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Larval morphology of selected species of families Lampyridae and Silphidae

Larvální morfologie a ekologie vybraných druhů čeledí Lampyridae a Silphidae

Doctoral thesis (*compilation of published works*)

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Declaration

I hereby declare that this thesis called "Larval morphology of selected species of families Lampyridae and Silphidae", is my own work, all co-authors of the manuscripts are properly listed, and only the sources listed in the References were used.

Martin Novák

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ABSTRACT

Larvae and pupae are notoriously overlooked and underestimated immature stages of beetles. While information about adults including their descriptions are common, not much is known about the larvae. However, knowledge about morphology and ecology of the juvenile stages can provide us with valuable information in various fields of science. Details about body features can help us in comparative taxonomy and phylogenetics, when the data gathered from studying adults are not sufficient. Knowledge about ecology can help us understand the interactions of the larvae with other animals and the environment, thus giving us valuable clues in possible conservation efforts or even pest control. Consequently, the combination of information about morphology and ecology can have practical utilization in other fields of human efforts, such as forensic entomology within criminal science.

This work presents four published articles and one manuscript accepted for review, predominantly dealing with larval morphology of five selected species of Lampyridae (fireflies) and Silphidae (carrion beetles), and summarizing and expanding information about their ecology. Furthermore, they provide extensive and detailed image documentation using scanning electron microscope. Additionally, general ecology of the families Lampyridae and Silphidae is compared and discussed in this work, as well as differences between the two families on one hand and evolutionary convergences on the other. Morphological structures are given in context with ecology, defensive behavior and feeding habits of the larvae. Bioluminescent display of Lampyridae as well as and ontogeny of larvae and pupae of both families are discussed.

First group of articles deals with all species of Lampyridae occurring in the Czech Republic and surrounding countries. Last instar larvae of *Lampyris noctiluca*, *Lamprohiza splendidula* and *Phosphaenus hemipterus* are described in great detail and discovered morphological features are given in context with their ecology and feeding habits. Bioluminescent display of *L. noctiluca* and *L. splendidula* is discussed. Additionally, pupae of *L. noctiluca* and *L. splendidula* are described, with notes on their development. Larva of *L. splendidula* possesses a unique feature behind stemmata not found in other firefly genera. Larva o *P. hemipterus* possesses numerous sensoria on its head appendages, which is probably connected with its unique ecology.

A key to larvae of all three species is provided, together with detailed comparative table of individual features.

Second group of articles deals with both species of *Thanatophilus* occurring in the Czech Republic. *Thanatophilus rugosus* and *T. sinuatus* are forensically important beetles and their potential in utilization in criminalistics is undisputable. However, besides last instar larvae, no identification key has been so far provided to distinguish between them. All larval instars of both species are thus described in great detail and a key to identify any larval instar within or between the species is provided. Additionally, developmental lengths for *T. rugosus* and *T. sinuatus* are presented for the needs of forensic science and their utilization in criminalistics discussed together with notes on laboratory rearing.

Key words

Lampyris noctiluca; *Lamprohiza splendidula*; *Phosphaenus hemipterus*; *Thanatophilus rugosus*; *Thanatophilus sinuatus*; Silphidae; Lampyridae; larval morphology; forensic entomology

ABSTRAKT

Larvy a kukly jsou notoricky přehlížená a podceňovaná nedospělá stádia brouků. Zatímco informace o dospělcích, včetně jejich popisů, jsou relativně běžné, o larvách se toho příliš neví. Znalost morfologie a ekologie juvenilních stádií nám však může poskytnout cenné informace v různých oblastech vědy. Detailní popisy tělesných struktur nám mohou pomoct v komparativní taxonomii a fylogenezi v případech, kdy studium dospělých stádií nedostačuje. Znalost ekologie nám může pomoct porozumět interakcím mezi larvami, jinými živočichy a okolním prostředím a tak poskytnout cenné informace pro jejich případnou ochranu nebo dokonce pro biologický boj proti škůdcům. Kombinované znalosti o morfologii a ekologii pak nakonec můžou mít praktické využití v dalších oborech vědeckého bádání, jakými jsou například forenzní entomologie v rámci kriminalistiky.

Tato práce představuje komentovaný soubor čtyř publikovaných článků a jednoho manuskriptu přijatého k recenzi, zabývajících se převážně larvální morfologií pěti vybraných druhů čeledí Lampyridae (světlušky) a Silphidae (mrchožroutovití). Dále pak sumarizují a rozšiřují znalosti o jejich ekologii. Vše je doplněno detailní obrazovou dokumentací včetně fotografií pořízených skenovacím elektronovým mikroskopem. Tato práce následně diskutuje obecnou ekologii obou čeledí a rozdíly mezi nimi na jedné straně a společné evoluční konvergence na straně druhé. Nalezené morfologické struktury jsou dány do kontextu s ekologií, obranným chováním a potravními nároky larev. Diskutována je rovněž ontogeneze larev a kukel obou čeledí a bioluminiscenční chování Lampyridae.

První skupina článků pojednává o všech druzích světlušek, které se vyskytují na území České republiky a v okolních zemích. Lze v nich nalézt detailní popisy posledních instarů larev druhů *Lampyris noctiluca*, *Lamprohiza splendidula* a *Phosphaenus hemipterus* a nalezené znaky jsou dány do kontextu s jejich ekologií a potravními nároky. Popisy kukel s poznámkami o jejich vývoji lze nalézt v článcích o druzích *L. noctiluca* a *L. splendidula*. Dále jsou diskutovány světlené projevy larev těchto dvou druhů. U larev *L. splendidula* lze nalézt neobvyklou strukturu blízko stemmat, která nebyla dosud nalezena v jiných rodech světlušek. Larvy *P. hemipterus* mají mnohem větší množství sensorií na přívěscích hlavy, než je tomu o zbylých druhů. Tento fenomén pravděpodobně souvisí s odlišnou ekologií tohoto druhu. Práce nakonec

přináší klíč k určování larev všech tří druhů, včetně detailní komparativní tabulky jednotlivých znaků.

Druhá skupina článků pojednává o obou druzích rodu Thanatophilus, které lze nalézt v České republice. *Thanatophilus rugosus* a *T. sinuatus* jsou forenzně významnými druhy brouků a jejich potenciál ve využití v rámci kriminalistiky je neoddiskutovatelný. Kromě třetího larválního instaru však dosud neexistoval žádný klíč k rozlišení těchto druhů ve všech juvenilních stádiích. To je v těchto článcích napraveno detailním popisem všech instarů. Dále byl sestaven klíč k jejich rozlišení jak mezi druhy, tak v rámci druhů samotných. Uvedené vývojové délky všech nedospělých stádií *T. rugosus* i *T. sinuatus* pak mohou sloužit potřebám forenzní entomologie a jejich využití v kriminalistice je diskutováno společně s poznámkami o chovu těchto brouků v laboratorních podmínkách.

Klíčová slova

Lampyris noctiluca; *Lamprohiza splendidula*; *Phosphaenus hemipterus*; *Thanatophilus rugosus*; *Thanatophilus sinuatus*; Silphidae; Lampyridae; larvální morfologie; forenzní entomologie

Table of Contents

"About your father. If it's any help, he's in the ground now. Sure it's bad news for him. But on the other hand it's party time for all the little worms."

Cat (Red Dwarf)

"We are all worms, but I do believe that I am a glow-worm."

Winston Churchill

1. INTRODUCTION

Fireflies (Lampyridae) and carrion beetles (Silphidae) are two well-known families of polyphagan beetles generally recognized in behalf to their unique life histories. While fireflies gained fame thanks to their ability of light production (bioluminescence), the intimate connection of carrion beetles with cadavers earned them a much less glamorous reputation (Newton 1991; Branham 2010). The adults of most fireflies usually do not feed anymore (although their larvae are always predatory), use light as means of sexual communication and have a slow development, whilst the adults of carrion beetles are usually necrophagous, widely use pheromones and develop relatively quickly (Schwalb 1961; Sikes 2008; Branham 2010; Ikeda et al. 2013). At first glance, these two groups of beetles seem to have very little in common, especially in adult stages, however their larvae share many evolutionary convergences, especially from morphological point of view (within the Silphidae especially in the subfamily Silphinae). The larvae of both families are epigeic; of oniscoid shape; dorsoventrally flattened; with dorsal plates laterally overreaching the body; with expressive pigmentation and strong body sclerotization; and with an eversible organ scattered with numerous microscopic protuberances – pygopod, that is placed inside the last abdominal segment of their body and helps them with locomotion (Newton 1991; Labella & Lloyd 1991).

Studies of beetle larvae are generally underestimated and neglected, however the information of their morphology and ecology provides us with beneficial information, that can be used not only in theory but also in practice.

Descriptions of larval morphology as well as information about ecology are outdated or completely missing in both Lampyridae and Silphidae (Newton 1991; De Cock 2009), however this kind of knowledge can be valuable in many aspects. Within the Lampyridae family, it would help both in taxonomy of species, as well as in their protection. The characteristics of many European species of fireflies are recently mostly based on adult male descriptions only; often using inadequately defined traits. That is why to this day, there are new species being discovered within already established species on one hand, and completely different species have been merged together in the past on the other (McDermott 1966; De Cock & Geisthardt 2007; Geisthardt et al. 2008; De Cock 2009). Additionally, the knowledge about habitat preferences and ecology of larvae, that are the only feeding stage of Lampyridae on the European continent, represents the fundamental information for potential conservation measures. Fireflies are generally threatened by habitat destruction and light pollution, which disrupts their light communication. Decrease in their abundance can be recently seen mainly in Southeast Asia (Lewis 2016).

In Silphidae, the knowledge of larval morphology and ecology is, besides taxonomy, crucial especially for forensic entomology within the criminalistics. The utilization of carrion beetles in criminal science helps detect possible post-mortem body manipulation or estimation of post-mortem interval (PMI) (Bourel et al. 2001; Matuszewski et al. 2013; Ridgeway et al. 2014; Charabidze et al. 2016). The PMI assessment based on entomological evidence (the developmental stages of beetles and flies (Diptera) found on the body) is important especially in cases, where the body was found after more than 72 hours after death. At this point, the state of the body (stiffness, body temperature etc.) does not allow for precise medical time-of-death estimation (Goff 2010). Identification of both species and its developmental stage while combined with information of average developmental length of this specific stage in a specific temperature then allows for determination of the time-of-death of the body the species was found on.

The aim of this thesis is to provide basic information about the species of interest, starting with morphology, which should supply an information bedrock for the organisms and can be further build upon. A thorough summary of current information about basic ecology, expanded by own observations and put in context with morphological features is the next step. With basic morphology and ecology

covered, the door is open to studies of specific structures, behavior and other more detailed research.

The articles presented here describe larvae and pupae of two beetle families occurring in the territory of the Czech Republic and in the central Europe in general. First group of articles represents a trilogy describing and discussing all species of fireflies that can be found in this region. The first article involves *Lampyris noctiluca* (Linnaeus, 1767), the largest firefly species of the area. Second article describes *Lamprohiza splendidula* (Linnaeus, 1767), the most common species of the Czech Republic and at the same time the only species whose winged adults are capable of spontaneous light production. The last article is about a diurnal species *Phosphaenus hemipterus* (Goeze, 1777) that is to this day an almost unknown firefly. Additionally, it provides a key and a feature comparison table to all of the abovementioned species. The second group of articles takes a little bit more sombre note since its main purpose is to serve criminal science. It describes both species of *Thanatophilus* that recently occur in the Czech Republic, providing key to distinguish between the similar beetles and forensically important information that can be used in criminal practice; first article involving *Thanatophilus rugosus* (Linnaeus, 1978) and second article describing *Thanatophilus sinuatus* (Fabricius, 1775). *Thanatophilus dispar* (Herbst, 1793), a third species of this genus occurring in the Czech Republic is recently considered extinct, since its last reliable report comes from the 40’s of the previous century (J. Růžička, unpublished).

2. AIMS OF THESIS

The aim of the thesis is a thorough redescription of larval morphology of selected species of Lampyridae and Silphidae and summary of differences among the specific species and instars within each family. In addition, the thesis outlines and updates information about larval ecology of Lampyridae. Lastly, it provides evaluation of developmental lengths of specific juvenile stages under constant temperature in selected silphid species for the purpose of forensic entomology.

2.1. Specific aims of the thesis:

1. Detailed description of larval morphology of the last instars of all three species of fireflies in the Czech Republic (*Lampyris noctiluca*, *Lamprohiza splendidula* and *Phosphaenus hemipterus*), together with detailed images using scanning election microscope. Summary and expansion of knowledge about their ecology.
2. Compiling of modern identification key to the larvae of central European lampyrids.
3. Detailed description of all larval instars and pupae of species *Thanatophilus rugosus* and *T. sinuatus*, together with detailed images using scanning election microscope, to enable qualitative-character species determination including specific larval instar.
4. Evaluation of developmental lengths of all juvenile stages of the selected species of *Thanatophilus* under constant temperature and finding parameters of the linear model.
5. Summary of the differences among specific larval instars of *T. rugosus* and *T. sinuatus*.

3. LITERATURE REVIEW

3.1. Larval morphology and ecology of Lampyridae

The family Lampyridae consists of approximately 2000 species in 83 genera and 12 subfamilies, distributed predominantly in relatively humid areas of Southeastern Asia and Latin America, with only a handful of species found in arid regions (Branham 2010). There is a recent rise of interest in studies of morphology of both adults and larvae, especially in genera from Oriental and Neotropical Realms (Archangelsky 2004, 2010; Deheyn & Ballantyne 2009; Fu et al. 2012; Ballantyne et al. 2013), where new species are being discovered and descriptions of the known ones are being extended with utilization of modern technologies not available in the past (Ballantyne & Menayah 2002; Fu et al. 2012). A digital camera can provide high quality images of the whole body habitus with reasonable level of detail. Additionally, high depth of field of these images, which was difficult to achieve in higher magnifications can now be reached with help of stacking software, combining multiple images with low depth of field into one final sharp photo. Electron microscopy can then provide detailed images of various microstructures that could not be observed with usual optical equipment. Enhanced digital microscopes represent a bridge between these two approaches, providing images of higher detail than optical tools, yet preserving the color and optical properties that are lost in electron microscopy. Moreover, a micro CT machine can scan a 3D image of the whole insect including its inner organs, thus providing the most realistic illustration to date (Wipfler et al. 2016). All these tools provide contemporary researchers with means to create high-detailed image files for descriptions that will not turn obsolete in a few decades and will be still relevant even for future scientists.

Fireflies can be found on the majority of the European continent, but their distribution is scattered. This is due to their preference of warm, humid environments and open landscape (Hůrka 2005; Branham 2010). There is approximately 64 recognized European species of fireflies in eight genera (Geisthardt & Satô 2007), however this number may likely change in future due to many ambiguities in outdated taxonomy (De Cock & Geisthardt 2007; Geisthardt et al. 2008; De Cock 2009). Our knowledge about European fireflies comes predominantly from the first half of the 20th century, later expanded by next generation of authors in 60’s and 70’s (Vogel 1912; Schwalb

1961; Papi 1969), when latest taxonomic revisions within the continent have been published (Viviani 2001). Complex phylogeographic studies and taxonomic information concerning European species are nevertheless incomplete and often hard to find, since specific firefly genera are usually distributed across more than one continent (Stanger-Hall et al. 2007).

The descriptions, observations and experiments performed on these beetles in the past should be revisited, updated and expanded, considering the larger possibilities, advanced knowledge and more effective tools of research we have at hand in the 21st century (De Cock 2009). Various types of high definition cameras capable to make videos of high frame rate to observe detailed movements during insect flight, IR cameras to observe behavior in low light conditions, gas chromatographs to detect volatile compounds like pheromones, light sources like LEDs of different spectra or tritium-based betalights that do not require power to perform ethology experiments on bioluminescent animals etc. enable for easier and more sophisticated studies of these beetles.

Within the superfamily Elateroidea, the Lampyrid larvae are characterized by presence of the epicranial suture and the light organ, usually located on ventral side of the seventh abdominal segment (Stehr 1991). The other characteristics include falcate (sickle-shaped) mandibles lacking molar region, either cleft longitudinally or with an inner channel; reduced articulated areas of maxillae; ill-defined labrum that can be part of nasale; pygopod, helping with movement; and five-segmented legs with tarsus and claw conjoined in pretarsus (Stehr 1991).

The larvae prefer environment with mean values of ecological factors, for example balanced level of humidity and moisture. They can be found along watercourses and water bodies, in leaf litter, on decomposing wood or under the stones. In arid regions, they usually stay hidden underground and resurface at night or immediately after rain (Grimaldi & Engel 2005; Branham 2010). The number of larval instars is probably correlated with the length of photoperiod and depending on the species, the overall duration of larval stage takes from several months up to two or three years, followed by pupal stage (Schwalb 1961; Tyler 2002; Branham 2010). During pupation, some species dig small underground chambers, others build mud chambers on the soil called „igloo“ or prefer pupating in decomposing wood or leaf litter (Grimaldi & Engel 2005; Branham 2010).

Whereas adults of fireflies often do not feed anymore, their larvae are fierce predators. Their prey predominantly consists of terrestrial snails or earthworms, however aquatic firefly larvae of some Asian species hunting water snails have been known too (Fu et al. 2006; Branham 2010). The larvae are able to track the slime trail of their prey as well as its polarization, thus never pursuing the target in the wrong direction (Branham 2010). The higher activity of larvae in moist environment is commonly regarded as a consequence of higher activity of their prey (Viviani 2001).

All known firefly larvae are capable of bioluminescence (Branham 2010) and some species are even able to produce light even before hatching from the egg (Hůrka & Čepická 1978). The majority of species produce light by a paired light organ (lantern) situated ventrally on the seventh abdominal segment, with the only known exceptions in genera *Lamprohiza* Motschulsky, 1853 and *Phausis* Leconte, 1851, that produce light by lanterns placed dorsally on second to sixth abdominal segments (Grimaldi & Engel 2005; Hůrka 2005; Branham 2010).

In the Czech Republic, we can find only three, often sympatric, species of fireflies (De Cock 2004), that belong to subfamily Lampyrinae and its tribes Lampyrini and Lucidotini. First one is *Lampyris noctiluca*. Genus *Lampyris* Geoffroy, 1762 belongs to tribe Lampyrini and consists of 60 described species distributed in Palearctic and Afrotropical Regions, of which 29 species can be found in Europe. Second species is *Lamprohiza splendidula*, in older literature known as *Phausis splendidula*. Genus *Lamprohiza* belonging to tribe Photinini, consists of only eight described species distributed exclusively in Europe; from southwestern parts of the continent, to its central and southeastern part. The last species of concern is *Phosphaenus hemipterus*, the only member of genus *Phosphaenus* Laporte, 1833, that belongs to the tribe Photinini and is currently distributed in Europe and eastern part of North America (Burakowski 2003; Geisthardt & Satô 2007). An interesting conclusions may come from future research of a poorly known genus *Phosphaenopterus* Schaufuss, 1870 in regard to *Phosphaenus*, with which it shares many common traits. *Phosphaenopterus* houses only two species; *Phosphaenopterus montadoni* Bourgeois, 1900, that can be found in Romania and *P. metzneri* Schaufuss, 1870 found in Portugal and French part of Pyrenees mountains. There have been no new reports regarding these species since the time they have been discovered for the first time (De Cock 2009). It is quite possible, that these are in fact just macropterous

forms of *Phosphaenus hemipterus* (Mikšić 1982), especially since they occur in the outer borders of its distribution area (De Cock 2009). However, thorough studies of museum material including types and sufficient fresh material for genetic and morphological analysis will be necessary to make any phylogenetic relationships clear.

Lampyris noctiluca is distributed across the almost the whole Palearctic Region, in Europe being absent only in Iceland and Ireland, in Asia reaching all the way to Mongolia and Russian Far East. In Europe, the species occupies warm and moist habitats on limestone substrate, close to deciduous forests (Schwalb 1961; Burakowski 2003). It can be found in lowlands but also in mountains in altitudes up to 1800 m a.s.l. (Hůrka 2005). Both geographical and vertical distribution of *Lampyris noctiluca* reveals general relatively high tolerance of this species to temperature and miscellaneous environment (Schwalb 1961).

Lamprohiza splendidula can be found almost over the whole Europe except its western part, distributed predominantly in its central and southeastern part (Burakowski 2003; Geisthardt & Satô 2007). It is the most common firefly in the Czech Republic (Hůrka 2005). This species prefers humid, shady and opened habitats of lowlands and highlands with deciduous forests. It can be found in thickets, glades, banks of brooks and creeks, on meadows or even in gardens (Schwalb 1961; Burakowski 2003; Hůrka 2005).

Phosphaenus hemipterus is distributed from England, Denmark, southern Sweden, Finland and Karelia through central Europe up to Pyrenees mountains, northern Italy, western part of the Balkan Peninsula, Transylvania and Ukraine (Burakowski 2003; Geisthardt & Satô 2007). To this day, it is the only European species of firefly that has been known to be imported to another continent, since it has been found as far as in Nova Scotia in Canada (Tyler 2002). This unintended introduction was probably facilitated by high tolerance or even preference of this species to human-altered environments. *Phosphaenus hemipterus* was considered a rare and little known species until only recently. The reason for this belief was probably the fact, that while this firefly can be found mainly in areas with high level of human-caused disturbance like gardens, parks, edges of fields or even parking lots, most of the previous research was performed in areas minimally influenced by humans (De Cock 2000). It is possible that this species is not as rare as expected after all.

Moreover, it can be even found in habitats that are considered irrelevant from conservation management point of view (De Cock 2000).

Despite its briefness, the morphology of adults of the three species of interest is relatively known. On the other hand, descriptions of the larvae are outdated and detailed information regarding ecology along with quality images are either insufficient or missing (Reitter, 1911; Korschefsky, 1951; Kratochvíl, 1957; Medvedev & Ryvkin, 1992; Klausnitzer, 1994; Burakowski, 2003).

3.2. Larval morphology and ecology of Silphidae

The Silphidae family consists of only about 185 species worldwide, divided into two subfamilies – Silphinae and Nicrophorinae. Distribution of this family is probably determined by combination of its ecological demands and evolutionary history. Silphidae prefer simultaneously colder and humid environment and most species can be found in the eastern part of Palearctic Region, where this family probably originated (Sikes 2008; Sikes et al. 2002; Růžička 2015). Their distribution is therefore quite limited in Afrotropical and Australian Regions. The worldwide revision of Silphidae was created in the 20's of the last century (Portevin 1926) and recent works include a worldwide catalogue published for subfamily Nicrophorinae only (Sikes et al. 2002). There are 30 species of Silphidae occurring in central Europe, from which 20 species are members of the Silphinae (carrion beetles) and 10 are members of Nicrophorinae (burying beetles). Much less work has been dedicated to the carrion beetles compared to the burying beetles and not much is known about their ecology (Růžička & Jakubec 2016). Yet their utilization in forensic entomology is undisputable, thus the focus of this theses lies on the members of the Silphinae subfamily only.

Carrion beetles together with flies (Diptera) represent the two most significant groups of insects that live on carrion of large vertebrates, including human (Midgley et al. 2010). It is the tight connection of obligate necrophages on carrion as a food source that enables their utilization in forensic entomology. However, not all members of this subfamily are as specialized as the common name suggests, as they include predators and phytophages, limiting their use in criminal science only to the facultative and obligate necrophages, represented by genera *Oiceoptoma* Leach, 1815, *Silpha*

Linnaeus, 1758 (some species only), *Necrodes* Leach, 1815 and *Thanatophilus* Leach, 1815 (Sikes 2008; Ikeda et al. 2013).

The usual features of larvae of Silphinae are mandibles without molar region; maxillae with broad mala with dense shrub of setae on its apex; and articulated, usually trimerous urogomphi (Newton 1991). The overall development takes only up to several months and larvae go through three instars. They overwinter either as adults, or in the last larval stage called prepupa, while pupation takes place in spring. The primary food source of larvae of the most species is carrion of vertebrates (Sikes 2008).

Species identification of necrophagous beetle larvae in forensics has been problematic since morphological descriptions and identification keys even for common species are not available. So far this issue has been dealt with by rearing the animal to adulthood. At that stage, the abundance of literature can be used for morphological identification, however this method is quite time-consuming and the results uncertain (Amendt et al. 2004). Another option of identification is provided by molecular methods (e.g. barcoding) which is currently a standard method in forensic investigations. Nevertheless, this is not always easily achievable due to possible degradation of DNA of old or incorrectly preserved specimens or a close kinship of species, especially when the data on the level of their intraspecific and interspecific DNA variability are scarce (Wells & Sperling 2001; Wells et al. 2001; Willows-Munro & Schoeman 2015; Cho et al. 2016).

Instar identification represents another problem. The most of the published morphological descriptions of larvae are based on mature larvae only, meaning individuals of the third instar. To overcome this issue, several statistical models dealing with size of various body parts of the larva in relation to the specific instar have been created (Midgley & Villet 2009; Velásquez & Viloria 2010; Frątczak & Matuszewski 2014; Frątczak et al. 2016; Jakubec 2016). The results of these models are nonetheless unreliable, since the larval body-size can significantly vary among the individuals of the same species coming from different populations or environments, or even depending on the chemical used to fix the specimen (Stillwell & Fox 2009; Midgley & Villet 2009).

Solution to both of these problems seems to lie in composing of a detailed morphological description of all larval instars of a specific species and finding

qualitative features or their combinations that would enable for reliable determination of the specific stages.

3.3. Comparison of general ecology of Lampyridae and Silphidae

Even though both families are semelparous (i.e. reproducing only once in a lifetime), they differ proportionately in lengths of larval and adult stages. The combination of resource exploitation and niche occupation in juvenile and adult stages seems to have an essential effect on this ratio (Lawrence & Newton 1982).

Fireflies of central Europe (and Elateriformia in general) have long lived larvae and short lived adults, with maximum lifespan up to three weeks (Burakowski 2003; Hůrka 2005). Different authors present different data on the length of the developmental cycle of two out of three species studied in this work and it varies between two years (with two overwinterings; Tyler 2002) and three years (and three overwinterings; Schwalb 1961). On the other hand, adults of Silphidae (and Staphilynoidea in general) are generally long-lived while larvae complete development in a few weeks (Lawrence & Newton 1982).

In fireflies, adults and larvae occupy different food niches; larvae are predators, while adults usually do not feed anymore or predate other firefly species and generally dwell in the shrub levels of the habitat (Lawrence & Newton 1982; Gronquist et al. 2006; De Cock 2009). According to Crowson (1981), where only the larvae live in the litter, the adults are usually short lived and markedly seasonal in occurrence. However, where the entire life cycle occurs in the litter, adults are normally long lived and may be found in the litter at all seasons whereas larvae occur at restricted seasons.

Adults of Silphidae generally live long, while larvae can complete their development in several weeks (Lawrence & Newton 1982). In the member species of Silphinae that are necrophagous, both immature and adult stages occupy the same habitat and exploit the same temporary resource (Lawrence & Newton 1982; Sikes 2005). The difference in juvenile-adult lifespan between Lampyridae and Silphidae is supported by the difference in availability of their preferred food source, when relative abundance of phytophagous prey for the Lampyrid predator is compared to the transience of carrion food source for the Silphid necrophage.

Number of larval instars in Coleoptera varies from one to almost 30, but is usually three to five. In Silphidae, the number is three (Lengerken 1929; Stehr 1991). In contrast to carrion beetles, the number of instars in central European species of Lampyridae varies according to different authors from five (Hůrka & Čepická 1978) to four to six (Schwalb 1961). Depending on the sex, it can also be five for males with possibly more for females (Tyler 2002), and the variability is believed to be dependent on environmental conditions and food availability. In larger species of fireflies, the number of instars can be even five to six for males and eight for females (unidentified *Lampyris* species from the Balkans; M. Novák, unpublished data). So far, no study has been performed to test the relations between environmental conditions and number of instars in Lampyridae.

Flightlessness in insects is generally thought to have evolved due to changes in habitat environment or habitat isolation. Brachypterous or apterous species are fairly common in the leaf litter fauna of many regions, and naturally such species tend to have more limited geographical ranges than their fully winged relatives (Crowson 1981; Ikeda 2008). Female neoteny or inability of flight is typical for all European species of fireflies (De Cock 2009). Within the Silphidae, only predatory species in relatively stable environments can afford flightlessness, since food availability for predators is relatively stable compared to carrion feeders. The most flight-capable species are the ones with obligate necrophagy and the evolutionary connection between flight capability and food habit, relative reproductive investment, and egg size has been shown for Silphidae by Ikeda (2008). There is a trade-off between ontogenetic development of female flight ability and egg production. Flightless females can invest more resources into egg production. On the other hand, with less eggs produced, the female can invest more resources into the egg itself, thus producing larger eggs and consequently larger, livelier larvae. Progeny that hatch from larger eggs are likely to have higher juvenile survivorship because they can better withstand environmental stresses such as starvation and desiccation, are superior in larval competition, and have a large range of food sizes (Ikeda 2008).

The opposite effect is shown in Lampyridae, where larvae are predatory, adults usually do not feed anymore and females lay hundreds of eggs (Ikeda 2008, Branham 2010; M. Novák, unpublished obs.). However, for obligate necrophagous species, flight capability and investment into egg quality rather than quantity seems like a better (or

the only possible) evolutionary strategy, considering unpredictable food availability together with its temporal limitation and considerable competition with other species or orders of carrion insects (e.g. Diptera) (Ikeda 2008).

Large dependence on olfactory signals and pheromone communication has been known in the Nicrophorinae (burying beetles) subfamily of Silphidae. Searching behavior for food is guided by olfaction and the burying beetles have sensitive chemosensors located on their antennae adapted to detect the smell of a recently dead animal. If a male discovers a suitable carcass for reproduction, it emits a sexual pheromone to attract the female (Dekeirsschieter et al. 2011b). Unfortunately, not much is known about the ecology of Silphinae, even though it is generally established, that the sense of smell and sexual pheromones in necrobiont species in general play a large role in their life histories (Kalinová et al. 2009; Podskalská et al. 2009; Dekeirsschieter et al. 2013; Fockink et al. 2013; Hoermann et al. 2013, 2016; Dubie et al. 2017). Moreover, the capability to find the carrion in short amount of time is crucial in highly competitive environments, considering the unpredictability of the food source in both space and time (Barton et al., 2013).

The adults of the majority of firefly species are not able to produce light and their courtship behavior is facilitated by pheromone communication, too (Lloyd 1973; De Cock & Matthysen 2005). However, there is a general assumption that the pheromone communication has been altogether switched for light communication in night-active firefly species, where adults are capable of bioluminescence (Branham 2010). The ability of light production originated several times independently within the Lampyridae family as well as several times disappeared to be replaced back with pheromone communication again (Grimaldi & Engel 2005; Lewis & Cratsley 2008; Gullan & Cranson 2010). It is hypothesized, that bioluminescence originally served only as means of defense in all developmental stages of the insect and the utilization in courtship is secondary (Case 2004). However, a recent study of a Nearctic firefly of the genus *Phausis* shows, that pheromones might play a certain role even in species that normally use light to find a mate (De Cock et al. 2014).

Unlike adults, larvae of all known firefly species are capable of bioluminescence (Branham 2010). Its manifestation is species-specific and can be simple or consist of several different types of display. Besides aposematism, other possible reasons for light production in larval stages are unknown to this day and only hypotheses exist.

For instance, larva of *Lampyris noctiluca* can produce light in three different ways (Tyler 2002). One, when disturbed, it will sometimes switch on its lights for a few seconds and then turn them off again. This seems to be a defensive mechanism to scare off potential predators. Two, some larvae have been known to glow continuously for hours, without any apparent provocation. These are, according to Tyler (2002), often fully grown larvae ready to pupate. He suspects this glow, which is very similar to that of an adult female, might be just part of the preparation for adulthood, at a time when the larva's body is undergoing internal changes. A third type of photic display described by Tyler (2002) is sometimes produced during movement and larvae displaying this behavior are called "walkabouts". It consists of definite pulses of light lasting ca. 2 seconds, separated from the next one by a longer interval of darkness lasting ca. 4 seconds, although Dreisig (1974) reports glows lasting 7.3 seconds on average and the interval of darkness of 20.2 seconds on average. The intensity of each pulse gradually builds up, followed by a period of steady brightness and then a final period, during which the light fades and goes out altogether (Schwalb 1961; Tyler 2002). Very similar walkabout behavior (but with different frequency and length of pulses) has been observed also in other European firefly genera (*Phosphaenus hemipterus*, De Cock 2003; *Luciola novaki* Müller, 1946, M. Novák, unpublished obs.). The simple type of larval photic display can be seen in e.g. *Lamprohiza splendidula*, where larvae appear reluctant to glow without external provocation. They emit a weak continuous glow when handled or even approached. They react both to vibrations and to loud noises (which, as a side effect, easily facilitates collecting them in the field). However, the light intensity may weaken or completely stop in certain cases, usually as a result of overstimulation (Schwalb 1961).

3.4. Evolutionary convergences of Lampyrid and Silphid larvae

Considering central European species, Lampyridae generally prefer open landscape at the edge of a forest, so that adults can effectively perform their luminous courtship, while larvae can stay hidden under the bushes and hunt prey in the humid environment (Branham 2010). Silphidae prefer either open landscape or forest environment, with their larvae staying close to decomposing carrion, using it both as a shelter and a food source (Jakubec & Růžička 2016). All in all, epigeic lifestyle of larvae of both groups reflects on their external morphology.

The popular image of a beetle as a black insect is not altogether without foundation. There is probably a greater proportion of black species in Coleoptera than in any other major order (Crowson 1981). Silphidae are no exception, being composed very largely of black or dark species and larvae being heavily sclerotized and pigmented. Moreover, in adult beetles, there is a general correlation of black color with nocturnal activity that is innate to most members of the Silphidae family (Crowson 1981, Newton 1991; Kočárek 2001), even though some genera are diurnal (e.g. *Thanatophilus*; Kočárek 2001). For largely nocturnal Lampyridae, it is not surprising that their adults and larvae are also predominantly dark-colored, at least within the European continent (Burakowski 2003).

Onisciform shape is defined as somewhat flattened body form, resembling terrestrial Isopoda (Caipinera 2008). In both fireflies and carrion beetles, this shape, combined with the abovementioned heavy sclerotization of the cuticle is considered an antipredatory adaptation, making the larvae difficult to grasp by a predator (Crowson 1981) and possibly making them move more easily in the accumulation of dead leaves, twigs, branches etc., which the litter layer of the ground is composed of, or in the decaying carrion. Additionally, paratergites as lateral parts of terga overreaching the body are very similar to those seen in terrestrial isopods (e.g. woodlice), where they clearly serve defensive purpose (Tuf et al. 2015).

Anal proleg, or pygopod is another feature common to surface-active type of larvae (Crowson 1981). It is composed of branching eversible membranous holdfast organs of various number depending on the species, and can be bare or hold various protuberances on the surface (Stehr 1991). It is generally believed this organ helps larva with locomotion, but in Lampyridae it bears numerous small spines and hooks and in addition to movement, the larva uses it to clean itself after feasting on its prey (Tyler 2002). In Silphinae, the pygopod bears numerous fine teeth (Stehr 1991), but it has yet to be found, if it serves another purpose besides locomotion.

Lampyrids are known to use extraoral digestion when hunting for prey (Branham 2010). This ability has been also reported for some members of the necrophagous Silphidae (Lawrence & Newton 1982), although there has been no thorough research concerning this topic. Firefly larvae generally feed on soft, slimy invertebrates, predominantly snails, with some species preferring earthworms as their diet. Some species can also exhibit facultative necrophagy, when they feed on fresh animal

cadavers that offer access through wounds to soft body parts (Schwalb 1961; Lloyd 2008). They are reported to follow 2-day-old slime-trails forward when tracking the snails, and can detect polarization in dry, if not stale, trails (Lloyd 2008). The snail *Cepaea nemoralis* (Linnaeus, 1758) (Pulmonata: Helicidae) is one of the preferred prey of central European species and is known to occur in three color variations; brown, yellow and banded yellow (Honěk 1995; Peltanová et al. 2012), with significant larval preference for non-banded types (O'Donald 1968). The reason for this preference remains unknown to this day (De Cock 2009). Larvae pierce the anterior part of the snail with their channeled mandibles and inject them with a dark secretion produced by a paired gland in foregut, possibly a neurotoxin, thus being able to incapacitate individuals with several time larger body mass (Schwalb 1961; Klots & Klots 1963; Hůrka & Čepická 1978; LaBella & Lloyd 1991; Branham 2010). They are also known to "ride" the snails, i.e. mounting the shell attaching themselves to it by the pygopod and assuming a favorable position to attack the head of the snail or its upper tentacles. In either case this is the best way to get the poison as close as possible to the center of the snail's nervous system (Schwalb 1961; Tyler 2002). Contrary to a widespread belief (Klots & Klots 1963; Hůrka & Čepická 1978), the toxin itself does not appear to predigest the prey, but the larva rather seems to chop out pieces of flesh with its mandibles while using digestive intestinal secretion, as was observed by Schwalb (1961). The resulting liquid is then ingested by maxillae and labium, while simultaneously being sifted from larger particles thanks to fine dense setation of these structures (Klots & Klots 1963; Hůrka & Čepická 1978). The hunting style of earthworm feeding species has not been described to this day.

In both families Lampyridae and Silphidae, mandibles are completely missing mola and prostheca, which is considered to be connected with extraoral digestion and intake of partially liquefied food (Crowson 1981; Branham 2010).

Defensive chemicals and chemical defense mechanisms have been discovered in some members of both families. Fireflies possess defensive chemicals called lucibufagins that make them generally unpalatable to predators (Eisner et al. 1978; De Cock & Matthysen 2003; Fu et al. 2007). Moreover, reflexive bleeding of larvae of *Nyctophila heydeni* (Olivier, 1884), a genus closely related to *Lampyris*, has been documented (De Cock et al. 2017). Chemical defenses are common also in Silphidae, either providing

distastefulness or ability to secrete defensive spray or ooze to deter predators (Eisner & Meindwald 1982; Eisner et al. 1985; 1986; J. Růžička pers. comm.).

3.5. Utilization of larval morphology and ecology of Silphid larvae in criminalistics

For the most precise calculation of the Post Mortem Interval (PMI) in forensic entomology criminal science, it is essential to correctly identify the specific necrophagous beetle species found on the body, its larval stage and relate this information to the presumed developmental length given by a thermal summation model. The rate of development of the animal is influenced by the temperature of its surrounding environment. Thermal-summation models thus generalize the effect of the specific temperature on the developmental length of the studied organism (Richards & Villet 2008). Since they are specific for each species, a correct taxonomic determination is crucial. At the same time, these models are specific for each developmental stage, i.e. egg, specific larval instar and pupa (Ridgeway et al. 2014). Thus, given the known length of development of the oldest of these stages found on the body, the approximate time of death can be estimated from the course of temperatures on the crime scene. However, incorrect identification of the species or its developmental stage, especially in larvae, can lead to a significant distortion of such estimate.

Selected species of genus *Thanatophilus* of subfamily Silphinae have been chosen as ideal organisms for application of the abovementioned approach. Members of this genus share not only general appearance, but they also have a very similar ecology. All known species are necrophagous in all active stages of development (larvae and adults) and they flourish on larger carrions of vertebrates, including humans. They appear to prefer earlier stages of decomposition and can commence to breed in the first 24 hours after death (Midgley & Villet 2009). Genus *Thanatophilus* recently consists of 23 valid species, of which fourteen are distributed in Palearctic Region, four in Nearctic, two are Holarctic in distribution and three occur in Afrotropical Region (Anderson & Peck 1985; Navarette-Heredia 2009; Růžička 2015). Taxonomy and classification of the *Thanatophilus* based on adult morphology has been revised in the 80's (Schawaller 1981) and is being expanded to this day (Kozminykh 1994; Růžička 2002; Ji 2012). They are phylogenetically postulated as a

sister group to the remaining Holarctic genera of Silphinae, altogether forming a larger cluster, which is a sister group to Neotropical and Australian genera *Oxelytrum* Gistel, 1848 and *Ptomaphila* Kirby & Spence, 1828 (Dobler & Müller 2000; Sikes et al. 2005).

Adult specimens of this genus can be identified based on several available identification keys (e.g., Anderson & Peck 1985; Navarette-Heredia 2009; Schwaller 1981; Šustek 1981; Ratcliffe 1996; Nikolaev & Kozminykh 2002), however, not much is known about the larvae of this genus and so far they have been described for eight species only. Moreover, these descriptions are often based on an unknown larval instar and many of them are quite brief and lacking images (e.g. Xambeau 1892, 1900; Lengerken 1929, 1938; Paulian 1941; Anderson 1987). The recognition of their usefulness nonetheless sparked a new interest among researches and larvae of two member species (*T. capensis* Wiedemann, 1812 [junior synonym *T. mutilatus* Laporte de Castelnau, 1840] and *T. micans* (Fabricius, 1794)) were re-described in the past few years as a result (Daniel et al. 2017). Along with morphological description of some of the larvae, the thermal summation models and instar identification models were developed (Ridgeway et al. 2014; Midgley & Villet 2009; Daniel et al. 2017; Frątczak & Matuszewski 2016). All this information is essential to estimate the time of colonization of the carrion, which is widely used as a proxy for PMI.

Two recently described larvae belong to species occurring almost exclusively in Africa (*T. micans* was also reported from Yemen [Růžička 2015]), which is somewhat disproportional to the fact that the center of biodiversity of the genus *Thanatophilus* is in the Palearctic Region (Růžička 2015; Schawaller 1981). To help covering this knowledge gap, *Thanatophilus rugosus* and *T. sinuatus* were chosen as focal species. They belong among abundant central European beetles, prefer open landscape environments, often occurring on carrion of vertebrates (Kočárek 2003; Matuszewski et al. 2010; Dekeirsschieter et al. 2011a; Jakubec & Růžička 2012).

Thanatophilus rugosus is widespread in the Palearctic Region, with distribution reaching from Europe to Japan (Růžička 2015), being considered a very common necrophagous beetle in Europe (Kočárek 2003; Matuszewski et al. 2010; Dekeirsschieter et al. 2011a). Similar to other carrion beetles (Silphidae) (Ridgeway et al. 2014; Charabidze et al. 2016; Velásquez & Viloria 2009, Matuszewski 2011) this species might become a very valuable forensic indicator, as its presence has been

detected in 16% of cases of entomological evidence collections on human remains in the Czech Republic (23 of 144 cases between years 2003 and 2016, H. Šuláková, unpublished data). However, only little is known about the immature stages of *T. rugosus*. The first description of unknown larval instar with brief notes regarding biology of adults was published at the end of the 19th century (Xambeau 1900). The description is nonetheless not detailed enough to guarantee correct species identification. Second and at the same time the last description of larval morphology of this species originated in the first half of the 20th century (Lengerken 1929), this time containing description of all three larval instars. The author of this work however confesses, that reliable species identification and distinction among the larval stages of *T. rugosus* and its sibling species *Thanatophilus dispar* and *Thanatophilus sinuatus* is possible only with comparative material at hand. He saw the body-size difference as the only solution to instar determination, even though he admits that due to high level of phenotypic plasticity in different environments such method is not very reliable.

Thanatophilus sinuatus has a very wide trans-Palearctic distribution (occurring across Europe, Asia and North Africa) and as well as *T. rugosus* is a very common beetle on the European continent (Růžička 2015; Jakubec & Růžička 2012). Presence of adults and larvae of *T. sinuatus* was recorded on 13.27 % (26 out of 196 between years 2003 and 2016, H. Šuláková, unpublished data) of human remains that were investigated by forensic entomologists in the Czech Republic. Moreover, the species is known to replace blowflies, one of the crucial groups of insects in forensic entomology, during the colder parts of the year (Bonacci et al. 2011). These findings show that *T. sinuatus* is yet another important indicator in the field of forensic entomology.

The need for its thorough redescription comes from the fact that it often co-occurs with *T. rugosus* (Linnaeus, 1758) (Jakubec & Růžička 2015; Frątczak-Łagiewska & Matuszewski 2018). Even though the larvae of this species have been described in the past by several authors (Xambeu 1892; Lengerken 1929, 1938; Paulian 1941), their reliable distinction from *T. rugosus* as well as instar identification is very problematic and only the third instar larvae can be identified to the species level (Díaz-Aranda et al. 2018; Frątczak-Łagiewska & Matuszewski 2018). Whilst these species possess many similarities, it is generally not safe to assume the information they provide are interchangeable. This was already proven for *T. capensis* and *T. micans* and the

potential error can be highly significant (Ridgeway et al. 2014). Finding unique morphological characters that would allow for precise identification is therefore essential for possible practical applications.

4. PUBLISHED WORK

The list of scientific articles published in peer-reviewed journals together with a manuscript in review that represent the core of this thesis is presented below. The articles and the manuscript can be found in the Annexes section of this work.

Annex 1

Novák M. (2017): Redescription of central European firefly larvae, Part 1: *Lampyris noctiluca* (Linnaeus, 1758) (Coleoptera: Lampyridae). *Zootaxa*, 4247: 429-444.

Annex 2

Novák M. (2018): Redescription of immature stages of central European fireflies, Part 2: *Lamprohiza splendidula* (Linnaeus, 1767) larva, pupa and notes on its life cycle and behaviour (Coleoptera: Lampyridae). *Zootaxa*, 4378: 516-532.

Annex 3

Novák M. (2018): Redescription of immature stages of central European fireflies, Part 3: *Phosphaenus hemipterus* (Goeze, 1777) larva, and notes on its life cycle and behaviour, with a key to three central European lampyrid larvae (Coleoptera: Lampyridae). *Zootaxa*, 4382: 450-464.

Annex 4

Novák M., Jakubec P., Qubaiová J., Šuláková H. & Růžička J. (2018): Revisited larval morphology of *Thanatophilus rugosus* (Coleoptera: Silphidae). *International Journal of Legal Medicine*, 132: 939-954.

Annex 5: Manuscript under review

Jakubec P, **Novák M.**, Qubaiová J., Šuláková H & Růžička J. (2018): Description of immature stages of *Thanatophilus sinuatus* (Coleoptera: Silphidae). *International Journal of Legal Medicine.* (accepted for review 07/2018)

5. DISCUSSION

5.1. Morphology of sensory organs

Detailed observation of the larval cuticle under scanning electron microscope revealed different types of *sensilla* and sensory organs in all studied species of Lampyridae and Silphidae. Since the exact determination of type and function of the observed sensory organs would merit a separate work, the following paragraphs will be dedicated to only a brief description and speculations on possible functions, with regards to the ecological aspects.

Sensilla trichodea and *sensilla chaetica* were the most abundant sensilla observed in all of the studied larvae. They resemble hairs of various length and thickness, freely movable in basal membrane or growing from a socket (Shileds 2008). Chemosensitivity and olfactory function of these structures could be possible for sensilla situated on the antennae, mechanoreceptive and thermosensitive function on legs, thorax and abdomen. Additionally, setae on ventrites generally have an auxiliary function during molting (Tyler 2002).

Sensilla coeloconica are defined as basiconic pegs or cones set in a shallow pit, most often chemo-, thermo-, or hygrosensitive (Shields 2008). They were observed on the epicranial plate and antennae of *Lampyris noctiluca*, where chemo-, thermo-, or hygrosensitive function seems plausible. Additionally, this sensillum seems to have a different form on the apex of antennomere II of this species than the rest of its body. In *Phosphaenus hemipterus*, these sensilla were also observed on antennae and uniquely on mandibles, where their presence might be connected with either a different type of prey, or pheromone communication in adults of this species, as will be discussed below. In *Thanatophilus rugosus* and *T. sinuatus*, this this type of sensillum was observed on the distal segments of labial and maxillary palps, where their olfactory or chemoreceptive function in connection with feeding seems likely. In *Lamprohiza splendidula*, this type sensillum was completely missing.

A unique type of sensillum was observed in *Phosphaenus hemipterus* on antennae, legs, and sclerotized parts of dorsum and venter. It is composed of a fibrous, weak seta set in a shallow toroidal socket. There is uncertainty, whether this process is just a modification of sensillum trichodeum or sensillum coeloconicum. Arguments for sensillum trichodeum are the wide occurrence on the body of the larva and

mechanoreceptive function, together with a fact that the observed sensillum is fibrous, instead of peg- or cone-shaped. Arguments for sensillum coeloconicum are the shallow socket, and the fact that the sensilla occurs with numerous modifications, together with unique prey type and ecology of *Phosphaenus hemipterus*, which may result in a need for different sensory organs.

The cuticle of the sclerotized areas of the legs, dorsum and venter in larvae of *Lampyris noctiluca* is densely littered with microscopic granulose protuberances. This unique characteristic could be the reason why the body of this species gives the impression of being "velvet" like. Whether the function of these protuberances is sensory, insulatory or other is nonetheless unknown.

The third antennomere (flagellum) in the observed lampyrid larvae is very short compared to the size of scape and pedicel; in some cases even shorter than the antennal sensorium. In all three firefly species, it has three articulated setae and two setae without articulation growing from its apex. Moreover, there is one more articulated sensillum on its base. In *Phosphaenus hemipterus*, this sensillum is replaced with an additional small vesicle, possibly a sensorium. The extra sensoria on flagellum are described in larvae of Scarabaeidae and are called *dorsal sensory spots* by Ritcher in 1966 (Lawrence & Ślipiński 2013). In *Thanatophilus rugosus* and *T. siunatus*, the flagellum is well developed and of similar length compared to the remaining antennomeres; bearing two articulated setae and two setae without articulation on its apex. The whole antennomere is scattered with sensilla chaetica, mainly in its distal half.

The antennal sensoria were found to have unique features in some of the studied species. Sensorium of *Thanatophilus sinuatus* is bearing a circle of small button-like sensilla placed around its apex with another circle of small sensillar pits closer to its base. We have expected to find a similar feature in the sibling species *T. rugosus*, however none was observed. Unfortunately the quality of the larval material of *T. rugosus* designated for the electron microscopy was not as good as in the former species, so it is still possible, that this structure of sensorium is common for the *Thanatophilus* genus as a whole and we just failed to see it on the low quality specimens. Hopefully, this question will be answered in future studies. Additionally, a typical group of three peg-like sensilla growing next to the sensorium was observed in both species. This feature is missing in lampyrid larvae, although occasional solitary

pig-like sensilla growing close to the sensorium were observed in some of the larvae. Sensorium of *Phosphaenus hemipterus* firefly bears very fine helical ridges from its bottom to the apex. It can be connected with special sensory function or sensory augmentation, however it can just be a side effect of ontogenetic growth of this structure, since the same surface texture can be generally observed on sensilla chaetica or any larger stout setae.

Phosphaenus hemipterus adults, unlike most lampyrids, prefer pheromone communication to visual communication. In larvae of this species, a striking amount of *sensoria* were observed compared to other sympatric firefly species. While *Lampyris noctiluca*, *Lamprohiza splendidula* and *Thanatophilus* larvae have single sensoria on second antennomeres only, *Phosphaenus hemipterus* bears additional sensoria on the distal palpomeres of the maxillary palpus and the labial palpus plus on the already mentioned third antennomere. Moreover, the second antennomere bears a longitudinal sensory slot throughout its whole length. A possible explanation for this phenomenon could be the broad pheromone utilization within this species. Whereas the larvae do not participate in sexual communication, the sensoria may simply be undeveloped functional organs of the adults. Another explanation may lie in the unique diet of this species, and possible use in prey tracking, in connection with the aforementioned higher number of sensilla coeloconica.

Lamprohiza splendidula possesses a unique feature, not found in other central European species and to my knowledge never observed in any other firefly larvae, with exception of sister species *Lamprohiza delarouzei* Jacquelin du Val, 1859 (figures in Bugnion [1929] suggest a similar feature). It is a membranous spot placed posteriorly behind each stemma of the larva. The function of this organ seems sensory, nevertheless the exact purpose is unknown. It is possible that this organ is light-sensitive and helps the larva determine favorable light conditions within its environment. Among non-beetles, certain species of Blattodea possess vestigial ocelli degenerated to a pair of transparent areas in the cuticle called *fenestra* (Gillot 2005). Within beetles, vestigial paired dorsal ocelli occur in adults in some taxa of Staphylinoidea and in Derodontidae, so their presence is not excluded within Coleoptera and Polyphaga (Leschen & Beutel 2004). Nevertheless, in both previous cases, the vestigial ocelli are placed on the head dorsally, while in the firefly larva, they are positioned laterally behind stemmata. Another assumption may come from

the fact that *Lamprohiza splendidula* is believed to react to disturbance in its surroundings by light emission (De Cock 2003). During my *in vivo* observations of larvae of this species, I have never observed a larva "turning-on" its light due to direct disturbance; in contrast, the light emitted by larvae was always observed from several meters away. This could mean that larvae of this species have a well-developed ability to detect vibration, perhaps by some kind of primitive tympanal organ represented by the membranous spot placed behind their eyes. However, the same sensory service could be provided by a standard sensilla without need of any specialized membrane. Until a tissue analysis and further experiments are conducted, the precise function of this structure will nonetheless remain unknown.

In conclusion, the firefly larvae seem to bear a larger number of unique sensory organs compared to the studied carrion beetles. Not only do they have more different types of sensilla on the surface of their body, but some species possess more types of sensoria or other specific structures compared to the relatively monotonous cuticular surface of *Thanatophilus* species. The difference may be connected with the diverse life histories of the two families. Fireflies as active predators probably need more sophisticated sensory equipment when searching for the prey, notably since their ability to track old snail trails is well known. In contrast, the *Thanatophilus* larvae hatch right next to their food source leaving it only when they are ready to pupate.

5.2. Morphological characteristics of *Thanatophilus* larvae and their utilization

Previous descriptions of developmental stages of *T. rugosus* and *T. sinuatus* were rather brief (not including some important morphological features like labium, maxillae, nor tentorium), and most of the characters are only mentioned in the text form without accessory images (Xambeu 1892, 1900; Lengerken 1929, 1938; Paulian 1941). Furthermore, the descriptions of developmental stages did not find any reliable characters that would allow for identification of larval instars between the two species. Lengerken (1929) attempted to include a description of differences among larval instars of all three central European species of the genus *Thanatophilus* (*T. dispar*, *T. rugosus*, and *T. sinuatus*) and offered several size-based characteristics. However, he acknowledged that these values are highly variable and may not be reliable. To our knowledge, the only reliable way of how to identify larvae of *T. sinuatus* and *T.*

rugosus was proposed in the articles included in this thesis and in the work of Frątczak-Łagiewska & Matuszewski (2018). According to our findings, some species characteristics like overall body shape or size of protergites recognized by Lengerken (1929) are highly variable among specimens and thus of limited use. Lengerken's description also did not mention differences in coloration between *T. rugosus* and *T. sinuatus* as their third instars can be very easily distinguished by white markings along the margins of the body of the latter.

When dealing with larval material in forensic entomology, species identification is only the first part of the challenge as specimens have to be identified to instars as well. Without such knowledge it is impossible to estimate the larval age accurately, which is crucial for the estimation of PMI in forensic entomology. Head width and other size-based characteristics with accompanying statistical models are often suggested as means to easily identify larval instars of necrophagous beetles (Midgley & Villet 2009; Velásquez & Viloria 2010; Fratczak & Matuszewski 2014, 2016; Jakubec 2016). One of the reasons of quantitative characters for instar identification being developed for forensically important beetle species is the belief that majority of their larvae lack qualitative identifying characters (Fratczak & Matuszewski 2014). Kilian and Madra (2015) challenged this idea by finding several qualitative characters for instar identification of *Sciodrepoides watsoni* (Spence, 1813) (Coleoptera: Leiodidae: Cholevinae).

Larval instars of both studied *Thanatophilus* species can be so far identified based on statistical models when three character measurements (head size expressed by distance between dorsal stemmata, and width of protergum and mesonotum) are provided (Frątczak & Matuszewski 2016). This approach is very popular thanks to its accessibility, but the accuracy of the results is doubtful (Lengerken 1929). Although we did not find an overlap among head widths of all three examined larval instars in both species, we agree with the idea that geographical region, temperature, quality, and abundance of food and other variables can have a profound effect on larval size (Stillwell & Fox 2009; Lengerken 1929). Qualitative characters like proportions of body parts, chaetotaxy, coloration, and other traits not affected by the size of the individual seem to be more reliable, and their utilization minimizes the probability of error. We believe that future morphological redescriptions of larvae will prove us right.

Many of the characters for instar identification are shared between *T. sinuatus* and *T. rugosus.* These characters are provided in the form of a dichotomous identification key in the attached article *Description of immature stages of Thanatophilus sinuatus (Coleoptera: Silphidae)* chapter *Identification key to T. sinuatus and T. rugosus larval instars***.** Additionally, *T. sinuatus* instars can be easily distinguished based on the coloration of their dorsal side. The third instar has white distal ends of almost all paraterga, the second instar has only small white marks in the middle of protergal paraterga. This pattern can sometimes be observed on other tergites of the second instar larvae, but it is individually variable. The first instar is always uniformly dark and closely resembling the first instar of *T. rugosus*. However, they appear to differ in the length of the first segment of labial and maxillary palpi.

In addition, we have gathered some evidence that qualitative characters for instar identification could be shared among the species of genus *Thanatophilus* as a whole. Besides the reported similarities between *T. sinuatus* and *T. rugosus,* we also examined a limited larval material of *T. dentigerus* (Semenov, 1891). All three species share the allometry in length of urogomphi segments (length of the first segment increases rapidly in the second instar, while the second segment does not). In addition, the femur of all legs becomes bicolored (dorsal side is darker than ventral part) in the third instar (the femurs of first and second instars are monochromatic). On the other hand, the color of tergites remains consistent throughout the larval development, which is the same as in *T. rugosus*, but different from *T. sinuatus* as discussed above. We could not confirm if the shape of tentorium changes between instars in *T. dentigerus* due to limited number of specimens in the first and second instar category.

We also found several uncommon characters that could be used for instar determination such as differences in appearance of claws, length of sagittal line, or relative position of presternal medial plate. However, these characters are rather crude and may be hard to observe in some individuals, thus we did not include them into the final differential diagnose of species and larval instars in the article *Description of immature stages of Thanatophilus sinuatus (Coleoptera: Silphidae)*. Nonetheless, we consider them worth mentioning additional to other more obvious differences.

The difference in appearance of claws is very slight and more prominent in larvae of *T. rugosus* than in *T. sinuatus,* nevertheless it can be possibly applicable to other species as well. The length of the sagittal line seems to be closely related to individual

development and can be observed even on the larval exuviae. Additionally, it seems to differ between the two studied species. Our unpublished data suggest that this character could be applicable to other species of *Thanatophilus* as well.

One of the less obvious and more challenging characters to use for instar identification could be the relative position of the presternal medial plate, which differs among instars of *T. rugosus*. In the first instar, it does not reach up to the anterior or posterior edge while in other two instars it reaches either the posterior edge (second instar) or both edges (third instar). However, this character can be rather unreliable, as the presternal median plate is flexible and may not be fully visible in some individuals.

The qualitative approach in larval and instar identification has its advantages and disadvantages, however the morphological characters that we found could be used to provide validation of the model or even replace it altogether.

It is a common belief among taxonomists that color is rarely a good character for identification, as it can change in time and vary among specimens. We have to partially agree with that statement as the color stability of *Thanatophilus* larvae can vary and is strongly affected by how the specimens were treated and preserved. During the preparation of larval specimens of *T. sinuatus* we noticed that the larvae killed in ethyl acetate fumes and directly stored in alcohol tend to darken on otherwise white features such as distal ends of paraterga of third and second instar, ventral sides of femur in third instar and all desclerotized parts on ventral side of all instars. The color change complicates not only instar identification, but also species identification as darker specimens of *T. sinuatus* could be mistaken for *T. rugosus*.

The process of darkening is quite rapid and can be observed after only a few days of storage. To prevent this deterioration or at least prolong the color stability of the specimen, it is necessary to place them in hot water right after death and only then store them in alcohol. The water should be slightly below boiling point (90–95°C) for desired effect to occur. Higher temperature could result in rupture of softer parts of cuticle and cause irreversible damage to the specimen. We also do not recommend skipping the first step of killing the animals in ethyl acetate fumes as they have tendency to curl and stiffen in that position when killed directly by hot water (Midgley & Villet 2009), which is inconvenient for handling and measuring of the specimens.

5.3. Predation and corresponding morphological modifications in Lampyridae

It has been established, that the prey is incapacitated by injecting of a poisonous compound through larva's channeled mandibles, partially predigested and subsequently consumed. However, specific experiments still need to be performed to understand the details of this feeding process in firefly larvae, thus the next lines represent conjecture only.

An all three studied species, a special solitary sensilla were observed on the mandibles positioned apically before the opening of the inner channel. It may be possible, that the function of these sensilla is mechanoreceptive. While the larva attempts a successful bite into its prey's body, the seta triggers the discharge of the toxin. Furthermore, a hyaline appendage, which gives the impression of a "shutter" can be found at the base of the mandibular channel opening. This hyaline appendage however takes different form in each of the species, resembling a blunt thick seta in *Lampyris noctiluca*, a feather-like structure in *Lamprohiza splendidula* and a subtriangular valve with fringing at the distal end in *Phosphaenus hemipterus*.

The flow of the deadly liquid, theoretically triggered by the mechanoreceptive sensilla, may be either controlled by this "shutter" or by the gland producing the fluid. On the other hand, this structure may just prevent the channel opening from getting blocked by small particles. Compared to the blunt thick seta found in *Lampyris noctiluca*, the form of the hyaline appendage in the other studied species indeed suggests a function more connected with protection of the mandibular channel from clogging, rather than a trigger for the toxin or a seal.

The same structures or at least one of them can be observed in other firefly species in various modifications and seem to be common within the family. Similar sensilla and fringing hyaline appendages were observed on larvae of *Lucidota atra* (Olivier, 1790) (Lampyrinae) (Branham & Archangelsky 2000). In *Pyractomena nigripennis* Solier (Lampyrinae), the hyaline appendage is missing but a strong seta is present anteriorly to the base of retinaculum (Archangelsky 2010) and in *Pyractomena borealis* (Randall, 1838) (Lampyrinae) (Archangelsky & Branham 1998), the solitary seta is missing, but a large protruding hyaline appendage is present at the mandibular channel opening. It is possible that in the latter species, the structure also possesses triggering function, especially considering its protruding position. The effect of these structures, their form

or presence or absence given in context with diet and feeding habits of the larvae might show interesting evolutionary convergences in connection with feeding habits or even help with taxonomical positioning in future research.

Phosphaenus hemipterus is an obligate earthworm predator (Majka & MacIvor 2009). Compared to other sympatric fireflies, preferring snails as their diet, this species is unique in its differently shaped mandibles, large number of sensoria and lack of dorsoventrally flattened body. While in snail feeders the mandibles are sickle shaped, in *Phosphaenus* they are more hooked. This shape may enable the larva to bite and remain attached to its prey. Since earthworms do not overproduce mucus as a defense, the larva could hypothetically hold on its prey and wait for the full dosage of the toxin to get into its body – an alternative to snail-riding observed in other taxa. The larva's use of tarsal claws to attach itself to the body of the prey as discussed by Majka & MacIvor (2009) supports this hypothesis.

The round cross-section of the body of *P. hemipterus* may be an adaptation to earthworm hunting, enabling easier capture of prey that is retreating into its ground tunnel. The significance of body shape is supported by the fact that Nearctic species of *Photinus* Laporte, 1833, which are also reported to prey on earthworms (Lloyd 2008), have a very similar general body-shape. Moreover, certain diurnal fireflies are also known to hunt earthworms, such as *Lucidina* Gorham, 1883 and *Stenocladius* Deyrolle & Fairmaire, 1878 (De Cock 2009). In both cases, the cross-section of the larva is oval, but no surveys regarding the presence of special sensilla have been carried out to my knowledge. Additionally, no detailed *in vivo* study of *Phosphaenus hemipterus* predation has been undertaken and therefore the presumed advantages of their morphology remain merely assumptions.

5.4. Ontogeny and life history

The general difference in the lengths of the larval stages between the studied fireflies and carrion beetles can be explained by the varying ecology. The predatory lifestyle of firefly larvae demands constant search for suitable prey and additionally for prey of the correct size in earlier instars. Even though the larvae possess a strong toxin to immobilize the victim a small larva still needs to avoid biting off more than one can chew, especially considering the defensive mucus production of the hunted

snails. Moreover, the strong dependency of snail activity on favorable weather conditions make the prey availability unpredictable. Dry periods in (not only) summer months, when snails hide in various unreachable locations can even cause some larvae to switch into a non-feeding pseudo-aestivation even though the food is offered to them on regular basis (M. Novák, pers. obs. of *Luciola novaki*). A steady income of food is therefore uncertain and rapid growth to the necessary size is difficult. Additionally, the larva needs to gain enough nutrients to create a sufficient fat body that will sustain it in adulthood.

The one year difference in the lengths of the developmental cycle in *Lampyris noctiluca* and *Lamprohiza splendidula* presented by different authors (two years [Tyler 2002] versus three years [Schwalb 1961]) may be caused by the effect of different geographical regions in which they performed their observations. While Tyler's research was conducted in the United Kingdom with a more humid and warmer climate, Schwalb's was from Germany with a more continental climate. However, both Schwalb (1961) and Tyler (2002) present similar developmental lengths for the pupal stage, which is not influenced by the environment, but by the internal settings of the organism. It is thus possible, that both authors are correct about the cycle length in terms of the regional climate and that *Lampyris noctiluca* and *Lamprohiza splendidula* can prolong their life by an additional one year and one overwintering, if the yearly temperatures are low or food sources are scarce. Unfortunately no research in this manner has yet been conducted on *Phosphaenus hemipterus*, partly because of the difficulty of rearing of its larvae. They are critically sensitive to any abrupt temperature changes, thus making it hard to make them successfully overwinter and survive to adulthood in laboratory conditions (R. De Cock, pers. comm.).

As mentioned before, the adults of European fireflies do not feed anymore and most of their energy reserve is spent on flight in males and egg production in females which are crucial for the survival of the population. All of these aspects favor long-lived larvae, steadily searching for unpredictable food sources, with the ability to repeatedly outlast unfavorable conditions in a diapause.

All the hardships of finding food in predatory juvenile stages have been nonetheless overcome by switching to necrophagy in the carrion beetles and the burden of finding a sufficient food source has moved to the adults. They lay their eggs in the ground very close or under the carrion and the young larvae feed from the same food

source until pupation. The rapid development of the young stages is not only enabled by the steady food availability on one hand, but also forced by its temporality. It is necessary for the larva to turn into adult in the shortest time possible, while the food is still in the favorable state of decomposition and available due to high interspecies competition of necrophagous animals.

We have observed several cases of cannibalism among the larvae of both studied *Thanatophilus* species. These cases were rather limited but they occurred in spite of the fact that food was provided ad libitum. Cannibalized specimens were often smaller or at some disadvantage (freshly molted specimens) and in some cases probably related (siblings). The cannibalistic behavior could have been promoted by the laboratory breeding conditions (i.e. limited space of Petri dishes), but not necessarily so. Such behavior could also suggest some nutritional needs that are not met when feeding strictly on decaying meet, thus being compensated by cannibalism or predation of smaller competitors under natural conditions (Matuszewski et al. 2011). Cannibalistic behavior was also observed among larvae of *Lampyris noctiluca*, when kept in small space with no food provision (P. Šípek, pers. comm.). In any case, disposing of the competition, even composed of members of one's own kin can be beneficial for the survivor in terms of food preservation or its alteration.

It is in the adult stage of necrophagous Silphidae, when the unpredictability of the food availability and large distances among the food sources become an issue. The adults feed from the same source as their larvae to provide themselves with energy to overcome long distances in the air and have and to sustain their longer lifespan. Only as a long-lived winged adult, the beetle has a chance and time to locate another suitable carrion, breed with a mate and continue the lineage. Longer life-span is not needed in adult fireflies. The populations occur in a relatively small area, due to flightlessness of the females. The dispersal is thus facilitated mainly by the larvae. Search for food is not an issue for firefly adults anymore, since all the nutrient gathering was completed in the juvenile stages.

Firefly larvae which are ready to pupate seem to switch into diurnal activity, and can often be seen striding along in broad daylight (Tyler 2002). The adult female rarely moves far before she dies, so Tyler (2002) presumes, that it may be that this final larval stage is the one in which fireflies are able to spread out in search of new habitats. Larvae preparing to pupate the same year often gather together in small

groups, and it is fairly common to find six or more side by side under one log (Tyler 2002). The result of the aggregating of pupating larvae may well be the reason why clusters of two to six glowing females can sometimes be found within a few centimeters of each other (Tyler 2002; M. Novák, pers. obs.). Imagines can therefore find a mate in a relatively short amount of time.

This behavior could be explained as a means of being more conspicuous for the males and thus having a higher chance of successful mating. Another possibility is finding the "perfect" spot in terms of environmental conditions for the larvae to successfully pupate, for the females to successfully lay eggs and for newly hatched larvae to survive. However, such a phenomenon might prevent the spreading of the population further in the biotope. In any case, this behavior must provide more evolutionary advantages than disadvantages. The manner in which the larvae gather together is unknown, but may potentially support the intraspecific communication hypothesis (see below) if light manifestations are involved.

In conclusion, feeding habits connected with food availability go hand in hand with different life strategies that make the most of every aspect of the beetle's life. Fireflies moved all the hardships of predatory food gathering to their larvae, so the adult stages can be used exclusively to secure reproduction. The food source of predators usually renews itself in a relatively predictable area that can be reached without the necessity of overcoming long distances using flight. The larvae nonetheless need enough time to slowly collect all the nutrients from the environment. On the other hand, the adults need much shorter time to find a mate and produce progeny. In contrast, the necrophagy of Silphidae puts the burden of finding food on the winged adults, often forced to travel long distances to find a solitary carrion where they reproduce and lay eggs to secure provision for the larvae. The larvae then have to quickly develop into adults before the food source gets depleted and the cycle continues. The flexible life-span of firefly larvae might consequently explain the different data of notoriously problematic, but relatively high number of lampyrid larval instars presented by different authors. Nonetheless a proper research focused on this issue must be conducted. In contrast, the fixed and reliable number of silphid larval instars (three) complies with the need of rapid development within relatively short amount of time.

5.5. Pupal development

According to Schwalb (1961), the larvae of both *Lampyris noctiluca* and *Lamprohiza splendidula* enter the stage of prepupa 8 – 20 days prior to pupation. From my observations, it was noticed that this period can be even shorter (1 – 6 days) for both species. The range presented by Schwalb seems to be quite wide. Besides different rearing conditions and especially varying temperature of the environment, that has a profound impact on the rate of development in poikilothermic organisms, a possible explanation may lie in a biological need of different prepupal stage period between the sexes. On the other hand, the difference between the lengths of the subsequent pupal stage between sexes is not that distinctive or is even identical (8 – 12 days for a female and 11 – 15 days for a male in *Lampyris noctiluca* and 7 days for both sexes in *Lamprohiza splendidula*; Schwalb 1961; Tyler 2002). Additionally, a male pupa of *L. noctiluca* was observed turning into an imago after eight days only, further widening the range of the reported pupal development lengths (M. Novák, pers. obs.). It is possible that individuals staying in prepupa for 20 days might have fed more recently than individuals staying in prepupa for eight days, i.e. needing a longer time to process the ingested food and expel the undigested waste. The prepupa is the stage of preparation of the larval body for entering the pupal stage, thus the time needed for processing and elimination of food intake might influence the length of the prepupal stage.

The longer period of the male pupal development of *Lampyris noctiluca* could be explained by the larger amount of changes taking place in a male's body compared to the neotenous female. This raises the question of why the time of pupation in *Lamprohiza splendidula* is identical in both sexes. It is possible that the production of eggs in the developing female (Schwalb 1961) prolongs the pupation period, thus compensating for metamorphosis into a winged imago in male. On the other hand, the same egg production happens in *Lampyris noctiluca* (Schwalb 1961) and yet the periods differ between the sexes. The reason for the same pupal period in *Lamprohiza splendidula* may be its overall small size in comparison with the much larger *Lampyris noctiluca*. The gap between sexes in the rebuilding times might thus be reduced in the former thanks to its relatively small body volume in relationship to the changes taking place.

In firefly pupae, the somatic changes include enhancement of the light producing organs and often their expansion in those cases, where the adult uses them actively in the courtship. For instance in *Lampyris* adults, the change generally takes place in females, while males retain the larval lights only. Males of *Lampyris noctiluca* do not produce light when searching for a mate and seem to glow only when disturbed. The possible reasons for male light production in this species may be aposematism or a simple vestigiality of the organ from previous developmental stage. Females on the other hand actively lure the males to breed by their bright enhanced adult lanterns. In *Lamprohiza splendidula*, both males and females undergo lantern change; males develop a completely new adult organ while females expand their larval lights with additional ones. However, an intriguing phenomenon was observed in the male pupa of *L. splendidula* on the fifth day of its development, prior to the activation of the adult lantern. The glow intensity of the larval lights had weakened and the next day when the adult lantern activated, the intensity of the larval lights went back to normal. It is possible that the final phase of activation of the male lantern is accompanied by the transfer of compounds essential for light production (i.e. luciferin, luciferase) into the newly developed organ, thus temporarily weakening the light production in the larval lanterns. To exclude the possibility that such a phenomenon was not a coincidental exception, more male pupae will have to be investigated, since only a single specimen was observed for this study. Moreover, the larval lights were still active several hours after the male emerged from the pupa and disappeared only after its cuticle hardened and darkened. It is possible that due to very low energy requirements of bioluminescence (Woods et al. 2007; Sharkley et al. 2010) the larval lights are still active in adult males, although not visible under the cuticle. The potential energy cost of their deactivation in the pupal stage could be after all higher than the overall cost of retaining them in adulthood in an unchanged state.

Pupation in fireflies under natural conditions occurs under fallen leaves, pieces of wood or stones, in dug out surface hemispherical chambers with the opening at the top (Schwalb 1961; Burakowski 2003). It was reported, that the larva of *Lamprohiza splendidula* can also pupate in a cell created of small pieces of dead leaf litter (R. De Cock, pers. comm.). However, this cell might be created by chance due to pupal defensive reflexes. When disturbed, the larva starts to repeatedly bend its abdomen forward and back, similar to “crunches”, thus moving small pieces of litter away from

its body as a side effect. The same may be true for the reported dug out chambers, but the construction process details of these structures have not been yet reported.

Pupation of *Thanatophilus* larvae occurs in a dug up underground pupation chamber, created by the third instar larva where it subsequently turns into pupa after a few days. When disturbed, the pupa exhibits the same defensive behavior as described in fireflies. However in *Thanatophilus*, the "crunches" are faster and more erratic, almost giving an impression of a fish washed-up on the shore. It seems likely, that in case the pupation chamber walls start crumbling inside, the pupa pushes away the debris and strengthens them by the "crunching" movement.

In comparison with studied firefly pupae, the *Thanatophilus* pupa has pairs of long stout hairs growing from its pronotum and sides of each abdominal segments. These setae are probably preventing the pupa directly touching its pleural regions with the wall of the pupation chamber, thus leaving the spiracles vital for pupa's breathing unblocked. Additionally, we observed that disturbance of the chamber before pupation of the *Thanatophilus* larvae (by other larvae or by human investigators) often resulted in its abandonment. Such larvae often resumed feeding and postponed pupation beyond the normal period or even died before reaching it. Pupation thus seems to be limited by a biologically preset optimal time frame rather than optimal level of gathered nutrients by the larva. Another explanation may be that larva in a dug up chamber already starts to undergo processes leading to prepupation even though it still exhibits normal larval behavior (i.e. feeding) after the disturbance. The triggered metamorphosis processes then malfunction due to excess activity and mortality levels raise significantly.

Young pupae of both studied families are creamy white or pale yellow. In the studied *Thanatophilus* species, the older pupae turn ochre to light brown in both sexes, especially on the "hard parts" like pronotum and elytra. Tyler (2002) describes the color of pupa of *Lampyris noctiluca* as pale yellow, turning olive green after several hours, but this only applies for the older male pupae, where the olive color is especially distinct in dimmer light conditions. The female pupa stays yellowish white with pink regions throughout the whole pupal period. Adult males are overall much darker compared to their female counterparts which is probably caused by the neoteny of the females and general lack of body parts with thick cuticle. There is one more distinction between male and female firefly pupae, which probably plays an important role in the

color difference. The pupal skin in the male seems to darken and harden as the pupa gets older. After the adults emerge, the exuvia left by a female pupa is extremely delicate and without any coloration. The exuvia of a male, on the other hand, is brown and still retains its original shape. The reason for this difference is unknown, yet it may be just a side effect of development of the thick cuticular features in the male body. However, in the winged *Thanatophilus* pupae, the exuvia have a similar delicate quality as in lampyrid females, thus disproving the previous argument.

5.6. Ontogeny of *Thanatophilus* larvae in laboratory conditions

Adults of *T. sinuatus* breed willingly when provided with food and material for egg laying. We did not encounter many setbacks following the breeding methodology suggested by Ridgeway et al. (2014). The constant temperature and photoperiod (20°C and 16/8 light cycle) in the climatic chambers, where the breeding took place, resulted in steady production of eggs, thus we did not have to experiment with different environmental conditions or different photoperiod. Nonetheless, this is in contrast to our experiences with its sibling species, *T. rugosus* as it was unable to breed under the abovementioned conditions and a change of light cycle to 12/12 was necessary to promote the production of eggs.

Both studied species were considered very similar in their occurrence patterns, both spatial and temporal. Although, Frątczak-Łagiewska & Matuszewski (2018) recently suggested that these two species differ in their seasonality in order to promote resource partitioning and therefore lower the resource competition between them. They observed larvae of *T. sinuatus* occurring throughout the year up to August, but *T. rugosus* larvae were not recorded past mid-June. Our findings about different photoperiod requirements support their hypothesis stating that *T. sinuatus* is able to breed later in the season when the photoperiod is closer to 18:6 (light:dark) hours ratio, while *T. rugosus* restricts its breeding to earlier months of the year when the photoperiod is closer to 12:12 ratio.

The total length of development of the immature stages under the same temperature (20°C) was very similar in both species, *T. sinuatus* (41.85 days) and *T. rugosus* (45.48 days). Both of them spend major parts of their juvenile life as third instar larvae and pupae but progress very rapidly through earlier stages (egg, first and second larval

instars). The larvae of *T. sinuatus* hatch after approximately three days (2.83 days) at 20°C. This period can be considered short compared to some larger beetles (genus *Dermestes* Linnaeus, 1758), which take around eight days on average at the same temperature (Coombs 1979, 1981). However, the length of egg development at 20°C is very similar to *T. rugosus* (3.34 days), *T. mutilatus [T. capensis]* (3.58 days) and *T. micans* (3.66 days) (Ridgeway et al. 2014). This somewhat shared trait, among the members of the genus could be caused by a number of factors including ecological ones. One of the possible explanations could be timing of the hatching of larvae in the optimal stage of carrion decay, as all these species share the preference for the same type of food source in the same stage of decomposition, even though they compete for it with other species (e.g., blowflies from family Calliphoridae) (Ridgeway et al. 2014; Dekeirsschieter et al. 2011a; Matuszewski et al. 2010, 2011).

5.7. Defensive behavior

The larvae of *Lamprohiza splendidula* as well as *Thanatophilus* have conspicuous laterally extended tergal plates on both the thorax and abdomen. This body shape is typical for larvae of Silphinae and is by no means an exception within the larvae of the Lampyridae (e.g. *Pyrogaster* Motschulsky, 1853, *Cratomorphus* Motschulsky, 1853, *Photuris*, etc.), nor within the order Coleoptera as a whole (e.g. Lycidae, some Carabidae). However, the exact advantage of this morphological architecture is unknown. When collecting firefly larvae in nature, I have often found the individuals pressed against moist dead foliage, with their head and legs hidden under tightly constricted tergal plates directly touching the substrate. In this way, the vulnerable appendages and delicate parts of the body are protected against potential predators, while the pigmentation of the larva matches perfectly with the color of dead leaves. Nonetheless, the camouflage is probably just an added value to the protective function, since in other taxa, larvae of similar shape are often brightly colored.

In the observed *Thanatophilus* larvae, the individuals picked up by pincers always exhibited sudden quick wriggling movements and subsequent agile attempt to flee. If the disturbance of the larva continued, it often retracted its appendages and rolled up its body. The latter defensive behavior can typically be observed in woodlice (Isopoda). Dark color of the dorsal plates may play a role in crypsis, even though

Thanatophilus has a diurnal activity. However, dark color can help the larva hide inside or around the carrion or in shady parts of the soil surface.

Compared to agile *Thanatophilus*, the firefly larvae are rather lethargic. They are known to possess defensive chemicals that make them distasteful to predators that either generally avoid them or quickly learn to avoid them in connection with their luminescence, which thus serves as aposematic display (Eisner et al. 1978; De Cock & Matthysen 2003; Fu et al. 2007). Reflexive bleeding discovered in *Nyctophila* sp. may protect the larva under the daylight, when the glow becomes unrecognizable (De Cock et al. 2017). Moreover, the larvae of this species are almost identical to *Lampyris*, where the distinctive body coloration quite possibly serves aposematic function too. It thus seems that mechanisms of chemical defense together with warning color/glow make the need for the ability of rapid escape unnecessary in this family.

Although chemical defenses are common in Silphidae, many of members of this family and especially Silphinae lack bright colors and are generally uniformly dark. The larvae of *Thanatophilus* are known to roll in a ball protecting soft parts of their bodies when disturbed, ooze a deterrent secretion, or just quickly escape to safety (J. Růžička & P. Jakubec, pers. comm; M. Novák, pers. obs.). Compared to lampyrid strategy of deterring the predator before the attack, the carrion beetle larvae seem to rely rather on defensive thanatosis and startle-and-flight strategy.

5.8. Photic behavior of lampyrid larvae

Larvae of *Lampyris noctiluca* and *Phosphaenus hemipterus* have been reported to display a variety of glow behaviors, including the so called “walkabout” display. In contrast, *Lamprohiza splendidula* larvae show only a simple type of glow. The “walkabout” behavior is expressed while the larva walks, consisting of definite pulses of light separated from the next one by a longer interval of darkness. Definite reason this type of photic manifestation has not been yet proven, however Tyler (2002) proposes five possible causes; 1) the glow has no purpose and is just a by-product of the light organs’ development; 2) the larva uses the light while tracking the prey; 3) the larva uses the light to attract the prey; 4) interspecific communication and 5) aposematic defense connected with unpalatability, which seems to be the only hypothesis supported by evidence (Sivinski 1981; Underwood et al. 1997; De Cock &

Matthysen 2003; De Cock 2009; Moosman et al. 2009). Since Tyler (2002) adequately explains the pros and cons for each of the five points, I would like to address intraspecific communication only. Viviani (2001) supposedly witnessed possible intraspecific communication in an unidentified *Bicellonychia* sp., where larvae reacted to flashes emitted by adults, hypothesizing the cause of this behavior could be informing the adults of an occupied food niche. This nonetheless cannot be the case with *Lampyris noctiluca*, where the egg-bearing females are flightless, thus communication of larvae with adult males would be pointless. The way larvae glow differs from that of the females. If this was indeed a signal for the males to distinguish between the larvae and females, a simple non-utilization of light by the larvae seems like a more effective solution. Furthermore, larvae do not usually glow during the adult mating season (Schwalb 1961) and evidence suggests that luminescence in Lampyridae seems to have arisen first in larvae and was only subsequently used for sexual communication in adults (Branham & Wenzel 2003). Yet, the flash display may serve in communication among larvae, for example for effective division of a food niche. On the other hand, larvae have only simple ocelli and not developed eyes like adults, so this assumption seems doubtful. In conclusion, the utilization of larval light in intraspecific communication with adults or even with other larvae seems unlikely and aposematic function still seems like the most plausible explanation.

Interestingly, low level luminescence was recently observed by Tisi et al. (2014) in the pairs of lightly-pigmented posterolateral spots on each tergite and even in the inactive light organ of larvae of *Lampyris noctiluca*. Nevertheless, according to the authors, this luminescence is so weak, that it is most probably unrelated to aposematic signalization.

A simpler type of luminescent display can be found in larvae of *Lamprohiza splendidula*, as they emit a weak continuous glow only when disturbed. However, the light intensity may weaken or completely stop in certain cases, usually as a result of overstimulation (Schwalb 1961; M. Novák, pers. obs.). This type of behavior seems similar to that described by Viviani (2001) in the Neotropical genera *Pyrogaster*, *Photuris* Dejean, 1833, and *Aspisoma* Laporte, 1833; the larvae respond to vibrations by glowing, but do not respond to mechanical manipulation. According to Viviani (2001), this behavior is probably a collective defense against predators, which lies in distraction and confusion of the “enemy”. In conclusion, what may seem like a

collective defense of larvae of *Lamprohiza splendidula* may either be just a by-product of larval tolerance to a certain amount of stimuli or indeed a tool of collective defensive behavior. This begs the question: why do the larvae – when in danger – only reduce their glow, and not stop it abruptly? It may be explained by an inability of the larval stage to alter its glow swiftly, as a result of the differing physiology of their photic organ compared to that of the adults (Timmins et al. 2001).

Unlike in adults, the spectrum of light emitted by the larvae of all three sympatric firefly species of the Czech Republic is very similar, conserving the green emission (De Cock 2003). This agrees with the lack of an intraspecific function (mating) and increased importance of an interspecific function such as defense, as stated by Viviani (2001). On top of that, lampyrids are reported to be unpalatable prey in general (Underwood et al. 1997; De Cock & Matthysen 2003; Moosman et al. 2009). With regards to the abovementioned species, the tests of unpalatability have only been performed on *Lampyris noctiluca*. There is a possibility that Batesian or Müllerian mimicry could have evolved within and between these taxonomic groups.

Schwalb (1961) states that adults of *Lamprohiza splendidula* are often found in spider webs, sucked dry. I have often witnessed males of this species stress-glowing from spider webs myself, but the question is whether they are indeed consumed by the spider. The glowing of individuals in webs (often for hours) suggests that they had not been approached and killed by the spider. On the other hand, males are often flying (and consequently found in the webs) in such large numbers that the spider may not be capable of attending to all its captures. If they are consumed, the spider is either immune to the defensive compounds of the firefly or the firefly does not possess them. In both cases, the Batesian or Müllerian mimicry has no effect. If they are avoided by the spider, it is possible that Schwalb (1961) had mistaken dried-up individuals caught in the web on previous days with the remains of other spider prey, and Batesian or Müllerian mimicry could still be operating. Which type of luminous mimicry, if any, is used in these fireflies will still have to be determined by further experiments. Larvae and adults must be tested individually, since the existence of defensive compounds in the adults may not guarantee the existence of the same compounds in juvenile stages and vice versa.

6. CONCLUSIONS

This thesis presents the most detailed redescriptions to-date of the immature stages of all members of Lampyridae and genus *Thanatophilus* occurring in the Czech Republic.

Within Lampyridae, last-instar larvae have been redescribed for all three species. *Lampyris noctiluca* is a well known widespread species of the Palearctic Region, but so far there is no way to distinguish its larva from other species of the genus *Lampyris*, or even from larvae of a closely related genus *Nyctophila*. Knowledge about detailed larval morphology will thus provide a good starting point in future comparative studies and add valuable morphological insight in the age of phylogenetics. Additionally, male and female pupae have been thoroughly described for this species.

The recently known differences among some larvae of genus *Lamprohiza* consist only of different pattern of the light organ and no comparative work concerning larvae of this genus has been published so far. The thorough redescription of larva of *Lamprohiza splendidula*, the most widespread of the eight species of the genus will provide the same starting point for larval comparative morphology as in the former species. More interesting information may come from possible future comparison with little known sibling genus *Phausis*, which is either a Nearctic form of *Lamprohiza* or a strongly convergent genus. Additionally, a strange feature that is to my knowledge uncommon in all other firefly genera so far, has been found on the head of this species. The latest phylogenetic studies (Martin et al. 2016) show, that both *Lamprohiza* and *Phausis* represent taxonomically problematic groups not only thanks to their unique morphology (especially of the light organs in larvae and adult females), but also genetically, and have been recently marked as genera of *incertae sedis*. A proper knowledge of morphology and comparative studies within and between these genera may help to resolve this issue. In addition, male and female pupae have been described for *L. splendidula*. In male pupa, a day-to-day development and transformation of the light organ has been outlined. Such information may be valuable in ontogenetic studies and provide insight into evolution of bioluminescence of these beetles and transformation of its utilization from assumed aposematic defense to sexual communication.

Redescription of larvae of *Phosphaenus hemipterus* has shown species-specific features not found in the other studied lampyrid species. The large amount of specific sensory organs and unique features are probably related to their different feeding habits and ecology. Possible convergences of these features may be found in future comparisons among the other earthworm feeders or pheromone communicators, thus explaining their relation to ecology. In addition, larval description of *P. hemipterus* will help in deciphering the connection of this species to genus *Phosphaenopterus*, which is by some scientists suspected of being just a macropterous form of *Phosphaenus*.

Within Silphidae, detailed redescriptions of all developmental stages (all larval instars and pupa) of *Thanatophilus* occurring in the Czech Republic have been provided. Both *T. rugosus* and *T. sinuatus* belong among the most common and widely spread necrophagous species of beetles. Their utility in the field of forensic entomology is undeniable as they have tight ecological associations with their food source (development can take place only on carrion) and are reported frequently from human remains or other large vertebrates. In addition, larval descriptions will once again provide a solid base for further redescriptions of other members of this genus, thus widening its utilization in criminalistics. Furthermore, information about biology of both species, including the developmental length of eggs, all instars and pupae under constant laboratory conditions, and notes on their behavior are provided. The lengths of development are similar in both species. These results can help increase the value of *T. rugosus* and *T. sinuatus* as bioindicators for forensic entomology.

To provide the accessibility of the text to non-professional entomologists a key for species identification of central European lampyrid larvae is presented, together with separate key for larvae of genus *Thanatophilus* within the Czech Republic, where *T. rugosus* and *T. sinuatus* can be identified in every larval instar.

I beleieve this work will provide other researches with valuable information and will help advancing the knowledge of these fascinating beetles in future.

7. REFERENCES

Amendt, J., Krettek, R. & Zehner, R. (2004): Forensic entomology. *Naturwissenschaften*, 91, 51-65.

Anderson R.S. & Peck S.B. (1985): *The insects and arachnids of Canada, part 13: the carrion beetles of Canada and Alaska (Coleoptera: Silphidae and Agyrtidae).* Agriculture Canada, Ottawa.

Anderson R.S. (1987): Scientific note. The larva of *Thanatophilus trituberculatus* (Kirby) (Coleoptera: Silphidae). *Coleopterist Bulletin,* 41: 34.

Archangelsky M. (2004): Description of the last larval instar and pupa of *Aspisoma fenestrata* Blanchard, 1837 (Coleoptera: Lampyridae) with brief notes on its biology. *Tijdschrift voor Entomologie*, 147: 49-56.

Archangelsky M. (2010): Larval and pupal morphology of *Pyractonema nigripennis* Solier (Coleoptera: Lampyridae: Photinini) and comparative notes with other Photinini larvae. *Zootaxa*, 2601: 37-44.

Archangelsky M. & Branham M.A. (1998): Description of the preimaginal stages of *Pyractomena borealis* (Randall, 1838) (Coleoptera: Lampyridae) and notes on its biology. *Proceedings of the Entomological Society of Washington*, 100: 421-430.

Ballantyne L.A. & Menayah R. (2002): A description of larvae and redescription of adults of the firefly *Pteroptyx valida* Olivier in Selangor, Malaysia (Coleoptera: Lampyridae: Luciolinae), with notes on Luciolinae larvae. *The Raffles Bulletin of Zoology*, 50: 101-109.

Ballantyne L., Fu X., Lambkin C., Jeng M.-L., Faust L., Wijekoon W.M.C.D., Li D. & Zhu T. (2013): Studies on South-east Asian fireflies: *Abscondita*, a new genus with details of life history, flashing patterns and behavior of *Abs. chinensis* (L.) and *Abs. terminalis* (Olivier) (Coleoptera: Lampyridae: Luciolinae). *Zootaxa*, 3721: 1-68.

Barton P.S., Cunningham S.A., Lindenmayer D.B. & Manning A.D. (2013): The role of carrion in maintaining biodiversity and ecological processes in terrestrial ecosystems. *Oecologia*, 171: 761-772.

Bonacci T., Greco S. & Brandmayr T.Z. (2011): Insect Fauna and Degradation Activity of *Thanatophilus* species on Carrion in Southern Italy (Coleoptera: Silphidae). *Entomologia Generalis,* 33: 63–70.

Bourel B., Tournel G., Hédouin V., Goff M.L. & Gosset D. (2001): Determination of drug levels in two species of necrophagous Coleoptera reared on substrates containing morphine. *Journal of Forensic Sciences*, 46, 600-603.

Branham M.A. (2010): Lampyridae Latreille, 1817, pp. 141-149. In: Leschen R.A.B., Beutel R.G. & Lawrence J.F. (eds.): *Handbook of zoology: Coleoptera, Beetles. Volume 2: Morphology and Systematics (Elateroidea, Bostrichiformia, Cucujiformia partim)*. Walter de Gruyter, Berlin, New York, 786 pp.

Branham M.A. & Archangelsky M. (2000): Description of the last larval instar and pupa of *Lucidota atra* (G.A. Olivier 1790) (Coleoptera: Lampyridae), with a

discussion of abdominal segment homology across life stages. *Proceedings of the Entomological Society of Washington*, 102: 869-877.

Branham M.A. & Wenzel J.W. (2001): The evolution of bioluminescence in cantharoids (Coleoptera: Elateroidea). *Florida Entomologist*, 84: 565-586.

Bugnion E. (1929): *Le ver-luisant provençal et la luciole niçoise. Mémoire, supplément au "Riviera Scientifique" (année 1929)*. Association des naturalistes de Nice et des Alpes-Maritimes, Nice, 131 pp.

Burakowski B. (2003): *Klucze do oznaczania owadów Polski, Czesc XIX Chraszcze – Coleoptera, Zeszyt 29, 30 Karmazynkowate – Lycidae, Swietlikowate – Lampyridae. [Key to identification of insects of Poland, Part XIX – Beetles (Coleoptera), Issue 29, 30 Lycidae, Lampyridae]*. Polskie Towarzystwo Entomologiczne, Torun, 39 pp. [in Polish]

Case, J.F. (2004): Flight studies on photic communication by the firefly Photinus pyralis. *Integrative & Comparative Biology*, 44: 250-258.

Charabidze D., Vincent B., Pasquerault T. & Hedouin V. (2016): The biology and ecology of *Necrodes littoralis*, a species of forensic interest in Europe. *International Journal of Legal Medicine*, 130: 273-280.

Cho S., Epstein S.W., Mitter K., Hamilton C.A., Plotkin D., Mitter C. & Kawahara A.Y. (2016): Preserving and vouchering butterflies and moths for large-scale museum-based molecular research. *PeerJ*, 4, e2160.

Coombs C.W. (1981): The development, fecundity and longevity of *Dermestes ater* Degeer (Coleoptera: Dermestidae). *Journal of Stored Products Research,* 17: 31-36.

Coombs C.W. (1979): The effect of temperature and humidity upon the development and fecundity of *Dermestes haemorrhoidalis* Küster and *Dermestes peruvianus* Laporte de Castelnau (Coleoptera: Dermestidae). *Journal of Stored Products Research,* 15: 43-52.

Crowson R.A. (1981): *The Biology of the Coleoptera*. Academic Press, London, 802 pp.

Daniel C.A., Midgley J.M. & Villet MH (2017): Determination of species and instars of the larvae of the afrotropical species of *Thanatophilus* Leach, 1817 (Coleoptera, Silphidae). *African Invertebrates*, 58: 1-10.

De Cock R. (2000): Rare, or simply overlooked? Practical notes for survey and monitoring of the small glow-worm *Phosphaenus hemipterus* (Coleoptera: Lampyridae). *Belgian Journal of Zoology*, 130: 93-101.

De Cock R. (2003): Larval and Adult Emission Spectra of Bioluminescence in Three European Firefly Species. *Photochemistry and Photobiology*, 79: 339-342.

De Cock R. (2004): Larval and Adult Emission Spectra of Bioluminescence in Three European Firefly Species. *Photochemistry and Photobiology*, 79: 339-342.

De Cock R. (2009): Biology and behavior of European lampyrids, pp. 161-200. *In*: Meyer-Rochow V.B. (ed.): *Bioluminescence in Focus – A Collection of Illuminating Essays*. Research Signpost, Trivandrum, 385 pp.

De Cock R. & Geisthardt M. (2007): Description of the adult female and larval stages of *Lampyris sardiniae* Geisthardt, 1987 (Coleoptera: Lampyridae). *Entomologische Zeitschrift*, 117: 99-102.

De Cock R. & Mathyssen E. (2003): Glow-worm larvae bioluminescence (Coleoptera: Lampyridae) operates as an aposematic signal upon toads (*Bufo bufo*). *Behavioral Ecology*, 14: 103-108.

De Cock R. & Matthysen E. (2005): Sexual communication by pheromones in a firefly, *Phosphaenus hemipterus* (Coleoptera: Lampyridae). *Animal Behavior*, 70: 807-818.

De Cock, R., Faust, L. & Lewis, S. (2014): Courtship and mating in *Phausis reticulata* (Coleoptera: Lampyridae): Male flight behaviors, female glow displays, and male attraction to light traps. *Florida Entomologist*, 97: 1290-1307.

De Cock R., Guzmán-Álvarez J.R., Luque F.R. & Fernández M. (2017): Presence of eversible organs in two *Nyctophila* species from Spain: *N. reichii* and *N. heydeni*. Poster, International Firefly Symposium 2017 (IFS 2017) during April 24-28, 2017 at Taipei, Taiwan. http://www.ifs2017taipei.org

Deheyn D.D. & Ballantyne L.A. (2009): Optical characterization and redescription of the South Pacific firefly *Bourgeoisia hypocrita* Olivier. *Zootaxa*, 2129: 47-62.

Dekeirsschieter J., Verheggen F.J., Haubruge E., Brostaux Y. (2011a): Carrion beetles visiting pig carcasses during early spring in urban, forest and agricultural biotopes of Western Europe. *Journal of Insect Science*, 11: 73.

Dekeirsschieter J., Verheggen F., Logany G. & Haubruge E. (2011b): Large carrion beetles (Coleoptera, Silphidae) in Western Europe: a review. *Biotechnology, Agronomy, Society and Environment*, 15: 435-447.

Dekeirsschieter J., Frederickx C., Lognay G., Brostaux Y., Verheggen F.J. & Haubruge E. (2013): Electrophysiological and Behavioral Responses of Thanatophilus sinuatus Fabricius (Coleoptera: Silphidae) to Selected Cadaveric Volatile Organic Compounds. *Journal of Forensic Sciences*, 58: 917-923.

Díaz-Aranda L.M., Martín-Vega D., Baz A. & Cifrián B. (2018): Larval identification key to necrophagous Coleoptera of medico-legal importance in the western Palaearctic. *International Journal of Legal Medicine*, in press.

Dobler S. & Müller J.K. (2000): Resolving phylogeny at the family level by mitochondrial cytochrome oxidase sequences: phylogeny of carrion beetles (Coleoptera, Silphidae). *Molecular Phylogenetics and Evolution*, 15: 390-402.

Dreisig H. (1975): Environmental control of the daily onset of luminescent activity in glow-worms and fireflies (Coleoptera: Lampyridae). *Oecologia*, 18: 85-99.

Dubie T.R., Talley J.L., Payne J.B., Wayadande A.W., Dillwith J. & Richards C. (2017): Filth Fly Activity Associated With Composted and Noncomposted Beef Cadavers and Laboratory Studies on Volatile Organic Compounds. *Journal of Medical Entomology*, 54: 1299-1304.

Eisner T., Goetz M.A., Hill D.E., Smedley S.R. & Meinwald J. (1978): Firefly „femmes fatales“ accquire defensive steroids (lucibufagins) from their firefly prey. *Proceeding of the Nation Academy of Sciences*, 94: 9723-9728.Frątczak K. & Matuszewski S. (2014): Instar determination in forensically useful beetles *Necrodes littoralis* (Silphidae) and *Creophilus maxillosus* (Staphylinidae). *Forensic Science International*, 241: 20-26.

Fockink D.H., Mise K.M. & Zarbin P.H. (2013): Male-Produced Sex Pheromone of the Carrion Beetles, *Oxelytrum discicolle* and its Attraction to Food Sources. *Journal of Chemical Ecology*, 39: 1056-1065.

Frątczak K. & Matuszewski S. (2016): Classification of forensically-relevant larvae according to instar in a closely related species of carrion beetles (Coleoptera: Silphidae: Silphinae). *Forensic Science, Medicine, and Pathology*, 12: 193-197.

Frątczak-Łagiewska K. & Matuszewski S. (2018): Resource partitioning between closely related carrion beetles: *Thanatophilus sinuatus* (F.) and *Thanatophilus rugosus* (L.) (Coleoptera: Silphidae). *Entomologia Generalis,* 37: 143-156.

Fu X., Ballantyne L. & Lambkin C. (2012): The external larval morphology of aquatic and terrestrial Luciolinae fireflies (Coleoptera: Lampyridae). *Zootaxa*, 3405: 1-34.

Fu X., Ohba N., Zhang Y. & Lei L. (2006): Rearing apparatus and diet for the aquatic firefly *Luciola leii* (Coleoptera: Lampyridae). *Canadian Entomologist*, 138: 399-406.

Fu X., Vencl F.V., Nobuyoshi O., Meyer-Rochow W.B., Lei C. & Zhang Z. (2007): Structure and function of the eversible glands of the aquatic firefly *Luciola lei* (Coleoptera: Lamypridae). *Chemoecology*, 17: 117-124.

Geisthardt M. & Satô M. (2007): Lampyridae, pp. 225-234. *In*: Löbl I. & Smetana A. (eds.): *Catalogue of Palearctic Coleoptera, Vol. 4*. Apollo Books, Sternstrup, 935 pp.

Geisthardt M., Figueira G., Day J.C. & De Cock R. (2008): A review of Portuguese fireflies with the description of a new species *Lampyris iberica* sp. nov. *Revista de Entomología*, 8(2): 147-152.

Gillot C. (2005): *Entomology. 3rd Edition*. Springer, Dordrecht, 831 pp.

Goff L.M. (2010): Early Postmortem Changes and Stages of Decomposition. *In*: Amendt J., Goff M.L., Campobasso C.P. & Grassberger M. (eds): *Current Concepts in Forensic Entomology*. Springer, Dordrecht, pp. 1-24.

Grimaldi D. & Engel M.S. (2005): *Evolution of insects*. Cambridge University Press, New York, 755 pp.

Gronquist M., Schroeder F.C., Ghiradella H., Hill D., McCoy E.M., Meinwald J. & Eisner T. (2006): Shunning the light to elude the hunter: diurnal fireflies and the “femmes fatales”. *Chemoecology*, 16: 39-43.

Gullan P.J. & Cranson P.S. (2010): *The insects: an outline of entomology, 4th edition*. Wiley-Blackwell publishing, Oxford, 565 pp.

Hoermann C. von, Steiger S., Müller J.K. & Ayasse M. (2013): Too Fresh Is Unattractive! The Attraction of Newly Emerged Nicrophorus vespilloides Females to Odour Bouquets of Large Cadavers at Various Stages of Decomposition. *PLoS ONE*, 8: 1-11.

Hoermann C. von, Ruther J. & Ayasse M. (2016): Volatile Organic Compounds of Decaying Piglet Cadavers Perceived by *Nicrophorus vespilloides*. *Journal of Chemical Ecology*, 42: 756-767.

Honěk A. (1995): Distribution and shell colour and banding polymorphism of the *Cepea* species (Gastropoda. Helicelidae) in Bohemia. *Acta Societatis Zoologica Bohemoslovaca*, 59: 63-77.

Hůrka K. (2005): *Brouci České a Slovenské republiky*. Nakladatelství Kabourek, Zlín, 391 pp.

Hůrka K. & Čepická A. (1978): *Rozmnožování a vývoj hmyzu.* Státní pedagogické nakladatelství, Praha, 224 pp.

Ikeda H., Kagaya T., Kubota T. & Abe T. (2013): Evolutionary relationships among food habit, loss of flight, and reproductive traits: life-history evolution in the Siphinae (Coleoptera: Silphidae). *Evolution*, 62: 2065-2079.

Ikeda H., Kagaya T., Kubota K. & Abe T. (2008): Evolutionary relationships among food habit, loss of flight and reproductive traits: life history evolution in the Silphinae (Coleoptera: Silphidae). *International Journal of Organic Evolution*, 62: 2065-2079.

Ikemoto T. & Takai K. (2000): A new linearized formula for the law of total effective temperature and the evaluation of line-fitting methods with both variables subject to error. *Environmental Entomology*, 29: 671-682.

Jakubec P. (2016): Thermal summation model and instar determination of all developmental stages of necrophagous beetle, *Sciodrepoides watsoni* (Spence) (Coleoptera: Leiodidae: Cholevinae). *PeerJ*, 4, e1944.

Jakubec P. & Růžička J. (2012): Distribution of open landscape carrion beetles (Coleoptera: Silphidae) in selected lowlands of the Czech Republic. *Klapalekiana*, 48: 169-189.

Jakubec P. & Růžička J. (2015): Is the type of soil an important factor determining the local abundance of carrion beetles (Coleoptera: Silphidae)? *European Journal of Entomology*, 112: 747-754.

Jakubec P. & Růžička J. (2016): *Coleoptera: Agyrtidae, Silphidae. Folia Heyrovskyana, Icones Insectorum Europae Centralis 26*. Kabourek Publishing, Zlín, 17 pp.

Ji Y. (2012): *The carrion beetles of China (Coleoptera: Silphidae)*. China Forestry Publishing House, Beijing, 309 pp.

Kalinová B., Podskalská H., Růžička J. & Hoskovec M. (2009): Irresistible bouquet of death--how are burying beetles (Coleoptera: Silphidae: Nicrophorus) attracted by carcasses. *Die Naturwissenschaften*, 96: 889-899.

Kilian A. & Mądra A. (2015): Comments on the biology of *Sciodrepoides watsoni watsoni* (Spence, 1813) with descriptions of larvae and pupa (Coleoptera: Leiodidae: Cholevinae). *Zootaxa*, 3955: 45-61.

Klausnitzer B. (1994): 50. Familie: Lampyridae, pp. 228-230. *In*: Klausnitzer B. (ed.): *Die Larven der Käfer Mitteleuropas, 2. Band, Myxophaga, Polyphaga, Teil 1*. Goecke & Evers, Krefeld, 325 pp.

Klots A.B. & Klots E.B. (1963): *Living Insects of the World*. Doubleday & Company, Inc, New York, 304 pp.

Kočárek P. (2001): Diurnal Activity Rhythms and Niche Differentiation in a Carrion Beetle Assemblage (Coleoptera: Silphidae) in Opava, the Czech Republic. *Biological Rhythm Research*, 32: 431-438.

Kočárek P. (2003): Decomposition and Coleoptera succession on exposed carrion of small mammal in Opava, the Czech Republic. *European Journal of Soil Biology*, 39: 31-45.

Korschefsky R. (1951): Bestimmungstabelle der bekanntesten deutschen Lyciden-, Lampyriden- und Drilidenlarven. *Beiträge zur Entomologie*, 1: 60-64, table 1.

Kozminykh V.O. (1994): A new species of carrion beetles of the genus *Thanatophilus* (Coleoptera, Silphidae) from the southern Urals. *Zoologicheskii Zhurnal,* 73: 161-165.

Kratochvíl J. (1957): *Klíč zvířeny ČSR, Díl II, Třásnokřídlí, blanokřídlí, řásnokřídlí, brouci.* Nakladatelství Československé akademie věd, Praha, 746 pp.

LaBella D.M. & Lloyd J.E. (1991): Lampyridae (Cantharoidea), pp. 427-428. *In*: Stehr F.W. (ed.): *Immature Insects, Vol. 2*. Kendall/Hunt Publishing Company, Iowa, 974 pp.

Lawrence J.F. & Newton A.F., Jr. (1982): Evolution and classification of beetles. *Annual Review of Ecology and Systematics*, 13: 261-290.

Lawrence J.F. & Ślipiński A. (2013): *Australian beetles, Volume 1. Morphology, Classification and Keys*. CSIRO Publishing, Collingwood, 561 pp.

Leschen R.A.B. & Beutel R.G. (2004): Ocellar atavism in Coleoptera: plesiomorphy or apomorphy? *Journal of Zoological Systematics and Evolutionary Research*, 42: 63–69.

Lengerken H. von (1929): Studien über die Lebenserscheinungen der Silphini (Col.). XI-XIII. *Thanatophilus sinuatus* F., *rugosus* L. und *dispar* Hrbst. *Zeitschrift für Morphologie und Ökologie der Tiere*, 14: 654-666.

Lengerken H. von (1938): Beziehungen zwischen der Ernährungsweise und der Gestaltung der Mandibeln bei den Larven der Silphini (Coleopt.). *Zoologischer Anzeiger*, 122: 171-175.

Lewis S. (2016): *Silent Sparks, The Wondrous World of Fireflies*. Princeton University Press, New Jersey, 223 pp.

Lewis S.M. & Cratsley C.K. (2008): Flash signal evolution, mate choice and predation in fireflies. *Annual Review of Entomology*, 53: 293-321.

Lloyd J. E. (1972): Chemical communication in fireflies. *Environmental Entomology*, 1: 265-266.

Lloyd J.E. (2008): Fireflies (Coleoptera: Lampyridae). In: Capinera, J.L. (Ed.), *Encyclopedia of Entomology. 2nd Edition*. Springer, London, pp. 1429-1452.

Majka C.G. & MacIvor J.S. (2009): The European lesser glow worm, *Phosphaenus hemipterus* (Goeze), in North America (Coleoptera, Lampyridae). *ZooKeys*, 29: 35-47.

Martin G.J., Branham M.A., Whiting M.F. & Bybee S.M. (2016): Total evidence phylogeny and the evolution of adult bioluminescence in fireflies (Coleoptera: Lampyridae). *Molecular Phylogenetics and Evolution*, 107: 564-575.

Matuszewski S., Bajerlein D., Konwerski S. & Szpila K (2010): Insect succession and carrion decomposition in selected forests of Central Europe. Part 2: composition and residency patterns of carrion fauna. *Forensic Science International*, 195: 42-51.

Matuszewski S. (2011): Estimating the pre-appearance interval from temperature in *Necrodes littoralis* L. (Coleoptera: Silphidae). *Forensic Science International*, 212: 180-188.

Matuszewski S., Bajerlein D., Konwerski S. & Szpila K. (2011): Insect succession and carrion decomposition in selected forests of Central Europe. Part 3: Succession of carrion fauna. *Forensic Science International*, 207: 150-163.

Matuszewski S., Szafałowicz M. & Jarmusz M. (2013): Insects colonising carcasses in open and forest habitats of Central Europe: search for indicators of corpse relocation. *Forensic Science International*, 231: 234-239.

McDermott F. A. (1966): Lampyridae. *In*: Steel W.O. (Ed.) *Coleopterorum Catalogus Supplementa Edita, Pars 9 (Edition Secunda)*. W. Junk, Gravenhage, 149 pp.

Medvedev L.N. & Ryvkin A.B. (1992): 45. Semeystvo Lampyridae, pp. 26-29. *In*: Ler P. A. (ed.): *Opredeliteľ nasekomykh Daľnego Vostoka SSSR v shesti tomach. Tom 3. Zhestokrylye ili zhuki. Chasť 2. [A key to the insects of Far Eastern USSR in six volumes. Vol. 3. Coleoptera or beetles. Part 2.]*. Nauka, Sankt Peterburg, 704 pp. [in Russian].

Midgley J.M. & Villet M.H. (2009): Effect of the killing method on post-mortem change in length of larvae of *Thanatophilus micans* (Fabricius 1794) (Coleoptera:

Silphidae) stored in 70% ethanol. *International Journal of Legal Medicine*, 123: 103-108.

Mikšić R. (1982): Die Lampyriden Europas (Coleoptera, Malacodermata). *Acta Entomologica Jugoslavica*, 17 (1-2): 19-26.

Moosman P.R. Jr., Cratsley C.K., Lehto S.D. & Thomas H.D. (2009): Do courtship flashes of fireflies (Coleoptera: Lampyridae) serve as aposematic signals to insectivorous bats? *Animal Behaviour*, 78: 1019-1025.

Navarette-Heredia J.L. (2009): *Silphidae (Coleoptera) de México: diversidad y distribución*. Universidad de Guadalajara, Guadalajara.

Newton A.F. (1991): Silphidae (Staphylinoidea), pp. 339-341. *In*: Stehr F.W. (ed.): *Immature Insects, Vol. 2*. Kendall/Hunt Publishing Company, Iowa, 974 pp.

Nikolaev G.V. & Kozminykh V.O. (2002): The carrion beetles (Coleoptera: Agyrtidae, Silphidae) of Kazakhstan, Russia and adjacent countries. Kazak Universiteti, Almaty, 166 pp. [in Russian]

Papi F. (1969): Light emission, sex attraction and male flash dialogues in a firefly, *Luciola luisitanica* Charp. *Monitore Zoologico Italiano (N.S.)*, 3: 135-184.

Paulian R. (1941): Les premiers états des Staphylinoidea. Étude de morphologie comparée. *Mémoires du Muséum National d'Histoire Naturelle (Nouvelle Série)*, 15: 1-361.

Peltanová A., Dvořák L. & Juřičková L. (2012): The spread of non-native *Cepaea nemoralis* and *Monacha cartusiana* (Gastropoda: Pulmonata) in the Czech Republic with comments on other land snail immigrants. *Biologia*, 67: 384-389.

Podskalská H., Růžička J., Hoskovec M. & Šálek M. (2009): Use of infochemicals to attract carrion beetles into pitfall traps. *Entomologia Experimentalis et Applicata*, 132: 59-64.

Portevin G. (1926): *Les grands nécrophages du globe. Silphini-Necrodini-Necrophorini. Encyclopédie Entomologique (Série A), Vol. 6*. Lechevalier, Paris, 269 pp.

Ratcliffe B.C. (1996): The carrion beetles (Coleoptera: Silphidae) of Nebraska. *Bulletin of University of Nebraska State Museum*, 13: 1-100.

Reitter E. (1911): *Fauna Germanica, Die Käfer des Deutschen Reiches, 3. Band*. K. G. Lutz' Verlag, Stuttgart, 436 pp.

Richards C.S. & Villet M.H. (2008): Factors affecting accuracy and precision of thermal summation models of insect development used to estimate post-mortem intervals. *International Journal of Legal Medicine*, 122: 401-408.

Ridgeway J.A., Midgley J.M., Collett I.J. & Villet M.H. (2014): Advantages of using development models of the carrion beetles *Thanatophilus micans* (Fabricius) and *T. mutilatus* (Castelneau) (Coleoptera: Silphidae) for estimating minimum post mortem intervals, verified with case data. *International Journal of Legal Medicine*, 128: 207-220.

Růžička J. (2002): Taxonomic and nomenclatorial notes on Palaearctic Silphinae (Coleoptera: Silphidae). *Acta Societatis Zoologicae Bohemicae*, 66: 303-320.

Růžička J. (2015): Silphidae, pp. 291-304. *In*: Löbl I. & Löbl D. (Eds.): *Catalogue of Palaearctic Coleoptera Vol. 2/1. Hydrophiloidea–Staphylinoidea*, Revised and Updated Edition. Brill, Leiden & Boston, xxvi + 1702 pp.

Sikes D.S., Madge R.B. & Newton A.F. (2002): A Catalog of the Nicrophorinae (Coleoptera: Silphidae) of the world. *Zootaxa*, 65: 1-304.

Sikes D.S., Trumbo S.T. & Peck S.B. (2005): Silphinae. *In*: *Tree of Life Web Project*. http://tolweb.org/Silphinae/26994/2005.02.07. Accessed July 26, 2017.

Sikes D.S. (2008): Carrion Beetles (Coleoptera: Silphidae), pp. 749-758. *In*: Capinera J.L. (Ed.): *Encyclopedia of Entomology, Second Edition*. Springer, London, 4346 pp.

Schwalb H.H. (1961): Beiträge zur Biologie der einheimischen Lampyriden *Lampyris noctiluca* Geoffr. und *Phausis splendidula* Lec. und experimentelle Analyse ihres Beutefang- und Sexualsverhaltens. *Zoologische Jahrbücher, Abteilung für Systematik, Geographie und Biologie der Tiere*, 88: 399-550.

Schawaller W. (1981): Taxonomie und Faunistik der Gattung Thanatophilus (Coleoptera: Silphidae). *Stuttgarter Beiträge zur Naturkunde, Serie A*, 351: 1-21.

Sharkley E.K, South A., Moosman P.R., Cratsley Ch.K. & Lewis S.M. (2010): Assesing condition-dependance of male flash signals of Photinus fireflies. Journal of Insect Behavior, 23: 215-225.Shields V.D.C. (2008): Ultrastructure of insect sensilla. In: Capinera, J.L. (Ed.), *Encyclopedia of Entomology. 2nd Edition*. Springer, London, pp. 4009-4023.

Sivinski J. (1981): The nature and possible functions of luminescence in Coleoptera larvae. *Coleopterists Bulletin*, 35: 167-179.

Stanger-Hall K.F., Lloyd J.E. & Hillis D.M. (2007): Phylogeny of North American fireflies (Coleoptera: Lampyridae): Implications for the evolution of light signals. *Molecular Phylogenetics and Evolution*, 45: 33-49.

Stillwell R.C. & Fox C.W. (2009): Geographic variation in body size, sexual size dimorphism and fitness components of a seed beetle: Local adaptation versus phenotypic plasticity. *Oikos*, 118: 703-712.

Stehr F.W. (1991): *Immature Insects, Volume 2*. Kendall/Hunt Publishing Company, Dubuque, 974 pp.

Šustek Z. (1981): Keys to identification of insects 2: Carrion beetles of Czechoslovakia (Coleoptera: Silphidae). *Zprávy Československé Společnosti Entomologické při ČSAV,* 2: 1-47. [in Czech]

Timmins G.S., Jackson S.K. & Swartz H.M. (2001): The evolution of bioluminescent oxygen consumption as an ancient oxygen detoxification mechanism. *Molecular Evolution*, 52: 321-332.

Tisi L.C., De Cock R., Stewart A.J.A., Booth D. & Day J. (2014): Bioluminescent leakage throughout the body of the glowworm *Lampyris noctiluca* (Coleoptera: Lampyridae). *Entomologia Generalis*, 35: 47-51.

Tuf I.H., Drábková L. & Šipoš J. (2015): Personality affects defensive behavior of *Porcellio scaber* (Isopoda, Oniscidea). *Zookeys*, 515: 159-171.

Tyler J. (2002): *The glow-worm*. Lakeside, Sevenoaks, 76 pp.

Velásquez Y. & Viloria A.L. (2009): Effects of temperature on the development of the Neotropical carrion beetle *Oxelytrum discicolle* (Brullé, 1840) (Coleoptera: Silphidae). *Forensic Science International*, 185(1-3): 107-109.

Velásquez Y. & Viloria A.L. (2010): Instar determination of the neotropical beetle *Oxelytrum discicolle* (Coleoptera: Silphidae). *Journal of Medical Entomology*, 47: 723-726.

Viviani V.R. (2001): Fireflies (Coleoptera: Lampyridae) from southeastern Brazil: Habitats, Life history, and Bioluminescence. *Annals of the Entomological Society of America*, 94: 129-145.

Vogel R. (1912): Beiträge zur Anatomie und Biologie der Larve von *Lampyris noctiluca*. *Zoologicher Anzeiger*, 39: 515-519.

Underwood T.J., Tallamy D.W. & Pesek J.D. (1997): Bioluminescence in firefly larvae: a test of the aposematic display hypothesis (Coleoptera: Lampyridae). *Journal of Insect Behavior*, 10: 365-370.

Wells J.D. & Sperling F.A.H. (2001): DNA-based identification of forensically important Chrysomyinae (Diptera: Calliphoridae). *Forensic Science International*, 120: 110-115.

Wells J.D., Pape T. & Sperling F.A.H. (2001): DNA-Based Identification and Molecular Systematics of Forensically Important Sarcophagidae (Diptera*). Journal of Forensic Sciences*, 45: 1098-1102.

Willows-Munro S. & Schoeman M.C. (2015): Influence of killing method on Lepidoptera DNA barcode recovery. *Molecular Ecology Resources*, 15: 613-618.

Wipfler B., Pohl H., Yavorskaya M.I. & Beutel R.G. (2016): A review of methods for analysing insect structures — the role of morphology in the age of phylogenetics. *Current Opinion in Insect Science*, 18: 60-68.

Woods W.A. Jr., Hendrickson H., Mason J. & Lewis S.M. (2007): Energy and predation costs of firefly courtship signals. *The American Naturalist*, 170: 702-708.

Xambeu P.J.V. (1892): Moeurs et métamorphoses d'insectes (Suite). *Annales de la Société Linnéenne de Lyon*, 39: 137-194.

Xambeu P.J.V. (1900): Moeurs et métamorphoses d'insectes, 9e mémoire, deuxieme partie. *Revue d'entomologie Caen*, 19: 1-56.

8. ANNEXES

Annex 1: Redescription of central European firefly larvae, Part 1: Lampyris noctiluca (Linnaeus, 1758) (Coleoptera: Lampyridae)

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Redescription of immature stages of central European fireflies, Part 1: *Lampyris noctiluca* (Linnaeus, 1758) larva, pupa and notes on its biology (Coleoptera: Lampyridae: Lampyrinae)

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Abstract

The mature larva of *Lampyris noctiluca* (Linnaeus, 1758) is redescribed and illustrated in detail, including scanning electron microscope images. Male and female pupae are briefly described, including notes on behaviour as well as light production of the immature stages. Observed structures, life cycle and behaviour of larvae and pupae are discussed.

Key words: *Lampyris noctiluca*, larva, pupa, morphology, ecology

Introduction

The genus *Lampyris* Geoffroy, 1762 (Lampyrinae) consists of 60 described species, distributed predominantly in the Palaearctic Region, of which 29 occur in Europe and only one, *Lampyris noctiluca* (Linnaeus, 1758), in the Czech Republic (Burakowski 2003; Geisthardt & Satô 2007). Descriptions of this species are brief and the morphology, particularly that of larvae, is poorly known. Schematic illustrations of variable quality are given in many works, but detailed images and descriptions are missing (e.g. Reitter 1911; Vogel 1913; Korschefsky 1951; Kratochvíl 1957; Medvedev & Ryvkin 1992; Klausnitzer 1994; Burakowski 2003). There are nine synonyms assigned to *Lampyris noctiluca* (Geisthardt & Satô 2007).

This firefly mostly inhabits warmer humid areas with limestone substrate, bordered by deciduous forest (Schwalb 1961; Burakowski 2003). It can be found in lowlands as well as in mountains of high-altitudes, of up to 1800 m a.s.l. (Hůrka 2005). According to Schwalb (1961), the geographical and vertical distribution of this species show a rather high tolerance with regards to temperature and exposure. The species may occur syntopically with other firefly species (e.g. *Lamprohiza splendidula* (Linnaeus, 1767); M. Novák, pers. obs.).

This work is the first of a proposed trilogy focusing on the immature stages of firefly species occurring in central Europe and at the same time the only species occurring in the author's homeland, the Czech Republic. The outdated knowledge of their morphology needs to be revisited. The second part will redescribe the larva and pupae of *Lamprohiza splendidula* and, the final part the larva of the diurnal *Phosphaenus hemipterus* (Goeze, 1777), and will present a dichotomous key and comparison of features of all three species.

Material and methods

Larvae of *Lampyris noctiluca* were collected in Ljubljana, Slovenia, in the first half of September 2013 and 2015. Overall six specimens were collected at the forest edge next to Koseze Pond (Koseški Bajer; 46°3'58.4"N, 14°28'10.7"E), on decomposing wood and on leaf litter, two and a half hours after sunset. The area of Koseze Pond is a landscape park, geologically mostly comprised of slates and limestones, with predominantly acidic soil. The area has many small streams and sources. The climate is continental with rainfall mostly in the summer and autumn months. Average annual rainfall is 1350 mm. Average yearly temperature is 9.7 °C, in summer the average is 19.6 °C (Anonymous 2012).

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The collected specimens were identified using the key in Burakowski (2003). All collected larvae were identified as higher instars and selected for subsequent analysis. Regarding the distinction of the individual instars, no work describing either morphological or biometric traits nor chaetotaxy exists. Furthermore, the total number of instars is not yet reliably determined. To solve this problem, individuals approaching the maximum species length limit were selected. Additionally, Schwalb's figure for the average larval sizes in each year of the 3-year cycle was used for confirmation of higher instar status (Schwalb 1961: p. 51, fig. 34).

Three of the larvae were fixed and stored in 60% ethanol and kept at a low temperature for subsequent morphological investigation. The material is housed in the author's personal collection. The remaining three specimens were kept alive for observations on their behaviour and obtaining pupae and adults. The larvae were kept separately, in round plastic containers 10 cm diameter and 4 cm high, padded with thick layers of moist tissues to prevent desiccation. The containers were placed in a ventilated room with a natural light source to provide the appropriate temperature and light period for the season. Each container was also provisioned with a *Cepaea* sp. snail as a food source. Cleaning of the containers with antiseptic cleaner and replacement of tissues took place every week. Two female pupae and one male pupa were obtained. They were kept alive during the whole pupal and consequent imaginal stage for developmental period verification, sex determination, behavioural observations and the possibility of photographing their glow. Since adults of this species no longer feed, a simple replacement of wet tissues every week was sufficient to keep pupae and imagines alive. The adults were additionally moved into deeper containers to provide them with larger comfort.

Optical imaging. The fixed specimens were cleaned using a small fine hair brush and then placed in a Digital Ultrasonic Cleaner PS-06A. The detached heads were afterwards boiled in 10% KOH (potassium hydroxide) for clearer visibility of delicate parts. Habitus was photographed while the specimen was submerged in ethanol, heads were photographed while submerged in glycerol (due to better optical properties and higher stability owing to the greater density of the glycerol). Images were taken using a Canon macro photo lens MP-E 65 mm and EF-S 60 mm on a Canon 550D body, attached to a sliding frame, using EOS Utility program. Sets of pictures of each habitus taken were consequently stacked into a final image with a high depth of field in Zerene Stacker (64-bit) by Zerene Systems LLC.

Living pupae were placed in small glass bowls and their habitus was photographed using the same equipment and consequently stacked into final images. Pupae were photographed in a dark environment, since the light of the reflectors causes discomfort and agitates them to move.

For morphological studies, two specimens were dissected and their body parts examined separately using Olympus SZX7 stereo microscope. The images of isolated maxillae and mandibles submerged in ethanol were taken using an Olympus XC30 Digital Colour Camera attached to Olympus CX41 biological microscope and consequently stacked into a sharp final image in Zerene Stacker (64-bit) by Zerene Systems LLC.

Electron imaging. Larvae were examined in the Faculty of Science of Charles University in Prague. The specimens were first dehydrated by passing them through a series of increasing alcohol concentrations. The samples were transferred sequentially through 60%, 70%, 80%, 90% and 95% alcohol for ca. 0.5 h each. Dehydrated samples were then dried by the Critical Point Drying method. Dry samples were subsequently attached to an aluminium disk target and coated with gold in a Bal-Tec Sputter Coater SCD 050, to ensure conductivity. The electron imaging was performed using JSM-6380LV (JEOL) Scanning Electron Microscope (SEM) with a high resolution of 3.0 nm (30 kW).

Interpretation and terminology of larval and pupal descriptions follows Archangelsky (2004) and LaBella & Lloyd (1991).

Interpretation of sensory organs follows Shields (2008) as follows: *Sensilla trichodea* can vary greatly in length and are freely moveable on a basal membrane, can be solely mechanosensitive, dually mechano- and contact chemosensitive, olfactory, or thermosensitive; *sensilla chaetica* usually takes form of bristles or spines similar to the sensilla trichodea, is typically set in a socket and can be mechano- or contact chemosensitive; *sensilla coeloconica* is defined as a basiconic peg or cone set in a shallow pit, most often chemo-, thermo-, or hygrosensitive.

Results

Lampyris noctiluca (Linnaeus, 1758)

Material examined. Ljubljana (Slovenia), three higher instar larvae out of six collected in the first half of September 2013 and 2015, three pupae reared from remaining collected larvae.

Diagnosis. Larvae robust, dark brown or black; with light coloured spots on posterolateral margins on pronotum and every tergite except caudal segment; pronotum concave on posterior margin; maxillary palpomere II with inner-lateral sagittal slot; mandibles with retinaculum forming a sharp inner tooth; mandibular channel covered with thick blunt seta; microscopic granulose protuberances, densely occurring on sclerites and legs; photic organ represented by a pair of conspicuous whitish spots located on pleurites of abdominal segment VIII.

Description of mature larva (Figs 1–8). Fusiform and robust; slightly flattened dorsoventrally. Body length 20–23 mm (from the anterior margin of pronotum to the apex of caudal segment); with 3 thoracic and 10 abdominal segments. Tergites from pronotum to abdominal segment IX extending laterally to cover the rest of the body and divided by sagittal line in dorsal view. Colouration: body dark brown or black, with distinct pinkish or yellowish spots on posterolateral margins on pronotum and every tergite except caudal segment. Spiracles on pleural plates of light colouration. Photic organ represented by a pair of whitish spots located on pleurites of abdominal segment VIII.

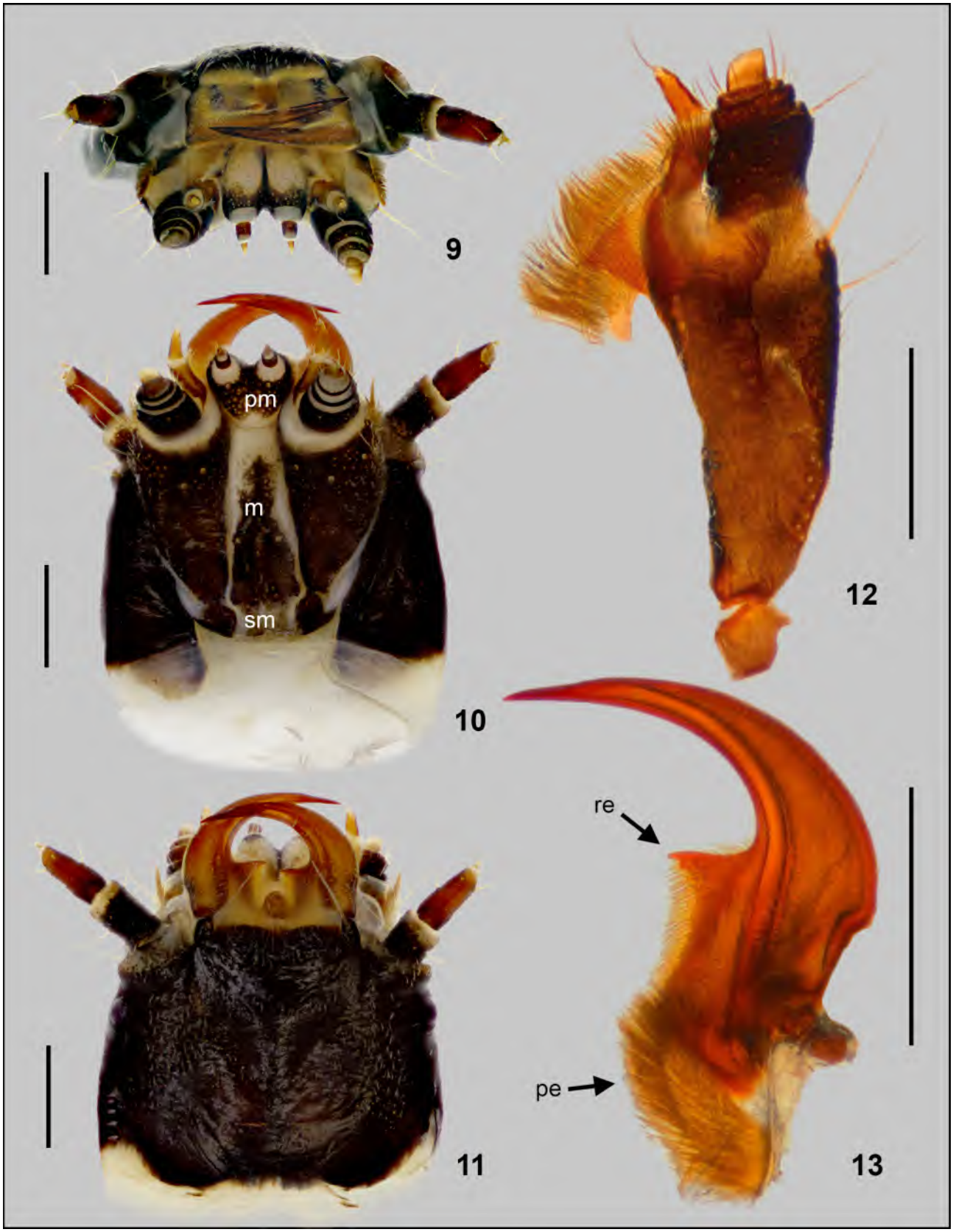
Cuticular outgrowths). 1. Stout, short, blunt, oblique setae (Figs 14, 20, 26; st); 2. dense granulose protuberances (Fig. 26; gp); 3. long stout setae (Figs 20, 23, 25; sch); 4. coeloconical receptors (Figs 14, 20, 21; sc).

Head capsule (Figs 9–11, 14–16). Prognathous; retractable within prothorax, extensible neck membrane forming a two layered envelope around retracted head; of equal width and length; slightly widening posteriorly. Epicranial plate laterally about the same size as width of head capsule at its shortest width, with one stout seta anterolateraly close to the base of antenna. Head capsule dorsally covered with short blunt setae lying on surface and coeloconical receptors (Figs 14, 21; sc). Epicranial suture hardly distinguishable for its dark pigmentation, but present (Fig. 11). One stemma on each side of head. Labrum fused to clypeus forming a clypeolabrum, covering base of mandibles in dorsal view. Clypeolabrum slightly double-arched in anterior view, with one long seta on each lateral corner, reaching the apex of mandibles (Fig. 14). Epipharynx formed by two plates, and an anterior pair of brushes of long setae on each plate, which project centrally past anterior margin of head. Hypopharynx covered with long setation. Gula absent (Fig. 10).

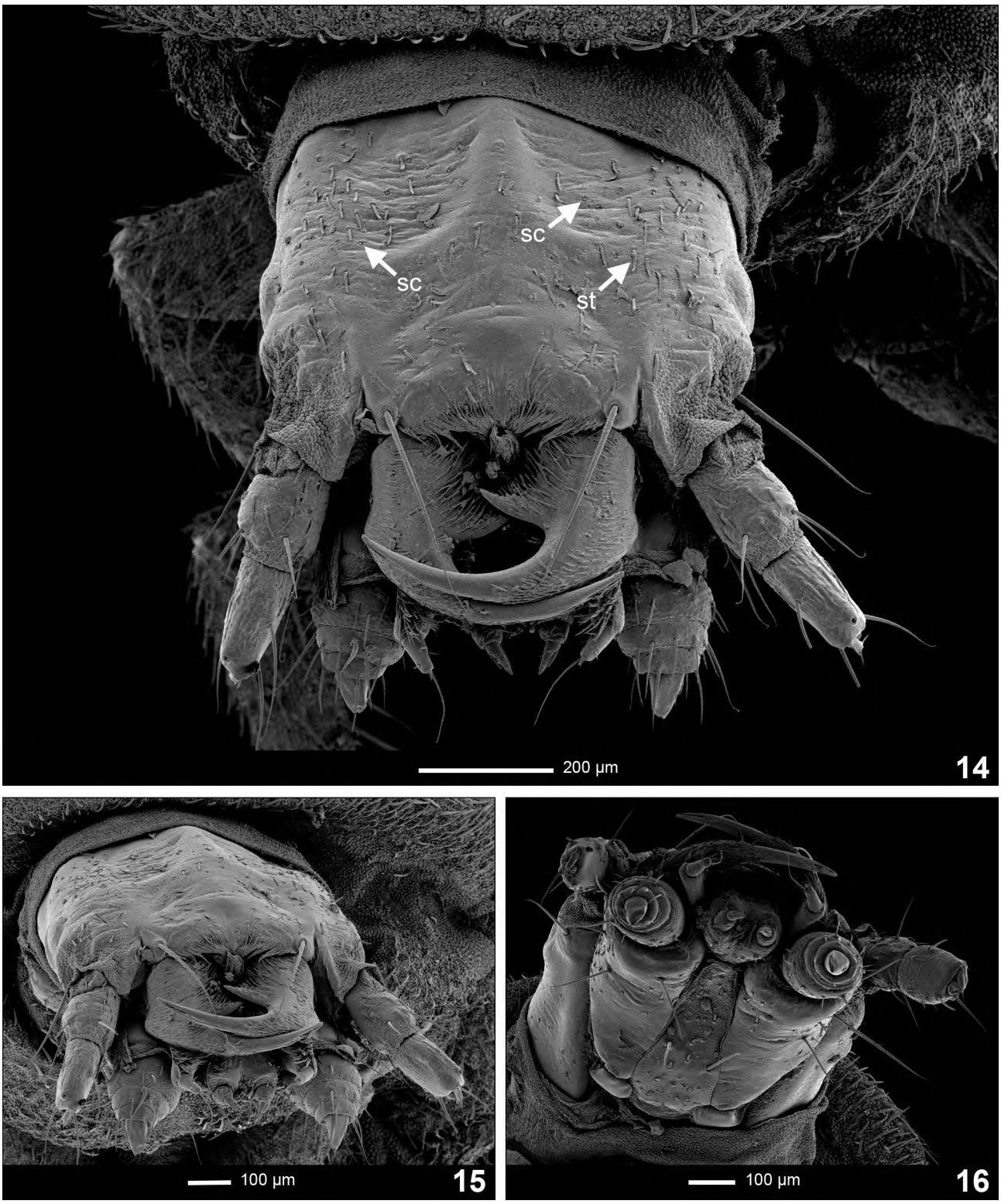
Antenna (Figs 20–22). Trimerous, inserted on lateral distal margin of epicranial plate; partially retractable within membranous socket. Basal antennomere widest, fully sclerotized, bearing short setae lying on surface, coeloconical receptors and several long oblique setae near the apical region (Fig. 20). Several long stout setae placed radially on the anterior margin, with a distinct seta on inner lateral area of antennomere. Second antennomere slightly shorter than first, laterally flattened; bearing numerous short setae lying on surface irregularly scattered over the antennomere, together with several coeloconical receptors (Fig. 21; sc) and four stout setae—first three in the middle and on apex of inner margin of antennomere, fourth on apical region of outer lateral margin of antennomere. Sensorium of second antennomere (Figs 21, 22; as) oval, widest at the base, closely touching second antennomere, shorter than third antennomere with no visible surface pattern. Third antennomere (Figs 21, 22; aIII) shortest, bearing four short setae; one basal and three apical, together with a pair of short cuticular projections; ventrally apical thick and dorsal-apical thin.

Maxilla (Fig. 12). Consisting of five parts, attached by membrane to labium forming a maxillo-labial complex (Fig. 17). Cardo transverse, sub-rectangular, slightly wider than long. Stipes elongated, ventrally relatively glabrous, setae mainly on distal half, with three long stout setae placed radially on ventral apical region; dorsolaterally covered with short setation lying on surface. Galea bimerous, basal part sub-cylindrical, slightly wider than distal, with long dorsal setation partially covering distal part; distal part sub-cylindrical, inclined centrally, with short setae and one apical seta longer than body of the distal part. Lacinia with a brush of long setae on outer lateral margin. Palpifer (Fig. 17; pf) large, rectangular, about the same length and width. Maxillary palpus trimerous (Figs 17, 18; mpI, mpII, mpIII), basal and second palpomere short and wide. Palpifer and palpomeres I–II covered with several setae mainly on outer dorsolateral margin; palpomere III (Fig. 18; mpIII) irregularly sub-conical, thick, blunt, with an inner longitudinal lateroapical sensory slot (Fig. 18; ses), small seta on outer lateral region and short outer lateral longitudinal sensory slot covered with thin seta lying on surface.

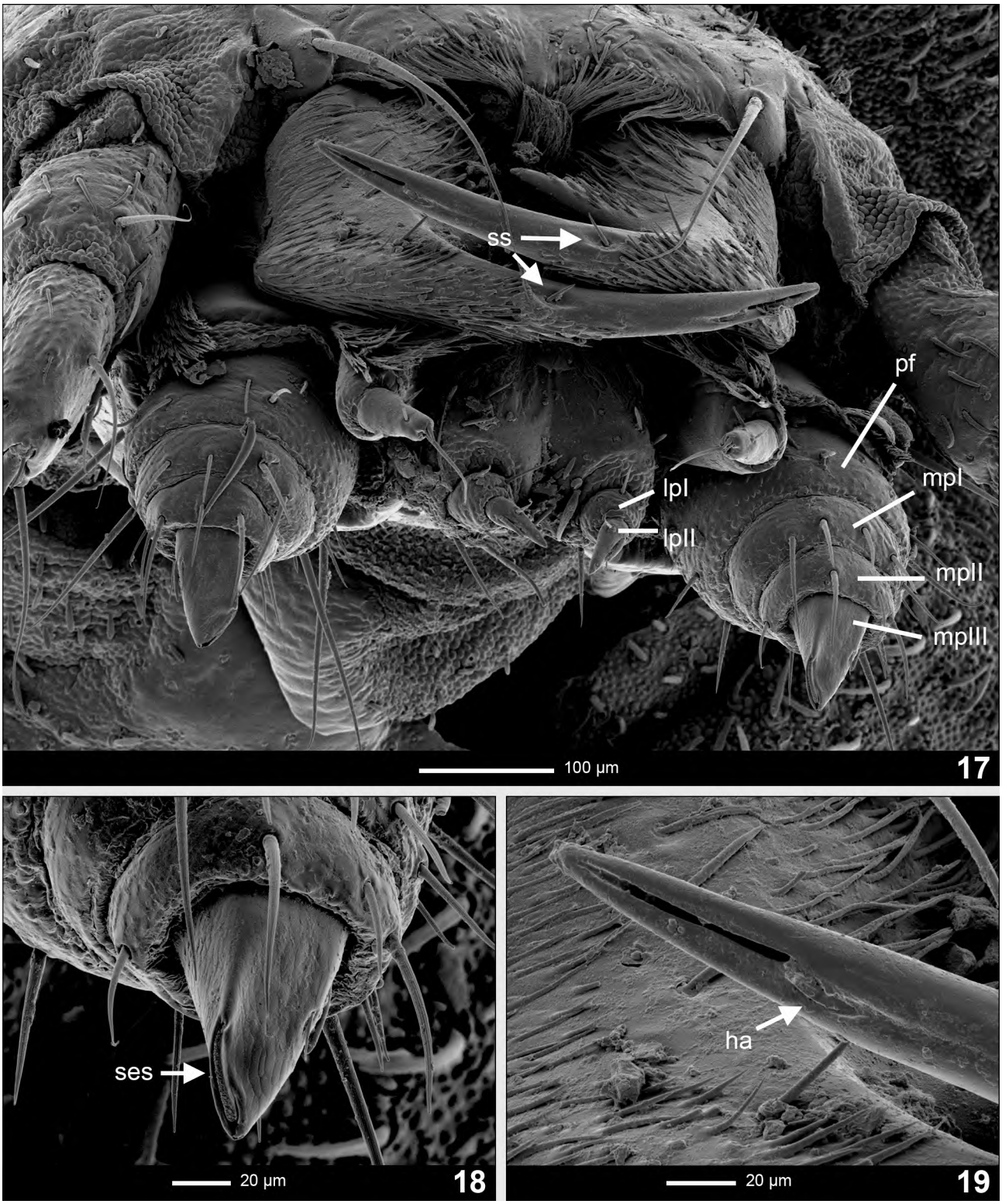
FIGURES 1–8. *Lampyris noctiluca*. General habitus of mature larva photographed in alcohol in dorsal (1) and ventral (2) views; abdominal segments VII to X in ventral view (3); lateral view (4). General habitus of mature larva photographed dry in dorsal (5) and ventral (6) views; abdominal segments VII to X in ventral view (7); lateral view (8). Scale bar: 5 mm.



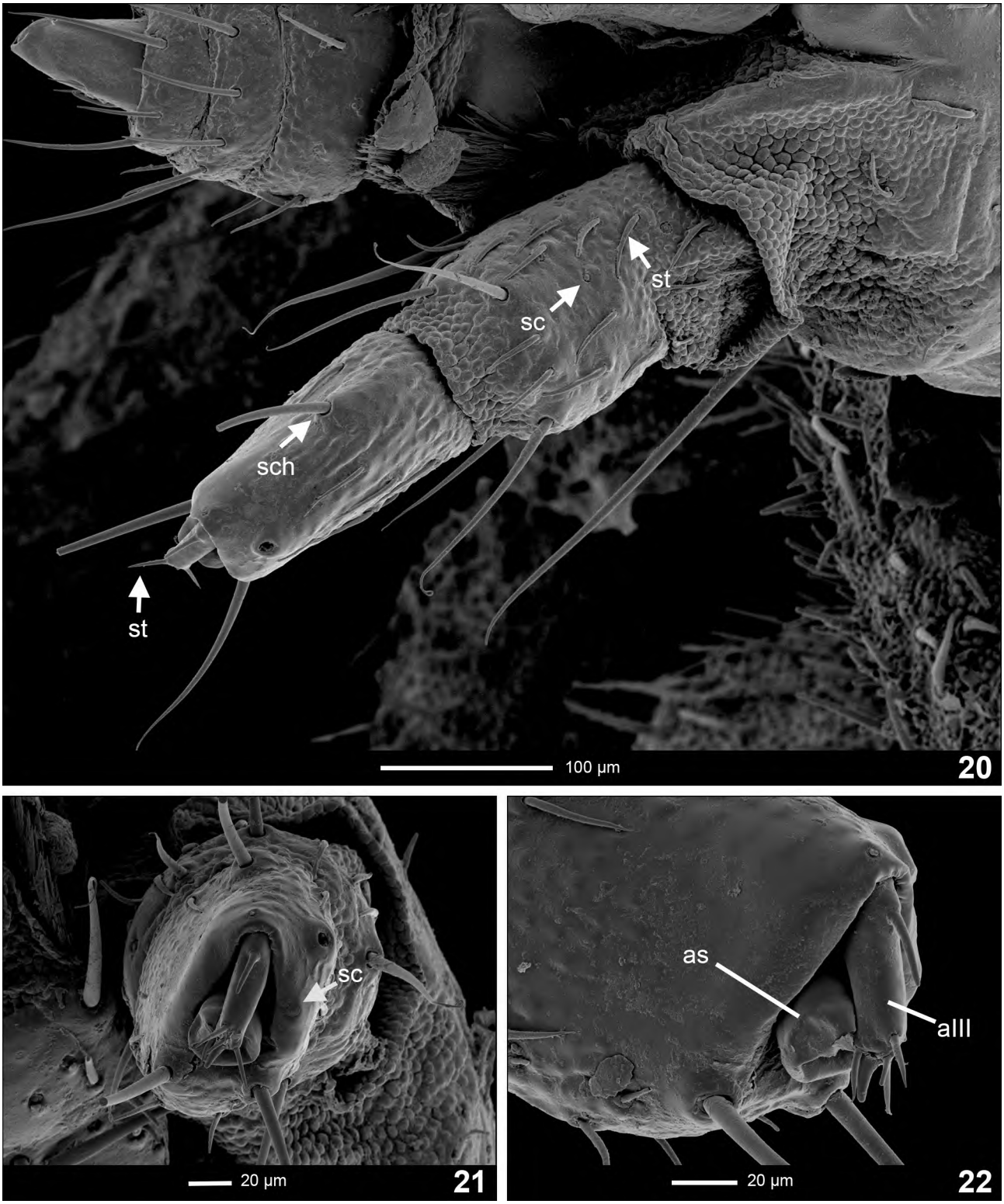
FIGURES 9–13. *Lampyris noctiluca*. Detail of head in anterior (9); ventral (10) and dorsal (11) views; right maxilla in dorsal view (12); right mandible in dorsal view (13). Abbreviations: pe—penicillus; re—retinaculum; p—prementum; m—mentum; sm—submentum. Scale bars: 0,5 mm.



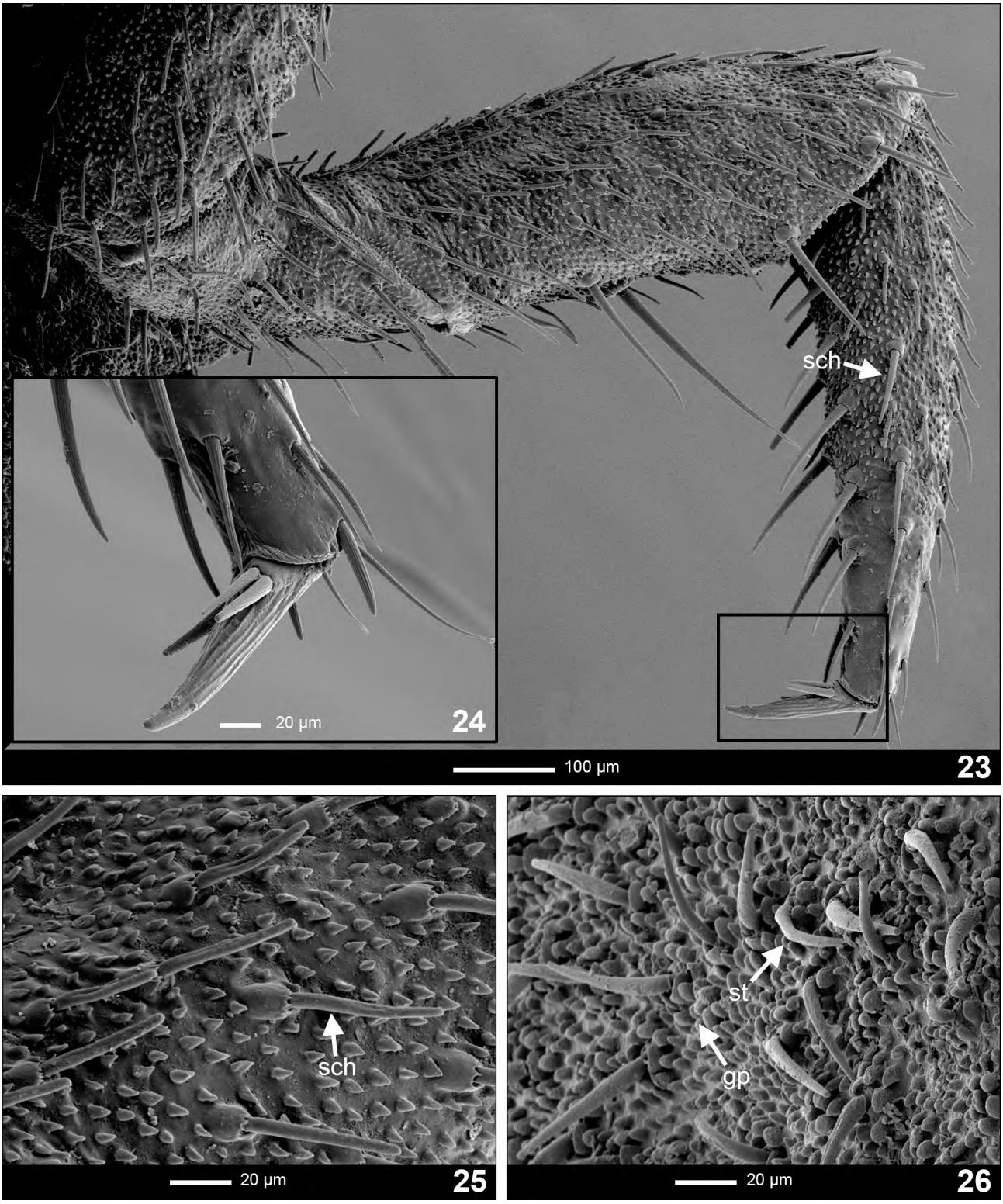
FIGURES 14–16. *Lampyris noctiluca*. SEM image of head in dorsal (14); anterior (15) and ventral (16) views. Abbreviations: sc—sensillum coeloconicum; st—sensillum trichodeum; sch—sensillum chaeticum.



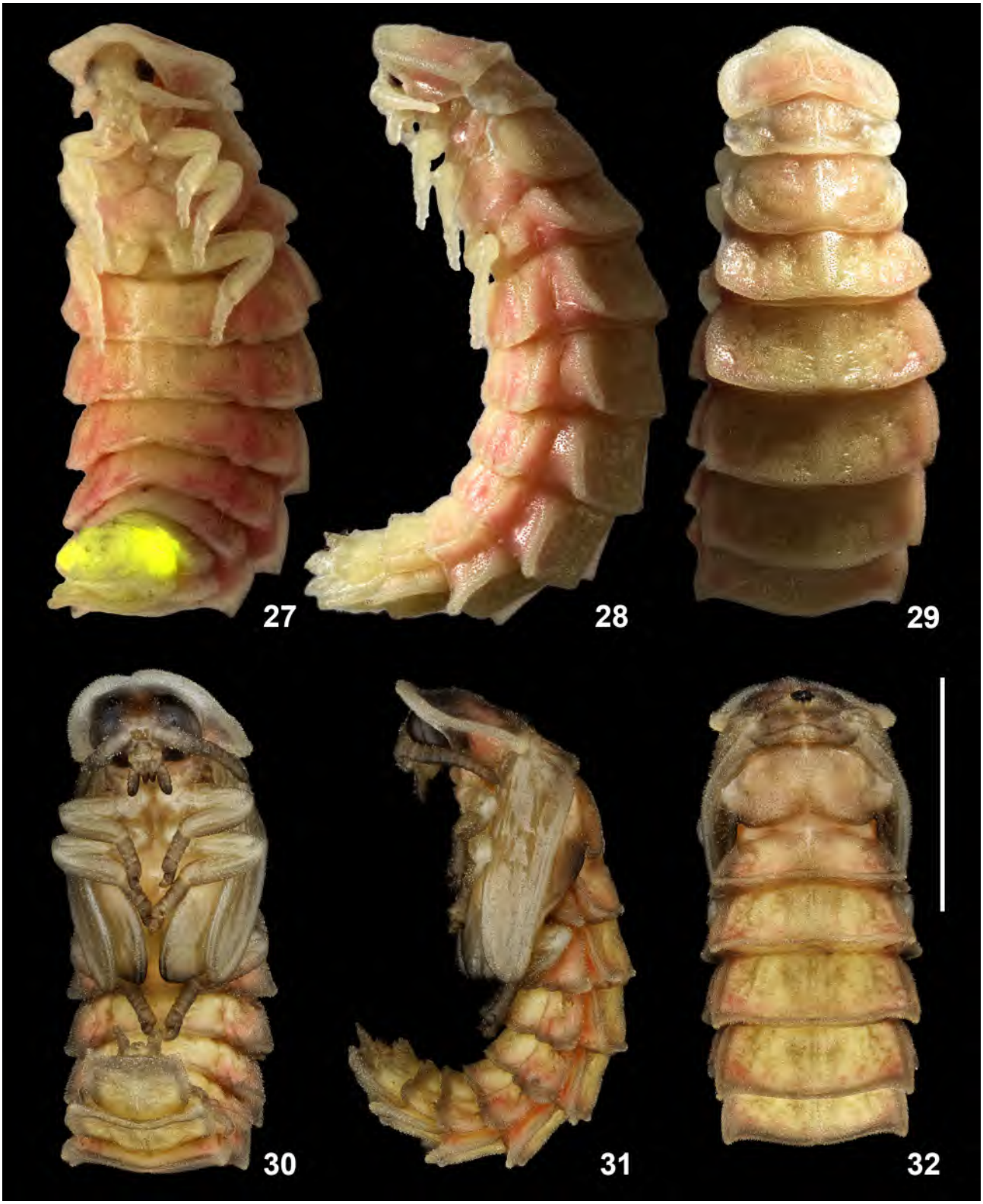
FIGURES 17–19. *Lampyris noctiluca*. SEM image of maxillolabial complex in anterior view (17); maxillary palpus (18); channel opening on the left mandible (19). Abbreviations: ha—hyaline appendage; lp1, lp2—labial palpus 1, 2; mpI–III—maxillary palpus I–III; pf—maxillary palpifer; ses—sensory slot; ss—solitary seta.



FIGURES 20–22. *Lampyris noctiluca*. SEM image of antenna in general view (20); anterior view (21); detail of sensorium and third antennomere (22). Abbreviations: aIII—third antennomere; as—antennal sensorium; sc—sensillum coeloconicum; st—sensillum trichodeum; sch—sensillum chaeticum.



FIGURES 23–26. *Lampyris noctiluca*. SEM image of leg in general view (23, framed area enlarged in Fig. 24); pretarsus (24). Detail of body surface on legs (25) and pronotum (26). Abbreviations: st—sensillum trichodeum; sch—sensillum chaeticum; gp—granulose protuberances.



FIGURES 27–32. *Lampyris noctiluca*. Glowing female pupa in ventral view (27). Female pupa in lateral (28) and dorsal (29) views. Male pupa in ventral (30), lateral (31) and dorsal (32) views. Scale bar: 5 mm.

Labium (Fig. 17). Closely attached to maxilla, formed by a short and strongly sclerotized prementum, mentum and weakly sclerotized submentum (Fig. 10; pm, m, sm). Glossae absent. Prementum heart-shaped in ventral view; covered with very short setation; bearing several longer blunt setae, and a pair of long stout setae, placed centrally on ventral region. Labial palpus bimerous (Fig. 17; lp1, lp2); basal palpomere wider than long, bearing several

setae; distal palpomere conical, longer and narrower than basal, bearing a short thin erect seta on basal half dorsally, a longer, stout and blunt seta covering a sagittal slot positioned on outer margin and sensillum coeloconicum on outer ventrolateral side of apex. Mentum elongated, sub-triangular, unsclerotized on lateral margins, ventrally bearing numerous short setae lying on surface and a pair of long, erect setae centrally.

Mandible (Figs 13, 19). Symmetrical, falcate, with an internal channel opening subapically on outer edge (Fig. 19). Penicillus well developed (Fig. 13; pe). Retinaculum present, forming one sharp inner tooth on basal half of mandible (Fig. 13; re). Inner margin of mandible from retinaculum to the base covered with stout setae (Fig. 13). Basal two-thirds of mandible ventrally with dense setation lying on surface and aimed centrally. Dorsally, mandible covered with several strong setae lying on surface, aiming medially on proximal two-thirds of each mandible. Lateral margin covered by brush of short setae lying on surface of basal two-thirds (Fig. 17). Sensory (hyaline) appendage on outer margin of mandible before channel opening consists of a blunt thick seta (Fig. 19; ha). A distinct short, stout seta set in a shallow depression present dorsally at anterior end of lateral setation (Fig. 17; ss).

Thorax (Figs 1, 2, 4–6, 8). Three-segmented, thoracic tergites divided by sagittal line (Figs 1, 5). Pronotum of equal length and width, sub-trapezoidal, wider posteriorly, rounded at posterolateral corners, strongly concave on posterior margin. Meso- and metanotum sub-rectangular, wider than long, mesonotum longer than metanotum. Lateral areas of meso- and metathorax formed by episternum and epimeron; episternum of mesothorax bearing an annular spiracle. Prosternum rounded, wider than long, robust, well sclerotized, subdivided into three plates; lateral ones extending above and to the sides of coxae; medial plate sub-pentagonal. Meso- and metasternum subdivided by transverse fold into poorly sclerotized basisternum and well sclerotized sternellum; sternellum subdivided into three plates, lateral ones extending above and to the sides coxae, representing large episterna and smaller epimera, medial plate less sclerotized on margins, heart-shaped with wider margin posteriorly.

Legs (Figs 23, 24). Pentamerous, all pairs similar in shape and size. Coxa large, stout, covered by short sharp setae. Coxal-trochanteal membrane (Figs 2, 6) reaching less than 1/2 of coxal length. Trochanter small, sub-triangular in lateral view, shorter than femur, covered by short sharp setae. Femur slightly fusiform, widening towards apex in lateral view, covered by short sharp setae, with several large setae ventrally. Tibiotarsus as long as femur, narrower, tapering towards distal end, bearing stout short sharp setae dorsally and strong sharp erect setae ventrally. Pretarsus (Fig. 24) composed of a claw with distinct ridges, ventrally bearing three short stout setae with fine ridges. Cuticle of leg (Fig. 25) densely covered with grainy protuberances except for apical half of tibiotarsus (Fig. 23).

Abdomen (Figs 1–8). Ten-segmented, tapering towards posterior end, segments I to VIII subdivided by fine sagittal line in dorsal view. Tergites of segments I to VIII sub-trapezoidal, similar in shape and colouration, wider than long; tergite of segment IX sub-rectangular; segment X forming a narrow, incompletely sclerotized dark ring, bearing the holdfast organ—pygopod (Fig. 3)—with several eversible processes. Ventrites of segments I to VIII sub-rectangular, slightly wider than long, well sclerotized, with a pair of long stout setae on posterolateral margins; ventrite of segment IX sub-trapezoidal. Pleural areas well sclerotized, pleural suture of segments I to V subdivide lateral areas into large sub-rectangular upper pleurite, bearing an annular spiracle, and narrow lower pleurite anteriorly covered; pleural segments VI to VIII with only upper pleurite bearing an annular spiracle. Segment VIII bearing photic organs ventrally on pleurites, appearing as two whitish spots (Fig. 3).

Larval behaviour. Larvae of *Lampyris noctiluca* were observed to "ride" the snails, which means mounting the shell and assuming a favourable position to inject the head of the snail or its upper tentacles with lethal toxin. The larva then remains on the shell until the snail shows no signs of life. Feeding can take up to two days. The larva avoids feeding on digestive organs and can sometimes remove these organs from the shell before resuming feeding on the rest of the body. After feeding, the larva remains passive for certain time and after ca. one day excretes a dark brown fluid from the back of its abdomen.

While collecting larvae in nature, a several seconds lasting glow was often observed, followed by a long period of darkness. In captivity, the larvae demonstrated this behaviour when disturbed. When undisturbed, the larvae occasionally showed photic behaviour of definite pulses of light lasting ca. 2 seconds, separated from the next one by a longer interval of darkness lasting ca. 4 seconds. The other photic manifestation consisted of continuous glow of weaker intensity, which could take several minutes.

Description of female pupa (Figs 27–29). Type of pupa: *adectica exarata libera*. Curved, ventrally concave. Length 20 and 23 mm. Colouration: yellowish white on tergites and parts that will become sclerotized in adult stage, pink especially in pleural region. Surface covered by short setae.

Head capsule. Completely covered by pronotum in dorsal view. Eyes small. Antennae short, extending laterally, without reaching posterolateral corners of pronotum. Mouthparts visible in ventral view.

Thorax. Pronotum similar in shape to that of larva, with more acute posterolateral corners and less elongated. Meso- and metanotum smaller, sub-rectangular; mesonotum significantly shorter than metanotum, bearing very short elytra which become vestigial in adult individual. All pairs of legs free, visible in ventral view, proportionally shorter to the overall body size than those of males. Spiracles present on pleural areas of mesothorax.

Abdomen. Abdominal segments sub-rectangular, wider than long. Ventrites on segments II–IV bearing a double sagittal line of depression in cuticle. Spiracles present on abdominal pleural areas of segments I–VIII. Segment VIII bearing functional larval photic organs (Fig. 27).

Description of male pupa (Figs 30–32). Type of pupa: *adectica exarata libera*. Curved, ventrally concave. Length 17 mm. Colouration on young pupae: ochred yellow on tergites and parts that will become sclerotized in adult stage, pink especially in pleural region in young pupae. Colouration on older pupae: beige to brown with a tint of olive or army green on pronotum, head, elytra and legs; inner surface of dorsal and abdominal tergites ochred yellow. Surface covered by short setae.

Head capsule. Completely covered by pronotum in dorsal view. Eyes distinctly large, on sides of the head. Antennae short, extending laterally towards distal end of prothoracic femur. Mouthparts visible in ventral view.

Thorax. Pronotum semicircular and proportionally longer than that of female when compared to overall body size (length of pronotum to body length ratio is 0.23 while in female the ratio is 0.15). Small narrow mesonotum and large wide metanotum sub-rectangular, bearing beige wing pads with dark brown apices, covered by beige elytra of about the same length; wing pads reaching distal end of second abdominal segment, when pupa is relaxed. Pro- and mesothoracic legs free, visible in ventral view; metathoracic legs almost completely covered by wing pads except for distal segments of tarsi, which extend past second ventrite. Spiracles present on pleural areas of mesothorax.

Abdomen. Abdominal segments sub-rectangular, wider than long. Spiracles present on abdominal pleural areas of segments I–VIII. Segment VIII bearing functioning larval photic organs.

Pupal behaviour. Both male and female pupae are commonly idle, either lying on their side or back, responding only to disturbance. A luminescent response can be induced by handling the animal or even by vibrations. It consist of one short glow, lasting several seconds , with a peak intensity lasting ca. 1 second and then quickly fading away. When under a strong light source, the pupa starts to move its abdomen, twiddling from side to side or doing “crunches”. This behaviour suggests an effort to move into darker area, i.e. positive thigmotaxis. The same behaviour can be observed in prepupae.

Development period and ontogenetic morphological changes in pupae. From the reared larvae, future male entered the stage of prepupa 1 day prior to pupation and future females 3–6 days prior to pupation. The pupal period lasted 8 days for male and 7–10 days for females.

The mesonotum of a female is laterally blunt in the first few days of pupal development, but later the lateral margins begin to sharpen to form vestigial elytra. On the other hand, in male pupae, the elytra and wings are already semi developed since the first day of the pupal stage. Therefore, the sex of future adult can be determined in any time of the pupal period.

Discussion

External sensory organs. Close observation of larval anatomy revealed different types of *sensilla* and sensory organs. Since the exact determination of type and function of observed sensory organs would merit a separate work, the following paragraphs will be dedicated to only a brief description and speculations on possible functions, with regards to the ecological aspects.

Sensilla trichodea were mostly found on sclerotized parts of dorsum and venter (Fig. 26; st), head capsule (Fig. 14; st) and antennae (Fig 20; st). It seems plausible, that specifically on the third antennomere, some of these sensilla are in fact *flagella*. In addition, setae on ventrites have an auxiliary function during moulting (Tyler 2002).

Probably the most abundant sensilla observed on this species were the *sensilla chaetica*. Being found on the antennae (Fig. 20; sch) and legs (Figs 23, 25; sch), the chemosensitivity function could be possible for sensilla situated on the antennae, the mechanoreceptive function on legs.

Sensilla coeloconica was observed on the epicranial plate and antennae (Figs 14, 20, 21; sc), where chemo-, thermo-, or hygrosensitive function seems plausible. Additionally, this sensillum seems to have a different form on the apex of antennomere II (Fig. 21; sc) than the rest of the body (Figs 14, 20; sc).

Granulose protuberances. Sclerotized areas of the legs, dorsum and venter are densely littered with microscopic granulose protuberances (Figs 25, 26). This unique characteristic of the cuticle could be the reason why the body of *Lampyris noctiluca* gives the impression of being "velvet" like. Whether the function of these protuberances is sensory, insulatory or other is nevertheless unknown.

Ontogeny. Different authors present different data on the length of the developmental cycle of this species. According to Tyler (2002) the cycle takes 2 years with 2 overwinterings, while Schwalb (1961) states 3 years and 3 overwinterings. The possible difference in their conclusions may lie in the different geographical regions in which these authors conducted their observations. While Tyler's observations are of species in the United Kingdom with a more humid and warmer climate, Schwalb's are from Germany with a more continental climate. Additionally, the number of instars also vary according to different authors from 5 (Hůrka & Čepická 1978) to 4–6 (Schwalb 1961). Depending on the sex, it can also be 5 for males with possibly more for females (Tyler 2002), and the variability is believed to be dependent on environmental conditions and food availability. Both Schwalb (1961) and Tyler (2002) present similar developmental lengths for the pupal stage, which is not influenced by the environment, but by the internal settings of the organism. It is therefore possible, that both authors are correct about the cycle length in terms of the regional climate and that *Lampyris noctiluca* can prolong its life by an additional year and one overwintering, if the conditions are not favourable.

Larvae which are ready to pupate seem to switch into diurnal activity, and can often be seen striding along in broad daylight (Tyler 2002). The adult female rarely moves far before she dies, so Tyler (2002) presumes, that it may be that this final larval stage is the one in which glow-worms are able to spread out in search of new habitats. Larvae preparing to pupate the same year often gather together in small groups, and it is fairly common to find six or more side by side under one log (Tyler 2002). The result of the aggregating of pupating larvae may well be the reason why clusters of 2–6 glowing females can sometimes be found within a few centimetres of each other (Tyler 2002). This behaviour could be explained as a means of being more conspicuous for the males and thus having a higher chance of successful mating. Another possibility is finding the "perfect" spot in terms of environmental conditions for the larvae to successfully pupate, for the females to successfully lay eggs and for newly hatched larvae to survive. However, such a phenomenon might prevent the spreading of the population further in the biotope. In any case, this behaviour must provide more evolutionary advantages than disadvantages. The manner in which the larvae gather together is unknown, but may potentially support the intraspecific communication hypothesis (see below) if light manifestations are involved.

The larva enters the stage of prepupa 8–20 days prior to pupation according to Schwalb (1961), but from my observations this period can be shorter (1–6 days). During this time the larva lies curled up in a semicircle either on its side or back. The pupation lasts ca. 8–12 days for a female and 11–15 days for a male (Schwalb 1961; Tyler 2002), which concurs with my observations for females only. The discrepancy in the pupation period may nevertheless be caused by a small sample size.

Hunting for prey. *Lampyris noctiluca* larvae are reported to follow 2-day-old slime-trails forward, tracking the snails, and can detect polarization in dry, if not stale, trails (Lloyd 2008). The snail *Cepaea nemoralis* (Linnaeus, 1758) (Helicidae) is one of the preferred prey of this species and is known to occur in three colour variations; brown, yellow and banded yellow, with significant larval preference for non-banded types (O'Donald 1968). The reason for this preference remains unknown to this day (De Cock 2009). Larvae pierce the anterior part of the snail with their mandibles and inject them with a dark secretion, possibly a neurotoxin, which kills their victim (Schwalb 1961; Klots & Klots 1963; Hůrka & Čepická 1978). They are also known to "ride" the snails, i.e. mounting the shell and assuming a favourable position to attack the head of the snail or its upper tentacles. In either case this is the best way to get the poison as close as possible to the centre of the snail's nervous system (Schwalb 1961; Tyler 2002). Contrary to a widespread belief (Klots & Klots 1963; Hůrka & Čepická 1978), the toxin itself does not appear to predigest the prey, but the larva rather seems to chop out pieces of flesh with its mandibles while using digestive intestinal secretion, as was observed by Schwalb (1961).

Specific experiments still need to be performed to understand the details of feeding in these animals, so the next lines represent conjecture only. Special, solitary sensilla were observed on the mandibles positioned apically before the opening of the inner channel (Fig. 17; ss). It may be possible, that the function of this sensilla is

mechanoreceptive. While the larva attempts a successful bite into its prey's body, the seta triggers the discharge of deadly liquid. Furthermore, a hyaline appendage, which gives the impression of a "shutter", composed of a blunt thick seta can be found at the base of the mandibular channel opening (Fig. 19; ha). This hyaline appendage nevertheless takes different form in each of the abovementioned species. The flow of the deadly liquid, theoretically triggered by the mechanoreceptive sensilla, may be controlled by this "shutter" or by the gland producing the fluid. On the other hand, this structure may just prevent the channel opening getting blocked by small particles. Similar sensilla and hyaline appendages were observed on larvae of *Lamprohiza splendidula* and *Phosphaenus hemipterus* (M. Novák, pers. obs.), as well as in *Lucidota atra* (Olivier, 1790) (Lampyrinae) (Branham & Archangelsky 2000). Still, in each of the abovementioned species, the sensilla and especially the hyaline appendage take different form. Additionally, one of the structures can be missing in other lampyrid species. In *Pyractomena nigripennis* Solier (Lampyrinae), the hyaline appendage is missing but a strong seta is present anteriorly to the base of retinaculum (Archangelsky 2010) and in *Pyractomena borealis* (Randall, 1838) (Lampyrinae) (Archangelsky & Branham 1998), the solitary seta is missing, but a large protruding hyaline appendage is present at the mandibular channel opening. It is possible that in the latter species, the structure also posesses triggering function, escpecially considering its protruding position.

Photic behaviour. According to Tyler (2002), the larva of *Lampyris noctiluca* can produce light in three different ways. One, when disturbed, it will sometimes switch on its lights for a few seconds and then turn them off again. This seems to be a defensive mechanism to scare off potential predators. Two, some larvae have been known to glow continuously for hours, without any apparent provocation. These are, according to Tyler (2002), often fully grown larvae ready to pupate. Therefore, this glow, which is very similar to that of an adult female, might be just part of the preparation for adulthood, at a time when the larva's body is undergoing internal changes. A third type of photic display described by Tyler (2002) is sometimes produced during movement. It consists of definite pulses of light lasting ca. 2 seconds, separated from the next one by a longer interval of darkness lasting ca. 4 seconds, although Dreisig (1974) reports glows lasting 7.3 seconds on average and the interval of darkness of 20.2 seconds on average. The intensity of each pulse gradually builds up, followed by a period of steady brightness and then a final period, during which the light fades and goes out altogether (Schwalb 1961; Tyler 2002). All three types of photic display were also observed in the described specimens.

The true cause of the last type of photic manifestation remains a mystery. Tyler (2002) proposes five possible causes; 1) the glow has no purpose and is just a by-product of the light organs' development; 2) the larva uses the light while tracking the prey; 3) the larva uses the light to attract the prey; 4) interspecific communication and 5) aposematic defence connected with unpalatability, which seems to be the only hypothesis supported by evidence (Sivinski 1981; Underwood *et al.* 1997; De Cock & Matthysen 2003; De Cock 2009; Moosman *et al.* 2009). Since Tyler (2002) adequately explains the pros and cons for each of the five points, I would like to address intraspecific communication only. Viviani (2001) supposedly witnessed possible intraspecific communication in an unidentified *Bicellonychia* sp., where larvae reacted to flashes emitted by adults, hypothesising the cause of this behaviour could be informing the adults of an occupied food niche. This nonetheless cannot be the case with *Lampyris noctiluca*, where the egg-bearing females are flightless, thus communication of larvae with adult males would be pointless. The way larvae flash differs from that of the females. If this was indeed a signal for the males to distinguish between the larvae and females, a simple darkness of the larvae seems like a more effective solution. Furthermore, larvae do not usually glow during the adult mating season (Schwalb 1961) and evidence suggests that luminescence in Lampyridae seems to have arisen first in larvae and then subsequently in the adults who use it for sexual communication (Branham & Wenzel 2003). Yet, the flash display may serve to communicate between larvae, for example for effective division of a food niche. On the other hand, larvae do not have developed eyes like adults, only simple ocelli, so this assumption seems doubtful. In conclusion, the utilisation of larval light in intraspecific communication with adults or even with other larvae seems unlikely.

Interestingly, low level luminescence was recently observed by Tisi *et al.* (2014) in the pairs of lightly-pigmented posterolateral spots on each tergite and even in the inactive light organ. Nevertheless, according to the authors, this luminescence is so weak, that it is most probably unrelated to aposematic signalization.

Pupa and prepupa. According to Schwalb (1961), the larva enters the stage of prepupa 8–20 days prior to pupation. From my observations, it was noticed that this period can be even shorter (1–6 days). The range presented by Schwalb seems to be quite wide. Besides different rearing conditions, a possible explanation may be a different period of prepupal stage depending on the sexes. But even in the pupal stage the difference between sexes is not that distinctive (8–12 days for a female and 11–15 days for a male; Schwalb 1961; Tyler 2002). Moreover,

the male pupa raised for the purpose of this article turned into an imago after 8 days only (M. Novák, pers. obs.). It may be that individuals staying in prepupa for 20 days might have fed more recently than individuals staying in prepupa for 8 days, i.e. needing a longer time to process the ingested food and expel the undigested waste. The prepupa is the stage of preparation of the larval body for entering the pupal stage, therefore the time needed for processing and elimination of food intake might influence the length of the prepupal stage.

Tyler (2002) describes the colour of pupa as pale yellow, turning olive green after several hours, but this only applies for the older male pupae (Figs 30–32), where the olive colour is especially distinct in dimmer light conditions. The female pupa (Figs 27–29) stays yellowish white with pink regions throughout the whole pupal period. Adult males are overall much darker compared to their female counterparts, but there is one more distinction between male and female pupae, which probably plays an important role in the colour difference. The pupal skin in the male seems to darken and harden as the pupa gets older. After the adults emerge, the exuvia left by a female pupa is extremely delicate and without any colouration. The exuvia of a male, on the other hand, is brown and still retains its original shape.

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References

Anonymous (2012) *Gozdnogospodarski načrt gozdnogospodarskega območja Ljubljana (2011–2020)* [*Forest management plan for Ljubljana forest management area 2011–2020*]. Zavod za gozdove Slovenije, območna enota Ljubljana, 1200 pp. [in Slovenian]

Archangelsky, M. (2004) Description of the last larval instar and pupa of *Aspisoma fenestrata* Blanchard, 1837 (Coleoptera: Lampyridae) with brief notes on its biology. *Tijdschrift voor Entomologie*, 147, 49–55. https://doi.org/10.1163/22119434-900000138

Archangelsky, M. (2010) Larval and pupal morphology of *Pyractonema nigripennis* Solier (Coleoptera: Lampyridae: Photinini) and comparative notes with other Photinini larvae. *Zootaxa*, 2601, 37–44. https://doi.org/10.5281/zenodo.197621

Archangelsky, M. & Branham, M.A. (1998) Description of the preimaginal stages *of Pyractomena borealis* (Randall, 1838) (Coleoptera: Lampyridae) and notes on its biology. *Proceedings of the Entomological Society of Washington*, 100, 421–430.

Branham, M.A. & Archangelsky, M. (2000) Description of the last larval instar and pupa of *Lucidota atra* (G.A. Olivier 1790) (Coleoptera: Lampyridae), with a discussion of abdominal segment homology across life stages. *Proceedings of the Entomological Society of Washington*, 102, 869–877.

Branham, M.A. & Wenzel, J.W. (2001) The evolution of bioluminescence in cantharoids (Coleoptera: Elateroidea). *Florida Entomologist*, 84, 565–586. https://doi.org/10.2307/3496389

Burakowski, B. (2003) *Klucze do oznaczania owadow Polski, Czesc XIX Chraszcze—Coleoptera, Zeszyt 29, 30 Karmazynkowate—Lycidae, Swietlikowate—Lampyridae*. [*Key to identification of insects of Poland, Part XIX—Beetles (Coleoptera), Issue 29, 30 Lycidae, Lampyridae*]. Polskie Towarzystwo Entomologiczne, Torun, 39 pp. [in Polish]

De Cock, R. (2009) Biology and behaviour of European lampyrids. *In*: Meyer-Rochow, V.B. (Ed.), *Bioluminescence in Focus—A Collection of Illuminating Essays*. Research Signpost, Trivandrum, pp. 161–200.

De Cock, R. & Matthysen, E. (2003) Glow-worm larvae bioluminescence (Coleoptera: Lampyridae) operates as an aposematic signal upon toads (*Bufo bufo*). *Behavioral Ecology*, 14, 103–108. https://doi.org/10.1093/beheco/14.1.103

Dreisig, H. (1975) Environmental control of the daily onset of luminescent activity in glow-worms and fireflies (Coleoptera: Lampyridae). *Oecologia*, 18, 85–99. https://doi.org/10.1007/bf00348090

Geisthardt, M. & Satô, M. (2007) Lampyridae. *In*: Löbl, I. & Smetana, A. (Eds.), *Catalogue of Palearctic Coleoptera. Vol. 4. Elateroidea – Derodontoidea – Bostrichoidea – Lymexyloidea – Cleroidea – Cucujoidea.* Apollo Books, Sternstrup, pp.

225–234.
Geoffroy, E.L. (1762) *Histoire abrégée des Insectes qui se trouvent aux environs de Paris; dans laquelle ces animaux sont rangés suivant un ordre méthodique. Tome I.* Durand, Paris, 523 pp.
https://doi.org/10.5962/bhl.title.14710
Goeze, J.A.E. (1777) *Entomologische beyträge zu des ritter Linné zwölften ausgabe des natursystems von Joh. Aug. Ephraim Goeze. Erster theil.* Weidmanns Erben und Reich, Leipzig, 736 pp.
https://doi.org/10.5962/bhl.title.45974
Hůrka, K. (2005) *Brouci České a Slovenské republiky.* (*Beetles of Czech and Slovak Republics*). Kabourek, Zlín, 391 pp. [in Czech and English]
Hůrka, K. & Čepická, A. (1978) *Rozmnožování a vývoj hmyzu.* [*Reproduction and ontogeny of insects*]. Státní pedagogické nakladatelství, Praha, 224 pp. [in Czech]
Klausnitzer, B. (1994) 50. Familie: Lampyridae. *In*: Klausnitzer, B. (Ed.), *Die Larven der Käfer Mitteleuropas, 2. Band, Myxophaga, Polyphaga. Teil 1*. Goecke & Evers, Krefeld, pp. 228–230.
Korschefsky, R. (1951) Bestimmungstabelle der bekanntesten deutschen Lyciden-, Lampyriden- und Drilidenlarven. *Beiträge zur Entomologie*, 1, 60–64, table 1.
Klots, A.B. & Klots, E.B. (1963) *Living Insects of the World*. Doubleday & Company, New York, 304 pp.
Kratochvíl, J. (1957) *Klíč zvířeny ČSR, Díl II, Třásnokřídlí, blanokřídlí, řásnokřídlí, brouci.* [*Key to Czechoslovakian fauna. Part II. Thysanoptera, Strepsiptera, Coleoptera*]. Nakladatelství Československé akademie věd, Praha, 746 pp. [in Czech]
LaBella, D.M. & Lloyd, J.E. (1991) Lampyridae (Cantharoidea). *In*: Stehr, F.W. (Ed.), *Immature Insects, Volume 2*. Kendall/ Hunt Publishing Company, Dubuque, pp. 427–428.
Linnaeus, C. (1758) *Systema naturae per regna tria naturae: secundum classes, ordines, genera, species, cum characteribus, differentiis, synonymis, locis. Tomus I. Editio decima, reformata.* Laurentius Salvius, Holmiae, 824 pp.
https://doi.org/10.5962/bhl.title.542
Linnaeus, C. (1767) *Systema naturae per regna tria naturae: secundum classes, ordines, genera, species, cum characteribus, differentiis, synonymis, locis. Tomus I. Pars II. Editio duodecima reformata.* Laurentius Salvius, Holmiae, 1327 pp.
https://doi.org/10.5962/bhl.title.68927
Lloyd, J.E. (2008) Fireflies (Coleoptera: Lampyridae). *In*: Capinera, J.L. (Ed.), *Encyclopedia of Entomology. 2nd Edition*. Springer, London, pp. 1429–1452.
Medvedev, L.N. & Ryvkin, A.B. (1992) 45. Semeystvo Lampyridae. *In*: Ler, P. A. (Ed.): *Opredeliteľ nasekomykh Daľnego Vostoka SSSR v shesti tomach. Tom 3. Zhestokrylye ili zhuki. Chasť 2.* [*A key to the insects of Far Eastern USSR in six volumes. Vol. 3. Coleoptera or beetles. Part 2*]. Nauka, Sankt Peterburg, pp. 26–29. [in Russian]
Moosman, P.R. Jr., Cratsley, C.K., Lehto, S.D. & Thomas, H.D. (2009) Do courtship flashes of fireflies (Coleoptera: Lampyridae) serve as aposematic signals to insectivorous bats? *Animal Behaviour*, 78, 1019–1025.
https://doi.org/10.1016/j.anbehav.2009.07.028
O'Donald, P. (1968) Natural Selection by Glow-worms in a Population of *Cepaea nemoralis*. *Nature*, 217, 194.
https://doi.org/10.1038/217194a0
Olivier, G.A. (1790) *Tableau encyclopédique et méthodique des trois règnes de la nature. Entomologie, ou, histoire naturelle des Crustacés, des Arachnides et des Insectes*. Panckoucke, Imprimeur-Libraire, hôtel de Thou, rue des Poitevins, Paris, 368 pp.
https://doi.org/10.5962/bhl.title.82248
Randall, J.W. (1838) Description of new species of coleopterous insects inhabiting the State of Maine. *Boston Journal of Natural History*, 2, 1–33.
Reitter, E. (1911) *Fauna Germanica, Die Käfer des Deutschen Reiches, 3. Band*. K. G. Lutz, Stuttgart, 436 pp.
Schwalb, H.H. (1961) Beiträge zur Biologie der einheimischen Lampyriden *Lampyris noctiluca* Geoffr. und *Phausis splendidula* Lec. und experimentelle Analyse ihres Beutefang- und Sexualsverhaltens. *Zoologische Jahrbücher, Abteilung für Systematik, Geographie und Biologie der Tiere*, 88, 399–550.
Shields, V.D.C. (2008) Ultrastructure of insect sensilla. *In*: Capinera, J.L. (Ed.), *Encyclopedia of Entomology. 2nd Edition*. Springer, London, pp. 4009–4023.
https://doi.org/10.1007/978-1-4020-6359-6_2295
Sivinski, J. (1981) The nature and possible functions of luminescence in Coleoptera larvae. *Coleopterists Bulletin*, 35, 167–179.
Solier, A.J.J. (1841) Essai sur les collaptérides (suite). *Annales de la Société Entomologique de France*, 10, 46–51.
Tisi, L.C., De Cock, R., Stewart, A.J.A., Booth, D. & Day, J. (2014) Bioluminescent leakage throughout the body of the glow-worm *Lampyris noctiluca* (Coleoptera: Lampyridae). *Entomologia Generalis*, 35, 47–51.
https://doi.org/10.1127/0171-8177/2014/0003
Tyler, J. (2002) *The glow-worm*. Lakeside, Sevenoaks, 76 pp.
Underwood, T.J., Tallamy, D.W. & Pesek, J.D. (1997) Bioluminescence in firefly larvae: a test of the aposematic display hypothesis (Coleoptera: Lampyridae). *Journal of Insect Behavior*, 10, 365–370.
https://doi.org/10.1007/bf02765604
Viviani, V.R. (2001) Fireflies (Coleoptera: Lampyridae) from southeastern Brazil: Habitats, Life history, and Bioluminescence. *Annals of the Entomological Society of America*, 94, 129–145.
Vogel, R. (1913) Zur Topographie und Entwicklungsgeschichte der Leuchtorgane von *Lampyris noctiluca*. *Zoologischer Anzeiger*, 41, 325–332.

Annex 2: Redescription of immature stages of central European fireflies, Part 2: Lamprohiza splendidula (Linnaeus, 1767) larva, pupa and notes on its life cycle and behaviour (Coleoptera: Lampyridae)

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Redescription of immature stages of central European fireflies, Part 2: *Lamprohiza splendidula* (Linnaeus, 1767) larva, pupa and notes on its life cycle and behaviour (Coleoptera: Lampyridae)

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Abstract

The mature larva of the firefly *Lamprohiza splendidula* (Linnaeus, 1767) is thoroughly redescribed and illustrated with detailed images, including scanning electron microscope figures. External sense organs, structure of tergal plates and their significance are discussed, as well as photic manifestation of the larvae and pupae and prey hunting in larvae. Male and female pupae are briefly described, with notes on their developmental changes and behaviour, and the life cycle outlined.

Key words: Elateroidea, Lampyrinae, bioluminescence, morphology, ecology

Introduction

There are only eight described species in the genus *Lamprohiza* Motschulsky, 1853 (Lampyrinae) in Europe. Seven occur in the southwestern part of the continent and one across almost all of Europe (Burakowski 2003; Geisthardt & Satô 2007). Descriptions of the latter species, *Lamprohiza splendidula* (Linnaeus, 1767) are brief and the morphology, in particular the morphology of larvae, is poorly known. Schematic illustrations in many prior works are of variable quality, and none contain detailed images (Reitter 1911; Vogel 1913; Korschefsky 1951; Kratochvíl 1957; Medvedev & Ryvkin 1992; Klausnitzer 1994; Burakowski 2003), with exception of Geisthardt (1979), describing only larval thorax and Bugnion (1929) describing larva of related species *Lamprohiza delarouzei* Jacquelin du Val, 1859.

Lamprohiza splendidula (Linnaeus, 1767) is distributed in southeastern and central Europe, reaching the River Rhine to the west, the Caucasus Mountains to the east, the southern shore of Baltic Sea to the north, and central Italy and the Balkan Peninsula to the south (Burakowski 2003). It is the most common firefly in the Czech Republic (Hůrka 2005). The species inhabits moist and shaded open habitats of lowlands and uplands with deciduous forests, thickets, clearings, banks of rivers and streams, meadows and gardens (Schwalb 1961; Burakowski 2003; Hůrka 2005). It may occur syntopically with other firefly species (e.g. *Lampyris noctiluca* (Linnaeus, 1758), *Luciola* sp.; M. Novák, unpublished observation).

This paper represents the second part of a trilogy focusing on the immature stages of firefly species occurring in central Europe. The first part (Novák 2017) redescribes the larva together with male and female pupae of *Lampyris noctiluca* and the third final part (Novák 2018) will deal with *Phosphaenus hemipterus* (Goeze, 1777) and provide a dichotomous key to all three species and a comparative table of morphological features.

Material and methods

Larvae of *Lamprohiza splendidula* were collected from two localities in Prague, Czech Republic. Sixteen specimens were collected at the end of August 2013 from a hillside next to the Kunratický stream, behind the Thomayer Hospital (50°01'47.6"N, 14°27'47.8"E), commencing an hour after sunset. Subsequently, eleven

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additional specimens were collected in the same season the following year (2014) for rearing. The area is inside the deciduous Kunratický forest, where larvae are found mostly under bushes among decomposing moist leaves. The local rock consists mainly of slate, the climate is temperate, mildly arid characteristic for the Prague plain. The average yearly temperature is 8.8 °C; average annual rainfall is 476 mm (Dostálek 2009).

Three further specimens were collected at the beginning of September 2013 at Petřín hill, near a stairway under the statue of K. H. Mácha (50°04'54.4"N, 14°24'7.6"E). Petřín is a recently landscaped hill in the centre of Prague, an anthropoecosystem with a large amount of park greenery, although in higher parts, remains of the original thermophilic oak forest can still be found. Local rock consists of slates and siltstone, climate is continental with the majority of rainfall in the summer and autumn months. Average annual rainfall is 625 mm. Average yearly temperature is 7.6 °C, in summer the average is 18.5°C (Bratka *et al.* 2011). Specimens were found two hours after sunset, under bushes and low herbaceous vegetation.

From the eleven individuals collected in August 2014 only two survived and entered the stage of pupa in June 2015. One male and one female pupa were obtained.

Methods of identification and storing of the larvae and rearing of the pupae as well as methods of optical and electron microscope imaging are described in Novák (2017). Interpretation and terminology of larval and pupal descriptions follows Archangelsky & Fikáček (2004), LaBella & Lloyd (1991), description of thoracic and abdominal sclerites follows Ballantyne & Menayah (2002) and Lawrence & Ślipiński (2013).

Lamprohiza splendidula (Linnaeus, 1767)

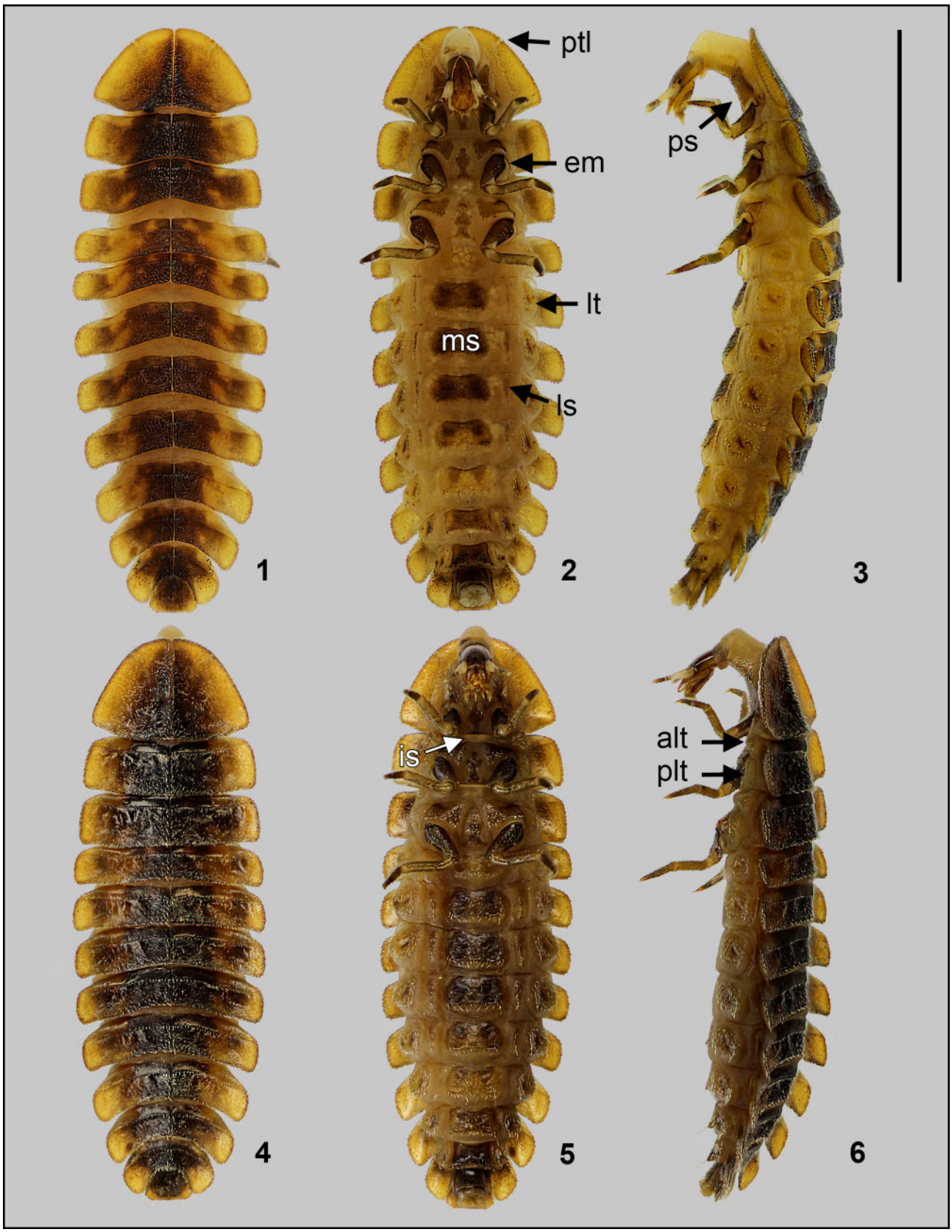
Material examined. Prague (Czech Republic), ten higher-instar larvae out of sixteen collected at the end of August 2013, one male and one female pupa were reared from eleven specimens collected at the end of August 2014.

Diagnosis. Larvae flat, laterally explanate, brown and ochre towards the lateral edges of tergites with pairs of lighter pigmented spots on abdominal tergites I–VI; protergum with narrow emargination anteromedially; light-coloured spot posteriorly behind each stemma; antennal sensorium with distinct basal constriction; retinaculum absent; mandibular channel opening covered by long feather-like or rounded-trapezium hyaline appendage; pretarsal claw ventrally bearing two long setae; photic organ of variable pattern, usually consisting of paired larger spots ventrolaterally on abdominal segments II and VI, sometimes with additional smaller spots ventrolaterally on abdominal segments III–V.

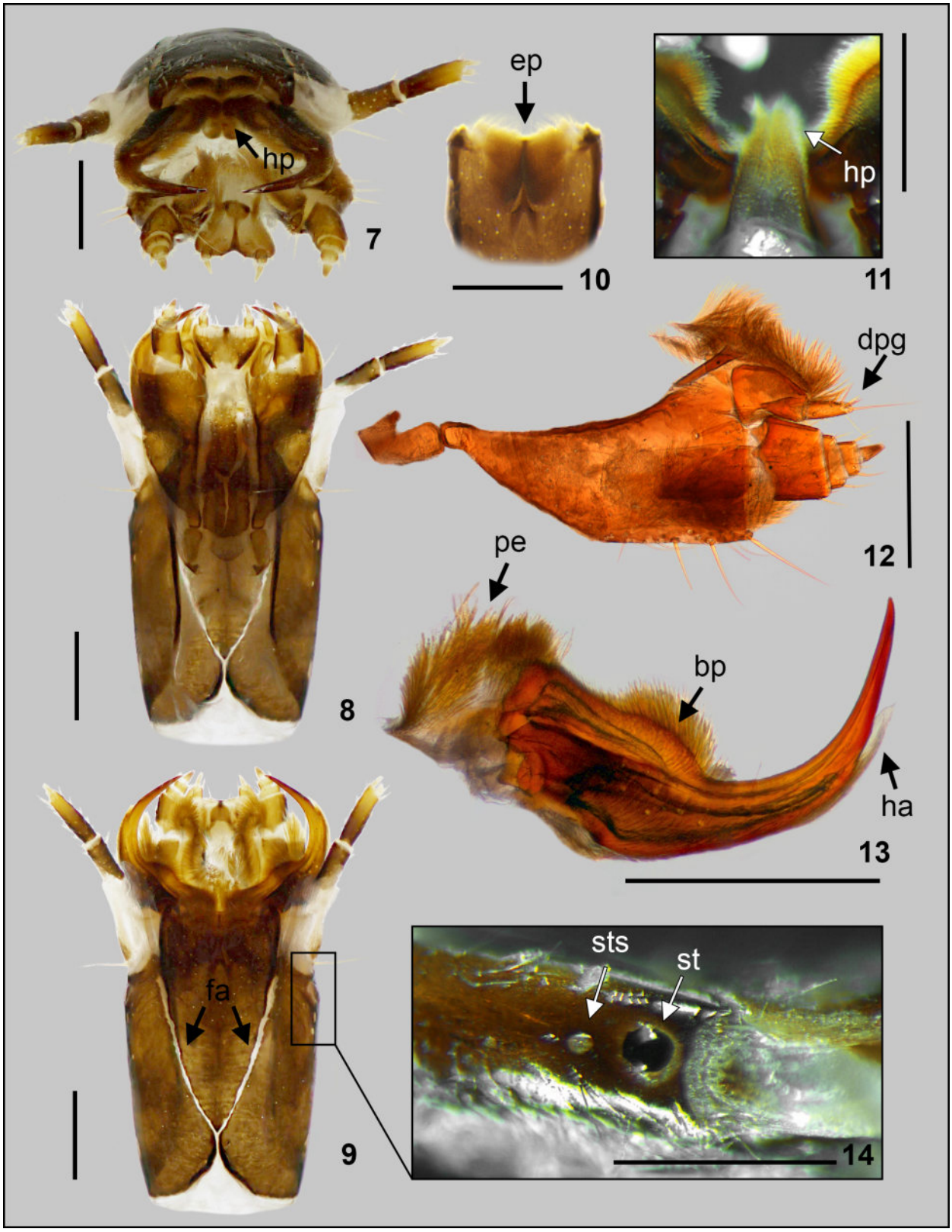
Description of mature larva (Figs 1–6). Elongate and onisciform; dorsoventrally flattened, tergites of thorax and abdomen finely setose on the edges (Figs 4, 15, 28), and strongly laterally explanate (Figs 1, 2, 4, 5). Body length ca. 11–12 mm (from the anterior margin of protergum to the apex of caudal segment); with 3 thoracic and 10 abdominal segments. Tergites from protergum to abdominal segment IX divided by sagittal line in dorsal view (Fig. 1). Colouration: dorsally brown and ochre towards the lateral edges of tergites; with pairs of lighter pigmented spots on abdominal tergites I–VI (Fig. 1), under which a variable number of localized photic organs may occur. Ventral region much lighter than dorsal, with ochre to light brown colouration, except central parts of sternal sclerites which are darker and more sclerotized. Spiracles on pleural plates are of dark brown colouration.

Types of general cuticular outgrowth observed. 1. Stout, long, erect setae (Figs 21, 22; les); 2. thin, short, erect setae (Figs 21, 22; tes); 3. flat setae, lying on/adjacent to the surface (Figs 15, 21, 29; fs).

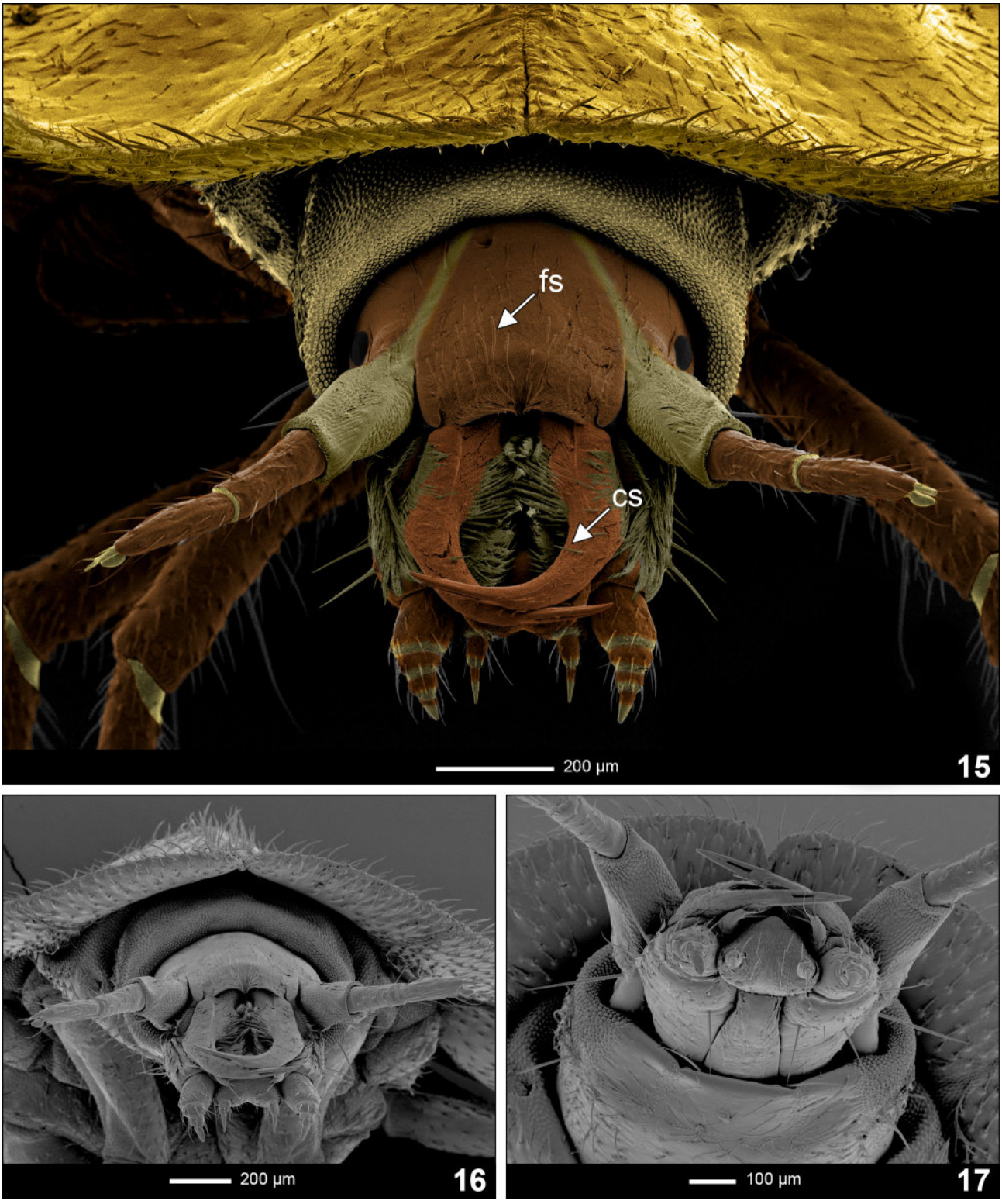
Head capsule (Figs 7–9, 15–17). Prognathous; retractable within prothorax, extensible neck membrane covered in extremely short spines and forming a two-layered envelope around retracted head; longer than wide, slightly tapering posteriorly. Epicranial plate laterally about 1.2 times longer than head capsule at its longest width, with one stout seta anterolateraly close to the base of antennae. Head capsule dorsally covered with long setae lying on surface (Fig. 15; fs). Epicranial suture of light colouration, Y-shaped, with a very short epicranial stem, frontal arms V-shaped (Fig. 9; fa). Gula not present (Fig. 8). One stemma on each side of the head, with a light-coloured spot placed posteriorly behind the stemma (Fig. 14; sts), possibly being a sensory organ. Labrum fused with clypeus forming clypeolabrum, covering the base of mandibles in dorsal view. Clypeolabrum double-arched in anterior view (Figs 15, 16), with no distinguishable setae on lateroapical margins. Epipharynx (Fig. 10; ep) formed by two plates and an anterior brush of long setae, which project centrally past anterior margin of the head. Hypopharynx (Figs 7, 11; hp) with long setation.



FIGURES 1–6. *Lamprohiza splendidula*. General habitus of mature larva photographed in alcohol in dorsal (1); ventral (2) and lateral (3) views. General habitus of mature larva photographed dry in dorsal (4); ventral (5) and lateral (6) views. Abbreviations: alt—anterior laterotergite; em—epimeron; is—intersternite; ls—laterosternite; lt—laterotergite; ms—median sternite; plt—posterior laterotergite; ps—prosternum; ptl—light-pigmented lines on protergum. Scale bar: 5 mm.



FIGURES 7–14. *Lamprohiza splendidula*. Detail of head in anterior (7); ventral (8) and dorsal (9, framed area enlarged in Fig. 14) views; detail of clypeolabrum in ventral view with epipharynx (10); detail of head with hypopharynx, after removal of maxillolabial complex, in ventral view (11); right maxilla in dorsal view (12); right mandible in dorsal view (13); detail of stemmal area (14). Abbreviations: bp—blunt protuberance on mandible; dsg—distal segment of galea; ep—epipharynx; fa—frontal arms; ha—hyaline appendage; hp—hypopharynx; pe—penicillus; st—stemma; sts—spot behind stemma. Scale bars: 0.25 mm.



FIGURES 15–17. *Lamprohiza splendidula*. SEM image of head in dorsal view (15, false colour); anterior (16) and ventral (17) views. Abbreviations: cs—distinct mandibular seta; fs—flat seta lying on surface.

Antenna (Figs 21–23). Trimerous, inserted on lateral distal margin of epicranial plate; partially retractable within membranous socket. Basal antennomere slightly wider than second antennomere, unsclerotized on posterolateral margin, bearing long flat setae lying on surface and erect setae lengthening towards the apical region. Second antennomere slightly narrower and longer than basal; bearing only erect setae equally spread across the antennomere, and with two longer setae on the outer apical region, next to sensorium. Sensorium of second

antennomere (Figs 22, 23; as) oblong, potato-shaped, with distinct basal constriction as a connection with the second antennomere; with no visible surface pattern. Third antennomere (Fig. 22; a3) of similar size to sensorium of the second antennomere, bearing three setae on the apex, one seta on its body, and three cuticular projections (Fig. 23); first longer and thick (cp1), second longer and thin (cp2), third one placed on the body of antennomere forming a small bulge (cp3).

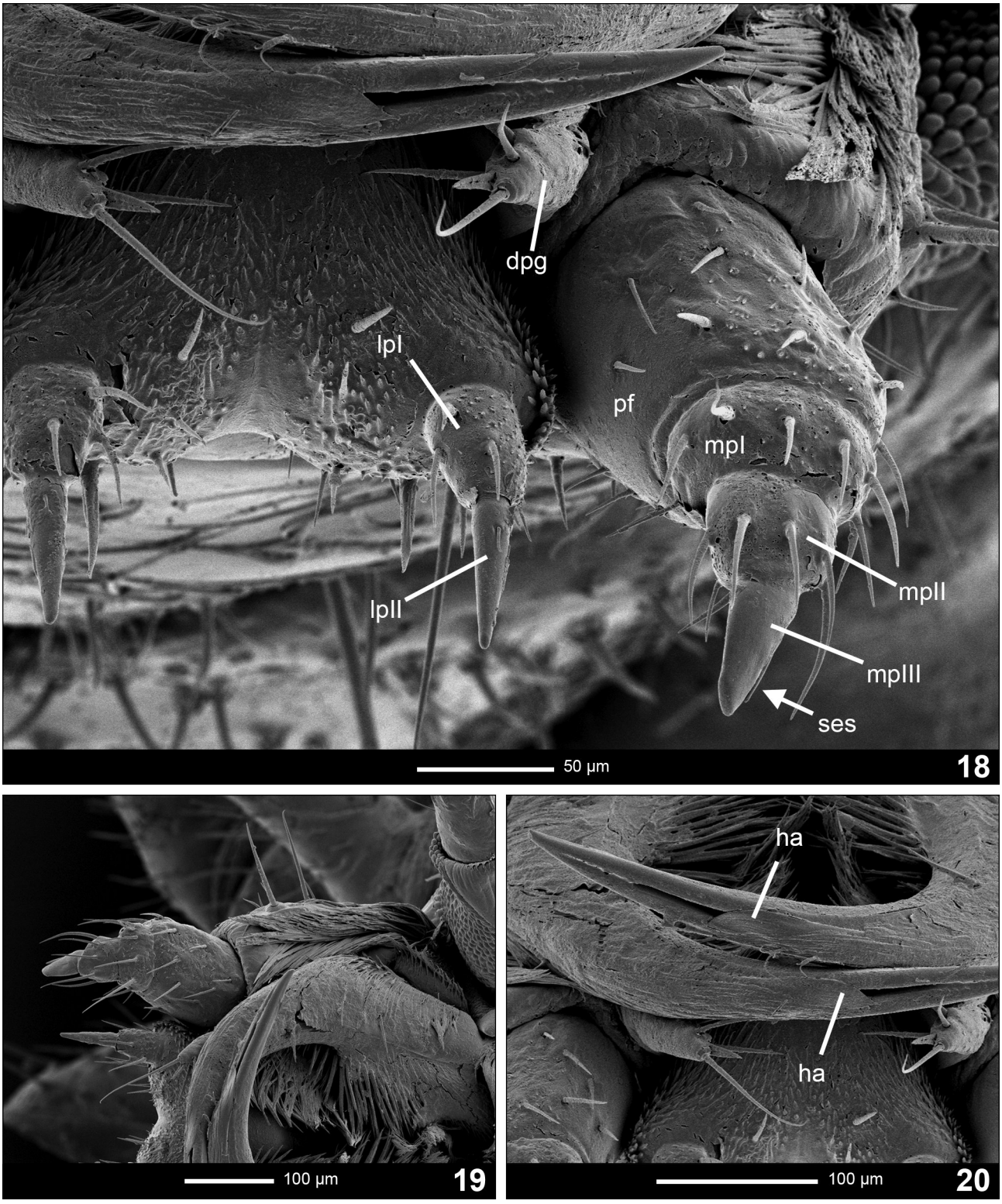
Maxilla (Fig. 12). Consisting of five parts, attached to lateral margins labium forming a maxillo-labial complex. Cardo vertical, subrectangular, about twice as long as wide. Stipes elongated, ventrally covered with erect setae, with three long stout setae placed radially on the ventral apical region. Outer dorsolateral area covered with long dense setation reaching the base of maxillary palpus (Fig. 19). Galea bimerous, with basal part larger than distal; distal part subcylindrical, pointing centrally, with short setae and one apical seta longer than body of the distal part and a blade-like flat cuticular projection on the apex (Figs 12, 18; dpg). Lacinia covered with brush of long setae on outer lateral margin (Fig. 12). Maxillary palpifer (Figs 18, 19; pf) large, subrectangular, of similar length and width. Maxillary palpus trimerous (Figs 18, 19; mp1, mp2, mp3), basal and second palpomeres short and wide. Palpifer and palpomeres I–II covered with setae; palpomere III subconical, narrow, sharp, bare, with outer lateral longitudinal depression, possibly a sensory slot, covered with thick blunt seta (Fig. 18; ses).

Labium (Figs 7, 8, 17, 18). Closely attached to maxilla, formed by a short and strongly sclerotized prementum, mentum and mostly membranous submentum (Fig. 8). Glossae absent. Prementum subtriangular, slightly heart-shaped in ventral view; covered with brush of short setae and bearing several pairs of longer setae along sagittal line of the apex, shortening towards ventral region and with one pair of longer, stout setae on central regions of ventral part. Labial palpus bimerous (Fig. 18; lp1, lp2); basal palpomere rectangular, longer than wide, bearing several setae; distal palpomere conical, longer and narrower than basal, bearing one short, thin seta lying on surface placed dorsally on basal part. Mentum elongated and subtriangular, unsclerotized on lateral margins, bearing numerous long setae lying on surface ventrally and a pair of long, erect setae posteromedially.

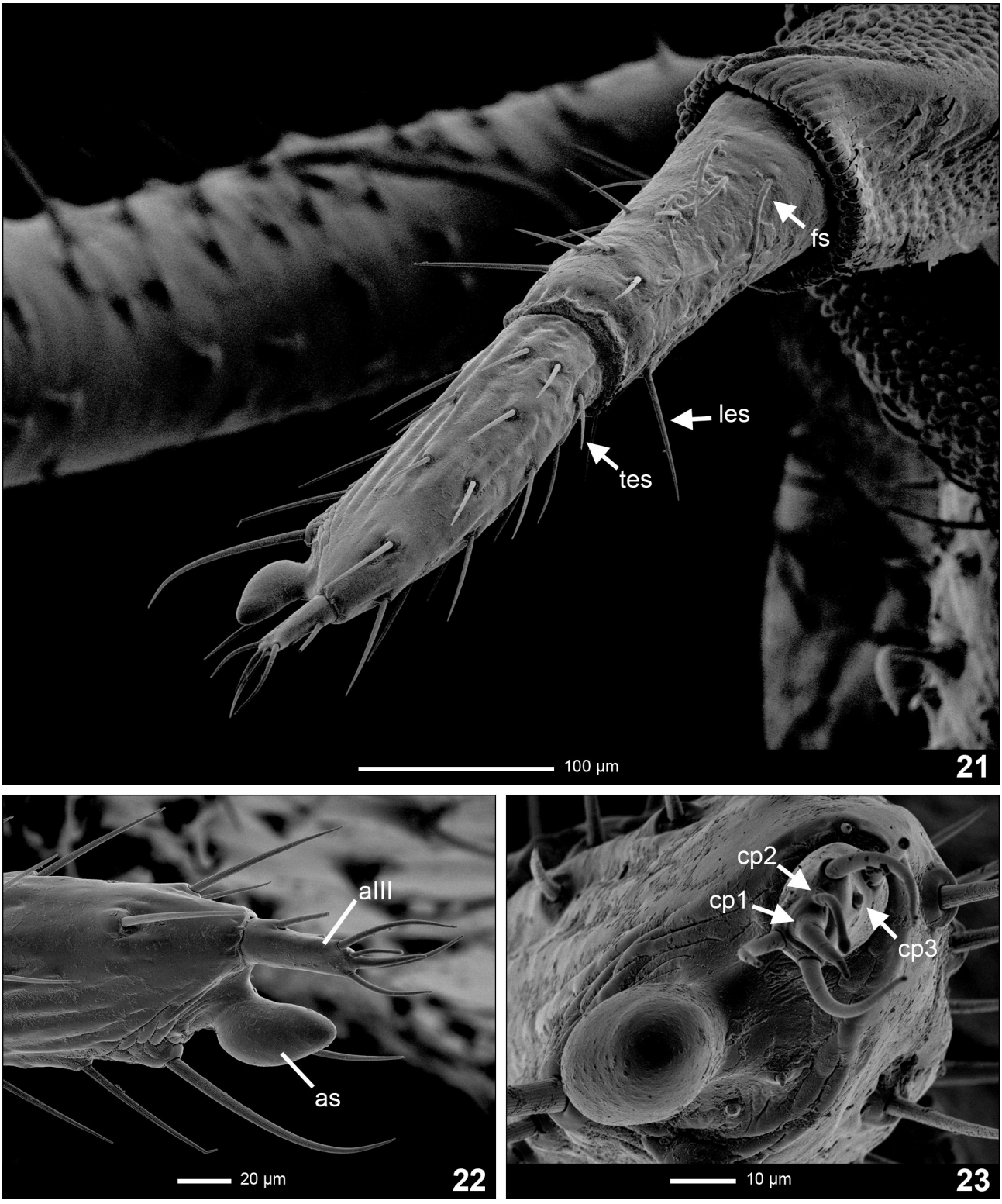
Mandible (Figs 7-9, 13, 15-17, 20). Symmetrical, falcate, with an internal channel opening subapically on outer edge (Fig. 20). Penicillus well developed (Fig. 13; pe). Retinaculum featureless, present only as a blunt protuberance on basal third of the mandible (Fig. 13, bp). Basal half on inner margin of mandible covered with a brush of stout setae, being longest on the retinaculous protuberance. Basal two-thirds of mandible ventrally with dense setation adjacent to the surface aimed centrally. Dorsal part of mandibles with several stout setae aiming centrally and a strong distinct seta aimed centrally (Fig. 15; cs), approximately in the central dorsal region of mandible. Lateral margin covered by brush of short setae lying on surface of basal two-thirds. Channel opening is partly covered by a feather-like or rounded-trapezium hyaline appendage with longer trapezoidal base situated ventrally (Figs 13, 20; ha).

Thorax (Figs 1–6). Three-segmented, thoracic tergites divided by sagittal line in dorsal view. Protergum subtriangular, wider than long, rounded at posterolateral corners, with narrow emargination anteromedially; ventrally with two light-pigmented lines, leading diagonally from the margin towards anterolateral corners of prosternum (Fig. 2; ptl). Meso- and metatergum subrectangular with round corners, ca. 4 times wider than long. Venter of prothorax composed of subrectangular, longer than wide prosternum (Fig. 3; ps), subdivided into three well sclerotized areas; lateral ones extending above and to the sides of coxae fusing with episterna; medial area arrow-shaped (tip aiming anteriorly). Epimera forming thin sclerotized strands (Fig. 2; em). Lateral areas of meso- and metathorax poorly sclerotized, composed of two laterotergites (Fig. 3; alt, plt); anterior one bearing a well developed bilabiate spiracle in mesothorax. Anterior ventral area of meso- and metathorax formed by membranous intersternite (Fig. 5; is) margined by paired anterior laterotergites (Fig. 3; alt). Posterior ventral area subrectangular, wider than long, subdivided into three well sclerotized areas; lateral ones extending anteriorly and laterally to coxae, joining episterna; medial area hourglass-shaped. Epimera forming thin sclerotized strands.

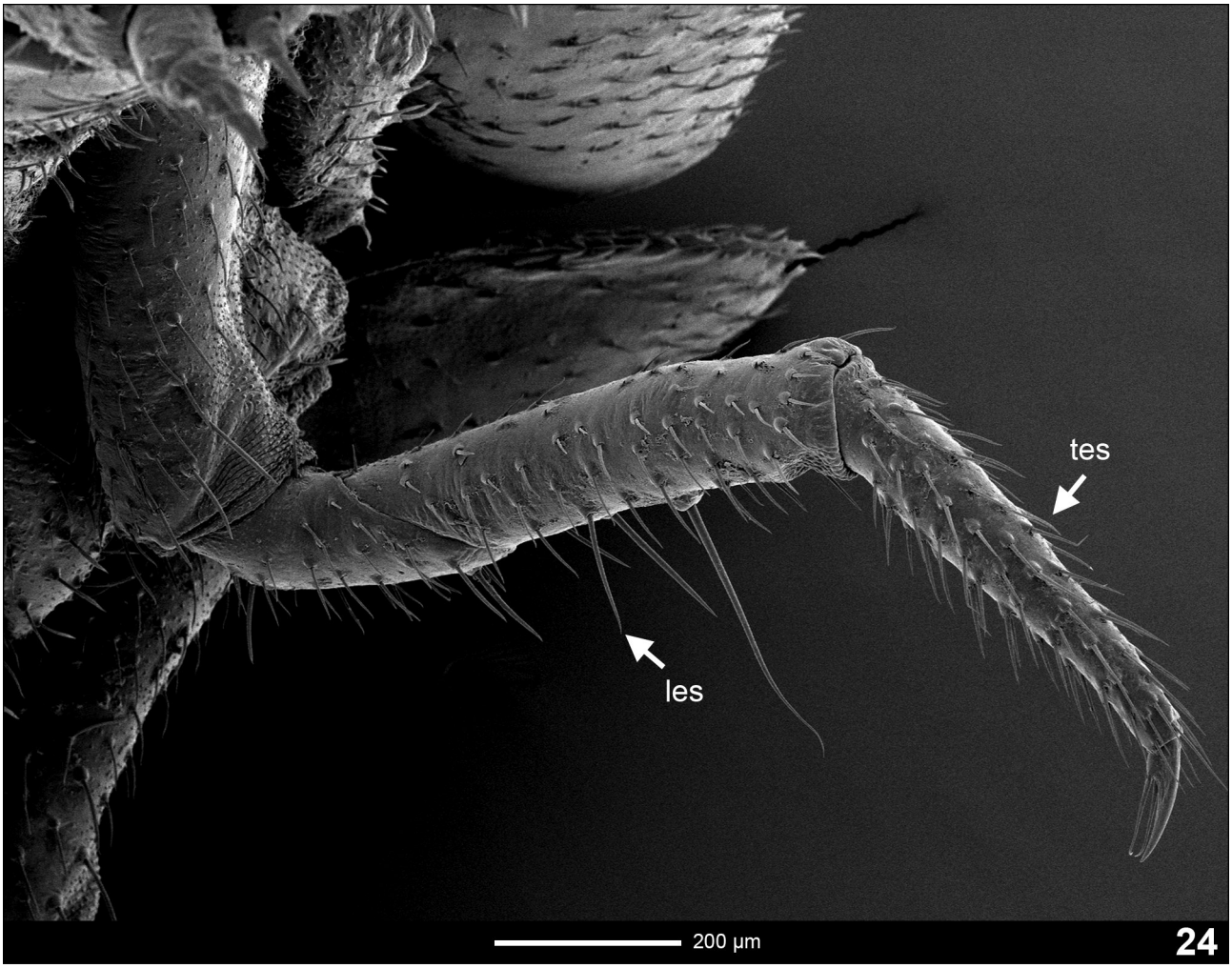
Legs (Figs 24–26). Pentamerous, all pairs similar in shape and size. Coxa large, stout, bearing stout setae. Coxal-trochanteral membrane reaching ca. 1/3 of coxal longitudinal length (Fig. 2). Trochanter smaller, elliptical in lateral view, shorter than femur, bearing shorter setae inclining to its surface and long stout setae, lengthening towards distal apex. Femur narrow and cylindrical in lateral view, bearing shorter setae inclining to its surface and long stout setae, lengthening ventrally, with one very long stout seta ventrally (Fig. 24). Tibiotarsus as long as femur, narrower, tapering towards distal end, bearing stout setae (Fig. 26). Pretarsus composed of a claw with fine ridges, ventrally bearing two long setae hooked apically towards each other, reaching the apex of the claw (Fig. 25).

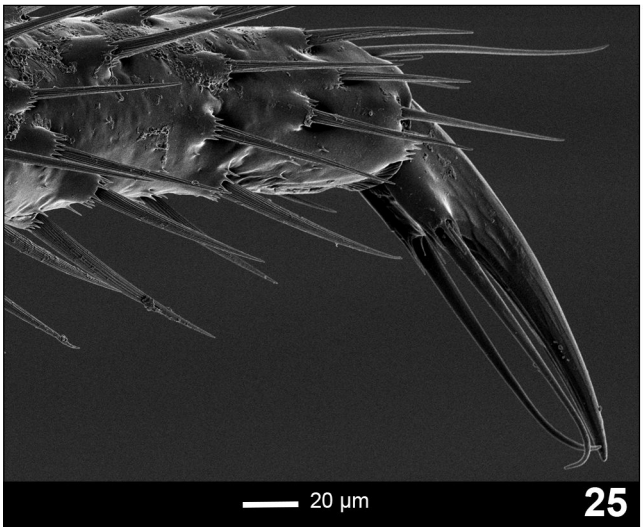


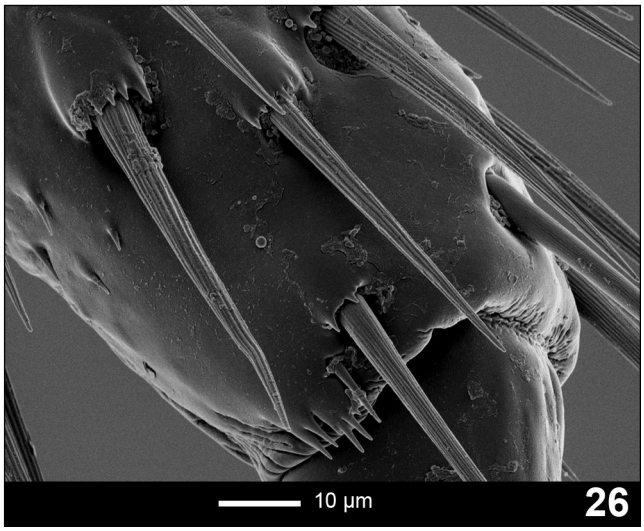
FIGURES 18–20. *Lamprohiza splendidula*. SEM image of maxillolabial complex in anterior (18) and dorsal (19) views; detail of mandibles (20). Abbreviations: dpg—distal part of galea; ha—hyaline appendage; lp1, lp2—labial palpus 1, 2; mp1–3—maxillary palpus 1–3; pf—maxillary palpifer; ses—sensory slot.



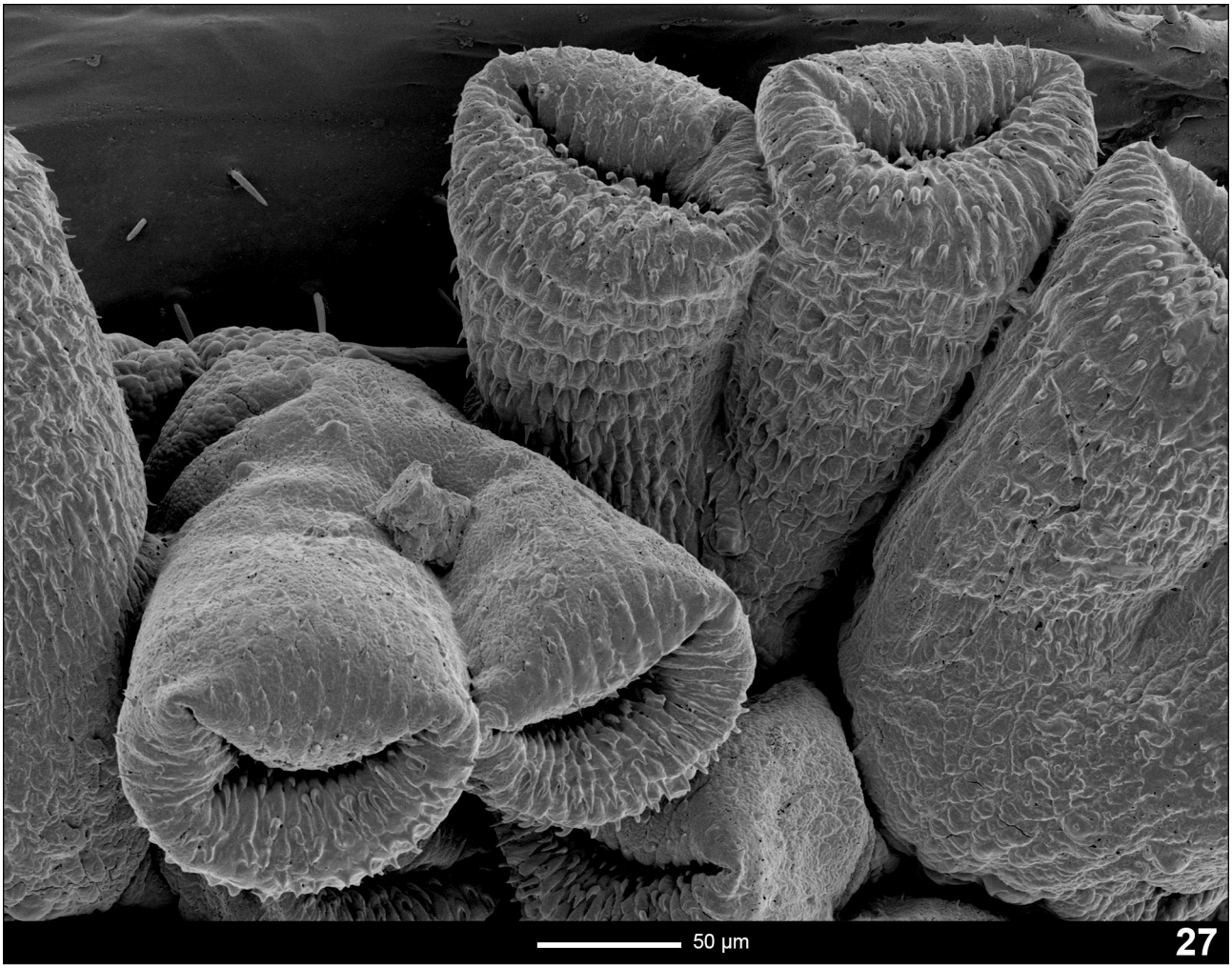
FIGURES 21–23. *Lamprohiza splendidula*. SEM image of antenna in general view (21); detail of sensorium and third antennomere (22); anterior view (23). Abbreviations: a3—third antennomere; as—antennal sensorium; cp1–3—cuticular projections 1–3; fs—flat seta lying on surface; les—long erect seta; tes—thin erect seta.

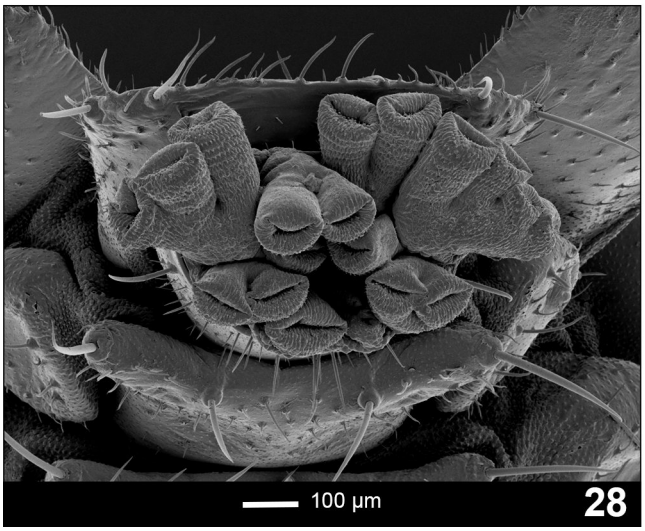


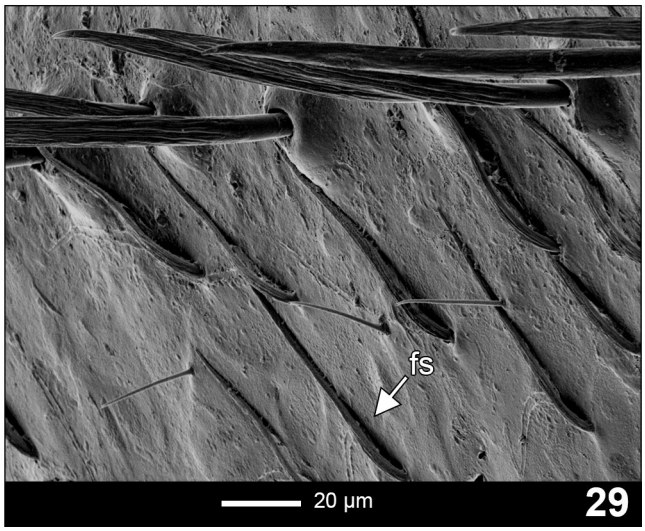




FIGURES 24–26. *Lamprohiza splendidula*. SEM image of left prothoracic leg in general view (24); pretarsus (25); detail of distal end of tibiotarsus (26). Abbreviations: les—long erect seta; tes—thin erect seta.







FIGURES 27–29. *Lamprohiza splendidula*. SEM image of pygopod in detailed (27) and general (28) views; detail of body surface of protergum (29). Abbreviation: fs—flat seta lying on surface.

FIGURES 30–37. *Lamprohiza splendidula*. General habitus of male pupa in ventral (30); dorsal (31); and lateral (32) views. Glowing male pupa in ventral (33) and lateral (34) views. General habitus of glowing female pupa in ventral (35); dorsal (36) and lateral (37) views. Abbreviations: mp—male photic organ. Scale bars: 5 mm.

Abdomen (Figs 1–6). Ten-segmented, tapering towards posterior end, segments I to VIII subdivided by fine sagittal line in dorsal view. Tergites of segments I to VII subrectangular, similar in shape and colouration, ca. 4 times wider than long; tergite of segment VIII subcrescentiform; tergite of segment IX subsemicircular; segment X forming a narrow, incompletely sclerotized dark ring, bearing the holdfast organ—pygopod (Figs 27, 28)—with several eversible processes. Segments I to VIII have single laterotergites (Fig. 2; lt) on each side with poorly sclerotized plates bearing bilabiate spiracles; the venter of segments I to VIII consists of median sternite (Fig. 2; ms), on segments I to V margined by paired narrow laterosternites (Fig. 2; ls). Median sternites of segments I to VIII subrectangular, wider than long, bearing a pair of long stout setae posterolaterally; median sternite and laterosternites of segment I membranous. Sternite of segment IX well sclerotized, rectangular and dark. Sternites of segment V and VI less sclerotized. Functioning photic organs do not follow a solid pattern; composed of paired or single spots placed ventrolaterally on abdominal segments II to VI in variable number; most common configuration consisting of larger, usually paired spots on abdominal segments II and VI; possible additional smaller spots, if present, paired or single, on abdominal segments III—V.

Notes on larval behaviour. During the collecting of larvae, specimens were, thanks to their glow, frequently spotted from several metres away. While searching between bushes, the collecting team caused noise and vibrations, which were probably perceived by the larvae. However, when collected from the ground and handled, larvae often substantially reduced their glow, thus sometimes making the actual collecting almost impossible without a flashlight.

Description of female pupa (Figs 35–37). Type pupa adectica exarata libera. Curved, ventrally concave. Length 11–12 mm. Colouration: light yellow, with translucent protergum and abdominal tergites. Surface covered in short setae.

Head capsule. Completely covered by protergum in dorsal view. Eyes small, on sides of the head. Antennae short, extending laterally towards mid-femur of the prothoracic legs. Mouthparts visible in ventral view.

Thorax. Protergum similar in shape to larva, with long setae on the anterior edge. Meso- and metatergum smaller, subrectangular, bearing vestigial wing pads. All pairs of legs free, visible in ventral view. Spiracles present on pleural areas of mesothorax.

Abdomen. Abdominal segments subrectangular, wider than long. Tergites on segments I–IX covered with long setae on the edges, on segments I–VIII strongly laterally overlapping the body. Tergal plates on segments I–VII bearing pairs of depressions in cuticle, placed laterally around the sagittal line. Spiracles present on abdominal pleural areas of segments I–VIII. Segments II–VI bearing functioning larval photic organs with additional female photic organs on segments VII and VIII (Figs 35–37).

Description of male pupa (Figs 30–32). Type pupa adectica exarata libera. Curved, ventrally concave. Length 10–11 mm. Colouration: light beige on head, thorax and elytra, light yellow on abdomen, with distinctively black eyes and membranous wings (Fig. 30) and translucent protergum and abdominal tergites. Surface covered in fine short setae.

Head capsule. Completely covered by protergum in dorsal view. Eyes distinctly large, on sides of the head. Antennae short, extending laterally towards the distal end of femur of the prothoracic legs. Mouthparts visible in ventral view.

Thorax. Protergum similar in shape to larva, with long setae on the anterior edge. Meso- and metatergum smaller, subrectangular, bearing black wing pads covered externally with shorter elytra; wing pads reaching distal end of second abdominal segment when pupa is relaxed. Pro- and mesothoracic legs free, visible in ventral view; metathoracic legs almost completely covered by wing pads except for distal segments of tarsi, which extend past second ventrite. Spiracles present on pleural areas of mesothorax.

Abdomen. Abdominal segments subrectangular, wider than long. Tergites on segments I–IX covered with long setae on the edges, on segments I–VIII strongly laterally explanate, on segments I–VII bearing pairs of depressions in cuticle, placed laterally around the sagittal line. Spiracles present on abdominal pleural areas of segments I–VIII. Segment II–VI bearing functioning larval photic organs with additional male photic organs on ventrites of segments VI and VII (Figs 33, 34).

Notes on prepupae. The prepupal period begins when larva becomes inactive, lying curled up in a semicircle either on its side or back. Nevertheless, it still responds to any disturbance by activating its light organ and contracting its abdomen, a behaviour typical for pupae. Stage of prepupa in observed specimens lasted 3 days in female and 7 days in male.

Notes on ontogenetic changes in pupae. In the male, the pupa is generally indistinguishable from the female pupa (Figs 35–37) during the first three days. Moreover, as in the female, it glows from the same places as the larva. On the fourth day the wings begin forming and darkening in colour, signalling the beginning of the period when male and female pupae can be distinguished. On the fifth day the glow intensity of the larval lights is weaker than in the previous days. On the sixth day the male light organ activates and begins to glow (Fig. 34; mp). During this time larval lights still function and their glow intensity is back to normal. By this time the membranous wings, protruding under incipient elytra, are completely black (Fig. 30). The thorax starts to sclerotize, slowly darkening in colour. At the same time, the pupal eyes are turning into large male eyes, but their development is not yet complete, since they resemble a half-inflated balloon at this stage (Fig. 30). On the seventh day the male emerges. Its larval lights still operate and their glow is visible through the still incompletely sclerotized cuticle (this was also observed by Schwalb 1961). On the eighth day the cuticle of the adult male finally hardens and the larval lights are no longer visible.

The female pupa does not go through such obvious changes as the male does. It appears more or less similar throughout the whole pupal period (Figs 35–37), with the exception of slowly darkening small eyes and overall shift of the body colour towards a darker shade of yellow. The reason for this is neoteny as well as the paler pigmentation of the female. As in the male, the female emerges on the seventh day.

Notes on behaviour observed in pupae. The pupa is inactive, either lying on its side or back, responding only to disturbance. A response consisting of a ca. 10-second glow with quick intensification and gradual fading can be induced by handling the animal or even by vibrations. In the male pupa with the male photic organ already developed (Figs 33, 34), the response is similar, with exception of the newly developed lantern, which can still glow feebly at least three times longer than the larval lights. When under a strong light source, the pupa reacts in the same manner as pupa of *Lampyris noctiluca*, as described in Novák (2017).

Discussion

External sensory organs. Similar to *Lampyris noctiluca,* sensilla chaetica, and sensilla trichodea were observed on the same parts of the body. These types of sensilla, together with speculations of their possible functions, were already described in Novák (2017).

Lamprohiza splendidula possesses a unique feature, not found in other central European species and to my knowledge never observed in any other firefly larvae, with exception of sister species *Lamprohiza delarouzei* (figures in Bugnion (1929) suggest similar feature). It is a membranous spot placed posteriorly behind each stemma of the larva (Fig. 14; sts). The function of this organ seems sensory, nevertheless the exact purpose is unknown. It is possible that this organ is light-sensitive and helps the larva determine favourable light conditions within its environment. Among non-beetles, certain species of Blattodea possess vestigial ocelli degenerated to a pair of transparent areas in the cuticle called *fenestra* (Gillot 2005). Within beetles, vestigial paired dorsal ocelli occur in adults in some taxa of Staphylinoidea and in Derodontidae, so their presence is not excluded within Coleoptera and Polyphaga (Leschen & Beutel 2004). Nevertheless, in both previous cases, the vestigial ocelli are placed on the head dorsally, while in the firefly larva, they are positioned laterally behind stemmata. Another assumption may come from the fact that *Lamprohiza splendidula* is believed to react to disturbance in its surroundings by light emission (De Cock 2003). During my *in vivo* observations of larvae of this species, I have never observed a larva "turning-on" its light due to direct disturbance; in contrast, the light emitted by larvae was always observed from several metres away. This could mean that larvae of this species have a well-developed ability to detect vibration, perhaps by some kind of primitive tympanal organ represented by the membranous spot placed behind their eyes. However, the same sensory service could be provided by a standard sensilla without need of any specialized membrane. In conclusion, until a tissue analysis and further experiments are conducted, the precise function of this structure will remain unknown.

Walking in tergite armour. The larvae of *Lamprohiza splendidula* have conspicuous laterally extended tergal plates on both the thorax and abdomen (Figs 1, 2, 4, 5). This body shape is by no means an exception within the larvae of the Lampyridae (e.g. *Pyrogaster* Motschulsky, 1853, *Cratomorphus* Motschulsky, 1853, *Photuris*, etc.) nor within the order Coleoptera (e.g. Lycidae, Silphidae). However, the exact advantage of this morphological architecture is unknown. While collecting larvae in nature, I have often found the individuals pressed against moist dead foliage, with their head and legs hidden under tightly constricted tergal plates directly touching the substrate.

In this way, the vulnerable appendages and delicate parts of the body are protected against potential predators, while the pigmentation of the larva matches perfectly with the colour of dead leaves. However, this camouflage is probably just an added value to the protective function, since in other taxa, larvae of similar shape are often brightly coloured.

Mandibles, pygopod and hunting for prey. Larvae of *Lamprohiza splendidula* generally feed on soft, slimy invertebrates, predominantly snails, but they can also feed on fresh animal cadavers that offer access through wounds to soft body parts (Schwalb 1961; Lloyd 2008). The style of hunting is almost the same, as in *Lampyris noctiluca* (Novák 2017) and, in the larvae of both species, a hyaline appendage resembling a "shutter" on the base of mandibular channel opening (Fig. 20; ha) was observed. These structures, however, take different forms, as the "shutter" resembles a feather-like structure in *Lamprohiza splendidula*. Compared to the blunt thick seta found in *Lampyris noctiluca*, the form of this hyaline appendage suggests a function more concerned with protection of the mandibular channel from clogging, rather than a trigger for a toxin as speculated in Novák (2017).

The holdfast organ—pygopod (Figs. 27, 28)—helps the larva with locomotion, but also serves during hunting, being used for fastening the snail-attacking larva to the snail's shell as has been observed in *Lampyris noctiluca* (Tyler 2002; M. Novák, unpublished observation), thus giving it a safe position and room for biting manoeuvres. In addition, the pygopod is used also for body cleaning (among other uses) after feeding (Tyler 2002; M. Novák, unpublished observation), especially of the head appendages with dense setation.

Life cycle and pupal development. As well as in sympatric *Lampyris noctiluca*, the information about the developmental cycle of *Lamprohiza splendidula* differs among authors including my observations, perhaps because of certain level of flexibility in adjusting the life cycle to specific environmental conditions. According to Schwalb (1961) the biology of the two species differs only slightly. The development of *Lamprohiza splendidula* takes 2 years according to Hůrka (2005) or possibly 3 years, during which the larva overwinters 3 times according to Schwalb (1961). The number of instars varies between 4 and 6 (Schwalb 1961; Hůrka 2005). The larva enters the stage of prepupa ca. 8–20 days prior to pupation according to Schwalb (1961), however this period may be as short as 3 days (M. Novák, unpublished observation). The wide range of duration of the prepupal stage stated by Schwalb (1961) raises the same questions as in *Lampyris noctiluca*. These are already discussed in Novák (2017) and will not be addressed again here. Pupation in natural conditions occurs in the spring after hibernation; under fallen leaves, pieces of wood or stones, in dug out hemispherical chambers with the opening at the top (Schwalb 1961; Burakowski 2003) or possibly in a cell of small pieces of dead leaf litter (R. De Cock, pers. com.). The pupal stage period lasts 7 days on average in both sexes according to Schwalb (1961), which accurately corresponds with my observations. In the sympatric *Lampyris noctiluca*, pupation lasts ca. 8–12 days for a female and 11–15 days for a male (Schwalb 1961; Tyler 2002; Novák 2017). The longer period of the male pupal development could be explained by the larger amount of changes taking place in a male's body compared to the neotenous female. This raises the question of why the time of pupation in *Lamprohiza splendidula* is also not longer for males. It is possible that the production of eggs in the developing female (Schwalb 1961) prolongs the pupation period. On the other hand, the same egg production happens in *Lampyris noctiluca* (Schwalb 1961) and yet the periods differ. The reason for the same pupal period may in fact be the small size of *Lamprohiza splendidula*, especially in comparison with the much larger *Lampyris noctiluca*. The gap between sexes in the rebuilding times might be reduced in the former thanks to its relatively small body volume in relationship to the changes taking place.

An intriguing phenomenon was observed in the male pupa (Figs 30–32) on the fifth day of development, prior to the activation of the adult lantern. The glow intensity of the larval lights had weakened and the next day when the adult lantern activated (Fig. 34; mp), the intensity of the larval lights went back to normal. It is possible that the final phase of activation of the male lantern is accompanied by the transfer of compounds essential for light production (i.e. luciferin, luciferase) into the newly developed organ, thus temporarily weakening the light production in the larval lanterns. To exclude the possibility that such a phenomenon was not a coincidental exception, more male pupae will have to be observed, since only a single specimen was observed for this study.

Depending on the conditions of the local climate the mature forms appear starting May–July and can be seen until September (Burakowski 2003; Hůrka 2005). The glowing adult females can sometimes be found in clusters, which make them quite distinctive (M. Novák, unpublished observation). According to Tyler (2002), the same phenomenon occurs in females of the sympatric *Lampyris noctiluca* and is probably the result of larval pre-pupation "ganging up", as he calls it. It is thus possible that similar behaviour may be found in the larvae of this particular species.

Photic behaviour. The interpretation of emission of light due to disturbance or manipulation is complicated, according to my *in vivo* observations. The larvae of *Lamprohiza splendidula* appear reluctant to glow without external provocation. They emit a weak continuous glow when handled or even approached. They react both to vibrations and to loud noises (which, as a side effect, easily facilitates collecting them in the field). However, the light intensity may weaken or completely stop in certain cases, usually as a result of overstimulation (Schwalb 1961; M. Novák, unpublished observation). This type of behaviour seems similar to that described by Viviani (2001) in the Neotropical genera *Pyrogaster*, *Photuris* Dejean, 1833, and *Aspisoma* Laporte, 1833; the larvae respond to vibrations by glowing, but do not respond to mechanical manipulation. According to Viviani (2001), this behaviour is probably a collective defence against predators, which lies in distraction and confusion of the "enemy". In conclusion, what may seem like a collective defence of larvae of *Lamprohiza splendidula* may either be just a by-product of larval tolerance to a certain amount of stimuli or indeed a tool of collective defensive behaviour. This begs the question: why do the larvae—when in danger—only reduce their glow, and not stop it abruptly? This may be explained by an inability of the larval stage to alter its glow swiftly, as a result of the differing physiology of their photic organ compared to that of the adults (Timmins *et al.* 2001).

According to De Cock (2003), the spectrum of light emitted by the larvae of *Lamprohiza splendidula* and its sympatric counterparts *Lampyris noctiluca* and *Phosphaenus hemipterus*, unlike that of adults, is very similar, conserving the green emission. This agrees with the lack of an intraspecific function (mating) and increased importance of an interspecific function such as defence, as stated by Viviani (2001). On top of that, lampyrids are reported to be unpalatable prey in general (Underwood *et al.* 1997; De Cock & Matthysen 2003; Moosman *et al.* 2009). With regards to the abovementioned species, the tests of unpalatability have only been performed on *Lampyris noctiluca*. There is a possibility that Batesian or Müllerian mimicry could have evolved within and between these taxonomic groups. Schwalb (1961) states that adults of *Lamprohiza splendidula* are often found in spider webs, sucked dry. I have often witnessed males of this species stress-glowing from spider webs myself, but the question is whether they are indeed consumed by the spider. The glowing of individuals in webs (often for hours) suggests that they had not been approached and killed by the spider. On the other hand, males are often flying (and consequently found in the webs) in such large numbers that the spider may not be capable of attending to all its captures. If they are consumed, the spider is either immune to the defensive compounds of the firefly or the firefly does not possess them. In both cases, the Batesian or Müllerian mimicry has no effect. If they are avoided by the spider, it is possible that Schwalb (1961) had mistaken dried-up individuals caught in the web on previous days with the remains of other spider prey, and Batesian or Müllerian mimicry could still be operating. Which type of mimicry, if any, is used in these fireflies will still have to be determined by further experiments. Larvae and adults must be tested individually, since the existence of defensive compounds in the adults may not guarantee the existence of the same compounds in juvenile stages and vice versa.

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References

Archangelsky, M. & Fikáček, M. (2004) Descriptions of the egg case and larva of *Anacaena* and a review of the knowledge and relationships between larvae of Anacaenini (Coleoptera: Hydrophylidae: Hydrophylinae). *European Journal of Entomology*, 101, 629–636.

https://doi.org/10.14411/eje.2004.086
Ballantyne, L.A. & Menayah, R. (2002) A description of larvae and redescription of adults of the firefly *Pteroptyx valida* Olivier in Selangor, Malaysia (Coleoptera: Lampyridae: Luciolinae), with notes on Luciolinae larvae. *Raffles Bulletin of Zoology*, 50 (1), 101–109.
Bratka, J., Pokorný, J., Roub, T. & Bratková, J. (2011) *Plán péče pro přírodní památku Petřín na období 2013–2022.* [*Management plan for Petřín Natural Monument for period 2013–2022*]. Zelený svět, Praha, 26 pp. [in Czech]
Bugnion, E. (1929) *Le ver-luisant provençal et la luciole niçoise. Mémoire, supplément au "Riviera Scientifique" (année 1929)*. Association des naturalistes de Nice et des Alpes-Maritimes, Nice, 131 pp.
Burakowski, B. (2003) *Klucze do oznaczania owadow Polski, Czesc XIX Chraszcze—Coleoptera, Zeszyt 29, 30 Karmazynkowate—Lycidae, Swietlikowate—Lampyridae*. [*Key to identification of insects of Poland, Part XIX—Beetles (Coleoptera), Issue 29, 30 Lycidae, Lampyridae*]. Polskie Towarzystwo Entomologiczne, Torun, 39 pp. [in Polish]
De Cock, R. (2003) Larval and Adult Emission Spectra of Bioluminescence in Three European Firefly Species. *Photochemistry and Photobiology*, 79, 339–342.
https://doi.org/10.1111/j.1751-1097.2004.tb00018.x
De Cock, R. & Matthysen, E. (2003) Glow-worm larvae bioluminescence (Coleoptera: Lampyridae) operates as an aposematic signal upon toads (*Bufo bufo*). *Behavioral Ecology*, 14, 103–108.
https://doi.org/10.1093/beheco/14.1.103
Dejean, P.F.M.A. (1833) *Catalogue des coléoptères de la collection de M. le comte Dejean*. Mequignon-Marvis père et fils, Paris, 96 pp.
Dostálek, J. (2009) *Plán péče o přírodní památku Údolí Kunratického potoka 2010–2019.* [*Management plan for Kunratický stream Valley Natural Monument for period 2010–2019*]. Ministerstvo životního prostředí ČR, Praha, 57 pp. [in Czech]
Geisthardt, M. (1979) Skelet und Muskulatur des Thorax der Larven und Imagines von *Lamprohiza splendidula* (L.) unter Berucksichtigung der Larve und der weiblichen Imago von *Lampyris noctiluca* (L.) (Coleoptera: Lampyridae). *Zoologische Jahrbucher. Abteilung fur Anatomie und Ontogenie der Tiere*, 101, 472–536.
Geisthardt, M. & Satô, M. (2007) Lampyridae. *In*: Löbl, I. & Smetana, A. (Eds.), *Catalogue of Palearctic Coleoptera. Vol. 4. Elateroidea—Derodontoidea—Bostrichoidea—Lymexyloidea—Cleroidea—Cucujoidea.* Apollo Books, Sternstrup, pp. 225–234.
Goeze, J.A.E. (1777) *Entomologische beyträge zu des ritter Linné zwölften ausgabe des natursystems von Joh. Aug. Ephraim Goeze. Erster theil.* Weidmanns Erben und Reich, Leipzig, 736 pp.
https://doi.org/10.5962/bhl.title.45974
Gillot, C. (2005) *Entomology. 3rd Edition*. Springer, Dordrecht, 831 pp.Hůrka, K. (2005) *Brouci České a Slovenské republiky.* (*Beetles of Czech and Slovak Republics*). Kabourek, Zlín, 391 pp. [in Czech and English]
Jacquelin Du Val, P.N.C. (1859) *Glanures entomologiques: recueil des notes monographiques, descriptions, critiques, remarques et synonymies diverses. Cahier 1. Chez M.A. Deyrolle, naturaliste*. Imprimerie de E. Donnaud, Paris, 164 pp.
https://doi.org/10.5962/bhl.title.66008
Klausnitzer, B. (1994) 50. Familie: Lampyridae. *In*: Klausnitzer, B. (Ed.), *Die Larven der Käfer Mitteleuropas. Band 2. Myxophaga, Polyphaga. Teil 1*. Goecke & Evers, Krefeld, pp. 228–230.
Korschefsky, R. (1951) Bestimmungstabelle der bekanntesten deutschen Lyciden-, Lampyriden- und Drilidenlarven. *Beiträge zur Entomologie*, 1, 60–64, table 1.
Kratochvíl, J. (1957) *Klíč zvířeny ČSR, Díl II, Třásnokřídlí, blanokřídlí, řásnokřídlí, brouci.* [*Key to Czechoslovakian fauna, Part II, Thysanoptera, Strepsiptera, Coleoptera*]. Nakladatelství Československé akademie věd, Praha, 746 pp. [in Czech]
LaBella, D.M. & Lloyd, J.E. (1991) Lampyridae (Cantharoidea). *In*: Stehr, F.W. (Ed.), *Immature Insects. Vol. 2*. Kendall/Hunt Publishing Company, Dubuque, pp. 427–428.
Laporte, F.L. (1833) Essai d'une revision du genre *Lampyre. Annales de la Société entomologique de France*, 1833, 122–153.
Lawrence, J.F. & Ślipiński, A. (2013) *Australian beetles, Volume 1. Morphology, Classification and Keys*. CSIRO Publishing, Collingwood, 561 pp.
Leschen, R.A.B. & Beutel, R.G. (2004) Ocellar atavism in Coleoptera: plesiomorphy or apomorphy? *Journal of Zoological Systematics and Evolutionary Research*, 42, 63–69.
https://doi.org/10.1046/j.0947-5745.2003.00241.x
Linnaeus, C. (1758) *Systema naturae per regna tria naturae: secundum classes, ordines, genera, species, cum characteribus, differentiis, synonymis, locis. Tomus I. Editio decima, reformata.* Laurentius Salvius, Holmiae, 824 pp.
https://doi.org/10.5962/bhl.title.542
Linnaeus, C. (1767) *Systema naturae per regna tria naturae: secundum classes, ordines, genera, species, cum characteribus, differentiis, synonymis, locis. Tomus I. Pars II. Editio duodecima reformata.* Laurentius Salvius, Holmiae, 1327 pp.
https://doi.org/10.5962/bhl.title.68927
Lloyd, J.E. (2008) Fireflies (Coleoptera: Lampyridae). *In*: Capinera, J.L. (Ed.), *Encyclopedia of Entomology. 2nd Edition*. Springer, London, pp. 1429–1452.
Medvedev, L.N. & Ryvkin, A.B. (1992) 45. Semeystvo Lampyridae. *In*: Ler, P.A. (Ed.), *Opredelitel' nasekomykh Dal'nego Vostoka SSSR v shesti tomach. Tom 3. Zhestokrylye ili zhuki. Chast' 2.* [*A key to the insects of Far Eastern USSR in six volumes. Vol. 3. Coleoptera or beetles. Part 2*]. Nauka, Sankt Peterburg, pp. 26–29. [in Russian]
Moosman, P.R. Jr., Cratsley, C.K., Lehto, S.D. & Thomas, H.D. (2009) Do courtship flashes of fireflies (Coleoptera:

Lampyridae) serve as aposematic signals to insectivorous bats? *Animal Behaviour*, 78, 1019–1025. https://doi.org/10.1016/j.anbehav.2009.07.028
Motschulsky, V. de (1853) Nouveautés. *Études Entomologiques*, 1, 77–80.
Novák, M. (2017) Redescription of immature stages of central European fireflies, Part 1: *Lampyris noctiluca* (Linnaeus, 1758) larva, pupa and notes on its biology (Coleoptera: Lampyridae: Lampyrinae). *Zootaxa*, 4247 (4), 429–444. http://doi.org/10.11646/zootaxa.4247.4.5
Novák, M. (2018) Redescription of immature stages of central European fireflies, Part 3: *Phosphaenus hemipterus* (Goeze, 1777) larva, and notes on its life cycle and behaviour, with a key to three central European lampyrid larvae (Coleoptera: Lampyridae). *Zootaxa*. [in review]
Reitter, E. (1911) *Fauna Germanica, Die Käfer des Deutschen Reiches. Band 3.* K. G. Lutz, Stuttgart, 436 pp.
Schwalb, H.H. (1961) Beiträge zur Biologie der einheimischen Lampyriden *Lampyris noctiluca* Geoffr. und *Phausis splendidula* Lec. und experimentelle Analyse ihres Beutefang- und Sexualsverhaltens. *Zoologische Jahrbücher, Abteilung für Systematik, Geographie und Biologie der Tiere*, 88, 399–550.
Timmins, G.S., Jackson, S.K. & Swartz, H.M. (2001) The evolution of bioluminescent oxygen consumption as an ancient oxygen detoxification mechanism. *Molecular Evolution*, 52, 321–332. https://doi.org/10.1007/s002390010162
Tyler, J. (2002) *The glow-worm*. Lakeside, Sevenoaks, 76 pp.
Underwood, T.J., Tallamy, D.W. & Pesek, J.D. (1997) Bioluminescence in firefly larvae: a test of the aposematic display hypothesis (Coleoptera: Lampyridae). *Journal of Insect Behavior*, 10, 365–370. https://doi.org/10.1007/bf02765604
Viviani, V.R. (2001) Fireflies (Coleoptera: Lampyridae) from southeastern Brazil: habitats, life history, and bioluminescence. *Annals of the Entomological Society of America*, 94, 129–145. https://doi.org/10.1603/0013-8746(2001)094[0129:FCLFSB]2.0.CO;2

Annex 3: Redescription of immature stages of central European fireflies, Part 3: Phosphaenus hemipterus (Goeze, 1777) larva, and notes on its life cycle and behaviour, with a key to three central European lampyrid larvae (Coleoptera: Lampyridae)

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Redescription of immature stages of central European fireflies, Part 3: *Phosphaenus hemipterus* (Goeze, 1777) larva, and notes on its life cycle and behaviour, with a key to three central European lampyrid larvae (Coleoptera: Lampyridae)

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Abstract

The mature larva of the elusive firefly *Phosphaenus hemipterus* (Goeze, 1777) is thoroughly redescribed and illustrated with detailed images, including scanning electron microscope figures. The external sense organs and their significance is discussed, as well as the predatory behaviour and specific lifestyle of the larva. A key to the central European lampyrid larvae (viz. *P. hemipterus*, *Lamprohiza splendidula* (Linnaeus, 1767) and *Lampyris noctiluca* (Linnaeus, 1758)) is provided as well as a comparative table of their morphological features.

Key words: Elateroidea, Lampyrinae, morphology, ecology, pheromone communication

Introduction

The genus *Phosphaenus* Laporte, 1833 (Lampyrinae) is represented by a single European species, widely distributed from England, Denmark, southern Sweden, Finland and Karelia through the central part of Europe to the Pyrenees, northern Italy, west Balkan Peninsula, Transylvania and Ukraine (Burakowski 2003; Geisthardt & Satô 2007). It seems to be the only European firefly that has been imported into another continent, since it is also present in Nova Scotia, Canada (Tyler 2002). The unintentional introduction was probably facilitated by its high tolerance of, or even preference for, human-altered environments. Published descriptions of this species are brief and the morphology, particularly the morphology of the larvae, is poorly known. Schematic illustrations in many published works are of variable quality, and detailed images and descriptions are missing (Reitter 1911; Korschefsky 1951; Kratochvíl 1957; Klausnitzer 1994; Burakowski 2003).

Phosphaenus hemipterus (Goeze, 1777) was considered a rare and poorly known species until recently. De Cock (2000) presumes that the reason for this is the fact that this firefly can be found mainly in areas with high levels of human disturbance. These include gardens, parks, parking lots and field edges, while much of the previous research has been conducted in areas mostly unaffected by humans. De Cock (2000) concludes that this species might not be as rare as previously thought, and furthermore, it can be found in areas which are not considered important from a conservation management point of view.

Interesting information may come from future studies of the poorly documented genus *Phosphaenopterus* Schaufuss, 1870 consisting of two species, one occurring in Romania and the other in Portugal and the French Pyrenees. Since the time of their descriptions, these species have not been reported again (De Cock 2009). Mikšić (1982) suggests they might be just macropterous forms of *Phosphaenus hemipterus*. Interestingly, these taxa occur on the outer borders of the distributional range of *Phosphaenus hemipterus* (De Cock 2009), but to be clear about their phylogenetic relationship, available type specimens will need to be examined or additional fresh material collected.

This paper represents the third and final part of a trilogy focusing on immature stages of firefly species occurring in central Europe. The larva together with male and female pupae of *Lampyris noctiluca* (Linnaeus,

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1758) were redescribed in the first part (Novák 2017), followed by a redescription of larva with male and female pupae of *Lamprohiza splendidula* (Linnaeus, 1767) in the second part (Novák 2018).

Material and methods

Specimens of *Phosphaenus hemipterus* were loaned from Petr Švácha, from the collection of the Institute of Entomology within the Biology Centre of the Academy of Sciences of the Czech Republic in České Budějovice. The specimens examined were found in forest litter in the Lednice area (48°47'58.8"N, 16°48'6.0"E), south Moravia, in April 1987, and stored in 80% alcohol. The area of Lednice is predominantly composed of quaternary sediments, with long temperate and dry summers and short mildly temperate dry winters. Average yearly rainfall is 1000 mm, average yearly temperature is 8.5 °C (Hučík *et al.* 2013).

Higher instar individuals were selected for subsequent analysis. Regarding the distinction of the individual instars, no work describing either morphological or biometric traits or chaetotaxy exists. To solve this problem, individuals approaching the maximum species length limit were selected.

Methods of optical and electron microscope imaging are described in Novák (2017). Interpretation and terminology of larval and pupal descriptions follows Archangelsky & Fikáček (2004), LaBella & Lloyd (1991), description of thoracic and abdominal sclerites follows Ballantyne & Menayah (2002) and Lawrence & Ślipiński (2013).

Phosphaenus hemipterus (Goeze, 1777)

Material examined. Lednice (Czech Republic), three higher-instar larvae out of eleven collected in April 1987.

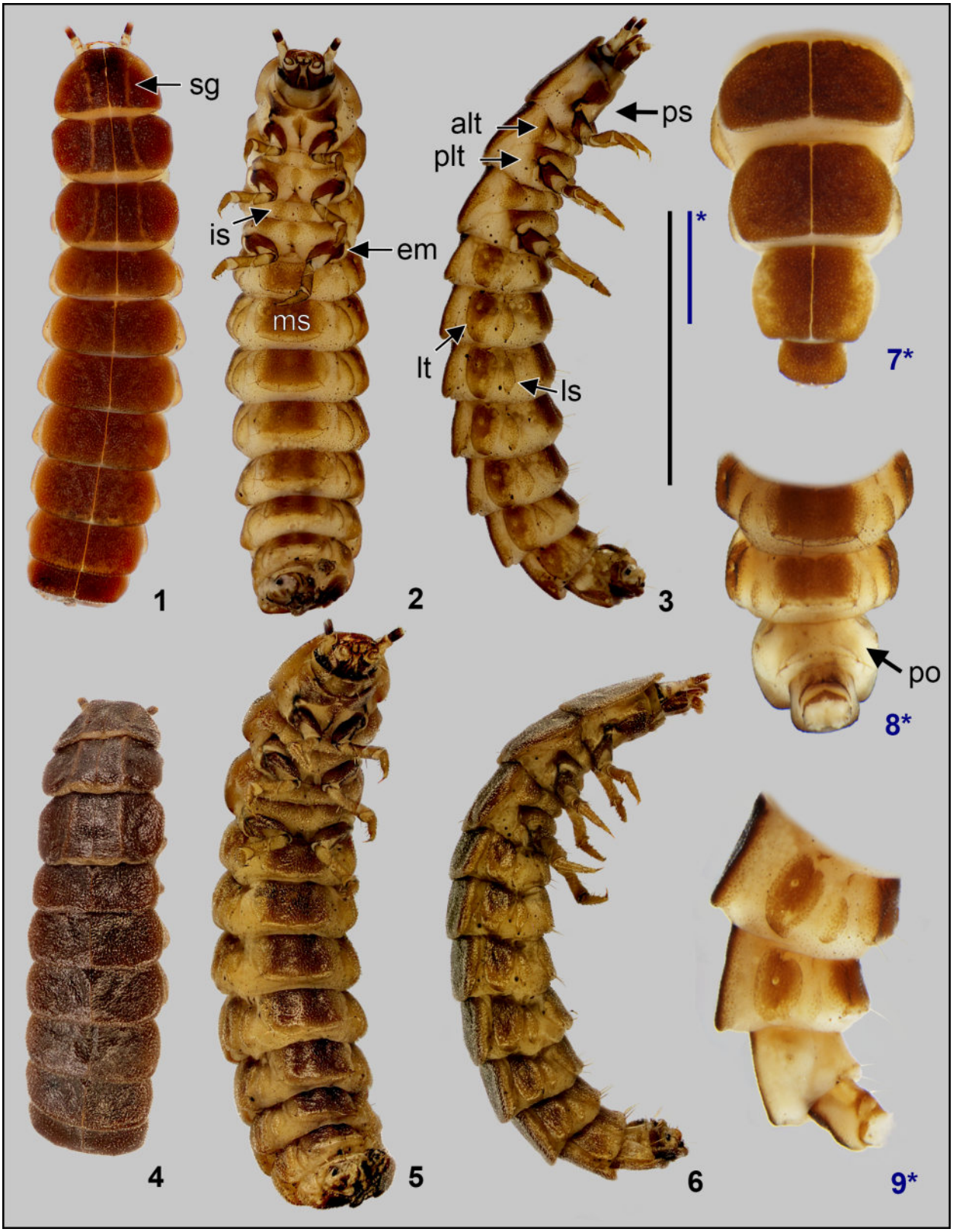
Diagnosis. Larvae oblong and slender; thoracic tergites subdivided with one parasagittal line of light pigmentation on each side subparallel to sagittal line; inner ventrolateral area of second antennomere with distinct longitudinal cleft; in addition to second antennomere, sensoria present also on third antennomere and distal segments of maxillary and labial palpi; mandible with short and blunt retinaculum; mandibular channel opening covered by a small hyaline appendage forming a subtriangular valve with fringing at the distal end; short fibrous setae growing from slightly sunken toroidal base covering antennae, legs and sclerites; photic organ consisting of a pair of white spots placed laterally on abdominal segment VIII.

Description of mature larva (Figs 1–6). Oblong and slender, cylindrical. Body length ca. 10–11 mm (from the anterior margin of protergum to the apex of caudal segment); with 3 thoracic and 10 abdominal segments. Tergites from protergum to abdominal segment VIII divided by sagittal line in dorsal view (Fig. 1). Thoracic tergites then subdivided with one parasagittal line of light pigmentation on each side, subparallel to sagittal line (Fig. 1; sg). Colouration: dorsally dark reddish-brown, ventrally pink/ochre/light brown with darker plates on laterotergites and sternum. Spiracles on laterotergites of light colouration. Paired photic organs placed laterally on venter of abdominal segment VIII.

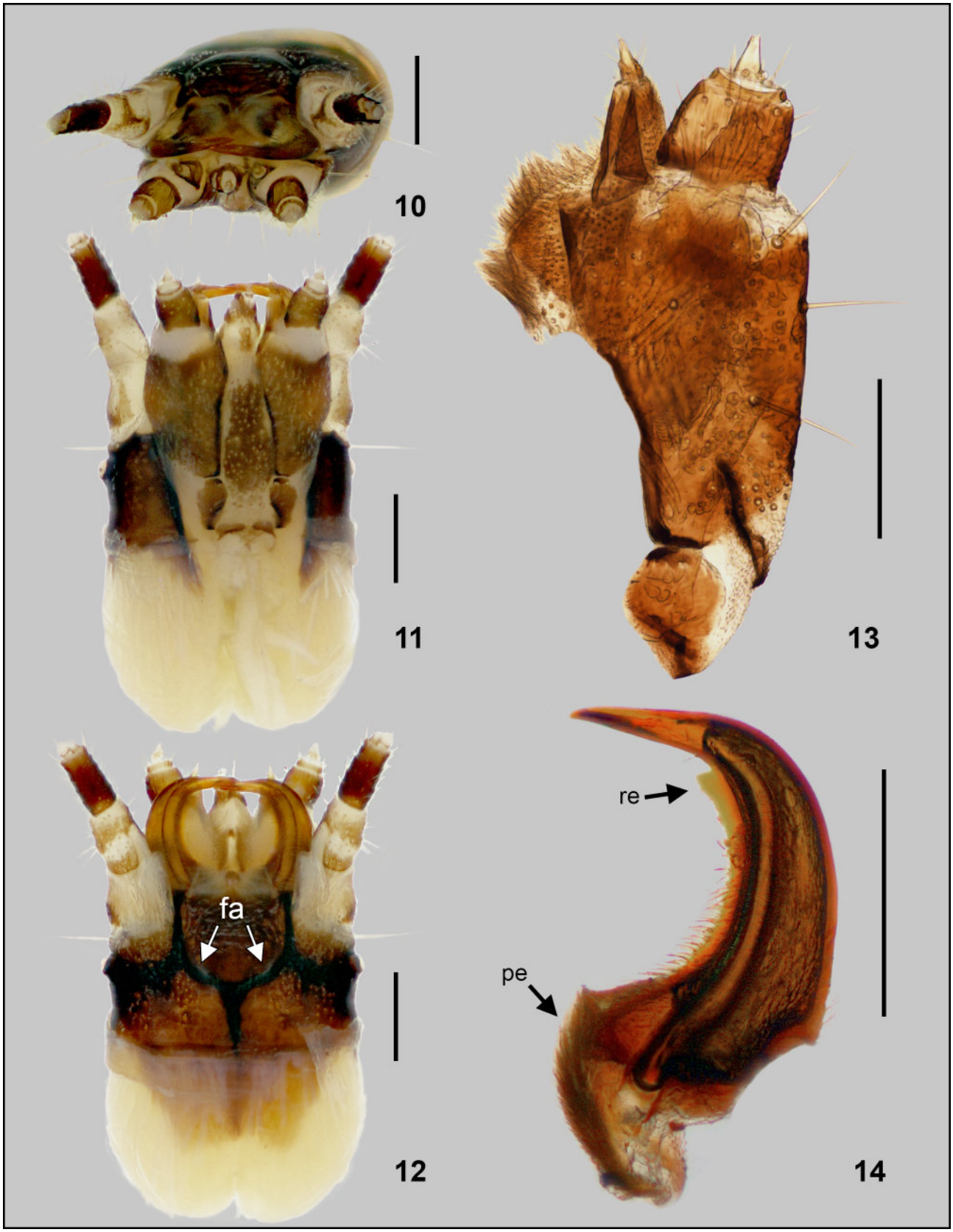
Types of general cuticular outgrowth observed. 1. Short, blunt, setae lying on surface (Figs 15, 29; bs); 2. stout, long setae (Figs 15, 27; ls); 3. flagellar setae growing from a slightly sunken toroidal socket (hereafter called toroidal setae; Figs 15, 20, 29; ts).

Head capsule (Figs 10–12, 15–17). Prognathous; retractable within prothorax, extensible neck membrane covered in extremely short spines and forming a two-layered envelope around retracted head; wider than long. Epicranial plate laterally about 1/3 of the width of the head capsule, slightly concave, with one stout seta anterolateraly, close to the base of antennae. Head capsule dorsally covered with short blunt setae lying on its surface (Fig. 15; bs). Epicranial suture dark, Y-shaped, frontal arms U-shaped (Fig. 12; fa). Gula not present (Fig. 11). One stemma on each side of the head. Labrum fused with clypeus forming clypeolabrum, covering base of mandibles in dorsal view. Clypeolabrum flat in anterior view, with two setae reaching one fourth of the length of mandibles, positioned on outer lateral sides (Fig. 15). Epipharynx formed by two plates, and an anterior brush of long setae, which project centrally past anterior margin of the head. Hypopharynx with short setation.

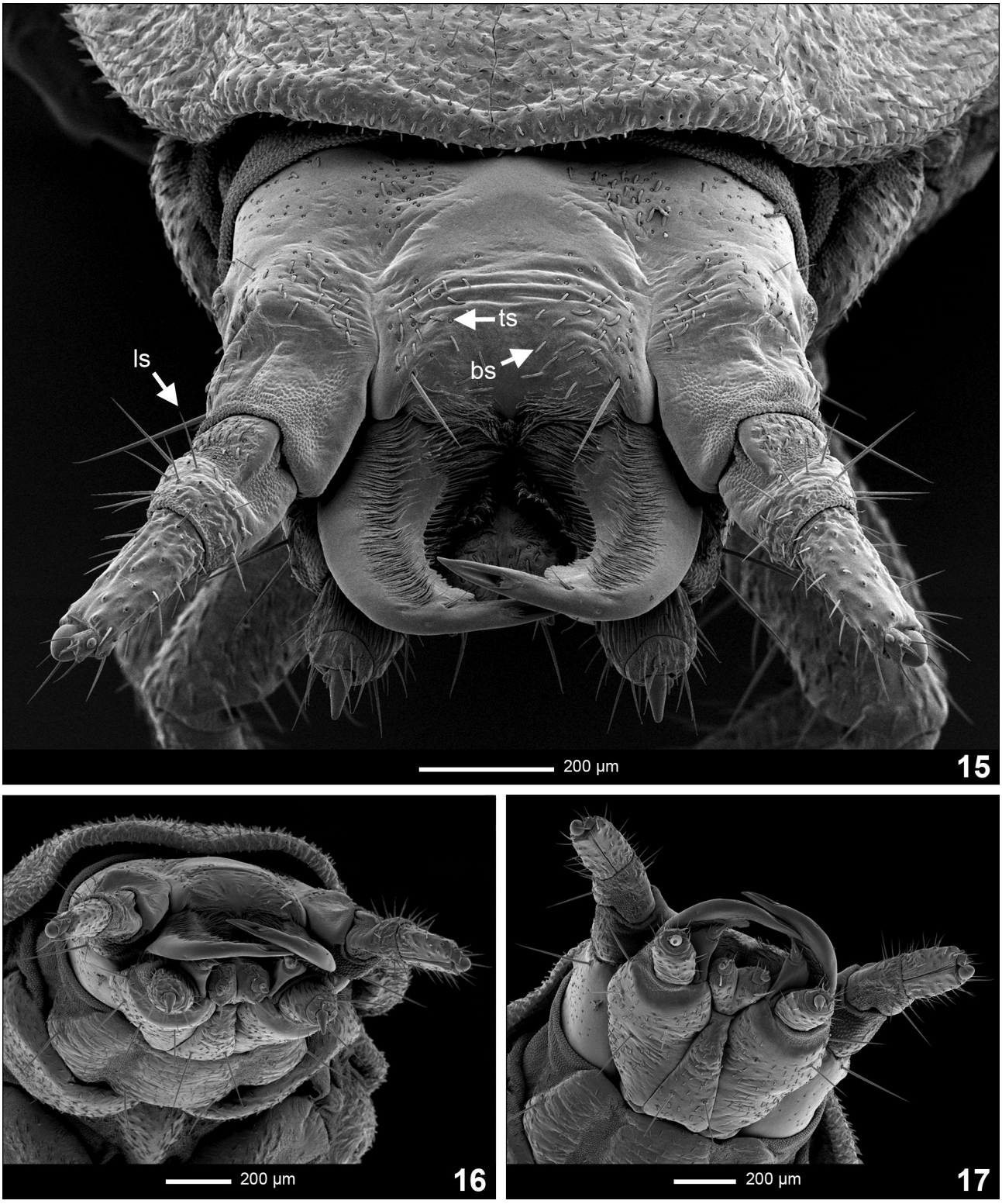
Antenna (Figs 24–26). Trimerous, inserted on lateral distal margin of epicranial plate; partially retractable within membranous socket. Basal antennomere widest, poorly sclerotized, slightly bulging on the dorsal side,



FIGURES 1–9. *Phosphaenus hemipterus*. General habitus of mature larva photographed in alcohol in dorsal (1); ventral (2) and lateral (3) views. General habitus of mature larva photographed dry in dorsal (4); ventral (5) and lateral (6) views. Detail of distal abdominal segments VI to X in dorsal (7); ventral (8) and lateral (9) views. Abbreviations: alt—anterior laterotergite; em—epimeron; is—intersternite; ls—laterosternite; lt—laterotergite; ms—median sternite; plt—posterior laterotergite; po—photic organ; ps—prosternum; ptl—light-pigmented lines on protergum; sg—parasagittal line. Scale bars: Figs 1-6, 5 mm; Figs 7-9, 1 mm.



FIGURES 10–14. *Phosphaenus hemipterus*. Detail of head in anterior (10); ventral (11) and dorsal (12) views; right maxilla in dorsal view (13); right mandible in dorsal view (14). Abbreviations: fa—frontal arms; pe—penicillus; re—retinaculum. Scale bars: 0.25 mm.



FIGURES 15–17. *Phosphaenus hemipterus*. SEM image of head in dorsal (15); anterior (16) and ventral (17) views. Abbreviations: bs—short, blunt, setae lying on surface; ls—stout, long setae; ts—toroidal setae.

densely covered by three types of setae; short blunt setae lying on surface and toroidal setae mainly posterolaterally, and several stout, almost perpendicular long setae around apical region (which are longest on this antennomere in comparison with the other antennomeres) easily observable under high magnification (Fig. 24). Second antennomere slightly longer, narrower and laterally flattened in comparison to basal antennomere; bearing only toroidal setae and blunt setae equally and abundantly spread across the antennomere. Inner ventrolateral area

of second antennomere with sensillum consisting of distinct longitudinal cleft; a sensory slot (Fig. 24; ses). Several sensilla coeloconica are present apically (Fig. 26; sc). Sensorium of second antennomere oval (Figs 24–26; as1), widest at the base, closely adhering to the second antennomere, slightly longer than the third antennomere, with very fine helical ridges from apex to bottom. Third antennomere (Fig. 24; a3) shortest, reaching about 1/2 of length of the sensorium of second antennomere and adjoining it, bearing a small sensorium, three short setae and three cuticular projections (Fig. 25); first longer and thick (cp1), second longer and thin (cp2) and third very short (cp3).

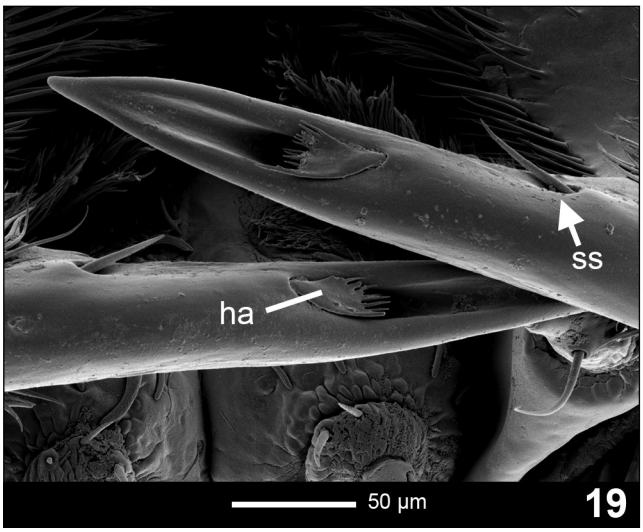
Maxilla (Fig. 13). Consisting of five parts, attached to labium forming a maxillo-labial complex. Cardo vertical, bulbous, with wider side adjacent to stipes. Stipes elongated, subtrapezoidal, ventrally covered with short blunt setae lying on its surface and four long and stout setae, three anteriorly and one medially. Galea bimerous, with basal part larger than distal, subtriangular in anterior view (with the tip of subtriangle aiming ventrally). Distal part conical, rotated medially with setae shorter than its body. Lacinia covered with brush of long setae on outer lateral margin. Maxillary palpifer large (Fig. 21; pf), subrectangular, slightly longer than wide. Maxillary palpus bimerous (Fig. 21; mp1, mp2), basal palpomere short and wide. Palpifer and basal palpomere covered with setae. Palpomere II (Fig. 22; mp2) bearing two setae, one thin and sharp placed dorsally and second slightly thicker and blunt, paired with sensorium near base on lateral surface (Fig. 22; ms).

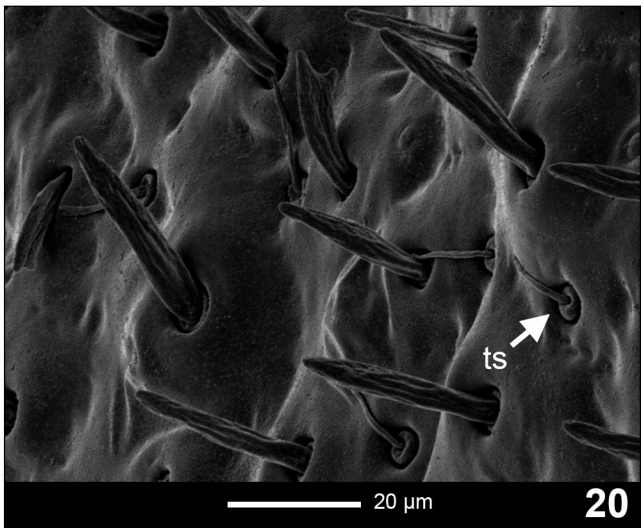
Labium (Fig. 21, 23). Closely attached to maxilla, formed by a short prementum, mentum and mostly membranous submentum (Fig. 11). Glossae absent. Prementum narrow, heart-shaped in ventral view; bearing three types of setae: blunt short setae lying on surface, sensory setae and a pair of long and stout setae underneath the palpi. Labial palpus bimerous (Fig. 23; lp1, lp2); basal palpomere wide and short, bearing several setae dorsally; second palpomere short, bearing a large sensorium ventrally (Fig. 23; ls), one thin seta between the apex and the sensorium, and two stout setae laterally around the apex: one sharp on the inner side and one blunt on the outer side. Mentum elongated, subtriangular, unsclerotized on lateral margins, ventrally bearing numerous short, blunt, setae lying on surface, numerous toroidal setae and a pair of large, stout setae posteromedially.

Mandible (Fig. 14). Symmetrical, falcate, with an internal channel opening subapically on outer edge (Fig. 19). Penicillus well developed (Fig. 14; pe). Retinaculum present, forming one thin and blunt hyaline process on apical third of mandible (Fig. 14; re). Inner margin of mandible from retinaculum to the base covered with stout setae, lengthening towards the base of mandible (Fig. 14). Ventrally, basal two-thirds of mandible covered with dense setation lying on surface, aimed medially. Dorsally, basal two thirds with sagittal line of dense, stout, setation lying on surface, of equal length, erect in its last third, aiming medially (Fig. 15). Lateral margin without setation. Sensory (hyaline) appendage on outer margin of mandible present before channel opening, forming a subtriangular valve with fringing at the distal end (Fig. 19; ha). A thin stout short seta present dorsally in retinaculum region on both mandibles (Fig. 19; ss). Several sensilla coeloconica present on the post-retinaculum apical part (Fig. 18; sc).

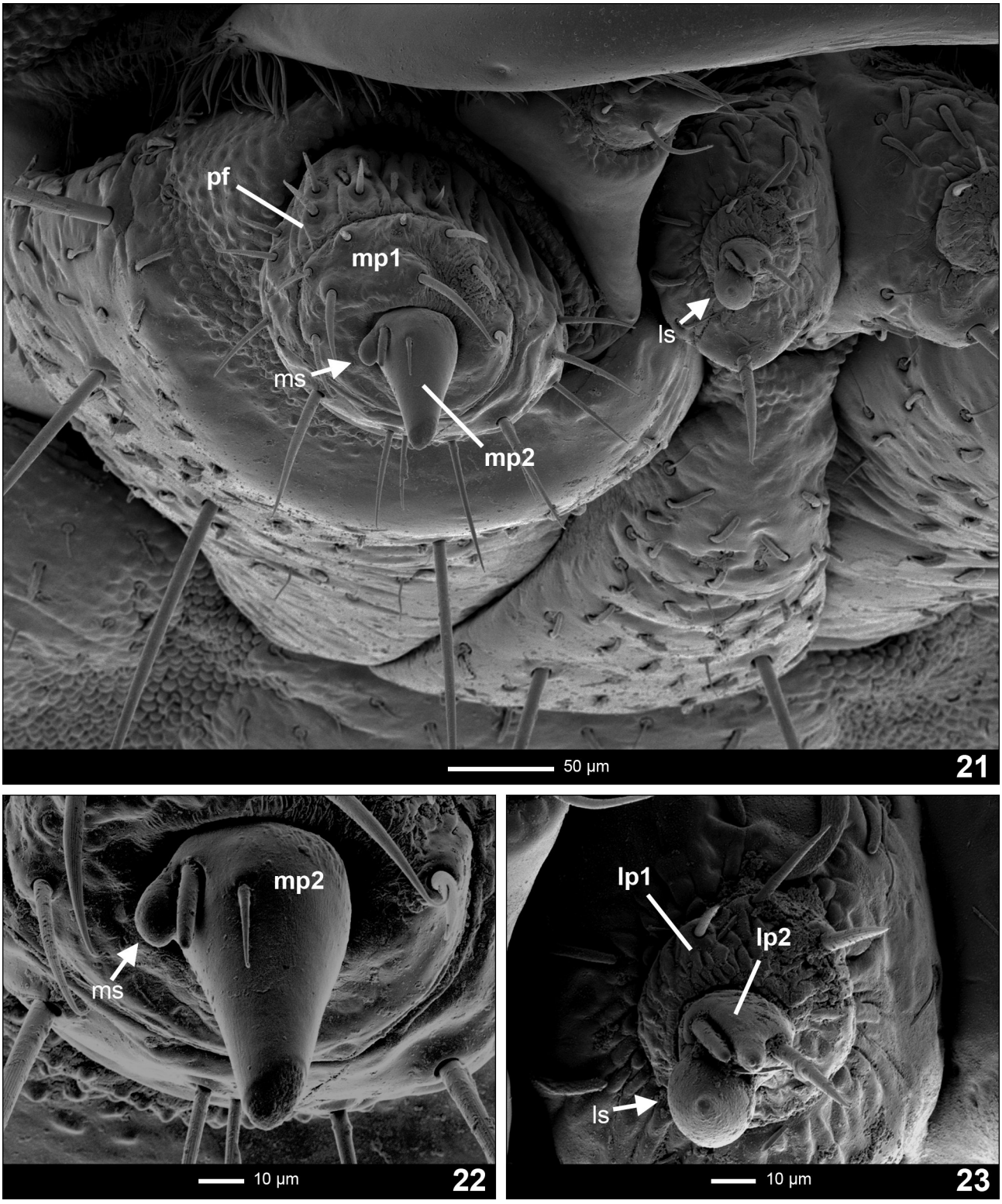
Thorax (Figs 1–6). Protergum wider than long, subsemicircular, wider posteriorly. Meso- and metatergum suboval, wider than long, with rounded margins. Venter of prothorax composed of subquadrate prosternum (Fig. 2; ps), subdivided into three well sclerotized areas; lateral ones narrow and transverse, extending above and to the sides of coxae fusing with episterna; medial area subrhomboid. Epimera forming thin sclerotized strands (Fig. 2; em). Lateral areas of meso- and metathorax composed of two laterotergites; anterior one sclerotized (Fig. 2; alt), bearing a well-developed bilabiate spiracle in mesothorax; posterior one membranous (Fig. 2; plt). Anterior ventral area of meso- and metathorax formed by mostly membranous intersternite (Fig. 2; is) with two darker-pigmented sagittal bands centrally, margined by paired anterior laterotergites. Posterior ventral area subdivided into triangular, wider than long basisternum and smaller sternellum. Basisternum subdivided into three darker-pigmented areas; lateral ones extending anteriorly and laterally to coxae, joining episterna; medial subrhomboid. Sternellum membranous, with sclerotized triangular centre touching with medial subrhomboid area of basisternum with its tip.

Legs (Figs 27–29). Pentamerous, all pairs similar in shape and size. Coxa large, stout, bearing short blunt setae lying on its surface, toroidal setae and stout long setae. Coxal-trochanteral membrane reaching more than 1/2 of coxal longitudinal length (Fig. 2). Trochanter smaller, subtriangular in lateral view, about the same size as femur, bearing short blunt setae lying on its surface, toroidal setae, and stout long setae, with long stout seta on distal venter, together with several shorter stout setae radially on distal end. Femur fusiform in lateral view, bearing short blunt setae lying on its surface, toroidal setae, and stout long setae, with one very long stout seta on the centre of ventral area (Fig. 27) and several shorter stout setae radially on distal end. Tibiotarsus as long as femur, narrower, tapering towards distal end, covered predominantly by stout sharp setae, lengthening dorsally. Pretarsus composed of a claw with fine ridges, ventrally bearing three short setae (Fig. 28).

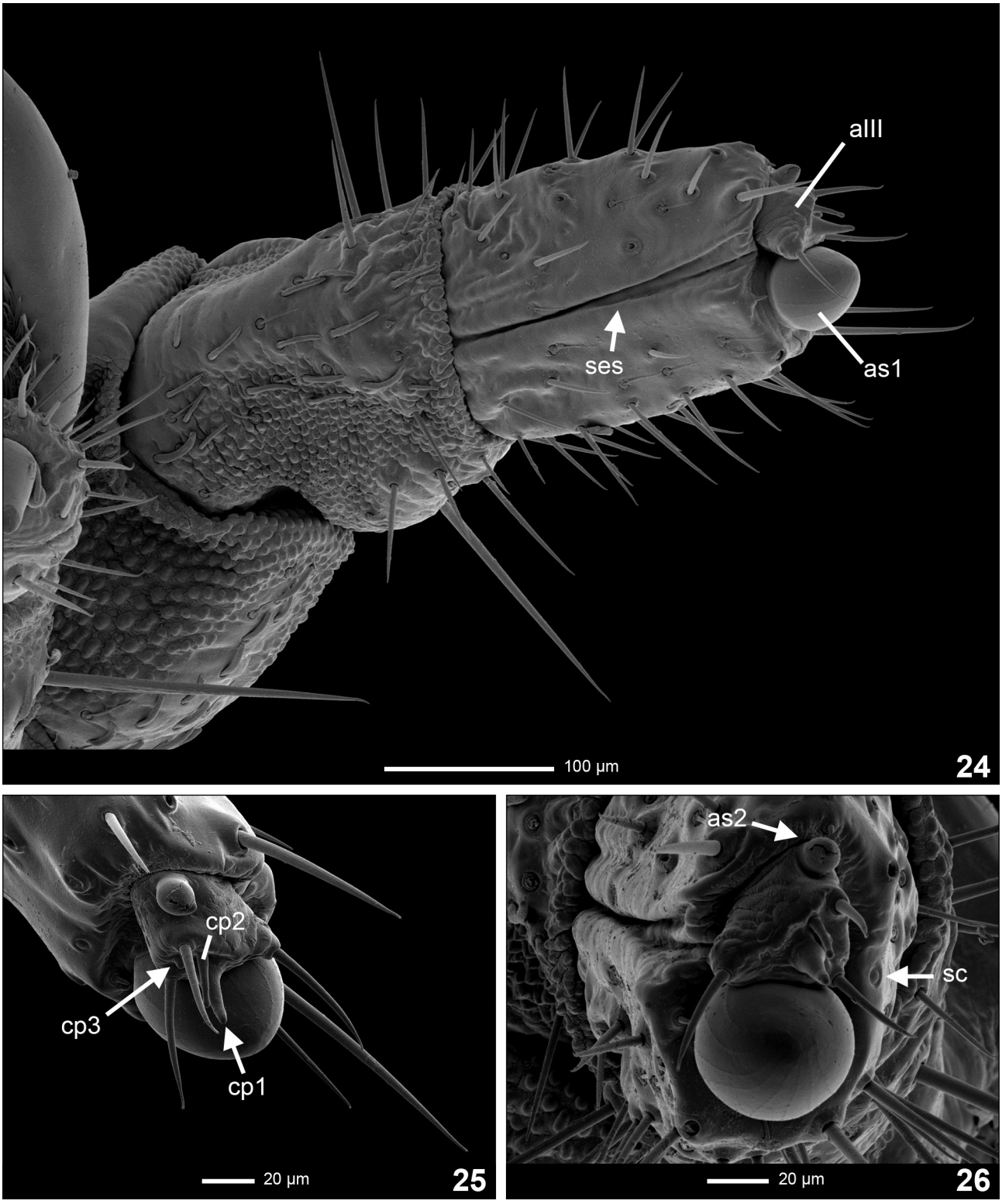




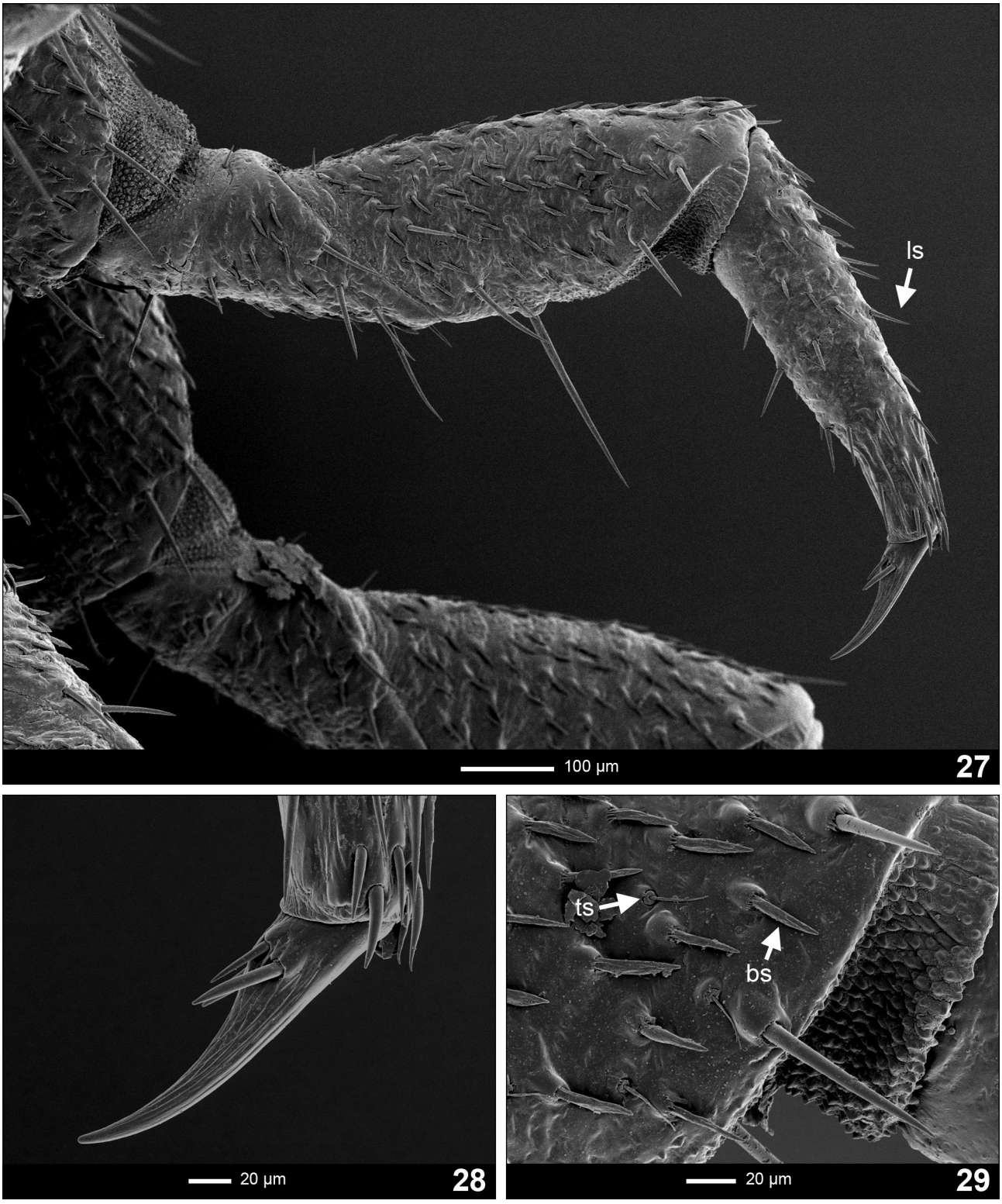
FIGURES 18–20. *Phosphaenus hemipterus*. SEM image of maxillolabial complex in anterior view (18); detail of mandibles (19); detail of body surface on protergum (20). Abbreviations: ha—hyaline appendage; lp1, lp2—labial palpus 1, 2; ls—labial sensorium; mp1, 2—maxillary palpus 1, 2; ms—maxillary sensorium; pf—maxillary palpifer; sc—sensillum coeloconicum; ss—solitary seta, ts—toroidal seta.



FIGURES 21–23. *Phosphaenus hemipterus*. SEM image of detail of maxillolabial complex in anterior view (21); detail of maxillary palpus 2 (22); detail of labial palpus (23). Abbreviations: lp1, 2—labial palpus 1, 2; ls—labial sensorium; mp1, 2—maxillary palpus 1, 2; ms—maxillary sensorium; pf—maxillary palpifer.



FIGURES 24–26. *Phosphaenus hemipterus*. SEM image of antenna in general view (24); detail of sensorium and third antennomere (25); anterior view (26). Abbreviations: a3—third antennomere; as1, 2—antennal sensorium 1, 2; cp1–3—cuticular projections 1–3; ses—sensory slot; sc—sensillum coeloconicum.



FIGURES 27–29. *Phosphaenus hemipterus*. SEM image of left prothoracic leg in general view (27); pretarsus (28); detail of distal end of femur (29). Abbreviations: bs—short, blunt, setae lying on surface; ls—stout, long setae; ts—toroidal setae.

Abdomen (Figs 1–9). Ten-segmented, slightly tapering towards posterior end, segments I to VIII subdivided by fine sagittal line in dorsal view (Fig. 1). Tergites of segments I to VII subrectangular, similar in shape and colouration, wider than long; tergite of segment VIII suboval; segment IX subrectangular, wider than long; segment X forming a narrow sclerotized dark ring, bearing the holdfast organ—pygopod—with several eversible processes. Segments I to VIII have single laterotergites (Fig. 3; lt) on each side with sclerotized plates bearing

bilabiate spiracles; the venter of segments I to VII consists of median sternite, margined by paired narrow weakly sclerotized laterosternites (Fig. 3; ls). Sternites of segments I to VIII subrectangular, well sclerotized (except segment VIII) wider than long, with a pair of long stout setae on posterolateral margins. Venter and laterotergites of segment VIII membranous, bearing laterally a pair of white spots representing a functioning photic organ (Fig. 8; po). Sternite of segment IX sclerotized posteriorly, forming a thin ring supporting segment X.

Notes on life cycle and behaviour. The life cycle of *Phosphaenus hemipterus* lasts two or three years. Eggs are white, spherical, with a diameter of ca. 0.6 mm (De Cock 2000). According to De Cock, late-instar female larvae tend to be larger and fatter than males and are easily recognised in the field (De Cock 2003). Pupation takes place in April–May, the pupal stage lasts ca. 2 weeks. Mature individuals are collected occasionally and rarely from July to August (Burakowski 2003).

Unlike most of the firefly species, whose larvae feed on snails and slugs, larvae of *Phosphaenus hemipterus* are obligate earthworm (*Lumbricus* spp., Lumbricidae) predators (Majka & MacIvor 2009). Majka & MacIvor (2009) observed the larvae while feeding, using tarsal claws of the legs to anchor themselves to the body of the earthworm and their extended antennae moving over the surface of the earthworm's body. The process of injecting a toxin into its prey in order to kill it, as observed in other species of fireflies (Schwalb 1961), has not yet been observed for this species. However, the presence of a mandibular channel suggests that a toxin is also used by this species.

Like *Lampyris noctiluca*, *Phosphaenus hemipterus* larvae also glow spontaneously by emitting bioluminescent pulses while active at night (De Cock 2003).

Both adult sexes are flightless. Neotenic females stay in litter or lower parts of plants, are active mainly at dusk (De Cock 2000; Burakowski 2003) and are very rarely found (Burakowski 2003). In contrast, the males have vestigial elytra, are diurnal, and can often be found on herbaceous plants and shrubs (Burakowski 2003). The larvae are predominantly nocturnal. Both adult sexes are feebly bioluminescent, although they appear only to glow in response to disturbance (Majka & MacIvor 2009).

Discussion

External sensory organs. The abundance and positioning of sensilla chaetica and sensilla trichodea in *Phosphaenus hemipterus* corresponds with the findings in both *Lampyris noctiluca* and *Lamprohiza splendidula* (Novák 2017, 2018). Sensilla coeloconica (Figs 18, 26; sc), on the other hand, were observed both on antennae and apical parts of mandibles, while in *Lampyris noctiluca* they were observed on antennae and epicranial plate and in *Lamprohiza splendidula* not observed at all. This sensillum is defined by Shields (2008) as a basiconic peg or cone set in a shallow pit, most often chemo-, thermo-, or hygrosensitive. The presence of this type of sensilla on the mandibles of *Phosphaenus hemipterus* might be connected either with a different type of prey, or pheromone communication in adults, as will be discussed below.

A unique type of sensilla was observed on antennae, legs, and sclerotized parts of dorsum and venter. This is a fibrous, weak seta set in a shallow toroidal socket (Fig. 20; ts). There is uncertainty, whether this process is just a modification of sensillum chaeticum or is the sensillum coeloconicum. Arguments for sensillum chaeticum are the wide occurrence on the body of the larva and mechanoreceptive function, together with a fact that the observed sensillum is fibrous, instead of peg- or cone-shaped. Arguments for sensillum coeloconicum are the shallow socket, and the fact that the sensilla occurs with numerous modifications, together with unique prey type and ecology of *Phosphaenus hemipterus*, which may result in a need for different sensory organs.

Phosphaenus hemipterus adults, unlike most lampyrids, prefer pheromone communication to visual communication. In larvae of this species, a striking amount of *sensoria* were observed compared to other sympatric firefly species. While *Lampyris noctiluca* and *Lamprohiza splendidula*, sympatric species of fireflies with *Phosphaenus*, have single sensoria on second antennomeres only, *Phosphaenus hemipterus* bears sensoria on the distal palpomere of the maxillary palpus (Figs 21, 22; ms), the labial palpus (Figs 21, 23; ls) and the third antennomere (Fig 26; as2), in addition to the previously mentioned second antennomere (Fig. 24; as1). Additionally, the second antennomere bears a sensory slot throughout its whole length (Fig. 24; ses). A possible explanation for this phenomenon is broad pheromone utilization within this species. Whereas the larvae do not participate in sexual communication, the sensoria may simply be undeveloped functional organs of adults. Another

explanation may be the unique diet of this species, and possible use in prey tracking, in connection with the aforementioned higher number of sensilla coeloconica.

Hunting for prey. *Phosphaenus hemipterus* is an obligate earthworm predator. Compared to other sympatric fireflies, preferring snails as their diet, this species is unique in its large number of sensoria and lack of dorsoventrally flattened body (Figs 3, 6). The possible intraspecific pheromone function has been mentioned above, as well as possible interspecific chemoreceptive function for tracking of prey. The round cross-section of the body may be an adaptation to earthworm hunting, enabling easier capture of prey that is retreating into a ground tunnel. The significance of body shape is supported by the fact that Nearctic species of *Photinus* Laporte, 1833, which are also reported to prey on earthworms (Lloyd 2008), have a very similar general body-shape. Moreover, certain diurnal fireflies are also known to hunt earthworms, such as *Lucidina* Gorham, 1883 and *Stenocladius* Deyrolle & Fairmaire, 1878 (De Cock 2009). In both cases, the cross-section of the larva is oval, but no surveys regarding the presence of special sensilla have been performed to my knowledge. Additionally, no detailed *in vivo* study of *Phosphaenus hemipterus* predation has been undertaken and therefore the presumed advantages of their morphology remain merely assumptions.

As well as in *Lampyris noctiluca* and *Lamprohiza splendidula*, a special, solitary sensillum was observed on the mandibles positioned apically before the opening of the inner channel together with a hyaline appendage resembling a "shutter" found at the base of the mandibular channel opening (Fig. 19; ha; Novák 2017, 2018). As in the previously mentioned species, both structures also possess a unique shape in *Phosphaenus hemipterus*. The "shutter", formed by a subtriangular valve with fringing at the distal end, gives the impression of preventing the channel opening from being blocked by rough particles, rather than serving as a seal.

The overall shape of the mandibles (Figs 14, 15) is yet another feature that distinguishes *Phosphaenus hemipterus* from its snail-hunting counterparts. While in snail feeders the mandibles are sickle shaped, in *Phosphaenus* they are more hooked. This shape may enable the larva to bite and remain attached to its prey. Since earthworms do not overproduce mucus as a defence, the larva could hypothetically hold on its prey and wait for the full dosage of toxin to get into its body—a variation on snail-riding observed in other taxa. The larva's use of tarsal claws (Fig. 28) to attach itself to the body of the prey as discussed by Majka & MacIvor (2009) supports this hypothesis.

A key to three species of central European lampyrid larvae

The key presented in this work consists of two parts. The first part is a dichotomous key assembled from the most distinct morphological features, enabling quick orientation and determination of the genus and species. The second part is represented by a comparative table that goes into greater detail, addressing interspecific differences in specific body parts (Table 1).

1 Tergal margins on thorax and abdomen laterally explanate ("trilobite" larva) . . . . . *Lamprohiza splendidula*
- Tergal margins on thorax and abdomen scarcely project 2
2 Thoracic and abdominal tergites dark brown or black, with pairs of light-pigmented spots on posterolateral margins of every segment of thorax and abdomen except IX and X . . . . . *Lampyris noctiluca*
- Tergites of thorax and abdomen fully dark brown or black; tergites of thorax subdivided by fine light-pigmented parasagittal lines on each side of sagittal line. . . . . *Phosphaenus hemipterus*

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I would like to thank Jan Růžička (Czech University of Life Sciences in Prague, Czech Republic) for his excellent professional guidance as my mentor, providing useful sources of information and tools for research, and great patience. I would also like to thank Raphaël De Cock (University of Antwerp, Belgium) for his priceless advice and help with the Lampyridae family, Petr Švácha (Institute of Entomology, Czech Academy of Sciences, České Budějovice, Czech Republic) for providing larval specimens of *Phosphaenus hemipterus* for this article and Max Barclay (Natural History Museum, London) and Christopher A. Harding (United Kingdom) for text revision. This study was funded by the Internal Grant Agency of the Faculty of Environmental Sciences, CULS Prague (IGA 2015, project no. 4211013123135, registration no. 20174221).

TABLE 1. Comparison of larvae of central European Lampyridae.

Character/Species	*Lampyris noctiluca*	*Lamprohiza splendidula*	*Phosphaenus hemipterus*
Body shape			
Habitus	fusiform and robust; tergal margins scarcely project	elongate and fusiform; tergal margins laterally explanate	oblong and slender; tergal margins scarcely project
Cross-section	slightly dorsoventrally flattened	dorsoventrally flattened	Oval
Head capsule			
Epicranial suture colour	dark-coloured or indistinguishable	light-coloured	dark-coloured
Frontal arms shape	U-shaped	V-shaped	U-shaped
Clypeolabrum lateroapically	with 2 distinguishable long setae	no distinguishable setae; light-coloured spot posteriorly, behind each stemma	with 2 distinguishable long setae
Longitudinal length of epicranial plate laterally	larger or same length as 1/2 of width of head capsule	larger than width of head capsule	shorter than 1/2 of width of head capsule
Hypopharynx setation	long	long	short
Antenna			
Second antennomere	no distinct longitudinal cleft	no distinct longitudinal cleft	inner ventrolateral area with distinct longitudinal cleft
Sensorium	smooth surface, closely adhering to antennomere	smooth surface, distinct basal constriction	fine helical ridges from apex to bottom, closely adhering to antennomere
Maxilla			
Maxillary palpus	trimerous	trimerous	bimerous
Terminal maxillary palpomere	irregularly subconical, thick and blunt, with inner-lateral sagittal slot	subconical, narrow and sharp	subconical, with outer-lateral sensorium
Labium			
Terminal palpomere of labial palpus	bearing stout blunt seta covering outer-lateral sagittal slot	bearing short thin setae only	bearing sensorium ventrally
Mandible			
Retinaculum	present, forming distinguishable sharp inner tooth	absent	present, short and blunt
Mandibular channel opening	covered by blunt thick seta	covered by a distinguishable long feather-like or rounded-trapezium hyaline appendage	covered by a small hyaline appendage forming a subtriangular valve with fringing at the distal end
Thorax			
Thoracic tergites	divided by sagittal line into two parts; with distinct pinkish or yellowish spots on posterolateral margins	divided by sagittal line into two parts	divided by sagittal line into two parts, which are then subdivided with another clear line subparallel to sagittal line
Protergum	subtrapezoidal, strongly concave on posterior margin	triangular, convex posteriorly on each half divided by sagittal line, with narrow emargination anteriorly	semicircular, more or less straight posteriorly
Meso- and metatergum	ca. 2 times wider than long	ca. 4 times wider than long	ca. 2 times wider than long

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TABLE 1. (Continued)

Character/Species	*Lampyris noctiluca*	*Lamprohiza splendidula*	*Phosphaenus hemipterus*
Legs			
Pretarsus	claw ventrally bearing 3 short setae	claw ventrally bearing 2 long setae	claw ventrally bearing 3 short setae
Coxa	coxal-trochanteral membrane reaching less than 1/2 of coxal longitudinal length	coxal-trochanteral membrane reaching less than 1/2 of coxal longitudinal length	coxal-trochanteral membrane reaching more than 1/2 of coxal longitudinal length
Abdomen			
Abdominal tergites I–VI	ca. 2 or 3 times wider than long	ca. 4 times wider than long	ca. 2 or 3 times wider than long
Sternum of segment I	sclerotized	not sclerotized	sclerotized
Laterosternites	present on segments I to VII	present on segments I to V	present on segments I to VII
Spiracle colouration	light	dark	light
Specific cuticular processes	granulose protuberances, densely occurring on sclerites and legs	no granulose protuberances nor setae growing from toroidal base	short fibrous setae growing from slightly sunken toroidal base
Photic organ shape and position	conspicuous pair of white spots ventrally on abdominal segment VIII	barely distinguishable paired or single spots ventrolaterally on abdominal segments II and VI, with possible additional spots ventrolaterally on abdominal segments III–V	dull and barely distinguishable pair of spots, ventrally on abdominal segment VIII

References

Archangelsky, M. & Fikáček, M. (2004) Descriptions of the egg case and larva of *Anacaena* and a review of the knowledge and relationships between larvae of Anacaenini (Coleoptera: Hydrophylidae: Hydrophylinae). *European Journal of Entomology*, 101, 629–636. https://doi.org/10.14411/eje.2004.086

Ballantyne L.A. & Menayah R. (2002) A description of larvae and redescription of adults of the firefly *Pteroptyx valida* Olivier in Selangor, Malaysia (Coleoptera: Lampyridae: Luciolinae), with notes on Luciolinae larvae. *Raffles Bulletin of Zoology*, 50 (1), 101–109.

Burakowski, B. (2003) *Klucze do oznaczania owadow Polski, Czesc XIX Chraszcze—Coleoptera, Zeszyt 29, 30 Karmazynkowate—Lycidae, Swietlikowate—Lampyridae*. [*Key to identification of insects of Poland, Part XIX—Beetles (Coleoptera), Issue 29, 30 Lycidae, Lampyridae*]. Polskie Towarzystwo Entomologiczne, Torun, 39 pp. [in Polish]

De Cock, R. (2000) Rare, or simply overlooked? Practical notes for survey and monitoring of the small glow-worm *Phosphaenus hemipterus* (Coleoptera: Lampyridae). *Belgian Journal of Zoology*, 130, 93–101.

De Cock, R. (2003) Larval and Adult Emission Spectra of Bioluminescence in Three European Firefly Species. *Photochemistry and Photobiology*, 79, 339–342. https://doi.org/10.1111/j.1751-1097.2004.tb00018.x

De Cock, R. (2009) Biology and behaviour of European lampyrids. *In*: Meyer-Rochow, V.B. (Ed.), *Bioluminescence in Focus—A Collection of Illuminating Essays*. Research Signpost, Trivandrum, pp. 161–200.

Deyrolle, H. & Fairmaire, L. (1878) Description de coléoptères recueillis par M. l'abbé David dans la Chine centrale. *Annales de la Société Entomogique de France*, 5 (8), 87–140.

Geisthardt, M. & Satô, M. (2007) Lampyridae. *In*: Löbl, I. & Smetana, A. (Eds.), *Catalogue of Palearctic Coleoptera. Vol. 4. Elateroidea—Derodontoidea—Bostrichoidea—Lymexyloidea—Cleroidea—Cucujoidea.* Apollo Books, Sternstrup, pp. 225–234.

Goeze, J.A.E. (1777) *Entomologische beyträge zu des ritter Linné zwölften ausgabe des natursystems von Joh. Aug. Ephraim Goeze. Erster theil.* Weidmanns Erben und Reich, Leipzig, 736 pp. https://doi.org/10.5962/bhl.title.45974

Gorham, H.S. (1883) Descriptions of Malacodermata in the Civic Museum of Natural History at Genua. *Annali del Museo Civico di Storia Naturale di Genova*, 18, 591–606.

Hučík, M., Kolibová, M., Cikánová-Konečná, G. & Furchová, H. (2013) *Územní plán Lednice—okres Břeclav.* [*Land-use plan Lednice—Břeclav region*]. Městský úřad Břeclav, odbor rozvoje a správy, oddělení úřad územního plánování, Břeclav, 45 pp. [in Czech]

Klausnitzer, B. (1994) 50. Familie: Lampyridae. *In*: Klausnitzer, B. (Ed.), *Die Larven der Käfer Mitteleuropas. Band. 2. Myxophaga, Polyphaga. Teil 1*. Goecke & Evers, Krefeld, pp. 228–230.

Korschefsky, R. (1951) Bestimmungstabelle der bekanntesten deutschen Lyciden-, Lampyriden- und Drilidenlarven. *Beiträge zur Entomologie*, 1, 60–64, table 1.

Kratochvíl, J. (1957) *Klíč zvířeny ČSR, Díl II, Třásnokřídlí, blanokřídlí, řásnokřídlí, brouci.* [*Key to Czechoslovakian fauna, Part II, Thysanoptera, Strepsiptera, Coleoptera*]. Nakladatelství Československé akademie věd, Praha, 746 pp. [in Czech]

LaBella, D.M. & Lloyd, J.E. (1991) Lampyridae (Cantharoidea). *In*: Stehr, F.W. (Ed.), *Immature Insects. Vol. 2*. Kendall/Hunt, Dubuque, pp. 427–428.

Laporte, F.L. (1833) Essai d'une revision du genre *Lampyre. Annales de la Société entomologique de France*, 1833, 122–153.

Lawrence, J.F. & Ślipiński, A. (2013) *Australian beetles, Volume 1. Morphology, Classification and Keys*. CSIRO Publishing, Collingwood, 561 pp.

Linnaeus, C. (1758) *Systema naturae per regna tria naturae: secundum classes, ordines, genera, species, cum characteribus, differentiis, synonymis, locis. Tomus I. Editio decima, reformata.* Laurentius Salvius, Holmiae, 824 pp. https://doi.org/10.5962/bhl.title.542

Linnaeus, C. (1767) *Systema naturae per regna tria naturae: secundum classes, ordines, genera, species, cum characteribus, differentiis, synonymis, locis. Tomus I. Pars II. Editio duodecima reformata.* Laurentius Salvius, Holmiae, 1327 pp. https://doi.org/10.5962/bhl.title.68927

Lloyd, J.E. (2008) Fireflies (Coleoptera: Lampyridae). *In*: Capinera, J.L. (Ed.), *Encyclopedia of Entomology. 2nd Edition*. Springer, London, pp. 1429–1452.

Majka, C.G. & MacIvor, J.S. (2009) The European lesser glow worm, *Phosphaenus hemipterus* (Goeze), in North America (Coleoptera, Lampyridae). *ZooKeys*, 29, 35–47. https://doi.org/10.3897/zookeys.29.279

Mikšić, R. (1982) Die Lampyriden Europas (Coleoptera, Malacodermata). *Acta Entomologica Jugoslavica*, 17 (1–2), 19–26.

Novák, M. (2017) Redescription of immature stages of central European fireflies. Part 1. *Lampyris noctiluca* (Linnaeus, 1758) larva, pupa and notes on its biology (Coleoptera: Lampyridae: Lampyrinae). *Zootaxa*, 4247 (4), 429–444. http://doi.org/10.11646/zootaxa.4247.4.5

Novák, M. (2018) Redescription of immature stages of central European fireflies, Part 2: *Lamprohiza splendidula* (Linnaeus, 1767) larva, pupa and notes on its life cycle and behaviour (Coleoptera: Lampyridae). *Zootaxa*. [in review]

Reitter, E. (1911) *Fauna Germanica, Die Käfer des Deutschen Reiches. Band 3.* K. G. Lutz, Stuttgart, 436 pp.

Schaufuss, L.W. (1870) *Nunquam otiosus. Zoologische Mittheilungen von Dr. L. W. Schaufuss, 1. Band.* Carl Adler's Buchhandlung, Dresden, 478 pp.

Shields, V.D.C. (2008) Utrastructure of insect sensilla. *In*: Capinera, J.L. (Ed.), *Encyclopedia of Entomology. 2nd Edition*. Springer, London, pp. 4009–4023.

Tyler, J. (2002) *The glow-worm*. Lakeside, Sevenoaks, 76 pp.

Annex 4: Revisited larval morphology of Thanatophilus rugosus (Coleoptera: Silphidae)

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ORIGINAL ARTICLE

Revisited larval morphology of Thanatophilus rugosus (Coleoptera: Silphidae)

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Abstract

Determination of insect species and their instars, occurring on human remains, is important information that allows us to use insects for estimation of postmortem interval and detect possible manipulation with the body. However, larvae of many common species can be identified only by molecular methods, which is not always possible. The instar determination is even more challenging, and qualitative characters that would allow a more precise identification are mostly unknown. *Thanatophilus rugosus* (Linnaeus, 1758) is a common necrophagous beetle in the whole Palaearctic region from Europe to Japan. The species is often encountered on corpses of large vertebrates including humans, and its potential to become a useful bioindicator for forensic entomology is therefore high. Adults can be easily distinguished from other species; however, larvae were never thoroughly described to allow species and instar identification. The aim of this study was to provide reliable morphological characters that would allow species and instar identification of *T. rugosus* larvae. The material for morphological study was obtained from rearing under controlled conditions (20 °C and 12:12 h of light/dark period), and specimens that were not studied morphologically were allowed to complete their development. Quantitative and qualitative morphological characters for instar and species identification are described and illustrated. Additionally, we report observations of biology and developmental length for all stages of the species.

Keywords *Thanatophilus rugosus* · Larval instar identification · Morphology · Forensic entomology

Introduction

Beetles (Coleoptera) are one of the most diverse groups of animals, which can be commonly encountered on vertebrate carcasses around the world [1]. Despite this well-known fact, their value for forensic entomology was not fully recognized until recently [2]. The tight association between food source (corpse) and beetles can provide a lot of information regarding toxicological profile of the deceased, possible postmortem body manipulation, and approximate time of death (postmortem interval (PMI)) itself [3–6]. The last one is an especially important feature for homicide investigations. If a body is discovered after more than 72 h, the state of the body itself cannot provide accurate data for such an estimate [7].

When estimating the PMI based on insect evidence, it is crucial to establish how old are the earliest stages that were developing on the body at the time of its discovery [8]. The most common way of how to calculate such an estimate is to use a thermal summation model [9]. These models are species- and stage-specific [5]; hence, to give an accurate estimate of PMI, the species and development stage have to be identified correctly. However, the identification of larval instars or even beetle species can be challenging.

Amendt et al. [10] offer two approaches that can be used to resolve the issue of species identification. The first is to rear the eggs and larvae to adulthood. At that stage, the abundance of literature can be used for morphological identification. The disadvantage of this method is that the development takes time and the results are highly uncertain due to possible mortality

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during rearing. The second approach recommended by the authors is to analyze specimens' DNA, which is currently a standard method in forensic investigations. Nevertheless, closely related or hybrid species can be difficult to distinguish, especially when the data on the level of their intraspecific and interspecific DNA variability are scarce [11, 12]. It is also worth mentioning that DNA extraction from old or badly preserved specimens could be difficult, expensive, or completely impossible [13, 14].

The second part of the problem is the issue of precise and accurate stage identification. A simple and elegant solution was provided by the discovery of Dyar's Rule, which states that the size of morphological characters between successive instars follows geometrical progression [15, 16]. This idea and its derivatives were used to create body size frequency distribution models and other models for identification of all larval instars of some forensically important species [17–21]. The simplicity of this approach, nonetheless, comes with a cost, because it is prone to errors when applied to animals from different geographical populations and breeding conditions other than the ones originally measured [22].

The solution to all of these problems could be a detailed morphological description of all developmental stages, which would reveal stage-specific qualitative characters and thus allow species and instar identification.

The genus *Thanatophilus* Leach, 1815, has currently 23 valid species: 14 are Palaearctic, four Nearctic, two Holarctic, and three Afrotropical in distribution [23–25]. The phylogenetic position of *Thanatophilus* is a sister branch to remaining Holarctic genera of Silphinae, together forming a cluster which is a sister group to Neotropical and Australian *Oxelytrum* Gistel, 1848, and *Ptomaphila* Kirby & Spence, 1828 [26, 27]. Taxonomy and classifications of *Thanatophilus* species based on adult morphology were reviewed by Schawaller [28], with later additions from Kozminykh [29], Růžička [30], and Ji [31]. The larvae of this genus are poorly known with only eight described species. These descriptions are often based on an unknown larval instar, and many of them are rather brief and without illustrations (see Table 1).

We choose *Thanatophilus rugosus* (Linnaeus, 1978) as our focal species because it is a widely distributed Palaearctic beetle, known to occur from Europe to Japan [25]. In Europe, it is considered a very common necrophagous beetle [41–43]. Similar to other carrion beetles (Silphidae) [5, 6, 44, 45], this species could also become a highly valuable forensic indicator as its presence was detected on 16% of the cases in the Czech Republic when the entomological evidence was collected (Šuláková, unpublished data) ($N = 23$ out of 144 cases between 2003 and 2016).

Little is known about the immature stages of *T. rugosus*. The first description of an unknown larval stage and some remarks about the biology of adults were published by Xambeu [38], but the description is not detailed enough to allow species identification. The second and last description of its larval morphology was done by Lengerken [33], and it depicts all three instars of *T. rugosus*. His portrayal was much more thorough than Xambeu's, although he himself admits that species identification of the larval stages of *T. rugosus*, *T. dispar*, and *T. sinuatus* requires comparative material and that the size-based instar identification, which he recognized as the only solution, is prone to errors due to the high morphological plasticity under different breeding conditions.

The aim of this paper is to provide a morphological description of all larval stages with special regard to characters and parts that were omitted in previous reports and to identify the characters that are species- and instar-specific. These characters would allow the identification of even badly preserved specimens when DNA analysis is difficult or does not answer the questions (e.g., to which instar the specimen belongs).

Table 1 List of species in genus *Thanatophilus* with described larvae. Only original morphological descriptions were included

Species	Author	Described stage	Comments
Thanatophilus capensis (Wiedemann, 1821)	Daniel et al. [32]	All three instars	Described and illustrated
Thanatophilus coloradensis (Wickham, 1902)	Anderson and Peck [23]	Probably 3rd instar	Described and partially illustrated
Thanatophilus dispar (Herbst, 1793)	von Lengerken [33, 34]	All three instars	Brief description and illustration
Thanatophilus lapponicus (Herbst, 1793)	Dorsey [35]	3rd instar	Described and illustrated
Thanatophilus micans (Fabricius, 1794)	Paulian [36]	Probably 3rd instar	Described and illustrated
Thanatophilus micans (Fabricius, 1794)	Prins [37]	Egg, three larval instars and pupa	Described and illustrated
Thanatophilus micans (Fabricius, 1794)	Daniel et al. [32]	All three instars	Described and illustrated
Thanatophilus rugosus (Linnaeus, 1758)	Xambeu [38]	Probably 3rd instar	Brief description
Thanatophilus rugosus (Linnaeus, 1758)	von Lengerken [33]	All three instars and pupa	Described and illustrated
Thanatophilus sinuatus (Fabricius, 1775)	Xambeu [39]	Probably 3rd instar	Brief description
Thanatophilus sinuatus (Fabricius, 1775)	von Lengerken [33 34]	All three instars and pupa	Described and illustrated
Thanatophilus sinuatus (Fabricius, 1775)	Paulian [36]	Probably 3rd instar	Brief description and illustration
Thanatophilus trituberculatus (Kirby, 1837)	Anderson [40]	Probably 3rd instar	Brief description and illustration

Material and methods

Adult specimens of *T. rugosus* were collected by baited pitfall traps around Albeř (Czech Republic) (49° 01′ 35.7″ N 15° 08′ 54.9″ E) between 16th and 20th May 2016. Subsequently, they were transferred to the laboratory, where they were kept in small breeding groups of five to eight individuals. These groups were placed inside well-ventilated plastic boxes (85 × 110 × 45 mm) with a 1-cm-thick layer of rough sand (diameter 1–4 mm) and were provided with fish meat (*Scomber scombrus* Linnaeus, 1758) ad libitum. Breeding and development of immature stages took place inside a climatic chamber with constant temperature of 20 °C and a 12-h light and 12-h darkness photoperiod regime, maintained by fluorescent light (Osram L 8 W/640).

Breeding boxes were thoroughly inspected at least once every 24 h, and the eggs were removed and separated and their development observed at the same frequency. Developmental milestones were recognized by the presence of exuvia. During each developmental stage (except pupa), we removed 6 to 10 specimens for morphological study and measurements. All selected specimens were killed in ethyl acetate fumes, fixed with hot water (90–95 °C), and stored in 75% alcohol solution. The rest was followed to record their development time, but only individuals with reliable known starting and finishing point are reported in Table 2. Due to this restriction, we had to exclude observations of several eggs, as the starting point of their development was unclear, because they were hidden among substrates (unlike majority of egg clutches that were found around the edges of breeding boxes).

Optical and electron imaging methodology follows Novák [46].

Optical imaging The fixed specimen were cleared by simple brush and then placed in Digital Ultrasonic Cleaner PS-06A. The detached heads were afterwards boiled in 10% potassium hydroxide (KOH) for clearer visibility of the delicate parts. Habitus was photographed while the specimen was submerged in ethanol; heads were photographed while being submerged in glycerol (due to better optical properties and higher stability thanks to higher viscosity of glycerol). Images were taken by a Canon macro photo lens MP-E 65 mm on a Canon 550D body, mounted on an automated macro rail for focus stacking (Cognisys StackShot). Smaller details were photographed using an Olympus BX53 microscope with an Olympus DP73 digital camera. The sets of pictures were consequently stacked into a final image with a high depth of field in Zerene Stacker 1.04 (64-bit) by Zerene Systems LLC.

Electron imaging For a detailed view of the morphology and body structure of the larvae, the samples were examined at the Faculty of Science of Charles University in Prague. The specimens were first dehydrated through a series of increasing alcohol concentrations. The samples were transferred sequentially to 60, 70, 80, 90, and 95% alcohol for ca. 0.5 h each. Dehydrated samples were then dried by a critical point drying method. Dry samples were subsequently attached to an aluminum disk target and coated with gold in Bal-Tec Sputter Coater SCD 050, to ensure conductivity. Electron imaging was performed using a JSM-6380LV (JEOL) scanning electron microscope (SEM) with a high resolution of 3.0 nm (30 kW).

Final figures from both optical and electron imaging were compiled using the GIMP ver. 2.8.16 graphic program; graphs were compiled using R ver. 3.4.1 statistical computing program.

Terminology and measurements Interpretation and terminology of larval and pupal descriptions follow Lawrence and Slipinski [47].The measurements were made by placing specimens under an Olympus SZX16 stereo microscope and measured with cellSens Entry 1.6 program. The following abbreviations are used in the text:

AI	length of antennomere I
AII	length of antennomere II
AIII	length of antennomere III
A1L	length of abdominal segment I
A1W	width of abdominal segment I
HL	head length (without labrum)
HW	head width (at the widest point)
LPI	length of labial palpomere I
LPII	length of labial palpomere II
MPI	length of maxillary palpomere I
MPII	length of maxillary palpomere II
MPIII	length of maxillary palpomere III
N1L	pronotal Length
N1W	pronotal width (at the widest point)
N2L	mesonotal length
N2W	mesonotal width (at the widest point)

Table 2 Development length of *Thanatophilus rugosus* at 20 °C and under a 12:12-h photoperiod

Stage	Egg	Instar I	Instar II	Instar III	Pupae
Mean length of development (in days)	3.397	3.061	5.583	19.777	13.659
Standard deviation (in days)	0.630	0.452	1.241	0.498	0.472
Number of observations	12	15	14	5	4

N3L	metanotal length
N3W	metanotal width (at the widest point)
URI	length of urogomphal segment I
URII	length of urogomphal segment II
URS	length of urogomphal terminal seta
N1L/W	ratio of pronotal length to pronotal width
N2L/W	ratio of mesonotal length to mesonotal width
N3L/W	ratio of metanotal length to metanotal width
HW/HL	ratio of head width to head length
A1L/W	ratio of abdominal segment I length to abdominal segment width

Results

Biology

We observed that breeding pairs did not reproduce in a photoperiod longer than 12:12 h (dark/light phases) such as 8:16 h. Females lay their eggs under the substrate in small clusters (usually around nine eggs per cluster) mainly along the edges and bottom of the breeding box. Before hatching, larvae are visible through the egg membrane, and shortly after hatching, they search for food in the proximity. They have a tendency to stay close to the food source most of the time.

In 20 °C, the development from egg to adult took on average more than 45 days. The mean length of development for each stage is given in Table 2. Species develops through three larval instars, and no variation was observed in this trait. Prolonged pupation and higher mortality were observed in individuals of the third instar if they are disturbed while preparing a pupation chamber. The disturbance was on our part motivated by the need for constant surveillance of the development progression. We solved the issue by limiting the available space for the chamber, which forced the larva to pupate next to the wall of the dish, so we could easily observe them without physically searching through the substrate.

Larvae often took the opportunity to cannibalize smaller or newly molted individuals, although the level of cannibalism was not very high as we limited the number of specimens in each dish and also provided the food ad libitum.

Morphometry

Online Resource 1 provides mean values of important morphological measurements and ratios for all three larval instars. We observed that some body parts of *T. rugosus* are not growing isometrically, but rather allometrically. This relationship is very prominent in the case of the size relationship between urogomphal segments I and II (Figs. 1a, b and 9c–e) and also between palpomeres I and II of labium (Figs. 1m–o and 2a, b). Figure 2a, b shows that one of the segments grows isometrically and the second one does not. This creates a proportional difference between their lengths and could be used as a character for instar identification.

The measurements of other body parts could also be used for instar identification. The full list is provided in Online

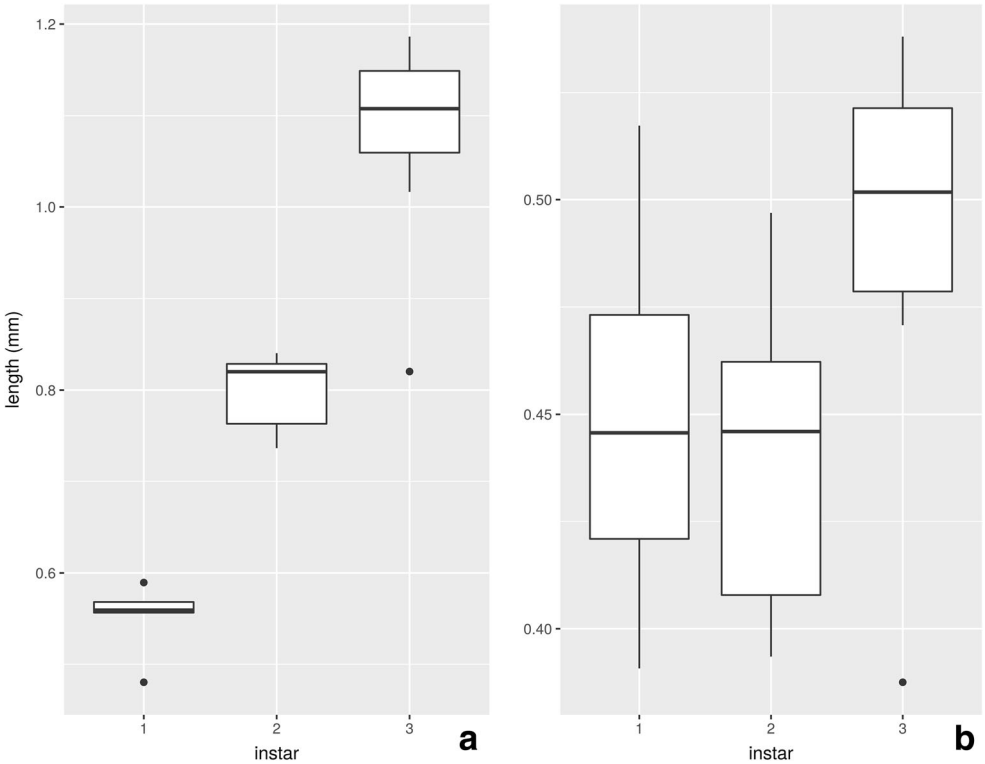

Fig. 1 Boxplots of length of urogomphal segments: segment I (**a**) and segment II (**b**) of all three larval instars of *Thanatophilus rugosus*. Horizontal lines within the boxes indicate median values; upper and lower boxes indicate the 75th and 25th percentiles, respectively; whiskers indicate the values with the 1.5 interquartile ranges; small, black dots are outliers

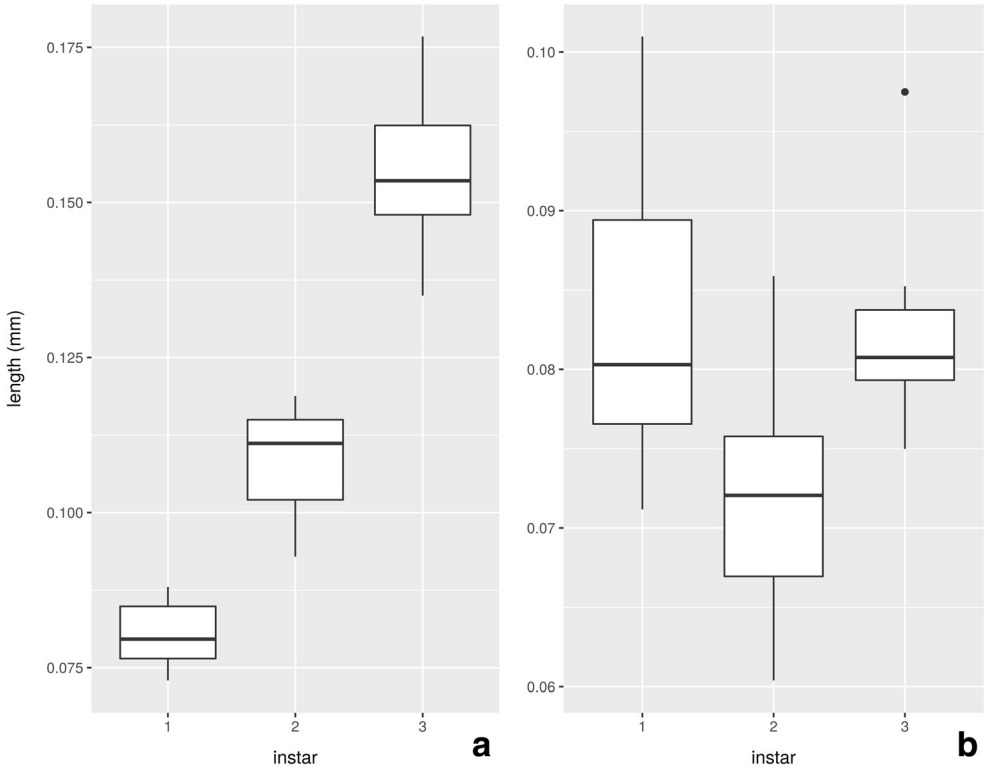

Fig. 2 Boxplots of length of labial palpomers: palpomere I (**a**) and palpomere II (**b**) of all three larval instars of *Thanatophilus rugosus*. Horizontal lines within the boxes indicate median values; upper and lower boxes indicate the 75th and 25th percentiles, respectively; whiskers indicate the values with the 1.5 interquartile ranges; small, black dots are outliers

Resource 1. The two measurements that are widely used for instar identification are head and pronotal width. We suggest to use head width (Fig. 3) for *T. rugosus* instar identification, as the supposed overlap among the instars was not observed in our dataset. For further comments on utilization of measurement-based characters, see the "Discussion" section.

Description of immature stages of *Thanatophilus rugosus*

Family SILPHIDAE Latreille, 1806
Sub-family Silphinae Latreille, 1806
Genus *Thanatophilus* Leach, 1815
Species *Thanatophilus rugosus* (Linnaeus, 1758)

Larvae

Body (Fig. 4 (a–i)): *Instar III* (Fig. 4 (a, d, g)). Mean value of total length: 13.25 ± 1.488 mm. Campodeiform larvae, more or less fusiform, widest at the metathorax and slightly narrowing towards both ends. Slender body only slightly dorsoventrally flattened. Head and all terga strongly sclerotized, and covered by vestiture of scattered setae. All terga on lateral margin with few long setae that can be clearly visible in dorsal or ventral view. Whole dorsal side with dark brown to black coloration. Abdominal paratergites small and pointed posteriorly with longest seta protruding in the direction of their apex. Ventral side of thorax white. Ventral area of segment I poorly sclerotized, white, with dark pigmentation only in the central area and far lateral edges. Segments II to VIII centrally dark with speckled dark lateral areas divided from the medial pigmentation by incomplete lighter stripe. Segment IX ventrally dark with lighter patches laterally. Segment X uniformly brown. Distal segments ventrally overall darker than proximal. *Instar II* (Fig. 4 (b, e, h)). Mean value of total length: 9.22 ± 1.45 mm. Ventral side of thorax white. Segment II ventrally sclerotized with two distinguishable white lines dividing central and lateral dark pigmented areas. Segments III to IX ventrally fully sclerotized and dark with slightly lighter central areas. *Instar I* (Fig. 4 (c, f, i)). Mean value of total length: 5.96 ± 0.898 mm. Ventral side of thorax white. Segment II ventrally sclerotized with two fine white lines dividing central and lateral dark pigmented areas. Segments III to IX ventrally fully sclerotized and dark.

Head capsule (Fig. 5a, d, e): *Instar III* (Figs. 4 (a, d, g) and 5e). Prognathous and protracted; HW 1.953 ± 0.15 mm, HL 1.144 ± 0.044 mm, HW/HL 1.711 ± 0.161; reniform in ventral view; gena short, about one fourth of the width of the head capsule in its longest width in dorsal view; head capsule dorsally covered with few long and many short stout setae. Epicranial stem present, frontal arms V-shaped, but with U-base as the angle changes in the middle of suture (Fig. 5a, d; *fa*), median endocarina absent. Six stemmata on each side of the head separated into two groups; four stemmata forming a trapezoid placed posteriorly behind antennal base, and two stemmata placed ventrally under antennal base, parallel to

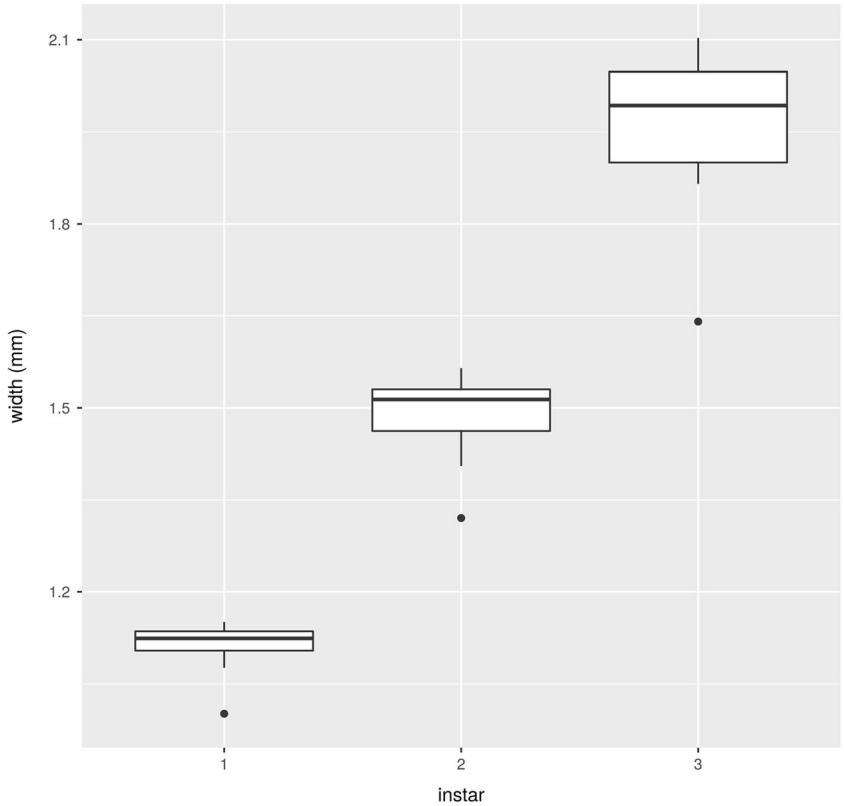

Fig. 3 Boxplots of head width of all larval instars of *Thanatophilus rugosus*. Horizontal lines within the boxes indicate median values; upper and lower boxes indicate the 75th and 25th percentiles, respectively; whiskers indicate the values with the 1.5 interquartile ranges; small, black dots are outliers

the wider base of the trapezoid (Fig. 6c). Frontoclypeal suture absent; consisting only of linear tentorial pits, parallel to the posterior edge of clypeus (Fig. 5a; *ftp*). Clypeus rectangular, ca. four times as wide as long, partially covering mandibles in dorsal view; dorsally with six stout setae placed lengthwise anteriorly and many short thin setae. Labrum (Fig. 5b) subtrapezoidal, dorsally with 8 long stout setae aimed anteriorly. Labral apex (Fig. 8e) double-arched, bearing two very short setae on the anterior edge. Epipharynx (Fig. 5c) anteriorly covered with rows of bulbous processes and a pair of two large bulbous sensoria anteromedially (Figs. 5c and 8e; *bs*) and a pair of differently shaped sensoria placed laterally. Pharynx covered with rows of setae and spines which project up to posterior edge of clypeus, with oblique transverse cibarial plates (Fig. 5c; *cp*) in labral-clypeal membrane area and a pair of sensoria placed posteromedially behind these plates. Ventral mouthparts retracted, forming a maxillo-labial complex (Fig. 8a). Hypostomal rods absent. Ventral epicranial ridges roughly reaching beyond the level of the posterior edge of the maxillo-labial complex. Gular region short with gular sutures converging anteriorly. Tentorium (Fig. 6) consisting of a pair of sclerotized anterior arms, hyaline dorsal arms and sclerotized posterior arms connected with posterior tentorial bridge. A pair of short sclerotized arms connected with filamentous secondary bridge growing dorsally from the middle of posterior arms. *Instar II* (Fig. 4 (b, e, h)). HW 1.484 ± 0.082 mm; HL 1.073 ± 0.082 mm; HW/HL 1.391 ± 0.145; length of gena about one third of the width of the head capsule in its longest width in dorsal view. Head capsule dorsally covered with long and several short stout setae. Epicranial suture and epicranial stem of light coloration. *Instar I* (Figs. 4 (c, f, i) and 5a, d). HW 1.108 ± 0.049 mm; HL 0.836 ± 0.103 mm; HW/HL 1.34 ± 0.139 times wider than long; length of gena about half of the width of the head capsule in its longest width in dorsal view.

Antennae (Fig. 7a–c): *Instar III.* Trimerous, inserted on lateral distal margin of gena; inserted in membranous socket. All antennomeres fully sclerotized and of similar length (AI 0.403 ± 0.034 mm, AII 0.414 ± 0.04 mm, AIII 0.353 ± 0.039 mm). Antennomere I cylindrical, slightly wider on distal end, sloping laterally towards the longitudinal axis of the larva, bearing no setae. Antennomere II club shaped, wider on distal end, sloping laterally towards the longitudinal axis of the larva bearing several stout setae unequally and scarcely scattered across the surface. Sensorium of antennomere II (Fig. 7c) placed on inner lateral area of its distal end together with three small but bulky sensilla lacking a socket, the longest one and the shortest one growing from the same base. Sensorium egg-shaped, widest at the base, encircled by a sclerotized ring, closely annealing to the second antennomere. Antennomere III placed on outer lateral area of antennomere II, bearing several stout setae mainly on its distal half (Fig. 7b). *Instar II.* All antennomeres fully sclerotized and of similar length (AI 0.294 ± 0.030 mm, AII 0.373 ± 0.022 mm, AIII

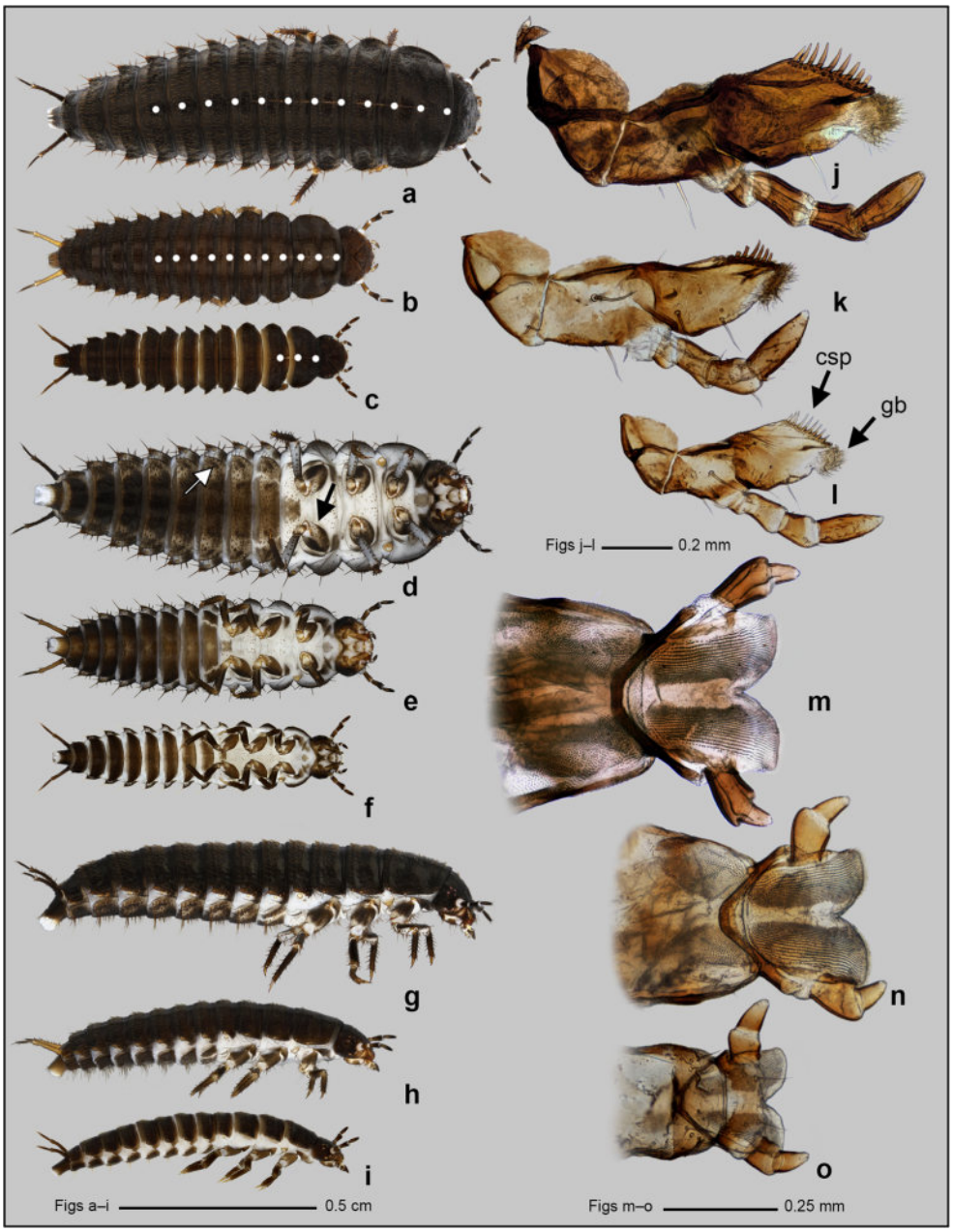

Fig. 4 *Thanatophilus rugosus*: dorsal habitus of third instar (a); second instar (b) and first instar (c) larva (sagittal line marked by white dots). Ventral habitus of third instar (d) (characteristic spots on abdominal ventrites (white arrow) and white inner side of coxa (black arrow)); second instar (e) and first instar (f) larva. Lateral view of third instar (g); second instar (h) and first instar (i) larva. Left maxilla of third instar (j); second instar (k) and first instar (l) larva. Labium of third instar (m); second instar (n) and first instar (o) larva. Abbreviations: csp—cuticular spines on lacinia; gb—brush of setae on galea

0.351 ± 0.015 mm). *Instar I.* All antennomeres fully sclerotized, antennomere I shortest (AI 0.213 ± 0.009 mm), antennomere II and III of similar length (AII 0.287 ± 0.025 mm, AIII 0.313 ± 0.016 mm).

Maxilla (Figs. 4 (j–l) and 8a): *Instar III* (Fig. 4j). Consisting of five parts (cardo, stipes, palpus, lacinia, and galea), attached to labium forming a maxillo-labial complex. Maxillary articulating areas present, completely unsclerotized. Lacinia and galea partly fused together. Cardo transverse, sub-triangular, ca. two times wider than long, with one short seta ventrolaterally close to the base of stipes. Stipes sub-rectangular, longer than wide, ventrally bearing one long stout seta in the center, one long stout seta outer-laterally and several short setae. Galea fixed, bearing two long setae outer-laterally, with a brush of very dense setation on its apex (Figs. 4 (l) and 8d; *gb*). Lacinia fixed, bearing 8 to 10 visible stout spines on its outer lateral margin (Fig. 4 (l); *csp*) together with an apical lobe bearing a short cuticular projection composed of several shorter spines grown together. Palpifer very short, sclerotized mainly on outer lateral margin. Maxillary palpus trimerous, palpomere I (Fig. 8a; *mpI*) cylindrical (MPI 0.201 ± 0.026 mm), ca. two times longer than wide; palpomere II (Fig. 8a; *mpII*) cylindrical (MPII 0.209 ± 0.011 mm), sloping laterally towards the longitudinal axis of the larva, palpomere III (Fig. 8a; *mpIII*) conical (MPIII 0.32 ± 0.033 mm), longest of the palpomeres. Palpifer and palpomeres sparsely covered by setae, palpomere III

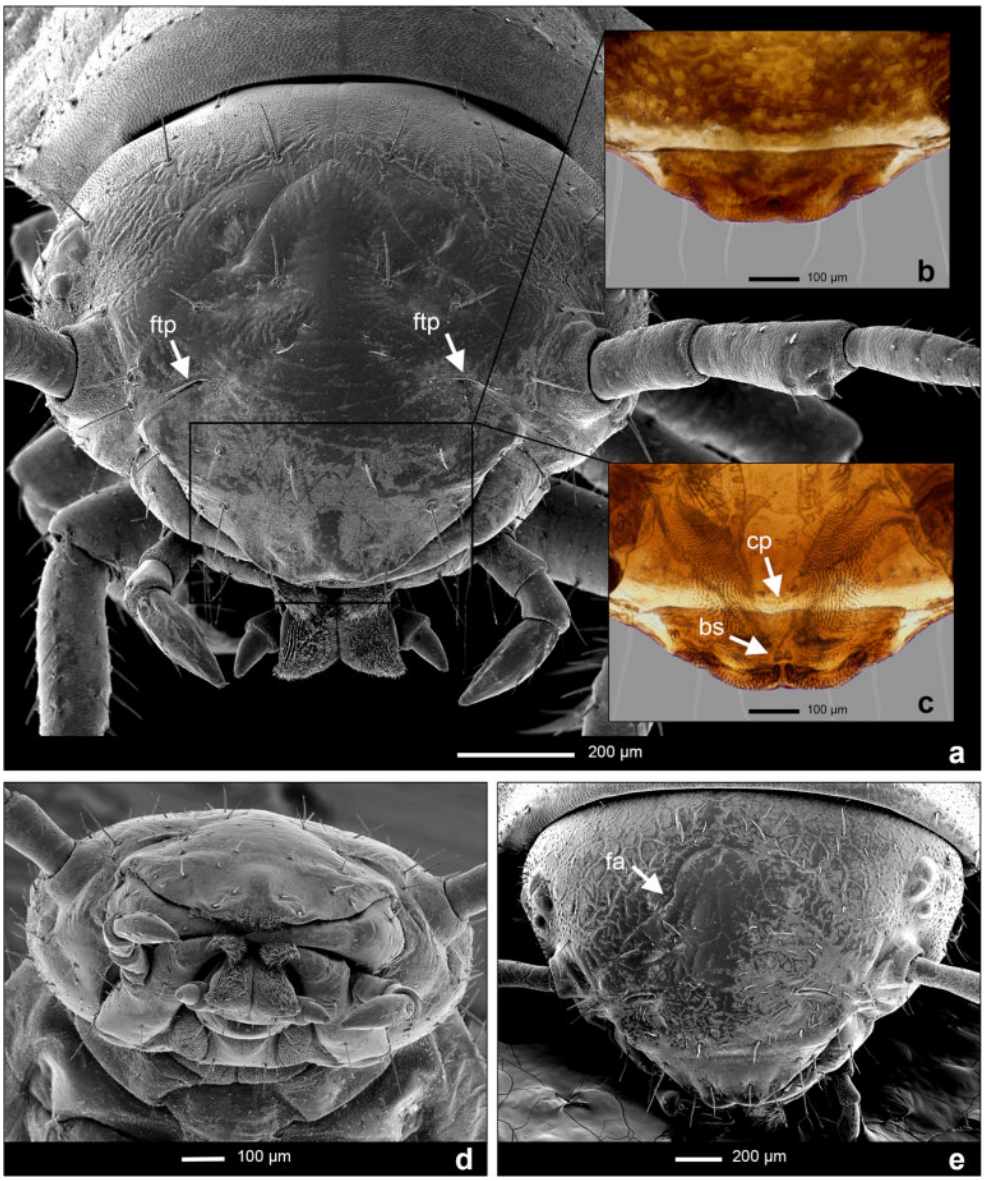

Fig. 5 *Thanatophilus rugosus*: head of first instar larva in dorsal view (**a**); detail of labrum of third instar larva in dorsal (**b**) and ventral (**c**) view. Head of first instar larva in frontal view (**d**); head of third instar larva in dorsal view (**e**). Abbreviations: bs—bulbous sensorium on epipharynx; cp—cibarial plates on pharynx; fa—frontal arm; ftp—frontal tentorial pit

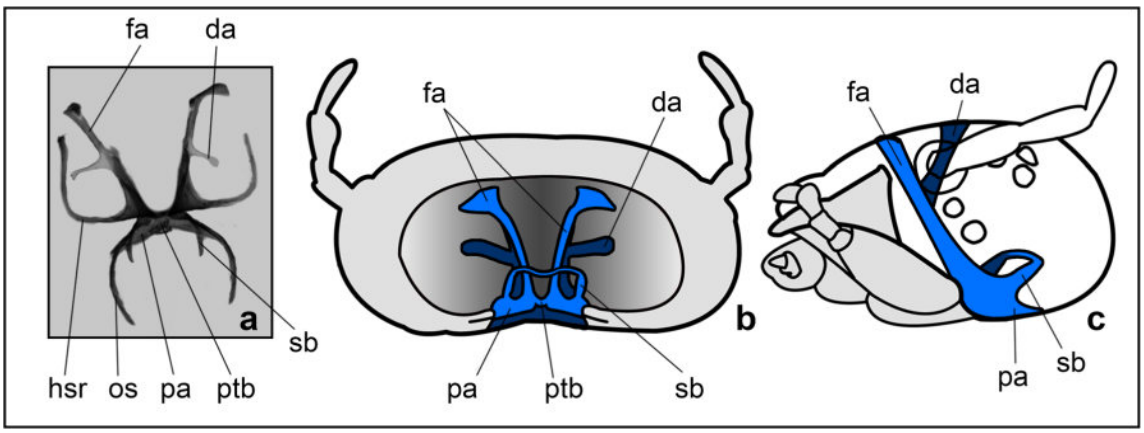

Fig. 6 *Thanatophilus rugosus*: tentorium in dorsal (**a**); posterior (**b**) and lateral (**c**) view. Abbreviations: da—dorsal arm; fa—frontal arm; hsr—hypostomal ridge; os—occipital suture; pa—posterior arm; ptb—posterior bridge; sb—short sclerotized arms connected with filamentous secondary bridge growing dorsally from the middle of posterior arms

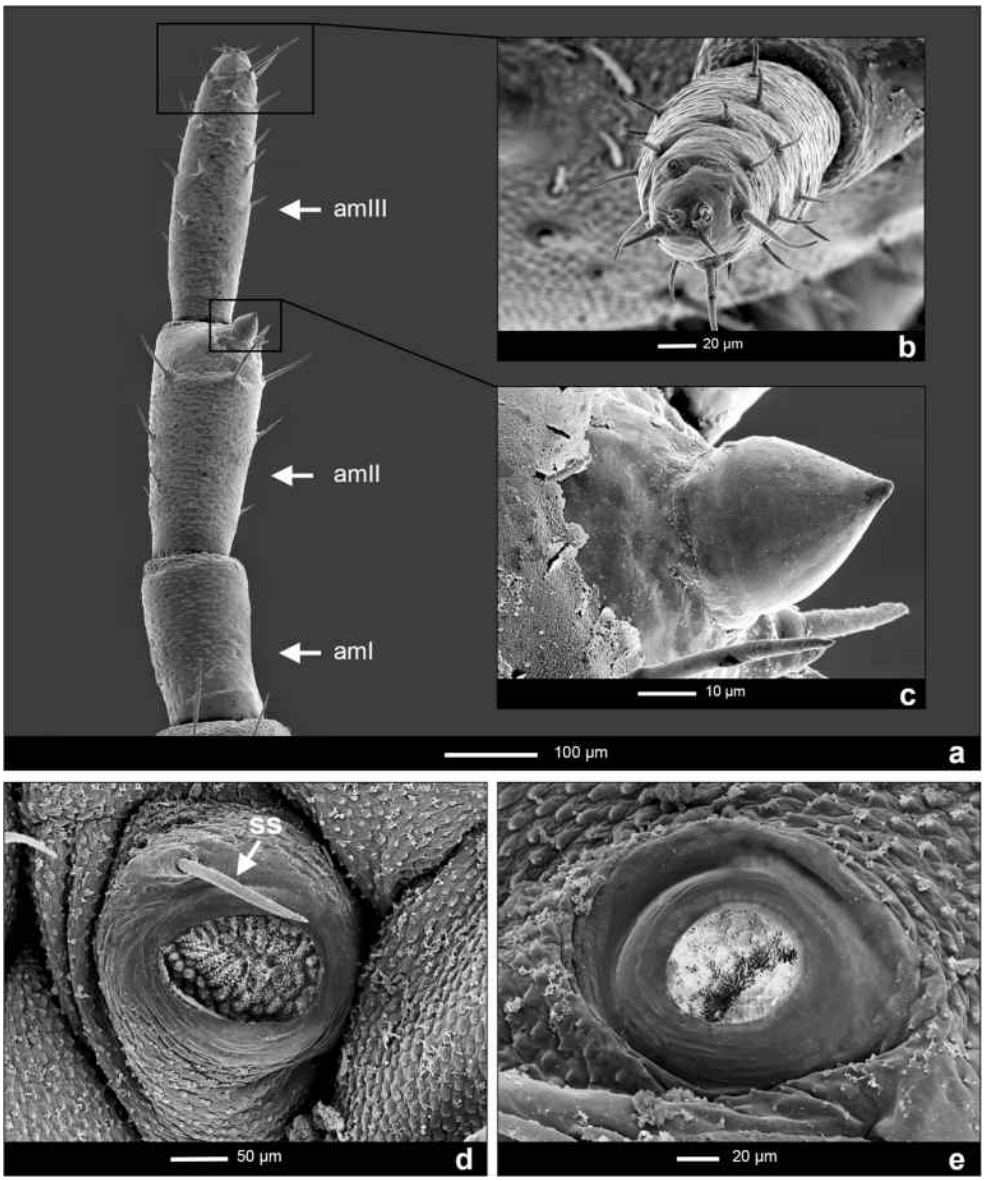

Fig. 7 *Thanatophilus rugosus*: left antenna of first instar larva in dorsal view (**a**); detail of third antennomere in frontal view (**b**); detail of antennal sensorium (**c**). Metathoracic (**d**) and abdominal (**e**) spiracle. Abbreviations: amI—antennomere I; amII—antennomere II; amIII—antennomere III; ss—seta on metathoracic spiracle

having a short stout seta in an articulated protuberance placed on outer-lateral edge of its base. The apex of palpomere III covered by short blunt peg-like sensilla (Fig. 8b). *Instar II* (Fig. 4 (k)). Palpomere lengths: MPI 0.146 ± 0.009 mm; MPII 0.133 ± 0.012 mm; MPIII 0.226 ± 0.021 mm. *Instar I* (Fig. 4 (l) and 8a). Palpomere lengths: 0.105 ± 0.01 mm; MPII 0.1 ± 0.011 mm; MPIII 0.181 ± 0.024 mm.

Labium (Figs. 4 (m–o) and 8a): *Instar III* (Fig. 4 (m)). Formed by prementum, mentum and submentum, all sclerotized on their basal areas (Fig. 8a; *pm*, *m*, *sm*). Ligula bi-lobed (Fig. 8a, d; *lig*); each lobe along the sagittal plane covered dorsally by a group of numerous longitudinal lines of fine short setation and dense bulbous projections apically and centrally between the two groups; ventrally, a pair of long setae is present in the lateral parts of the central area of prementum, as well as a pair of shorter setae in the basal half. Labial palpus bimerous (LPI 0.156 ± 0.014 mm, LPII 0.083 ± 0.007 mm), with no setation; basal palpomere (Fig. 8a; *lpI*) club-shaped, sloping laterally towards the longitudinal axis of the larva, distal palpomere (Fig. 8a; *lpII*) conical, blunt, ca. one third of the length of basal palpomere, bearing a group of short blunt peg-like sensilla on its apex (Fig. 8c). Mentum longer than wide, sub-oval, with dark pigmentation on its base; ventrally bearing two pairs of long setae on its posterior half. Submentum bearing a pair of long stout and several shorter thin setae, paired or irregularly scattered posterolaterally, alongside its sclerotized distal half when viewed ventrally. *Instar II* (Fig. 4 (n)). Labial palpi lengths: LPI 0.109 ± 0.009 mm, LPII 0.072 ± 0.008 mm. *Instar I* (Fig. 4 (o)). Labial palpi lengths: LPI 0.080 ± 0.006 mm, LPII 0.083 ± 0.01 mm.

Mandibles (Fig. 9a, b, f–i): *Instar III* (Fig. 9a, b). Symmetrical, simple without mola or prostheca, basal half

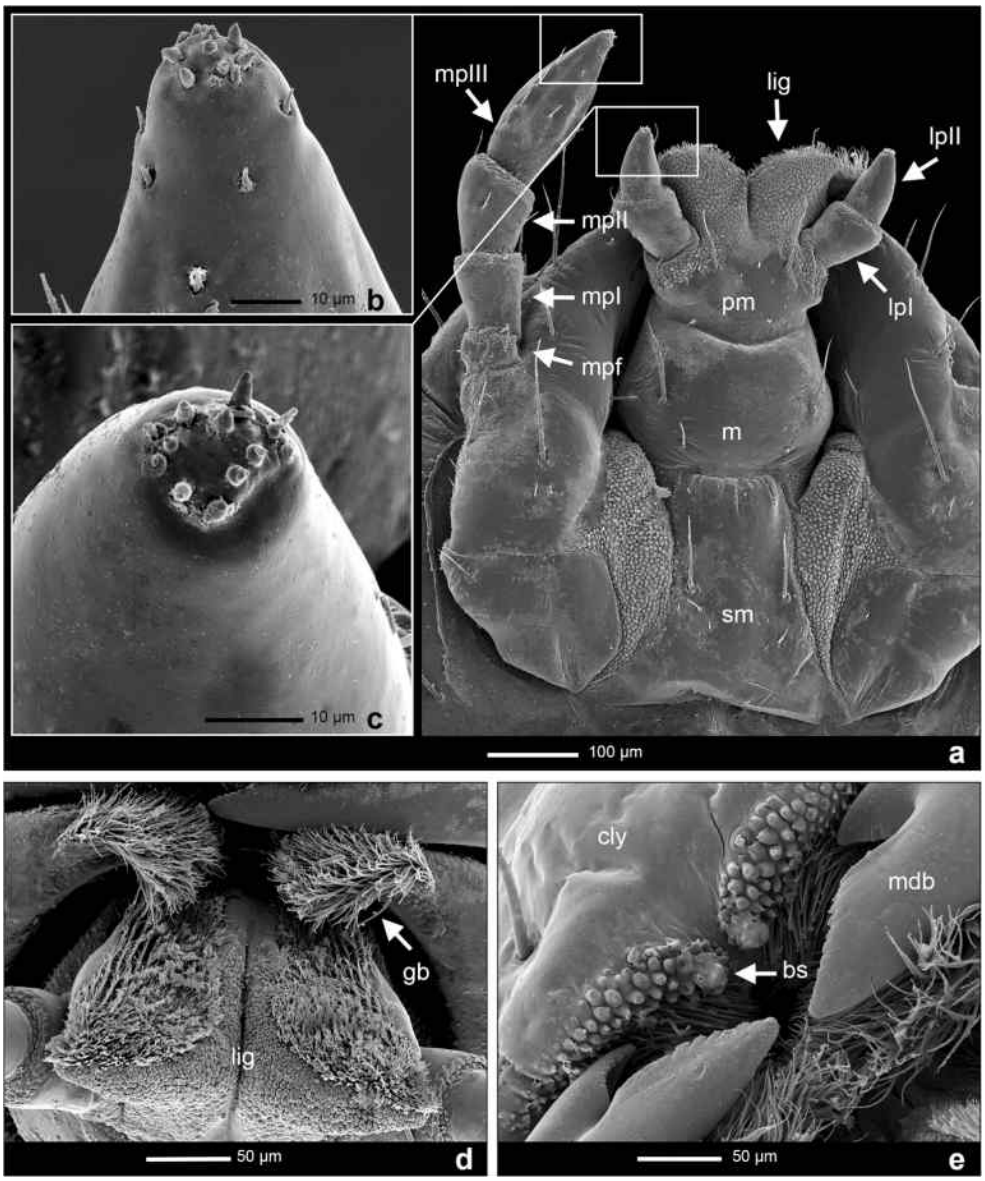

Fig. 8 *Thanatophilus rugosus*: maxillo-labial complex of first instar larva in ventral view (**a**); detail of apices of labial palpomere II (**b**) and maxillary palpomere III (**c**). Detail of ligula (**d**) and labrum (**e**) in frontal view. Abbreviations: bs—bulbous sensorium on epipharynx; gb—brush of setae on galea; lig—ligula; lpI—labial palpomere I; lpII—labial palpomere II; m—mentum; mdb—mandible; mpI—maxillary palpomere I; mpII—maxillary palpomere II; mpIII—maxillary palpomere III; mpf—maxillary palpifer; pm—prementum; sm—submentum

consisting of wide triangular base in dorsoventral cross section, distal half more dorsoventrally flattened, apex consisting of two scissorial teeth lying obliquely, perpendicular to the plane of movement of the mandible, apical tooth (Fig. 9g; *at*) longer and serrated laterointernally, sub-apical tooth (Fig. 9g–i; *st*) shorter, positioned dorsally towards the outer tooth and serrated lateroexternally towards the serrated area of the outer tooth. One long stout seta present laterodorsally on mandibular base (Fig. 5d) and one short stout seta present outerlaterally in the mid-length of the mandible (Fig. 9f; *ms*). Left mandible larger, covering the apex of the right mandible when clenched (Fig. 5d). *Instar II* (Fig. 9f, g). Same as Instar III. *Instar I*. Same as Instar III.

Thorax (Fig. 4 (a–i)): *Instar III* (Fig. 4 (a, d, g)). Three-segmented, thoracic tergites divided by sagittal line; paraterga slightly overlapping the body forming irregular semicircles. Pronotum (N1W 3.054 ± 0.377 mm, N1L 1.186 ± 0.154 mm) sub-oval, wider posteriorly, rounded at posterolateral corners. Mesonotum (N2W 3.413 ± 0.511 mm; N2L 0.698 ± 0.106 mm) and metanotum (N3W 3.515 ± 0.459 mm; N3L 0.631 ± 0.068 mm) sub-oval, similar in shape and size. Ventrolateral areas of prothorax, mesothorax, and metathorax formed by sclerotized episternum and epimeron; spiracular sclerite of mesothorax mostly membranous (except for inner anterior edge), bearing a large (relative to abdominal spiracles) annular spiracle (Figs. 4 (d, g) and 7d) with yellow-colored peritreme and bearing one long stout seta on its inner lateral margin. The atrium (inner chamber) padded with shrub-like filtration hairs. Presternum short, semi-lens shaped, wider than long, subdivided into three plates; lateral ones well sub-triangular, well sclerotized; medial plate sub-rectangular, semi-sclerotized, reaching edges of the presternum both anteriorly and

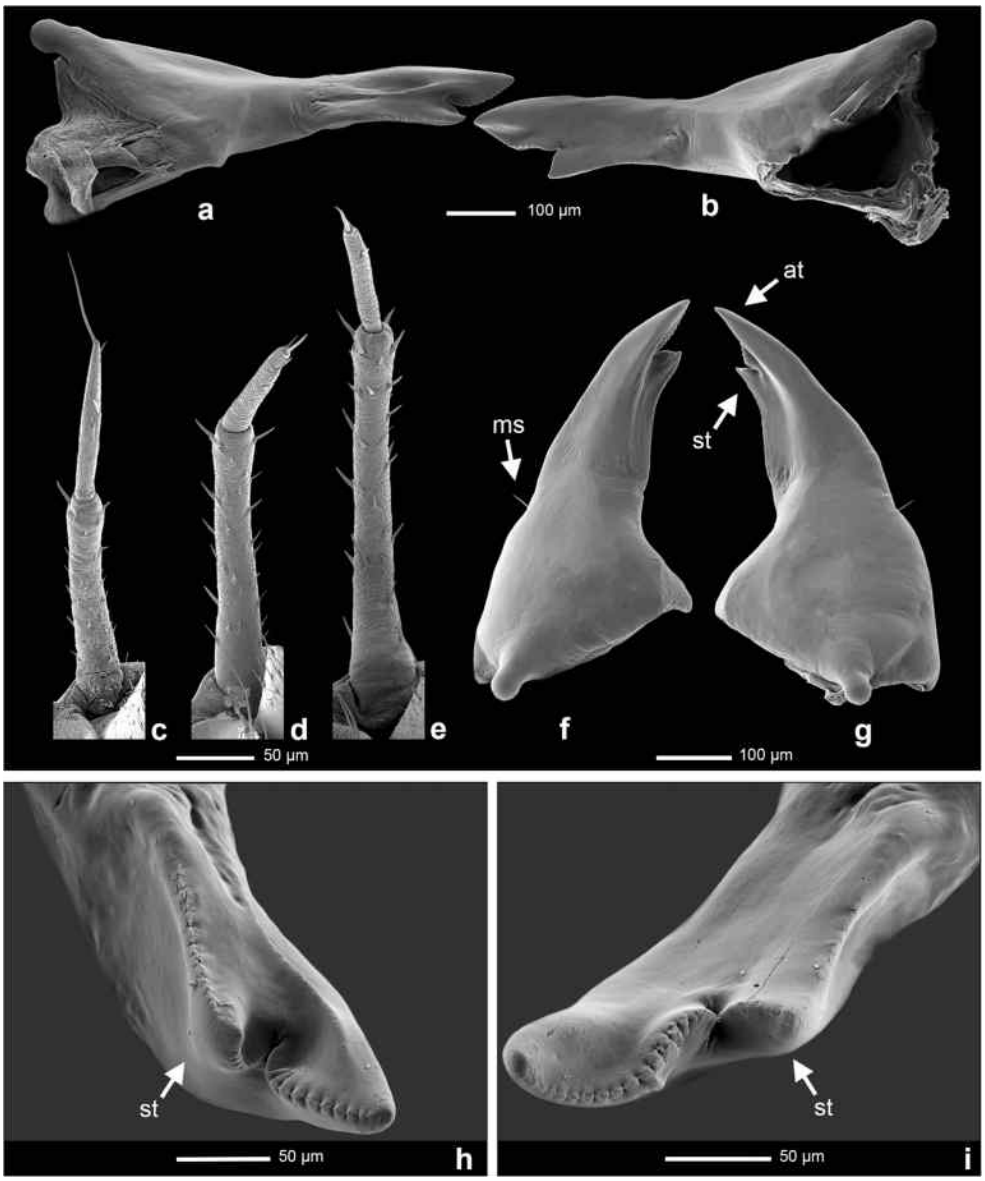

Fig. 9 *Thanatophilus rugosus*: left (**a**) and right (**b**) mandible of third instar larva in posterior view. Urogomphi of first (**c**), second (**d**), and third (**e**) instar larva. Left (**f**) and right (**g**) mandible of second instar larva in dorsal view. Detail of apices of left (**h**) and right (**i**) mandible of third instar larva in frontal-ventral view. Abbreviations: at—apical tooth; ms—seta present outer-laterally in the mid-length of the mandible; st—sub-apical tooth

posteriorly. Mesosternum and metasternum sub-divided by transverse fold into membranous basisternum and sternellum. *Instar II* (Fig. 4 (b, e, h)). Pronotum sub-oval (N1W 2.038 ± 0.193 mm; N1L 0.946 ± 0.089 mm), wider posteriorly, rounded at posterolateral corners. Mesonotum (N2W 2.26 ± 0.231 mm; N2L 0.474 ± 0.029 mm) and metanotum (N3W 2.363 ± 0.321 mm; N3L 0.398 ± 0.062 mm) sub-oval, similar in shape and size. Presternum medial plate sub-rectangular, poorly sclerotized, reaching only posterior edge of the presternum. *Instar I* (Fig. 4 (c, f, i)). Pronotum (N1W 1.476 ± 0.064 mm; N1L 0.622 ± 0.08 mm) semicircular, wider posteriorly, rounded at posterolateral corners. Mesonotum (N2W 1.595 ± 0.077 mm; N2L 0.303 ± 0.024 mm) and metanotum sub-oval, similar in shape and size. Metanotum (N3W 1.655 ± 0.094 mm; N3L 0.261 ± 0.015 mm). Presternal medial plate sub-circular, not reaching posterior nor anterior edge of the presternum.

Abdomen (Fig. 4 (a–i)): *Instar III* (Fig. 4 (a, d, g)). Ten-segmented, tapering posteriad, segments I to IV dorsally sub-divided by fine sagittal line anteriorly (Fig. 4 (a)), on segment IV barely visible. Tergites of segments I to VIII sub-rectangular, narrow, A1W 3.384 ± 0.473 mm; A1L 0.454 ± 0.072 mm, similar in shape and coloration, with posteriorly pointed paratergites. Tergite of segment IX sub-rectangular, bearing paired, well-developed two-segment urogomphi (Fig. 9e) that are inserted dorsolaterally. Basal segment of urogomphi narrow (URI 1.078 ± 0.118 mm), wider on proximal and distal ends, slightly bent posteromedially, bearing short stout setae; distal segment slender (URII 0.49 ± 0.051 mm), cylindrical, with one seta inserted on the apex (URS 0.117 ± 0.012 mm) and two setae inserted slightly below the apex; first dorsally and second inner-ventrolaterally. Segment X dorsally sub-trapezoidal, forming a well-sclerotized cylinder; distal central

half of dorsal area with two longitudinal lines of white pigmentation; segment X holding the hold-fast organ (pygopod) with several eversible processes. Ventrites of segments I to VIII sub-trapezoidal; ventrite of segment IX sub-rectangular. Spiracles (Figs. 4 (d) and 7e) annular, with yellow-colored peritreme and bearing no setae. Spiracle on segment I is the largest of abdominal spiracles. *Instar II* (Fig. 4 (b, e, h)). Tergites of segments I to VIII sub-rectangular, narrow, A1W 2.351 ± 0.293 mm; A1L 0.279 ± 0.043 mm, similar in shape and coloration. Basal segment of urogomphi (Fig. 9d) narrow (URI 0.8 ± 0.040 mm), distal segment slender (URII 0.442 ± 0.039 mm), cylindrical, ca. half as long as basal segment, with one seta inserted on the apex (URS 0.131 ± 0.2 mm). *Instar I* (Fig. 4 (c, f, i)). Tergites of segments I to VIII sub-rectangular, A1W 1.568 ± 0.108 mm, A1L 0.184 ± 0.015 mm, basal segment of urogomphi (Fig. 9c) narrow, URI 0.554 ± 0.035 mm, distal segment slightly slender and cylindrical, but almost the same length URII 0.449 ± 0.043 mm, with long terminal seta (URS 0.207 ± 0.066 mm).

Legs (Figs. 4 (d–f) and 10a–d): *Instar III* (Figs. 4 (a) and 10a). Pentamerous including pretarsus, all pairs similar in shape and size. Coxa large, stout, covered by stout setae; with white pigmentation on the posterior and anterior area of the apex; coxal-trochanteal membrane reaching ca. one third of longitudinal length. Trochanter small, sub-triangular in lateral view, centrally white pigmented and sclerotized only basally and distally, covered by several stout setae of the same length as coxa and one seta ca. three to four times longer than the rest, placed ventrally on the distal end. Femur cylindrical, dorsally sclerotized. Ventrally completely white, bearing two longitudinal lines of sharp stout setae (number of setae in these lines vary from 5 to 10 and no systematic difference was revealed)

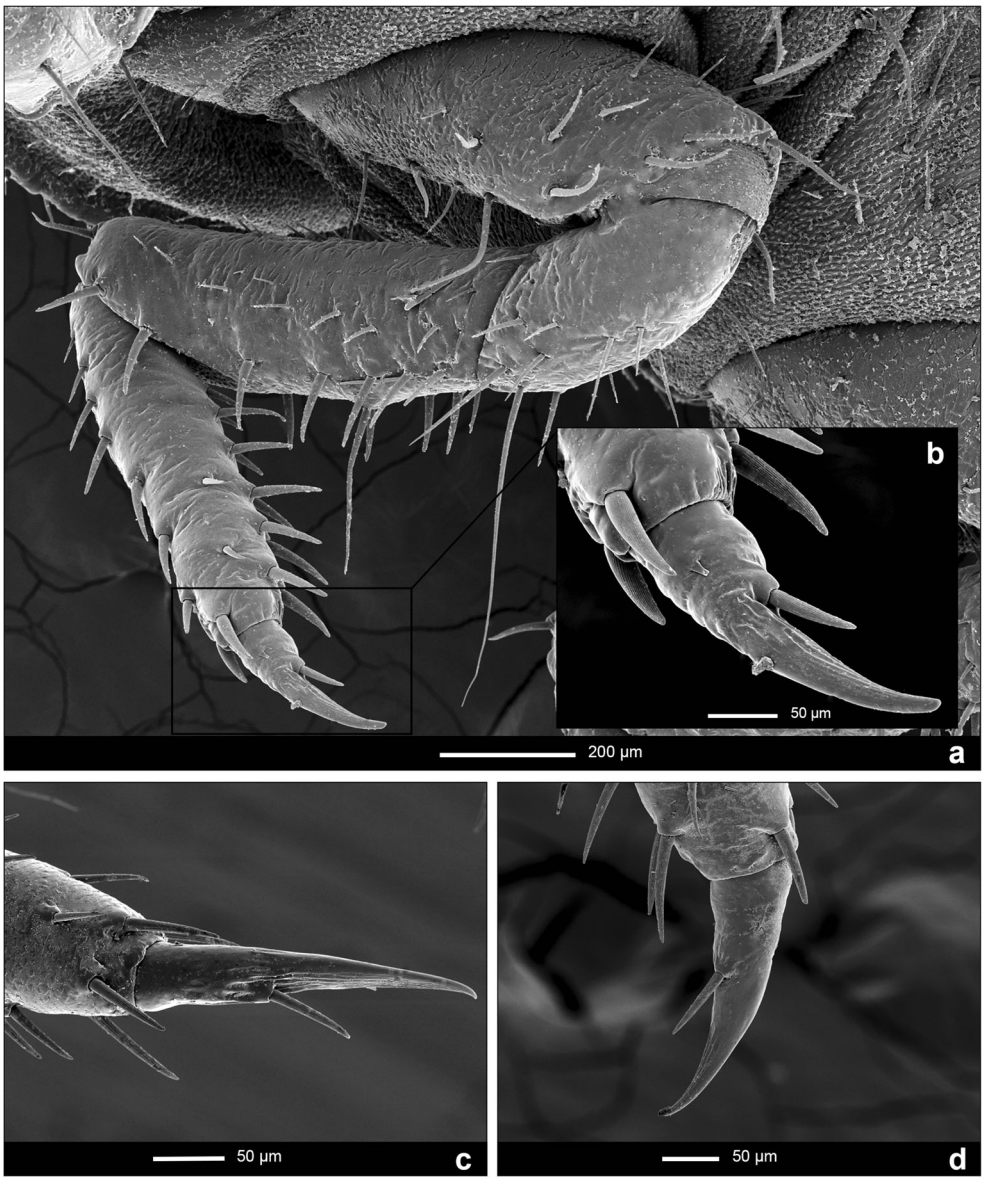

Fig. 10 *Thanatophilus rugosus*: leg of third instar larva in lateral view (**a**). Detail of tarsal claw of third instar (**b**); first instar (**c**) and second instar (**d**) larva

and a very long seta (ca. two times the length of neighboring setae) between these lines; several other irregular longitudinal lines with shorter setation placed laterally and dorsally. Tibiotarsus ca. as long as femur, narrower, tapering towards distal end, bearing several longitudinal lines of stout sharp setae around its circumference followed by less regular lines of shorter setae. Pretarsus (Fig. 10b) composed of a claw with bulky base, ventrally bearing one stout seta of ca. one third of the length of pretarsus, placed in the mid-length of the claw. Common setae on coxa and trochanter generally thinner and slightly longer than stout strong setae on femur and tibiotarsus. *Instar II* (Fig. 4 (e)). Trochanter small, sub-triangular in lateral view, centrally white pigmented and darker only proximal and distal ends. Femur cylindrical, fully sclerotized and dark pigmented, bearing two longitudinal lines of sharp stout setae ventrally with one ca. two to three times longer seta centrally between the two lines. Claw (Fig. 10d) appears to be more slender than in instar III. *Instar I* (Fig. 4 (f)). Trochanter small, sub-triangular in lateral view, dark pigmented with weak lighter patch on the central area. Claw (Fig. 10c) more slender than in later instars, with narrow base.

Pupa (Fig. 11)

Type of pupa: *adectica exarata libera*. Curved, ventrally concave. Length 9.3 mm. Coloration: cream white body with dark-brown setae.

Head capsule: partially covered by pronotum in dorsal view. Antennae short, extending laterally, without reaching posterolateral corners of pronotum. Mouthparts visible in ventral view.

Thorax: surface of pronotum covered by numerous short brown hairs, with two pairs of long stout dark-brown setae on its anterolateral edge (Fig. 11b; *ps*). Pronotum similar in shape to that of adult by wavy cutting of its posterior margin, but less convex anteriorly. Mesonotum shorter but wider than metanotum, with distinct triangular protuberance posteromedially representing future scutellum of adult. Wing pads and rectangular elytra completely white and about the same length; wing pads reaching fourth abdominal segment. Prothoracic and mesothoracic legs free, visible in ventral view; tibiae of metathoracic legs partially covered by wing pads, distal segments of tarsi extend to seventh abdominal segment. Spiracles present on pleural areas of mesothorax.

Abdomen: abdominal segments sub-rectangular, wider than long. Segments II–VII bearing pairs of long stout dark-brown setae (Fig. 11a; *as*). Urogomphi on segment VIII short and bulky, white, with dark-brown apices bearing medium-long stout dark-brown setae (Fig. 11b, c; *us*). Spiracles present on abdominal pleural areas of segments I–VIII, on segments I–IV light-brown, otherwise white.

Differential diagnose of larval instars

Instar I. Body length 5.96 ± 0.868 mm, head width 1.108 ± 0.49 mm. Basal segment of urogomphi almost as long as the second one and terminal seta half of the length of the second

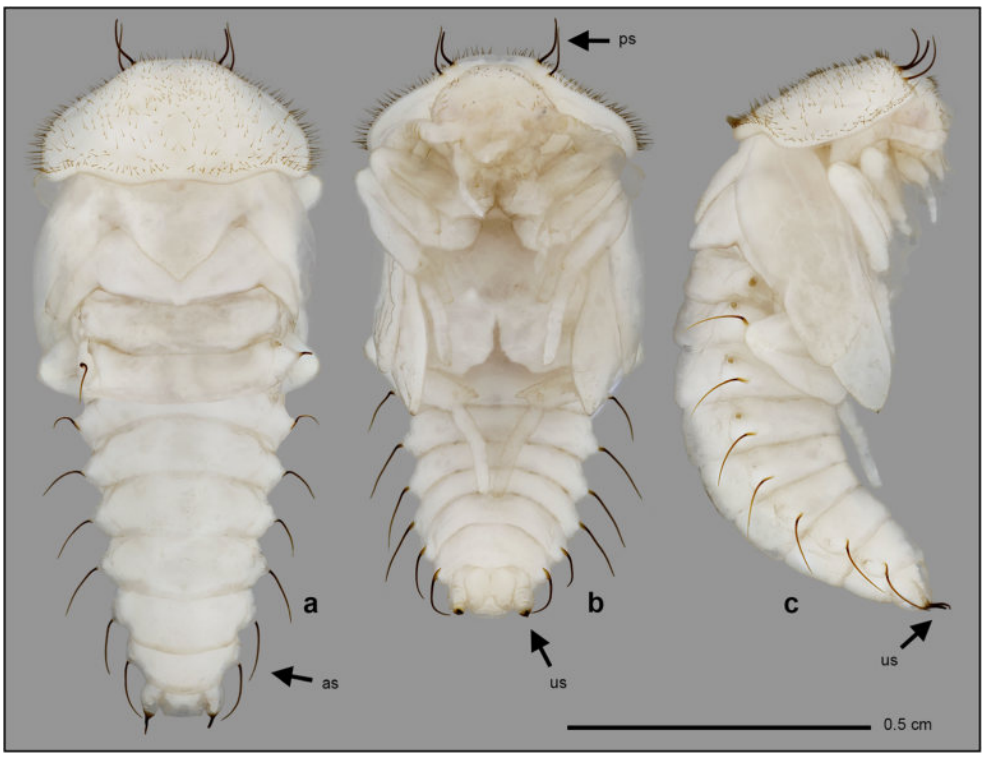

Fig. 11 *Thanatophilus rugosus*: pupa in dorsal (**a**), ventral (**b**) and lateral (**c**) view. Abbreviations: as—abdominal setae; ps—pronotal setae; us—urogomphal setae

segment (Fig. 9c). Abdominal ventrites 2–9 uniformly brown (Fig. 4 (f)). Sagittal line terminated on metathorax (Fig. 4 (c)). Inner side of all coxa brown (Fig. 4 (f)).

Instar II. Body length 9.22 ± 1.450 mm, head width 1.484 ± 0.082 mm. Basal segment of urogomphi approximately twice the length of the second segment and terminal seta less than one third of the length of the second segment (Fig. 9d). Abdominal ventrites 2–9 uniformly brown with slight discoloration in the middle (Fig. 4 (e)). Sagittal line terminated on the abdominal segment III or IV (Fig. 4 (b)). Inner side of all coxa brown (Fig. 8).

Instar III. Body length 13.25 ± 1.488 mm, head width 1.953 ± 0.15 mm. Basal segment of urogomphi approximately twice the length of the second segment and terminal seta less than one third of the length of the second segment (Fig. 9e). Abdominal ventrites 2–8 light brown with dark brown spots on the lateral edges and darker more uniform line in the middle (Fig. 4 (d)). Sagittal line terminated on abdominal segment V or VI (Fig. 4 (a)). Inner side of all coxa white (Fig. 4 (d)).

Discussion

Previous descriptions of developmental stages of *T. rugosus* by Xambeu [38] and von Lengerken [33] were rather brief (not including some important morphological features like labium, maxillae, nor tentorium), and most of the characters are only mentioned in the text form without accessory images. Von Lengerken [33] attempted to include a description of differences among larval instars of all three species of the genus *Thanatophilus* (*T. dispar*, *T. rugosus*, and *T. sinuatus*) and offered several size-based characteristics. Nevertheless, he acknowledged that these values are highly variable and may not be reliable. According to our findings, some species characteristics like overall body shape or size of protergites recognized by von Lengerken [33] are highly variable among specimens and thus of limited use. Von Lengerken's description also did not mention differences in coloration between *T. rugosus* and *T. sinuatus* as their third instars can be very easily distinguished by white markings along the margins of the body of the latter.

Head width and other size-based characteristics with accompanying statistical models are often suggested as a means to easily identify larval instars of necrophagous beetles [17–21]. This approach is very popular thanks to its accessibility, but the accuracy of the results is doubtful [33]. Although we did not find an overlap among head widths of all three examined larval instars, we agree with the idea that geographical region, temperature, quality, and abundance of food and other variables can have a profound effect on larval size [22, 33]. Qualitative characters like proportions of body parts, cheatotaxy, coloration, and other traits not affected by the size of the individual seem to be more reliable, and their utilization minimizes the probability of error.

One of the reasons of quantitative characters for instar identification being developed for forensically important beetle species is the belief that majority of their larvae lack qualitative identifying characters [19]. Kilian and Madra [48] challenged this idea by finding several qualitative characters for instar identification of *Sciodrepoides watsoni* (Spence, 1813) (Coleoptera: Leiodidae: Cholevinae). Our results also contradict the idea and we reckon that future morphological re-descriptions of larvae will prove us right. We found several uncommon characters that could be used for instar determination such as differences in appearance of claws, length of sagittal line, or relative position of presternal medial plate.

The difference in appearance of claws is very slight, nonetheless possibly applicable to other species as well. We believe it is worth mentioning additional to other more obvious differences. The length of the sagittal line seems to be closely related to individual development and can be observed even on the larval exuvia. Our unpublished data suggest that this character could be applicable also to other species of the genus *Thanatophilus*.

One of the less obvious and more challenging characters to use for instar identification could be the relative position of the presternal medial plate, which differs among instars. In the first instar, it does not reach up to the anterior or posterior edge while in other two instars it reaches either the posterior edge (Instar II) or both edges (Instar III). This character is rather crude as the presternal median plate is flexible and may not be fully visible in some individuals, thus we did not include it into the differential diagnose of larval instars. Nonetheless, it is worth mentioning.

Our article provides detailed morphological re-description of all larval instars of *T. rugosus*. This will allow identification of the species and all its instars regardless of their size or stage of development. The results can be further used in basic and applied fields of science such as developmental biology and forensic entomology.

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Compliance with ethical standards

Conflict of interest H. Šuláková is an employee of the Faculty of Environmental Sciences and Police of the Czech Republic.

Ethical approval All applicable international, national, and/or institutional guidelines for the care and use of animals were followed.

References

1. Midgley JM, Richards CS, Villet MH (2010) The utility of Coleoptera in forensic investigations. In: Amendt J, Goff ML, Campobasso CP, Grassberger M (eds) Curr. Concepts Forensic Entomol. Springer Netherlands, Dordrecht, pp 57–68
2. Midgley JM, Villet MH (2009) Development of *Thanatophilus micans* (Fabricius 1794) (Coleoptera: Silphidae) at constant temperatures. Int J Legal Med 123(4):285–292. https://doi.org/10.1007/s00414-008-0280-0
3. Bourel B, Tournel G, Hédouin V, Lee Goff ML, Gosset D (2001) Determination of drug levels in two species of necrophagous Coleoptera reared on substrates containing morphine. J Forensic Sci 46(3):600–603. https://doi.org/10.1520/JFS15010J
4. Matuszewski S, Szafałowicz M, Jarmusz M (2013) Insects colonising carcasses in open and forest habitats of Central Europe: search for indicators of corpse relocation. Forensic Sci Int 231(1-3): 234–239. https://doi.org/10.1016/j.forsciint.2013.05.018
5. Ridgeway JA, Midgley JM, Collett IJ, Villet MH (2014) Advantages of using development models of the carrion beetles *Thanatophilus micans* (Fabricius) and *T. mutilatus* (Castelneau) (Coleoptera: Silphidae) for estimating minimum post mortem intervals, verified with case data. Int J Legal Med 128(1):207–220. https://doi.org/10.1007/s00414-013-0865-0
6. Charabidze D, Vincent B, Pasquerault T, Hedouin V (2016) The biology and ecology of *Necrodes littoralis*, a species of forensic interest in Europe. Int J Legal Med 130(1):273–280. https://doi.org/10.1007/s00414-015-1253-8
7. Goff LM (2010) Early postmortem changes and stages of decomposition. In: Amendt J, Goff ML, Campobasso CP, Grssberger M (eds) Curr. Concepts Forensic Entomol. Springer, Dordrecht, pp 1–24
8. Rivers D, Dahlem G (2014) The science of forensic entomology. John Wiley & Sons, Inc., Chichester
9. Richards CS, Villet MH (2008) Factors affecting accuracy and precision of thermal summation models of insect development used to estimate post-mortem intervals. Int J Legal Med 122(5):401–408. https://doi.org/10.1007/s00414-008-0243-5
10. Amendt J, Krettek R, Zehner R (2004) Forensic entomology. Naturwissenschaften 91(2):51–65. https://doi.org/10.1007/s00114-003-0493-5
11. Wells JD, Sperling FAH (2001) DNA-based identification of forensically important Chrysomyinae (Diptera: Calliphoridae). Forensic Sci Int 120(1-2):110–115. https://doi.org/10.1016/S0379-0738(01)00414-5
12. Wells JD, Pape T, Sperling FAH (2001) DNA-based identification and molecular systematics of forensically important Sarcophagidae (Diptera). J Forensic Sci 45(5):1098–1102. https://doi.org/10.1520/JFS15105J
13. Willows-Munro S, Schoeman MC (2015) Influence of killing method on Lepidoptera DNA barcode recovery. Mol Ecol Resour 15(3): 613–618. https://doi.org/10.1111/1755-0998.12331
14. Cho S, Epstein SW, Mitter K, Hamilton CA, Plotkin D, Mitter C, Kawahara AY (2016) Preserving and vouchering butterflies and moths for large-scale museum-based molecular research. Peer J 4: e2160. https://doi.org/10.7717/peerj.2160
15. Dyar HG (1890) The number of molts of Lepidopterous larvae. Psyche A J Entomol 5:420–422
16. Klingenberg CP, Zimmermann M (1992) Dyar rule and multivariate allometric growth in 9 species of waterstriders (Heteroptera, Gerridae). J Zool 227(3):453–464. https://doi.org/10.1111/j.1469-7998.1992.tb04406.x
17. Midgley JM, Villet MH (2009) Effect of the killing method on post-mortem change in length of larvae of *Thanatophilus micans* (Fabricius 1794) (Coleoptera: Silphidae) stored in 70% ethanol. Int J Legal Med 123(2):103–108. https://doi.org/10.1007/s00414-008-0260-4
18. Velásquez Y, Viloria AL (2010) Instar determination of the neotropical beetle *Oxelytrum discicolle* (Coleoptera: Silphidae). J Med Entomol 47(5):723–726. https://doi.org/10.1603/me09058
19. Fratczak K, Matuszewski S (2014) Instar determination in forensically useful beetles *Necrodes littoralis* (Silphidae) and *Creophilus maxillosus* (Staphylinidae). Forensic Sci Int 241:20–26. https://doi.org/10.1016/j.forsciint.2014.04.026
20. Frątczak K, Matuszewski S (2016) Classification of forensically-relevant larvae according to instar in a closely related species of carrion beetles (Coleoptera: Silphidae: Silphinae). Forensic Sci Med Pathol 12(2):193–197. https://doi.org/10.1007/s12024-016-9774-0
21. Jakubec P (2016) Thermal summation model and instar determination of all developmental stages of necrophagous beetle, *Sciodrepoides watsoni* (Spence) (Coleoptera: Leiodidae: Cholevinae). Peer J 4:e1944. https://doi.org/10.7717/peerj.1944
22. Stillwell RC, Fox CW (2009) Geographic variation in body size, sexual size dimorphism and fitness components of a seed beetle: local adaptation versus phenotypic plasticity. Oikos 118(5):703–712. https://doi.org/10.1111/j.1600-0706.2008.17327.x
23. Anderson RS, Peck SB (1985) The insects and arachnids of Canada, part 13: the carrion beetles of Canada and Alaska (Coleoptera: Silphidae and Agyrtidae). Agriculture Canada, Ottawa
24. Navarette-Heredia JL (2009) Silphidae (Coleoptera) de México: diversidad y distribución. Universidad de Guadalajara, Guadalajara
25. Růžička J (2015) Silphidae. In: Löbl I, Löbl D (eds) Cat. Palaearct. Coleopt. Vol. 2/1. Hydrophiloidea–Staphylinoidea, Revis. Updat. Ed. Brill, Leiden & Boston, pp 291–304
26. Dobler S, Müller JK (2000) Resolving phylogeny at the family level by mitochondrial cytochrome oxidase sequences: phylogeny of carrion beetles (Coleoptera, Silphidae). Mol Phylogenet Evol 15: 390–402. https://doi.org/10.1006/mpev.1999.0765
27. Sikes DS, Trumbo ST, Peck SB (2005) Silphinae. In: Tree Life Web Proj. http://tolweb.org/Silphinae/26994/2005.02.07. Accessed 26 Jul 2017
28. Schawaller W (1981) Taxonomie und Faunistik der Gattung *Thanatophilus* (Coleoptera: Silphidae). Stuttg Beitr Naturkunde, Ser A 351:1–21
29. Kozminykh VO (1994) A new species of carrion beetles of the genus *Thanatophilus* (Coleoptera, Silphidae) from the southern Urals. Zool Zhurnal 73:161–165
30. Růžička J (2002) Taxonomic and nomenclatorial notes on Palaearctic Silphinae (Coleoptera : Silphidae). Acta Soc Zool Bohemicae 66:303–320
31. Ji Y (2012) The carrion beetles of China (Coleoptera: Silphidae). China Forestry Publishing House, Beijing
32. Daniel CA, Midgley JM, Villet MH (2017) Determination of species and instars of the larvae of the afrotropical species of *Thanatophilus* Leach, 1817 (Coleoptera, Silphidae). African Invertebr 58(2):1–10. https://doi.org/10.3897/AfrInvertebr.58.12966
33. von Lengerken H (1929) Studien über die Lebenserscheinungen der Silphini (Col.). XI-XIII. *Thanatophilus sinuatus* F., *rugosus* L. und *dispar* Hrbst. Z Morphol Ökol Tiere 14:654–666
34. von Lengerken H (1938) Beziehungen zwischen der Ernährungsweise und der Gestaltung der Mandibeln bei den Larven der Silphini (Coleopt.) Zool Anz 122:171–175
35. Dorsey CK (1940) A comparative study of the larvae of six species of Silpha (Coleoptera, Silphidae). Ann Entomol Soc Am 33(1): 120–134. https://doi.org/10.1093/aesa/33.1.120
36. Paulian R (1941) Les premiers états des Staphylinoidea. Étude de morphologie comparée Mém Mus Natl Hist Nat (Nouv Ser) 15:1–361
37. Prins AJ (1984) Morphological and biological notes on some South African arthropods associated with decaying organic matter. Part 2.

The predatory families Carabidae, Hydrophilidae, Histeridae, Staphylinidae and Silphidae (Coleoptera). Ann South African Mus 92:295–365
38. Xambeu PJV (1900) Moeurs et métamorphoses d'insectes, 9e mémoire, deuxieme partie. Rev Entomol Caen 19:1–56
39. Xambeu PJV (1892) Moeurs et métamorphoses d'insectes (Suite). Ann Soc Linn Lyon 39:137–194
40. Anderson RS (1987) Scientific note. The larva of *Thanatophilus trituberculatus* (Kirby) (Coleoptera: Silphidae). Coleopt Bull 41:34
41. Kočárek P (2003) Decomposition and Coleoptera succession on exposed carrion of small mammal in Opava, the Czech Republic. Eur J Soil Biol 39(1):31–45. https://doi.org/10.1016/S1164-5563(02)00007-9
42. Matuszewski S, Bajerlein D, Konwerski S, Szpila K (2010) Insect succession and carrion decomposition in selected forests of Central Europe. Part 2: composition and residency patterns of carrion fauna. Forensic Sci Int 195(1-3):42–51. https://doi.org/10.1016/j.forsciint.2009.11.007
43. Dekeirsschieter J, Verheggen FJ, Haubruge E, Brostaux Y (2011) Carrion beetles visiting pig carcasses during early spring in urban, forest and agricultural biotopes of Western Europe. J Insect Sci 11:73. https://doi.org/10.1673/031.011.7301
44. Velásquez Y, Viloria AL (2009) Effects of temperature on the development of the Neotropical carrion beetle *Oxelytrum discicolle* (Brullé, 1840) (Coleoptera: Silphidae). Forensic Sci Int 185(1-3):107–109. https://doi.org/10.1016/j.forsciint.2008.12.020
45. Matuszewski S (2011) Estimating the pre-appearance interval from temperature in *Necrodes littoralis* L. (Coleoptera: Silphidae). Forensic Sci Int 212(1-3):180–188. https://doi.org/10.1016/j.forsciint.2011.06.010
46. Novák M (2017) Redescription of immature stages of central European fireflies, part 1: *Lampyris noctiluca* (Linnaeus, 1758) larva, pupa and notes on its biology (Coleoptera: Lampyridae: Lampyrinae). Zootaxa 4247:429–444. https://doi.org/10.11646/zootaxa.4247.4.5
47. Lawrence JF, Slipinski SA (2013) Australian beetles. Volume 1: morphology, classification and keys. CSIRO Publishing, Collingwood
48. Kilian A, Mądra A (2015) Comments on the biology of *Sciodrepoides watsoni watsoni* (Spence, 1813) with descriptions of larvae and pupa (Coleoptera: Leiodidae: Cholevinae). Zootaxa 3955(1):45–61. https://doi.org/10.11646/zootaxa.3955.1.2 10.11646/zootaxa.3955.1.2

Annex 5 (Manuscript in review): Description of immature stages of Thanatophilus sinuatus (Coleoptera: Silphidae)

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Abstract

Necrophagous beetles of genus *Thanatophilus* are well recognized as a group of beetles with a high potential utility in forensic entomology. They can be used to estimate postmortem interval (PMI) or validate the value for other groups of insects commonly encountered on human remains, like blowflies (Calliphoridae). However, reliable tools for instar and species identification of their larvae are needed as such information is crucial for allowing accurate PMI estimate. One of the most common species of the genus *Thanatophilus* in Europe is *Thanatophilus sinuatus*. This species occurs frequently on human remains and its larvae feed on decaying tissues throughout their development. Therefore the larvae could become useful bioindicators for forensic entomology, although their current description does not allow reliable instar or species identification. Our goal was to provide morphological characters for species and instar identification of all larval stages of *T. sinuatus*. The larvae were obtained from laboratory rearing under controlled conditions (20°C and 16:8 h of light/dark period). Qualitative and quantitative morphological instar and species-specific characters are described and illustrated. Additionally, we report observations of biological and developmental length for all stages of the species. We also compared these morphological characters with recent description of *T. rugosus* and provided an identification key of these two similar and often co-occurring species. We noticed that some characters for instar identification were shared between *T. sinuatus* and *T. rugosus* and confirmed by comparison with larvae of *T. dentigerus* that they can be applied to other species of the genus.

Keywords: *Thanatophilus sinuatus*, Larval Instar Identification, Morphology, Forensic Entomology.

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Introduction

Necrophagous beetles are currently well established as a useful ecological group of insects in forensic entomology [1–3]. In some cases they can provide us with estimates of the postmortem interval (PMI) as accurate as other groups of insects (e.g. blowflies (Calliphoridae)) [3], or at least they can allow for validation of these estimates. The list of such species is long, but only fraction of them can be currently be used as we lack the necessary basic information about their morphology (species and instar identification) and biology (thermal summation models).

Genus *Thanatophilus* Leach, 1815 belongs to the family Silphidae and contains 23 described valid species, distributed in Holarctic and Afrotropical Realms [4–6]. Most of these species occur in the North Hemisphere. Members of this genus share not only general appearance, but they also have a very similar ecology. All known species are necrophagous in all active stages of development (larvae and adults) and they flourish on larger carrions of vertebrates, including humans. They appear to prefer earlier stages of decomposition and can commence to breed in the first 24 hours after death [7]. These features make them a very promising group of beetles that could be used as bioindicators in the field of forensic entomology.

Adult specimens of this genus can be identified based on several available identification keys (e.g., [5, 6, 8–11]). Morphology of their larvae was mostly neglected in the past as was pointed out by Novák et al. 2018; (Table 1) [12]. However, the recognition of their usefulness sparked a new interest and larvae of three member species (*T. capensis* Wiedemann, 1812 [junior synonym *T. mutilatus* Laporte de Castelnau, 1840], *T. micans* (Fabricius, 1794) and *T. rugosus* (Linnaeus, 1758)) were re-described in the past few years as a result [12, 13]. Along with morphological description of some of the larvae, the thermal summation models and instar identification models were developed [1, 7, 12–14]. All these information are essential to estimate time of colonization, which is widely used as a proxy for PMI.

Two out of the three recently described larvae belong to species occurring almost exclusively in Africa (*T. micans* was also reported from Yemen [4]), which is somewhat disproportional to the fact that the center of biodiversity of the genus *Thanatophilus* is in the Palearctic Region [4, 8]. To help covering this knowledge gap we chose *Thanatophilus sinuatus* (Fabricius, 1775) as our focal species. This beetle has a very wide trans-Palaearctic distribution (occurring across Europe, Asia and North Africa) and is very common especially on the European continent [4, 15]. Adults and larvae of *T. sinuatus* were specifically recorded on 13.27 % (26 out of 196) of human remains that were investigated by forensic entomologists in the Czech Republic (between the years 2003 – 2018) (Šuláková, unpublished data). Additionally, the species is known to replace blowflies, one of the crucial groups in forensic entomology, during the colder parts of the year [16]. These findings support our view that *T. sinuatus* is indeed an important species in the field of forensic entomology.

The need for its thorough redescription comes from the fact that it often co-occurs with *T. rugosus* (Linnaeus, 1758) [17, 18]. It is very difficult to separate these two species morphologically in the larval stages [19]. Only the third instar can be identified to the species level [12, 18]. Even though these species possess many similarities, it is generally not safe to assume the information they provide are interchangeable. This was already proven for *T. capensis* and *T. micans* and the potential error can be highly significant [1]. Finding unique morphological characters that would allow for precise identification is therefore important for potential practical applications.

Our re-description also aims to establish characters for larval instar identification. The main focus is on the qualitative characters that are independent of the larval size, as they provide more accurate insight. The advantage of these characters lies in the fact,that they are much more straightforward to apply and only a microscope is necessary to detect them. This is in contrast to statistical methods where accurate measurements of several features are needed and data has to be processed in a specialized statistical software. We would like to test if such qualitative morphological characters are shared among other species of the same genus and compare them with characters that were already identified for larval instars of *T. rugosus* [12].

Material and Methods

We collected adults of *T. sinuatus* by baited pitfall traps placed in an arable fields around Albeř (Czech Republic) (49°01'35.7"N 15°08'54.9"E) between 16th and 20th of May, 2016. All collected beetle specimens were transported into the laboratory, identified to species and sexed using identification key [9]. Adults of *T. sinuatus* were afterwards divided into groups of six, with equal number of male and female specimens. We followed a breeding protocol outlined in Novák et al. 2018 [12]. The breeding took place inside a climatic chamber with constant temperature and photoperiod regime (20°C and 16 hours of light followed by 8 hours of dark) to mimic the natural environmental conditions during breeding season of this species.

The breeding boxes were inspected at least once a day and new eggs were removed and placed in vertically positioned Petri dishes (9 cm diameter) filled up to 2/3 with soil and with a small piece of fish meat (*Scomber scombrus* Linnaeus, 1758) as described by Ridgeway et al. 2014 [1]. These Petri dishes were secured with rubber bands to prevent unwanted opening and stored on trays for easier handling. Moisture was provided by submerging the bottom part of the dish into three centimeter layer of tap water for a few seconds. The advantage of this breeding methodology lies in larger mass of soil regulating and stabilizing moisture. In addition to that, larvae are also provided with enough space to create their pupation chambers. The layer of soil, however, is very thin, thus forcing larvae to create pupal chambers right next to the wall, making them visible and easily observable without any disturbance.

Clutches of eggs from the same day were kept together and hatching larvae were further separated based on the time they hatched or entered next stage, allowing us to observe them and collect data about their development rate in different developmental periods (egg, 1st - 3rd instar larvae and pupa). At each developmental stage we removed few representative specimens and fixed them. The fixation process involved killing the specimen in ethyl acetate fumes, fixing it in hot water bath (90-95°C) and storing in 75% alcohol solution.

Optical imaging. The fixed specimens were cleared from soil and food particles by fine brush and placed in Digital Ultrasonic Cleaner PS-06A for a short period of time (up to one minute, depending on the fragility of the specimen and its dirtiness). To improve the visibility of internal parts we detached heads of some specimens and boiled them in 10% potassium hydroxide (KOH). In case this process failed to clear the specimen to a desirable level, we bleached it in 4% hydrogen peroxide to remove excess pigments and prevent the specimen from further loss of firmness by the effect of KOH. Images of external morphology were taken while specimens or their body parts were submerged in ethanol or

glycerol to prevent drying. Glycerol additionally improves image quality by its favorable optical properties and by stabilizing the sample due to its viscosity. Images were taken by Canon macro photo lens MP-E 65 mm on a Canon 550D body, mounted on automated macro rail for focus stacking (Cognisys StackShot). Smaller details were photographed using Olympus BX53 microscope with Olympus DP73 digital camera. The sets of pictures were consequently stacked into a final image with a high depth of field in Zerene Stacker 1.04 by Zerene Systems LLC.

Electron imaging. Fine details of external morphology and body structure were examined at the Faculty of Science of Charles University in Prague by JSM-6380LV (JEOL) Scanning Electron Microscope (SEM) with a high resolution of 3.0 nm (30 kW). The methodology follows Novák, 2017 [20]. Before imaging the specimens were first dehydrated through a series of increasing alcohol concentrations. The samples were transferred sequentially to 60%, 70%, 80%, 90% and 95% alcohol for ca. 0.5 h each. Dehydrated samples were then dried by Critical Point Drying method. Dry samples were subsequently attached to an aluminium disk target and coated with gold in Bal-Tec Sputter Coater SCD 050, to ensure conductivity.

Final images from both optical and electron imaging were compiled using GIMP ver. 2.8.16 graphic program; graphs were compiled using R ver. 3.4.1 statistical computing program.

Terminology and measurements. Interpretation and terminology of larval and pupal descriptions follows Lawrence & Ślipiński, 2013 [21]. To obtain precise measurements of key morphological structures, we photographed them under Olympus SZX16 stereo microscope and measured them by graphics program EidosMicro. The following abbreviations are used in the text:

AI—Length of Antennomere I
AII—Length of Antennomere II
AIII—Length of Antennomere III
A1L—Length of Abdominal Segment I
A1W—Width of Abdominal Segment I
HL—Head Length (without labrum)
HW—Head Width (at the widest point)
LPI—Length of Labial Palpomere I
LPII—Length of Labial Palpomere II
MPI—Length of Maxillary Palpomere I
MPII—Length of Maxillary Palpomere II
MPIII—Length of Maxillary Palpomere III
N1L—Pronotal Length
N1W—Pronotal Width (at the widest point)
N2L—Mesonotal Length
N2W—Mesonotal Width (at the widest point)
N3L—Metanotal Length
N3W—Metanotal Width (at the widest point)
URI—Length of Urogomphal Segment I

URII—Length of Urogomphal Segment II

URS—Length of Urogomphal Terminal Seta

N1L/W—Ratio of Pronotal Length to Pronotal Width

N2L/W—Ratio of Mesonotal Length to Mesonotal Width

N3L/W—Ratio of Metanotal Length to Metanotal Width

HW/HL—Ratio of Head Width to Head Length

A1L/W—Ratio of Abdominal Segment I Length to Abdominal Segment Width

Results

Biology

Females of *T. sinuatus* were able to reproduce under long day photoperiod of 16:8 hours (light:dark phases) and at temperature of 20° C. They laid small clusters of eggs (7.6 ±1.45 eggs per cluster) in the substrate. They were probably unable to reach the desired depth under our laboratory setup,, resulting in the clutches being deposited at the very bottom of the breeding box.

Development from egg to adult took on average 41.85 ±3.08 days. The mean duration of each developmental stage is given in Table 1. The first instar larvae started searching for food right after emerging from the egg and stayed very close to the food source even throughout the second and partly the third instar. During the third instar they stopped feeding and dug up a pupation chamber where they subsequently turned into pupa after a few days. The third instar seems to be a critical period in the development of this species as many specimens were unable to transition into pupae. We observed that disturbance of the chamber before pupation (by other larvae or by human investigators) often resulted in its abandonment. Such larvae often resumed feeding and postponed pupation beyond the normal period or even died before reaching it.

We observed cannibalistic behavior, very likely also among the sibling larvae that was not driven by starvation, as the food was provided *ad libitum*.

Table 1

Development length of *Thanatophilus sinuatus* at 20°C and under 16:8 photoperiod.

Stage	**egg**	**instar I**	**instar II**	**instar III**	**pupae**
Mean Length of development (in days)	2.83	2.97	3.26	19.30	13.56
Standard deviation (in days)	0.50	0.50	1.28	2.27	2.44
Number of observations	46	41	38	24	20

Morphometry

As expected, most measured body parts grow isometrically (see Online Resource 1). However, certain characters grow allometrically. These are certain segments of urogomphi and labial palpi. The best example of allometric growth in *T. sinuatus* larvae is a change in the length of the first and second labial palpomeres. The first segment grows in an approximately linear way, though the second segment stays the same and even shrinks in the third instar (Figs 1a, b and 4o–q). The discrepancy in growth rates of these two segments translates into a proportional difference that can be used as an instar identification character. There is a similar relationship between the first and second segments of urogomphi (Fig. 6e–

g). The trend is clearly visible from graphs of length ratios between those segments throughout the subsequent instars as shown on Figures 3a and b.

Our analysis suggests that head width (Fig. 2) could also be used for instar identification. We did not observe any overlap in the values among the instars, although there are some limitations of this method (see Novak at al. 2018 [12]).

Description of immature stages of *Thanatophilus rugosus*

Family SILPHIDAE Latreille, 1806
Subfamily Silphinae Latreille, 1806
Genus *Thanatophilus* Leach, 1815
Species *Thanatophilus sinuatus* (Fabricius, 1775)

Description of immature stages of *Thanatophilus sinuatus*

Larvae. **Body** (Fig. 4a–i):

Instar III (Fig. 4a, d, g) . Mean value of total length: 14.84 ±1.72 mm. Campodeiform larvae, more or less fusiform, widest at the metathorax and slightly narrowing towards both ends. Slender body only slightly dorso-ventrally flattened. Head and all terga strongly sclerotised, and covered by vestiture of scattered setae. All terga on lateral margin with few long setae that can be clearly visible from above or below. All thoracic and abdominal tergites I to VIII laterally explanate, dark brown to black, with translucent paratergites, completely creamy-white pigmented on their distal ends (Fig. 4j). Thoracic paratergites rounded, abdominal paratergites small and pointed posteriorly with longest seta protruding in the direction of its apex. Ventral side of thorax mostly white. Ventral area of abdominal segment I poorly sclerotized, white, with dark pigmentation only in the central area of the medial sternite and far lateral edges of laterosternites. Sclerites of abdominal segments II to VIII anteriorly with light-pigmented rim, with dark medial sternites and speckled dark laterosternites divided from the former by narrow lighter area (Fig.- 4d; sls). Segment II with two distinguishable white lines dividing medial sternite and laterosternites. Venter of segment IX and X uniformly brown. Posterior segments ventrally overall darker than anterior. *Instar II* (Fig. 4b, e, h). Mean value of total length: 9.56 ±1.20 mm. Paratergites of thorax and abdomen with translucent spots of light pigmentation that are the most visible on protergum, but never with full creamy-white pigmentation on the distal ends of paratergites (Fig. 4k). Abdominal segment II ventrally sclerotized with distinguishable white lines dividing medial sternite and laterosternites. Sternites of abdominal segments III to VIII with narrow rim of light pigmentation anteriorly (Fig. 4e; flr), otherwise uniformly dark. *Instar I* (Fig. 4c, f, i). Mean value of total length: 6.21 ±0.93 mm. All thoracic and abdominal tergites uniformly dark brown to black (Fig. 4l). Abdominal segment II ventrally sclerotized with white lines dividing medial sternite and laterosternites. Sternites of abdominal segments III to VIII uniformly dark. Posterior segments ventrally overall darker and more sclerotized than anterior.

Head capsule (Fig. 5): *Instar III*. Prognathous and protracted; HW 2.13 ±0.08 mm, HL 1.21 ±0.16 mm, HW/HL 1.78 ±0.24; reniform from ventral view; gena short, approximately half of the width of the head capsule in dorsal view. Head capsule dorsally covered with infrequent long setae mainly on labrum and clypeus and scattered short stout setae mainly on frons and vertex. Surface of frons and vertex covered by shallow cracks, with sparse convex nodules scattered along the cracklines mainly on vertex (Fig. 5c). Epicranial stem present, frontal arms V-shaped, with U-shaped base in 1/3 of their length from the stem (Fig. 5a; es, fa and 5f; esfa). Epicranial suture and epicranial stem of light coloration. Median endocarina absent. Six stemmata on each side of the head separated into two groups; four stemmata forming a trapezoid placed posteriorly behind antennal base and two stemmata placed ventrally under antennal base, parallel to the wider base of the trapezoid. Frontoclypeal suture absent; consisting only of linear anterior tentorial pits (Fig. 5a, f; atp), parallel to the posterior edge of clypeus. Clypeus subrectangular, ca. three and a half times as wide as long, partially covering mandibles in dorsal view; dorsally with six stout setae placed lengthwise anteriorly and many short thin setae, growing in elliptical pattern stretched over the area of clypeus. Labrum subtrapezoidal, dorsally with 8 long stout setae aimed anteriorly (two pairs on lateral-posterior margins and four on the anterior half of labrum). Labral apex double-arched, bearing two very short setae on the anterior edge (Fig. 7e; las). Epipharynx anteriorly covered with rows of bulbous processes and a pair of two large bulbous sensoria anteromedially (Fig. 5d, 7e; bs) and a pair of short conical spines placed laterally. Pharynx covered with rows of setae and spines which project up to posterior edge of clypeus, with oblique transverse cibarial plates (Fig. 5d; cp) in labral-clypeal membrane area and a pair of sensoria placed posteromedially behind these plates. Ventral mouthparts retracted, forming a maxillo-labial complex. Hypostomal rods absent. Ventral epicranial ridges present, roughly reaching beyond the level of the posterior edge of the maxillo-labial complex. Gular region short with gular sutures converging anteriorly. Tentorium consisting of a pair of sclerotized anterior arms (Fig. 5e–g; aa), laterally extended by fine hyaline lobes (Fig. 5e; ihl, ohl) in their basal 2/3, before the dorsal arms connecting to them; hyaline dorsal arms (Fig. 5e–-g; da) connected to frons near the beginning of the U-shaped base of the frontal arms; and sclerotized posterior arms (Fig. 5e–g; pa) connected with posterior tentorial bridge (Fig. 5e; ptb). A pair of short sclerotized arms connected with filamentous secondary bridge (Fig. 5 e–g; sb) growing dorsally from the middle of posterior arms. *Instar II*. HW 1.57 ±0.08 mm; HL 1.02 ±0.15 mm; HW/HL 1.57 ±0.23. Vertex covered by scattered convex nodules (Fig. 5a). *Instar I*. HW 1.09 ±0.06 mm; HL 0.70 ±0.12 mm; HW/HL 1.62 ±0.30. Vertex covered with dense small convex nodules (Fig. 5b).

Antennae (Fig. 6a–d): *Instar III*. Trimerous, inserted on lateral distal margin of gena; inserted in membranous socket. All antennomeres fully sclerotized and of more or less similar length (AI 0.33 ±0.04 mm, AII 0.37 ±0.03 mm, AIII 0.42 ±0.04 mm). Antennomere I (Fig. 6a; amI) cylindrical, sloping laterally towards the longitudinal axis of the larva, bearing several stout setae on its distal half. Antennomere II (Fig. 6a; amII) club shaped, wider on distal end, sloping laterally towards the longitudinal axis of the larva, bearing several stout setae unequally and scarcely scattered across the surface. Sensorium of antennomere II (Fig. 6c, d) placed on inner lateral area of its distal end together

with three small but bulky sensilla lacking a socket (Fig. 6c; bs), placed ventrally from the sensorium, the longest and the shortest sensilla growing from the same base, closer to the third antennomere compared to the medium-sized sensilla. Sensorium egg-shaped with conical top, widest at the base, encircled by a sclerotized ring, closely annealing to the second antennomere; a circle of small button-like sensilla placed around the apex of the sensorium (Fig. 6d; cs), with another circle of small sensillar pits (Fig. 6c; sp) closer to the base. Antennomere III (Fig. 6a; amIII) placed on outer lateral area of antennomere II, bearing several stout setae mainly on its distal two thirds and 4 setae on its apex; 2 articulated and short (Fig. 6b; sas), and 2 without articulation, one short and peg-like (Fig. 6b; sps), one long (Fig. 6b; las). *Instar II.* All antennomeres fully sclerotized, with first antennomere the shortest: AI 0.28 ±0.04 mm, AII 0.37 ±0.02 mm, AIII 0.40 ±0.03 mm. *Instar I.* All antennomeres fully sclerotized, antennomere I shortest: AI 0.19 ±0.02 mm; antennomere II and III of similar length: AII 0.27 ±0.04 mm, AIII 0.34 ±0.04 mm.

Maxilla (Figs 4m, 7a). *Instar III*. Maxillary articulating areas present, completely unsclerotized. Lacinia and galea partly fused together. Cardo transverse, subtriangular, ca. two times wider than long, with one medium long seta ventro-laterally close to the base of stipes. Stipes sub-rectangular, longer than wide, ventrally bearing one long stout seta centrally, one long stout seta outer-laterally and several short setae roughly in between the two. Galea fixed; bearing two long setae outer-laterally and one short seta ventrally close to its base; with a brush of very dense setation on its apex (Figs 4m, 7d; gb). Lacinia fixed, bearing eight to eleven visible stout spines on its outer lateral margin together with an apical lobe bearing a short cuticular projection composed of several shorter spines grown together (Fig. 4m; csp). Palpifer very short (Fig. 7a; mpf), sclerotized mainly on outer lateral margin. Maxillary palpus trimerous, palpomere I (Fig. 7a; mpI) cylindrical (MPI 0.11 ±0.03 mm), ca. two times longer than wide; palpomere II (Fig. 7a; mpII) cylindrical (MPII 0.13 ±0.02 mm), sloping laterally towards the longitudinal axis of the larva, palpomere III (Fig. 7a; mpIII) conically elongate (MPIII 0.22 ±0.03 mm), longest of the palpomeres. Palpifer and palpomeres sparsely covered by several setae, palpomere III having a short stout seta in an articulated protuberance placed on outer-lateral edge of its base. The apex of palpomere III covered by numerous short blunt peg-like sensilla (Fig. 7b). *Instar II.* palpomere lengths: MPI 0.10 ±0.02 mm; MPII 0.11 ±0.02 mm; MPIII 0.23 ±0.02 mm. *Instar I.* palpomere lengths: MPI 0.06 ±0.01 mm; MPII 0.08 ±0.01 mm; MPIII 0.19 ±0.02 mm.

Labium (Figs 4n–q, 7a, d) *Instar III.* Prementum, mentum and submentum are all sclerotized on their basal areas. Ligula bi-lobed (Figs 4n, 7a, d; lig); each lobe along the sagittal plane covered dorsally by a group of numerous longitudinal lines of fine short setation and dense bulbous projections apically and centrally between the two groups; ventrally, a pair of long setae is present in the lateral parts of the central area of prementum (Fig. 7a; pm), as well as two pairs of shorter setae in between them centrally and a pair of shorter setae on the basal half. Labial palpus bimerous (Fig. 4 q), LPI 0.12 ± 0.02 mm, LPII 0.06 ±0.01 mm; basal palpomere (Fig. 7; lpI) club-shaped, sloping laterally towards the longitudinal axis of the larva, with no setation; distal palpomere (Fig. 7, lpII) conical, blunt, ca. 1/3 of the length of basal palpomere, sparsely covered in small thin peg-like sensilla on distal half and bearing

a group of short blunt peg-like sensilla on its apex (Fig. 7c). Mentum (Fig. 7a; m) as long as wide, sub-oval, with dark pigmentation on its base; ventrally bearing two pairs of long setae on its posterior half. Submentum (Fig. 7a; sm) bearing a pair of long stout and several shorter thin setae, paired or irregularly scattered posterolaterally, alongside its sclerotized distal half when viewed ventrally. *Instar II.* Labial palpus bimerous (Fig. 4 p), LPI 0.08 ±0.02 mm, LPII 0.07 ±0.01 mm. *Instar I.* Labial palpus bimerous (Fig. 4o), LPI 0.05 ±0.01 mm, LPII 0.08 ±0.01 mm.

Mandibles (Fig. 8) *Instar III.* Symmetrical (with slightly asymmetrical apex), simple without mola or prostheca, basal half consisting of wide triangular base in dorsoventral cross section, distal half more dorsoventrally flattened (Fig. 8a–d), apex consisting of two scissorial teeth lying obliquely, perpendicular to the plane of movement of the mandible; apical tooth (Fig. 8f; at) longer and serrated laterointernally, sub-apical tooth (Fig. 8f; sat) shorter, positioned dorsally towards the outer tooth and serrated lateroexternally towards the serrated area of the outer tooth. One long stout seta present laterodorsally on mandibular base (Fig. 8e; lds) and one short stout seta present outer-laterally in the mid-length of the mandible (Fig 8a, e; ms). Left mandible larger, covering the apex of the right mandible when clenched (Fig. 8e). In higher instars, the scissorial teeth of mandibles more worn out with apices and serrated areas more blunt (Fig. 8g). *Instar II*. and *Instar I.* same as Instar III.

Thorax (Fig. 4a–l) *Instar III.* Three-segmented, thoracic tergites divided by sagittal line; paratergites creamy white and translucent, slightly overlapping the body forming irregular semicircles (Fig. 4j). Protergum (N1W 3.41 ±0.2 mm, N1L 1.43 ±0.20 mm) suboval, wider posteriorly, rounded at posterolateral corners. Mesotergum (N2W 3.79 ±0.23 mm; N2L 0.72 ±0.08 mm) and metatergum (N3W 3.99 ±0.24 mm; N3L 0.65 ±0.06 mm) suboval, similar in shape and size. Venter of prothorax composed of short, semi-lens shaped, wider than long prosternum, subdivided into three dark-pigmented areas; lateral ones subtriangular, well sclerotized; medial area subrhomboid (or subpentagonal), semi-sclerotized. Ventrolateral areas of pro-, meso- and metathorax composed of episternum and epimeron forming thin well-sclerotized strands and semi-sclerotized pre- and postcoxale. Lateral areas of thorax membraneous, mesothorax bearing a large (relative to abdominal spiracles) annular spiracle with yellow colored peritreme (Fig. 4d, g) and bearing one long stout seta on its ventral posterior margin. The atrium (inner chamber) padded with shrub-like filtration hairs. Meso- and metasternum subdivided by transverse fold into membraneous basisternum and sternellum. Basisternum covered by “freckles” of dark pigmentation, from which stout setae grow. Anterior ventral area of meso- and metathorax formed by membranous intersternite, laterally bounded with pair of sclerotized patches. *Instar II.* Thoracic praterga with translucent spots of light pigmentation but never with full creamy-white pigmentation on the distal end; most visible on protergum (Fig. 4k). Protergum and mesotergum subdivided by sagittal line, metatergum subdivided by sagittal line on its anterior edge only. Protergum (N1W 2.31 ±0.14 mm; N1L 0.95 ±0.11 mm) suboval, wider posteriorly, rounded at posterolateral corners. Mesotergum (N2W 2.55 ±0.15 mm; N2L 0.43 ±0.06 mm) and metatergum (N3W 2.70 ±0.13 mm; N3L 0.34 ±0.07 mm) suboval, similar in shape and size. *Instar I.* Thoracic terga of uniform dark brown or black coloration (Fig. 4l). Protergum and mesotergum subdivided by sagittal line, metatergum subdivided by sagittal line

on its anterior edge only. Protergum (N1W 1.46 ±0.11 mm; N1L 0.57 ±0.05 mm) semicircular, wider posteriorly, rounded at posterolateral corners. Mesotergum (N2W 1.56 ±0.13 mm; N2L 0.28 ±0.04 mm) and metatergum suboval, similar in shape and size. Metatergum (N3W 1.65 ±0.11 mm; N3L 0.23 ±0.04 mm).

Abdomen (Fig. 4a–i) *Instar III*. Ten-segmented, tapering towards posterior end, segments I to VI dorsally subdivided by fine sagittal line anteriorly, on segment VI barely visible. Tergites of segments I to VIII subrectangular, narrow (A1W 3.93 ±0.22 mm; A1L 0.50 ±0.09 mm), similar in shape and coloration, with posteriorly pointed, creamy-white translucent paratergites. Tergite of segment IX subrectangular, bearing paired, well developed two-segmented urogomphi (Fig. 6g) that are inserted dorsolaterally. Basal segment of urogomphi narrow (URI 1.22 ±0.21 mm), wider on proximal and distal ends, slightly bent posteromedially, bearing short stout setae. Distal segment slender (URII 0.52 ±0.08 mm), ca. 2.3 time shorter than basal, cylindrical, with one short seta inserted on its apex (URS 0.12 ±0.04 mm) and two prominent setae inserted on its body; the first thin and inserted dorsally slightly below the apex, the second stout and inserted inner-ventrolaterally on the apical third of the segment. Segment X dorsally subtrapezoidal forming a well sclerotized cylinder; distal central half of dorsal area with two longitudinal lines of white pigmentation; segment X holding the hold-fast organ (pygopod) with several eversible processes, sparsely covered in short spines on their distal ends (Fig. 6h). Segments I to VIII have membraneous laterotergites with poorly sclerotized plates bearing annular spiracles with yellow colored peritreme, bearing no setae and placed on the venter of paratergites; the venter of segments I to VIII consists of subtrapezoidal, wider than long median sternite, margined by paired subtriangular laterosternites; median sternite and laterosternites of segment I membranous, but pigmented. Sternite of segment IX sub-rectangular. Spiracle on segment I largest of abdominal spiracles. *Instar II*. No abdominal tergites subdivided by sagittal line. Tergites of segments I to VIII sub-rectangular, narrow, A1W 2.67 ±0.14 mm; A1L 0.26 ±0.05 mm, similar in shape and coloration. Paratergites of abdomen with translucent spots of light pigmentation, but never with full creamy-white pigmentation on the distal ends. Venter of abdomen visually subdivided to median sternite and laterosternites on segments I and II only. Basal segment of urogomphi (Fig. 6f) narrow (URI 0.94 ±0.11 mm), slightly bent posteromedially, distal segment slender (URII 0.50 ±0.07 mm), cylindrical, ca. half as long as basal segment, with one seta inserted on the apex (URS 0.15 ±0.01 mm) and bearing no seta on its base. *Instar I.* No abdominal tergites subdivided by sagittal line. Tergites of segments I to VIII sub-rectangular (A1W 1.63 ±0.10 mm, A1L 0.16 ±0.02 mm). Venter of abdomen visually subdivided to median sternite and laterosternites on segments I and II only. Basal segment of urogomphi (Fig. 6e) narrow (URI 0.55 ±0.04 mm), bent posteromedially, distal segment slightly slender and cylindrical, but almost the same length as basal (URII 0.41 ±0.07 mm), with long terminal seta (URS 0.24 ±0.04 mm), and bearing no seta on its base.

Legs (Figs 4a–i and 9) *Instar III*. Pentamerous including pretarsus, all pairs similar in shape and size. Coxa large, stout, covered by stout setae; with white pigmentation on the posterior and anterior area of the apex; coxal-trochanteal membrane reaching ca. 1/3 of longitudinal length. Trochanter small,

subtriangular in lateral view, centrally white pigmented and sclerotized only basally and distally, covered by several stout setae of the same length as coxa and one seta ca. three to four times longer than the rest, placed ventrally on the distal end (Fig. 9a; ts). Femur cylindrical, dorsally sclerotized. Ventrally completely white (Fig. 4d; wf), bearing two longitudinal lines of sharp stout setae and a very long seta (ca. two times the length of neighboring setae) between these lines (Fig. 9a; fs); several other irregular longitudinal lines with shorter setation placed laterally and dorsally. Tibiotarsus ca. as long as femur, narrower, tapering towards distal end, bearing several longitudinal lines of stout sharp setae around its circumference followed by less regular lines of shorter setae. Pretarsus composed of a claw with stout base (Fig. 9b), ventrolaterally bearing a pair of stout setae placed in the mid-length of the claw. Common setae on coxa and trochanter generally thinner and slightly longer than stout strong setae on femur and tibiotarsus. *Instar II*. Femur cylindrical, fully sclerotized and dark pigmented. Claw (Fig. 9c) appears to be more slender than in instar III. *Instar I.* Claw (Fig. 9d) more slender than in instar III, with narrow base.

Pupa. (Fig. 10) Type of pupa: *adectica exarata libera*. Curved, ventrally concave. Length 22.2 mm. Coloration: cream white body with dark-brown setae.

Head capsule: Partially covered by protergum in dorsal view. Antennae short, extending laterally to half of the lateral length of protergum. Mouthparts visible in ventral view.

Thorax: Surface of protergum covered by numerous scattered short brown hairs, with a distinct line of hairs around its edge (Fig. 10; es) and with two pairs of long stout dark-brown setae on its anterolateral edge (Fig. 10; ps). Protergum oval, convex. Mesonotum less wide than metanotum, with distinct triangular protuberance posteromedially representing future scutellum (Fig. 10; sc). Wing pads (Fig. 10; wi) and rectangular elytra (Fig. 10; el) completely white and about the same length; wing pads reaching fourth abdominal segment. All pairs of legs free, visible in ventral view; femurs and tibiae of metathoracic legs partially covered by wing pads, distal segments of tarsi extend to fifth abdominal segment when pupa straightened, if curved reaching seventh abdominal segment. Spiracles present on pleural areas of mesothorax.

Abdomen: Abdominal segments sub-rectangular, wider than long. Segments II–VII bearing a pair of long stout brown setae (Fig. 10; as). Urogomphi on segment VIII short and bulky, white, with dark-brown apices bearing medium-long stout brown setae (Fig. 10; us). Spiracles present on abdominal pleural areas of segments I–VIII, on segments I-IV light-brown, otherwise white.

Identification key to larval instars of *T. sinuatus* and *T. rugosus*

1(2) Ventral part of femur on all legs white (Fig. 4d, wf); abdominal sclerites speckled with dark spots (Fig. 4d, sls). Anterior arms of tentorium with both, inner and outer hyaline lobes (Fig. 5e; aa, ohl and ihl). = **Third instar**

2(1) Ventral part of femur on all legs dark and of similar shade as the rest of the femur (Fig. 4e and f); abdominal sclerites uniformly brown (Fig. 4e and f). Anterior arms of tentorium without or only with inner hyaline lobes (Fig. 5e; aa, ihl).

3(4) First segment of urogomphi ca. two times longer than the second (Fig. 6f); first segment of labial palpi longer and larger than the second segment (Fig. 4p). Anterior arms of tentorium with inner hyaline lobes only (Fig. 5e; aa and ihl). = **Second instar**

4(3) First segment of urogomphi less than 1.5 times longer than the second (Fig. 6e); first segment of labial palpi as long as the second and of similar bulk (Fig. 4o). Anterior arms of tentorium without any hyaline lobes (Fig. 5e; aa). = **First instar**

Identification key to *T. sinuatus* and *T. rugosus* larval instars

Third instar

1(2) Tergites dark brown, with paratergites fully creamy white on distal ends (Fig. 4a and j). = ***T. sinuatus***

2(1) Tergites uniformly dark brown (Novák et al. 2018; fig. 4a [12]). = ***T. rugosus***

Second instar

1(2) Tergites dark brown, protergum (and possible other tergites) with lighter translucent spots on paratergites, never fully reaching distal ends (Fig. 4b and k). = ***T. sinuatus***

2(1) All tergites uniformly dark brown (Novák et al. 2018; fig. 4b [12]). = ***T. rugosus***

First instar

1(2) Length of the first segment of labial palpus approximately 0.05 ±0.01 mm, length of the first segment of maxillary palpus approximately 0.057 ±0.01 mm. = ***T. sinuatus***

2(1) Length of the first segment of labial palpus approximately 0.08 ±0.006 mm, length of the first segment of maxillary palpus approximately 0.105 ±0.01 mm. = ***T. rugosus***

Discussion

Previous descriptions of developmental stages of *T. sinuatus* did not find any reliable characters that would allow for species identification of its larval instars [19, 22–24]. To our knowledge, the only reliable way of how to identify larvae of *T. sinuatus* and *T. rugosus* was proposed in Novák at al. 2018 [12] and Frątczak-Łagiewska & Matuszewski 2018 [18]. However, both of them are limited to the third instar only, thus limiting its practical use. When dealing with larval material in forensic entomology, species identification is only the first part of the challenge as specimens have to be identified to instars as well. Without such knowledge it is impossible to estimate the larval age accurately, which is crucial for the estimation of PMI in forensic entomology. Larval instars of *T. sinuatus* can be so far identified based on statistical models when three character measurements (distance between dorsal stemmata, and width of protergum and mesonotum) are provided [14]. This approach has its advantages and disadvantages as we argued previously [12]. Nonetheless, the morphological characters that we report in this article could be used to provide validation of the model or even replace it altogether.

Many of the characters for instar identification are shared between *T. sinuatus* and *T. rugosus.* The latter was already described in detail [12]. These characters are provided in the form of a dichotomous identification key in chapter **Identification key to *T. sinuatus* and *T. rugosus* larval instars** above**.**

Additionally, *T. sinuatus* instars can be easily distinguished based on the coloration of their dorsal side. The third instar has white distal ends of almost all paraterga (Fig. 4j), the second instar has only small white marks in the middle of protergal paraterga (Fig. 4k). This pattern can sometimes be observed on other tergites of the second instar larvae, but it is individually variable. The first instar is always uniformly dark (Fig. 4l) and closely resembling the first instar of *T. rugosus*. However, they appear to differ in the length of the first segment of labial and maxillary palpi (Fig. 1).

We have gathered some evidence that qualitative characters for instar identification could be shared among the species of genus *Thanatophilus*. Besides the reported similarities between species of *T. sinuatus* and *T. rugosus*, we also examined a limited larval material of *T. dentigerus*. All three species share the allometry in length of urogomphi segments (length of the first segment increases rapidly in the second instar, while the second segment does not). In addition, the femur of all legs becomes bicolored (dorsal side is darker than ventral part) in the third instar(the femurs of first and second instars are monochromatic). On the other hand, the color of tergites remains consistent throughout the larval development, which is the same as in *T. rugosus*, but different from *T. sinuatus* as we discussed earlier. We could not confirm if the shape of tentorium changes between instars in *T. dentigerus* due to limited number of specimens in the first and second instar category.

Adults of *T. sinuatus* breed willingly when provided with food and material for egg laying. We did not encounter many setbacks following the breeding methodology suggested by Ridgeway at al. 2014 [1]. The constant temperature and photoperiod (20°C and 16/8 light cycle) in the climatic chambers, where the breeding took place, resulted in steady production of eggs, thus we did not have to experiment with diferent environmental conditions. the photoperiod. Nonetheless, this is in contrast to our experiences with its sibling species, *T. rugosus* [12] as it was unable to breed under the abovementioned conditions and a change of light cycle to 12/12 was necessary to promote the production of eggs.

Both species (*T. sinuatus* and *T. rugosus*) were considered very similar in their occurrence patterns, both spatial and temporal. Although, Frątczak-Łagiewska & Matuszewski 2018 [18] recently suggested that these two species differ in their seasonality in order to promote resource partitioning and therefore lower the resource competition between them. They observed larvae of *T. sinuatus* occurring throughout the year up to August, but *T. rugosus* larvae were not recorded past mid-June. Our findings about different photoperiod requirements support their hypothesis stating that *T. sinuatus* is able to breed later in the season when the photoperiod is closer to 18:6 (light:dark) hours ratio, while *T. rugosus* restricts its breeding to earlier months of the year when the photoperiod is closer to 12:12 ratio.

The length of the development under the same temperature was very similar in both species, *T. sinuatus* (41.85 days) and *T. rugosus* (45.48 days) [12]. Both of them spend major parts of their life as third instar larvae and pupae but progress very rapidly through earlier stages (egg, first and second larval instars). The larvae of *T. sinuatus* hatch after approximately three days (2.83 days) at 20°C. This period can be considered short compared to some larder beetles (genus Dermestes), which take around eight days on average at the same temperature [25, 26]. However, the length of egg development at 20°C is very similar to *T. mutilatus [T. capensis]* (3.58 days), *T. micans* (3.66 days) and *T. rugosus* (3.34 days) [1, 12]. This somewhat shared trait, among the members of the genus could be caused by a number of factors including ecological ones. One of the possible explanations would be timing of the hatching of larvae in the optimal stage of carrion decay, as all these species share the preference for the same type

of food source in the same stage of decomposition, even though they compete for it with other species (e.g., blowflies from family Calliphoridae) [1, 27–29].

We have observed several cases of cannibalism among the larvae of *T. sinuatus*. These cases were rather limited but they occurred in spite of the fact that food was provided *ad libitum*. Cannibalized specimens were often smaller or at some disadvantage (freshly molted specimens). It should also be noted that some of these specimens were probably related (siblings). We observed this pattern among the larvae of *T. rugosus* as well [12]. This behavior could be promoted by breeding conditions within the limited space of Petri dishes, but not necessarily so. Such behavior could also suggest some nutritional needs that are not met when feeding strictly on decaying meet. Thus, this could be compensated by cannibalism or predation of smaller competitors (like Calliphoridae) under natural conditions [28]. Further studies of feeding habits under natural conditions are nevertheless needed to provide support for either of such hypotheses.

It is a common belief among taxonomists that color is rarely a good character for identification, as it can change in time and vary among specimens. We have to partially agree with that statement as the color stability of *T. sinuatus* larvae can vary and is strongly affected by how the specimens were treated and preserved. During the preparation of larval specimens we noticed that the larvae killed in ethyl acetate fumes and directly stored in alcohol tend to darken on otherwise white features such as distal ends of paraterga of third and second instar, ventral sides of femur in third instar and all desclerotized parts on ventral side of all instars. The color change complicates not only instar identification, but also species identification as darker specimens of *T. sinuatus* could be mistaken for *T. rugosus*.

The process of darkening is quite rapid and can be observed after only a few days of storage. To prevent this deterioration or at least prolong the color stability of the specimen, it is necessary to place them in hot water right after death and only then store them in alcohol. The water should be slightly below boiling point (90–95°C) for desired effect to occur. Higher temperature could result in rupture of softer parts of cuticle and cause irreversible damage to the specimen. We also do not recommend skipping the first step of killing the animals in ethyl acetate fumes as they have tendency to curl and stiffen in that position when killed directly by hot water [30], which is inconvenient for handling and measuring of the specimens.

This article presents re-description of all developmental stages of *T. sinuatus*, which is one of the most common and widely spread necrophagous species of beetles (see [4, 18, 27, 31, 32]). The utility of the species for the field of forensic entomology is undeniable as it has tight ecological association with its food source (development can take place only on carrion [33]) and is reported frequently from human remains or other large vertebrates (e.g., [2, 16, 34]). To increase the accessibility of the text to non-professional entomologists we provide a key for instar identification of the *T. sinuatus* and its close relative *T. rugosus*. Additionally, we present the key for identification of those two species in every larval instar along with information about the biology of *T. sinuatus*, including the developmental length of all stages under constant laboratory conditions, and notes on its behavior. We believe that these results can help increase the value of this species as a bioindicator for forensic entomology.

Figure captions

Fig. 1 Boxplots of labial palpomers length in all three larval instars of *Thanatophilus sinuatus*: palpomere I (**a**) and palpomere II (**b**). Horizontal lines within the boxes indicate median values; upper and lower boxes indicate the 75th and 25th percentiles, respectively; whiskers indicate the values with the 1.5 interquartile ranges; small, black dots are outliers.

Fig. 2 Boxplots presenting the head width of all larval instars of *Thanatophilus sinuatus.* Horizontal lines within the boxes indicate median values; upper and lower boxes indicate the 75th and 25th percentiles, respectively; whiskers indicate the values with the 1.5 interquartile ranges; small, black dots are outliers.

Fig. 3 Boxplots presenting the length ratio between: urogomphi (URII and URI) (**a**) and labial segments (LPII and LPI) (**b**) of all three larval instars of *Thanatophilus sinuatus*. Horizontal lines within the boxes indicate median values; upper and lower boxes indicate the 75th and 25th percentiles, respectively; whiskers indicate the values with the 1.5 interquartile ranges; small, black dots are outliers.

Fig. 4 *Thanatophilus sinuatus*: dorsal habitus of third instar (**a**); second instar (**b**) and first instar (**c**) larva. Ventral habitus of third instar (**d**); second instar (**e**) and first instar (**f**) larva. Lateral view of third instar (**g**); second instar (**h**) and first instar (**i**) larva. Differences of pigmentation of paratergites among third instar (**j**); second instar (**k**) and first instar (**l**) larva. Left maxilla of third instar larva in ventral view (**m).** Labium of third instar larva in dorsal view (**n**). Length differences among labial palpi of first instar (**o**); second instar (**p**) and third instar larva (**q**). Abbreviations: csp – cuticular spines on lacinia; flr – light-pigmented rim on frontal edge of sternites of second instar larvae; gb – brush of setae on galea; lig – ligula; sls – speckled laterosternites of third instar larva; wf – white venter of femur of third instar larva.

Fig. 5 *Thanatophilus sinuatus*: head of second instar larva in dorsal view (**a**); detail of head surface in dorsal view of first instar (**b**) and second instar (**c**) larva. Labrum, pharynx and epipharynx of third instar larva in ventral view (**d**). Tentorium of third instar larva in posterior (**e**); dorsal (**f**) and lateral (**g**) view (tentorium consisting of sclerotized features marked in dark-blue and hyaline/membranous features marked in light-blue). Abbreviations: aa – anterior arm; atp – anterior tentorial pit; bs – bulbous sensorium on epipharynx; cp – cibarial plates on pharynx; da – dorsal arm; esfa – epicranial stem with frontal arms (marked by dotted line); fa – frontal arm; ihl – inner hyaline lobe of anterior arms; ohl – outer hyaline lobe of anterior arms; os – occipital suture; pa – posterior arms; ptb – posterior tentorial bridge; sb – short sclerotized arms connected with filamentous secondary bridge growing dorsally from the middle of posterior arms.

Fig. 6 *Thanatophilus sinuatus*: right antenna of third instar larva in ventral view (**a**); apex of second instar antennomere III (**b**); detail of third instar antennal sensorium and neighboring sensilla in lateral (**c**) and dorsal (**d**) view. Urogomphus of first instar (**e**), second instar (**f**) and third instar larva (**g**). Pygopod (**h**). Abbreviations: amI – antennomere I; amII – antennomere II; amIII – antennomere III; bs – bulky sensilla next to sensorium; cs – circle of button-like sensilla on sensorium; las – long apical sensilla; sas – short articulated sensilla; sp – sensillar pit; sps – short, peg-like sensilla.

Fig. 7 *Thanatophilus sinuatus*: maxillo-labial complex of second instar larva in ventral view (**a**); apex of maxillary palpomere III (**b**) and labial palpomere II (**c**). Detail of ligula (**d**) and labral apex (**e**) in frontal view. Abbreviations: bs – bulbous sensorium on epipharynx; gb – brush of setae on galea; las – labral apical seta; lig – ligula; lpI – labial palpomere I; lpII labial palpomere II; m – mentum; mpI –

maxillary palpomere I; mpII – maxillary palpomere II; mpIII – maxillary palpomere III; mpf – maxillary palpifer; pm – prementum; sm – submentum.

Fig. 8 *Thanatophilus sinuatus*: third instar larva right mandible in posterior (**a**) and ventral (**b**) view and left mandible in ventral (**c**) and posterior (**d**) view. Deposition of mandibles in head capsule of third instar larva in frontal view (**e**). Detail of scissoral teeth and their abrasion on right (**f**) and left (**g**) mandible of third instar larva in posterior view. Abbreviations: at – apical tooth; lds – seta on laterodorsal area of mandibular base; ms – seta on outer-lateral area in the mid-length of the mandible; sat – sub-apical tooth.

Fig. 9 *Thanatophilus sinuatus*: leg of third instar larva in lateral view (**a**). Detail of tarsal claw of third instar (**b**); second instar (**c**) and first instar (**d**) larva. Abbreviations: fs – longest seta of femur; ts – longest seta of trochanter.

Fig. 10 *Thanatophilus sinuatus*: pupa in ventral (**a**), lateral (**b)** and dorsal (**c**) view. Abbreviations: as – abdominal setae; el – future elytron; es – line of setae on the edge of protergum; ps – pronotal setae; sc – scutellum; us – urogomphal setae; wi – future wing.

Compliance with Ethical Standards

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Conflict of Interest: H. Šuláková is employee of Faculty of Environmental Sciences and Police of the Czech Republic.

Ethical approval: All applicable international, national, and/or institutional guidelines for the care and use of animals were followed.

References

1. Ridgeway JA, Midgley JM, Collett IJ, Villet MH (2014) Advantages of using development models of the carrion beetles Thanatophilus micans (Fabricius) and T. mutilatus (Castelneau) (Coleoptera: Silphidae) for estimating minimum post mortem intervals, verified with case data. Int J Legal Med 128:207–220 . doi: 10.1007/s00414-013-0865-0

2. Charabidze D, Vincent B, Pasquerault T, Hedouin V (2016) The biology and ecology of Necrodes littoralis, a species of forensic interest in Europe. Int J Legal Med 130:273–280 . doi: 10.1007/s00414-015-1253-8

3. Midgley JM, Richards CS, Villet MH (2010) The Utility of Coleoptera in Forensic Investigations. In: Amendt J, Goff ML, Campobasso CP, Grassberger M (eds) Current Concepts in Forensic Entomology. Springer Netherlands, Dordrecht, pp 57–68

4. Růžička J (2015) Silphidae. In: Löbl I, Löbl D (eds) Catalogue of Palaearctic Coleoptera Volume 2/1. Hydrophiloidea – Staphylinoidea, Revised and Updated Edition. Brill, Leiden & Boston, pp 291–304

5. Anderson RS, Peck SB (1985) The Insects and Arachnids of Canada, Part 13: The carrion beetles of Canada and Alaska (Coleoptera: Silphidae and Agyrtidae). Agriculture Canada, Ottawa

6. Navarette-Heredia JL (2009) Silphidae (Coleoptera) de México: diversidad y distribución. Universidad de Guadalajara, Guadalajara

7. Midgley JM, Villet MH (2009) Development of Thanatophilus micans (Fabricius 1794) (Coleoptera: Silphidae) at constant temperatures. Int J Legal Med 123:285–292 . doi: 10.1007/s00414-008-0280-0

8. Schawaller W (1981) Taxonomie und Faunistik der Gattung Thanatophilus (Coleoptera: Silphidae). Stuttgarter Beiträge zur Naturkunde, Ser A 351:1–21

9. Šustek Z (1981) Keys to identification of insects 2: Carrion beetles of Czechoslovakia (Coleoptera: Silphidae). Zprávy Československé Společnosti Entomol při ČSAV 2:1–47

10. Ratcliffe BC (1996) The carrion beetles (Coleoptera: Silphidae) of Nebraska. Bull Univ Nebraska State Museum 13:1–100 . doi: 10.1093/aesa/90.3.399

11. Nikolaev GV, Kozminykh VO (2002) The carrion beetles (Coleoptera: Agyrtidae, Silphidae) of Kazakhstan, Russia and adjacent countries. Kazak Universiteti, Almaty

12. Novák M, Jakubec P, Qubaiová J, et al (2018) Revisited larval morphology of Thanatophilus rugosus (Coleoptera: Silphidae). Int J Legal Med 132:939–954 . doi: 10.1007/s00414-017-1764-6

13. Daniel CA, Midgley JM, Villet MH (2017) Determination of species and instars of the larvae of the afrotropical species of Thanatophilus Leach, 1817 (Coleoptera, Silphidae). African Invertebr 58:1–10 . doi: 10.3897/AfrInvertebr.58.12966

14. Frątczak K, Matuszewski S (2016) Classification of forensically-relevant larvae according to instar in a closely related species of carrion beetles (Coleoptera: Silphidae: Silphinae). Forensic Sci Med Pathol 12:193–197 . doi: 10.1007/s12024-016-9774-0

15. Jakubec P, Růžička J (2012) Distribution of open landscape carrion beetles (Coleoptera: Silphidae) in selected lowlands of the Czech Republic. Klapalekiana 48:169–189

16. Bonacci T, Greco S, Brandmayr TZ (2011) Insect Fauna and Degradation Activity of Thanatophilus species on Carrion in Southern Italy (Coleoptera: Silphidae). Entomol Gen 33:63–70 . doi: 10.1127/entom.gen/33/2011/63

17. Jakubec P, Růžička J (2015) Is the type of soil an important factor determining the local abundance of carrion beetles (Coleoptera: Silphidae)? Eur J Entomol 112: . doi: 10.14411/eje.2015.071

18. Frątczak-Łagiewska K, Matuszewski S (2018) Resource partitioning between closely related carrion beetles: Thanatophilus sinuatus (F .) and Thanatophilus rugosus (L .) (Coleoptera : Silphidae). Entomol Gen 37:143–156 . doi: 10.1127/entomologia/2017/0352

19. von Lengerken H (1929) Studien über die Lebenserscheinungen der Silphini (Col.). XI-XIII. Thanatophilus sinuatus F., rugosus L. und dispar Hrbst. Zeitschrift für Morphol und Ökologie der Tiere 14:654–666

20. Novák M (2017) Redescription of immature stages of central European fireflies, Part 1: *Lampyris noctiluca* (Linnaeus, 1758) larva, pupa and notes on its biology (Coleoptera: Lampyridae: Lampyrinae). Zootaxa 4247:429–444

21. Lawrence JF, Ślipiński SA (2013) Australian Beetles. Volume 1: morphology, classification and keys. CSIRO Publishing, Collingwood

22. von Lengerken H (1938) Beziehungen zwischen der Ernährungsweise und der Gestaltung der Mandibeln bei den Larven der Silphini (Coleopt.). Zool Anz 122:171–175

23. Paulian R (1941) Les premiers états des Staphylinoidea. Étude de morphologie comparée. Mémoires du Muséum Natl d'Histoire Nat (Nouvelle Ser 15:1–361

24. Xambeu PJ V. (1892) Moeurs et métamorphoses d'insectes (Suite). Ann la Société Linnéenne Lyon 39:137–194

25. Coombs CW (1981) The development, fecundity and longevity of Dermestes ater Degeer (Coleoptera: Dermestidae). J Stored Prod Res 17:31–36 . doi: 10.1016/0022-474X(81)90029-1

26. Coombs CW (1979) The effect of temperature and humidity upon the development and fecundity of Dermestes haemorrhoidalis Küster and Dermestes peruvianus Laporte de Castelnau (Coleoptera: Dermestidae). J Stored Prod Res 15:43–52 . doi: 10.1016/0022-474X(79)90011-0

27. Dekeirsschieter J, Verheggen FJ, Haubruge E, Brostaux Y (2011) Carrion beetles visiting pig carcasses during early spring in urban, forest and agricultural biotopes of Western Europe. J Insect Sci 11:Number 73

28. Matuszewski S, Bajerlein D, Konwerski S, Szpila K (2011) Insect succession and carrion decomposition in selected forests of Central Europe. Part 3: Succession of carrion fauna. Forensic Sci Int 207:150–163 . doi: 10.1016/j.forsciint.2010.09.022

29. Matuszewski S, Bajerlein D, Konwerski S, Szpila K (2010) Insect succession and carrion decomposition in selected forests of Central Europe. Part 2: Composition and residency patterns of carrion fauna. Forensic Sci Int 195:42–51 . doi: 10.1016/j.forsciint.2009.11.007

30. Midgley JM, Villet MH (2009) Effect of the killing method on post-mortem change in length of larvae of Thanatophilus micans (Fabricius 1794) (Coleoptera: Silphidae) stored in 70% ethanol. Int J Legal Med 123:103–8 . doi: 10.1007/s00414-008-0260-4

31. Jakubec P, Růžička J (2017) Spatial distribution modeling of Thanatophilus sinuatus (Coleoptera: Silphidae) in the Czech Republic. In: 17th International Multidisciplinary Scientific GeoConference SGEM 2017. International Multidisciplinary Scientific Geoconference, Sofia, pp 875–882

32. Matuszewski S, Szafałowicz M (2013) Temperature-dependent appearance of forensically useful beetles on carcasses. Forensic Sci Int 229:92–99 . doi: 10.1016/j.forsciint.2013.03.034

33. Sikes D (2008) Carrion beetles (Coleoptera: Silphidae). In: Capinera JL (ed) Encyclopedia of Entomology. Volume 1: A - C, 2nd ed. Springer, Berlin, pp 749–758

34. Matuszewski S, Frątczak K, Konwerski S, et al (2016) Effect of body mass and clothing on carrion entomofauna. Int J Legal Med 130:221–232 . doi: 10.1007/s00414-015-1145-y

Addendum – figures used in the article

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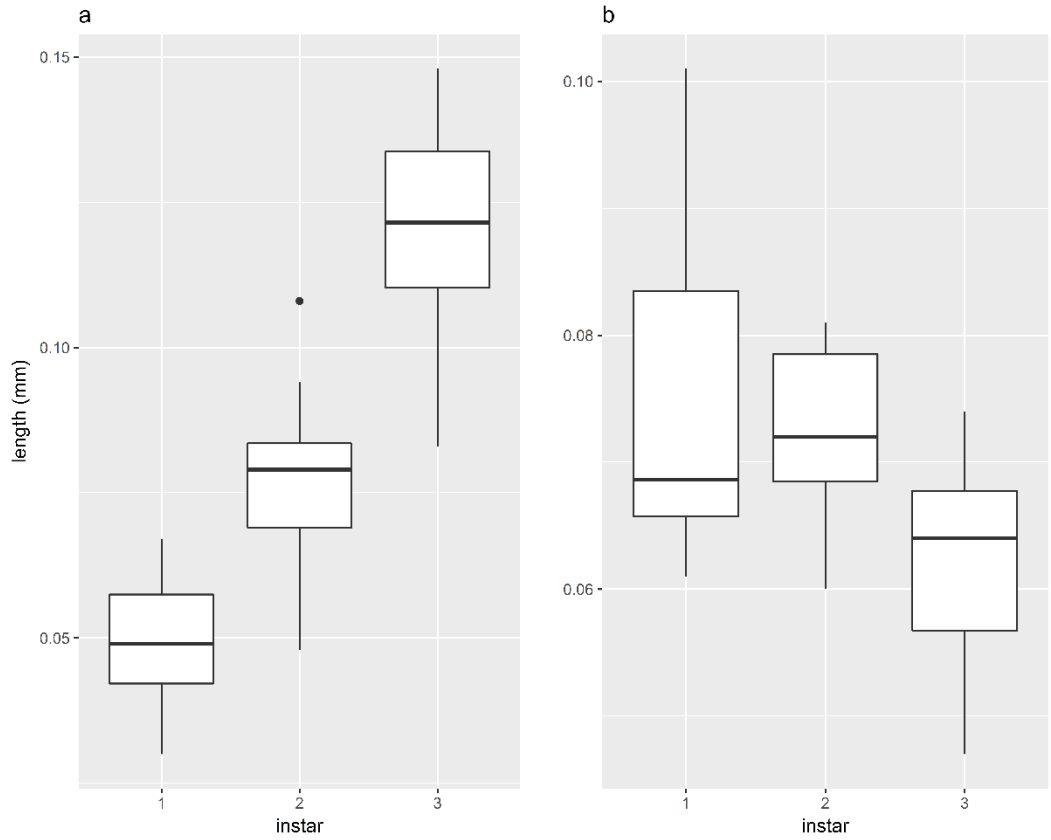

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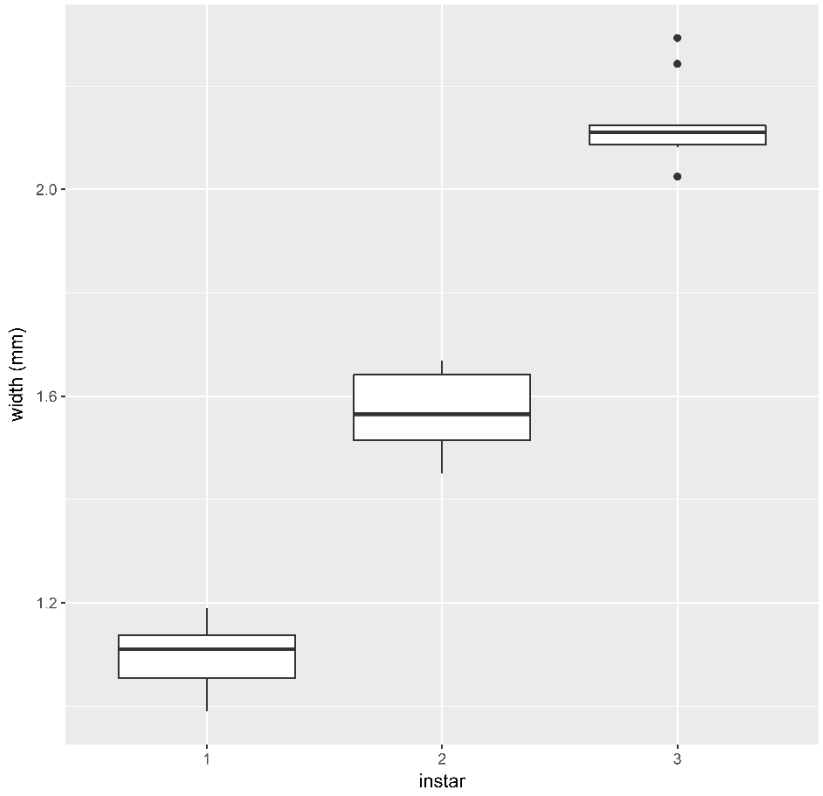

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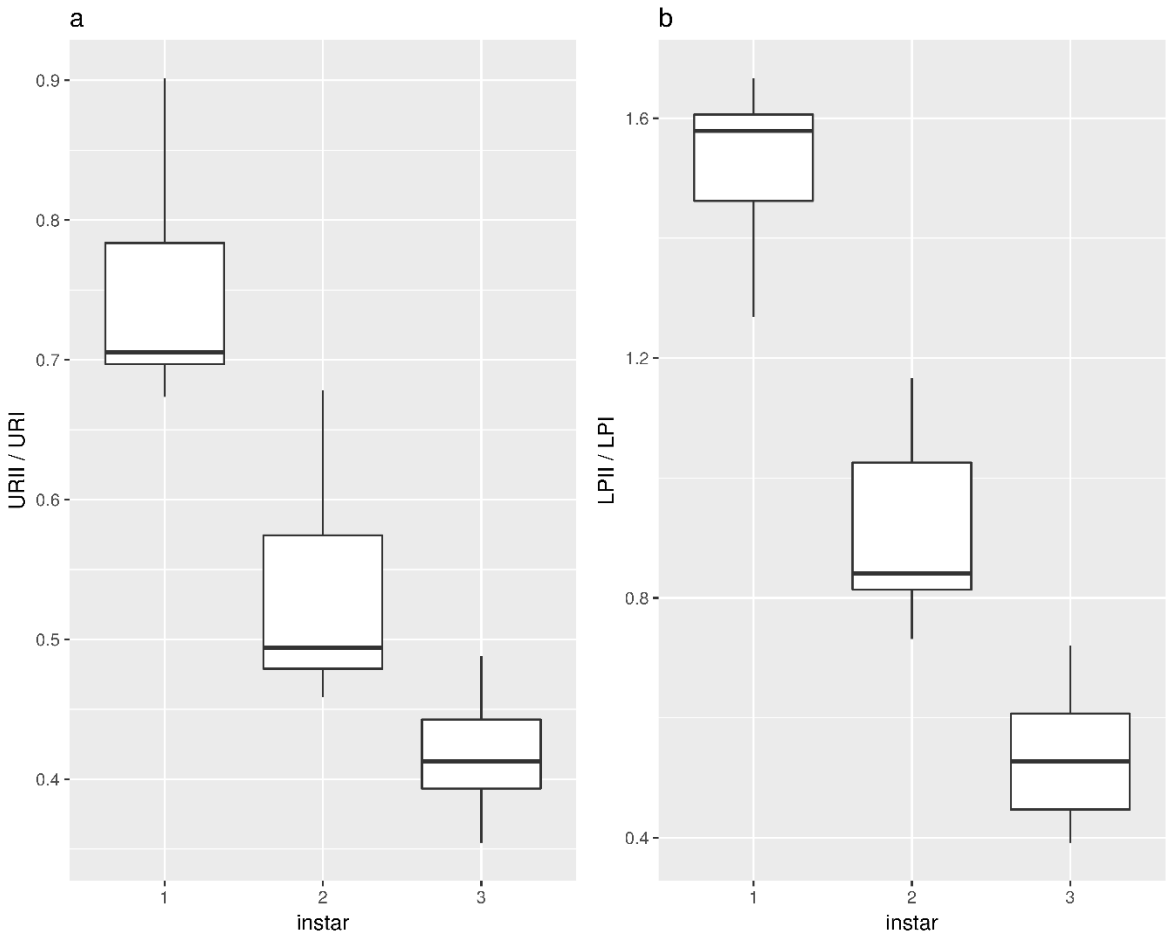
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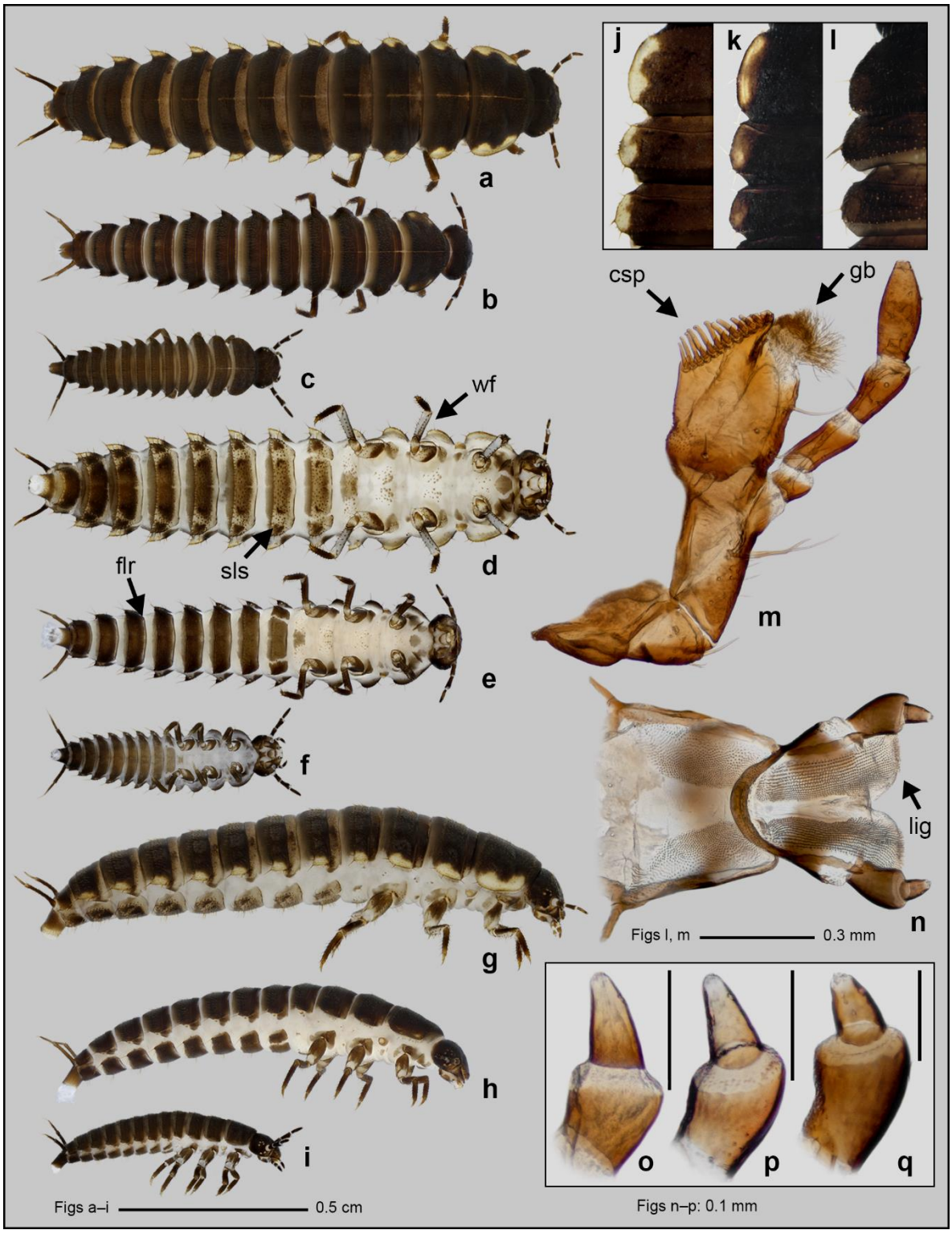
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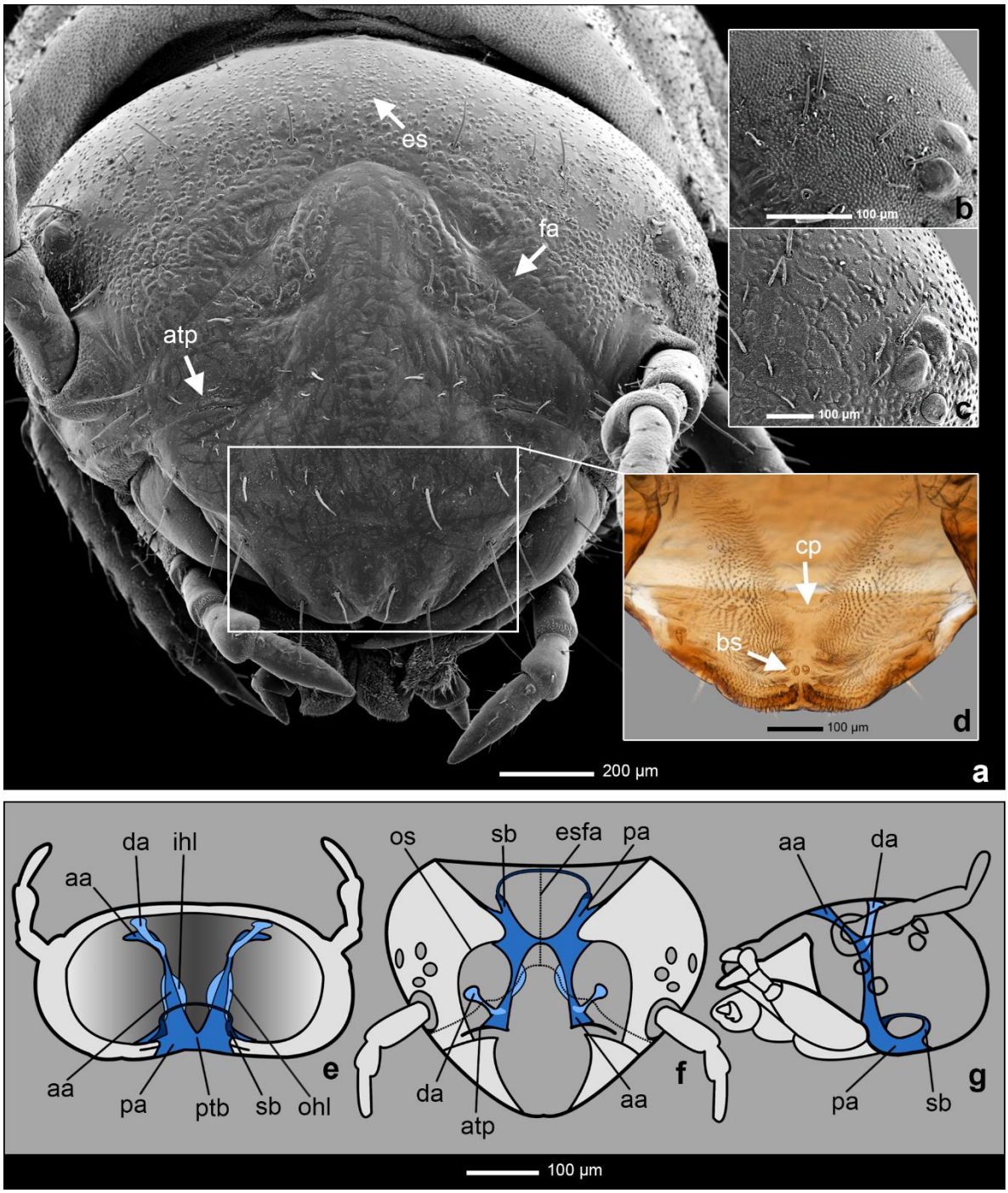
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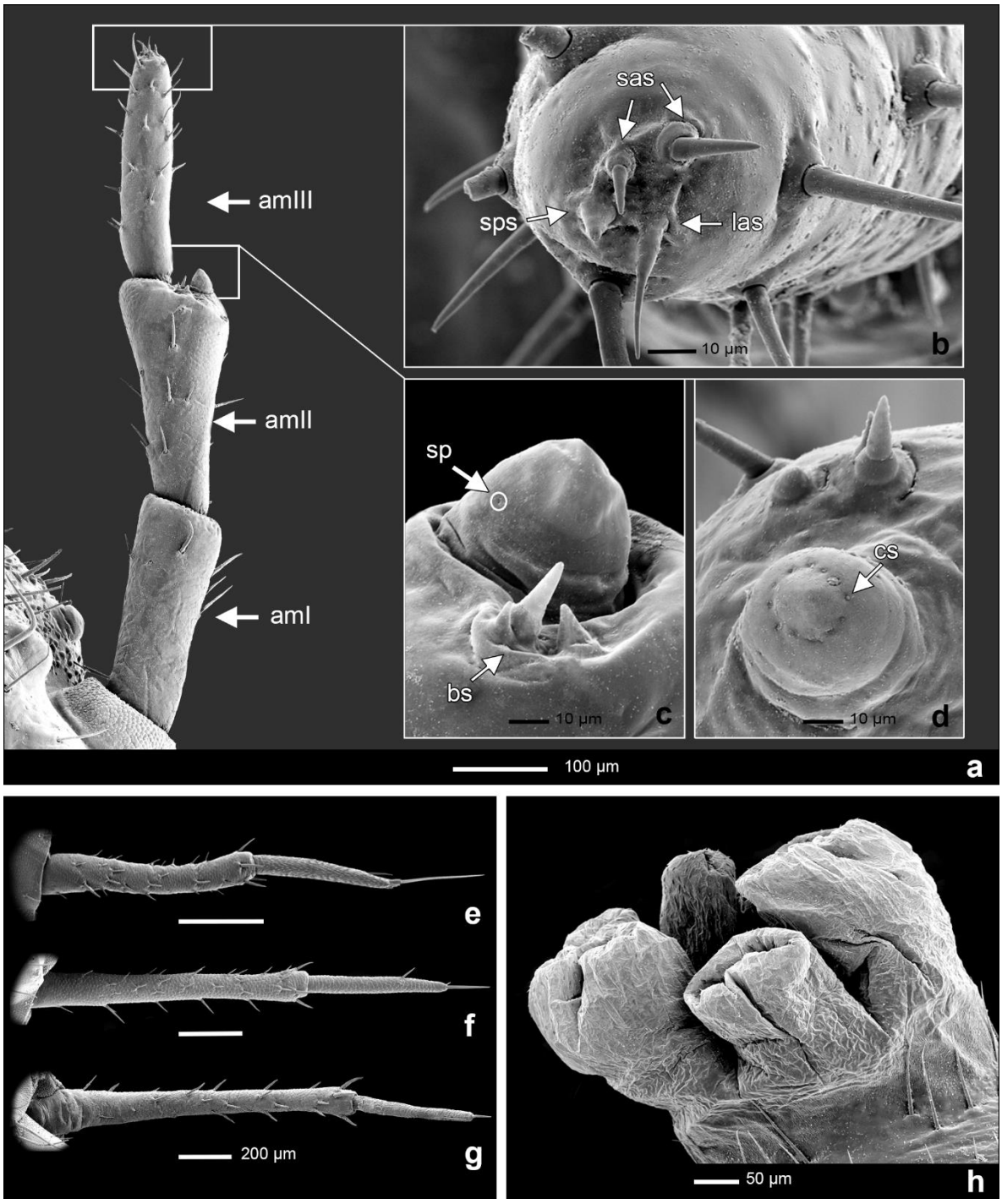
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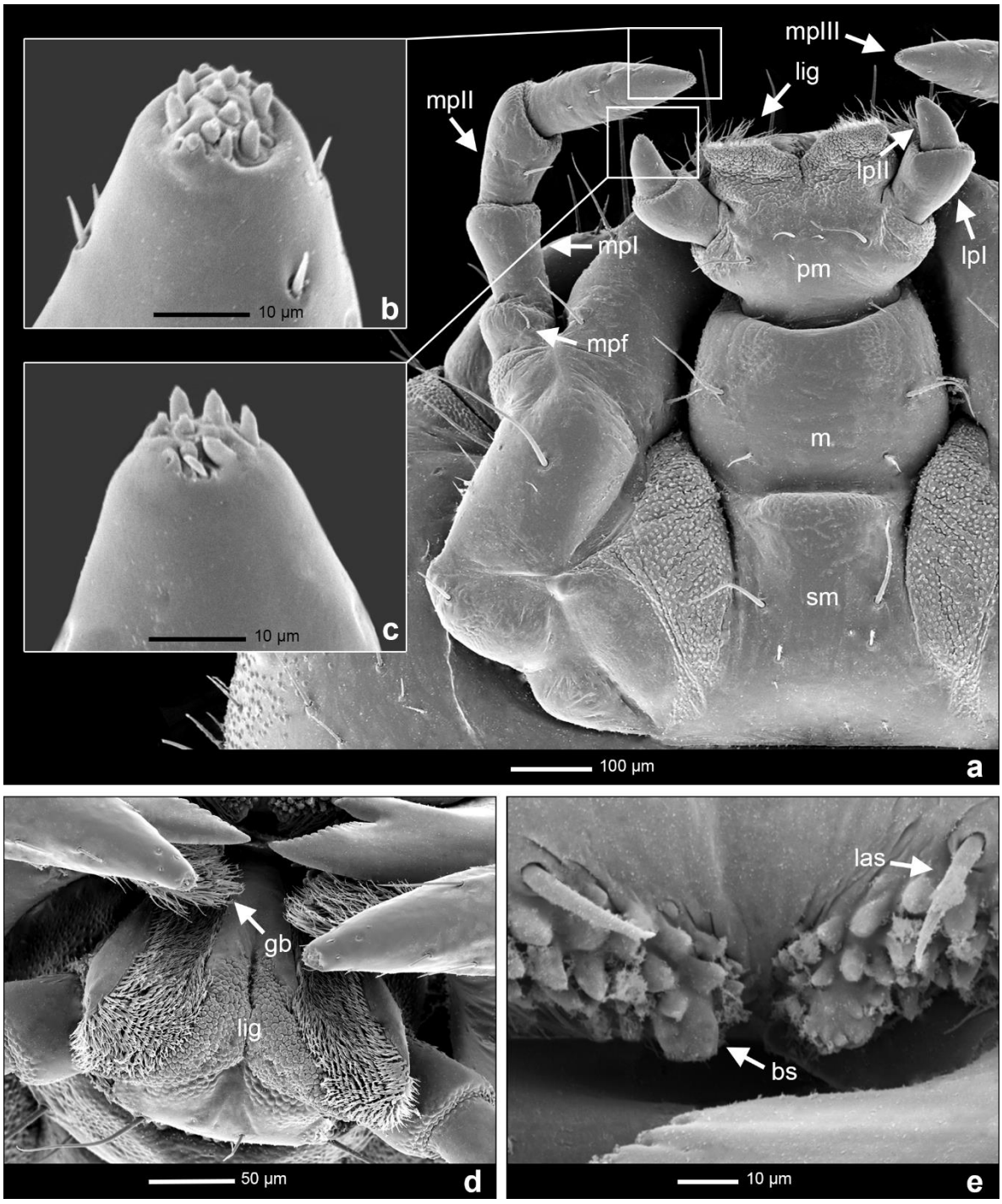
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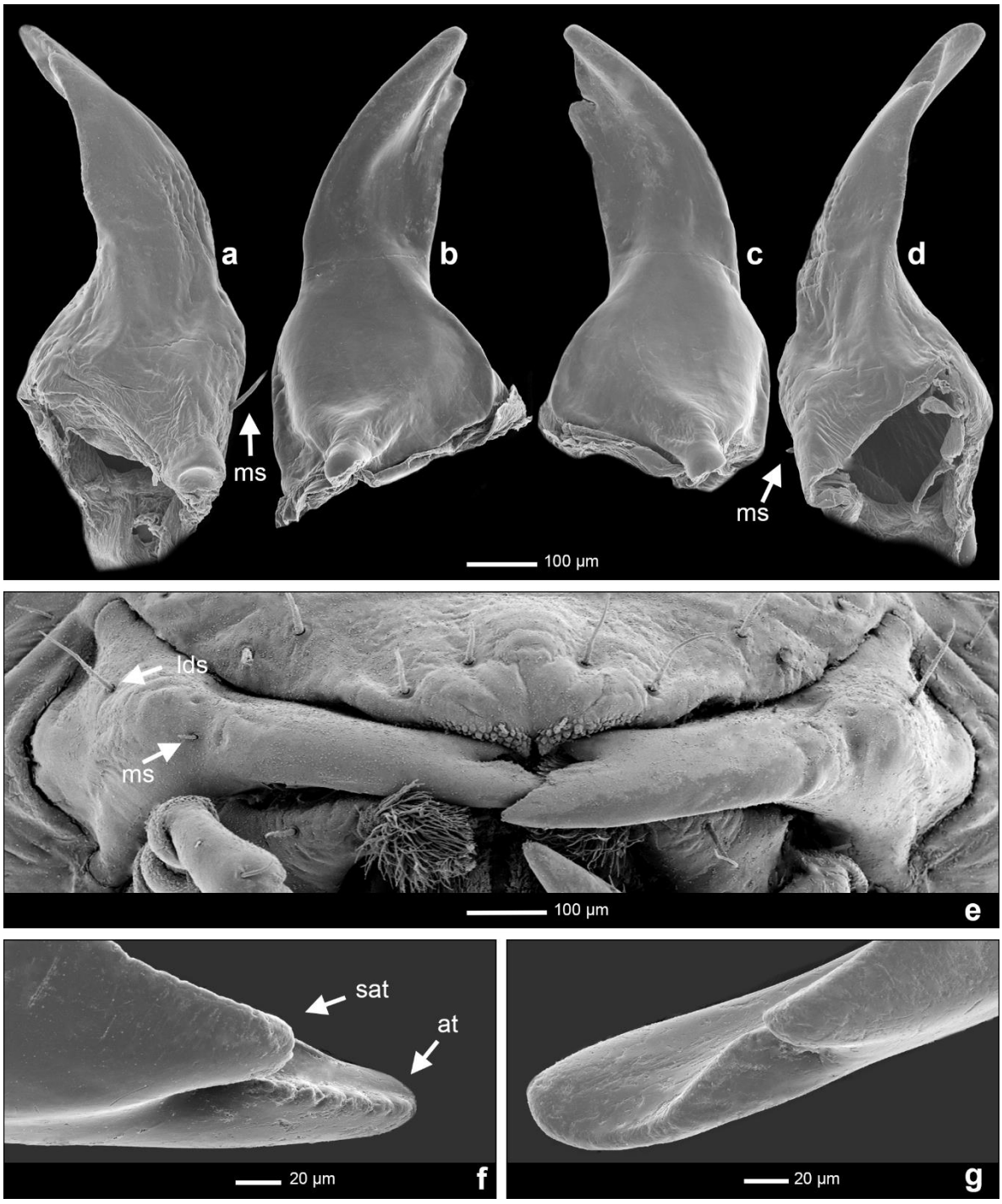

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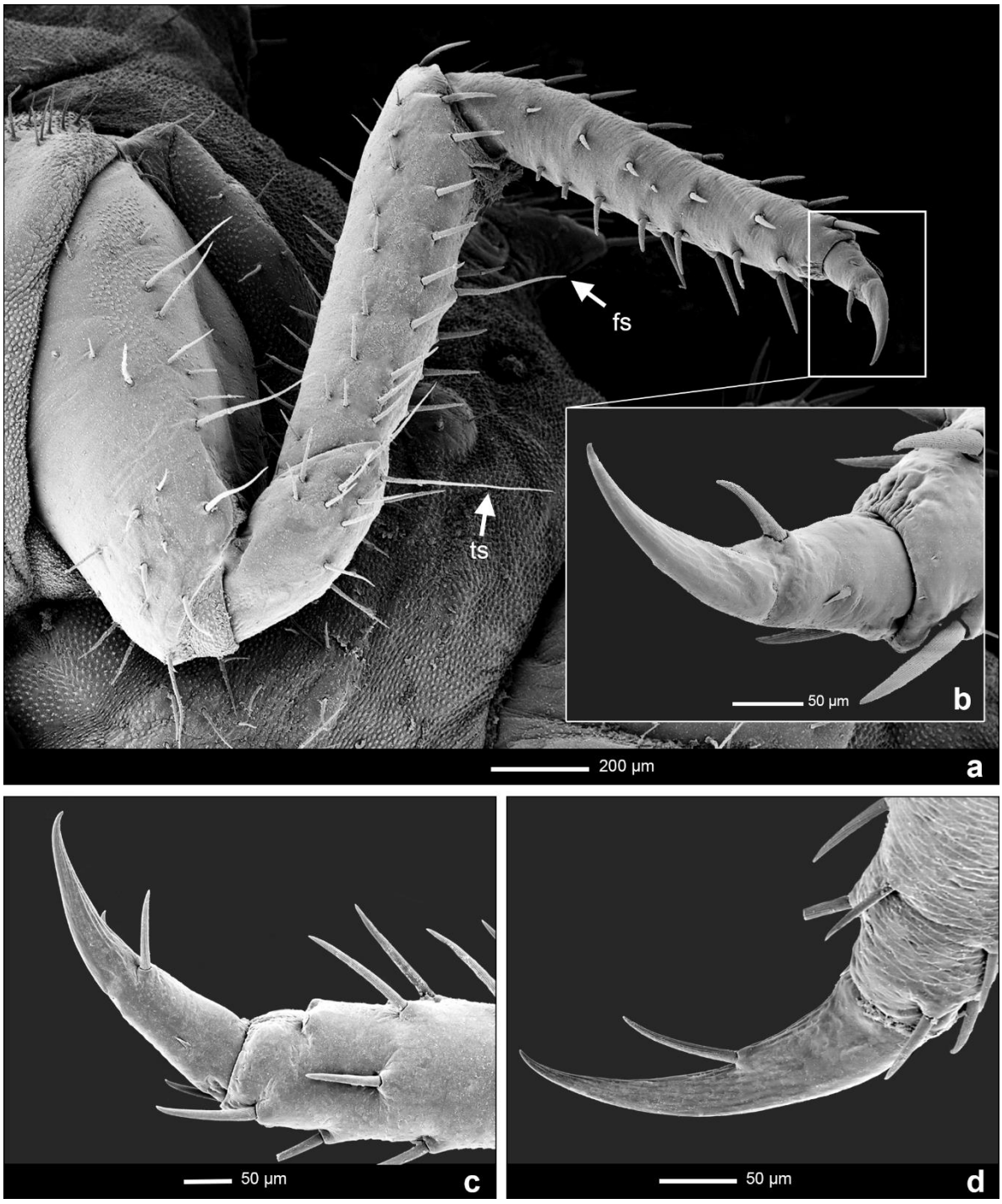

Figure 10: