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# The legs of “spider associated” parasitic primary larvae of *Mantispa aphavexelte* (Mantispidae, Neuroptera) – Attachment devices and phylogenetic implications

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ABSTRACT

The legs of the primary larva of *Mantispa aphavexelte*, parasite in egg sacks of spiders, were examined using scanning electron microscopy (SEM), histology and confocal laser scanning microscopy (CLSM). The leg morphology is described in detail, including intrinsic muscles. Functional adaptations of the leg attachment devices are discussed, especially regarding the material composition. For example, a sole-like flexible ventral tarsal surface containing resilin is combined with sclerotized pseudo-claws. This likely enables the larvae to cope with surface structures on the spider's body, with substrates on the ground, and also with various structural elements in the spider's nest. The leg morphology is evaluated with respect to phylogenetic affinities. A trumpet-shaped, elongated empodium has likely evolved early in the evolution of Neuroptera and may consequently belong to the groundplan of a large subgroup of the order. It characterizes most groups of the hemerobiform lineage and is also present in the myrmeleontiform Psychopsidae. The presence of a tarsal protrusion resembling a pretarsus confirms the monophyletic origin of Mantispoidea. A single fixed tooth and a specific surface structure are potential autapomorphies of Mantispidae. A distal tibial subunit partly separated from the main part of the leg segment is an apomorphy only described for larvae of *M. aphavexelte*.



## 1. Introduction

Mantispidae are an unusual and conspicuous group of Neuroptera (e.g. Redborg, 1998; Aspöck, 1999), with adults characterized by raptorial forelegs strikingly similar to those of Mantodea (Aspöck et al., 1980; Eggenreich and Kral, 1990; Kral et al., 2000). A characteristic feature shared by the family with Berothidae and Rhachiberothidae is hypermetamorphosis (Redborg and MacLeod, 1985; Aspöck et al., 2001), with highly mobile first instar larvae and scarabaeiform following immature stages (Redborg, 1998), which are partly immobilized and only equipped with shortened and simplified thoracic legs (Aspöck and Aspöck, 2007). Interestingly, Berothidae and Rhachiberothidae were nested within Mantispidae in a recent phylogenetic study (Winterton et al., 2017), rendering this long-recognized family paraphyletic.

Unlike immatures of their close relatives Berothidae and Rhachiberothidae, the agile primary larvae of *Mantispa* are characterized by a very unusual type of behaviour. They enter the egg cocoon of spiders and feed on their offspring (Kaston, 1938; Hungerford, 1939; Viets, 1941; Lucchese, 1955; Rice, 1986; Redborg, 1998; Aspöck and Aspöck, 2007). Three strategies are known in Mantispidae for locating the spiders' egg cocoon (Redborg, 1998): i) spider boarding, this includes a unique phoretic behaviour, here the primary larvae stretch out their legs and reache out to await a passing spider (Hoffmann, 1936; Batra, 1972; Redborg and MacLeod, 1983); ii) active searching for egg-sacs, where larvae are most likely attracted to spider silk and show no interest in adult spiders (Brauer, 1869; McKeown and Mincham, 1948); iii) opportunistic behaviour showing both before mentioned strategies (Redborg, 1998). The latter strategy is known for close relatives of *Mantispa aphavexelte* (Viets, 1941; Redborg, 1985; Rice and Peck, 1991; Hoffman and Brushwein, 1992); this strategy involves dealing with a great variety of substrates, to which the larvae have to attach.

The postcephalic body of scarabaeiform 2nd instar is weakly sclerotized and probably largely immobilized, with distinctly reduced legs (Tauber and Tauber, 1968).

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Pupation takes place within the egg sack of the spider in a cocoon produced by the larva (Brauer, 1869; Redborg, 1998; Aspöck and Aspöck, 2007).

The legs of primary larvae of Mantispidae and Berothidae were illustrated in Minter (1990) as well as in Redborg and MacLeod (1985), but were never examined and documented in detail. The details of the attachment devices were never studied adequately. Furthermore, the material composition of the cuticle, which can play a major role in attachment mechanisms of insects, was never investigated. Its relevance was shown in a number of studies, for instance for the ladybird beetle *Coccinella septempunctata* (Peisker et al., 2013), the orthopteran species *Tettigonia viridissima* and *Locusta migratoria* (Perez-Goodwyn et al., 2006), the stinky bug *Nezara viridula* (Rebora et al., 2018), a wide range of hymenopterans (Gladun et al., 2009), for brachyceran flies (Niederegger and Gorb, 2003) and the avian ectoparasite *Crataerina pallida* (Petersen et al., 2018).

The main purpose of the present study is to present a detailed description of the legs of the primary larva of *M. aphavexelte* (Fig. 1), with a main focus on the attachment system, including the material composition of its cuticle. The results are discussed with respect to the function of the unusual adhesive lobe in the context of parasitism. Finally, the leg morphology is evaluated with respect to its potential phylogenetic significance.

## 2. Materials and methods

All primary larvae of *M. aphavexelte* Aspöck and Aspöck, 1994 were reared from a female collected on 28.08.2012 in Umbria (Italy), south of Monte del Lago, Castello di Zocco, ruderal area with some olive trees and ruins, 260 m (43° 8′9.47″N, 12° 9′57.73″E). The mantispids were determined using the key of Aspöck and Aspöck (1994) by H.P.. Two primary larvae (fixed in Dubosq-Brasil) were embedded in Araldite CY 212® (Agar Scientific, Stansted/Essex, England) and sectioned with a microtome HM 360 (Microm, Walldorf, Germany) equipped with a diamond knife (cross sections and longitudinal sections, thickness 1 μm). The sections were stained with toluidine blue and pyronin G (Waldeck GmbH and Co.KG/Division Chroma, Münster, Germany). Other specimens were dried at the critical point (Emitech K850 critical point dryer), sputter-coated with gold (Emitech K500), and fixed on a rotatable specimen holder (Pohl, 2010); images were taken with a FEI (Philips) XL 30 ESEM at 10 kV.

All specimens of *M. aphavexelte* used for confocal laser scanning microscopy (CLSM) were stored in 70% ethanol. The legs were dissected and embedded in glycerine (99.9%) on a glass slide and covered with a high-precision cover slip prior to scanning. For CLSM visualization a Zeiss LSM 700 (Carl Zeiss Microscopy GmbH) was used. Four laser lines with the wavelengths of 405, 488, 555 and 639 nm and four emission filters of BP420–480, LP490, LP560, LP640 nm were used for visualizing the autofluorescences of the insect's cuticle (cf. Fig. 1 in Büsse and Gorb, 2018). Maximum intensity projections were combined using ZEN 2008 software (www.zeiss.de/mikroskopie) and all images were subsequently processed in Affinity Photo and Affinity Design (Serif Ldt., www.affinity.serif.com). For more information on using CLSM to determine the material properties of the insect cuticle, we refer to Michels and Gorb (2012).

The maximum intensity projections of the CLSM analysis result in visualization of combined autofluorescence signals in every single pixel and provide information about the presence of various components with different material properties of the cuticle (Andersen, 1979; Vincent, 2002; Michels and Gorb, 2012). It is therefore possible to estimate the material composition of the analysed cuticle. The following colour code can be assigned: (1) red autofluorescence – tough and sturdy, sclerotized cuticle; (2) blue, green and red combined, resulting in pink, brownish, yellow and green autofluorescence within the overlay - tougher but more flexible cuticle if compared with the previous one, chitin and/or resilin dominated; (3) characteristic blue autofluorescence - rubber-like cuticle, high proportion of resilin. The results of the CLSM analyses described in the following section allow for a qualitative assessment only and do not represent a quantitative measurement.

Additional material:

*Osmylus* sp. (final instar larva, fixed in 70% ethanol, Tabarz, Mühlbach, Thüringen, Germ., coll. R. Bellstedt)
*Sisyra* sp. (final instar larva, fixed in 70% ethanol, Georgenthal, unterster Klosterteich, Thüringen, Germ., coll. R. Bellstedt)

## 3. Results

### 3.1. General leg morphology

Three well-developed and similar pairs of thoracic legs are present in the 1st instar larvae (Fig. 1). The cuticular surfaces of coxa, trochanter, femur and parts of the tibia display varying patterns of

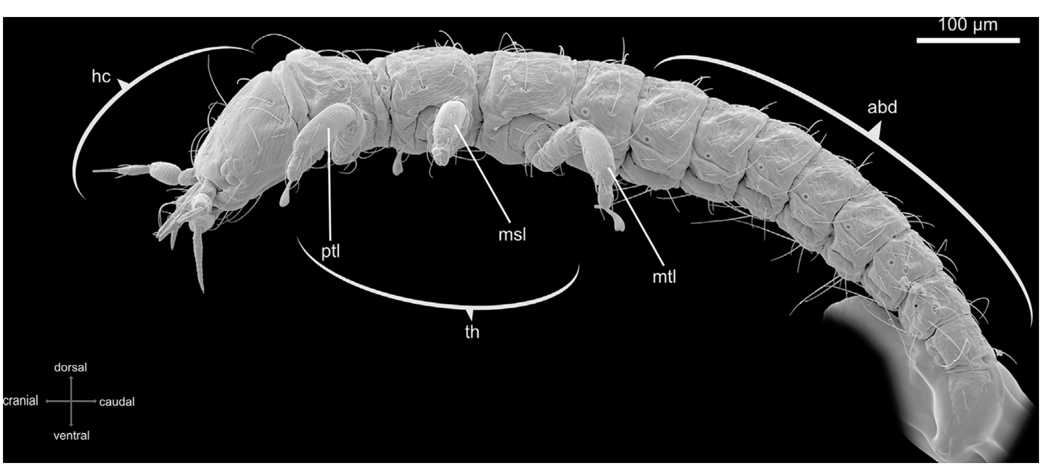


**Fig. 1.** SEM micrograph of *Mantispa aphavexelte* showing the entire primary larva. The lateral overview shows the position of the legs and the general configuration of the thoracic segments and other body regions. Abbreviations: abd – abdomen; hc – head capsule; msl – mesothoracic leg; mtl – metathoracic leg; ptl – prothoracic leg; th – thorax.

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