

## Description of the larva of *Mollerella calcarella* (Diptera: Chironomidae) and notes on life-cycle and biotope

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With 14 figures

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**Schlagwörter:** Mollerella, Limnophyes, Chironomidae, Diptera, Niederlande Northwest-Europa, Morphologie, Larve, Imago, Lebenszyklus, Biotop, Biologie, CO1 gen, DNA

*Mollerella calcarella* (Diptera: Chironomidae) is a terrestrial orthoclad, first discovered from the Netherlands (Saether and Ekrem, 1999). The conspecificity of the larva with the known adults is established by means of DNA barcoding of both stages (CO1-gen). The larva strongly resembles *Limnophyes*. The bright blue colour of the living larva, twisted antennal blade more than twice the length of the flagellum, the short basal antennal segment, the 4 inner mandibular teeth, placement of the setae submenti within, instead of below the mentum, the absence of a lateral bulge on the base of the mentum, the small procerus and the shorter anal setae separate the larva from *Limnophyes*. The larvae have been collected mostly in nature reserve areas in seepage zones between higher Pleistocene sands and lower Holocene deposits.

### 1 Introduction

The genus *Mollerella* Saether & Ekrem (1999) includes at this moment three species: *Mollerella calcarella* Saether & Ekrem in western Europe, *Mollerella algerica* (Moubayed, 1989) in Algeria and *Mollerella kaputu* Andersen (2014) in Tanzania.

The male and female can be separated from all other Orthocladiinae with macrotrichia on the wing membrane by the following combination of characters:

- Whole wing covered with macrotrichia
- Wing cuneiform (without anal lobe)
- Costa produced distinctly beyond wing vein  $R_{4+5}$
- Eyes bare
- Antenna with distinct apical seta.

Supplying characters, especially in comparison with *Paraphaenocladus*, are:

- Acrostichals absent
- Middle setae on the scutellum are inserted before the other setae
- Tibial spurs strongly reduced
- Colour darker and hairs denser than in *Paraphaenocladus*
- Male with parallel-sided anal point, slightly spatulate at apex, slanting upwards directed, well visible in side-view.

The pupa resembles that of *Limnophyes* because the posterior margin of the tergites is armed with long narrow spines. It runs to *Chaetocladus algericus* couplet 768 in Langton and Visser (2003). This species belongs to the genus *Mollerella* and is only known from Algeria. *M. calcarella* differs from this species because the posterior spines on tergites III-VIII are longer on each successive segment (Langton 2019).

## 2 Material and methods

In October 2017 the second author collected adults with a sweeping net (Tilburg, The Netherlands Buunderpad, Dutch grid X=13700; Y = 396800) and in November 2018, he also collected 2 larvae (1 in 3<sup>d</sup> and 1 in 4<sup>th</sup> stage) at the same location, with Tullgren method. DNA was extracted and amplified from adult legs or nondestructively from larvae according to standard protocol (van der Broek et al., 2018), using a cocktail of primers LCO1490 and HCO2198 (Folmer et al., 1994), and LepF1 and LepR1 (Hebert et al., 2004) to amplify the COI barcode region. The sequenced male and both larvae proofed conspecific with a 100 % match of the DNA CO1 gen.

The description is based on the sequenced larvae and supported by: HMP 57140 Twijzel July 29 2017 4<sup>th</sup> instar (2); HMP 58126 Tilburg Buunderpad Nov. 17 2018 4<sup>th</sup> instar (1); HMP 58153 Tilburg Buunderpad Nov. 8 2018: 4<sup>th</sup> (1) 3<sup>rd</sup> (8) and 2<sup>nd</sup> instar (2); HMP 59145 's Hertogenbosch, Moerputten Nov. 15 2019: 4<sup>th</sup> (5), 3<sup>rd</sup> (2) and 2<sup>nd</sup> instar (2). The sequenced adult and larvae are, slide-mounted in DMHF, deposited in National Museum of natural History Naturalis in Leiden, the Netherlands (vouchers: RMNH.5012474; L(4) RMNH.5086239; L(3) RMNH.5086240).

## 3 Description of the 4<sup>th</sup> instar larva

Small larvae with a body length of 2.8-3.3 mm. Fresh larvae are blue (fig. 1), but turn pale in ethanol (95 %). Anterior and posterior parapods normal developed. Both with groups of claws. Thorax and abdomen without conspicuous setation. The lateral setae on the abdomen are 20-40 µm long. Procercus (fig. 2) weakly developed, without chitinous parts and with about 6 dark setae (66-117 µm). Supraanal setae dark and appr. 70 µm long. Anal tubules shorter than posterior parapods, without constrictions and slightly attenuated apically.

Head about 0.25 mm long, yellow with a small eye-spot in front fused to the large central eye-spot (fig. 1). Antenna about 1/6 of the head length.

Antenna (fig. 3) 5 segmented (length chitinous parts: 18-8-1.3-4.3-1.3 µm) with a long twisted antennal blade (36 µm long) more than twice as long as the flagellum. Three large ring-organs of which the basal ones, situated in the middle, are higher than wide. The apical one smaller and more circular. Lauterborn organs present but small.

Labro-epipharyngeal region (fig. 4). S1 serrate, S2 and other S setae simple. No labral lamellae. Pecten epipharyngis (fig. 5) with 3 (sometimes 4) simple scales. Premandible (fig. 6) with 2 distal and 1 or 2 proximal teeth. Brush absent or vestigial.

Mandible (Fig. 7) with a long apical tooth and 4 internal teeth. SSD with pointed tip. Seta interna (fig. 8) consists of 3 brushes.

Mentum (fig. 9) With 2 large median teeth and 5 diminishing teeth laterally. The outermost tooth might be hidden beneath the adjacent tooth and thus simulating only 4 lateral teeth. Lateral on the mentum a pit is discernible bearing a blade-like appendage not yet seen in other Orthoclaadiinae (fig. 10). Setae submenti lie well within the mental plate (fig. 11).

## 4 Differential diagnosis of the fourth instar

In the key of Klink and Moller Pillot (2003) the larva keys out to *Limnophyes*, based on:

- Two median mental teeth
- S1 serrate
- Mentum with 5 pairs of lateral teeth
- Beard absent

The following characters set *M. calcarella* larvae apart from *Limnophyes*:

**4<sup>th</sup> Instar** (head length about 0.25 mm)

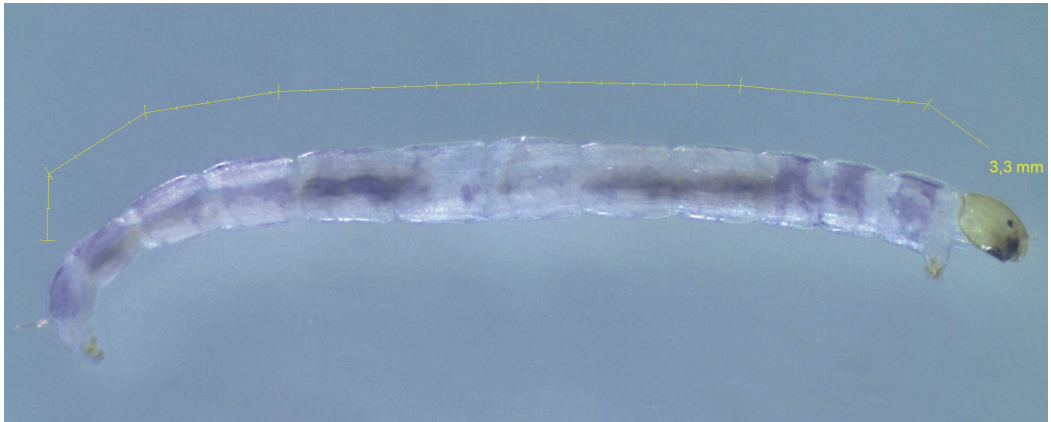
- Living larvae with violet spots on both thorax and abdomen (fig. 1). *Limnophyes* usually has a darker thorax than abdomen, where the greenish, blue or violet streaks are less intense.
- First antennal segment is about 1.5 x as long as broad (fig. 3). *Limnophyes* >2 x.
- Antennal blade twisted and more than twice the length of the flagellum (fig. 3). *Limnophyes*: blade at most slightly longer than flagellum (Saether 1990).
- Mandible with 4 true inner teeth (fig. 7). *Limnophyes*: 3 (sometimes 4) inner teeth.
- Setae submenti lie within the mental plate (fig. 9,11). *Limnophyes*: far beneath the mental plate (fig. 12).
- A lateral pit on the mentum bearing a blade-like appendage (seems unique in Orthoclaadiinae) (fig. 10)
- Bulbous extension on the base of the mentum absent (fig. 10). *Limnophyes*: present (fig. 12).
- Procercus small and anal setae short (66-110 µm) (fig. 2). *Limnophyes*: Procercus large and anal setae longer (150-450 µm) (fig. 13).

**3<sup>rd</sup> Instar** (head length 0.16-0.22 mm)

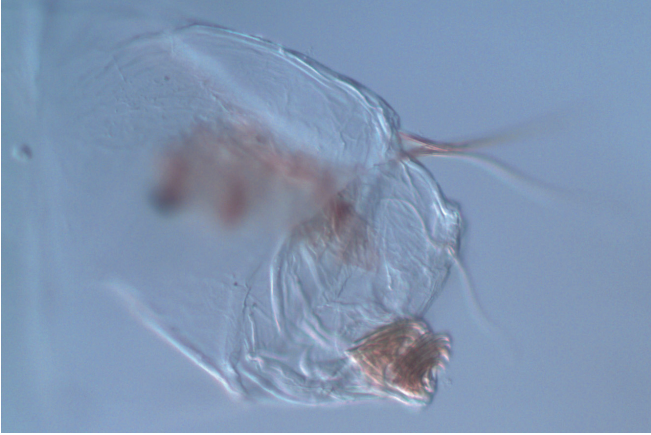
- Characters of mentum, setae submenti and mandible as in 4<sup>th</sup> instar
- Antenna with 1<sup>st</sup> segment barely longer than 2<sup>nd</sup> segment. In *Limnophyes* almost twice as long as the second.
- The other characters are less useful than in the 4<sup>th</sup> instar.

**2<sup>nd</sup> Instar**

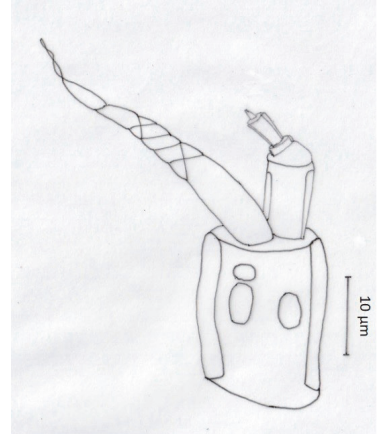
Identification seems unreliable at the moment.



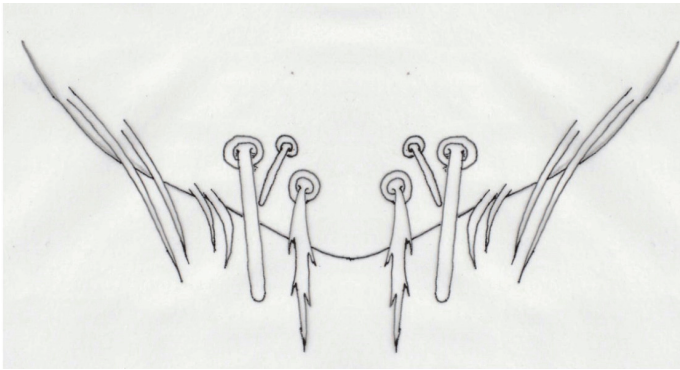
**Fig. 1** *Mollerella calcarella*. Larva overview



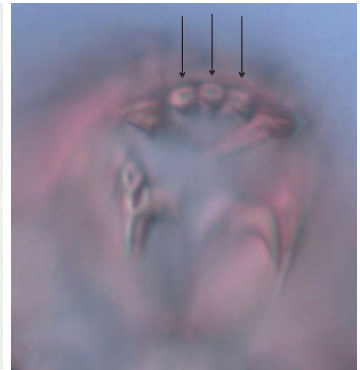
**Fig. 2** *Mollerella calcarella*. Procercus and anal setae



**Fig. 3** *Mollerella carella*. Antenna



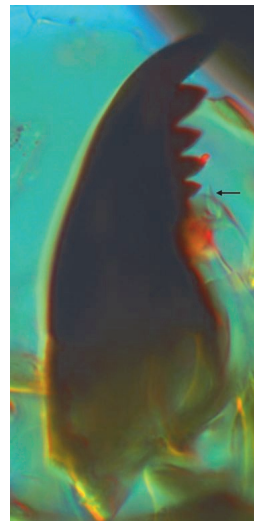
**Fig. 4** *Mollerella calcarella*. Labral region



**Fig. 5** *Mollerella calcarella*  
Pecten epipharyngis



**Fig. 6** *Mollerella calcarella*. Premandible



**Fig. 7** *Mollerella calcarella*. Mandible



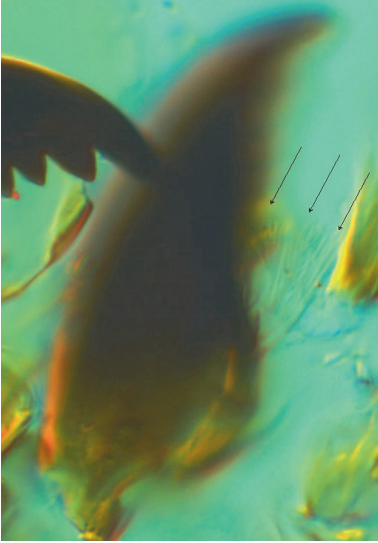


Fig. 8 *Mollerella calcarella*. Mandible with seta interna

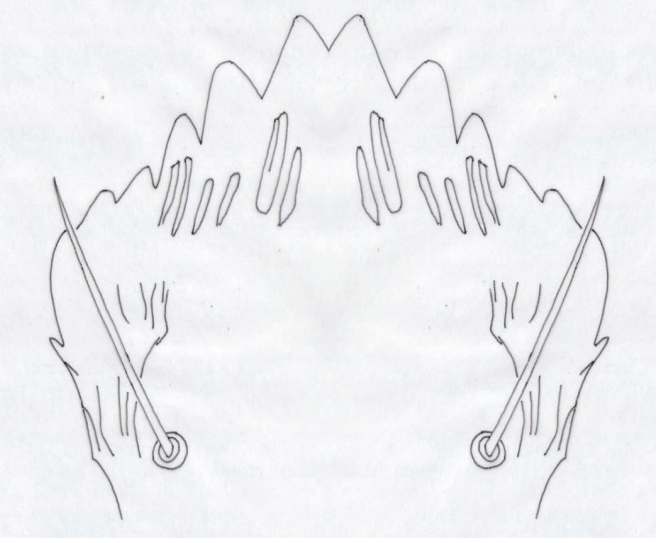


Fig. 9 *Mollerella calcarella*. Mentum

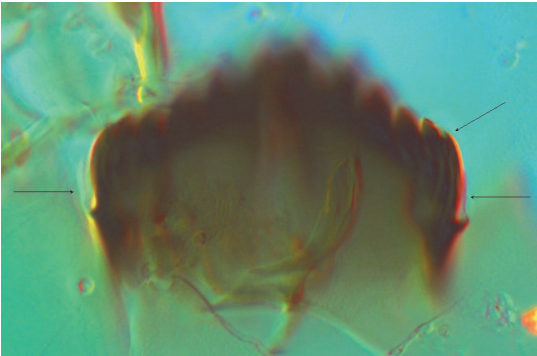


Fig. 10 *Mollerella calcarella*. Mentum lateral appendage

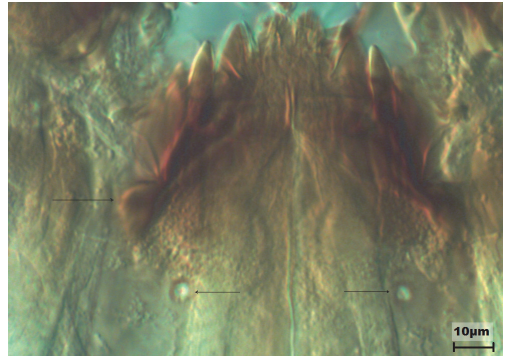


Fig. 11 *Mollerella calcarella*. Setae subdentales

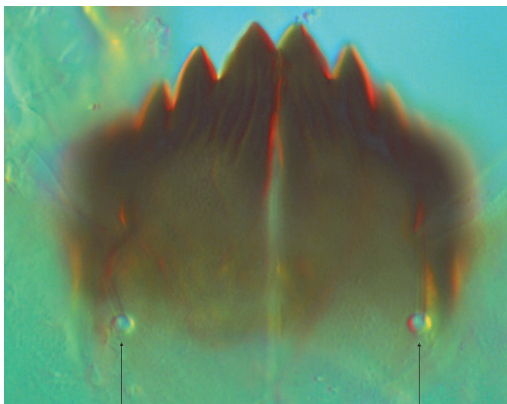


Fig. 12 *Limnophyes*. Mentum with setae subdentales and bulbous lateral swelling

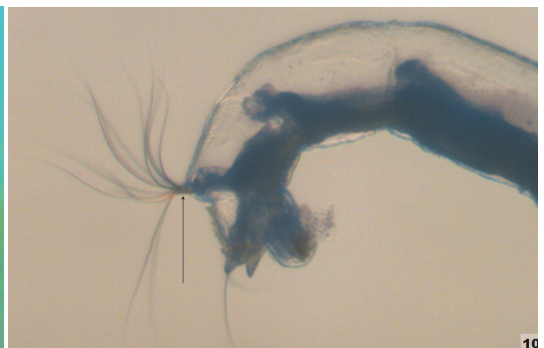
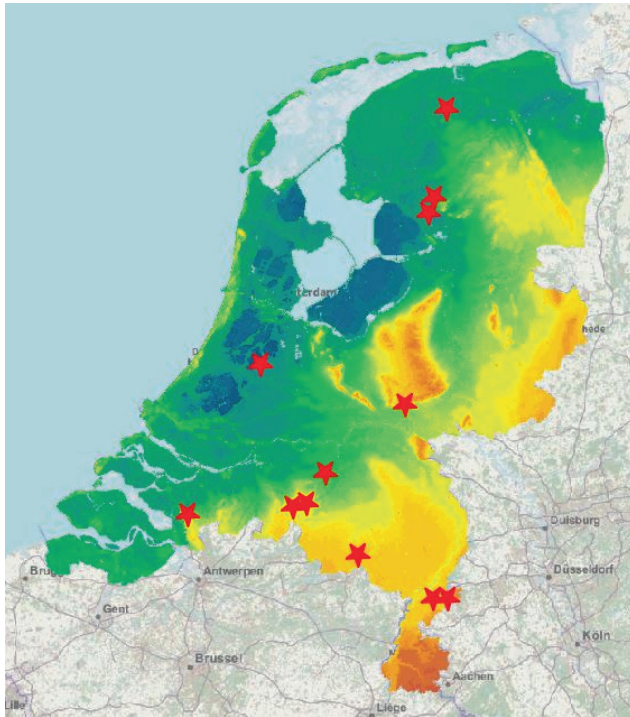


Fig. 13 *Limnophyes*. Procercus and anal setae

Most of the localities where the species was found in The Netherlands and France were woods, usually of alder trees (*Alnus glutinosa*) and gray willow shrubs (*Salix cinerea*). In some cases other tree species were dominant (*Betula*, *Fagus*, *Pinus*). A few localities were edges of small brooks or ponds, half or more shaded. Also *Molleriella kaputu* was caught in Tanzania along a stream in forest (Andersen, 2014). The findings in the Netherlands are projected in figure 14. Of these 12 locations, 7 are situated in a Nature 2000 conservation area, 2 are located in a nature reserve and 3 have no conservation status. Eleven of the 12 locations lay on higher Pleistocene sands, bordering lower Holocene deposits. The exception is a peat excavation landscape, where *M. calcarella* has been collected in a birch-marsh in the “Nieuwkoopse Plassen” area.



**Fig. 14 *Molleriella calcarella*. Distribution in the Netherlands (on Actual Hight Database Netherlands)**

### Acknowledgement

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