

# DNA barcoding resolves species complexes in *Stigmella salicis* and *S. aurella* species groups and shows additional cryptic speciation in *S. salicis* (Lepidoptera: Nepticulidae)

ERIK J. VAN NIEUKERKEN, MARKO MUTANEN & CAMIEL DOORENWEERD

Nieukerken, E.J. van, Mutanen, M. & Doorenweerd, C.: DNA barcoding resolves species complexes in *Stigmella salicis* and *S. aurella* species groups and shows additional cryptic speciation in *S. salicis* (Lepidoptera: Nepticulidae). [DNA analyser visar artuppdeleningen inom *Stigmella salicis*- och *S. aurella* grupperna och att det finns ytterligare kryptiska arter inom *S. salicis* (Lepidoptera: Nepticulidae).] – Entomologisk Tidskrift 132 (4): 235-255. Uppsala, Sweden 2012. ISSN 0013-886x.

We sequenced the mitochondrial barcoding marker COI and nuclear marker EF1-alpha for most Nordic and other European species of the *Stigmella salicis* and *S. aurella* species groups. In the *S. salicis* group both markers confirm the synonymy of *S. lappovimella* with *S. zelleriella*. Specimens previously identified as *Stigmella salicis* and *S. vimineticola* are shown to form a complex of several cryptic species for which the taxonomy needs to be worked out. The species previously recorded as *S. vimineticola* from Norway represents probably an unnamed species. In the *S. aurella* group, the oligophagous Rosaceae feeders *S. aurella* and *S. poterii* are confirmed to be each a single oligophagous species. The synonymy between *Stigmella ulmariae* from *Filipendula ulmaria* and *S. filipendulae* from *Filipendula vulgaris* is corroborated.

E.J. van Nieukerken & C. Doorenweerd, NCB Naturalis, PO Box 9517, NL-2300 RA Leiden, Netherlands, [nieukerken@naturalis.nl](mailto:nieukerken@naturalis.nl)

M. Mutanen, Zoological Museum, Department of Biology, PO Box 3000, FI-90014 University of Oulu, Finland, [marko.mutanen@oulu.fi](mailto:marko.mutanen@oulu.fi)

The Lepidoptera fauna of northern Europe, including Sweden, is amongst the best studied in the world and with a very long history of study as well thanks particularly to Linnaeus, but also other early naturalists. Whereas in Linnaeus' time the smallest moths were still mostly unknown, we now have an unprecedented knowledge of their taxonomy, biology and distribution as, for instance, shown in the first two volumes dealing with Microlepidoptera in the prestigious series Nationalnyckeln (Bengtsson et al. 2008, Bengtsson & Johansson 2011). Ingvar Svensson was a leading person in the study of Swedish smaller moths, with his constant stream of annual updates of the checklist (starting with Svensson

1974, the latest posthumously published: Svensson 2011). Furthermore he was interested in unravelling difficult species groups, resulting in a number of species descriptions (e.g. Svensson 1966, 1976), and had the strong opinion that a taxonomist should be careful when considering lumping different forms into one species: in this way one might lose important ecological information. He called himself a splitter at the species level, in contrast to a conservative attitude towards higher classification (a lumpner of genera) (Svensson 1992).

His interest in species complexes and host-plant races in leafminers often lead to heated debates, e.g. during the biannual conferences of the

Societas Europaea Lepidopterologica, almost all of which he visited between 1978 and 2009. In this paper we will consider some species complexes that had our joint interest and for which Ingvar also brought material together for molecular research. After it appeared to be impossible to get allozyme data from his material, it luckily could still be used for DNA analysis many years later.

The genus *Stigmella* Schrank, 1802 is the largest genus in the Nepticulidae, comprising to date 391 named species worldwide (Diškus & Puplesis 2003, van Nieuwerkerken 2010b), of which 107 are known from Europe (van Nieuwerkerken 2011). The genus is rather homogeneous in morphological characters, including the genitalia, making it difficult to separate it into clear-cut subgenera. Instead, informal species groups are widely recognised and used in European literature (Emmet 1976, Johansson 1971, Johansson & Nielsen 1990, van Nieuwerkerken 1986), and many of these probably represent monophyletic entities, although a full phylogenetic study of the genus is not yet available. Two of these species groups are the subject of this paper.

The *Stigmella salicis* and *aurella* groups contain several species or species complexes that have been the subject of many debates. Whereas Emmet (1976) still recognised all host races of *Stigmella aurella* (Fabricius, 1775) and of *S. poterii* (Stainton, 1857) as different species, later it became clear by conventional methods that these species had a broader host range than previously thought (Johansson & Nielsen 1990, Klimesch 1981). Here we corroborate these findings by DNA barcodes and confirm a further synonymy in the group. The *Stigmella salicis* group is a more complex one, with some species complexes feeding on the same range of *Salix* species and some on a different range. One of these was of particular interest to Ingvar Svensson: the pair *S. zelleriella* (Snellen, 1875) and *S. lappovimella* (Svensson, 1976). In this case, the DNA barcode confirms what has been thought by different authors (Johansson & Nielsen 1990, van Nieuwerkerken 1983), that this particular pair is just one variable species. Ingvar knew about these results and had accepted them. Interestingly, another species that he did not study so much in detail, *S. salicis*, appears to form a spe-

cies complex, as suggested earlier by finding different forms in Norway and Sweden (Aarvik et al. 2001, Aarvik et al. 2003, Bengtsson et al. 2008). The extent of this complex, however, is remarkable.

DNA barcoding has shown in the last seven years to be a useful addition for understanding species complexes and cryptic species in Lepidoptera (e. g. Hebert et al. 2004, Decaens & Rougerie 2008, Hausmann et al. 2009, Huemer & Hebert 2011, Ivanova et al. 2009, Segerer et al. 2010, Vaglia et al. 2008, Wilson et al. 2010). We have started building a DNA barcode database for Nepticulidae (van Nieuwerkerken 2007, 2010a), and in *Ectoedemia* Busck, 1907 its usefulness is shown for European species (van Nieuwerkerken et al. 2012), at the same time demonstrating that in one species complex, the *E. rubivora* complex, only the nuclear gene EF1-alpha was able to separate the species and not the common barcode marker COI. Here we combine two datasets of *Stigmella*, one covering a large part of the European fauna and also including sequences of EF1-alpha, and another basically covering the Finnish fauna. We have chosen to include EF1-alpha sequences to have an independent marker from a different genome (nuclear versus mitochondrial) in order to have a better supported pattern and to rule out artefacts and anomalies of one marker (van Nieuwerkerken et al. 2012).

This study should not be seen as a taxonomic revision of these groups since not enough material is yet available to study morphological characters properly. It merely serves to indicate the complex nature of the taxonomy of these groups and to encourage collecting and studying especially *Salix* feeding *Stigmella*. Only with a good representation of adult material from throughout Europe and from many hosts, paired with DNA sequences, such a revision is possible.

### Material and methods

**Material.** Material used is very diverse, many sequences were derived from larvae that were sampled from their leafmines, and for which the mines usually remain as voucher. Larvae were stored in ethanol 96-100%, usually in a minus 80 freezer, but those collected by Ingvar Svensson had been several years in a minus 20 freezer

in Lund University before they were sent to us (still in their mines) in ethanol 70%. These mines were dried after taking out the larvae. Adults used for sequencing were either reared or collected as adult. Two methods of extraction were used: in Leiden usually the DNA was extracted non-destructively from the abdomen when preparing genitalia slides (Knölke et al. 2005), but for the Finnish material usually one hindleg was used.

The labels of each specimen in the figured trees are composed of the voucher registry number, (where RMNH.INS. is the registry for insects in the collection of the NCB Naturalis (Leiden) and MM stands for Marko Mutanen collection), the (initial) species name, the ISO code for the country and the hostplant name for larvae and reared adults. In total we sequenced COI from 145 specimens (102 in the *salicis* group and 43 in the *aurella* group) and EF1- $\alpha$  from 63 specimens (50 in the *salicis* group and 13 only in the *aurella* group). No type material was included in the sequenced material.

Detailed data of the material and sequences (including GenBank accession numbers) are posted on the BOLD website (<http://www.bold-systems.org>), in the public project “*Stigmella* Ent. Tidskrift.” including photographs of many vouchers (free registration is needed for access to the details). The registry numbers link to BOLD process numbers. A datasheet abstracted from these data is available as online supplementary material on the journals website.

*Molecular methods.* COI sequences of MM’s material were generated at the Biodiversity Institute of Ontario, University of Guelph, Canada (<http://ibol.org/>). Protocol for DNA extraction, amplification, sequencing and sequence alignment are explained in detail in Ivanova et al. (2009).

In Leiden DNA was extracted from larvae or abdomens with the QIAGEN DNeasy Blood & Tissue kit. A PCR cycle consisted of 3 minutes initial denaturation at 94°C, 15 seconds cycle denaturation at 94°C, 30 seconds cycle at annealing temperature, 40 seconds cycle extension at 72°C for 40 cycles. A final extension at 72°C for 5 minutes occurred after all cycles had finished. The annealing temperature used for COI was 50°C, for EF1- $\alpha$  57°C. PCR was performed

in volumes of 25  $\mu$ l, containing 0.4  $\mu$ M of each primer, 50  $\mu$ M dNTP, 1x Qiagen PCR buffer, 1.25 units of Qiagen Taq polymerase and 1  $\mu$ L DNA template. All samples were sequenced in both directions on an ABI 3730 XL by MACROGEN EUROPE. SEQUENCHER 4.2 software was used to align the forward and reverse sequences, to manually check for ambiguities in the chromatograms and to export contigs. The sequences of both markers contain no gaps or stop codons. The sequences, primer details as well as all chromatograms are posted on the BOLD website. The EF1- $\alpha$  primers have been optimized for Nepticulidae (van Nieuwerkerken et al. 2012) and can amplify a section of 482 bp.

*Tree building.* Neighbor-joining trees were created in PAUP\* 4.0b10 (Swofford 2003) using uncorrected P distance rather than the frequently used K2P distance (Srivathsan & Meier 2011). Different trees were created for the two groups and two markers, using two representatives from the other group as outgroup. Neighbor-joining trees serve to display the sequences by similarity, where the scale bar at the bottom can be used to measure the difference between sequences. More similar sequences will be grouped together by the Neighbor-joining algorithm. When using barcoding markers, these are referred to as barcode clusters. In practice such clusters often represent different species, when far apart; clusters with very small distances may simply represent genetic variation within the species. There has been some discussion what method to use for calculating these trees, recent research has shown that it is probably better to use the simple method of uncorrected P distances than the often used so called K2P distance, that is assuming a certain evolutionary model (Srivathsan & Meier 2011). In practice we have seen that both methods result in very similar trees. The clusters as delimited by us are subjective, but the distance between them is larger than the distances seen within them, moreover they occur in both independent markers. No phylogenetic conclusions can be taken from these trees: two taxa that appear as sister taxa in a NJ tree do not necessarily appear as sister species in a cladistic analysis. Because the tree topology does not play an essential role in Neighbor-joining similarity trees, bootstrap support values for the nodes would be

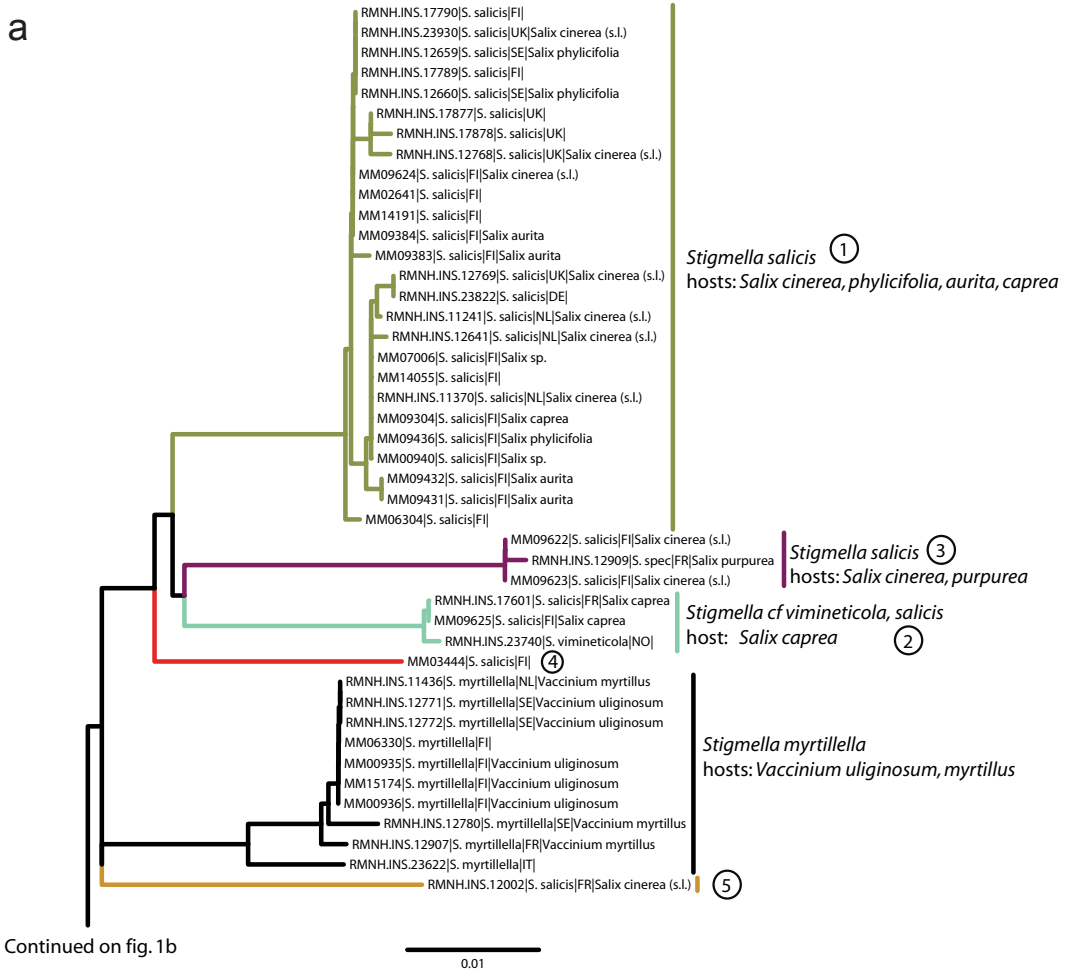


Figure 1. Neighbour-joining tree of the *Stigmella salicis* group based on COI sequences. For each sequence we provide the sample number, the original species name, ISO code for country and hostplant when reared or collected as larva. The numbers refer to the clusters of the *S. salicis-vimineticola* complex. – a) shows a large cluster *S. salicis*, tentatively regarded as the “real *salicis*”, *S. myrtillella* and the smaller “*salicis*” clusters 2, 3, 4 and 5, that may represent new species. – b) shows on top that all sequences from *S. zelleriella* and *S. lappovimella* are mixed, with hardly any differences, showing it to form a single species; further two “*salicis*” clusters: one (6) from *Salix caprea* in Britain and one (7) from *S. vimineticola* and finally four well recognised species: *S. obliquella*, *S. assimilella*, *S. trimaculella* and *S. benanderella*. Scale bar represents difference between sequences, 0.01=1 %.

Likhetsträd som visar hur lika olika individer inom *Stigmella salicis* gruppen är baserat på COI sekvenser. För varje sekvens anges provnummer, ursprungligt artnamn, ISO kod för land och värdväxt för de som insamlats som larver. Inringade nummer anger cluster av liknande individer inom *S. salicis-vimineticola* komplexet. – a) visar ett stort *S. salicis* kluster, preliminärt kallat “äkta *salicis*”; klustrena *S. myrtillella*, det mindre “*salicis*” samt 2, 3, 4 och 5 representerar troligen nya arter. I – b) ser man längst upp att alla sekvenser från *S. zelleriella* och *S. lappovimella* är blandade och har mycket små skillnader, vilket visar att det rör sig om en enda art; vidare finns två “*salicis*” kluster; ett (6) från *Salix caprea* i Storbritannien och ett (7) från *S. vimineticola*. Slutligen finns kluster av fyra välkända arter: *S. obliquella*, *S. assimilella*, *S. trimaculella* and *S. benanderella*. Skalan visar storleken på skillnaden, 0.01=1%.

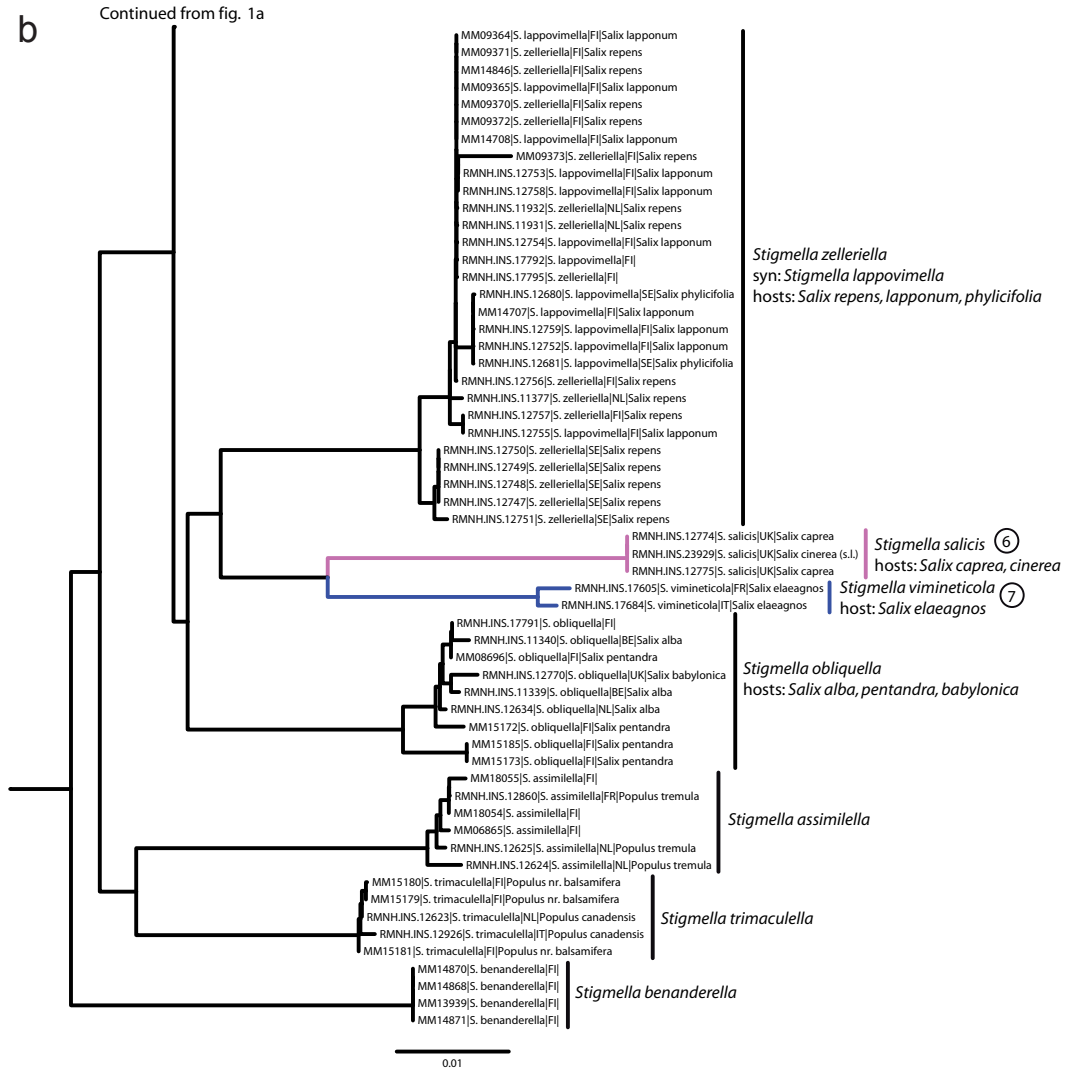


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rather meaningless, if not misleading, and for that reason we did not add them to the tree.

**Photography.** Adults were photographed by Ari Kakko (Oulu, Finland) and by CD (using a motorized Zeiss SteREO Discovery.V12 equipped with a PlanApo S 0.63x lens and an AxioCam MRc5 camera). Most specimens were from MM's collection; those from Leiden are indicated with RMNH. Leafmines (most from the

Leiden collection) were photographed by EJvN with a Zeiss Stemi SV11 and AxioCam HR, using dark field illumination; genitalia slides were photographed with a Zeiss Axioskop H and AxioCam (different types). Figure 4e was received from Kai Berggren.

Because both *Stigmella* and *Salix* are usually abbreviated as *S.*, we will not abbreviate the generic name *Salix* to avoid confusion.

## Results and Discussion

### The *Stigmella salicis* group

The *Stigmella salicis* group is one of the best-defined and recognisable species groups in the genus *Stigmella*, and undoubtedly monophyletic. Characters include the usually non-metallic forewing pattern, presence of cilia line on forewing, aedeagus with few cornuti, and female bursa with a band like signum running all around the bursa. Almost all feed on Salicaceae, except *S. myrtillella* (Stainton, 1857), which feeds on *Vaccinium* species (Ericaceae), and the group occurs widely throughout the Holarctic region. The European species recognised before this study and their hostplants are listed in Table 1. Puplesis & Robinson (2000) widened the concept of the group by including Neotropical species with different female genitalia and feeding on different hosts. We tentatively treat the group in its old narrow circumscription.

The similarity of the *Salix* feeding species has been known for a long time and has led to confusion of the species since the 19<sup>th</sup> century, when the hostplant was often used for the identification. Until Hering's study of the genitalia (Hering 1943), northern European *S. obliquella* (Heinemann, 1862) was frequently misidentified as *S. vimineticola* (Frey, 1856), an alpine species. Later, van Nieukerken (1986) synonymised many of the used names with *S. salicis*, which he considered to be an oligophagous *Salix* feeder. By more careful study of the genitalia, however, it later appeared that the al-

pine *S. vimineticola* was a different species on the basis of male and female genitalia and external characters (Johansson & Nielsen 1990) and the synonymy was rejected. The finding of more forms shed more doubt on the identity of *S. salicis* (Stainton, 1854), but traditional morphology has not yet been able to solve the riddle of the *salicis* group. A number of species was usually recognised (e.g. Bengtsson et al. 2008, Johansson & Nielsen 1990), and recently *Stigmella arbusculae* (Klimesch, 1951), was added to the Swedish fauna and taken out of synonymy with *S. salicis* (see Svensson 2010).

The following species are clearly recognisable by morphology and have clear barcode clusters with little variation: *S. trimaculella* (Haworth, 1828), *S. assimilella* (Zeller, 1848), *S. obliquella* (Fig. 7), *S. benanderella* (Wolff, 1955) and *S. myrtillella* (Fig. 6). Therefore, they are not discussed further. We have not been able to get fresh material of the species *S. arbusculae* (Klimesch, 1951) or *S. pallidiciliella* Klimesch, 1946. The remaining species are discussed by species or cluster recognised in the Neighbor-joining trees as illustrated in Fig. 1-2.

Taxonomy and identification of *Salix* can be troublesome, and current floras treat some species differently. Several floras nowadays recognise *Salix atrocinerea* next to *Salix cinerea* (Jalas & Suominen 1976, Rechinger & Akeroyd 1993, Zinovjev & Kadis 2009) with almost vicariant distribution: *Salix atrocinerea* occurs only in western Europe and the Iberian

Table 1. The European species of the *Stigmella salicis* group as recognised before this study, with their hostplants.

*De europeiska arterna i Stigmella salicis gruppen så som de uppfattades före denna studie, med sina värdväxter.*

Species	Hostplants
<i>Stigmella salicis</i> (Stainton, 1854)	<i>Salix aurita</i> , <i>cinerea</i> s.l., <i>caprea</i> , <i>phylicifolia</i>
<i>Stigmella arbusculae</i> (Klimesch, 1951)	<i>Salix retusa</i> , <i>reticulata</i> , <i>glabra</i> , <i>waldsteiniana</i>
<i>Stigmella vimineticola</i> (Frey, 1856)	<i>Salix elaeagnos</i> , <i>viminalis</i>
<i>Stigmella myrtillella</i> (Stainton, 1857)	<i>Vaccinium myrtillella</i> , <i>uliginosum</i>
<i>Stigmella zelleriella</i> (Snellen, 1875)	<i>Salix repens</i> s.l.
<i>Stigmella lappovimella</i> (Svensson, 1976)	<i>Salix lapponum</i> , <i>phylicifolia</i>
<i>Stigmella benanderella</i> (Wolff, 1955)	<i>Salix repens</i> s.l.
<i>Stigmella obliquella</i> (Heinemann, 1862)	<i>Salix alba</i> , <i>fragilis</i> , <i>triandra</i> , <i>babylonica</i> , <i>pentandra</i>
<i>Stigmella pallidiciliella</i> Klimesch, 1946	<i>Salix purpurea</i>
<i>Stigmella trimaculella</i> (Haworth, 1828)	<i>Populus nigra</i> , <i>canadensis</i> , <i>nr balsamifera</i>
<i>Stigmella assimilella</i> (Zeller, 1848)	<i>Populus tremula</i> , <i>alba</i> , <i>canescens</i>

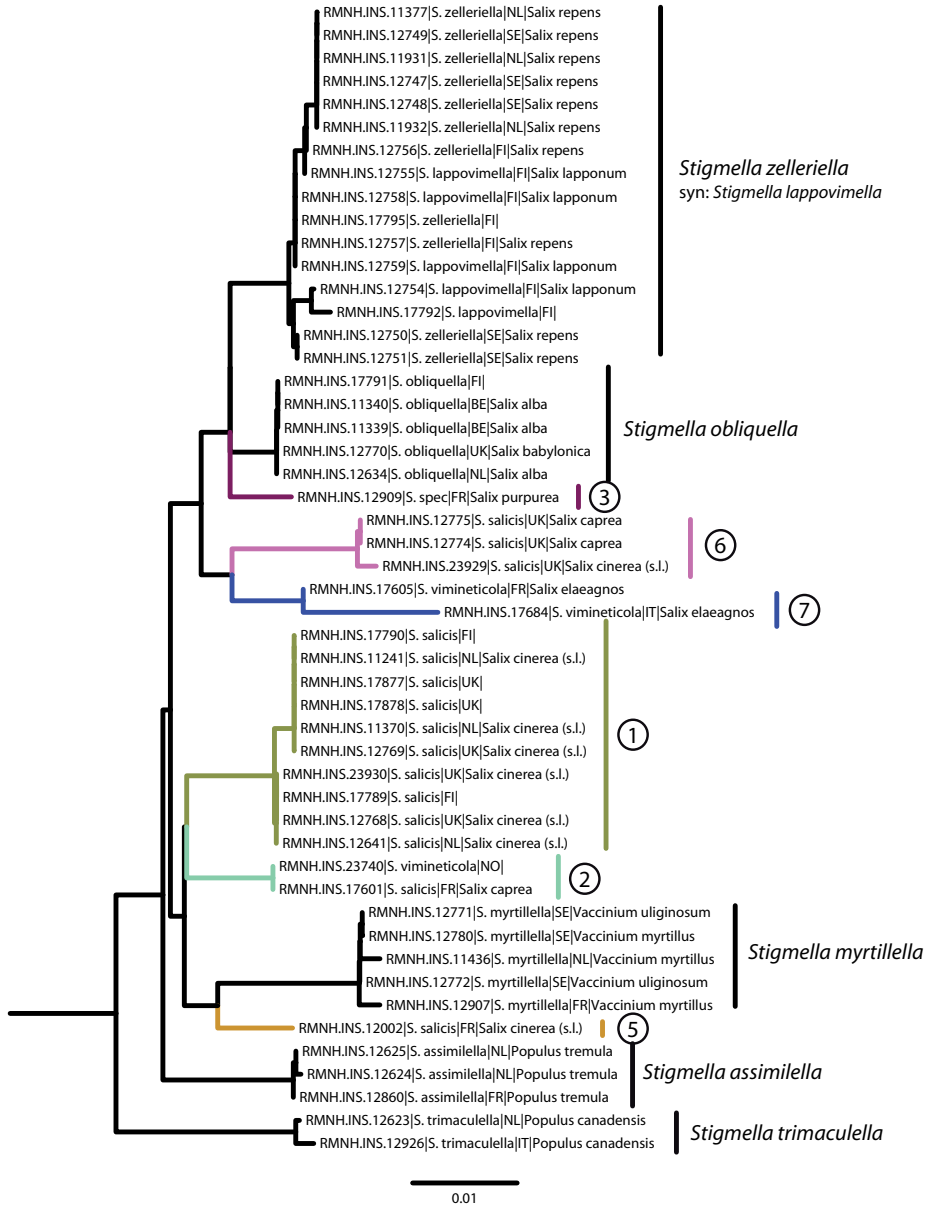


Figure 2. Neighbour-joining tree of the *Stigmella salicis* group based on *EF1-alpha* sequences. The numbers refer to the clusters of the *S. salicis-vimineticola* complex. Although the branching pattern is different, this gene recognises the same clusters as COI: 1, 2, 3, 5, 6 and 7 (there are no sequences for 4) and the species *S. obliquella*, *S. myrtillella*, *S. assimilella* and *S. trimaculella*. *S. lappovimella* and *S. zelleriella* again do not differ. Scale bar represents difference between sequences, 0.01 = 1 %.

Likhetsträd av *Stigmella salicis* gruppen baserad på *EF1-alpha* sekvenser. Inringade nummer visar kluster av liknande individer inom *S. salicis-vimineticola* komplexet. Även om förgreningarna skiljer en del identifieras samma kluster med denna gen som med COI (Fig. 1): 1, 2, 3, 5, 6 and 7 (det finns inga sekvenser för 4) och arterna *S. obliquella*, *S. myrtillella*, *S. assimilella* and *S. trimaculella*. Inte heller här fanns några skillnader mellan *S. lappovimella* and *S. zelleriella*. Skalan visar storleken på skillnaden, 0.01=1%.

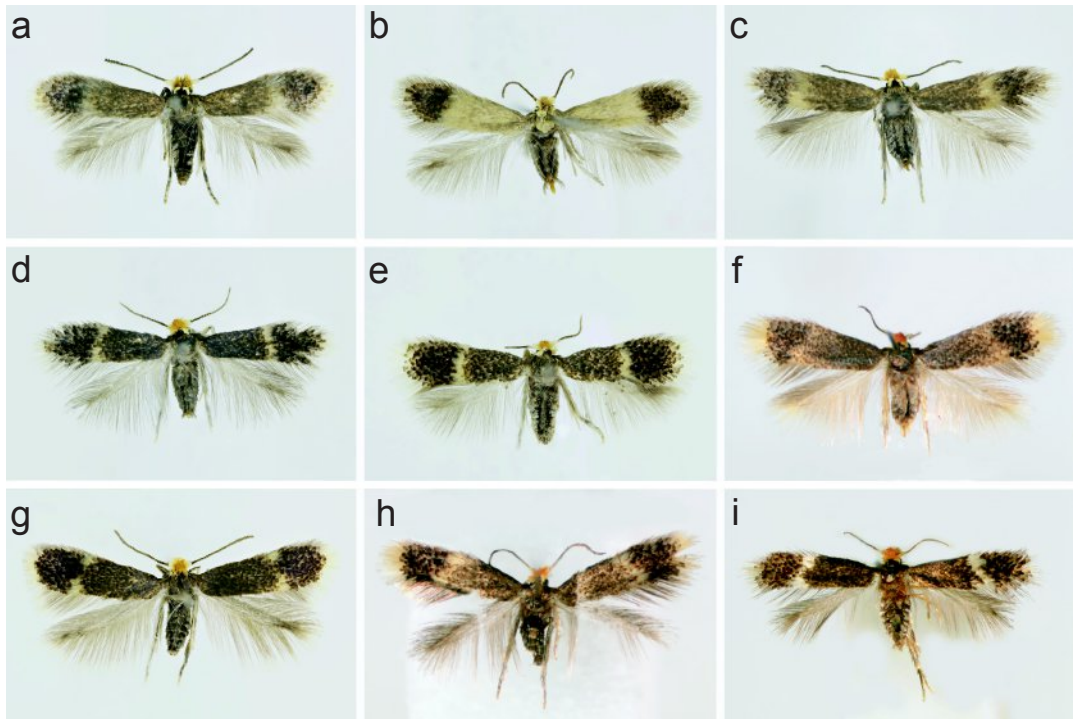


Figure 3. Species in the *Stigmella salicis* group, habitus. – a) *S. zelleriella*, male, Finland: Oba Kiiminki, e.l. 2008 *Salix repens*, M. Mutanen. – b) *S. zelleriella* (as *lappovimella*), female, Finland: Ks Salla, la. 15.viii.1994 *Salix lapponum*, M. Mutanen. – c) *S. zelleriella* (as *lappovimella*), female, Finland: Obb Rovaniemi, la. 13.ix.1995 *Salix lapponum*, J. Itämies. – d) *S. myrtillella*, female, Finland: Oba Kiiminki, e.l. 2008 *Vaccinium uliginosum*, M. Mutanen. – e) *S. obliquella*, male, Finland: Obb Rovaniemi e.l. 1993 *Salix pentandra*, M. Mutanen. – f) *S. vimineticola*, female, Germany: Baden Württemberg, Illerauen, Illertissen, e.l. 1989, *Salix elaeagnos*, A. Scholz (RMNH). – g) *S. salicis* (cluster 1), male, Finland: Oba Kiiminki, e.l. 2008 *Salix phylicifolia*, M. Mutanen. – h) *S. cf. salicis* (cluster 6), male, England: Hampshire, Portsmouth, el 2008, *Salix cinerea* s.l., J. Langmaid (RMNH). – i) *S. cf. salicis* (cluster unknown, maybe 5), female, Spain: Málaga, El Burgo, el 2001, *Salix atrocinnerea*, E.J. van Nieukerken (RMNH).

Arter inom *Stigmella salicis* gruppen.

Peninsula, where *Salix cinerea* is almost absent. Because not all floras treat the differences it is likely that identifications by entomologists' are often not reliable. We have used the indication *Salix cinerea* s.l. for records of that host from western Europe where also or only *Salix atrocinnerea* occurs. *Salix repens* as used here is also to be taken in the broad sense, including *Salix arenaria* and *Salix rosmarinifolia* (Jalas & Suominen 1976, Rechinger & Akeroyd 1993). Other *Salix* species can also be hard to identify, thus host records should be viewed with caution.

*Stigmella zelleriella* versus *lappovimella* (Fig. 3a-c)

*Stigmella zelleriella* was described as *Nepticula zelleriella* Snellen, 1875 from the coastal dunes in the Netherlands, flying around *Salix repens* (as *Salix fusca*: Snellen 1875). This name (*zelleriella*) was subsequently misinterpreted by most authors, and therefore the species was described again from the Danish dunes as *Nepticula repentiella* Wolff 1955, after rearing it from leafmines. Only much later (van Nieukerken 1983) was it realised that these two are the same



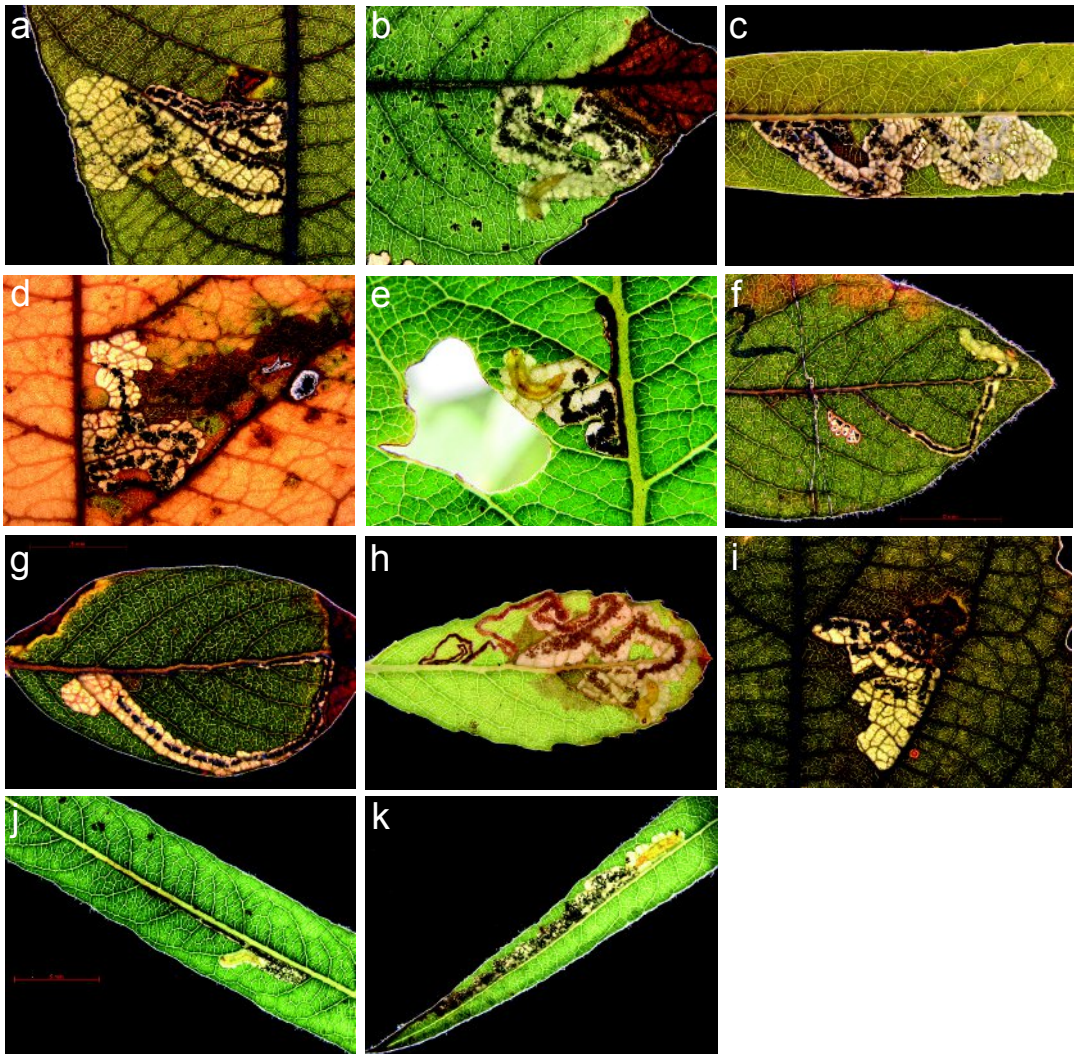


Figure 4. Leafmines and larvae of the *Stigmella salicis-vimineticola* complex. – a) *S. salicis* (cluster 1), England: Surrey, Charlwood *Salix cinerea* s.l., la 29.x.2006, J. Langmaid (RMNH.INS.12769) UK. – b) *S. salicis* (cluster 1), Netherlands, Zuid-Holland, Wassenaar, Meijendel, *Salix cinerea* s.l., la 11.x.2007, E.J. van Nieuwerkerken (RMNH.INS.12641). – c) *S. cf. salicis* (cluster 3), France: Alpes Maritimes, Casterino, *Salix purpurea*, la 11.x.2008, E.J. van Nieuwerkerken & C. Doorenweerd (RMNH.INS.12909). – d) *S. near vimineticola* (cluster 2), France: Alpes Maritimes, Les Mesches, *Salix caprea*, la 10.x.2008, E.J. van Nieuwerkerken & C. Doorenweerd (RMNH.INS.17602–4). – e) *S. near vimineticola* (cluster 2), Norway: AAY Arendal, Havsøy, *Salix caprea*, la 2010, K. Berggren (photo Berggren). – f) *S. cf. salicis* (cluster 5), France: Finistère, Fouesnant, *Salix atrocinearea*, la. 10.vii.2006, E.J. van Nieuwerkerken (RMNH.INS.12002). – g) *S. cf. salicis* (cluster 5), vacated mine from same sample as figure 4f. – h) *S. cf. salicis* (cluster 6), England, Hampshire, Portsmouth, *Salix cinerea* s.l., la. 14.x.2011, J. Langmaid & I. Thirlwell. – i) *S. cf. salicis* (cluster 6), England, Hampshire, Southsea, *Salix caprea*, la. 9.ix.2006, J. Langmaid (RMNH.INS.12774-5). – j, k) *S. vimineticola* (cluster 7), Italy: Cuneo, Entracque, Trinità, Ponte del Suffiet, *Salix elaeagnos*, la. 13.x.2008, E.J. van Nieuwerkerken & C. Doorenweerd (2 examples from sample of RMNH.INS.17684).

Bladminor och larver från *Stigmella salicis-vimineticola* komplexet.

species, distributed along Europe's western and northern coasts. Meanwhile Svensson (1977) had described *Nepticula lappovimella* Svensson, 1977 from adults caught on *Salix lapponum* in tundra peat moors in northern Sweden. On the basis of the examination of a number of Swedish specimens it was synonymised with *S. zelleriella* (van Nieukerken 1983), later followed in the faunal treatment of the Fennoscandian Nepticulidae (Johansson & Nielsen 1990). This synonymy was not accepted by Svensson and several other authors, and arguments were sought to support the specific identity, both in ecology, distribution, larval mine form and detailed studies of genitalia and scales (Bruun 1988, Bruun & Itämies 1997, Svensson 1986). Svensson (1986) also suggested the use of electrophoresis [of allozymes] to solve this problem. Although the electrophoretic study never actually took place, material that he collected for this purpose has now been used for DNA barcoding. The 28 specimens we sequenced show hardly any variation (Fig. 1b) and specimens from different hosts (*Salix repens*, *Salix lapponum*, *Salix phylicifolia*) from Finland, Sweden, and the Netherlands only differ by a maximum of five basepairs on the total of 658. The data of EF1-alpha (Fig. 2) show even less variation. These data unequivocally support the synonymy of *S. lappovimella* with *S. zelleriella*. The barcoding results are not so much contrasting with ecological and distribution data as might appear from previous studies. In one Finnish locality (Oba Kiiiminki), mines on both *Salix repens* and *Salix lapponum* occur at the same site, and mines are also found on apparent hybrids between these plants, suggesting that the ecology of the two taxa does not differ but is due to the usually separate habitats of both *Salix* species. The ranges of these plants also show little overlap, which is why the leafmining taxa do not usually occur in sympatry. The putative minor differences in leaf mines (Bruun & Itämies 1997) may simply be reflections of the different leaf structure. Similarly, the reported slightly smaller wingspan may refer to phenotypic plasticity, as leaves of *Salix repens* are likely often sub-optimally small for a larva. Overall, the mines on both plants are similar and differ from those of *S. salicis* s.s. Furthermore, the described difference in wing

coloration (Svensson 1985) is a sampling artefact. Our large reared material indicates that *S. lappovimella* shows an equal range of colour variation to that of 'typical' *zelleriella* (Fig. 3a-c). Leaves of *Salix repens* and *Salix lapponum* are both densely hairy, which may be the reason that *S. zelleriella* and some other lepidopteran species (e.g. *Anacamptis temerella* (Lienig & Zeller, 1846), *Ancylis subarcuana* (Douglas, 1847)) seem to have a preference for these two plants. The female of *S. zelleriella* has a relatively long ovipositor, which undoubtedly serves as an adaptation facilitating egg-laying on hairy leaves. *Salix phylicifolia* as a food plant for *S. zelleriella* is rather unusual, and the species also seems to avoid *Salix glauca* despite its hairiness and superficial similarity to *Salix lapponum* (MM, pers. obs.). It is interesting to note that *Salix lapponum* and *phylicifolia* are chemically rather similar (Julkunen-Tiitto 1989). The host range of *S. zelleriella* is thus relatively small, but a record in central Russia, where the species is assumed to feed on *Salix triandra* is of interest (van Nieukerken et al. 2004). As yet we do not know the barcode of that population.

#### *Stigmella salicis* complex

Specimens from a number of *Salix* species and identified as *S. salicis* or *S. viminicola* form at least seven barcode clusters with large genetic distances in both markers. We have not yet been able to include material of Alpine or Swedish *S. arbusculae*, feeding on alpine dwarf species of *Salix*, but see below under cluster 4.

#### Cluster 1: *Stigmella* cf. *salicis* s.s. (Fig. 3g, 4a, 4b, 5a, 5b)

The largest number of specimens from western and northern Europe (Finland, Sweden, United Kingdom, Netherlands and Germany) belong to this cluster. They have been found mostly on hairy *Salix* species (sallows): *Salix cinerea* s.l. (certainly also including misidentified *S. atrocinerea*), *Salix aurita*, *Salix caprea*, and one not hairy species: *Salix phylicifolia*. Whether this is indeed the real *S. salicis* needs to be verified by checking genitalia of types (from England) and designation of a lectotype. The type series has never been studied in detail and hostplants are only indicated by a more general "sallow" on the

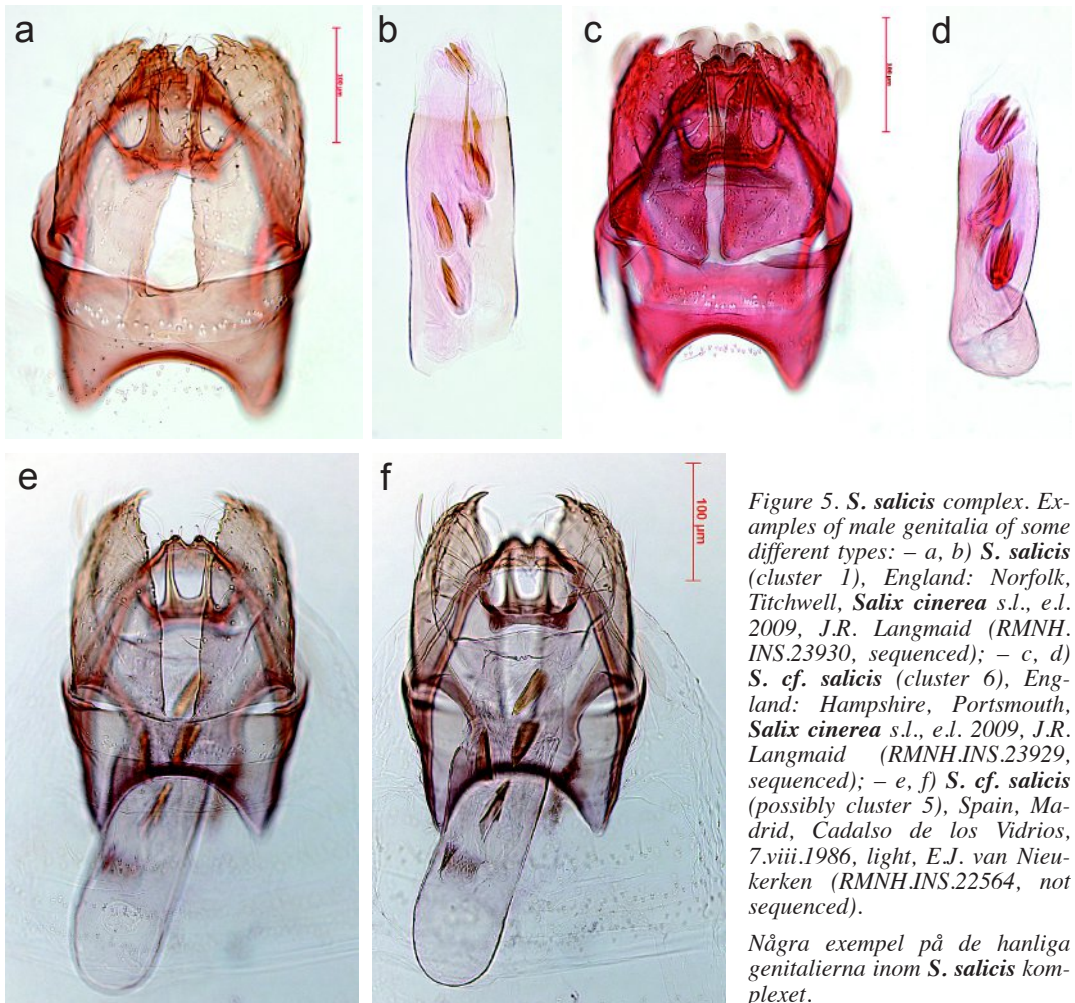


Figure 5. *S. salicis* complex. Examples of male genitalia of some different types: – a, b) *S. salicis* (cluster 1), England: Norfolk, Titchwell, *Salix cinerea* s.l., e.l. 2009, J.R. Langmaid (RMNH.INS.23930, sequenced); – c, d) *S. cf. salicis* (cluster 6), England: Hampshire, Portsmouth, *Salix cinerea* s.l., e.l. 2009, J.R. Langmaid (RMNH.INS.23929, sequenced); – e, f) *S. cf. salicis* (possibly cluster 5), Spain, Madrid, Cadalso de los Vidrios, 7.viii.1986, light, E.J. van Nieukerken (RMNH.INS.22564, not sequenced).

Några exempel på de hanliga genitalierna inom *S. salicis* komplexet.

labels (K.R. Tuck pers. comm.); the fact that at least two forms occur in England on these hosts (this and cluster 6) makes checking types a necessary step.

**Cluster 2: *Stigmella* near *vimineticola*** (Fig. 4d, e) This cluster comprises one female from Norway (RMNH.INS.23740), identified as *S. vimineticola* on the base of the long pointed ovipositor (Aarvik et al. 2001, Aarvik et al. 2003), but suggested to be another species later (Bengtsson et al. 2008). Two specimens respectively from Finland (MM09625) and the French Alps (RMNH.

INS.17601), share this barcode. Both were taken as larvae from *Salix caprea*. Meanwhile, in Norway, the species has also been reared from this host (Kai Berggren personal communication, see Fig. 4e), so we may conclude that this is an undescribed species feeding, possibly exclusively, on *Salix caprea*.

**Cluster 3: *Stigmella* from Åland and southern France** (Fig. 4c)

The specimens in this cluster seem to share little else than the barcode, one is taken from *Salix purpurea* in the Alps of southern France (RMNH.

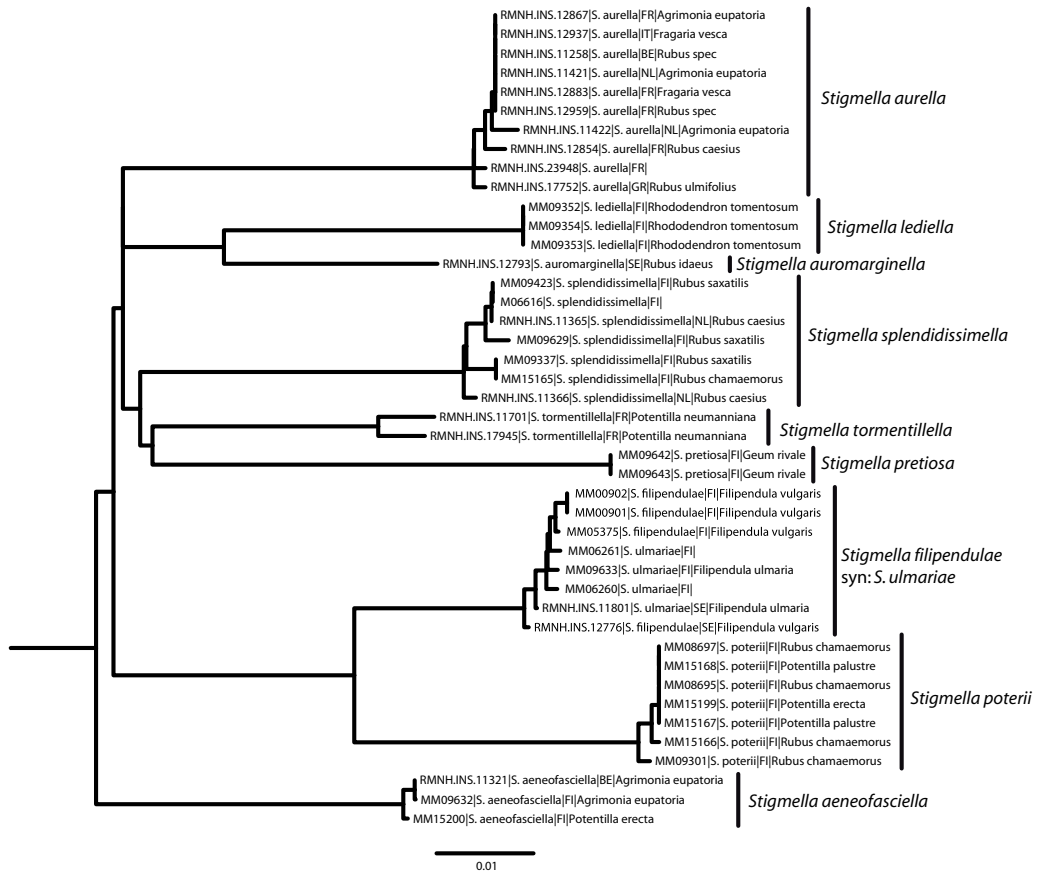


Figure 6. Neighbour-joining tree of the *Stigmella aurella* group based on COI sequences. This tree shows that all recognised species are separated by large distances, with the notable exception of *S. ulmariae* and *S. filipendulae*: the last two do not differ in the COI sequence and should be regarded as one species. For the species *S. aurella*, *S. splendidissima*, *S. poterii* and *S. aeneofasciella* the tree shows that specimens from different hostplants do not show differences. Scale bar represents difference between sequences, 0.01 = 1 %.

Likhetsträd som visar hur lika olika individer inom *Stigmella aurella* gruppen är baserat på COI sekvenser. Trädet visar att alla de kända arterna är åtskilda med stora skillnader, med ett intressant undantag: *S. ulmariae* and *S. filipendulae* som inett skiljer sig i COI sekvenser och därför bör betraktas som en art. För *S. aurella*, *S. splendidissima*, *S. poterii* and *S. aeneofasciella* visar trädet att individer från olika värdväxter inte skiljer sig åt. Skalan visar storleken på skillnaden, 0,01=1%.

INS.12909), and two (MM09622-3) are from the Åland islands (Finland) on *Salix cinerea*. *Stigmella pallidiciliella* is the only species known to feed exclusively on *Salix purpurea*. However, *S. pallidiciliella* has never been found as far north as Åland. It is mostly known from Central Europe with an unconfirmed record from Poland on the basis of leafmines (Michalska 1983, not accepted by Buszko & Nowacki 2000). Unfor-

tunately the barcode of *S. pallidiciliella* is still unknown. Our data are currently insufficient for any conclusion about the identity of this cluster. The Åland occurrence was exceptionally abundant as several hundred mining larvae were observed on the single willow bush. Such mass occurrences have not commonly been reported for *S. salicis*. Currently newly collected material from Åland is being reared.

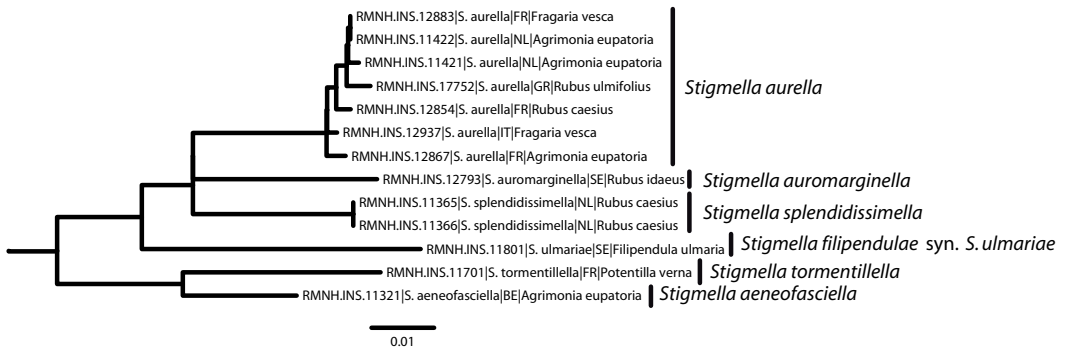


Figure 7. Neighbour joining tree of the *Stigmella aurella* group based on *EF1-alpha* sequences. This tree shows in principle the same as the *COI* tree in Fig. 6, but several species are missing, and we have no sequence for *S. filipendulae*. Scale bar represents difference between sequences, 0.01 = 1%.

Likhetsträd som visar hur lika olika individer inom *Stigmella aurella* gruppen är baserat på *EF1-alpha* sekvenser. Resultatet är i princip detsamma som med *COI* sekvenser (Fig. 6), men många arter saknas. Det finns till exempel ingen sekvens för *S. filipendulae*. Skalan visar storleken på skillnaden, 0,01=1%.

#### Cluster 4: *Stigmella Lapland*

This refers to a single specimen taken as an adult in Finnish Lapland (MM03444). The voucher specimen of this sample has been destroyed. It is, however, known that *S. salicis* includes two characteristic forms in North Scandinavia. Some specimens from Finnish Lapland show dark ground colour with bluish tinge and bright white broad fascia. Such specimens have been reared from *Salix myrtilloides* and also from other *Salix* species. Also, a paler form of *S. salicis* with indistinct fascia and less contrasting cilia has been reported from northernmost Finland and Sweden (Johansson & Nielsen 1990). In addition, *S. arbusculae* was recently reported from northern Sweden (Bengtsson et al. 2008, Svensson 2010). The locality where the latter was found, Björkliden, is about 100 km away from the locality in Finland where MM03444 was found in the Kilpisjärvi region. The possibility exists that these two belong to the same species. Swedish specimens of *S. arbusculae* have been reared on *Salix reticulata*, which has an abundant population in the Kilpisjärvi region, further supporting that MM03444 and Swedish *S. arbusculae* may be conspecific. It also seems possible the specimens reared from *Salix myrtilloides* in Finnish Lapland represent the same species as Swedish *S. arbusculae*, as both taxa seem to share similar external appearance with dark ground colour

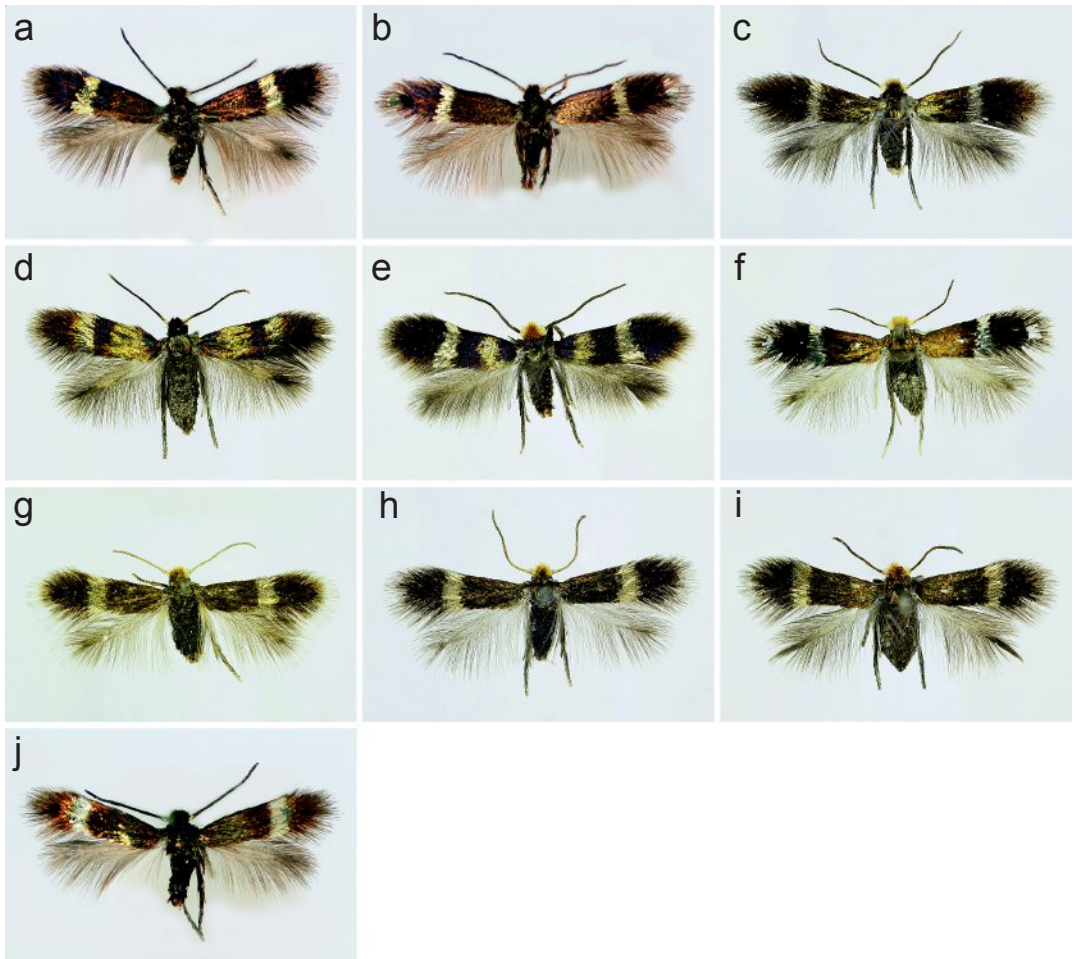
and broad white fascia. Specimens from *Salix myrtilloides* remain to be sequenced. It seems possible that the *S. salicis* group contains still more hidden species in northernmost Lapland, where *Salix* reaches the highest diversity in northern Europe.

#### Cluster 5: *Stigmella Salix atrocinerea* Brittany (Fig. 4f, g, possibly also: 3i, 5e, f)

The specimen (RMNH.INS 12002) was collected in Brittany, France on *Salix atrocinerea* (see above for identity of this host). The leafmines (Fig. 4f, g) show more a gallery character than other *salicis* forms. Material from French collections that EvN studied at least contains a second species next to *salicis* with different genitalia, collected near Bordeaux. It may be this species, but as yet it is impossible to link the two. Leafmines collected on *Salix atrocinerea* in southern Spain and Central Portugal resemble those from Brittany, the female depicted in Fig. 3i was reared from those. Male genitalia from “*S. salicis*” from Spain that we have seen differ in their cornuti from “normal” *S. salicis* (Fig. 5e, f).

#### Cluster 6: *Stigmella Salix caprea* Britain (Fig. 3h, 4h, i, 5c, d)

This refers to a series reared from *Salix caprea* or *cinerea* (possibly *atrocinerea*) with eggs in-



Figures 8. Species in the *Stigmella aurella* group, habitus. – a) *S. aurella* male, France: Haut-Rhin, Lapoutroie, el 2003, *Rubus* sp., E.J. van Nieukerken (RMNH), – b) *S. auromarginella*, male, Sweden: Blekinge, Almö, el 1990, *Rubus* sp., R. Johansson (RMNH). – c) *S. splendidissima*, male, Finland: Al Eckerö, e.l. 2008, *Rubus saxatilis*, M. Mutanen. – d) *S. aeneofasciella*, female, Finland: Al Föglö, e.l. 2009, *Agrimonia eupatoria*, M. Mutanen. – e) *S. pretiosa*, male, Finland: Al Eckerö, e.l. 2008, *Geum pratense*, M. Mutanen. – f) *S. lediella*, female, Finland: Oba Oulu, e.l. 2009, *Rhododendron tomentosum*, M. Mutanen. – g) *S. filipendulae*, male, Finland Al Lemland, e.l. 1996, *Filipendula vulgaris*, M. Mutanen. – h) *S. filipendulae* (as *ulmariae*), male, Finland: Oba Kiiminki, 22.-23.6.2007, M. Mutanen. – i) *S. poterii*, female, Finland: Oba Kiiminki, e.l. 2008, *Rubus chamaemorus*, M. Mutanen. – j) *S. tormentillella*, male, France: Meuse, Montmédy, el 2003, *Potentilla neumanniana*, E.J. van Nieukerken & C. van den Berg (RMNH).

Arter i *Stigmella aurella* gruppen.

variably on leaf upperside, found commonly by John Langmaid along the south coast of England (Hampshire). The male genitalia also differ slightly from the more typical *S. salicis* (Fig. 5c, d).

**Cluster 7 *Stigmella cf. vimineticola*** (Fig. 3f, 4j, k) This cluster is represented by two specimens, collected in the French and Italian Alps as larvae on *Salix elaeagnos* in mines showing the typical form of *S. vimineticola*. No adults were reared, so confirmation that this is the real *S. vimineticola* by sequencing adults is still needed, but we assume it is most likely that this actually represents that species. In the French and Italian alpine localities we found it together with larvae of *S. nivenburgensis* (Preissecker, 1942) (new record for France), identified by the barcode, but also easily recognisable by the much narrower and straight mines. This is the only *Salix* feeding *Stigmella* in Europe belonging to another species group (the *S. betulicola* group).

### The *Stigmella aurella* group

The *Stigmella aurella* group comprises a number of species that specialize on a group of rosaceous herbs and shrubs, notably the genera *Rubus*, *Fragaria*, *Potentilla*, *Agrimonia* and *Filipendula*, belonging to the subfamily Rosoideae (Rosaceae). The European species recognised before this study and their hostplants are listed

in Table 2. Strange enough none of the species in the group feed on the equally related *Rosa* or *Sanguisorba* (Potter et al. 2007), on which we find representatives of the *Stigmella anomalella* and *sanguisorbae* species groups. Obvious outliers are *Stigmella dryadella* (Hofmann, 1868) on *Dryas*, a rather isolated genus in the Rosaceae (Potter et al. 2007) and *S. lediella* (Schleich, 1867) on *Rhododendron tomentosum* (Ericaceae) (formerly *Ledum palustre*) in Europe. In Finland mines of *S. lediella* have also been found on non indigenous *R. canadense* and in the East Palearctic it occurs on some other *Rhododendron* species (Puplesis 1994). Most Rosaceae feeders show some degree of oligophagy, which has led in the past to confusion and a complicated taxonomy. In Britain Emmet (1976) was still uncertain about such “races” and treated them tentatively as separate species, both around the species *S. aurella* (on *Rubus*, *Geum*, *Fragaria* and *Agrimonia*) and *S. poterii* (on *Sanguisorba*, *Potentilla*, *Comarum* and *Rubus chamaemorus*). In central Europe various host races of *S. aurella* were considered as synonyms (Borkowski 1975, Klimesch 1981) and likewise in northern Europe *S. splendidissimella* (Herrich-Schäffer, 1855) was considered oligophagous on *Rubus*, *Fragaria*, *Agrimonia* and *Geum* (Johansson & Nielsen 1990). In the early 1980s the *aurella* complex was studied at the Free University of

Table 2. The European species of the *Stigmella aurella* group as recognised before this study, with their hostplants.

De europeiska arterna i *Stigmella aurella* gruppen så som de uppfattades före denna studie, med sina värdväxter

Species	Hostplants
<i>Stigmella aurella</i> (Fabricius, 1775)	<i>Rubus</i> , <i>Fragaria</i> , <i>Geum</i> , <i>Agrimonia</i> , ( <i>Geranium</i> )
<i>Stigmella auromarginella</i> (Richardson, 1890)	<i>Rubus</i>
<i>Stigmella splendidissimella</i> (Herrich-Schäffer, 1855)	<i>Rubus</i> , <i>Fragaria</i> , <i>Geum</i> , <i>Agrimonia</i>
<i>Stigmella pretiosa</i> (Heinemann, 1862)	<i>Rubus</i> , <i>Geum</i>
<i>Stigmella geimontani</i> (Klimesch, 1940)	<i>Geum</i>
<i>Stigmella aeneofasciella</i> (Herrich-Schäffer, 1855)	<i>Agrimonia</i> , <i>Potentilla</i> , <i>Fragaria</i>
<i>Stigmella tormentillella</i> (Herrich-Schäffer, 1860)	<i>Potentilla</i>
<i>Stigmella stelviana</i> (Weber, 1938)	<i>Potentilla</i>
<i>Stigmella dryadella</i> (Hofmann, 1868)	<i>Dryas</i>
<i>Stigmella poterii</i> (Stainton, 1857)	<i>Potentilla</i> , <i>Rubus chamaemorus</i> , <i>Sanguisorba</i>
<i>Stigmella filipendulae</i> (Wocke, 1871)	<i>Filipendula vulgaris</i>
<i>Stigmella ulmariae</i> (Wocke, 1879)	<i>Filipendula ulmaria</i>
<i>Stigmella lediella</i> (Schleich, 1867)	<i>Rhododendron tomentosum</i> , <i>canadense</i>

Amsterdam (see e.g. Wilkinson 1982), both with hybridisation and host choice experiments and by analysis of allozymes. The convincing result of this research was that the host races of *S. aurella* do not exist, but represent one oligophagous species. Unfortunately, it was never published in detail, but only mentioned briefly in a checklist (van Nieukerken 1986). Results of the DNA barcoding, supported by the EF1-alpha gene here, support this view, following the general trend of Rosaceae feeders to be oligophagous (Huemer 1988a, b). Figure 6 and 7 show the neighbor-joining trees of respectively COI and EF1-alpha. We will only discuss some species in more detail, but not *S. lediella* (Fig. 8f), *S. auromarginella* (Richardson, 1890) (Fig. 8b), or *S. tormentillella* (Herrich-Schäffer, 1860) (Fig. 8j). The species *S. pretiosa* (Heinemann, 1862) (Fig. 8e) is known to feed on *Geum* in northern Europe, but also on *Rubus* in the central European mountains. However, since we have not studied central European representatives we refrain from further discussion on this species. We were unable to get fresh material of the species *S. geimontani* and *S. stelviana*.

#### *Stigmella aurella* (Fig. 8a)

We have sequenced specimens taken from various *Rubus* species, *Fragaria* and *Agrimonia*, from a wide geographical range from the Netherlands to Greece. COI sequences show hardly any variation, the largest distance being a little more than 0.3%, the distance in EF1-alpha is even less. This species, not known from the Nordic countries, easily feeds on these host genera and on *Geum*. In Greece it was also found on another plant family, *Geranium versicolor* (Geraniaceae) (van Nieukerken 1986), although we have not yet checked the barcode of that population. The oligophagy of this species, as had been indicated by studies of the genitalia and allozymes, is now confirmed by the two DNA markers.

#### *Stigmella splendidissimella* (Fig. 8c)

Our material originates only from various *Rubus* species, showing hardly any variation. Since mines of this species are not always separable from *S. aurella* and *S. auromarginella* where they occur sympatrically (but see Koster et al.

1984), checking DNA barcodes forms a good additional method to separate leafmines when larvae or larval remains are available.

#### *Stigmella filipendulae* versus *S. ulmariae* (Fig. 8g, h)

*Stigmella filipendulae* (Wocke, 1871) was described from *Filipendula vulgaris* and *S. ulmariae* (Wocke, 1879) from *F. ulmaria*. Doubt about the separate identity has existed for a long time (Bengtsson et al. 2008, Johansson & Nielsen 1990, van Nieukerken & Johansson 1987, Waters 1924) but no consensus had been reached. Our barcode data show no difference between populations on both hosts, but unfortunately we have only a single EF1-alpha sequence to date. We have not seen sufficient genitalia slides to confirm the differences given by Bengtsson et al. (2008) as consistent, they seem to fall within what one would expect as normal intraspecific variation. The fact that both hosts have a rather different ecology, respectively growing in warm limestone grasslands and in marshes has supported the idea of different biological species. However, when looking at the habitats of these plants in central Europe, one will find them more often occurring within a distance of metres, and it is quite possible that populations use both hosts in such localities. Also in Öland both plants can be found in the same localities. Although cases of species with the same barcode exist (van Nieukerken et al. 2012, Bengtsson 2010b), overall the data support the synonymy of both species better than separate identities. A further analysis of nuclear genes, particularly EF1-alpha will be helpful to corroborate or refute this hypothesis.

#### *Stigmella poterii* (Fig. 8i)

Our data support the view that larvae feeding on *Rubus chamaemorus*, *Potentilla erecta* or *Comarum palustre* (= *Potentilla palustris*) are not different. The species was described from *Sanguisorba*, but we have only rarely seen it on that host and do not have any recent material suitable for barcoding. Records from *Sanguisorba* are very often misidentifications for other species, such as *S. anomalella*, *S. centifoliella* or *S. rolandi* (van Nieukerken et al. 2006). In fact a record of *S. poterii* by the senior author from



the Netherlands (van Nieuwerkerken 1982) is now believed to be almost certainly based on mines of *S. anomalella*. There is no reason, however, to doubt that genuine *poterii* from *Sanguisorba* in Britain is conspecific with the *Potentilla* and *Rubus* feeders.

*Stigmella aeneofasciella* (Fig. 8d)

Likewise *S. aeneofasciella* is known from *Agri-  
monia* and *Potentilla*, and, as expected, our data show that the barcodes of these do not differ.

### General discussion

European species, as currently recognised in the *Stigmella salicis* and *aurella* groups, are all easily separable by the DNA barcode; none share the same barcodes, except the pairs *S. zelleriella-lappovimella* and *filipendulae-ulmariae*. The first case is clearly shown to be a single variable species, feeding on a number of *Salix* species in the coastal dunes and in the tundra of northern Fennoscandia. The EF1-alpha gene corroborates this as do the other characters. The second case we also consider as a case of synonymy, but the corroboration by a nuclear gene is still lacking, and would be desirable to exclude the possibility of recent speciation, which has not yet resulted in differences in the COI sequence. In contrast, what formerly has been considered to be a pair of two variable species *Stigmella salicis* and *S. vimineticola*, has very divergent DNA barcodes, showing seven very different clusters in Europe, with similar divergence in EF1-alpha for those specimens where this sequence is available. Where other data are available, it is clear that these must represent different biological species. The taxonomy of these needs to be worked out by checking types of *S. salicis* and its synonyms, preferably including the barcodes. More reared material of these species is needed before a full revision is possible. This finding makes *Stigmella* suddenly one of the more diverse insect groups feeding on *Salix* with more than 10 European species (and many more in Asia, see Puplesis 1994), paralleling the genus *Phyllonorycter* which now has nine *Salix*-feeding species in Europe, including also some with rather similar hostplants in northern Fennoscandia (Bengtsson 2010a, Bengtsson & Johansson 2011, De Prins & De Prins 2011). Both genera provide

an interesting model for studying evolution and speciation within one group of hostplants, and in this way could form an interesting counterpart to the very diverse group of nematine sawflies, of which 200 species alone are gall-formers on Salicaceae, but there are also external feeders, catkin feeders and leafrollers on *Salix* (Nyman et al. 2000, Nyman et al. 2006).

In the Rosaceae feeding *S. aurella* group our study corroborates the fact that most species are oligophagous on a number of genera, and in this way resemble many other Rosaceae feeding insects (Huemer 1988a, b).

### Acknowledgements

We are deeply indebted to Ingvar Svensson for many lively discussions on these topics, joint fieldwork and providing specimens to test his hypotheses. We are honoured to be able to publish this in his memory. Roland Johansson (Växjö, Sweden) was the first to realise that *Stigmella salicis* might form a species complex, and kindly shared his thoughts and findings with us. Kees van den Berg (NCB Naturalis, Leiden) assisted us in many ways with rearing, preparation and fieldwork. Per Douwes (Lund, Sweden) is acknowledged for keeping and sending Ingvar's specimens. Leif Aarvik (Ås, Norway), Bengt Å. Bengtsson (Färjestaden, Sweden), Kai Berggren (Kristiansand, Norway), Willy Biesenbaum (Velbert-Langenberg, Germany), Rob Edmunds (Downham Market, UK), Roland Johansson (Växjö, Sweden), Juhani Itämies (Oulu, Finland), Ali Karhu (Viinijärvi, Finland), Ole Karsholt (Copenhagen, Denmark), Sjaak Koster (Lossier, Netherlands), John Langmaid (Southsea, UK) and Paolo Triberti (Verona, Italy) are acknowledged for providing specimens for our study. Many data in France and Italy were obtained during the EU funded EDIT WP 7 project "All Taxa Biodiversity Inventories in the Mercantour/Alpi Maritime natural parks", we thank Marta de Biaggi (Valdieri, Italy) and Marie-France Leggia (Nice, France) for arranging permits. Dick Groenenberg and Frank Stokvis (NCB Naturalis, Leiden) did part of the sequencing work in the NCB molecular facility. We are much indebted to Paul Hebert and the staff of Biodiversity Institute of Guelph, Canada, for conducting sequencing of MM's material through a grant provided by Genome Canada to BIO. MM thanks Petri Hirvonen, Anttoni Mutanen, Nestori Mutanen, Tomi Mutanen and Panu Välimäki for company and help during field trips. Dick Groenenberg (NCB Naturalis, Leiden) and John Langmaid (Southsea, UK) kindly corrected an earlier version of this manuscript.

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### Online supplementary material:

Specimen data of barcoded specimens.

### Sammanfattning

Författarna har analyserat två kritiska grupper av dvärgmalar med svensk eller nordisk anknytning, vilkas larver lever av olika viden *Salix* spp. resp. rosenväxter Rosaceae. I Sverige har flera dvärgmalstaxa av olika skäl behållits som goda arter då osäkerhet har rått om deras artsstatus. Det gäller framför allt artkomplexen *Stigmella zelleriella-lappovimella* och *S. filipendulae-ulmariae*. Genom att undersöka mitokondriegen COI (med 658 baspar) och kärn-DNA (EF1- $\alpha$  med 482 baspar, primer specialdesignat för Nepticulidae) samt genom att jämföra med genitalmorfologi och värdväxtval, har författarna kunnat visa att några av dessa taxa hör till samma art, medan det tycks finnas en del obeskrivna arter inom några andra taxa:

*Nepticula repentiella* Wolff, 1955 och *Nepticula lappovimella* Svensson, 1976 är synonymer med *Nepticula zelleriella* Snellen, 1875, nu i släktet *Stigmella*. Taxonet lappvidevärgmal *S. lappovimella* har i Sverige uppfattats som en nordlig art, förment bunden till lappvide *Salix lapponum*, medan dyndvärgmal *S. zelleriella* har ansetts vara en kustnära art, som är knuten till krypvide *Salix repens* s.l. Material från Sverige, Finland och Holland visar mycket liten variation i DNA (<0.8% i COI och ännu mindre i EF1- $\alpha$ ). *S. zelleriella* och *lappovimella* förekommer dessutom sympatiskt inom ett mindre område i mellersta Finland. Minor är där funna på *Salix repens* och *Salix lapponum* samt hybrider mellan dessa på åtminstone en lokal. Skillnader i minornas utseende kan tillskrivas de olika

bladstrukturerna hos olika viden eller mellan olika individer av samma videart. Morfologiska skillnader i genitalier och fjäll på framvingen bedöms ligga inom den naturliga variationen. Honornas utstickande äggläggningpapiller är anpassade för att lägga ägg på håriga blad, men i sällsynta fall kan även glattbladiga viden väljas, exempelvis grönvide *Salix phylicifolia*.

Komplexet kring sälgdvärgmal *Stigmella salicis* är fortfarande outrett, men DNA analyserna tyder på att gruppen innehåller flera arter, som ännu inte är identifierade. De är i huvudsak bundna till viden med håriga blad såsom sälg *Salix caprea*, bindvide *S. aurita* och gråvide *S. cinerea*. Även här tjänar undantagsvis grönvide *Salix phylicifolia* som värdväxt.

Taxonet *Stigmella vimineticola* är en god art med klart skilt DNA från *S. salicis*, men ett närstående taxon funnet i Norge och först förmodat vara *vimineticola*, representerar uppenbarligen en obeskriven art. Möjligen kan denna finnas också i Finland och Frankrike, då några exemplar därifrån visar samma DNA-profil.

Två exemplar från Åland, funna på *Salix cinerea*, står nära *Stigmella pallidiciliella* Klimesch, 1946 som är känd från ett fåtal länder i Mellan- och Sydeuropa, närmast i Tjeckien. Ålands-exemplarens identitet är ännu oklar och DNA från *S. pallidiciliella* har inte analyserats.

Den art som nyligen har anmälts som nätvidedvärgmal *Stigmella arbusculae* (Klimesch, 1951) från Björkliden i Torne lappmark och som lever på nätvide *Salix reticulata*, finns troligen också i Finland, då exemplar som påminner om denna är funna i Finska Lappland, där den också tycks kunna leva på odonvide *Salix myrtilloides*.

Osäkerhet har rått om brudbrödsdvärgmal *Stigmella filipendulae* (Wocke, 1871) och älgörtsdvärgmal *S. ulmariae* (Wocke, 1879) är en eller två arter. COI analyserna visar att dessa taxa med största sannolikhet är synonymer. Tyvärr har kärn-DNA ännu inte kunnat verifiera detta. Både på Öland och i Mellaneuropa kan man finna populationer av dessa "arter" på brudbröd och älgört på bara några meters avstånd från varandra. Dessutom är det typiskt att dvärgmalar som lever på växter inom Rosaceae är oligofaga.

Fingerörtsdvärgmal *Stigmella poterii* (Stainton, 1857) har tidigare varit skild från en närstående art, *Nepticula tengstroemi* Nolcken, 1871, som är bunden till hjortron *Rubus chamaemorus*. De flesta författare har under senare tid ansett dem vara synonymer. Undersökningar har nu definitivt visat att *poterii* är en oligofag art vars larv lever i bladen av hjortron, kråklöver *Comarum palustre* och blodrot *Potentilla erecta*. Den kan undantagsvis även leva på *Sanguisorba*.