



## The immatures of lauxaniid flies (Diptera: Lauxaniidae) and their taxonomical implications

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### Abstract

The immature stages of insects can provide valuable data both for taxonomy and phylogeny, but they are well known only for negligible proportion of the described species. Here we describe lauxaniid immatures for 17 species that were reared under laboratory conditions and subjected to morphological investigation. Following species were included in our study: *Cnemacantha muscaria*, *Homoneura biumbrata*, *Homoneura limnea*, *Minettia austriaca*, *Minettia fasciata*, *Minettia flaviventris*, *Minettia loewi*, *Minettia plumicornis*, *Peplomyza litura*, *Poecilolycia vittata*, *Pseudolyciella pallidiventrifera*, *Sapromyza apicalis*, *Sapromyza hyalinata*, *Sapromyza intonsa*, *Sapromyza sexpunctata*, *Sapromyzosoma quadripunctata*, *Sapromyzosoma quadricincta*. SEM images of the eggs are provided along with the illustrations of the cephaloskeleton and brief description of all three larval instars. The cephaloskeleton, as well as external morphology suggest that subgenus *Minettia* s. str. may not be monophyletic. Species *Sapromyza sexpunctata* and *Sapromyzosoma* spp., *Pseudolyciella pallidiventrifera* and *Poecilolycia vittata* are probably closely related. *Sapromyza apicalis*, *S. hyalinata* and possibly also *S. intonsa* form a separate clade from the previous group. These results clearly support the long-standing suspicion, that genus *Sapromyza* is not monophyletic. *Sapromyza sexpunctata* should be considered a separate genus related to *Sapromyzosoma*.

The spines on dorsal surface of labial lobe suggest relationships between *Peplomyza* and *Meiosimyza* species. Affinities of *Cnemacantha muscaria* remain uncertain. However, the extended Malpighian tubules suggest relationship to *Homoneura* or *Minettia*.

**Key words:** Diptera, Lauxaniidae, egg, larva, morphology, cephaloskeleton, SEM

## Introduction

An accurate taxonomy is a precondition for testing hypotheses about past history events, reconstructing ancestral character states, estimating the timing of historical events etc. Molecular characters have many advantages and the number of studies using molecular characters for estimation of phylogeny is growing rapidly (Pagel 1999). The number of morphology based studies is growing as well. However, only minor publications use also immature morphology, praxis advocated by Hennig (Meier & Lim 2009). Recent papers, e.g. Beutel *et al.* (2010), demonstrate that morphological characters represent a vital source of data in insect systematics. Except for the imagines, the holometabolous insects offer an extra source of phylogenetic data: the immatures (Meier & Lim, 2009). The pupal stage allowed the larval and mature stages to evolve independently. This line of research in Diptera was started by Willi Hennig and his “Larvenformen der Dipteren” (Hennig, 1948, 1950, 1952). Several recent studies have shown, that the immatures of holometabolous insects bear considerable amount of information (reviewed by Meier & Lim 2009), e.g. in the phylogenetic study of the butterfly family Nymphalidae (Freitas & Brown 2004) the adults were shown to be the main source of conflict. The immatures, by the definition, cannot be subject to direct sexual selection, which can be the cause of rapid divergence among closely related species (e.g. Arnquist 1998, Kopp & True 2002, Puniamoorthy *et al.* 2010). Consequently, the immatures are more suitable to reveal deeper nodes in the phylogeny (Meier & Lim 2009).

Within the Cyclorrhapha, the larval stages are generally poorly known. The main obstacle is obtaining identified immature stages which usually require rearing. Such rearing efforts have been made for relatively few taxa either because of an interest by specialists (Berg & Knutson, 1978; Meier, 1996, Rotheray & Gilbert, 1999) or because the species were of medical importance or forensic importance. Not even the third instar of *Drosophila melanogaster* had been properly documented until recently (Wipfler *et al.* 2013). Within the fly families where the immatures are known, the information refers often only to the third instar (Teskey 1981). However, in several recent papers Szpila & Pape (2005, 2007, 2008) demonstrated that the first instar may dramatically differ from the later instars, as well as between species.

The family Lauxaniidae represents a species-rich family of acalyptrate flies (up to 2000 valid species, Gaimari in verb). The family is currently divided into three subfamilies (Lauxaniinae, Homoneurinae and Eurychoromyiinae, Gaimari & Silva 2010a). Eurychoromyiinae are restricted to the Neotropics (Gaimari & Silva 2010b), while the next two subfamilies are cosmopolitan. The European fauna is relatively well known, in spite of that new species are described quite commonly. For example Merz (2001, 2003, 2007a, 2007b) described six new European species within years 2001–2007, what demonstrates, how understudied this group of flies is. Systematics of the family is unsatisfactory as well. Subfamily Lauxaniinae is supposed not to be monophyletic, as well as some large genera, such as *Sapromyza* (Shewell 1987, Papp & Shatalkin 1998).

The lauxaniid flies are almost omnipresent in woodland and mesic habitats. Summary of their biology and immature morphology is given by Gaimari & Silva (2010a) and Papp & Shatalkin (1998), original papers were published by Meijere (1909), Hennig (1952), Miller & Foote (1975, 1976), Miller (1977a, 1977b, 1977c), Sasakawa & Ikeuchi (1982, 1983) and Semelbauer & Kozánek (2011, 2012, 2013). Generally, the larvae are phytosaprophagous and are easily reared in laboratory conditions on decaying deciduous tree leaves. The aim of this paper is to provide thorough descriptions of immatures for relatively broad scale of European lauxaniid genera.

## Material and methods

Adult flies were obtained by sweeping the vegetation at several localities in western and northern Slovakia. The key of Shatalkin (2000) and its English translation (Schacht *et al.* 2004) were used for determination. Nomenclature follows Shatalkin (2000). Both males and females were kept in plastic jars filled with moss

*Sapromyzosoma* spp. is supported also by the adult character: black dots on the abdominal tergites. Given that *S. sexpunctata* and *Sapromyzosoma* spp. appear more closely related to *Pseudolyciella* and *Poecilolycia* than to any other species or genera, they should be no longer included in *Sapromyza*. This could be possibly applied also to close relatives of *S. sexpunctata*: *S. opaca* Becker 1895 and *S. zetterstedti* Hendel 1908.

The higher level relationships of lauxaniids remain poorly understood. The inflated Malpighian tubules (+ the calcareous deposit over the puparium) suggest a link between *Minettia*, *Homoneura* and possibly *Cnemacantha muscaria*. This is partly supported by the bumps on laterals of body segments in *M. plumicornis*, *M. loewi* and *Homoneura* spp. Another clade may be composed of *Meiosimyza* + *Tricholauxania* + *Aulogastromyia* and *Peplomyza*. These genera share a smooth body integument, simple spines on the inner surface of labial lobe and egg chorion characters (small pits between the ridges are developed in *T. praeusta*, *M. decempunctata*, *M. affinis* and *M. laeta*). A close relationship between *S. hyalinata*, *S. intonsa*, *S. apicalis* and *Calliopum* + *Lauxania* (Semelbauer & Kozánek 2012) is suggested by several larval features, e.g. the presence of dental sclerites in the first instar (except *S. intonsa*), the cirri-like hairs of labial lobe and the bare puparium (not known in *S. intonsa*) with darkened apical part (only in *S. hyalinata*, *Lauxania* and *Calliopum*, unpublished results). However, the immatures of many additional species will have to be studied before these characters can be fully evaluated. In particular, the larvae of suitable outgroups have to be studied in order to determine character polarities.

## Conclusions

Our results support the non-monophyly of *Sapromyza*, what has been suspected for a long time (Shewell 1987, Papp & Shatalkin 1998). It is likely that genus *Sapromyza* should be sampled much more intensely than was feasible in our study. We conclude that immatures are a very promising source of data in lauxaniid taxonomy and systematics. We believe that future research of larval morphology in combination with cladistic analysis and molecular characters may establish a firm base for the higher-level classification of lauxaniid flies.

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