

**Abstracts of the Immature Beetles Meeting 2007**  
**October 4-5, Prague, Czech Republic**

Martin FIKÁČEK, Jiří SKUHROVEC & Petr ŠÍPEK  
(editors)

The second Immature Beetles Meeting was held during October 4-5, 2007 in Prague as a continuation of a much smaller get-together of people interested in the immature stages of Coleoptera which was organized in Prague in 2006. The meeting took place at the Faculty of Science, Charles University, in cooperation with the National Museum in Prague and the Crop Research Institute in Prague. Altogether 27 participants from Europe and North America attended the meeting, including two pre-graduate students from the Montana State University, USA, and Charles University, Czech Republic.

The official programme consisted of 15 short lectures whose abstracts are presented below in alphabetical order. Coffee breaks and a joint lunch in a nearby restaurant provided a handy opportunity for informal discussions of the lectures and individual research projects and interests of the participants. During both evenings, most people continued in a slightly more relaxed setting in a pub over a glass (or perhaps two) of Czech beer.

To sum up, the meeting seemed to be very fruitful. Vasily Grebennikov wrote us in an e-mail just after the meeting: 'the meeting was large enough to be called a conference, and small enough to meet people in a family manner and talk to them, i.e. very good in both professional and personal dimensions', and we sincerely hope that everyone else thought favourably of it as well!

All participants agreed that the IBM meetings should be held regularly at two year intervals. The next one is planned for the autumn of 2009. Details about the forthcoming meeting will be available on the Immature Beetles Meeting web pages at [http://web.natur.cuni.cz/zoologie/entomologie/meeting/IBM\\_2007.htm](http://web.natur.cuni.cz/zoologie/entomologie/meeting/IBM_2007.htm), together with the photos and abstracts of the past meetings. Please contact us if you need further information.

Martin Fikáček (mfikacek@seznam.cz)  
Jiří Skuhrovec (jirislav@email.cz)  
Petr Šípek (sipek.petr@tiscali.cz)  
organizers of the Immature Beetles Meeting

### The list of participants of the Immature Beetles Meeting 2007

Ahrens, Dirk (Zoologische Staatssammlung, München, Germany)  
Bílý, Svatopluk (National Museum, Prague, Czech Republic)  
Bocák, Ladislav (Palacký University, Olomouc, Czech Republic)  
Bocáková, Milada (Palacký University, Olomouc, Czech Republic)  
Boháč, Jaroslav (University of South Bohemia, České Budějovice, Czech Republic)  
Delgado, Juan (Universidad de Murcia, Murcia, Spain)  
Di Giulio, Andrea (University "Roma Tre", Roma, Italy)  
Fikáček, Martin (National Museum, Prague, Czech Republic)  
Grebennikov, Vasily V. (Canadian Food Inspection Agency, Ottawa, Canada)  
Hájek, Jiří (National Museum, Prague, Czech Republic)  
Hlaváč, Peter (Košice, Slovakia)  
Ilčíková, Anna (Charles University, Prague, Czech Republic)  
Ivie, Michael A. (Montana State University, Bozeman, USA)  
Janšta, Petr (Charles University, Prague, Czech Republic)  
Jelínek, Josef (National Museum, Prague, Czech Republic)  
Kilian, Aleksandra (University of Wrocław, Wrocław, Poland)  
Knapp, Michal (Czech University of Life Sciences, Prague, Czech Republic)  
Knížek, Miloš (Forestry and Game Management Research Institute, Prague, Czech Republic)  
Kolibáč, Jiří (Moravian Museum, Brno, Czech Republic)  
Král, David (Charles University, Prague, Czech Republic)  
Maier, Crystal (Montana State University, Bozeman, USA)  
Perreau, Michel (Université Paris 7, Paris, France)  
Prokop, Jakub (Charles University, Prague, Czech Republic)  
Rezková, Kateřina (Charles University, Prague, Czech Republic)  
Saska, Pavel (Crop Research Institute, Prague, Czech Republic)  
Skuhrovec, Jiří (Crop Research Institute, Prague, Czech Republic)  
Solodnikov, Alexey Yu. (Natural History Museum of Denmark, Copenhagen, Denmark)  
Šípek, Petr (Charles University, Prague, Czech Republic)  
Švácha, Petr (Institute of Entomology, Czech Academy of Sciences, České Budějovice, Czech Republic)  
Volkovitsh, Mark G. (Zoological Institute, Russian Academy of Sciences, Sankt Petersburg, Russia)

The abstracts should be cited as follows:

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The abstracts are printed without any corrections.



Fig. 1: Participants of the Immaturation Meeting 2007 on October 4, 2007. From left to right: back row: D. Král, J. Prokop, J. Hájek, J. Skuhrovec, A. Kilian, P. Saska, M. Perreau, J. Kolibáč, D. Ahrens, J. A. Delgado, M. A. Ivie, A. Di Giulio, P. Hlaváč, and P. Švácha; front row: J. Boháč, V. V. Grebennikov, M. Fikáček, and C. Maier.

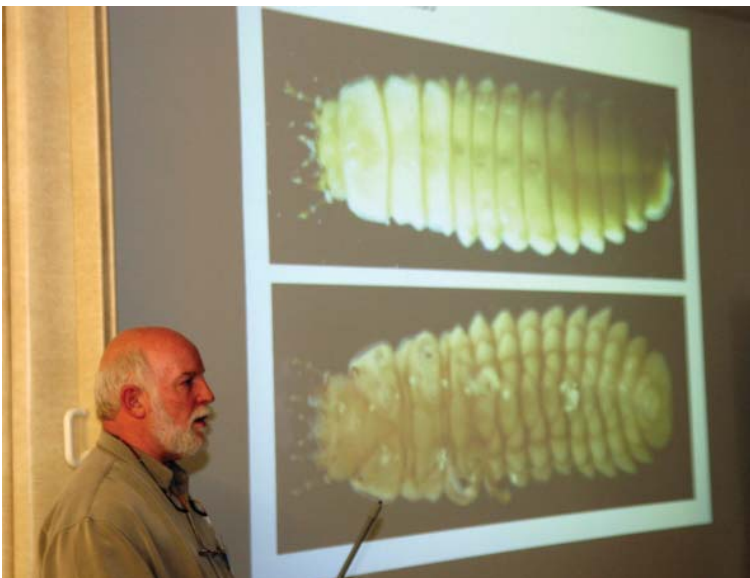
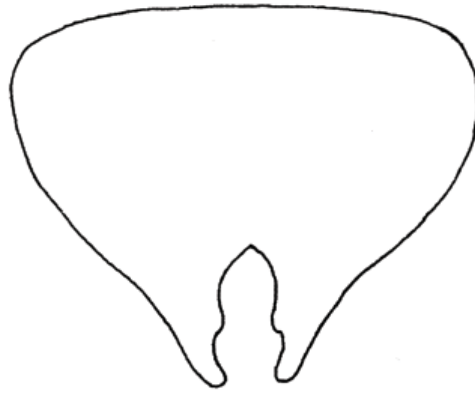
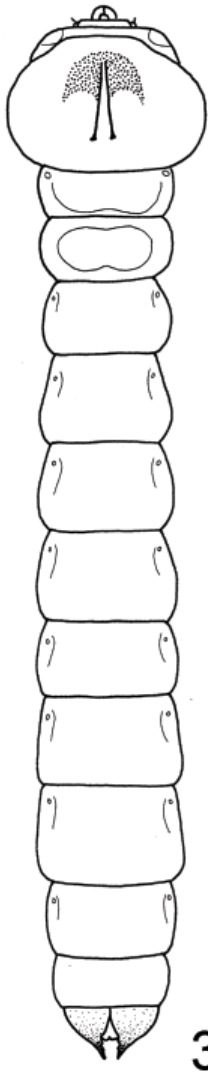


Fig 2: Michael Ivie during his lecture on the larval stages of the Omethidae.



Figs 3-5 (to the abstract by Bílý & Prokop, p. 292). 3: adult larva of *Coraebus undulatus* (F.); 4: sclerotization of the last abdominal segment of larva of *Coraebus florentinus* (Herbst, 1801); 5: fossil of the last abdominal segment of a larva of Agrilinae gen. et sp. indet. (spec. No. JD2080: Jaroslav Dolejš private collection, Prague), Late Eocene, Kučlín near Bílina (Czech Republic).

## Abstracts

### **DNA taxonomy and immature insects – the easy way of species identification?**

Dirk AHRENS<sup>1,2)</sup> & Alfried VOGLER<sup>2,3)</sup>

<sup>1)</sup> Zoologische Staatssammlung München, Münchhausenstr. 21, 81247 Munich, Germany;  
e-mail: ahrens.dirk\_col@gmx.de

<sup>2)</sup> Entomology Department, Natural History Museum, London SW7 5BD, United Kingdom

<sup>3)</sup> Division of Biology and NERC Centre for Population Biology, Imperial College London, Silwood Park Campus, Ascot, Berkshire SL5 7PY, United Kingdom

DNA sequences provide a character system that is universal to all developmental stages of organisms, but varying levels of genetic variation within and among species can make species association ambiguous. The problem is compounded where target groups are less well studied or incompletely represented in DNA databases. Here we investigate the utility of DNA for the species delimitation itself by establishing group membership on the molecular level and then linking the patterns of DNA variation to traditionally delimited species. We sequenced ca. 1600 bp of mitochondrial *cox1* and *rrnL* and ca 700 bp of nuclear 28S rRNA from 250 larval and adult chafers (Coleoptera: Scarabaeidae) from four sites in the tropical lowlands of Nepal. We tested for the presence of diagnosable groups within haplotype networks by applying population aggregation analysis (PAA) and we diagnosed shifts in branching rates on clock-constrained trees to assess the putative transition from speciation and population coalescence patterns as an indicator of the species level of genetic organisation. Using these two methods we delineated between 48 and 54 groups depending on the genetic marker chosen, and 86.1% - 92.7% of larval specimens could be assigned Linnaean names by their falling within diagnosable groups that contained identified adults. The remaining larvae were delineated as five species, four of which are *Anomala* or *Adoretus* based on their placement on a phylogenetic tree. We conclude that in a complex assemblage of chafers the sequence variation was highly structured and that any given individual (larval or adult) can be readily associated with a particular DNA group. The association of different developmental stages then becomes a matter of determining the extent of the groups, supporting the possibility of a purely sequence-based taxonomic system that integrates all life stages. Our findings are used to discuss the DNA based identification approach for a wider use in taxonomy of immature stages.

## Late Eocene Buprestid-larva (Coleoptera: Buprestidae), the first possible record from Tertiary of northern Bohemia

Svatopluk BÍLÝ<sup>1)</sup> & Jakub PROKOP<sup>2)</sup>

<sup>1)</sup>Department of Entomology, National Museum, Kunratic 1, CZ-148 00 Praha 4, Czech Republic;  
e-mail: svatopluk\_bily@nm.cz

<sup>2)</sup>Charles University in Prague, Faculty of Science, Department of Zoology, Viničná 7, CZ-128 44, Praha 2,  
Czech Republic; e-mail: jprokop@natur.cuni.cz

Larvae of all Buprestid beetles are very soft and poorly sclerotised except for the mouth parts and, in some cases, last abdominal sternite (for the detail larval morphology of Buprestidae see BÍLÝ 1999). Well-sclerotised last abdominal sternite of larva is developed only in the subfamily Agrilinae (world-wide distribution), namely in the tribes Agrilini Laporte, 1835 and Coraebini Bedel, 1921 and it forms two large, well-sclerotised, terminal teeth (Figs 3, 4). Very similar, sclerotised abdominal structure was observed also in the Neotropical genera *Pterobothris* Fairmaire & Germain, 1858 (Buprestinae) and *Philandia* Germain & Kerremans, 1906 (uncertain subfamilial/tribal status, most probably Agrilinae).

No fossil record of the Buprestid larva has been found so far, since their larvae are endophagous (mostly xylophagous) and very soft without distinct sclerotisation enabling a fossil record.

The enigmatic sample No. JD2080 (housed in Jaroslav Dolejš private collection, Prague) from the Kučlín was a little mysterious for a long time. After having compared the sample with the recent material of Buprestid larvae we are inclined to believe that it can represent the rest of the larval body of some large species of the subfamily Agrilinae (most probably *Coraebus* Laporte & Gory, 1839). The shape and size of the fossil sample corresponds with the shape and size of the sclerotised, last larval, abdominal segment of large, recent species of *Coraebus* or *Agrilus* Curtis, 1825 including the shape of inner teeth (Fig. 5). Of course, we cannot be sure and the present sample can represent a part of some other unknown organism.

Fossils from Kučlín locality situated at the Trupelník Hill near Bílina in the České středohoří volcanic areas are preserved in various types of diatomite dated to Late Eocene (Priabonian) (see KVAČEK 2002). Entomofauna with prevalence of terrestrial insects mostly belonging to Coleoptera families (particularly Curculionidae) is well known by pioneer work of DEICHMÜLLER (1881). Additional material of more than 100 insect specimens collected during last two decades extend insect diversity known to Deichmüller and reveal also new taxa of buprestid beetles (PROKOP 2003).

BÍLÝ S. 1999: Larvae of buprestid beetles (Coleoptera: Buprestidae) of Central Europe. *Acta Entomologica Musei Nationalis Pragae, Supplementum* **9**: 1-45 + 33 unpaginated plates.

DEICHMÜLLER J. V. 1881: Fossile Insecten aus dem Diatomeenschiefer von Kutschlin bei Bilin, Böhmen. *Nova Acta Leopoldina* **42**: 295-331.

KVAČEK Z. 2002: Late Eocene landscape, ecosystems and climate in northern Bohemia with particular reference to the locality of Kučlín near Bílina. *Bulletin of the Czech Geological Survey* **77**: 217-236.

PROKOP J. 2003: Remarks on palaeoenvironmental changes based on reviewed Tertiary insect associations from the Krušné hory piedmont basins and the České středohoří Mts. in northwestern Bohemia (Czech Republic). *Acta Zoologica Cracoviensia* **46 (Supplementum)**: 329-344.

## The larval characters and bionomy of the central European species of the tribe Staphylinini (Coleoptera: Staphylinidae)

Jaroslav BOHÁČ

Faculty of Agriculture, University of South Bohemia, Studentská 13, CZ-370 05 České Budějovice, Czech Republic; e-mail: jardaboh@seznam.cz

Larvae, pupae and eggs of the central European species of the tribe Staphylinini have been described in about 60 papers containing mostly morphological data and generally disregarding larval bionomics. New views on the morphology and biology of immature stages of the tribe were presented in papers by Potockaja, Szujecki, Kasule and Topp and summarized by BOHÁČ (1982). The additional larvae of the tribe were described from laboratory stocks (Boháč, unpublished data). The study of immature stages resulted in separation closely related species (e.g. *Ocypus tenebricosus* and *O. biharicus*), description of the differences in individual instars and assessing of periods of the duration of the development of individual stages under consideration of defined laboratory conditions. Two types of development were disclosed, i.e. type 1 reproducing in spring and type 2 reproducing in autumn and completing oviposition in spring. The evidence for two morpho-ecological types of larvae was obtained: the burrowing type of larva (genera *Platydracus* and *Staphylinus*) and the running type of larva (genera *Ocypus* and *Tasgius*). The generic concept of the tribe proposed by BOHÁČ (1982) based on the larval morphology is recently used in advanced monographies (e.g. LÖBL & SMETANA 2004).

BOHÁČ J. 1982: The larval characters of Czechoslovak species of the genera *Abemus*, *Staphylinus* and *Ocypus*. *Studie ČSAV* (Praha), **1982(4)**: 1-96 + 26 pls.

LÖBL I. & SMETANA A. 2004: *Catalogue of Palaearctic Coleoptera. Vol. 2. Hydrophiloidea-Staphyliniidea*. Apollo Books, Stenstrup, 942 pp.

## Immature stages in Hydraenidae: the state of the art

Juan A. DELGADO

Departamento de Zoología, Facultad de Biología, Universidad de Murcia, E-30100, Murcia, Spain; e-mail: jdelgado@um.es

The family Hydraenidae is one of the most speciose and interesting families of aquatic beetles. Traditionally, the phylogenetic position of the family Hydraenidae was linked to the family Hydrophilidae within the superfamily Hydrophiloidea. However it is now widely accepted that the sister group of Hydraenidae is the family Ptiliidae and thus, that Hydraenidae is a member of the Staphyliniidea. To support this phyletic placement, a number of autapomorphies have been proposed. However, among them, only a few characters refer to larvae. Unfortunately, most part of these traits can be questioned as good autapomorphies, although being good diagnostic characters. As in many other beetle groups, after decades of study, good larval traits were not available for phylogenetic studies until recently. This is largely a consequence of a generalized lack of interest in the knowledge of the preimaginal

stages which has lasted for decades. Trying to solve in part this problem, the immatures of the family Hydraenidae are being intensively investigated during the last ten years. These studies have two main objectives: 1) to develop the basal knowledge necessary to describe and identify the larvae of the different taxa and 2) to utilize these descriptions and the new found features in phylogenetic studies.

During this time we have made significant progress in understanding the preimaginal stages of this family. Our unpublished studies on the hydraenid egg phase suggest that in many members of the family the eggs are protected by a coat of an amorphous matter of unknown composition that clearly do not resembles silk-like strands. Some old descriptions of eggs covered by silken cocoons were surely biased by the old, traditional relation between Hydraenidae and Hydrophilidae.

Recent studies have provided the first detailed descriptions of the larvae of some hydraenid genera. A thoroughly description of several first instars larvae have demonstrated that not all the genera in this family have cephalic egg-bursters. In fact, these structures seem to be restricted to the subfamily Ochthebiinae, considered as a derived lineage within Hydraenidae. The lack of cephalic egg-bursters in some basal hydraenid genera such as *Limnebius* and *Hydraena* confirms our doubts about the close relation between Hydraenidae and Hydrophilidae, which was based in supposed similarities as the common use of cephalic egg-bursters upon hatching. It is now evident that such structures have evolved several times in different lineages in the order Coleoptera. Some other morphological features such as the morphology of cephalic appendages, sclerotization pattern, type of stigmata and legs morphology differs clearly from the morphology of the larvae of Hydrophilidae.

In our studies, a considerable effort has been put in the description of the chaetotaxic pattern in the described larvae. To do that, a chaetotaxic system has been proposed in order to standardize descriptions of different hydraenid larvae. Our system is based in three different and previously proposed systems: the BOUSQUET & GOULET (1984) system for Carabidae, the KOVARIK & PASSOA (1993) system for Histeridae and, mainly the ASHE & WATROUS (1984) system for Staphylinidae. Our system is in debt with all these excellent labelling systems. It closely follows the Ashe-Watrous system but was modified in order to accommodate several conceptions of the other proposed systems to the family Hydraenidae.

A central difference between our system and the system used in larval Staphylinidae is the larval instar used to base the starting of the descriptions: first instar in Hydraenidae, third instar in Staphylinidae. First instar larvae were also employed in the other two referred systems. This important difference implies that in Hydraenidae the increasing number of setae found in the second and third instars is considered as an addition of setae to the primary pattern of setae seen in the first instar. Conversely, in Staphylinidae the "primary" pattern of setae seem to be reached in the third instar larvae and the absence of some setae in first and second instars is considered as a secondary lack. We consider more congruent to see the pattern of setae found in first instars larvae as the primary pattern of setae in a lineage, considering the subsequent increase of setae in second and third instar as additions to the primary pattern. Using this "ontogenetic thinking" it is possible to differentiate between primary, subprima-



ry and secondary setae between larval instars as did WHEELER (1990) in Leiodidae. This is impossible in the Ashe-Watrous system. In any case, despite the considerable differences between the systems proposed to describe the chaetotaxy in several families of Coleoptera, all have been proved extremely useful in several fields of study. Today a general consensus is being reached about the need to a more general and widely applicable system for the whole order Coleoptera. This project should be carried out by a wide team of workers specialized in the larvae of different families of Coleoptera and, in this sense, the 2007 IBM meeting could be an excellent starting point for such a task.

Future studies should continue to investigate the morphology of different genera, mainly among the subfamilies and lineages little known or undescribed at larval level, as the African Prosthetopinae. We have also planned to expand our interest in hydraenid larvae in order to undertake studies of larval biology, ecological strategies, etc. Detailed studies on morphology of eggs shells and pupal morphology and pupal chaetotaxy will complete the increasing knowledge in this interesting family of water beetles.

ASHE J. S. & WATROUS L. E. 1984: Larval chaetotaxy of Aleocharinae (Staphylinidae) based on a description of *Atheta corticaria* Kraatz. *The Coleopterists Bulletin* **38**: 165-179.

BOUSQUET Y. & GOULET H. 1984: Notation of primary setae and pores on larvae of Carabidae (Coleoptera: Adephaga). *Canadian Journal of Zoology* **62**: 573-588.

KOVARIK P. W. & PASSOA S. 1993: Chaetotaxy of larval Histeridae (Coleoptera: Hydrophiloidea) based on a description of *Onthophilus nodatus* LeConte. *Annals of the Entomological Society of America* **86**: 560-576.

WHEELER Q. D. 1990: Morphology and ontogeny of postembryonic larval Agathidium and Anisotoma (Coleoptera: Leiodidae). *American Museum Novitates* **2986**: 1-46.

## **The first known larva of the Australian tribe Mystropomini (Coleoptera: Carabidae: Paussinae): morphology and phylogenetic implications within the subfamily**

Andrea DI GIULIO<sup>1)</sup> & Wendy MOORE<sup>2)</sup>

<sup>1)</sup>Department of Biology, University "Roma Tre", Viale G. Marconi 446, I-00146 Rome, Italy;  
e-mail: digiulio@uniroma3.it

<sup>2)</sup>Department of Entomology, California Academy of Sciences, 875 Howard Street, San Francisco, California  
94103, USA; wmoore@calacademy.org

The carabid subfamily Paussinae consists of five tribes: Metriini, Mystropomini, Ozaenini, Protopaussini, and Paussini. All Paussinae are characterised by a derived mode of chemical defense in adults (like the Brachininae they are "bombardier beetles") and by an unique type of larva. This larval type has been defined as "discotelic" for the presence of a wide, transverse, plate-like structure (terminal disk), resulting from the modification of the last three abdominal segments and urogomphi. This structure is used by free-living Metriini and Ozaenini to trap prey through an ambush strategy, while larvae of the myrmecophilous

tribe Paussini are thought to use this structure to supply pleasant chemicals to the ants. Within the subfamily Paussinae, larval morphological characters have proven useful for inferring phylogenetic relationships, though, to date, larvae were known for only three of the five tribes. The recent discovery of the larva of *Mystropomus subcostatus* Chaudoir fills an important gap in our knowledge of paussine larval morphology since it is the first known larva for the Australian tribe Mystropomini. While the discovery that *Mystropomus* larvae possess a terminal disk was expected, certain attributes of this structure confirm the prevailing hypothesis that Mystropomini is a basal member of the subfamily with many similarities with larvae of Metriini. No external structural features indicate that this larva lives with ants, as do many other members of the subfamily. The comparison of the structural features of this larva with those of other known larvae in the subfamily allows us to assess support from larval characters for competing phylogenies inferred from adult characters and molecular sequence data.

### **“Flying larvae” and phylogeny of Meloidae (Coleoptera)**

Andrea DI GIULIO & Marco A. BOLOGNA

Department of Biology, University “Roma Tre”, Viale G. Marconi 446, I-00146 Rome, Italy;  
e-mail: digiulio@uniroma3.it

The Meloidae (Coleoptera, Polyphaga, Tenebrionoidea), with about 120 genera and 3000 species, represents a monophyletic, cosmopolitan family of phytophagous beetles, divided into four subfamilies: Eleticinae, Meloinae, Tetraonycinae and Nemognathinae. This group of insects is well known for complex hypermetabolic development connected to larval parasitism on Hymenoptera (Aculeata) and Orthoptera (Acridoidea), and for the production of cantharidin, a terpenoid with protective and aggregating properties. Postembryonic development includes 7 larval instars and 5 distinctive phases (first instar, instars II-VI, coarctate phase, instar VII, pupa). The first instar larva shows the most striking morphological variety and it is known for all the subfamilies, most of the tribes and about 50% of genera. Later instars, scarcely mobile and of scarabeiform type, are largely unknown.

Several competing phylogenetic hypotheses have been proposed based on first-instar larval morphology. The disagreement was mostly due to different interpretations of convergent or shared derived status of adaptations to phoresy, mostly on hymenopterans. During the last five years we have performed several cladistic analyses by using morphological, behavioural, or molecular characters (also combined) in order to interpret with more certainty the phylogeny of Meloidae and to infer the evolution of larval morphology. The hypothesis here proposed is that a campodeiform highly mobile larva was derived from an orthosomatic first instar larva similar to that of other Tenebrionoidea, and still present in the basal meloid subfamily Eleticinae. This first instar larva has usually received the name of *triungulin* (more recently *planidium*) because of the peculiar shape of pretarsus, specialized in some genera for climbing and holding the host. The evolution of a similar type of larva, highly plastic from the adaptive point of view and pre-adapted to the parasitism on aculeate hymenopterans, may have produced the rapid differentiation of the other three subfamilies: Tetraonycinae, Nemognathinae and

Meloinae. In these subfamilies the phenomenon of phoresy evolved several times independently, giving rise to different larval types, with many morphological convergences: once in the Tetraonycinae, in which the basal type of triungulin is missing; once in the Nemognathinae, that still show a non-phoretic triungulinid larva in the basal tribe of Stenoderini; and at least 6 times in the large subfamily Meloinae. Within the Meloinae there are different types of non-phoretic triungulins in all 7 tribes, with a wide variety of morphological adaptations connected to different feeding strategies.

### **Larvae of small hydrophiloid families Spercheidae, Hydrochidae and Epimetopidae: troublemakers in phylogenetic studies**

Martin FIKÁČEK<sup>1,2)</sup>

<sup>1)</sup> Department of Entomology, National Museum, Kunratic 1, CZ-148 00 Praha 4, Czech Republic;  
e-mail: mfikacek@seznam.cz

<sup>2)</sup> Charles University in Prague, Faculty of Science, Department of Zoology, Viničná 7, CZ-128 44 Praha 2, Czech Republic.

The superfamily Hydrophiloidea consists of six well-defined families, of which the family Hydrophilidae comprises the majority of known species. Remaining families (Georissidae, Helophoridae, Epimetopidae, Hydrochidae and Spercheidae) are much less diverse in number of known species and morphology of adults. In spite of an extensive effort to clarify the phylogeny of the superfamily, phylogenetic relationships of mentioned families still remain unclear. Summary of published phylogenetic analyses based on adult and larval morphological characters as well as molecular data would result in an entirely unresolved strict consensus tree. Three hydrophiloid families seem to be especially responsible for problems when the phylogeny of the superfamily is inferred: Spercheidae, Hydrochidae and Epimetopidae.

Within the family Epimetopidae, larvae of two species of the genus *Epimetopus* are described. As nearly no larval material is available for this family, the larva remains very little known and in most studies concerning the larval morphology, the data are only adopted from published descriptions. Published descriptions of larva of *Epimetopus trogooides* (ROCHA 1967, COSTA et al. 1988, ARCHANGELSKY 1997) disagree to each other in some characters, especially in shape of the head capsule, morphology of abdominal segments 8 and 9, and number of segments of urogomphi. Detailed study of the immature stages of the Epimetopidae is therefore badly needed.

The family Spercheidae usually stand as sister-group to the Hydrophilidae (e.g. ARCHANGELSKY 1998), or as sister-group to all remaining Hydrophiloidea (BEUTEL 1994, 1999). When larval morphology is used, this ambiguity is caused mainly by the morphology of the larval head capsule, differing considerably from all other hydrophiloids in many aspects (larval head of *Spercheus* is generally of staphylinoid shape, subprognathous, with gula, non-prominent mouth parts and large lacinia of maxilla). These characters seem to correspond with the floating and filter-feeding habits of the larva. Based on the detailed study on the head chaetotaxy, the

interpretation of some characters has to be corrected in comparison with published studies: (1) head chaetotaxy is well differentiate and corresponding with that of other hydrophiloids in first instar, secondarily homonomous in higher instars; (2) nasale present, lacking teeth, but bearing nasal setae; (3) epistomal lobes probably reduced (homologues of epistomal setae seem to be present); (4) mandibular apex simple, retinaculum with two teeth; (5) lacinia developed as simple inner projection of stipes, with chaetotaxy completely corresponding with the stipes chaetotaxy of other hydrophiloids.

The knowledge on the morphology of larval Hydrochidae is very insufficient and the contrary published results (RICHMOND 1920, ARCHANGELSKY 1997, BEUTEL 1999, MAKHAN 2004) seem to be caused by considerable variability between various hydrochid larvae (differences were found mainly in shape of anterior margin of head capsule, relative size of antennomeres and sensorium and shape of stipes, mandibles and labium). This suggest that a detailed study of wider spectrum of hydrochid larvae is badly needed. Studies on head chaetotaxy of *Hydrochus megaphallus* confirm that the inner projection of stipes developed in some hydrochid larvae is homologous with lacinia of Spercheidae.

In a preliminary phylogenetic analysis of the Hydrophiloidea based on chaetotaxy of larval head (FIKÁČEK 2006), Spercheidae and Hydrochidae were placed together with larvae of the hydrophilid subfamily Sphaeridiinae, which are also quite derived because of their terrestrial life-style. Applying of methods avoiding the long-branch attraction effect resulted in the tree in which both Hydrochidae and Spercheidae are standing as sister-group to the Hydrophilidae. This confirms that even the careful evaluation of morphological characters can help to clarify the position of both families within the Hydrophiloidea.

- ARCHANGELSKY M. 1997: Studies on the biology, ecology, and systematics of the immature stages of New World Hydrophiloidea (Coleoptera: Staphyliniformia). *Bulletin of the Ohio Biological Survey, New Series* **12(1)**: 1-207.
- ARCHANGELSKY M. 1998: Phylogeny of the Hydrophiloidea (Coleoptera: Staphyliniformia) using characters from adult and preimaginal stages. *Systematic Entomology* **23**: 9-24.
- BEUTEL R. G. 1994: Phylogenetic analysis of Hydrophiloidea based on characters of the head of adults and larvae. *Koleopterologische Rundschau* **64**: 103-131.
- BEUTEL R. G. 1999: Morphology and evolution of the larval head of Hydrophiloidea and Histeroidea (Coleoptera: Staphyliniformia). *Tijdschrift voor Entomologie* **142**: 9-30.
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**Good-bye Scydmaenidae (Coleoptera): larval morphology  
and 18S rDNA sequence suggest that rove beetles are paraphyletic  
with respect to ant-like stone beetles**

Vasily V. GREBENNIKOV<sup>1,2)</sup> & Alfred F. NEWTON<sup>3)</sup>

<sup>1)</sup>Entomology Research Laboratory, Ontario Plant Laboratories, Canadian Food Inspection Agency,  
K. W. Neatby Bldg., 960 Carling Avenue, Ottawa, Ontario K1A 0C6, Canada;

email: grebennikovv@inspection.gc.ca

<sup>2)</sup>Institut für Spezielle Zoologie und Evolutionbiologie, Friedrich-Schiller-Universität Jena, Erbertstraße 1,  
D-07743 Jena, Germany

<sup>3)</sup>Zoology Department, Field Museum of Natural History, 1400 South Lake Shore Drive, Chicago, IL 60605,  
USA; email: anewton@fieldmuseum.org

Ant-like stone beetles (Coleoptera: Scydmaenidae) is a cosmopolitan group of more than 4600 described species in about 82 genera, although numerous new species remain to be named. These beetles are found in forest leaf litter and other relatively moist habitats where the adults or larvae prey on oribatid mites. The group has been maintained as a separate family since 1815. It appears increasingly convincing that Scydmaenidae, together with Silphidae and Staphylinidae, might form a monophyletic unit. It has even been hypothesised that the two former families might, in fact, be nested deep inside the Staphylinidae clade. This scenario, if true, would require synonymizing Scydmaenidae with Staphylinidae and treating the former as a subunit of the Staphylinidae.

To test the phylogenetic position of Scydmaenidae, a matrix of larval morphological characters was constructed to include 34 taxa and 90 parsimoniously informative characters. The following families/subfamilies were represented in the analysis, with the number of terminal taxa in parentheses: Leiodidae (1), Agyrtidae (1), Silphidae (3), Scydmaenidae (3), Omaliinae (1), Tachyporinae (1), Piestinae (1), Apateticinae (1), Trigonurinae (1), Oxyporinae (1), Megalopsidiinae (1), Leptotyphlinae (3), Steninae (2), Euaesthetinae (4), Pseudopsinae (3), Paederinae (3) and Staphylininae (4). The most parsimonious trees obtained were consistent in retaining monophyly of the Staphylinine Group of subfamilies (the latter seven subfamilies, either with or without Oxyporinae) plus Scydmaenidae. Monophyly of this group was not affected by character ordering or character re-weighting using successive approximation. These results support the hypothesis of paraphyly of Staphylinidae with respect to Scydmaenidae.

A separate analysis was performed, for which a matrix of 18S rDNA sequences data was constructed to include 52 terminal taxa and about 2100 aligned nucleotide positions using ClusX default settings. Neighbour joining, maximal parsimony, maximum likelihood, and Bayesian approximation were used as topology-building methods. Monophyly of the Staphylinine Group + Scydmaenidae was not retained, although Silphidae and Scydmaenidae were variably nested inside Staphylinidae on all unrooted dendrites, which also included representatives of Leiodidae and Agyrtidae. These results are consistent with the hypothesis that Staphylinidae are paraphyletic with respect to Scydmaenidae.

This study provides strong support for the hypothesis that ant-like stone beetles do not form an independent family, but are in fact rather modified members of Staphylinidae and, consequently, should logically be treated as a subfamily within the Staphylinine Group.

### **First known larva of the Omethidae**

Michael A. IVIE

Montana Entomology Collection, Montana State University, Marsh Labs, 1901 S. 19th Ave., Bozeman, MT 59717, USA

Abstract not available.

### **Pretergal and tergal glands in larvae of Staphyloidea – preliminary review of presence, classification and problems**

Aleksandra KILIAN

Department of Biodiversity and Evolutionary Taxonomy, University of Wrocław, ul. Przybyszewskiego 63/77, 51-148 Wrocław, Poland; e-mail: a.kilian@biol.uni.wroc.pl

Various, pore-like structures are present on dorsal side of thoracic and abdominal segments of larvae in order Coleoptera. Their function and anatomy are very often unknown and neglected in descriptions of larval morphology, even if chaetotaxy is given in detail. Here, I summarized preliminarily data from literature and my own studies about glands in larvae of superfamily Staphyloidea. Data on presence, number and arrangement of the tergal and pretergal glands, their diversified terminology for all known larvae of Leiodidae and some representatives of Staphylinidae and Hydraenidae are gathered. New data on their morphology – SEM photographs are provided. The research reveals a few morphological types of these pore-like, most probably glandular, structures on dorsal side of the larval body. Here are presented and illustrated. The problem of the identity of the larval glands and their arrangement as diagnostic character are discussed. Detailed histological, physiological, comparative studies are postulated. Such investigations seem to be essential to clarify their function and homology, and to make phylogenetic analysis on the basis of larval chaetotaxy and porotaxy.

### **Biology of *Brachinus* larvae (Coleoptera: Carabidae), coleopteran parasitoids**

Pavel SASKA & Alois HONĚK

Department of Entomology, Crop Research Institute, Drnovská 507, CZ-161 06 Praha 6 – Ruzyně, Czech Republic; e-mail: saska@vurv.cz

Parasitoid larvae develop at the expense of a single host, which is killed by the parasitoid. Parasitoid mode of life occurs mainly in Hymenoptera and Diptera, but they can be found also in other orders of insects. In Coleoptera, this life strategy is rare. In Carabidae, parasitoid

species are known from three tribes: Brachinini, Peleciini and Lebiini (ERWIN 1979).

North American wetland species of *Brachinus* parasitize the pupae of water beetles (Dytiscidae, Gyrinidae and Hydrophilidae), and develop through five instars (ERWIN 1979). So far the life history has been described for two European dry land species only: *Brachinus explodens* Duftschmid and *B. crepitans* (Linnaeus). Both species were reared on pupae of other carabids of the genus *Amara* (SASKA & HONĚK 2004, 2005). Up to now, four species of *Amara* have been successfully used as host for *Brachinus* larvae: *A. aenea* (DeGeer), *A. familiaris* (Duftschmid), *A. similata* (Gyllenhal) and *A. littorea* (Schiødte) (SASKA & HONĚK 2004, 2005; Saska, unpubl. data).

Females of *Brachinus* usually lay eggs singly on the substrate surface, stuck to the soil particles. Freshly laid eggs are narrowly oval and soft. Several hours before eclosion, the mandibles of the embryo became dark and visible through the chorion. Larval stage has three instars, but the larval development goes through three distinct behavioural phases different from the instars: the searching, feeding and resting phase. The newly hatched first instar larva is typically caraboid. After hatching it immediately starts searching for a host. On finding a suitable pupa, a larva starts feeding, usually on the host's appendages. The larva pierces the pupal cuticle and lick up the haemolymph that exuded from the wound. Larvae moult to the second instar without moving away from the host pupa. The second instar larva is erucoid, with a white soft body. The mode of feeding is as in the first instar, but the preferred feeding locations are at the intersegmental membrane connecting head and prothorax, prothoracic pleura, pronotal sternum or abdominal pleura. During feeding, the second instar larva is always attached to the host by its ventral side. The third instar larva is erucoid, with a deeply folded cuticle, which allows a great increase in volume during the short period of intensive feeding. After moulting, the larva adopts a typical feeding position, attached by its dorsal surface to the host's body and with its head and thorax bent backwards. In contrast to the first and second instars, larvae of the third instar chew the tissues of their hosts with their mandibles. The total feeding period (from the attachment of the first instar larva until the host is consumed by the third instar larva) lasts 6-7 days at 25°C. When the feeding is finished, the larva turns onto its ventral side with the remains of the host remaining on the dorsum and rest for another 3-5 days and thereafter pupate. During this period the shape of the larva changes from obovate to pear-shaped, reflecting the body shape of the adult. The fresh pupa is white. Two days before adult emergence the mandibles darken, and 12 h before emergence the head, pronotum and legs become pale orange. The pupal stage of both species lasts 8-10 days.

Since duration of all development phases except the searching phase varied with temperature, the thermal requirements for development of eggs, larvae and pupae of both species were established using three constant temperatures between 17.7-27.4°C. The LDT, the lower development threshold for eggs, is 9.4°C for *B. explodens* and 7.2°C for *B. crepitans*, respectively; the SET, the sums of effective temperatures, are 154.4 and 180.7 day degrees, respectively. LDT for the total postembryonic development (except the searching phase) is 12.3°C in *B. explodens* and 10.5°C in *B. crepitans*, respectively, and SET are 209.2 and 289.5 day degrees, respectively.

The conversion of ingested food during the pre-imaginal development is very high in both *Brachinus* species, as it ranges from 38.5 to 85.5% of the fresh mass for larval, and 33.5-

81.5% of the fresh mass for total pre-adult development. The efficiency of conversion does not change with temperature. Growth rate, consumption rate, relative growth rate and relative consumption rate are high for larval and moderate to high for total pre-adult development, and significantly increase with temperature in both species.

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**Larval morphology enhances phylogenetic reconstruction  
in Cetoniidae (Coleoptera: Scarabaeoidea)  
and allows the interpretation of the evolution of larval feeding habits**

Petr ŠÍPEK<sup>1)</sup>, Estefania MICÓ<sup>2)</sup>, Miguel Angel MORÓN<sup>3)</sup> and Eduardo GALANTE<sup>2)</sup>

<sup>1)</sup> Charles University in Prague, Faculty of Science, Department of Zoology, Viničná 7, CZ-128 44 Praha 2, Czech Republic; e-mail: sipek.petr@tiscali.cz

<sup>2)</sup> Centro Iberoamericano de la Biodiversidad (CIBIO), University of Alicante, Spain

<sup>3)</sup> Instituto de Ecología, A.C. Km. 2.5 antigua carretera a Coatepec, núm 351, Congregación El Haya, C.P. 91070, Veracruz, Mexico

Cetoniids or flower beetles represent one of the showiest groups of beetles, consisting of almost 3900 species and distributed almost worldwide, with exception of subpolar areas and remote islands (KRAJČÍK 1998, 1999). The beetles are diurnal, feeding on sap and ripe fruits, larvae develop in rotten wood or other decayed organic matter or are associated with social insects.

With many large and colorful forms, Cetoniids have been in focus of many collectors since the origin of entomology. However unless so popular only a little work was done in resolving their intra-group classification, nor in examination of their phylogenetic relationship to other Scarab beetles. The aim of our study was to test the monophyly of the group, to question the current intra-group classification of Cetoniidae (KRIKKEN 1984) and to find possible relationship between larval feeding habits and their phylogenetical position.

A total number of 76 morphological characters were used for the analysis, with 38 larval and 38 imaginal characters in 42 species of Cetoniidae. Six representatives of Rutelidae, Melolonthidae and Dynastidae were used as outgroup taxa. The results of our analysis strongly supports the monophyly of the Cetoniidae (with 99% bootstrap value), but do not support the monophyly of all subfamilies of Cetoniidae (sensu KRIKKEN 1984). We found an apparent paraphyly of Trichiinae, where the tribe Trichiini is forming a monophyletic group with Valginae, and the tribes Incaini and Osmodermatini are joined into a clade with Cetoniinae. The monophyly of *Osmoderma* and Cetoniinae is well supported.



The comparison of the larval feeding habits and the proposed phylogeny shows a tendency from rotten wood to a wide diet of organic matter, including compost, dung, vertebrate nests and debris piles of ants.

In our study larval characters have proven to be more informative than those of the adults for deeper phylogeny, and are also corresponding with current molecular analysis (SMITH et al. 2006). Indeed the most resolved clades (with more than 80% bootstrap support) are defined mainly by larval characters. Thus we conclude that larval morphology is more informative in investigation of phylogeny than adult morphology.

All results presented in this contribution are part of the recent paper by MICÓ et al. (in press).

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## Which strategy of weevil larvae brings more benefits, endophagy or ectophagy?

Jiří SKUHROVEC

Department of Herbiology, Crop Research Institute, Drnovská 507, CZ-161 06 Praha 6 – Ruzyně, Czech Republic;  
e-mail: jirislav@email.cz

Weevil (Curculionidae) larvae are mostly endophagans feeding in the host plant tissue. Endophagy of larvae is a plesiomorphic trait of the superfamily Curculionoidea (MARVALDI et al. 2002). In central Europe, the exceptions are only the genera *Cionus*, *Phytobius* and tribe Hyperini (DIECKMANN 1989) whose larvae are ectophagans feeding on the host plant surface, eating mostly leaves and sometimes the flowers.

Several weevils from the tribe Hyperini do not conform the above definition of ectophagy. Larvae of the red clover weevil, *Hypera nigrirostris*, develop inside of the clover inflorescence. Therefore, the development is outside of the plant tissue, but the closed inflorescence still provides the protection to larvae from parasites, predators or unfavourable conditions, benefits typical for endophagans. This switch to „endophagy“ is not unique for species of the tribe Hyperini.

The similar life strategy was observed in *Limobius borealis* at the inflorescences of the *Geranium*-species and *Hypera gracilentia* living on an unidentified species of Apiaceae in southern Portugal (WINKELMANN & SKUHROVEC 2007). SKUHROVEC (2005) also demonstrated more complicated situation in *Hypera arator*, where the larvae can combine ectophagous and also „endophagous“ mode of life depending on the development stage of its host plant, *Dianthus* spp. Weevil develops on *Dianthus arenarius bohemicus* inside the ovary. On *Dianthus carthusianorum*, larvae were observed to develop also on the surface of the ovary. The seeds of *D. carthusianorum* are tightly packed in contrast to *D. arenarius bohemicus*. It is possible that the decisive factor for the development of larvae is the space among seeds.

Several ectophagous weevils thus return to their original life strategy, endophagy, because of adaptation to living within the compact plant structure without penetration into plant tissues.

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## **Larval chaetotaxy as a source of characters in beetles: pitfalls and problems**

Alexey SOLODOVNIKOV

Natural History Museum of Denmark / University of Copenhagen, Zoological Museum, Universitetsparken 15,  
DK-2100 Copenhagen, Denmark; e-mail: asolodovnikov@snm.ku.dk

Chaetotaxy of beetle larvae is a significant source of characters for descriptive and phylogenetic aspects of systematics of this largest group of animals of comparable age. Survey of the mostly modern systematic literature employing larval chaetotaxy in Coleoptera reveals, however, that contrary to some general claims for the utility of this character set, use of larval chaetotaxy is rather limited. This is mostly because researchers find working with larval chaetotaxy difficult and time consuming. The presentation is focused on the methodological analysis of the factors that make exploration of chaetotaxy so cumbersome. They are divided into two categories: intrinsic and operational. It is revealed that the most dangerous of them are operational, which arise from the multiplication of inconsistencies coming from different levels of comparative morphological research. As a result, ill-defined assessments of larval chaetotaxy may bring more confusion than clarity to the systematics of beetles, especially

to its phylogenetic component which is intuitively avoided by researchers who refuse to use chaetotaxy. This presentation attempts to scrutinize the sources of these inconsistencies undermining studies of larval chaetotaxy in hope of eliminating them from present and future systematic studies of Coleoptera. Some methodological issues raised here are also applicable to adult Coleoptera, to other insects and invertebrates, or to the proper ways of exploring the comparative morphology of living organisms, underlying evolutionary and systematic research.

This presentation will be a digest of the respective recently published paper:  
SOLODOVNIKOV A. Yu. 2007: Larval chaetotaxy of Coleoptera (Insecta) as a tool for evolutionary research and systematics: less confusion, more clarity. *Journal of Zoological Systematics and Evolutionary Research* **45**: 120-127.

## Techniques for depicting soft-bodied unsclerotized larvae

Petr ŠVÁCHA

Institute of Entomology, Czech Academy of Sciences, Branišovská 31, CZ-370 05 České Budějovice, Czech Republic; e-mail: svacha@entu.cas.cz

White soft-bodied unsclerotized larvae with very low contrast and poorly defined body regions are difficult to depict without special illumination and/or staining. Lineart is often misleading (particularly when produced by illustrators). When the body wall is flattened in a slide, numerous artifacts result, and only details of small regions (not overviews) can be depicted.

Epicuticle is impermeable for stains that would be uptaken by inner cuticular layers. Local dye injection is possible, but the resulting staining is non-homogeneous and insufficient for overviews. Moreover, combined incident/transmitted light provides better results than any of those illuminations alone.

To enhance contrast and make fine details more distinct, soft tissues should be removed and the cuticle should be stained.

Removing soft tissues: A well-preserved non-contracted specimen is needed. In soft larvae, hydroxide will deform small pieces of cuticle (larvae shortly before moulting with already dissolved endocuticle are unusable). Larger “cutouts” of the larval body should be cleaned manually as much as possible, but without damaging the cuticle (damages take up stain later). A short treatment with hot 5% KOH may be used for final cleaning if necessary, and only then the cuticular cylinder should be cut in halves to enable use of transmitted light.

Staining: Quick and durable staining by chlorazole black E (CARAYON 1969); the dye dissolves in water, ethanol, and some other liquids. Parker black ink for fountain pens provides similar results and may contain the same dye. The stain does not wash out or bleach in spirit or glycerol and can be also used for staining temporary microscopic slides. The dye has great affinity to membranes (and is thus useful also for partly sclerotized larvae), often contrasts and makes visible muscle attachments, and enhances visibility of endoskeleton. It is also invaluable for dissections.

Specimens may be observed and photographed in a dish with 80% spirit. For transmitted or combined illumination, special transillumination stands are useful but not necessary – a glass plate with underlaid white paper illuminated obliquely by a spotlight is sufficient. Floating/drift of the specimen should be prevented, particularly for stacking purposes. Overviews usually have insufficient depth of focus and combining several differently focussed photographs may be necessary. Manual stacking provides best results but is very laborious. Stacking software: (1) CombineZ (Alan Hadley, <http://www.hadleyweb.pwp.blueyonder.co.uk/>), free and of reasonable quality for simpler objects; (2) HeliconFocus Lite and Pro, latter with added retouching tools (Danylo Kozub, <http://www.heliconsoft.com/>), excellent and reasonably priced, Mac version available; (3) Automontage (no personal experience, very expensive); (4) proprietary (supplied, e.g., by Olympus or Leica). Retouching is necessary in most cases and a graphical tablet is invaluable for that purpose.

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