RVO 2021-6; 1♂, same locality, ex pupa *Salix* 11.X.2021, genitalia slide RVO 3672, BOLD sample ID: NLON1100-22; 1♂, Uppland, Kista, 28.IX.2012, genitalia slide RVO 2021-7, BOLD sample ID: NLON947-21; 1♀, same locality, ex pupa *Salix* 9.X.2021, genitalia slide RVO 3673, BOLD sample ID: NLON1101-22; 1♀, same locality, 19.X.2011, genitalia slide RVO 2021-8, BOLD sample ID: NLON948-21; 1♀, ex pupa *Salix* 23.IX.2021, genitalia slide RVO 3674, , BOLD sample ID:

NLON1102-22. All specimens H. Hellberg leg. & coll.; 1♀, LATVIA, Rig. Carnikava, ex l. *Salix* 15–20.VII.2011, leg. N. Savenkov, BOLD sample ID: MM20616 coll. ZMUO; 1♂1♀, same data, coll. NHMO; 1 ex, FINLAND, Nylandia, Helsinki, Vuosaari 60.1729 N 25.1083 E, 21–22. VII.2011, leg. Jari Junnilainen, BOLD sample ID: MM22736, coll. ZMUO; 35 ex, FINLAND, Nylandia, Espoo 66814:83731, larvae/pupae on *Salix purpurea* 8.IX.2019, leg. Tomi Mutanen, coll. ZMUO; 8 ex FINLAND, Regio aboensis, Salo 6704:3287, larvae on *Salix purpurea* 28.VII.2020, leg. Marko Mutanen & Tomi Mutanen, coll. ZMUO.

**Molecular diagnosis.** *Ph. saligna* with BIN: BOLD:AAQ1589 forms a distinct cluster with the nearest neighbour being BIN: BOLD:AAL5482 (*Ph. asiatica*) with a p-distance of 4.49 %. Intraspecific variability of BIN of *Ph. saligna* is 1.28 %. In the phylogenetic tree (Fig. 18), *Ph. saligna* is a sister-species to *Ph. asiatica*, although

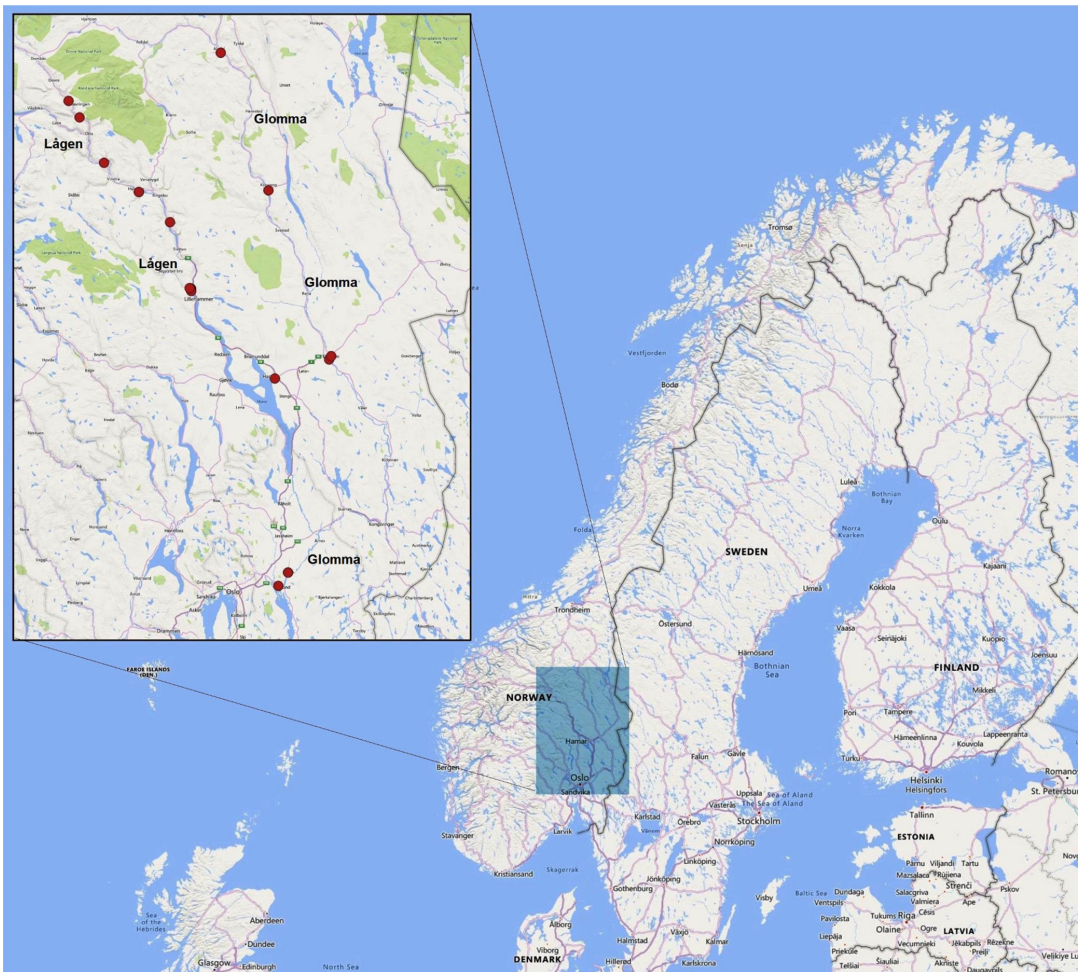


FIGURE 25. Distribution of *Phyllocnistis triandricola* sp. n. in Norway along the rivers Lågen and Glomma.

this association is only poorly supported. Together with *Ph. triandricola* sp. n., the triplet forms a strongly supported clade (BS=99 %). However, it is likely that this clade includes another undescribed species with BIN: BOLD:ACL6215 not included in our dataset.

**Observations on biology.** In Sweden bred from an imported or cultivated narrow leaved *Salix* species, probably *Salix purpurea* L. In Finland, numerous specimens have been reared from *Salix purpurea*, which occurs there only as an ornamental plant. Based on observations made there, the species may be monophagous on this *Salix*. Where present, it often occurs in great numbers as larvae. The oviposition probably takes place on the underside of the leaves, and the mines

are all observed there. This species mines both in the leaves and in the twig (Figure 26). The newly hatched larva mines the leaf in a zigzag manner forming a mine that eventually fills a noticeable part of the leaf. When most of the epidermis has been consumed it will leave the leaf through the petiole and sometimes continue in the twig for a new leaf some 10–20 cm away, or it enters a new leaf immediately through an adjacent petiole. The new leaf is mined in the same way as the first before pupation takes place in a cocoon spun in a cigar-shaped fold at the underside of the leaf margin. The larva, when mining the stem, has also been observed making a 180 degree turn like *Ph. triandricola* sp. n. The latter species mines mainly in the stem and uses the leaves only at the start and





**FIGURE 26.** Mines of *Phyllocnistris saligna* (Zeller, 1837) from Sweden: Kista. Photo: H. Hellberg.



**FIGURE 27.** Mines of *Phyllocnistris asiatica* Martynova, 1955 from Sweden: Lund. Photo: B.Å. Bengtsson.

end of the larval stage. In contrast, it seems that *Ph. saligna* uses the leaves as its main habitat, and that the stems are mined only occasionally. It appears that *Ph. saligna* has two or three generations a year. Fresh mines and imagines have been found as late as late September and October.

**Distribution.** According to our data *Ph. saligna* is present in Central Sweden, Finland, Latvia and in Austria. Data from the BOLD database indicate (BIN: BOLD:AAQ1589) occurrence also in France, Germany, Italy and Spain. It has also been recorded from the United Kingdom, but consideration will now have to be given as to whether *Ph. saligna* does indeed occur there as a result of *Ph. asiatica* being discovered in Cambridge (see below).

### Remarks on *Phyllocnistis asiatica*

**Examined material.** 1♀, DENMARK, LFM, Hyde Skov, ex pupa 17.IX.1980, genitalia slide BÅB 5072, leg. & coll. B.Å. Bengtsson; 1♂, DENMARK, LFM; Systofte, 15.VII.2018, genitalia slide RVO 3236, BOLD sample ID: NLON945-21, leg. & coll. R. Voith; 1♀ SWEDEN, Skåne, Lund, 22.VIII.2019, genitalia slide RVO 3510, BOLD sample ID: NLON950-21; 1♂, same locality, 20.VII.2011, genitalia slide RVO 3512, BOLD sample ID: NLON954-21; 1♀, same locality, 02.VIII.2011, genitalia slide RVO 3509, leg. & coll. B.Å. Bengtsson; 2♀♀, Sweden, Skåne, Lund, Høje Å, 20.VII.2013, genitalia slide BÅB 7438, 7437, leg. & coll. B.Å. Bengtsson; 1♀, AUSTRIA, Kärnten, Lassendorf, STG 10, 7.VIII.2002, genitalia slide RVO3741, BOLD sample ID: TIPSY389-12; 1♀, Obermösach, 16.VII.2014, genitalia slide RVO 3744, BOLD sample ID: PHLAJ372-14, KLM Lep 01987, leg. & coll. C. Wieser; 1♀, UNITED KINGDOM, Cambridgeshire, Wicken Fen, ex larva on *Salix alba*, 27.VIII.2018, imago 7.IX.2018, BOLD sample ID: NLON859-21, leg. & coll. R.J. Heckford.

**Molecular diagnosis.** *Ph. asiatica* with BIN: BOLD:AAL5482 forms a distinct cluster with the nearest neighbour being BIN: BOLD:ACL6215 (probably an undescribed species) with a

p-distance of 3.56 %. Intraspecific variability of BIN of *Ph. asiatica* is 1.25 %. In the phylogenetic tree (Figure 18), *Ph. asiatica* is a sister-species to *Ph. saligna*, although this association is only poorly supported. Together with *Ph. triandricola* sp. n., the triplet forms a strongly supported clade (BS=99 %). However, it is likely that this clade includes another undescribed species with BIN: BOLD:ACL6215 not included in our dataset.

**Observations on biology.** Observations from Lund, S Sweden (Bengtsson obs.) indicates that *Ph. asiatica* mines narrow leaved *Salix alba*, both on the upper- and underside of the leaves (Figure 27). Oviposition is on the leaf, and the young larva often mines along the central vein for some time before it enters a stem through the petiole. According to Liška *et al.* (2018) the larva creates underside mines in leaves, or only short mines in stems. The larva moves to a new leaf in the final mining stage and pupates in a fold at the leaf margin. Sobczyk (2019) reported that he bred the species from *Salix alba* and *S. fragilis* L., and that the larva usually mines the underside of the leaf forming a zigzag mine, filling most of it. The frass is deposited in the middle of the mine, forming a visible dark track. It will usually not mine the stem, and when it does, it is only for a short distance. Sobczyk (2019) reported that it has two generations, one in July–beginning of August and one in the autumn. This is similar to observations from South Sweden where it has one generation in July and a second in August/September where fresh mines have been found as late as 24 September (Bengtsson obs.). The second generation produces imagines which are somewhat darker and more strongly marked than the summer generation. This is shown in the illustrations in Laštůvka *et al.* (2018). Liška *et al.* (2018) reported *Salix euxina*, *S. purpurea* and *S. babylonica* L. as additional food-plants.

**Distribution.** Recorded from Kazakhstan, Uzbekistan and Turkmenistan in the original account by Martynova (1955). Subsequently found in Slovakia (Pastorális *et al.* 2018), Czech Republic (Liška *et al.* 2018), Germany (Sobczyk 2019) and Russia (Leontiev *et al.* 2021). In the present work Austria, England, Denmark and Sweden are added. In the BOLD database there



are several samples from France, Italy and the Netherlands, some identified as *Ph. saligna*, belonging to BIN: BOLD:AAL5482 and in our opinion conspecific with *Ph. asiatica*.

### Remarks on *Phyllocnistis ramulicola*

**Examined material.** 1♂, UNITED KINGDOM, Hampshire: Havant Thicket, mine on *Salix cinerea* L., IX.2007, leg. J.R. Langmaid, coll. NHMO.

**Molecular diagnosis.** *Ph. ramulicola* with BIN: BOLD:AAL5481 forms a distinct cluster with the nearest neighbour being BIN: BOLD:AAZ7400 with a p-distance of 2.73 %. No specimens of BIN: BOLD:AAZ7400 which may represent another undescribed species are accessible to us. Intraspecific variability of *Ph. ramulicola* is 0.48 %. In the phylogenetic tree (Figure 18), *Ph. ramulicola* is associated with the species feeding on *Populus* spp., although this association is only poorly supported.

**Observations on biology.** Bred from *Salix cinerea* in England (Langmaid & Corley 2007) and from *S. euxina* in the Czech Republic (Liška et al. 2018). Information from the BOLD database gives *S. atrocinerea* Brot. as a food-plant in France and *S. melanopsis* Nutt. and *S. pentandra* L. as food-plants in the Czech Republic. Data on the life history from the description of the species (Langmaid & Corley 2007): «Larval mine. From the oviposition site on the stem, at first either upwards or downwards, the downward-directed mines then turning upwards after a variable distance, sometimes crossing or coalescing with the earlier part of the track or the mines of other larvae and sometimes turning abruptly sideways to continue mining on the other side of the stem; finally, up the petiole of a leaf, ending at the leaf-base where the cocoon is spun. The mine is at first greenish ochreous, soon turning ochreous whitish and becoming whiter with age.»

**Distribution.** England, the Czech Republic, France, Italy, Portugal and Switzerland (Langmaid & Corley 2007, Liška et al. 2018).

### Discussion

The present study of the *Phyllocnistis saligna* complex demonstrates the usefulness of DNA barcoding as a tool in discovering cryptic species. However, because several evolutionary processes may cause deep intraspecific divergences (Mutanen et al. 2016), the possible presence of a new species should be verified by classical taxonomic methods based on morphology and biology or additional nuclear genetic markers. In this case the search for distinguishing morphological characters was successful and confirmed the molecular results. Previously published data of a large number of loci and SNPs further confirms the validity of the new species. The diagnostic morphological character is situated internally in the female ovipositor which is usually considered taxonomically uninformative and was not expected to be of taxonomic value.

Our data suggests that at least in part of their ranges species of the complex are monophagous. The complicated taxonomy and identification difficulties of their *Salix* host-plants may have contributed to the confused taxonomy of the group. Apart from *Ph. ramulicola* which feeds on *Salix cinerea* they feed on narrow-leaved *Salix* species. Furthermore, it seems likely that the species complex associated with *Salix* spp. includes additional undescribed species, each with a unique barcode BIN.

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