Description of immature stages of *Nemophora bellela* (Walker, 1863) (Lepidoptera: Adelidae)

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Little life history data is available on the larvae of Adelidae (Lepidoptera, Adeloidea). We provide information on the life history of *Nemophora bellela* (Walker, 1863), a circumpolar adelid species occurring in northern Europe on peat bogs and open tundra with *Betula nana*. The habitat is described and details of the larval behavior and larval diet are provided. The later instar larvae are case dwelling and feed on the ground on detritus. The larval case and chaetotaxy are described in detail. The female pupal exuviae is described. Our observations on the life history of *N. bellela* are in general agreement with the known details of the life history of related species, but some differences were also observed.

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1. Introduction

The non-ditrysian lepidopteran superfamily Adeloidea consists of 583 described and an estimated several hundred undescribed species that belong to six families: Cecidosidae, Prodoxidae, Tridentaformidae, Incurvariidae, Heliozelidae and Adelidae (Nieukerken *et al.* 2011, Regier *et al.* 2015). Little is known about the life history of Adelidae. Early instar larvae of most species of Adelidae are likely miners. Later instars regularly construct a flattened, oval case made of dead plant material (Heath & Pelham-Clinton 1976). Of the North European species of *Nemophora* (Hoffmannsegg, 1798), the life history of *N. amatella* (Staudinger, 1892) and, until now, *N. bellela* (Fig. 1) have remained almost completely unknown; even the larval cases had not been described previously. The former species occurs, sometimes in large numbers, in taiga forests with *Vaccinium myrtillus* L., while the latter is associated with open peat bogs and tundra habitats with *Betula nana* L. *Nemophora bellela* is the only species of *Nemophora* occurring in tundra habitat in northern Europe. Adults are diurnal and are flying relatively early in the summer, most often in June in northern Finland.

In July 2009, the authors J. I. and R. L. had an

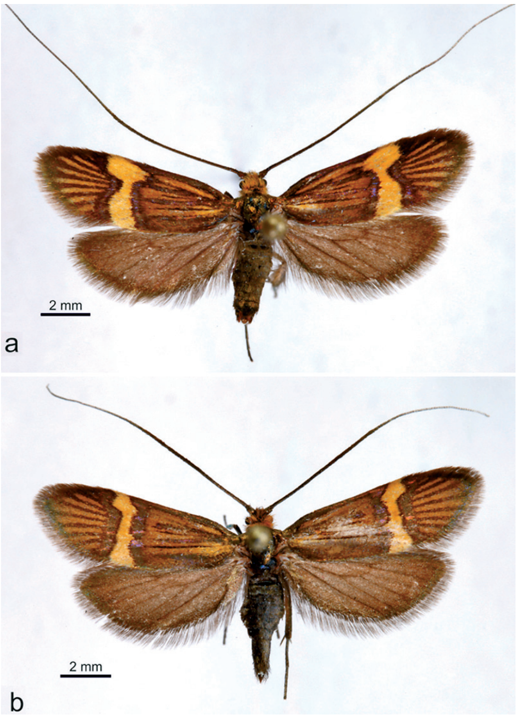

Fig. 1. Adult habitus of *Nemophora bellela*. – a. Male. – b. Female.

opportunity to collect insects in remote fields of NW Finland (EnL: Enontekiö, Doskalharji, WGS84 coordinates: 69.186 N 21.528 E). When trying to find larvae of arctic *Xestia* moths, they carefully examined the ground under clusters of *Empetrum nigrum* L. plants growing on stony soil. During these efforts they found three odd-looking adelid larval cases, believed to be those of *N. bellela*, which is the only adelid occurring in the area. Rearing attempts, however, failed to yield any adults. After four years (2013), they visited the area for a second time and concentrated on searching for more larval cases. This was successful and resulted in the discovery of 23 cases. The following spring, a female of *N. bellela* emerged from one of the cases. The identity of the species had also been confirmed by DNA barcoding of another larva. The DNA barcode of *N. bellela* is unique and differs by K2P distance of 1.5% from its closest relative, *N. amatella*.

In this article, we provide details on the habitat and larval behavior of *N. bellela* in the field. We continue by describing the larva and the larval case as well as the pupal exuviae and discuss our findings in the light of observations done in other species of Adelidae.

2. Materials and methods

Larval cases were visually searched on the ground in a suitable habitat. For rearing, the larvae were placed in a 10 l plastic bucket with living *Empetrum,* dead leaves of *Betula nana* and *Vaccinium uliginosum* L. as well as *Betula pubescens* Ehrh. The bucket was covered with air-penetrating fabric and was kept outside in Oulu (~500 km south of the collection locality) during the autumn and winter.

For larval description, we examined two last instar larvae. One of the larvae examined was removed live from the case in late autumn when hibernating. The second larva was removed from the case the next spring. Little variation was observed between the larvae. For describing the larvae, we used Gerasimov’s (1952) and Werner’s (1958) definition of setal arrangement for the prothoracic plate and ninth abdominal segment. According to this interpretation, prothoracic setae D1 and D2 arise on the middle of the plate behind XD1 and XD2 setae. In contrast, Hirowatari (2001) interpreted Werner’s seta XD1 as D1, D2 as D1 and D2 as SD2. In addition, the number of L and SV setae differ on abdominal segment 9. Werner has setae L1 + L2 and SV1 + SV2 but Hirowatari used the arrangement L1 + L2 + L3 and only one seta SV1.

3. Results

3.1. Field observations of *N. bellela* larvae and laboratory rearing

The habitat where larval cases of *N. bellela* were found represents typical lower arctic-alpine terrain. The habitat consists of nearly open tundra with single mountain birches (*Betula pubescens* ssp. *czerepanovii* (N. I. Orlova) Hämet-Ahti) (Fig. 2). Dominant plants are *Betula nana*, *Empetrum nigrum*, *Vaccinium uliginosum*, *V. myrtillus*, *V. vitis-idaea* L., *Salix herbacea* L. and *S. reticulata* L. In Finland, *N. bellela* also occurs, though more sparsely, in the subarctic and boreal

Fig. 2. Habitat of *Nemophora bellela* in Enontekiö, NW Finland.

Fig. 3. Larval case of *Nemophora bellela* (shown with white arrow) in its natural habitat.

Fig. 4. Pupal case of *Nemophora bellela* from lateral (top) and ventral (bottom) view.

Fig. 5. Variation among full-grown cases in *Nemophora bellela*.

zones on open peat bogs with dwarf pines (*Pinus sylvestris* L.).

At first, the larvae were searched more or less haphazardly on the ground below patches of *Empetrum* growing on stones. Later, it was noticed that larvae tend to gather at the tops of small sandy hills rising one to three meters above the surrounding terrain. The cases were often found under *Empetrum*, but also under twigs of B. nana and *V. uliginosum* lying on the ground. Some cases were found on bare sand or gravel (Fig. 3). Sometimes one end of the case was slightly buried in sand, but with most of the case being still visible. In the field, feeding behavior was not observed. In captivity, the larvae fed on various dead leaves. In a relatively small rearing jar with high density of larvae, they also ate fresh leaves of *B. nana* and *V. uliginosum*. After overwintering, there were nine cases in the rearing container that appeared to be full-grown and four in which the larvae had likely died during overwintering. Some larvae possibly died as a consequence of other larvae feeding on parts of their cases in the rearing bucket. One female specimen of *N. bellela* emerged after overwintering.

3.2. Description of the larval case

The length of full-grown cases (n=9) varied from 16.5 mm to 23 mm, the average being 19.5 mm. The case is relatively narrow and long (Figs. 4 and 5) and composed of several small fragments of dead leaves. The number of fragments varied from 9 to 13, the average being 10.3. Frequently, the dorsal and ventral sides of the cases were constructed of a slightly different number of leaf fragments. Compared to the case of *N. degeerella* (Linnaeus, 1758) (see Itämies 2013), the case is considerably longer (9 mm in *N. degeerella*), more elongated and made of a greater number of plant fragments than that of *N. degeerella* (which is made of 5 leaf fragments), but otherwise the case structure is similar.

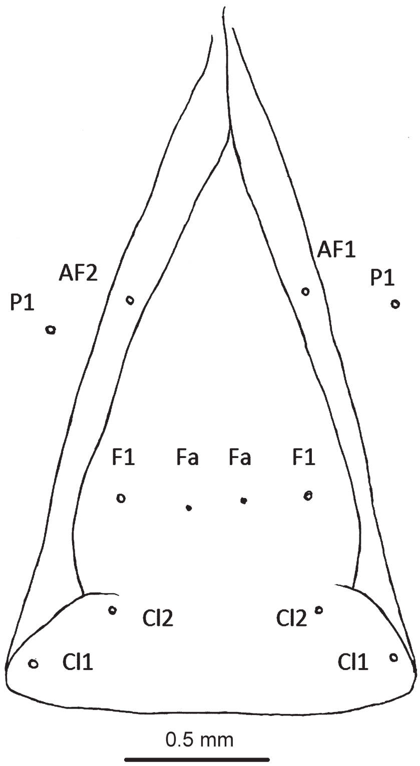

Fig. 6. Chaetotaxy of frontoclypeal area of head of *Nemophora bellela*.

3.3. Description of the last instar larva

The rudimentary prolegs of adelid larvae, including *N. bellela*, are unusual in being reduced with transverse rows of crochets situated midventrally. Most adelid larvae differ from larvae of other families in having fused thoracic coxal plates at least on the prothorax, seta AF2 absent on adfrontal sclerite on the head, and the presence of multiserial crochets on segments AIII–VI instead of prolegs, which are absent on AX. Crochets are arranged in several transverse rows that gradually decrease in size from the center (Werner 1958, Davis in Stehr 1987). The only described larva of *Ceromitia* is that of *C. tubulifolia* Parra and Ogden, 2011) which indicates moderately well-developed prolegs with uniordinal, circular crochets present in this genus.

Morphology of last instar *N. bellela* larva: Head prognathous, adfrontal suture terminating at epicranial suture. Six stemmata present, arranged in an uneven circle. Body dorsoventrally flattened, tapering caudad, all segments medially swollen and contracted between segments. Thoracic plates brown, present on segments T1–3; prothoracic plate large including all setae of XD, D, and SD groups. Meso- and metathoracic plates smaller, divided into three parts around D, SD and L setae. Segment AX with dorsal and ventral anal plates; thoracic legs present, prothoracic coxae fused together. Colour of larva whitish, head and plates brown.

Chaetotaxy of *N. bellela* larva, schematically depicted in Fig. 7: The names of the setae follow Hinton (1946). Setae of frontoclypeal area on head as in Fig. 6. Distances between setae Cl2–Cl2 and F1–F1 similar; distance between F1–AF1 about 2 × F1–Cl2; P1 adjacent to and slightly ventrad to AF1. Seta D1 situated on level with XD2; setae SD1 and SD2 in vertical line with XD2; spiracle separate from pronotum. Setae SV1 and SV2 on common pinaculum on prothorax. Seta V1 not found on thorax, position of setae D1, D2, SD1 and SD2 on meso- and metathorax close to frontal margin of segments,

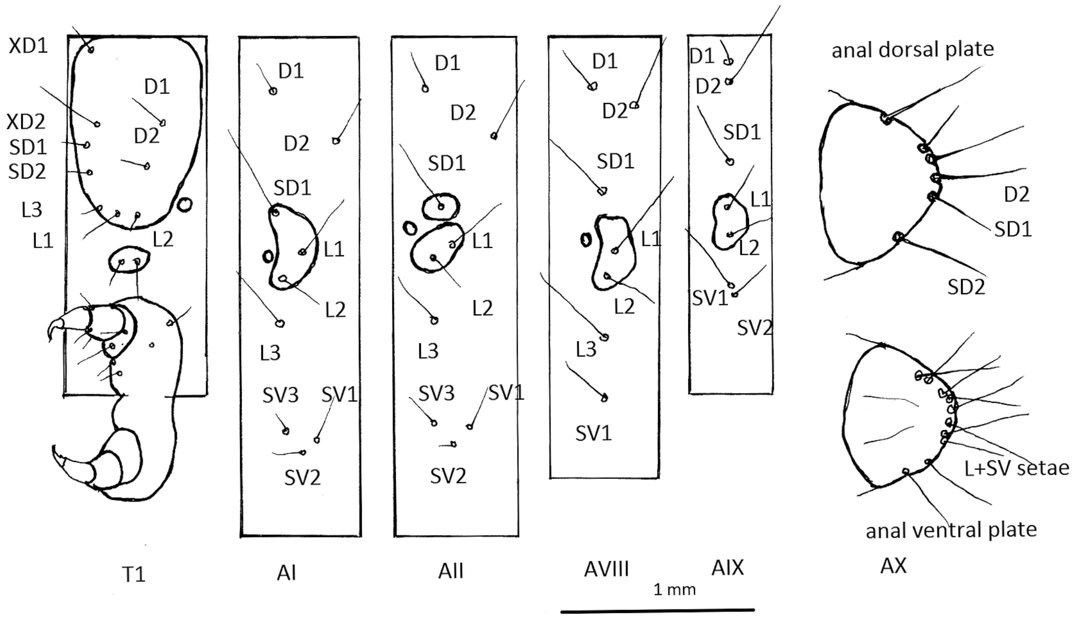

Fig. 7. Chaetotaxy on six segments of body of *Nemophora bellela*. Abbreviations: AI–AX = abdominal segments, T1 = prothorax. Segments not depicted are fully repetitive and would not provide any additional information of larval chaetotaxy.

and all three L setae on united pinaculum. Position of seta D2 on segments AI–AVIII caudad and more ventrad than D1. Setae SD2 and V1 absent on abdomen, two L setae present on AIX, position of setae SD1, L1 and L2 on AI–AII caudad of spiracle on common pinaculum; SD1 separate on other segments. Anal dorsal plate with three pairs of setae, apparently D1 absent; anal ventral plate with five pairs of L and SV setal groups.

3.4. Description of the pupal exuviae

Only one female pupal exuviae of *N. bellela* was examined in this study, for which reason a full description of the pupal characters cannot be given. The general pupal morphology seems to agree with characters provided by Patočka & Turčáni (2005) for Adeliidae. According to these authors, uniserial rows of spines on the 3rd to 7th tergites and maxillae extending approximately to the level of the forelegs are characteristic of *N. degeerella* among Adelidae. The same characters hold true for *N. bellela*. The antennae of the single female exuviae examined are long, extending to the eighth abdominal segment and then curved dorsally to wrap around the abdomen. The shape of the labrum and the shape of labial palpi differ to some extent as well (Fig. 8). Moreover, there seems to be a difference in the location of SV setae on the 6th tergite, the pair of SV1 in *N. bellela* being situated closer to the stigma than those in *N. degeerella*. The cremasters are distinctly different, but the pupae compared represent opposite sexes, which explains this difference (Patočka & Turčáni 2005). In *N. bellela* the female cremaster (Fig. 7) bears a single pair of minute spines arising from the extreme caudal-lateral margins of abdominal segment 10.

4. Discussion

In this paper, we provide new data on the life history of Adelidae. Details of their early life history are generally scanty and are known for only a few species. *Nematopogon metaxella* (Hübner, 1813) (Nematopogoninae) has been reported laying eggs on various herbaceous plants, and on hatching, the larvae drop to the ground where they construct a case and feed on living and dead leaves (Chretién 1894). Kuroko (1961) reports *Nemophora raddei* (Rebel, 1901) inserting eggs into the ovaries of *Salix sieboldiana* Blume, where

Fig. 8. Pupal exuviae of female *Nemophora bellela*.

larvae initially feed, and later descend to the ground and begin feeding on dead leaves from an oval case. Various, partly conflicting information on host plants for many other European species is reported in the literature (e.g. Emmet 1988, Bengtsson *et al.* 2008), but detailed descriptions are scarce. Larvae of the African *Nemophora acaciae* Agassiz & Kozlov, 2015 have been reared from the flowers of *Acacia* spp., but details of its life history remain unclear (Agassiz & Kozlov 2015). Several European species of *Nemophora*, including *N. minimella* (Denis & Schiffermüller, 1775), *N. cupriacella* (Hübner, 1819) and *N. metallica* (Poda, 1761), have been reported feeding on Dipsacaceae, first in flowers, later on the ground from a larval case (e.g. Huismann *et al.* 2009). In Finland, larvae of *Nemophora cupriacella* have frequently been found feeding on living leaves of *Succisa pratensis* Moench near the ground in the spring. Early instar larvae of *Nemophora violellus* (Denis & Schiffermüller, 1775) feed in flowers of *Gentiana pneumonanthe* L. (Gentianaceae) (Huismann *et al.* 2009). *Nemophora fasciella* has been reported feeding on *Ballota nigra* L. (Emmet 1988) and *N. congruella* (Fischer von Röslerstamm, 1840) and *N. ochsenheimerella* (Hübner, 1813) on needles of Norwegian spruce (*Picea abies* L.) (Bengtsson *et al.* 2008).

Itämies (2013) described the larval case and details of the life history of *N. degeerella*. The early life history of *N. degeerella* remains unknown, but after overwintering, the larva gnaws holes in various dead leaves on the ground. Our observations on the life history of *N. bellela*, including the fact that the later instars of the larvae are case dwellers feeding on dead plant material on the ground, agree with those observed in other species. For example, we still do not know on which plant the eggs are laid and whether the early larvae are miners of living plant tissues as is the case with all other species of Adelidae for which respective information is available. For the initial food plant, *Betula nana* is a good candidate since it always occurs in the habitat where *N. bellela* can be found.

The phylogenetic affinities of Adelidae with respect to other lepidopteran families, as well as those between the adeloid groups, have recently been studied in several connections (Mutanen *et al.* 2010, Regier *et al.* 2013, 2015, Heikkilä *et al.* 2015). According to these investigations, the sister group of Adeloidea is the South American superfamily Andesianoidea. Regier *et al.* (2015) found the monophyly of Adelidae strongly supported in nt123 data, but paraphyletic in degen1 data with respect to Heliozelidae. They concluded that "the weight of the evidence favours adelid monophyly". Phylogenetic affinities within Adelidae are in great need of clarification and detailed information of their early stages provides valuable information for that.

Larval and pupal characters of *N. bellela* are in good accordance with those observed in other species of Adelidae, supporting the integrity of the family in respect to Heliozelidae, which in turn differ in some respects of larval and pupal morphology. Gerasimov (1952) and Werner (1958) described the larva of *N. degeerella* based on following characters: Seta D1 of prothoracic plate located at level of XD2, seta SD1 of abdominal segment 8 dorsad to spiracle and seta L1 ventrocaudad from spiracle and seta L2 directly ventrad from spiracle. These characters agree with *N. bellela*, which differs in the frontoclypeal setae. Setal distance between setae F1–F1 is the same as Cl2–Cl2 while in *N. degeerella* F1–F1 distance is longer than Cl2–Cl2.

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