Morphology of caterpillars and pupae of European *Maculinea* species (Lepidoptera: Lycaenidae) with an identification table

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Of the four recognized *Maculinea* species that occur in Europe, three (*M. teleius*, *M. nausithous*, and *M. alcon*) are found on damp meadows, and may co-occur; sometimes their larval instars even occupy the same host ant nest. It is, therefore, important to be able to distinguish between the caterpillars of these species for effective conservation. We present the morphology of the larvae and pupae of these three species, and a simple key to their identification. Inter-specific differences among larvae and pupae, and within-species differences among larval instars, are underlined in order to enable their proper identification. The length, colour and distribution of bristles are considered the best features for species and instar identification. The morphology of the described species is compared with that of the other European species, *M. arion*.

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1. Introduction

Maculinea species are parasitic myrmecophilous butterflies in the family Lycaenidae. In Europe, the genus *Maculinea* includes four species, all of which are considered vulnerable or threatened. *M. arion* Linnaeus, *M. alcon* Denis & Schiffermüller and its ecological race *M. rebeli* Hirschke, *M. teleius* Bergsträsser and *M. nausithous* Bergsträsser. The distinction between *M. alcon* and *M. rebeli* has been suggested only recently and it is supported by ecological (Thomas *et al.* 1989, Munguira 1989, Munguira & Martin 1993) but not genetic (Als *et al.* 2004, Bereczki *et al.* 2005) differences.

All *Maculinea* species are characterized by an unusual life cycle. The adults lay eggs on specific foodplants. The caterpillars feed on or in the flowerheads of these plants for the first three larval instars. The fourth instar caterpillars leave the foodplant immediately after moulting, and drop to the ground, after which they are adopted by *Myrmica* ants. The larvae remain as fourth instars within the ant nest for approx. 10 or 22 months, during which time they increase their body mass almost one hundred times without moulting (Thomas 1984, Elmes *et al.* 1991). At that period they feed on ant grubs, trophic eggs and ant regurgitations. Pupation also takes place within the ant nest.

The ranges and habitats of three European *Maculinea* species, viz. *M. teleius*, *M. nausithous* and *M. alcon*, show considerable overlap (Wynhoff 1998) and they often occur sympatrically on wet lowland meadows. Females of *M. teleius* and *M. nausithous* oviposit on the same food plant, *Sanguisorba officinalis* L., although there are differences in the position and phenological stage of flowers used: *M. teleius* tends to oviposit on young flowerheads of *S. officinalis* with closed petals, located at medium plant height, whereas *M. nausithous* usually selects relatively old flower buds at the top of plants (Figurny & Woyciechowski 1998, Wynhoff 2001). *M. alcon* oviposits on *Gentiana pneumonanthe* L., although the species is also known to use other food plants of the genus *Gentiana* in other localities (Thomas 1984, Jutzeler 1988, Wynhoff 2001). The pattern of caterpillar co-occurrence in ant nests is even more complex, as in some populations two *Maculinea* species may use the same host ant and can be found together in a single nest (Sielezniew & Stankiewicz 2001, Stankiewicz & Sielezniew 2002).

As a consequence, it is not possible to distinguish between the caterpillars and pupae of these three co-occurring species only based on foodplant or host ant species used. On the other hand, correct species identification within this specialized genus is necessary for accurate studies of *Maculinea* ecology (e.g. host ant specificity, competition between co-occurring *Maculinea* species, population genetic research) and for conservation. The aim of this paper is to present simple morphological features that can be used to distinguish the species and larval instars of juveniles of *M. nausithous*, *M. teleius* and *M. alcon*. We also compare the larval morphology of these three species with the *M. arion* and *M. rebeli*.

The first to fourth larval instars will be referred to as LI to LIV, the larval thoracic segments as TI to TIII and the larval abdominal segments as AI to AIX (following Kitching & Luke 1985; see Fig. 1a).

2. Material and methods

To present simple morphological features that can be used to distinguish the species and larval instars of juveniles of *M. nausithous*, *M. teleius* and *M. alcon* in geographically wide range of occurence, we used material from three locations in Central Europe and Asia. To describe the morphology of larvae feeding on the food plant (LI, LII, LIII and LIV), we gathered material in southern Poland, in Novosibirsk (Russia) and in Mongolia within 2002–2004. To distinguish species of *M. teleius* and *M. nausithous*, we used material from Mongolia, the region where, within *Maculinea* genus, only *M. teleius* were observed as the imago (M. Woyciechowski, unpubl. data). The description of caterpillars found on *S. officinalis* flowerheads in Mongolia presented the control for our observations in other regions. We had 190 caterpillars of *M. teleius* from this region conserved in ethanol (72%). Finally, we identified live caterpillars of *M. teleius* found in flowerheads of *S. officinalis*: 270 larvae from southern Poland and 386 larvae from Novosibirsk region. For *M. nausithous* we described 691 alive caterpillars from southern Poland and 150 from Novosibirsk. To describe the morphology of *M. alcon* we used 10 alive larvae found in southern Poland within *G. pneumonanthe* flowers and 28 larvae conserved in ethanol 72% from Novosibirsk, found within flowers of *G. macrophylla*. Additionally, we obtained morphology of this species using scanning electron micrographs of LIII and LIV of *M. alcon* from Denmark that were compared with those from southern Poland and Novosibirsk. Because the examination of the larvae and pupae of *M. rebeli* revealed no diagnostic morphological features by which they can be distinguished from *M. alcon*, the description of *M. alcon* given here can apply equally well to *M. rebeli*.

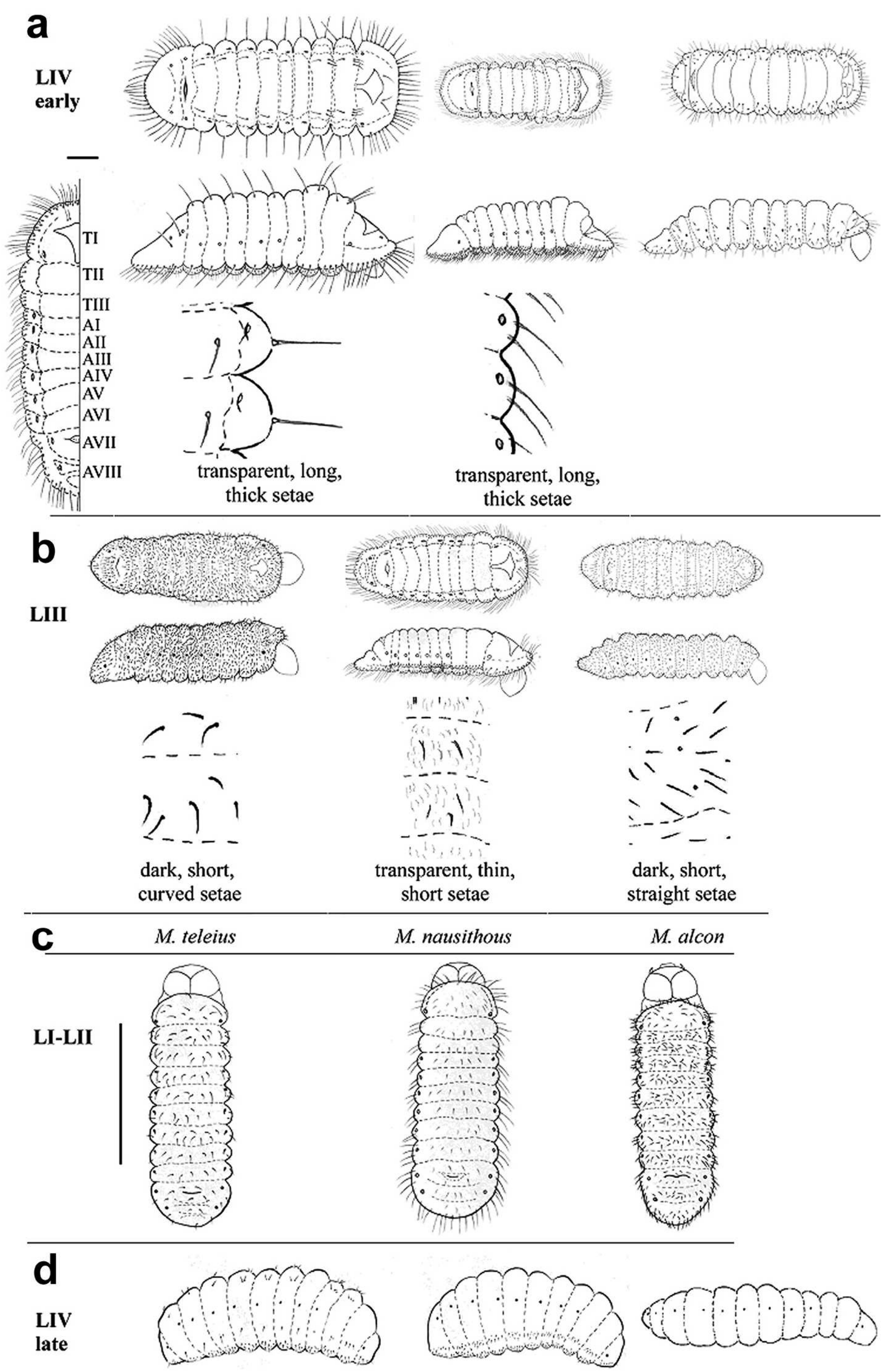

Fig. 1. – a. Dorsal and lateral views of the early fourth instar larvae, with insets showing the distribution and type of setae present on the cuticle, and the scheme used to label larval segments. – b. Dorsal and lateral views of the third instar larvae, with insets showing the distribution and type of setae present on the cuticle. – c. Dorsal views of the first and second instar larvae of three *Maculinea* species. – d. Lateral views of the late fourth instar larvae, showing their characteristic shape. Scale bar 1 mm.

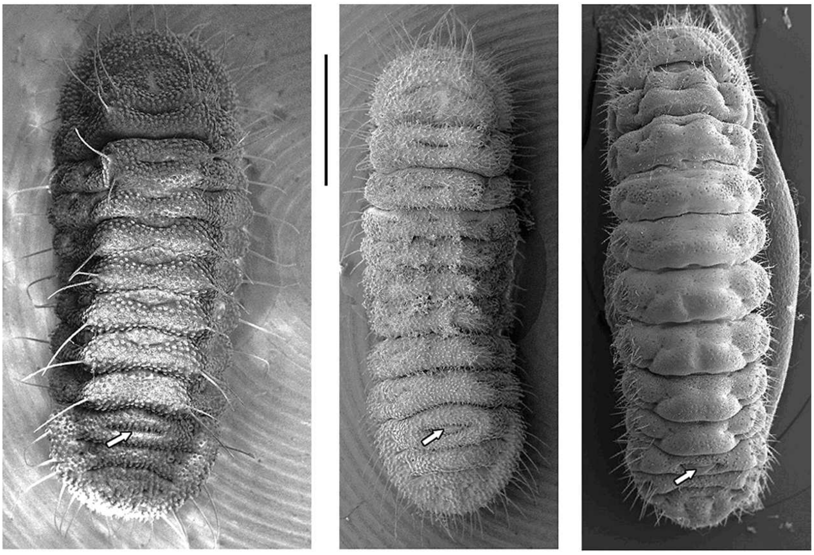

Fig. 2. Scanning electron micrographs of early fourth instar larvae of the three *Maculinea* species. The dorsal nectary organ is indicated with a white arrow. Scale bar 1 mm.

To describe the larval (LIV) and pupal morphology within *Myrmica* ant nests, we used material gathered in Southern Poland. The nests were opened in the field and *Maculinea* caterpillars and pupae taken to laboratory. Next, we described the morphology and when individuals reached the imago stage we identified the *Maculinea* species. We gathered 58 larvae and 54 pupae for *M. teleius*, *M. nausithous* and *M. alcon*. We described morphology of 36 larvae and 41 pupae of *M. teleius* and 4 larvae and 2 pupae of *M. nausithous*. For *M. alcon*, we verified species and described morphology of 18 larvae from the ant nests and 11 pupae.

For observations and measurements we used Nikon microscope SMZ 1500 and Nikon digital camera CoolPix 995. In addition, scanning electron micrographs were used in the questionable cases of species and instar description.

3. Caterpillar morphology

3.1. Ant-associated epidermal organs

The ant-associated epidermal organs of the fourth instar larva of *Maculinea arion* have been described by Kitching & Luke (1985). In short, there is a prominent Dorsal Nectary Organ (DNO) on the seventh abdominal segment (AVII) of the caterpillar, and several Pore Cupola Organs (PCOs) scattered over the dorsal surface of the caterpillar.

This description also holds for fourth instar caterpillars of all other *Maculinea* species. The DNO produces droplets of a sugar-rich secretion, which is sometimes offered to *Myrmica* ants during the adoption of fourth instar caterpillars. The PCOs on the other hand are present on all larval instars, although they are found at the highest density on the fourth instar (Malicky 1969), and it is not clear whether their structure and function is the same for all instars.

3.2. Setae and cuticle

Four types of setae can be found on the cuticle of *Maculinea* caterpillars (Fig. 1a–b): (1) Transparent, short (50–70 µm), thin setae. (2) Transparent, long (100–700 µm), thick setae. (3) Dark, short (50–120 µm), straight setae. (4) Dark, short (50–250 µm), slightly curved setae. The presence and distribution of these provides the best means to distinguish between the different *Maculinea* larvae. In addition, the general body shape and the presence or absence of enlarged segments and folds in the cuticle are useful cues.

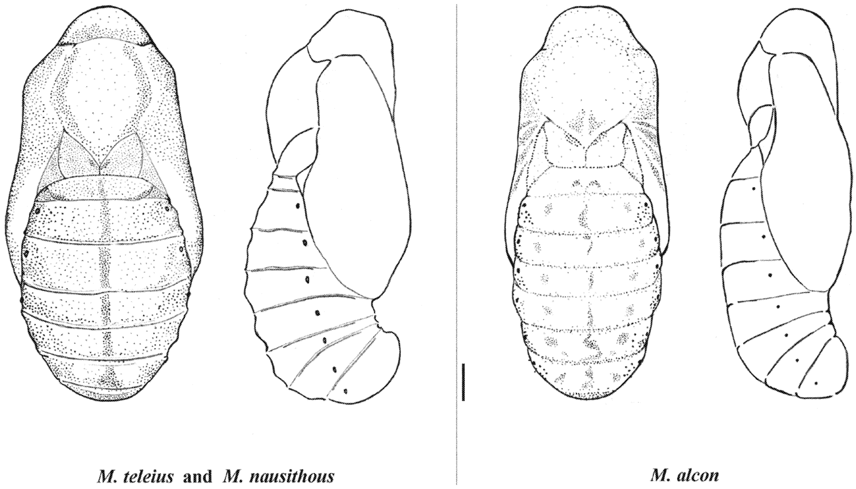

Fig. 3. Dorsal and lateral views of pupae of the three *Maculinea* species. There are no obvious morphological differences between the pupae of *M. teleius* and *M. nausithous*. Scale bar 1 mm.

3.3. Species description

Below we give the characteristics of the larvae of the three sympatrically occurring *Maculinea* species.

3.3.1. M. teleius

LI and LII have setae distributed uniformly over the entire surface (Fig. 1c). They are placed irregularly, with the average distance between them similar to the length of a single seta (50–100 μm). Setae are dark and slightly curved. LIII is generally similar, but the junction between the head and TI is covered by a small cuticular fold (Fig. 1b).

In LIV the setae are transparent, thick and longer than those found on LIII (300–700 μm). They grow sparsely but regularly on the dorsal surface of the thorax and abdomen, and in a fringe around the lateral margin of the caterpillar, with the average distance between setae on a recently moulted larva 200–300 μm (Fig. 1a). A cuticular fold of TI covers about 3/4 of the head, and completely covers the junction between the head and the thorax. As the larval cuticle expands during growth in the ant nest, the density of setae becomes lower. The setae often break off during growth, but their darker-coloured bases remain visible, and the single pair of setae on the dorsal surface of AI to AVII is characteristic of *M. teleius*. The general body shape is initially onisciform (woodlouse-shaped; Fig 1a, Fig. 2), and becomes barrel-like just before pupation (Fig. 1d). The cuticle of this species is highly sculptured (Fig. 2) and appears dull and unreflective.

3.3.2. M. nausithous

LI and LII are covered with short (50–70 μm), transparent setae growing densely on the dorsal surface of the body that are hardly visible, even at 100 x magnification (Fig. 1c). There are also setae that are longer (100–400 μm) in a fringe around the lateral margin and above the head. LIII is generally similar (Fig. 1b) although one or two long setae (200–400 μm) are also sometimes found on the dorsal surface of TII.

LIV of *M. nausithous* generally resembles the previous instar, but with long setae (100–400 μm) occurring in regular clusters along the lateral margins of the caterpillar (from seven to ten setae on TII and TIII, from four to five setae on AI–AVIII; Fig. 1a). Caterpillars of *M. nausithous* in this stage are often much smaller than those of *M. teleius*. After growth in the ant nest, *M. nausithous* caterpillars can be recognised by the presence of several (from four to ten) dark setal bases on each abdominal and thoracic segment along the lateral side (Fig. 1d). The general body shape is again initially onisciform, but more rounded than *M. teleius* (Fig. 1a, Fig. 2), becoming barrel-shaped just before pupation (Fig. 1d). The cuticle is again highly sculpted and unreflective (Fig. 2).

3.3.3. M. alcon

LI–LII of *M. alcon* have dark, short (50–120 µm) and straight setae covering the entire body at high density (the average distance between them is 30–40 µm) and distributed in no clear pattern (Fig. 1c). LIII differs from the earlier instars in the size and shape of segments: The thoracic segments (TI–TIII) are significantly longer and thinner than the abdominal segments, and have clear contractions between each segment (Fig. 1b).

LIV is characterised by transparent short setae (50–120 µm) occurring across the surface of the body (Fig. 1a). The caterpillar in initially cylindrical in general shape, and remains this way until pupation, with clearly visible and distinct body segments (Fig. 1d). The cuticle of this species is particularly smooth and shiny (Fig. 2), and this becomes much more noticeable as the caterpillar develops in the ant nest.

4. Pupal morphology

The pupae of *M. teleius* and *M. nausithous* cannot readily be distinguished from each other. Both are stout, with a thick, dull cuticle (Fig. 3). The pupa of *M. alcon* is more slender, with a transparent, shiny cuticle (Fig. 3). The developing pupa of this species appears yellowish brown. Just before eclosion the wing pattern and colour becomes visible through the cuticle of pupae of described *Maculinea* species.

5. Identification

This section presents the identification of caterpillars living on foodplant, and shortly after adoption, and identification of large caterpillars and pupae found in ant nests. Note that LIII and even sometimes LII may emerge from their food plants under stressful conditions. The section ends with general notes on *M. rebeli* and *M. arion*.

5.1. Larval surface

i. Covered only with short (50–250 µm), dark setae (Fig. 1b)
a) Dark, straight setae, densely distributed, with average distances between them 30–40 µm – *M. alcon* LI–LIII
– Thoracic segments thinner and longer than abdominal – *M. alcon*, LIII
b) Dark slightly curved setae, sparsely distributed, with average distances between them 50–100 µm – *M. teleius*, LI–LIII
ii. Fringed by long (100–400 µm) transparent setae above the legs and over the head (Fig. 1a–b)
a) No enlarged segments – *M. nausithous*, LI–LIII
b) Segment TIII enlarged – *M. nausithous*, LIV
iii. Covered with long (300–700 µm) transparent setae above the legs, over the head and on the dorsal part of the body (Fig. 1a)
a) Segments TIII and AVI enlarged – *M. teleius*, LIV
b) No enlarged segments – *M. alcon*, LIV

5.2. Caterpillar

i. Body barrel-like
– A single seta on the lateral side of each segment from AI to AVIII – *M. teleius*
– Several (from four to ten) setae on the lateral side of each segment – *M. nausithous*
ii. Body elongated, cylindrical – *M. alcon*

5.3. Pupa

i. Pupal cuticle – not transparent, thick, dark, dull – *M. teleius, M. nausithous*
ii. Pupal cuticle – transparent, thin, light-coloured, glossy – *M. alcon*

5.4. *M. rebeli* and *M. arion*

M. rebeli and *M. arion* generally occur in habitats and on food plants that are distinct from the other three European *Maculinea* species, so we have not included detailed morphological features here. The larvae and pupae of *M. arion*, on the other hand, are morphologically distinct from the other species. LI–LIII are covered by short, transparent setae at a medium density (distance between setae is similar to their length), with LIII having some longer setae fringing the body. LIV has sparse, but regularly distributed long, thick, transparent setae, distributed as for *M. teleius*. The general body shape of *M. arion* LIV is also similar to that of *M. teleius*.

6. Discussion

The morphological differences between the different species of *Maculinea*, described here, may reflect different life history strategies employed. The most characteristic differences are in the fourth instar larva, which is the stage at which the majority of interactions between *Maculinea* butterflies and their *Myrmica* ant hosts take place. This instar has highly developed myrmecophilous organs, and many of the differences in morphology of this instar probably reflect variations in the myrmecophilous strategy employed by the different species (Malicky 1969, Kitching & Luke 1985).

Maculinea butterflies develop in nests of *Myrmica* ants following one of two strategies. Predatory species (*M. arion*, *M. teleius* and *M. nausithous*) gain all their nutrition by feeding directly on the ant brood, and generally avoid contact with the worker ants in the nest as much as possible (Thomas & Wardlaw 1992), while "cuckoo" species (*M. alcon* and *M. rebeli*) receive most of their nutrition in the form of food regurgitated by worker ants, and appear to show much tighter integration into the ant colony (Elmes *et al.* 1991). The differences in morphology between LIV for these two groups are striking. The predatory species have an onisciform body shape and possess long, stout setae both of which have been regarded as adaptations to avoid predation or damage to vital organs by worker ants (Malicky 1969). The cuckoo species, on the other hand, are cylindrical in shape and largely unprotected by setae. In addition, they possess a smooth, shiny cuticle, which probably reflects an abundance of cuticular hydrocarbons. These compounds are thought to be important for integration into the Myrmica colony through chemical mimicry of the ant brood (Akino *et al.* 1999, Elmes *et al.* 2002). It is interesting that in the morphological features of *M. nausithous* the typical characteristics of the other predatory species are less well expressed (e.g. sculpted cuticle) and a trend to more cuckoo species characteristics might be found, for example the body shape is more cylindrical than onisciform. While this species seems to be entirely predatory in its lifestyle, it might be much more tightly integrated into the host colony than other predatory species, possibly because it may have evolved to specialize in eating ant eggs (Elfferich 1998).

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