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Larval morphology of Central European species of fireflies (Coleoptera: Lampyridae)

Larvální morfologie středoevropských druhů světlušek (Coleoptera: Lampyridae)

Diplomová práce

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Thesis title

Larval morphology of central European species of fireflies (Coleoptera: Lampyridae)

Objectives of thesis

Světlušky (Coleoptera: Lampyridae) jsou fascinující skupinou, známou složitými etologickými projevy a bioluminiscencí (Branham 2010). Larvy světlušek jsou predátoři s unikátní morfologií kusadel (LaBella & Lloyd 1991, Branham 2010), v poslední době roste zájem o detailní studium jejich morfologie zejména u orientálních a neotropických druhů (např. Archangelsky 2004, 2010, Deheyn & Ballantyne 2009, Fu et al. 2012, Ballantyne et al. 2013). Ve střední Evropě se vyskytují pouze tři druhy světlušek, reprezentující tři rody (Hůrka 2005, Geisthardt & Sato 2007), jejich morfologie je jen nedostatečně známá (Korschefsky 1951, Klausnitzer 1994, Burakowski 2003).

Cílem diplomové práce bude detailně popsat morfologii larev vzrostlých instarů všech tří našich druhů, dokumentovat detailní stavbu zejména přívěsků hlavy a celkového habitu těla pomocí barevných makrofotografií a fotografií z rastrovacího elektronového mikroskopu, a spojit nalezené rozdíly s literárními informacemi o jejich odlišné ekologii. Cílem je také revidovat existující klíče k určování středoevropských zástupců.

Methodology

Larvy byly sebrány v terénu v předchozím roce, další část larev je zapůjčena od dr. Petra Švách y (Entomologický ústav AVČR, České Budějovice). Pro morfologické studium bude část larev vyvařena v KOH a fotografována v glycerolu či alkoholu, část larev bude usušena v mírném vakuu, namontována na kovové disky, pozlacena a studována SEM na mikroskopickém servisním pracovišti PřFUK v Praze. Detailní morfologické popisy budou sepsány s terminologií používanou v recentních pracích o larvách této skupiny (viz výše), vypracován bude i vylepšený dichotomický klíč k určování středoevropských larev. Ilustrace budou smontovány do tabulí, plánované je pozdější použití textu i ilustrací v navazující publikaci.

Schedule for processing

léto - podzim 2013: shromáždění larválního materiálu z ČR a Slovinska, zapůjčení materiálu z EúU AVČR, rešerše literatury; prosinec 2013 - únor 2014: fotodokumentace, SEM; únor - březen 2014: morfologické popisy, tvorba dichotomického klíče; duben 2014: finalizace DP.

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Keywords

Coleoptera, Lampyridae, larvální morfologie, determinační klíč, střední Evropa

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DECLARATION

I hereby declare, that this Master's Thesis was elaborated independently under the guidance of Mgr. Jan Růžička, Ph.D., and that I quoted all the literary sources used.

In Prague, April 15 ……………………………………

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ABSTRACT

From approximately two-thousand known firefly species, only three occur in the Czech Republic. While the descriptions of the adult morphology of *Lampyris noctiluca* (Linnaeus, 1767), *Lamprohiza splendidula* (Linnaeus, 1767) and *Phosphaenus hemipterus* (Geoffroy, 1762), are fairly known, the descriptions of their larval morphology are out-dated and detailed information regarding their ecology is either scattered or missing. This work thus provides detailed re-description of mature-instar larvae of the three abovementioned species, together with photographic documentation. A general and a detailed key to Central European lampyrid larvae is compiled and provided in this work. Habitus macrophotography are included, together with detailed images from Scanning Electron Microscope. Information about life history, ecology and behaviour is then summarized for each of the species and correlated with the morphological features observed. All three lampyrid species of the Czech Republic occur sympatrically, but differ greatly in their morphology. The differences, next to general body shape, colouration, and position of photic organs, lie mainly in different types of setation, pattern of sensory organs on head appendages, morphology of mandibles and maxillae, layout of pleurites and many other small details described in this work. The significance of various morphological modifications are discussed in regard to the ecology of each species.

Keywords: Lampyridae, larvae, morphology, Lampyris, Lamprohiza, Phosphaenus

ABSTRAKT

Ze zhruba dvou tisíc známých druhů světlušek, se pouze tři druhy vyskytují na území České republiky. Zatímco morfologie dospělců druhů *Lampyris noctiluca* (Linnaeus, 1767), *Lamprohiza splendidula* (Linnaeus, 1767) a *Phosphaenus hemipterus* (Geoffroy, 1762), jsou relativně známé, popisy morfologie jejich larev jsou zastaralé a detailní informace týkající se ekologie jsou buď kusé, anebo chybí. Tato práce se zabývá detailní re-deskripcí vyšších instarů larev výše zmíněných druhů, společně s poskytnutím fotografické dokumentace. Je zde rovněž zpracován obecný i detailní klíč k určení středoevropských druhů. Součástí práce jsou makrofotografie habitů, společně s detailními fotografiemi pořízenými skenovacím elektronovým. mikroskopem. U každého druhu jsou shrnuty informace o jeho životním cyklu, ekologii a chování, a dány do souvislosti s pozorovanými znaky. Všechny tři druhy se sice v České republice vyskytují sympatricky, ovšem vzájemné rozdíly v morfologii jsou mezi nimi zřetelné. Kromě tvaru těla, barvy a umístění orgánů vyzařujících světlo, spočívají rozdíly především v odlišných typech ochlupení, smyslových orgánů, přívěsků hlavy, morfologie maxil a mandibul, pozice pleuritů a v mnoha dalších detailech popsaných v této práci. Význam různých morfologických modifikací je nakonec diskutován v souvislosti s rozdílnou ekologií každého druhu.

Klíčová slova: Lampyridae, larvy, morfologie, Lampyris, Lamprohiza, Phosphaenus

TABLE OF CONTENTS

1. INTRODUCTION

Fireflies (Coleoptera: Lampyridae) are a fascinating group of insects, known for their complex behavioural manifestations and the ability of bioluminescence (Fig. 1) (Branham 2010). Larvae of fireflies are fierce predators able to track down their prey, and with unique morphology of mandibles (LaBella & Lloyd 1991; Branham 2010). There has been a growing interest in morphology studies of both adults and larvae mainly in genera of Oriental and Neotropical regions (e.g. Archangelsky 2004, 2010; Deheyn & Ballantyne 2009; Fu et al. 2012; Ballantyne et al. 2013), where new species are discovered (Archangelsky 2004, 2010) and known species are being re-described in greater detail using modern technology (Ballantyne & Menayah 2002; Fu et al. 2012).

Figure 1 *Lamprohiza splendidula*, female © NEUROtiker 2006

In Central Europe, only three known lampyrid species occur, represented by three genera; *Lampyris noctiluca* (Linnaeus, 1767), *Lamprohiza splendidula* (Linnaeus, 1767); (also known as *Phausis splendidula*) and *Phosphaenus hemipterus* (Geoffroy, 1762) (Hůrka 2005; Geisthardt & Sato 2007). Descriptions of these species are brief and the morphology, namely morphology of larvae, is poorly known. Schematic illustrations are in many of these works present in variable quality, nevertheless detailed images are missing (Reitter 1911; Korschefsky 1951; Kratochvíl 1957; Medvedev & Ryvkin 1992; Klausnitzer 1994; Burakowski 2003).

2. GOALS OF THESIS

1. Re-describe higher-instar larval morphology of all Czech lampyrid species in greater detail than in previous works.
2. Document detailed composition of head appendages and the whole body habitus by colour macrophotography and images from Scanning Electron Microscope.
3. Connect the detected differences among examined species with information from literature about their different ecology.
4. Review existing keys to Central European lampyrid species recognition.

3. LAMPYRIDAE

3. 1. Taxonomy

The family Lampyridae belongs to the order of Coleoptera and superfamily Cantharoidea. This superfamily has not yet been comprehensively elaborated in the Czech Republic neither taxonomically nor faunistically. The last taxonomical research on Central European species was done by Freude in 1979 (Švihla 2005). The sister groups of Lampyridae are probably the Cantharidae and Lycidae (Bocáková et al. 2007).

Taxonomic position

The following classification was taken from Leschen et al. (2010):

Order: Coleoptera

Suborder: Polyphaga

Infraorder: Elateriformia

Superfamily: Cantharoidea (synonym Elateroidea)

Family: Lampyridae

3. 2. Phylogeny

The previous morphological studies within the Cantharoidea assumed a close monophyletic relationship among the families with the ability of bioluminescence. Apart from Lampyridae, the capability of light production can also be found in Phengodidae, Rhagophthalmidae and two independent genera of Elateridae (Branham 2010). Nevertheless, the recent studies show that although key features like the softness of body, neoteny (a state where the individual reaches sexual maturity while keeping juvenile traits) and bioluminescence are mainly present in beetles of abovementioned superfamily, their evolutionary origin is different. For instance, the glowing ability within Cantharoidea evolved at least in four independent ways according to molecular analysis. Therefore it is assumed, that closely related families can obtain similar traits independently (Bocáková et al. 2007; Branham 2010). This opinion is supported by molecular analysis which indicated biochemical differences in the composition of luciferase, mainly between families Lampyridae and Phengodidae (Viviani 2002).

Branham & Wenzel (2001), according to their morphological traits comparison, propose that Lampyridae themselves are not a monophyletic group and monophyly is probably present only in two out of seven recognized subfamilies, namely Photurinae and Luciolinae. The fact that lampyrids are not a monophyletic group is also supported by Stanger-Hall et al. (2007) stating, that North American lampyrid fauna did not originate from a single adaptive radiation, but is the result of several independent invasions instead. Different subgroups of Lampyridae in North America are in fact closely related to species from Europe, Latin America and Asia respectively (Stanger-Hall et al. 2007).

Nevertheless, disagreements still prevail regarding the question which species actually belong to Lampyridae family. Branham & Wenzel (2001) proposed a transfer of genera *Drilaster* Kiesenwetter, 1879; *Harmatelia* Olivier, 1910; and *Pterotus* LeConte, 1859 into Cantharoidea incertae sedis (of uncertain placement). Bocáková et al. (2007), on the other hand, claims that genus *Drilaster* belongs to Lampyridae family, although she doesn't propose any formal taxonomical changes. Stanger-Hall et al. (2007) then returns recently excluded genera *Pterotus* and *Rhagophthalmus* Motschulsky, 1854 back to Lampyridae. Even though the latest research of Bocáková (2007) and Stanger-Hall (2007) could be accepted as the most fresh insight into the problematic, the definite answer will be brought by future.

3. 3. Distribution

The Lampyridae include approximately 2000 species in 83 genera and 12 subfamilies, distributed mainly in relatively humid areas of Southeast Asia and Latin America (Branham 2010). Conversely, the species of this family are not very abundant in arid areas (Branham 2010). Lampyrid genera of North America are closely related to genera of Europe, Asia and Latin America (Stanger-Hall et al. 2007).

According to Viviani (2001), the last taxonomical reviews within the continents and subcontinents were done in 50's and 60's of the 20th century, while the work of potential taxonomists is being more and more complicated by expansion of civilization and consequent alteration of the landscape. Lloyd & Gentry (2009) state, that most problems seem to arise from habitat loss to development, lowering of water tables and light pollution (Fig. 2). An example of this drastic change is given by Lloyd (2006), recounting several undescribed species occurring in north central Florida in the years 1966 – 1980 which he has not sighted for more than a decade.

Figure 2 Light pollution © Thomas Hawk

In general, complex phylogeographic studies as well as taxonomical information of Lampyridae are fragmentary and difficult to access, since specific genera of fireflies are mostly found on more than only one continent (Stanger-Hall et al. 2007).

3. 4. Fireflies of Europe and Czech Republic

Lampyrids can be found in the most parts of Europe, although the presence is scattered thanks to this family's preference of humid warm sites and opened landscape (Hůrka 2005). The fireflies of Europe are classified within 2 subfamilies, 3 tribes, and 8 genera (Geisthardt & Sato 2007):

Subfamily: **Lampyrinae** Latreille, 1817

Tribe: **Lampyrini** Latreille, 1817

genus: *Lampyris* Geoffroy, 1762

genus: *Nyctophila* E. Olivier, 1884

genus: *Pelania* Mulsant, 1860

Tribe: **Photinini** E. Olivier, 1907

genus: *Lamprohiza* Motschulsky, 1853

(synonym *Phausis* LeConte, 1851)

genus: *Phosphaenopterus* Schaufuss, 1870

genus: *Phosphaenus* Laporte, 1833

Subfamily: **Luciolinae** Lacordaire, 1857

Tribe: **Luciolini** Lacordaire, 1857

genus: *Lampyroidea* A. Costa, 1875

genus: *Luciola* Laporte, 1833

Larvae of Czech lampyrid species generally have 5 instars, they overwinter and pupate in spring of the following year (Hůrka & Čepická 1978; Hůrka 2005). The adults emerge approximately after 10 days. These do not accept food anymore and are active during dusk and night (Hůrka & Čepická 1978; Hůrka 2005).

The adults of lampyrid species living in the Czech Republic live approximately 2 or 3 weeks (Hess 1920; Smith et al. 2009).

In the Czech Republic, three often sympatric lampyrid genera (De Cock 2003) of Lampyrinae, belonging to tribes Lampyrini and Lucidotini are present, as follows:

3. 4. 1. *Lampyris noctiluca* (Linnaeus, 1767)

Distribution

Genus *Lampyris* Linnaeus, 1767 consists of 60 described species, distributed predominantly in Palearctic region, of which 26 species occur in Europe and only one in the Czech Republic (Burakowski 2003; Geisthardt & Sato 2007). This firefly inhabits mostly warmer lowland areas with limestone substrate, but can be also found in mountains of high-altitudes, of up to 1800 m a.s.l. (Burakowski 2003; Hůrka 2005).

Life cycle

Developmental cycle takes 2 – 3 years. Pale yellow eggs are roughly spherical (with diameter ca. 1 mm) and may faintly glow for the first few days after oviposition (Tyler 2002). The time of hatching is negatively correlated with temperature of the environment and takes from 27 to 45 days. Towards the end of this period, an inner glow appears in the egg, signalling activation of the young larva's photic organ and upcoming hatching (Tyler 2002). The body length of first instar larva is ca. 5 mm and can be up to 23 mm long in the last instar. Larvae of *Lampyris noctiluca* overwinter twice and pupate in early summer. Pupa is olive green and will often glow in response to handling or vibration. The pupation lasts ca. 8 – 12 days for a female and 11 – 15 days for a male (Tyler 2002). Individuals appear mature in May and July, and in the mountainous regions, they can be found until September. Females usually appear a few days earlier than males. After mating, female lays approximately 50 – 100 eggs (depending on her size) and dies within a few days (Tyler 2002; Burakowski 2003; Hůrka 2005).

Adults

Members of this genus are characterized by small mandibles with a broad base and pointy narrow apex. Pronotum is almost semicircular, with poorly distinguishable transparent areas or lacking them completely. Distinct sexual dimorphism is developed (Burakowski 2003).

Male (Fig. 3)

Length 10 – 12 mm. Body pigmentation brown to dark brown, legs lighter coloured; pronotum with yellowish lateral edges, elytra brown. Eyes very large, tangential anteriorly. Pronotum with almost straight base and pointy posterior edges. Elytra with 3 – 4 ribs shortened in the posterior region, covering the whole abdomen. Photic organs form two luminous spots on ventrite VI (morphologically on sternite VII). Aedeagus with asymmetrical phallobase; parameres of subtriangular shape, with semi-oval appendage medially on the apical half; oblong phallus basally connected to parameres, with lateroapical plates (Burakowski 2003; Hůrka 2005).

Figure 3 *Lampyris noctiluca*, male © Josef Dvořák

Female (Fig. 4)

Length 15 – 20 mm, wingless, vermiform. The body and legs reddish brown, lateral edge of pronotum, rear edges of meso- and metanotum and the central line on tergites yellowish. Eyes small, non-tangential. The last segment of the maxillary palpus triangular. Pronotum bent dorsally on the edges, with round posterior edges. Photic organs on abdominal segments VI – VIII, consisting of large luminous bands on ventrite V and VI, and a luminous spot on either side of the ventrite VII. Tergite VIII rounded posteriorly, ventrite VII emarginated, with short membranous *spiculum ventrale* anteriorly. Ovipositor membranous; valvifers with short trabecular support, valves setose, with a small styliform appendage (Tyler 2002; Burakowski 2003; Hůrka 2005).

Figure 4 *Lampyris noctiluca*, female © Stanislav Krejčík

Behaviour

According to Tyler (2002), the larva of *Lampyris noctiluca* can produce light in three different ways. Firstly, 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 the potential predators. Secondly, 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 the adult female's, might be just part of the preparation for adulthood, at a time when the larva's body is undergoing all sorts of internal changes. Third type of photic display is sometimes produced during movement. It consists of definite pulses of light lasting ca. 2 seconds, separated from the next by a longer interval of darkness lasting ca. 4 seconds. 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 (Tyler 2002; M. Novák unpublished observation).

Lampyris noctiluca larvae are reported to follow 2-day-old slime-trails forward, tracking the snails, presumably and remarkably demonstrating that they are able to detect polarization in dry, if not stale, trails (Lloyd 2008).

The larvae which are ready to pupate the same year seem to switch into diurnal activity, and can often be seen striding purposefully 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 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 female usually begins to glow soon after dusk, with start of her display being triggered when the light intensity around her falls below 1 lux (Dreisig 1975; Hůrka 2005). The male does not generally become airborne until half an hour or more after the females begin their display, and usually finishes his search flight before many of the females have stopped glowing. During the display, the female may stay close to the ground, or may climb half a metre or more up a grass stem to make herself more conspicuous to searching males. Because the light organ is set on the ventral side of her body, the female has to twist her abdomen over so that the light can be seen from above. The female's display lasts usually for two or three hours. It is normally only virgin females which glow; after mating, they are unlikely to repeat their display. Clusters of between two and six glowing females can sometimes be found within a few centimetres of each other. These clusters may well be the result of the "ganging" of pupating larvae (Tyler 2002).

3. 4. 2. *Lamprohiza splendidula* (Linnaeus, 1767)

Distribution

There are only 8 described species in the genus *Lamprohiza* Linnaeus, 1767 in Europe; 7 occur in the south-western part of the continent, one in almost all of Europe (Burakowski 2003). It is the most common firefly in the Czech Republic (Hůrka 2005; Geisthardt & Sato 2007).

Lamprohiza splendidula is distributed in south-eastern and central Europe, on the west it reaches the Rhine River, on the east the Caucasus Mountains, on the north it extends to the central province of Fennoscandia, and on the south to central Italy and the Balkan Peninsula (Burakowski 2003). It inhabits moist and shaded habitats of lowlands and uplands, mainly in deciduous forests, thickets, clearings, banks of rivers and streams, meadows and the gardens (Burakowski 2003; Hůrka 2005).

Life cycle

The biology of *Lamprohiza splendidula* differs only slightly from *Lampyris noctiluca* (Schwalb 1961). Developmental cycle lasts three years; 6 – 8 days after fertilization, the female lays 60 – 90 yellowish, spherical eggs (having diameter of 0.6 – 0.8 mm and being luminescent) in shaded areas, on the ground surface, at the base of plants, mosses, on the lower side of fallen leaves, etc. (Burakowski 2003). Predatory larvae can grow up to 12 mm. Their bodies are strongly dorsoventrally flattened, which makes them similar to larvae of Silphidae (Burakowski 2003). Larvae feed mainly on snails, sometimes on other soft-bodied invertebrates. Pupation occurs in the spring after hibernation; under fallen leaves, pieces of wood or stones; in dug up chambers opened from the top (Burakowski 2003). Depending on the conditions of the local climate the mature forms appear in the May – July and can be seen until September (Burakowski 2003; Hůrka 2005).

Adults

The species is very distinctive thanks to two translucent, almost transparent “windows“ on anterior part of pronotum, under which the head is completely hidden. Mandibles are long, crossed above labrum. Distinct sexual dimorphism is present (Burakowski 2003).

Male (Fig. 5)

Length of 8 – 10 mm. Body dark brown, legs and antennae yellowish brown. Eyes very large, circular, close to each other in the front, and therefore on the ventral part of the head, the notch for the base of mandibles and labium is very narrow. The bases of the antennae are very close to each other. Elytra subparallel, with 3 – 4 fine ribs, shortened before the anterior region; surface covered with fine wrinkles is densely pilous, with short setation. Wings fully developed. Last visible tergite strongly cut out posteriorly. Photic organs in form of two rectangular bands on ventrite V and VI (morphologically on sternites VI and VII). Phallobase three times longer than aedeagus, parameres with apical spines, phallus protruding beyond parameres and aimed dorsally (Burakowski 2003; Hůrka 2005).

Figure 5 *Lamprohiza splendidula*, male © Stanislav Krejčík

Female (Fig. 6)

Length 6 – 10 mm. Body yellowish white to yellowish brown. Eyes small, non-tangential anteriorly. Head ventrally with wide notch for the base of the mandible and labium. Vestigial elytra short, protruding, reaching up the first abdominal tergite.

Vestigial wings strongly reduced, completely hidden under the elytra. Abdominal tergites laterally-widened. Intensively glowing photic organs in a form of 2 – 12, usually paired, ventrolateral spots (Schwalb 1961) can be found on ventrites III, V, VI and pleurites II – VI; these organs are difficult to observe on dead beetles. Ovipositor with sclerotized wide valvifers supported by short trabeculae, and elongated membranous valvae. Valvae and styliform appendages strongly setose (Burakowski 2003; Hůrka 2005).

Figure 6 *Lamprohiza splendidula*, female © Stanislav Krejčík

Behaviour

The larvae of *Lamprohiza splendidula* emit a weak continuous glow when handled or even approached, nevertheless, the light intensity may weaken or completely cease in certain cases (M. Novák, unpublished observation). During collection of specimen, cannibalistic behaviour was observed when two specimen were placed in a single container (M. Novák, unpublished observation).

Winged males are active and fly during dusk and night (Burakowski 2003; Hůrka 2005), emitting short lasting glows followed by longer lasting interval of darkness (M. Novák, unpublished observation). Apterous females are stationary on the ground (Burakowski 2003; Hůrka 2005) and emit continuous glow to lure partners (M. Novák, unpublished observation). As well as in *Lampyris noctiluca*, the females can be found in clusters, which make them quite distinct (M. Novák, unpublished observation).

3. 4. 3. *Phosphaenus hemipterus* (Geoffroy, 1762)

Distribution

The genus 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 of the Balkan Peninsula, Transylvania and Ukraine (Burakowski 2003; Geisthardt & Sato 2007).

Phosphaenus hemipterus has been considered a rare and not well known species until recently. De Cock (2000) presumes, that the reason of this is the fact, that the habitat of this firefly can be found mainly in areas with high level of human disturbance. These consist of gardens, parks, parking lots and field edges, while most of the previous research have been made in areas mostly unaffected by human. De Cock (2000) eventually states, 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.

This species is listed as vulnerable (VU) in Red List of the Czech Republic (Švihla 2005).

Life cycle

Life cycle lasts two or three years. Eggs are white, spherical, with diameter of ca. 0.6 mm (De Cock 2000). Larva reaches length of up to 11 mm in the last instar. According to De Cock, late-instar female larvae tend to be larger and fatter than males and easily recognised in the field (De Cock 2003). Length of pupa is 7 – 10 mm. Body white, dorsoventrally flattened, bent. Pupation takes place in April – May, the pupal stage lasts ca. two weeks. Mature individuals are caught occasionally and rarely from July to August (Burakowski 2003, Hůrka 2005).

Adults

The front edge of the head (nasale) is emarginated, forming small notches, and densely setose. Eyes small, widely spread out. Antennae long and thick, antennomeres II – X wider than long. Sickle-shaped mandibles are narrow, very hairy, with crossing apical parts. The male is short-winged, female lacks both elytra and wings (Burakowski 2003). *Phosphaenus hemipterus* is the only one of the more than 2,000 species of known lampyrids in which both females and males are flightless (De Cock 2000).

According to Burakowski (2003), emission of light by mature forms is negligible, with light bodies difficult to distinguish on dead specimens. Adults of both sexes retain the paired larval light organ in the eighth abdominal segment (De Cock & Matthysen 2005). The sexes differ in that males have dorsally transparent "windows" in the segment bearing light organs, through which the light shines, whereas females' light organs protrude laterally and the dorsolateral sides of the segment are less pigmented so that the light is visible from the side as well as from above as is seen in the larvae. Thus, in both sexes the bioluminescent emission is clearly visible (De Cock & Matthysen 2005).

Male (Fig. 7)

Length 6 – 8 mm. Body reddish brown, pronotum and elytra brown, the last abdominal segment paler. Body surface is covered with short setation. Pronotum semicircular, slightly stretched forward, basal part almost straight, with rounded posterior edges. Elytra short, protruding, reaching to the first abdominal tergite. Shortened wings completely hidden under the elytra. Scutellum tongue-shaped, cut on the posterior edge. Legs robust. Photic organs forming two small

Figure 7 *Phosphaenus hemipterus,* male © Ugis Piteräns

spots are located on the ventrite VII (morphologically on sternite VIII). Tergite VIII trapezoidal, emarginated on the posterior margin. Phallobase of half of the length of the aedeagus, parameres convex distally and hooked proximally, apex of phallus does not exceed the apexes of parameres (Burakowski 2003; Hůrka 2005).

Female (Fig. 8)

7 – 10 mm long, vermiform, without elytra or wings. Body reddish brown, poorly pilous with yellow setation. Antennae slightly shorter and more slender than in the male. Pronotum semicircular, with a small indentation on the frontal edge. Mesonotum with appendage on theposterior edge, metanotum with concavity in the frontal part. Scutellum very small. Photic organs on ventrites VI and VII (morphologically on sternites VII and VIII) (Burakowski 2003; Hůrka 2005). Tergite VIII with a small notch on the apex. Ventrite VII membranous. Ovipositor as shown in Burakowski (2003: 35, fig. 100).

Figure 8 *Phosphaenus hemipterus*, female © Raphael De Cock

Behaviour

Unlike most of the firefly species, whose larvae feed on snails and slugs, larvae of *Phosphaenus hemipterus* are obligate earthworm (*Lumbricus terrestris* Linneaus, 1758) 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.

As well as in *Lampyris noctiluca*, *Phosphaenus hemipterus* larvae also glow spontaneously by emitting glow pulses while active at night (De Cock 2003).

Females are staying in litter or mundane parts of plants, being active mainly during dusk (De Cock 2000, Burakowski 2003, Hůrka 2005) and are very rarely found (Burakowski 2003). In contrast, the males are diurnal, and can be often found on herbaceous plants and shrubs (Burakowski 2003). The larvae are predominantly nocturnal (Hůrka 2005).

Both sexes are feebly bioluminescent, although they appear only to glow in response to disturbance (Majka & MacIvor 2009).

3. 4. 4. Light emission spectra

According to De Cock (2003), larvae of all three species present in the Czech Republic have similar mean spectral values of bioluminescence, seen by human eye as lime green. The overview of mean peak wavelengths (λ_{max}) of larval and adult bioluminescence spectra according to De Cock (2003) is as follows:

Lampyris noctiluca:	$\lambda_{max} = 546 \pm 3$ nm
Lamprohiza splendidula (dorsal view):	$\lambda_{max} = 549 \pm 3$ nm
Lamprohiza splendidula (ventral view):	$\lambda_{max} = 546 \pm 3$ nm
Phosphaenus hemipterus:	$\lambda_{max} = 546 \pm 6$ nm

The emission spectra of *L. splendidula* were measured from dorsal and ventral views because the larval bioluminescence in this species comes from ventrolateral light organs that also shine through the dorsal cuticle (De Cock 2003).

De Cock (2003) adds, that emission spectra of adults have not been published yet, and the only descriptions found were for *Lampyris noctiluca* with *in vivo* adult female peak emission $\lambda_{max} = 551$ nm. Schwalb (1961) suggests that the spectral maxima in *Lamprohiza splendidula* and *Lamypris noctiluca* are identical for larvae and adults (Fig. 9, 10) of both species and lie within a bandwidth of 550 – 580 nm.

Nonetheless, this range is too wide and the spectral characteristics of Central-European species need a further study.

Figure 9 *Lamprohiza splendidula*, glowing female © Stanislav Krejčík

3. 5. Firefly larvae

Larvae prefer mesic environment (environment with intermediate values of ecological factors like balanced supply of moisture). They can be found along watercourses and water bodies as well as in fallen leaves, degrading wood or under the stones. In arid areas, the larvae usually remain under ground and emerge during night or immediately after rainfall (Grimaldi & Engel 2005; Branham 2010). The number of larval instars is probably negatively correlated with the length of photoperiod and varies between 4 and 9. Depending on the species, the larvae live from several months up to 2 years followed by pupation. For this purpose, some species dig small underground chambers, others build aboveground chambers from mud called “igloo” and some species prefer to pupate in decomposing wood (Grimaldi & Engel 2005; Branham 2010).

They are fierce predators with ability to follow a chemical trail of their prey (mostly snails) while determining its polarity so they never follow their victims in a wrong direction (Branham 2010). It is the higher activity of larvae in environments with higher humidity that is perceived as a result of higher activity of their prey (Viviani 2001). Larvae pierce the body of their prey with their long ditched mandibles and inject them with a dark secretion from a pair of glands, located in the front part of intestine, which kills and partially liquefies and digest the prey. The fluid is then aspirated by maxillae and labium, which are covered with fringes of fine setation that stops anything that does not turn into tiny particles (Klots & Klots 1963; Hůrka & Čepická 1978). In laboratory conditions, the larvae can feed on cheese, liver extract or dead gastropods (McLean et al. 1972 in Viviani 2001).

All known lampyrid larvae are capable of bioluminescence (Branham 2010) and in some species, they may be luminescent even before they emerge from egg (Hůrka & Čepická 1978). Most of the species glow by a pair of photic organs situated on abdominal segment VIII, while the only known exceptions are two species of genus *Lamprohiza*; *Lamprohiza delarouzei* (Jacquelin du Val, 1859), which has two pairs of photic organs on abdominal segments II and VI and *Lamprohiza splendidula* (Linnaeus 1767), which has 3 – 12 glowing spots on abdominal segments II – VI (Grimaldi & Engel 2005; Hůrka 2005; Branham 2010).

3. 5. 1. Light organ

Production of light as well as the anatomy of the glowing organ differs between larvae and adults. While the adults of many lampyrid species can control glowing and produce signals from occasional discrete flashes to stroboscopic serenades, larvae can produce only slowly varying glow (Timmins et al. 2001). In addition, even pupae and eggs can be luminescent. It is assumed, that this phenomenon is, among others, for reasons of aposematism and antioxidation. Protection from UV radiation is another assumption (Deheyn & Ballantyne 2009; Gullan & Cranson 2010). Although firefly larvae use the same biochemical reaction of the luciferase-luciferin system to produce light (Strause & Deluca 1981), their luminescence differs from adults because of the use of isozymes differences in location, morphology and physiology of the light organs (Christensen & Carlson 1982), often resulting in a different colour of bioluminescence and behavioural displays (Viviani 2001).

3. 5. 2. Bioluminescence utilisation by larvae

Lloyd (1978) and Sivinski (1981) state possible reasons of larval glow as follows:

- Attracting of prey
- Defence against predators
- Illumination of surrounding environment
- Communication between larvae and adults
- Marking of territory
- Intraspecific warning signals

Viviani (2001) adds three main patterns of bioluminescence observed in Brazilian species, among others, belonging to subfamilies Lampyrinae and Luciolinae:

1. Larvae can be mechanically stimulated to light response and glow abundantly;
2. Larvae can be mechanically stimulated to light response but generally glow sporadically;
3. Larvae cannot be mechanically stimulated to light response but may be able to communicate among each other by flashes.

The time period in which larva can emit a continuous glow (for example *Aspisoma lineatum* Gyllenhal, 1817) is generally longer then in adults, even though the single intervals can significantly differ among different species. This difference is probably caused by different utilization of light production depending on species-specific habitat (Grimaldi & Engel 2005). Viviani (2001) presumes that shorter flashes (*Photinus* sp.) could serve as a collective defence against predators; a simultaneous glow of several specimen might distract attention of a predator and therefore lower the risk of being caught.

Nevertheless the collective glow of larvae can also have an importance within the species. According to Viviani (2001), it can serve as a communication manner between juveniles and adult females, where larvae are signalling an occupied locality in order to prevent intraspecific competition for food resources. This behaviour can be assumed for example in west African species *Luciola discicollis* Kaufmann, 1965 (Kaufmann 1965 in Viviani 2001).

Figure 10 *Lampyris noctiluca*, glowing female © Henrik Kettunen 2009

In comparison with adults, the colour of light produced by larvae is generally shifted towards green. This fact supports the recent view on bioluminescence of juvenile specimen not having a function in sexual communication but having a function in defence. Viviani (2001) states, that the majority of the terrestrial animals is sensitive mainly to colours that can be found in the green spectrum. The colour of glow also supports the idea of larval bioluminescence being evolutionary older than the bioluminescence of adults and served as a mean of communication with predators; probably as aposematic defence. There are, however, also species where the spectrum of larval glow is identical to the one of adults. An example of such species can be *Aspisoma lineatum*, nevertheless since the colour emitted by this species is in the yellow-green spectrum, this fact is not in conflict with the abovementioned.

Larvae may emit glow from the whole body surface like in *Aspisoma lineatum* or localized light sources like in *Lampyris noctiluca*, while other species emit light only while moving (*Photinus* sp.) (Viviani 2001).

An interesting fact is that the larvae of many lampyrid species, namely from genera *Pyrogaster* Motschulsky, 1853; *Photuris* Dejean, 1833 or species *Aspisoma lineatum* respond to vibrations, most frequently caused and observed during research by scientists themselves (Underwood et al. 1997), toads (De Cock & Matthysen 2003) and insectivorous bats (Moosman et al. 2009), but do not respond to mechanical manipulation (Viviani 2001). This behaviour could be explained by abovementioned collective defence against predators which lies in the distraction. We can imagine a predator observing a number of light sources, but when this predator stumbles upon a specific specimen, this one ceases to glow and the attention of the predator is diverted to other still glowing individuals.

An even more interesting fact is what Viviani (2001) witnessed in unidentified *Bicellonychia* sp. (subfamily Photurinae) for several times. The larvae reacted to flashes emitted by adults while it seemed that they also synchronized their signals. The cause of this behaviour could be informing the adults of occupied food niche and the synchronicity could have been caused by vibrations created by the entomologist and collective defence mechanism of the larvae. Nevertheless, additional information on this behaviour is not available, therefore this phenomenon will need more study in the future.

3. 5. 2. 1. Aposematism and defence

The signals emitted by animals in order to distinguish its own species, search for a sexual partner or for the purpose of defence can be in some cases utilized in different ways. Courtship signals which are sought by predators and parasites can serve as an example. However, the predators also perceive aposematic warning signals, emitted by potential prey, which is chemically protected against them or generally not suitable for consumption (Page & Ryan 2005).

Lampyrid larvae are generally unpalatable to the most of predators including several mammals, amphibians and fishes, thanks to specific chemical compounds in their bodies (Lloyd 1973; Underwood et al. 1997; De Cock & Matthysen 2001; Fu et al. 2007). At the same time, the experience of an attack at light-emitting unpalatable prey lowers the probability of consecutive attack in mice (Underwood et al. 1997), toads (De Cock & Matthysen 2003) and insectivorous bats (Moosman et al. 2009). Hence, the ability of light production has in these cases similar meaning as warning colouration of vespids or poisonous sea gastropods, generally known as aposematic (De Cock & Matthysen 2003; Moosman et al. 2009). A curious phenomenon may occur in certain frogs which do not find lampyrids unpalatable; a frog can actually start glowing itself, if it eats enough fireflies (Klots & Klots 1963). Whether this phenomenon is purposeful mimicking of aposematic defence against the frog's predator or strictly random consequence remains a question.

In his research of Neotropical species, Viviani (2001) repeatedly observed, that individuals that have been caught or manipulated-with frequently started to glow or increased the intensity of light manifestation. An example of such species is *Cratomorphus concolor* Perty, 1830 (subfamily Lampyrinae), where adults commence to glow when disturbed. The larvae of this species react in the same manner; an intense glow lasting seven seconds. On the other hand, with increasing frequency of harassment the period of light emission shortens (Viviani 2001).

In other Neotropical species, *Photinus fuscus* Germar, 1824 (subfamily Lampyrinae), inhabiting ground levels of mesophylic forests, the adults often luminously react on vibrations caused by nearby pedestrians (Viviani 2001). A similar behaviour in larvae was observed by the same author in unspecified *Pyrogaster* sp. (subfamily Photurinae) of the same area, where the emitted glow of one individual was frequently followed by glows of other larvae. In many cases, Viviani observed a simultaneous glow produced by larvae in reaction to his progressing through the habitat, although mechanical stimulation of individuals never elicited a bioluminescent display.

3. 5. 2. 2. Illumination of the surroundings and search for prey

Although bioluminescence is in some cases used by adults in preying as it is known in "femmes fatales" (adult lampyrid females luring males of different lampyrid species by mimicking courtship signals; Gronquist et al. 2006), the light emission from predatory point of view has significance also in larvae of some species. Larvae of *Aspisoma* sp. (subfamily Lampyrinae) do not respond to mechanical manipulation by glowing and generally emit light sporadically, but they are able to produce relatively long lasting glow (up to five seconds). Viviani (2001) states that according to the fact that the photic organs of these larvae are positioned in a manner of directing the light beam into the area in front of the individual, the purpose may lie in tracking of glossy slime track of preyed gastropods. Given the fact, the individuals of this genus inhabit also water environment, it is plausible, that bioluminescence may be also used for luring the water snails on dry land, where they are more vulnerable against attack. It is important to say, that even the Central European lampyrid species, with photic organs placed posteroventrally are believed to be able to follow the "snail track" (Fig. 11) (Hůrka & Čepická 1978).

Figure 11 *Lampyris noctiluca*, preying larva © Stanislav Krejčík

Another lampyrid where Viviani (2001) proposes utilization of light for gastropod tracking is Neotropical *Cratomorphus* sp. (subfamily Lampyrinae). The larvae emit an intensive long lasting glow (sometimes longer than 10 seconds), which is observable in a distance of up to 50 meters (Viviani 2001).

4. MORPHOLOGY

4. 1. Family Lampyridae

4. 1. 1. General description of adults

The antennae consist from 11 antennomeres and may be pectinate or flagellate in male. Eyes are large in male and much reduced in female. Head is more or less concealed by pronotum. Males are winged and females mostly apterous and vermiform (Nayar et al. 1976). Elytra are soft and fit very loosely alongside the abdomen (Klots & Klots 1963). The abdomen with photic organs on segments VI and VII in male and segment VII only in female, the light emitted being stronger in the latter (Nayar et al. 1976). Adults of numerous species do not accept food anymore, however certain species nourish themselves by nectar or pollen (Klots & Klots 1963).

4. 1. 2. General description of larvae

According to Stehr (1991), larval characteristics of Lampyridae include: falcate mandibles which may be cleft longitudinally or channelled; a reduction in the articulated area of the maxillae; an ill-defined labrum which may be contained in a nasale; a pygopod which aids in locomotion; and legs that are pentamerous with tarsus and claw fused into a tarsungulus. Another characteristic is the lack of a molar region on mandibles.

The lampyrids can be distinguished from other cantharoids by the presence of an epicranial suture which is absent in similar families, and a photic organ which is usually situated on the venter of abdominal segment VIII (Stehr 1991).

5. MATERIAL AND METHODS

Larvae of *Lamprohiza splendidula* were collected from two localities in Prague, Czech Republic at the end of August and beginning of September 2013. Sixteen specimen were collected from a hillside next to Kunratický stream, behind Thomayer hospital (50°1'47.588"N, 14°27'47.793"E). The area is inside a deciduous Kunratický forest (Fig. 12), where larvae were found mostly under bushes among decomposing moist leaves. The specimen collection started one hour after the sunset, and resulted in finding of 16 specimen. Parent rock is composed mainly of slates, climate is temperate, mildly arid, typical for the Prague plain. The average yearly temperature is 8.8 °C; average annual rainfall is 476 mm (Dostálek not dated).

Figure 12 Kunratický forest © toulejse.cz

Three other specimen were collected at Petřín hill (Fig. 13), near stairway under the statue of K. H. Mácha (50°4'54.437"N, 14°24'7.604"E). Petřín is recently a landscaped hill in the centre of Prague; an anthropoecosystem with high amount of park greenery, although in higher parts, remains of original thermophilic oak forest can be found. Parent rock is composed of slates and siltstone,

Figure 13: Petřín hill, statue of K. H. Mácha © envis.praha-mesto.cz

climate is continental with the majority of rainfall in 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). Specimen were found two hours after sunset, under bushes and under low herbaceous vegetation.

Larvae of *Lampyris noctiluca* were collected in Ljubljana, Slovenia, in the first half of September 2013. Three specimen were collected in the forest edge next to Koseze Pond (Koseški Bajer; Fig. 14; 46°3'58.37"N, 14°28'10.73"E), on decomposing wood and on leaf litter, two and a half hours after the sunset. The area of Koseze Pond is a landscape park, geologically mostly comprised of slates and limestones, with mostly acidic soil. The area has many small streams and sources. The climate is continental with the majority of rainfall in 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 (Zavod za gozdove Slovenije 2012).

Figure 14 Koseze Pond

The collected specimens of *Lamprohiza splendidula* and *Lampyris noctiluca* were fixed and stored in 60% ethanol and kept in low temperature.

The species collected were identified using entomological key by Burakowski (2003).

Specimen of *Phosphaenus hemipterus* were borrowed from RNDr. Petr Švácha, CSc, from collection of Institute of Entomology within Biology Centre of the Academy of Sciences of the Czech Republic in České Budějovice. The specimen examined were found in forest litter in Lednice area (48°47'58.818"N, 16°48'6.036"E), south Moravia, in April of 1987, and stored in 80% alcohol. The area of Lednice is predominantly composed of quaternary sediments, with long temperate and arid summer and short mildly temperate dry winter. Average yearly rainfall is 1000 mm, average yearly temperature is 8,5 °C (Hulčík et al. 2013).

5. 1. Optical imaging

Specimen of all three species were cleared by simple brush and then placed in 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 being submerged in glycerol (due to better optical properties and higher stability thanks to larger density of glycerol). Images were taken by 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 programme. The sets of pictures of each habitus taken were consequently stacked into a sharp final image in Zerene Stacker (64-bit) by Zerene Systems LLC.

For detailed understanding of morphology, several specimens were dissected and their body parts examined separately using Olympus SZX7 stereo microscope. The images of isolated maxillae and mandibles were taken by Olympus XC30 Digital Colour Camera attached to Olympus CX41 biological microscope.

5. 2. Electron imaging

For detailed view of anatomy and body structure of the collected larvae, the samples were examined in the Faculty of Science of Charles University in Prague. The specimens examined were first dehydrated by 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. The electron imaging was performed using JSM-6380LV (JEOL) Scanning Electron Microscope (SEM) with a high resolution of 3.0 nm (30kW).

Figure 15 Scanning Electron Microscope

Interpretation and terminology of larval descriptions follows Archangelsky & Fikáček (2004).

6. RESULTS: RE-DESCRIPTION OF CZECH LAMPYRID LARVAE

6. 1. *LAMPYRIS NOCTILUCA* (Linnaeus, 1767)

General body description (Fig. 16; Annex 2, 3)

Fusiform and robust; slightly dorsoventrally flattened. Body length 5 – 23 mm (from the anterior margin of pronotum to the apex of caudal segment); with pronotum, mesonotum and metanotum and 10 abdominal segments. Pronotum of equal length and width. Tergites from pronotum to abdominal segment IX divided by sagittal line in dorsal view. Colouration: most of the 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 whitish patch on ventrite VII of abdominal segment VIII.

Types of general cuticular processes observed (Annex 23)

1. Stout, shorter, blunt, oblique setae;
2. Dense granulose protuberances;
3. Long filamentous setae;
4. Coeloconical receptors.

Head capsule (Annex 1, 4)

Prognathous; retractable within prothorax; of equal width and length; slightly widening posteriorly. Gena about the same size as the width of the head capsule in its shortest width, with one stout seta anterolateraly close to the base of antennae. Head capsule dorsally covered with short blunt adjacent setae and coeloconical receptors. Epicranial suture not distinguishable. One stemma on each side of the head. Labrum fused with clypeus forming labro-clypeus, covering base of mandibles in dorsal view. Labro-clypeus mildly double-arched in anterior view, with two long setae reaching the apex of mandibles, positioned on outer lateral sides. Epipharynx formed by two plates, and an anterior pair of brushes of long setae on each plate, which project centrally past anterior margin of the head. Hypopharynx covered with long setation.

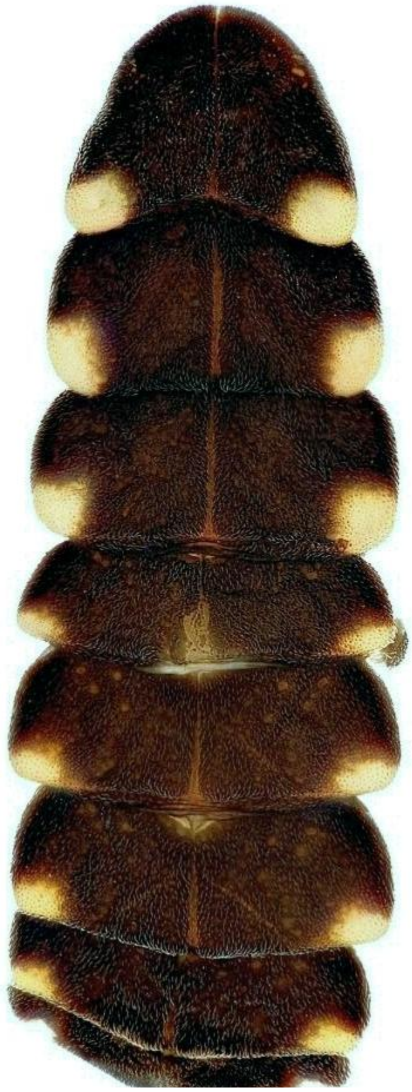

Figure 16: *Lampyris noctiluca*, larva

Antenna (Annex 15, 16, 17)

Trimerous, inserted on lateral distal margin of gena; partially retractable within membranous socket. Basal antennomere widest, fully sclerotised, bearing shorter adjacent setae, coeloconical receptors and several long oblique setae near the apical region. Several long stout setae placed radially on the anterior side, with a distinct seta on the inner lateral area of this antennomere. Second antennomere slightly shorter than the basal, laterally flattened; bearing both types of previously mentioned setae unequally and scarcely scattered across the surface, together with several coeloconical receptors; with four well observable stout setae – first three in the middle and on the apex of inner lateral part of the antennomere, third on apical region of the outer lateral part of the antennomere. A sensillum placodeum is present on the inner side of the apex (Annex 25). Sensorium of second antennomere oval, widest at the base, closely annealing to the second antennomere, shorter than the third antennomere with no visible surface pattern. Third antennomere shortest, bearing four short setae; one on its base and three on the apex, together with a pair of short cuticular projections; first thick and second thin.

Maxilla (Fig. 17; Annex 1, 18)

Consisting of five parts, attached to labium forming a maxillo-labial complex. Cardo subrectangular, slightly wider than long. Stipes elongated, ventrally relatively bald, setae mainly on distal half, with three long stout setae placed radially on the ventral apical region; dorsolaterally covered with short adjacent setation. Galea bimerous, with basal part subcylindrical, slightly wider than distal, with long dorsal setation partially covering distal part; distal part subcylindrical, rotated centrally, with short setae and one apical seta longer than body of the distal part. Lacinia covered with brush of long setae on outer lateral margin. Maxillary palpus tetramerous, basal palpomere largest, rectangular, about the same length and width, second and third palpomere short and wide. Palpomeres I – III covered with setae; palpomere IV (Annex 19) irregularly subconical, thick, blunt, with an inner longitudinal lateroapical sensory slot, small seta on outer lateral region and short outer lateral longitudinal sensory slot covered with thin adjacent seta.

Labium (Annex 1, 4, 18)

Closely attached to maxilla, formed by a short and strongly sclerotized prementum, mentum and weakly sclerotized submentum. 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; 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 outer-laterally and sensillum placodeum on outer ventrolateral side of the apex. Mentum elongated, subtriangular, unsclerotized on lateral margins, ventrally bearing numerous short, adjacent setae and a pair of long, erect setae centrally.

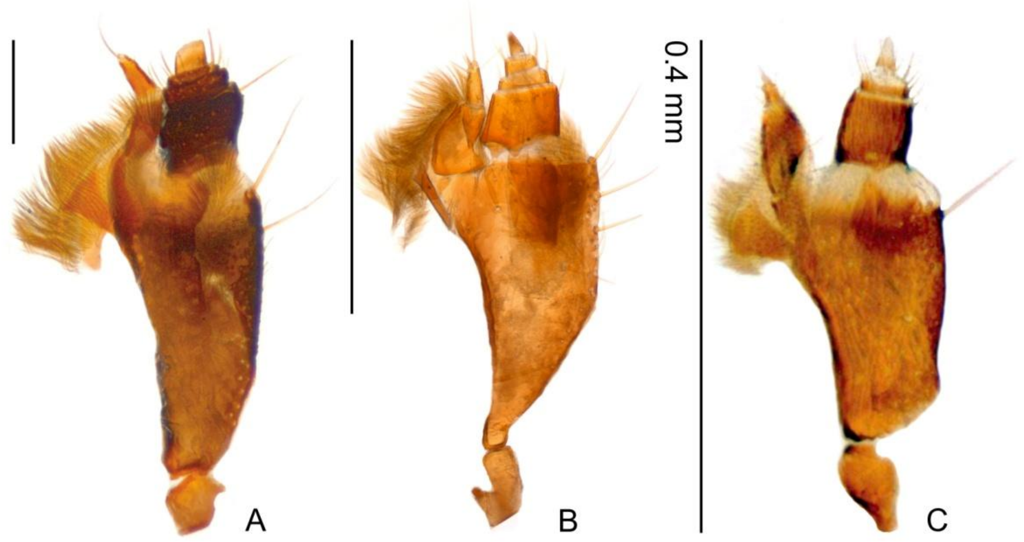

Figure 17: Maxillae. – A) *Lampyris noctiluca*; B) *Lamprohiza splendidula*; C) *Phosphaenus hemipterus.*

Mandible (Fig. 20; Annex 1)

Symmetrical, falcate, with an inner channel opening subapically on outer edge. Retinaculum present, forming one sharp inner tooth on basal half of mandible. Inner margin of mandible from retinaculum to the base covered with stout setae. Basal two-thirds of mandible ventrally with dense adjacent setation aimed centrally (Annex 14). Dorsally, mandible covered with several adjacent strong setae, aiming centrally on the proximal two-thirds of each mandible (Annex 14). Lateral margin covered by brush of adjacent, short setae on basal two-thirds. Sensory (hyaline) appendage (Annex 20) on outer margin of mandible before channel opening is missing or undistinguishable. A distinct short, stout seta present ventrally at the anterior end of lateral setation.

Thorax (Annex 2, 3)

Three-segmented, thoracic tergites divided by sagittal line in dorsal view. Pronotum subtrapezoidal, wider posteriorly, rounded at posterolateral corners, strongly concave on posterior margin. Meso- and metanotum subrectangular, wider than long, mesonotum longer than metanotum. Lateral areas of meso- and metathorax formed by episternum and epimeron; episternum of mesothorax bearing a bifurous spiracle. Presternum subrectangular, wider than long, rounded, robust, well sclerotized, subdivided into three plates; lateral ones extending above and to the sides of coxae, carrying episterna and epimera; medial plate of subpentagonal shape. 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, carrying large episterna and smaller epimera, medial plate less sclerotized on margins, heart-shaped with base posteriorly.

Legs (Annex 21)

Five-segmented, all pairs similar in shape and size. Coxa large, stout, dorsally sclerotized in more than a 1/2 of longitudinal length, covered by short sharp setae. Trochanter smaller, subtriangular 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 long fibrous setae ventrally. Tibiotarsus as long as femur, narrower, tapering towards distal end, bearing stout short sharp setae dorsally and strong sharp erect setae ventrally. Tarsungulus (Annex 22) composed of a claw with distinct ridges, ventrally bearing three short stout setae with fine ridges. Cuticle of leg densely covered with grainy protuberances except for pical half of tibiotarsus.

Abdomen (Annex 2, 3)

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

6. 2. *LAMPROHIZA SPLENDIDULA* (Linnaeus, 1767)

General body description (Fig. 18, Annex 6, 7)

Elongate and fusiform; dorsoventrally flattened, tergites of thorax and abdomen finely serrated on the edges, and strongly laterally overlapping the body. Body length ca. 5 – 12 mm (from the anterior margin of pronotum to the apex of caudal segment); with pronotum, metanotum and mesonotum and 10 abdominal segments. Pronotum wider than long, with deep emargination anteromedially. Tergites from pronotum to abdominal segment IX divided by sagittal line in dorsal view. Colouration: dorsally brown and ochrish towards the lateral edges of tergites; with pairs of lighter pigmented spots on abdominal tergites I – VI. Ventral region much lighter than dorsal, with ochrish to light brown colouration except darker more sclerotized central parts of ventrites. Spiracles on pleural plates of dark brown colouration. Photic organs placed ventrolaterally, localized under two pairs of distinct lighter pigmented spots on tergites of abdominal segments II and VI, with possible minor pairs of dull spots on tergites III – V of abdominal segments, which might bear additional photic organs.

Figure 18: *Lamprohiza splendidula*, larva

Types of general cuticular processes observed (Annex 23)

1. Stout, long, erect setae;
2. Thin, short, erect setae;
3. Stout flattened adjacent setae of ca. half the length of 1).

Head capsule (Annex 5, 8)

Prognathous; retractable within prothorax; longer than wide, slightly tapering posteriorly. Gena long, with one stout seta anterolateraly close to the base of antennae. Head capsule dorsally covered with long adjacent setae. Epicranial suture of light colouration, V-shaped. One stemma on each side of the head, with a light coloured spot placed posteriorly behind the stemma (Fig. 19), possibly being a sensory organ. Labrum fused with clypeus forming labro-clypeus, covering base of mandibles in dorsal view. Labro-clypeus double-arched in anterior view, with no distinguishable setae on lateroapical margins. Epipharynx formed by two plates, and an anterior brush of long setae, which project centrally past anterior margin of the head. Hypopharynx with long setation.

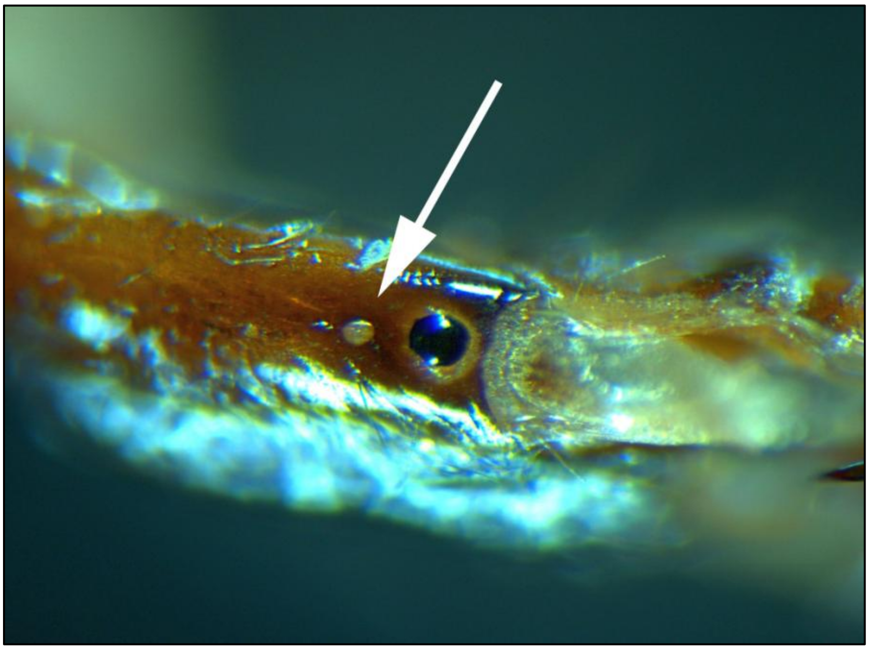

Figure 19: *Lamprohiza splendidula*, membraneous spot placed posteriorly behind stemma

Antenna (Annex 15, 16, 17)

Trimerous, inserted on lateral distal margin of gena; partially retractable within membranous socket. Basal antennomere slightly wider, unsclerotized on posterolateral margin, bearing long flat adjacent setae 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 oblong, potato-shaped, with distinct basal constriction as a connection with the second antennomere; slightly shorter than the third antennomere; with no visible surface pattern. Third antennomere shortest, bearing three setae on the apex, one seta on its body, and three cuticular projections; first longer and thick, second longer and thin, third one placed on the body of antennomere forming a small bulge.

Maxilla (Fig. 17; Annex 5, 18)

Consisting of five parts, attached to labium forming a maxillo-labial complex. Cardo 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. Galea bimerous, with basal part larger than distal; distal part subcylindrical, rotated 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. Lacinia covered with brush of long setae on outer lateral margin. Maxillary palpus tetramerous, basal palpomere largest, subrectangular, of similar length and width, second and third palpomeres short and wide. Palpomeres I – III covered with setae; palpomere IV (Annex 19) subconical, narrow, sharp, bare, with outer lateral longitudinal sensory slot covered with thick blunt seta.

Labium (Annex 5, 8, 18)

Closely attached to maxilla, formed by a short and strongly sclerotized prementum, mentum and mostly membranous submentum. 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; basal palpomere rectangular, longer than wide, bearing several setae; distal palpomere conical, longer and narrower than basal, bearing one short, thin, adjacent seta placed dorsally on basal part. Mentum elongated and subtriangular, unsclerotized on lateral margins, bearing numerous long adjacent setae ventrally and a pair of long, erect setae posteromedially.

Mandible (Fig. 20; Annex 5)

Symmetrical, falcate, with an inner channel opening subapically on outer edge. Retinaculum featureless, present only as a blunt bulgy projection on basal third of the mandible. Basal half on inner margin of mandible covered with stout setae, being longest on the retinaculous bulge. Basal two-thirds of mandible ventrally with dense adjacent setation aimed centrally (Annex 14). Dorsal part of mandibles with several stout setae aiming centrally and a strong distinct seta aimed centrally, approximately in the central dorsal region of mandible (Annex 14). Lateral margin covered by brush of adjacent, short setae on basal two-thirds. Sensory (hyaline) appendage (Annex 20) on outer margin of mandible before channel opening is missing, even though channel opening is covered by a feather-like or rounded-trapezium fold with longer trapezoidal base situated ventrally.

Figure 20: Mandibles. – A) *Lampyris noctiluca*; B) *Lamprohiza splendidula*; C) *Phosphaenus hemipterus*.

Thorax (Annex 6, 7)

Three-segmented, thoracic tergites divided by sagittal line in dorsal view. Pronotum subtriangular, wider than long, rounded at posterolateral corners, with deep emargination anteromedially. Meso- and metanotum subrectangular, ca. 4 times wider than long. Lateral areas of meso- and metathorax formed by episternum and epimeron; episternum of mesothorax bearing a bifurous spiracle. Presternum subrectangular, longer than wide, well sclerotized, subdivided into three plates; lateral ones extending above and to the sides of coxae, carrying episterna and epimera; medial plate subrhomboid, longer than wide. Meso- and metasternum poorly sclerotized, subdivided by transverse fold into unsclerotized basisternum and sternellum; sternellum subdivided into three plates, lateral ones extending above the coxae, carrying episterna and epimera, medial plate hourglass-shaped.

Legs (Annex 21)

Five-segmented, all pairs similar in shape and size. Coxa large, stout, dorsally sclerotized in ca. 2/3 of longitudinal length, bearing stout setae. Trochanter smaller, elliptical in lateral view, shorter than femur, bearing adjacent shorter setae and long stout setae, lengthening towards distal apex. Femur narrow and cylindrical in lateral view, bearing adjacent shorter setae and long stout setae, lengthening ventrally, with one very long stout seta ventrally. Tibiotarsus as long as femur, narrower, tapering towards distal end, bearing stout setae. Tarsungulus (Annex 22) composed of a claw with fine ridges, ventrally bearing two long setae hooked apically towards each other, reaching the apex of the claw.

Abdomen (Annex 6, 7)

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 subcrescent; tergite of segment IX subsemicircular; segment X forming a narrow, incompletely sclerotized dark ring, holding the holdfast organ – pygopod (Annex 24) – with several eversible processes. Ventrites of segments I to VIII subrectangular, wider than long, with no sclerotization on margins, which bear a pair of long stout setae posterolaterally; ventrite of segment IX well sclerotized, rectangular and dark. Ventrites of segment V and VI less sclerotized. Pleural segments weakly sclerotized, pleural suture of segments I to V subdivide lateral areas into subrectangular upper pleurite, bearing a bifurous spiracle, and narrow lower pleurite; segments VI to VIII only with upper pleurite bearing a bifurous spiracle. Photic organs placed ventrolaterally on II and VI abdominal segments, with possible additional photic organs ventrolaterally on abdominal segments III – V.

6. 3. *PHOSPHAENUS HEMIPTERUS* (Geoffroy, 1762)

General body description (Fig. 21; Annex 10, 11)

Oblong and slender, cylindrical. Body length ca. 3 – 11 mm (from the anterior margin of pronotum to the apex of caudal segment); with pronotum, mesonotum and metanotum and 10 abdominal segments. Pronotum wider than long, of semicircular shape. Tergites from pronotum to abdominal segment IX divided by sagittal line in dorsal view. Thoracic tergites then subdivided with one clear line on each side, subparallel to sagittal line. Colouration: dorsally dark reddish-brown, ventrally pinkish/ochrish/light brown with darker ventrites and dorsal plates of pleural region. Thoracic tergites subdivided by sagittal line, with one clear line on each side, subparallel to sagittal line. Spiracles on pleural plates of light colouration. Paired photic organs on ventrite VII of abdominal segment VIII.

Types of general cuticular processes observed

1. Stout, short, blunt, erect setae;
2. Stout, long setae;
3. Flagellar setae growing from a slightly sunken toroidal socket (hereafter called toroidal setae; Fig 22; Annex 23).

Figure 21: *Phosphaenus hemipterus*,larva

Head capsule (Annex 9, 12)

Prognathous; retractable within prothorax; wider than long. Gena short, slightly concave, with one stout seta anterolateraly close to the base of antennae. Head capsule dorsally covered with short blunt adjacent setae. Epicranial suture of dark colouration, Y-shaped. One stemma on each side of the head. Labrum fused with clypeus forming labro-clypeus, covering base of mandibles in dorsal view. Labro-clypeus flat in anterior view, with two setae reaching one fourth of the length of mandibles, positioned on outer lateral sides. 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. In ventral view, antennae overlay posterolateral margins of mandibles.

Antenna (Annex 15, 16, 17)

Trimerous, inserted on lateral distal margin of gena; partially retractable within membranous socket. Basal antennomere widest, poorly sclerotised, slightly bulgy on the dorsal side, densely covered by three types of setae; adjacent short blunt setae 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) well observable under high magnification. Second antennomere slightly longer, narrower and laterally flattened in comparison to basal antennomere; bearing only torodial setae and blunt setae equally and abundantly spread across the antennomere. Inner ventrolateral area of second antennomere with distinct longitudinal cleft (Fig. 22). Several sensilla placodea (Annex 25) are present apically. Sensorium of second antennomere oval, widest at the base, closely annealing to the second antennomere, slightly longer than the third antennomere, with very fine helical ridges from apex to bottom. Third antennomere shortest, adjoining the sensorium of second antennomere, bearing a small sensorium, three short setae and three cuticular projections; first longer and thick, second longer and thin and third very short.

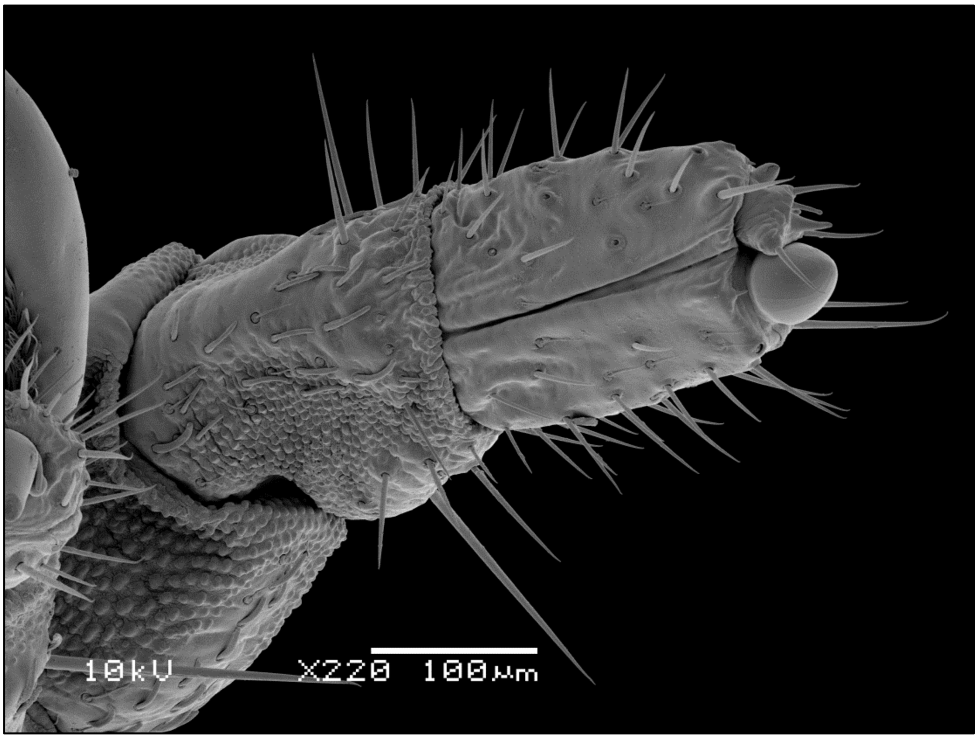

Figure 22 *Phosphaenus hemipterus*, longitudial cleft on second antennomere

Maxilla (Fig. 17, Annex 9, 18)

Consisting of five parts, attached to labium forming a maxillo-labial complex. Cardo bulbous, with wider side adjacent to stipes. Stipes elongated, subtrapezoidal, ventrally covered with adjacent short blunt setae 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 palpus trimerous, basal palpomere largest, subrectangular, equally long and wide, second palpomere short and wide. Palpomeres I and II covered with setae. Palpomere III (Annex 19) bearing two setae, one thin and sharp placed dorsally and second slightly thicker and blunt, paired with a bulgy sensorium on outer lateral surface.

Labium (Annex 9, 12, 18)

Closely attached to maxilla, formed by a short prementum, mentum and mostly membranous submentum. Glossae absent. Prementum narrow, heart-shaped in ventral view; bearing three types of setae: blunt short adjacent setae, sensory setae and a pair long and stout setae underneath the palpi. Labial palpus bimerous; basal palpomere wide and short, bearing several setae dorsally; second palpomere short, bearing ventrally a large sensorium, 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, adjacent setae, numerous toroidal setae and a pair of large, stout setae posteromedially.

Mandible (Fig. 20; Annex 9)

Symmetrical, falcate, with an inner channel opening subapically on outer edge. Retinaculum present, forming one thin and blunt inner tooth on apical third of mandible. Inner margin of mandible from retinaculum to the base covered with stout setae, lengthening towards the base of mandible. Ventrally, basal two-thirds of mandible covered with dense, adjacent setation, aimed centrally. Dorsally, basal two thirds with sagittal line of dense, stout, adjacent setation on equal length, erect in the last third of length, aiming centrally (Annex 14). Lateral margin without setation (Annex 14). Sensory (hyaline) appendage (Annex 20) on outer margin of mandible before channel opening is present, forming a subtriangular valve with fringing at the distal end. A thin stout short seta present dorsally in retinaculum region on both mandibles. Several sensilla placodea present on the post-retinaculum apical part.

Thorax (Annex 10, 11)

Three-segmented, thoracic tergites subdivided by sagittal line in dorsal view. Thoracic tergites divided by sagittal line into two parts, which are then subdivided with another clear line, subparallel to sagittal line. Pronotum subsemicircular, wider posteriorly. Meso- and metanotum suboval, wider than long, with rounded margins. Lateral areas of meso- and metathorax formed by episternum and epimeron; episternum of mesothorax bearing a bifurous spiracle. Presternum subquadrate, well sclerotized, subdivided into three plates; lateral ones narrow and wide, extending above and to the sides of coxae, carrying similar sized episternum and epimeron; medial plate subrhomboid, poorly sclerotized. Meso- and metasternum subdivided by a transverse fold into an unsclerotized anterior basisternum, and a poorly sclerotized sternellum, subdivided into three plates, lateral ones carrying the episterna and epimera, medial plate subtriangular.

Legs (Annex 21)

Five-segmented, all pairs similar in shape and size. Coxa large, stout, dorsally sclerotized in less than a 1/2 of longitudinal length, bearing adjacent short blunt setae, toroidal setae and stout long setae. Trochanter smaller, subtriangular in lateral view, about the same size as femur, bearing adjacent short blunt setae, 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 adjacent short blunt setae, toroidal setae, and stout long setae, with one very long stout seta on the center of ventral area 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 ventrally. Tarsungulus (Annex 22) composed of a claw with fine ridges, ventrally bearing three short setae.

Abdomen (Annex 10, 11)

Ten-segmented, slightly 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, wider than long; tergite of segment VIII heart-shaped with sharp posterolateral margins; segment IX subrectangular, longer than wide; segment X forming a narrow, incompletely sclerotized dark ring, holding the holdfast organ – pygopod – with several eversible processes. Ventrites of segments I to VIII subrectangular, wider than long and well sclerotized, with a pair of long stout setae on posterolateral margins. Ventrite of segment IX subrectangular, of similar width and length, well sclerotized and dark. Pleural suture of segments I to VII subdivide lateral areas into large, well sclerotized, subrectangular upper pleurites, bearing a spiracle, and narrow, small, poorly sclerotized lower pleurites, which are very narrow on segments VI and VII. Segment VIII with upper pleurite only. Bifurous spiracles present on pleurites I to VIII. Segment VIII bearing a paired photic organs ventrally.

7. KEY TO CENTRAL EUROPEAN LAMPYRID LARVAE

The key presented in this work consists of two parts. First part is assembled from the most distinct morphological features, enabling quick orientation and determination of the species. Second part goes into larger detail, addressing interspecific differences in particular body parts. The priority of features in the keys is primarily sorted by the level of their conspicuousness.

7. 1. General key to Central European lampyrid larvae

1) Distinct pinkish or yellowish spots on posterolateral margins of pronotum and on every tergite, except abdominal segment IX and X, present. Retinaculum present, forming one sharp inner tooth on basal half of mandible. .... *Lampyris noctiluca* (Linnaeus, 1767)

– Distinct pinkish or yellowish spots on posterolateral margins on pronotum and on every tergite, except abdominal segment IX and X, missing. Retinaculum featureless or thin and blunt. 2

2) Maxillary palpus trimerous, with bulgy sensorium on distal palpomere. Inner ventrolateral area of second antennomere with distinct longitudinal cleft. Epicranial suture not distinguishable or dark. ....
.... *Phosphaenus hemipterus* (Geoffroy, 1762)

– Maxillary palpus tetramerous, without bulgy sensorium on distal palpomere. Inner ventrolateral area of second antennomere without distinct longitudinal cleft. Epicranial suture of light colouration. ....
.... *Lamprohiza splendidula* (Linnaeus, 1767)

7. 2. Detailed key to Central European lampyrid larvae

7. 2. 1. Head capsule

1) Lateroapical margins of labro-clypeus with no distinguishable setae. Light-coloured spot posteriorly, behind each stemma. Epicranial suture light-coloured. .... *Lamprohiza splendidula* (Linnaeus, 1767)

– Lateroapical margins of labro-clypeus with two distinguishable long setae. No distinguishable light-coloured spot behind each stemma. Epicranial suture dark-coloured or undistinguishable. 2

2) Length of gena larger or same length as one-half of width of head capsule. .... *Lampyris noctiluca* (Linnaeus, 1767)

– Length of gena shorter than one-half of width of head capsule. .... *Phosphaenus hemipterus* (Geoffroy, 1762)

7. 2. 2. Antenna

1) Inner ventrolateral area of second antennomere with distinct longitudinal cleft. Sensorium of second antennomere longer than third antennomere, with fine helical ridges from apex to bottom. .... *Phosphaenus hemipterus* (Geoffroy, 1762)

– Inner ventrolateral area of second antennomere without distinct longitudinal cleft. Sensorium of second antennomere shorter than third antennomere, with smooth surface. 2

2) Sensorium of second antennomere with distinct basal constriction. .... *Lamprohiza splendidula* (Linnaeus, 1767)

– Sensorium of second antennomere closely annealing to the antennomere. .... *Lampyris noctiluca* (Linnaeus, 1767)

7. 2. 3. Maxilla

1) Maxillary palpus trimerous, with bulgy sensorium on distal palpomere. .... *Phosphaenus hemipterus* (Geoffroy, 1762)

– Maxillary palpus tetramerous, without bulgy sensorium on distal palpomere. 2

2) Maxillary palpomere IV subconical, narrow and sharp. .... *Lamprohiza splendidula* (Linnaeus, 1767)

– Maxillary palpomere IV irregularly subconical, thick and blunt. .... *Lampyris noctiluca* (Linnaeus, 1767)

7. 2. 4. Labium

1) Distal palpomere of labial palpus bearing a bulgy sensorium ventrally. .... *Phosphaenus hemipterus* (Geoffroy, 1762)

– Distal palpomere of labial palpus without bulgy sensorium. 2

2) Distal palpomere of labial palpus bearing stout blunt seta covering outer-lateral sagittal slot. .... *Lampyris noctiluca* (Linnaeus, 1767)

– Distal palpomere of labial palpus without sagittal slot, and without any stout blunt setae. .... *Lamprohiza splendidula* (Linnaeus, 1767)

7. 2. 5. Mandible

1) Retinaculum present, forming distinguishable sharp inner tooth. .... *Lampyris noctiluca* (Linnaeus, 1767)

– Retinaculum featureless, or blunt and dull. 2

2) Dorsal part of mandible covered with sagittal line of dense stout adjacent setation, aimed centrally. .... *Phosphaenus hemipterus* (Geoffroy, 1762)

– Dorsal part of mandible without sagittal line of dense setation, although several stout setae might be present. .... *Lamprohiza splendidula* (Linnaeus, 1767)

7. 2. 6. Thorax

1) Thoracic tergites divided by sagittal line into two parts, which are then subdivided with another clear line, subparallel to sagittal line. .... *Phosphaenus hemipterus* (Geoffroy, 1762)

– Thoracic tergites divided by sagittal line into two parts only. 2

2) Meso- and metanotum ca. four-times wider than long. Pronotum with deep emargination anteriorly. .... *Lamprohiza splendidula* (Linnaeus, 1767)

– Meso- and metanotum ca. two-times wider than long. Pronotum without deep emargination anteriorly, strongly concave on posterior margin. Thoracic tergites with distinct pinkish or yellowish spots on posterolateral margins. .... *Lampyris noctiluca* (Linnaeus, 1767)

7. 2. 7. Legs

1) Tarsungulus composed of claw, ventrally bearing two long setae. .... *Lamprohiza splendidula* (Linnaeus, 1767)

– Tarsungulus composed of claw, ventrally bearing three short setae. 2

2) Coxa large, stout, dorsally sclerotized in more than a 1/2 of longitudinal length. .... *Lampyris noctiluca* (Linnaeus, 1767)

– Coxa large, stout, dorsally sclerotized in less than a 1/2 of longitudinal length. .... *Phosphaenus hemipterus* (Geoffroy, 1762)

7. 2. 8. Abdomen

1) Abdominal tergites I – VI ca. four-times wider than long. Ventrite I unsclerotized. Spiracles of dark colouration. .... *Lamprohiza splendidula* (Linnaeus, 1767)

– Abdominal tergites I – VI ca. two- or three-times wider than long. Ventrite I sclerotised. Spiracles of light colouration. 2

2) Pleural suture subdivides lateral areas into upper pleurite bearing a spiracle and lower pleurite on pleural segments I to V. .... *Lampyris noctiluca* (Linnaeus, 1767)

– Pleural suture subdivides lateral areas into upper pleurite bearing a spiracle and lower pleurite on pleural segments I to VII. .... *Phosphaenus hemipterus* (Geoffroy, 1762)

7. 2. 9. Cuticular processes

1) Granulose protuberances, densely occurring on sclerites and legs, present. .... *Lampyris noctiluca* (Linnaeus, 1767)

– Granulose protuberances, densely occurring on sclerites and legs, missing. .. 2

2) Short fibrous setae growing from slightly sunken toroidal base present. .... *Phosphaenus hemipterus* (Geoffroy, 1762)

– Short fibrous setae growing from slightly sunken toroidal base missing. ....

.... *Lamprohiza splendidula* (Linnaeus, 1767)

7. 2. 10. Photic organs

1) Photic organs placed ventrolaterally on abdominal segments II and VI, with possible additional photic organs ventrolaterally on abdominal segments III – V. .... *Lamprohiza splendidula* (Linnaeus, 1767)

– Photic organ present ventrally on abdominal segment VIII. 2

2) Photic organs forming conspicuous white patch on venter of abdominal segment. .... *Lampyris noctiluca* (Linnaeus, 1767)

– Photic organs forming a pair of dull, hardly distinguishable spots ventrolaterally on abdominal segment. .... *Phosphaenus hemipterus* (Geoffroy, 1762)

8. DISCUSSION

8. 1. External sensory organs

During the close observation of larval anatomy, different types of possible *sensilla* and sensory organs were noted on the body-surface of each species (Annex 23). In addition, each of the species had a unique type of sensilla not observed in the others. Since the exact determination of type and function of observed sensory organs would be worth a separate thesis, the following paragraphs will be dedicated to brief description and speculations of possible function only, with connection to ecology of the particular species, where possible.

At least four types of sensilla defined by Shields (2008) were observed; sensilla *trichoidea*, sensilla *chaetica*, sensilla *placodea* and sensilla *campaniformia*. According to Shields (2008), sensilla trichodea vary greatly in length and are freely movable on a basal membrane. While sensilla chaetica are similar to sensilla trichodea, they can take form of bristles or spines, and are typically set in a socket. Both types have been probably observed in all three species, even though having different shapes.

Sensilla chaetica is probably the most abundant sensillum observed in studied species, being found on antennae, legs and sclerotized parts of dorsum and venter. According to Shields (2008), this type of sensillum can be either solely mechanosensitive, or dually mechano- and contact chemosenstitive. The chemosensitivity function is very probable for sensilla situated on the antennae, the mechanoreceptive function on the rest of the body, even though dual function with chemoreception on these body-parts is not excluded. The longest setae in all three species were observed on head capsule (gena, stipes, mentum, antennae), legs (ventral part of femur and tibiotarsus) and posterior parts of ventrites. The mechanoreceptive function is apparent. In addition, legs help lampyrids grasping their prey and setae on ventrites have auxiliary function during moulting (Tyler 2002).

Sensilla trichoidea – as a type of sensillum usually growing out of cuticle and lacking a socket – were mostly found on antennae of all described species, namely third antennomere. This type can be solely mechanosensitive, dually mechano- and contact chemosensitive, olfactory, or thermosensitive (Shields 2008). These sensilla take different forms in all three studied species, differing in length, thickness or shape (conical versus rod-like), therefore different sensory functions, like thermoreception, olfactory or chemoreception, are possible.

Sensilla placodea are defined as plate-like, with level slightly raised above, or depressed below the surface cuticle, being olfactory (Shileds 2008). This type was observed on *Lampyris noctiluca* and *Phosphaenus hemipterus* (Annex 25), but not on *Lamprohiza splendidula*; in *Lampyris noctiluca* solely on apex of second antennomere, while in *Phosphaenus hemipterus* both on antennae and apical parts of mandibles. The presence of this type of sensilla on mandibles of *Phosphaenus hemipterus* might be connected with different type of hunted prey. The lack of this type of sensilla on *Lamprohiza splendidula* is nevertheless confusing; although it is possible, that this species might just have a different-shaped sensory organs with the same function, for example campaniform.

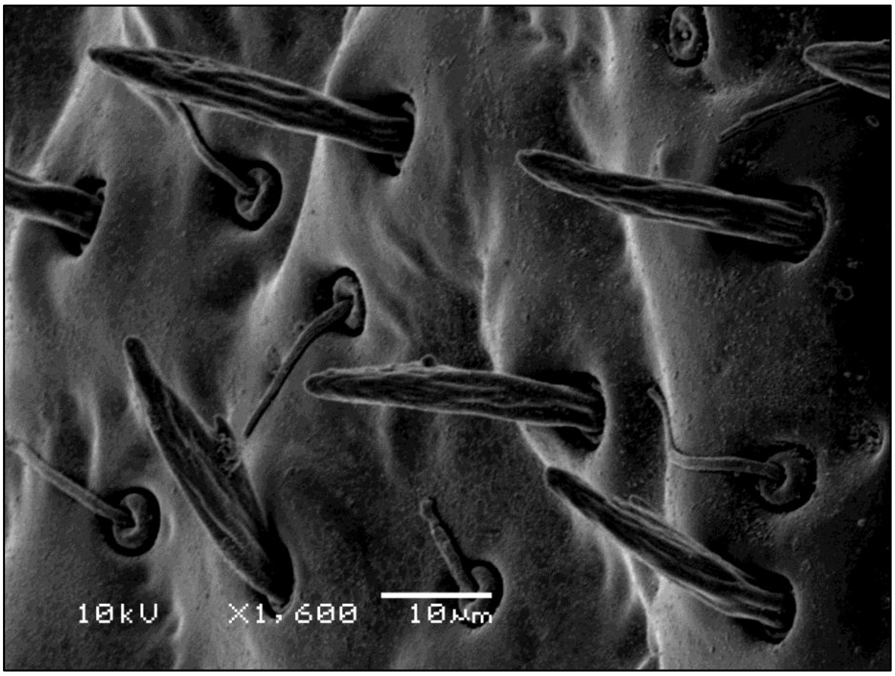

Figure 23 *Phosphaenus hemipterus*, toroidal setae

A very unique type of sensilla was observed on antennae, legs, and sclerotized parts of dorsum and venter of *Phosphaenus hemipterus*. This is a fibrous, weak seta set in a shallow toroidal socket (Fig. 23). Question is, if this process is just a modification of sensillum chaeticum or it is sensillum coeloconicum, defined by Shileds (2008) as a basiconic peg or cone set in a shallow pit, most often chemo-, thermo-, or hygrosensitive. Arguments for sensillum chaeticum are wide occurrence on the body of 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 shallow socket, and the fact that sensilla occur in numerous modifications, together with different prey type and ecology of *Phosphaenus hemipterus*, which may result in different need for sensory organs.

In *Lampyris noctiluca*, sensilla coeloconica, this time in a shape corresponding with the description of Shields (2008) was observed on head capsule and antennae. In case of this species, the function is more probably thermo- or hygrosensitive.

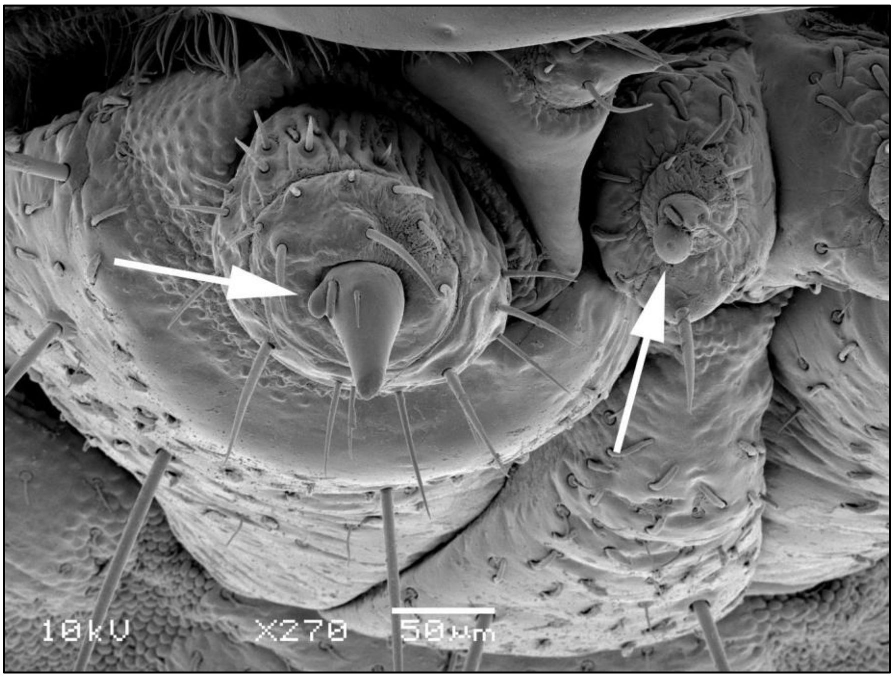

Figure 24 *Phosphaenus hemipterus*, sensoria on maxillary and labial palpi

Phosphaenus hemipterus adults, unlike most of the lampyrids, prefer pheromone communication to visual communication. In larvae of this species, a striking amount of *sensoria* was observed (Fig. 24). While *Lampyris noctiluca* and *Lamprohiza splendidula* have single sensoria on second antennomere only, *Phosphaenus hemipterus* bears sensoria on distal meres of maxillary palpi, labial palpi and third antennomere, in addition to previously mentioned second antennomere (Annex 13). The most obvious explanation for this phenomenon is the broad pheromone utilization within this species. Whereas the larvae cannot participate in sexual communication, the sensoria may be just undeveloped functional organs of adults. Another explanation may be the different diet of this species, and possible connection to prey tracking, with possible connection with abovementioned higher amount of sensilla placodea.

While *Lamprohiza splendidula* probably lacks sensilla placodea, this species possesses a unique feature, not found in the remaining two species. It is a *membranous spot* placed posteriorly behind each stemma of the larva (Fig. 19). The function of this organ is most probably sensory, nevertheless the exact purpose is unknown. *Lamprohiza splendidula* is believed to react by light emission to disturbance in its surroundings (De Cock 2003). During my *in vivo* observations of this species, I have never observed larva "turning-on" its light due to disturbance, the light emitted by larva was always vice versa spotted from several meters already (M. Novák, unpublished observation). This could mean, that larvae of this species have well developed sense of vibration-detection and the mysterious membranous spot placed behind their eyes could therefore be some kind of tympanal organ.

8. 2. Granulose protuberances of *Lampyris noctiluca*

The body of *Lampyris noctiluca* has been described as "velvet" many times. The "velvet" effect is probably caused by unique species-specific surface of cuticle. Sclerotized plates of legs, dorsum and venter are densely littered with microscopic granulose protuberances (Fig. 25). Whether their function is sensory, insulatory or other, or whether they are just an evolutionary remnant is unknown.

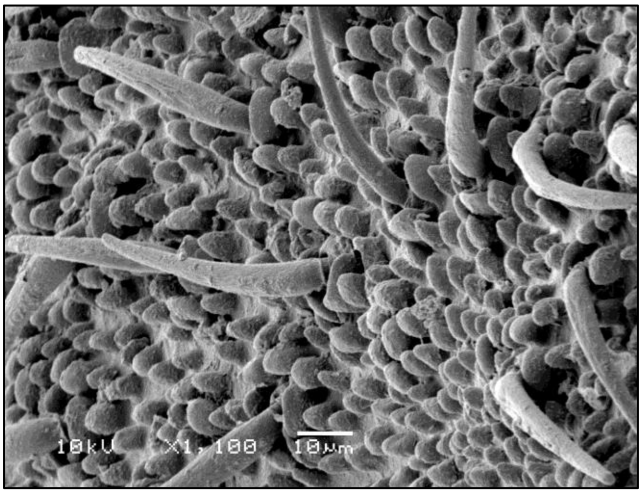

Figure 25: *Lampyris noctiluca*, granulose protuberances

8. 3. Hunting for prey

Lampyrid larvae are known to be predatory, as it is in the three species described in this work. *Lampyris noctiluca* and *Lamprohiza splendidula* are snail and slug specialists (Lloyd 2008), while *Phosphaenus hemipterus* is an obligate earthworm predator (Majka & MacIvor 2009). The largest difference between the two food-specialist groups observed, is probably both high amount of sensoria and lack of dorsoventrally flattened body in *Phosphaenus hemipterus*. 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 catch of prey, that is retreating into a ground tunnel. The significance of body shape is supported by the fact, that Nearctic *Photinus* sp. (Fig. 26), which is also reported to prey on earthworms (Lloyd 2008) has very similar general body-shape. Nevertheless, detailed *in vivo* study of *Phosphaenus hemipterus* predation is missing.

Figure 26 *Photinus* sp., larva © Antonio Liberta

If the body-shape has indeed a significant importance in feeding habits, another comparison with Neotropical species might be interesting. In the *Photuris* sp. (subfamily Photurinae, Fig. 27), with infamous "femmes fatales" females, the larva has a striking similarity to larvae of *Lamprohiza splendidula*. *Photuris* sp. larvae are considered to be omnivores and scavengers, and reported to eat snails, worms, insect larvae, dead insects, and ripe berries (Lloyd 2008). In addition, *Lamprohiza splendidula* larvae are clearly capable of cannibalism (M. Novák, unpublished observation), thus it is possible, that *Lamprohiza splendidula* may in fact be omnivorous, instead of being simply a gastropod specialist. While *Photuris* sp. does not belong to the same subfamily as *Lamprohiza splendidula*, convergent evolution of similar body features convenient for a specific way of life remains a possibility.

Figure 27 *Photuris* sp., larva © Andrew Williams

It is established, that lampyrid larvae pierce the body of their prey with long ditched mandibles and inject them with a dark secretion, which kills and partially liquefies and digest the prey (Klots & Klots 1963; Hůrka & Čepická 1978). On mandibles of all three species, special, mechanoreceptive sensilla were observed, positioned apically, before the opening of inner mandibular channel. It is quite possible, that while the larva attempts a successful bite into its prey's body, this seta triggers the discharge of digestive liquid. Furthermore, a shutter can be found in all three species – in each one having different form – located at the base of mandibular channel opening (Annex 20).

Nevertheless, if the flow of digestive liquid, theoretically triggered by the mechanoreceptive sensilla, is being controlled by the shutter or by the gland producing this fluid, remains a question.

The dense setation observed on mandibles, maxillae, hypopharynx and labium agrees with Klots & Klots (1963), stating, that its purpose is to filter pre-digested food from larger particles.

The holdfast organ – pygopod (Annex 24) – helps larva with movement, but also serves during hunting, being used for fastening the snail-attacking larva to snail's shell as has been observed at least in *Lampyris noctiluca* by Tyler (2002), thus giving it a safe position and room for biting manoeuvres. In addition, pygopod is used also for body cleaning (among other) after hunt (Tyler 2002; M. Novák, unpublished observation), especially of head appendages with dense setation, which filter pre-digested fluid as mentioned above.

The stout, strong, erect setae placed ventrally on tibiotarsus of all three species may represent another possible adaptation for prey hunting. The setae would be probably the most practical in earthworm hunting *Phosphaenus hemipterus*, in terms of grasping the prey, because snails tend to excrete huge amounts of mucus as a defence, and the snail-hunting larvae anxiously avoid getting in contact with this secretion. In addition, the strong, stout setae can be found in similar quality and quantity on tibiotarsi of all three pairs of legs of all here-described species. Therefore it is more likely, that their function is predominantly mechanoreceptive.

8. 4. Photic behaviour

According to observations of De Cock (2003), larvae of *Lampyris noctiluca* and *Phosphaenus hemipterus* emit spontaneous light pulses while moving, whereas *Lamprohiza splendidula* larvae do not show this behaviour and only glow when disturbed. From my personal observations, I can confirm this behaviour pattern only in *Lampyris noctiluca*, since I have not witnessed *in vivo* specimen of *Phosphaenus hemipterus* yet. As for *Lamprohiza splendidula*, emission of light due to disturbance or manipulation is not that clear according to my *in vivo* observations. During the collecting of larvae, specimen were, thanks to their glow, frequently spotted already from several meters. The fact is, that during the search between bushes, the collecting team caused noise and vibrations, which were probably perceived by the larvae. Nevertheless, in the moment the specimen were being collected from the ground and handled, larvae often strongly reduced their glow, thus sometimes making the collection almost impossible without a flashlight. This type of behaviour is similar to what describes Viviani (2001) in Neotropical genera *Pyrogaster*, *Photuris*, and *Aspisoma*; larvae respond to vibrations, but do not respond to mechanical manipulation. This behaviour is probably collective defence against predators, which lies in distraction and confusion of the "enemy". Question why do the larvae – when being in danger – only reduce their glow, and not cease it completely, may be explained by inability of larval stage to vary its glow swiftly, which is caused by different physiology of photic organ (Timmins et al. 2001). Even though, larvae of *Lampyris noctiluca* (and consequently *Phosphaenus hemipterus*) are reported to gradually "turn off" their light organ within few seconds, the situation may be different in larvae of *Lamprohiza splendidula*, which may be caused by different anatomy of their light organ compared to the two abovementioned species.

According to De Cock (2003), the spectrum of light emitted by larvae of all three lampyrid species, as opposed to adults, is very similar, conserving the green emission. This fact agrees with the lack of intraspecific function (mating) and increased importance of and interspecific function such as defence, as stated by Viviani (2001).

All three species described in this work, are reported to live sympatrically (De Cock 2003). This is supported by my observations at least in *Lampyris noctiluca* and *Lamprohiza splendidula*, which occurred in the same locality in Slovenia (specimen of *Lamprohiza splendidula* found in Slovenia are not mentioned in the methodology, since they have not been used for description in this work). On top of that, lampyrids are reported to be an unpalatable prey in general (Underwood et al. 1997; De Cock & Matthysen 2003; Moosman et al. 2009), which brings up a possibility that Batesian or Millerian mimicry could have evolved within and between these taxonomic groups.

It should be noted, that according to De Cock (2003), the spectrum of produced light by larvae of *Lamprohiza splendidula* is different in dorsal and ventral/lateral view. Since the light of ventrolaterally placed photic organs shines through abdominal tergites, its colour is slightly shifted. It is possible, that the slight shift of colour spectrum serves as intraspecific communication with adults who, unlike larvae, have well developed sight. Viviani (2001) witnessed possible intraspecific communication in unidentified *Bicellonychia* sp., where the larvae reacted to flashes emitted by adults, hypothesising the cause of this behaviour could be informing the adults of occupied food niche. Larvae of *Lamprohiza splendidula*, unlike *Bicellonychia* sp., are not capable of emitting pulses of light, but so are not the females of this species. From this point of view, the possibility of division of niche might be plausible. On the other hand, if the larvae truly react only to disturbances in their surroundings, this hypothesis is incorrect. Above that, Viviani probably describes communication between larvae and flying males, which has no effect on distribution of females on the locality in connection to the larvae. It is the female who produce offspring and consequently food competition. In addition, *Lamprohiza splendidula* females can be often found in clusters (M. Novák, unpublished observation) and are known to have more or less stationary way of life (Tyler 2002), which again does not agree with the hypothesis of intraspecific communication due equal division of food niche.

Display of green light may not be only adaptive in regard of mimicry but also for being as conspicuous as possible. This agrees with Viviani (2001), who states, that the majority of the terrestrial animals is sensitive mainly to colours that can be found in the green spectrum. The specific photic behaviour, sometimes produced during movement of larvae of *Lampyris noctiluca* and *Phosphaenus hemipterus*, consists of definite pulses of light lasting ca. 2 seconds, separated from the next by a longer interval of darkness lasting ca. 4 seconds (Tyler 2002; M. Novák, unpublished observation). Nevertheless the true cause of this 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) larva uses the light while tracking the prey; 3) larva uses the light to attract the prey; 4) interspecific communication; 5) aposematic defence. Since Tyler (2002) adequately explains the pros and cons for each of the five points, I would like to address the intraspecific communication only. Let me remind, that the colour spectrum of *Lampyris noctiluca* and *Phosphaenus hemipterus* larvae is identical. If the slightly shifted spectrum of *Lamprohiza splendidula* would indeed somehow be due to intraspecific communication, so might be the abovementioned photic manifestation of *Lampyris noctiluca* and *Phosphaenus hemipterus*, if the intervals of light pulses in these two differed. Nevertheless, detailed information of this behaviour in the case of *Phosphaenus hemipterus* is missing and described differences in lengths of the intervals, although probable, are not available. Therefore the question of possibility of intraspecific communication in the three here-described sympatric species remains unanswered.

9. CONCLUSION

The Lampyridae are a fascinating family of insects, with interesting ecology and behavioural manifestations. Unfortunately, in the past, they did not get as much scientific attention as they deserved, namely in regards to species occurring in the Czech Republic, while the most influential works on *Lampyris noctiluca*, *Lamprohiza splendidula* (Fig. 28) and *Phosphaenus hemipterus* were published abroad. In addition, the less glamorous larvae have been generally neglected compared to adults. The past trend has nevertheless changed. With help of modern scientific instruments, many articles describing new species and re-describing the old ones in greater detail started to appear. There is still a lot of work to be done with respect to the "Czech Trio" of fireflies, namely detailed study of sensory organs, life history, ecology, behaviour, and interspecific interactions. My hope nonetheless is, that the detailed revision of morphology and identification key, presented in this work, would provide a good starting point.

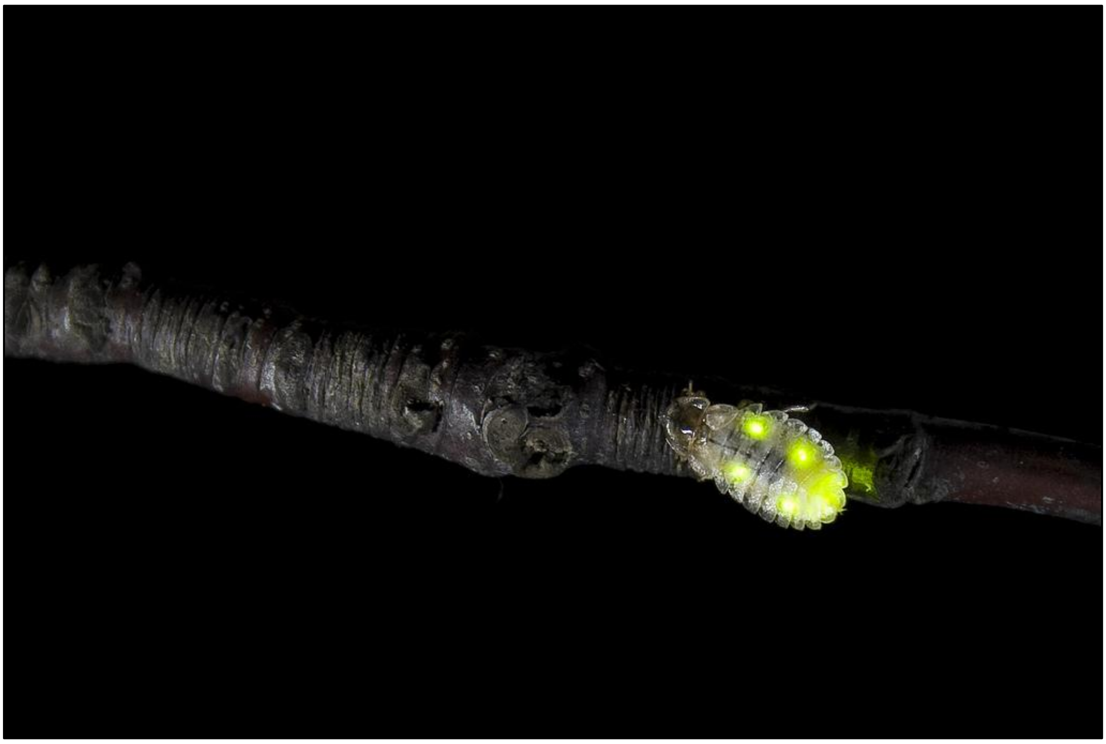

Figure 28 *Lamprohiza splendidula*, glowing female © Péter I. Pápics

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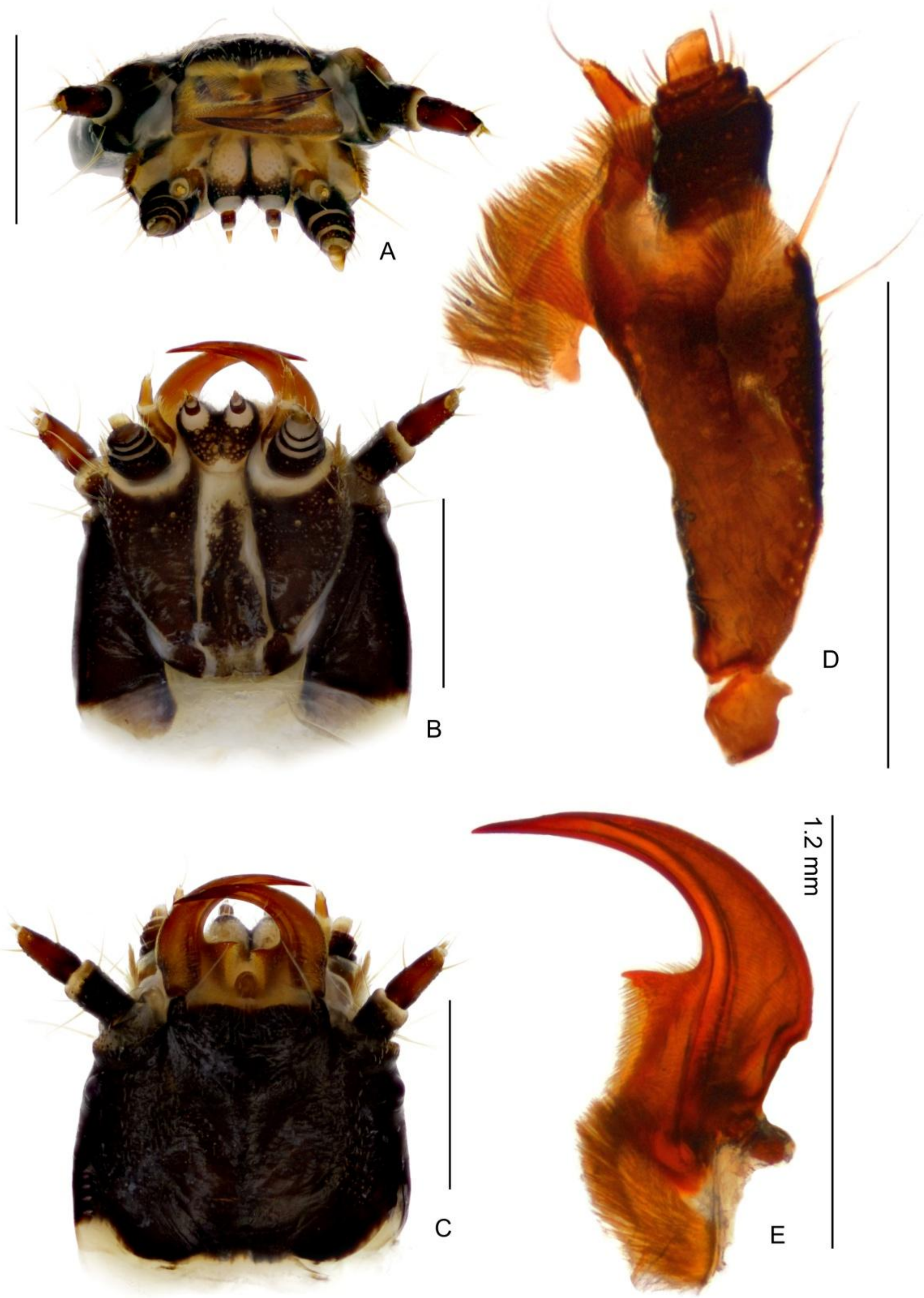
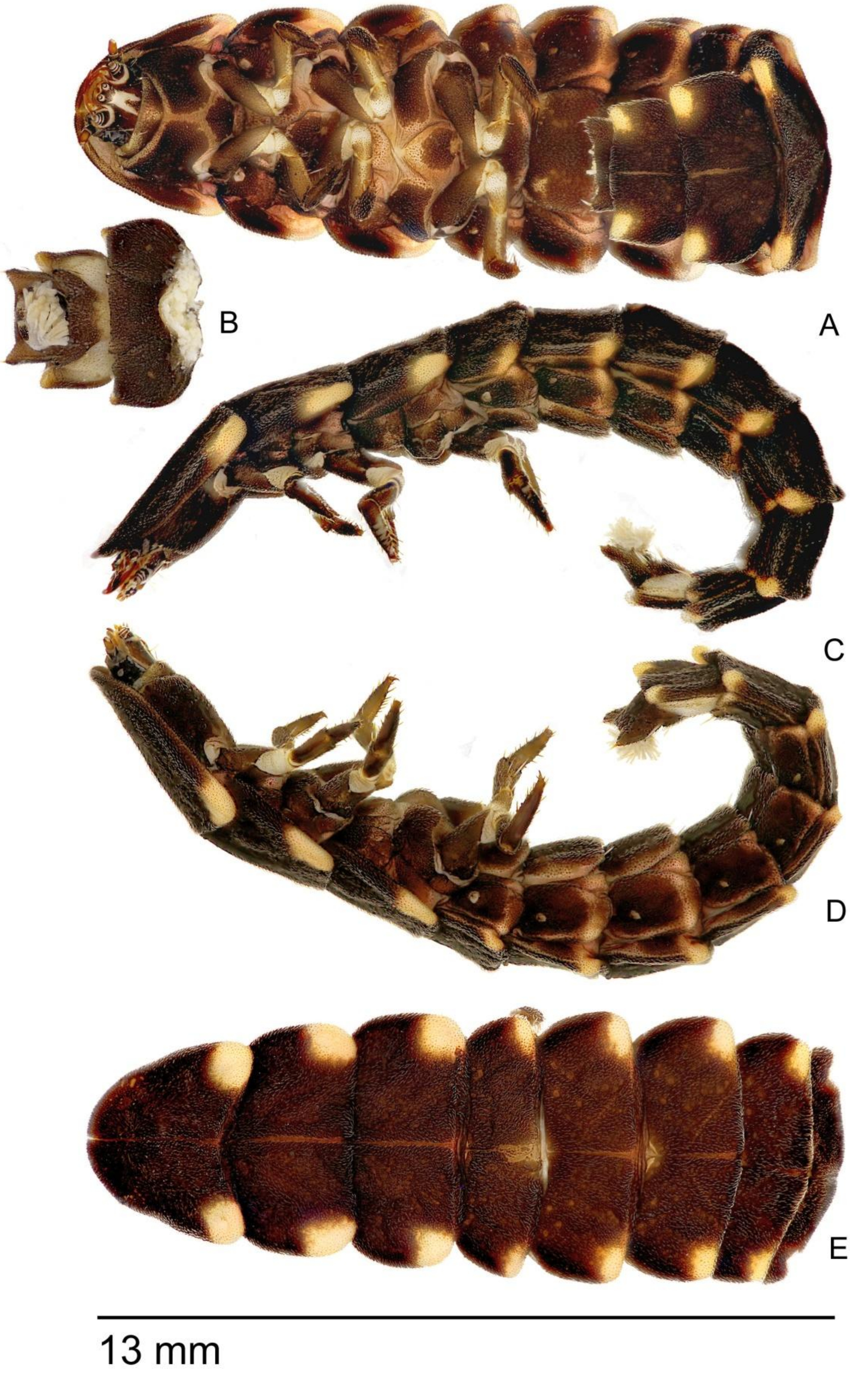
11. ANNEXES

Annex 1: *Lampyris noctiluca*, larva, head. – A) Anterior view; B) ventral view; C) dorsal view; D) maxilla; E) mandible.

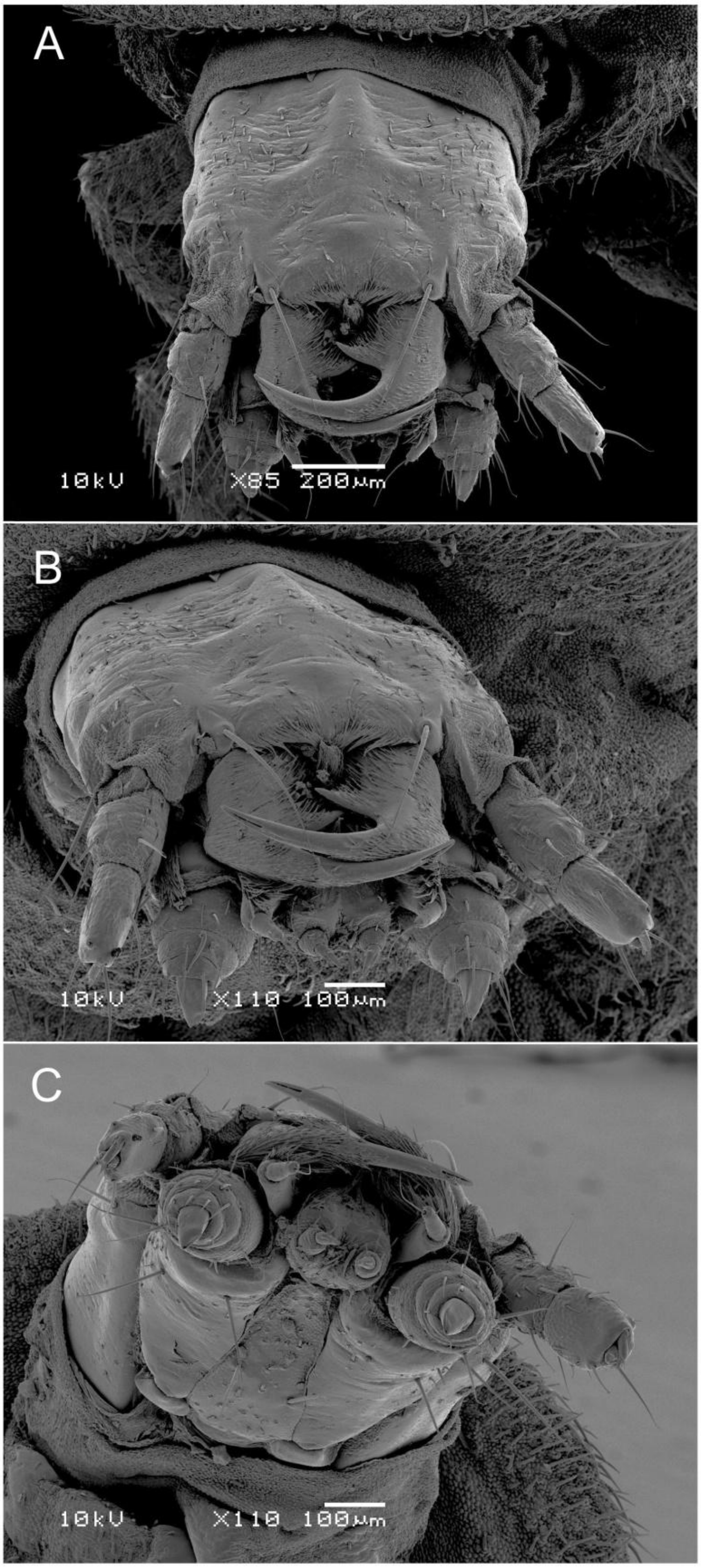


Annex 2: *Lampyris noctiluca*, larva, habitus. – A) Ventral view; B) final segments, ventral view; C) lateral view, left; D) lateral view, right; E) dorsal view.

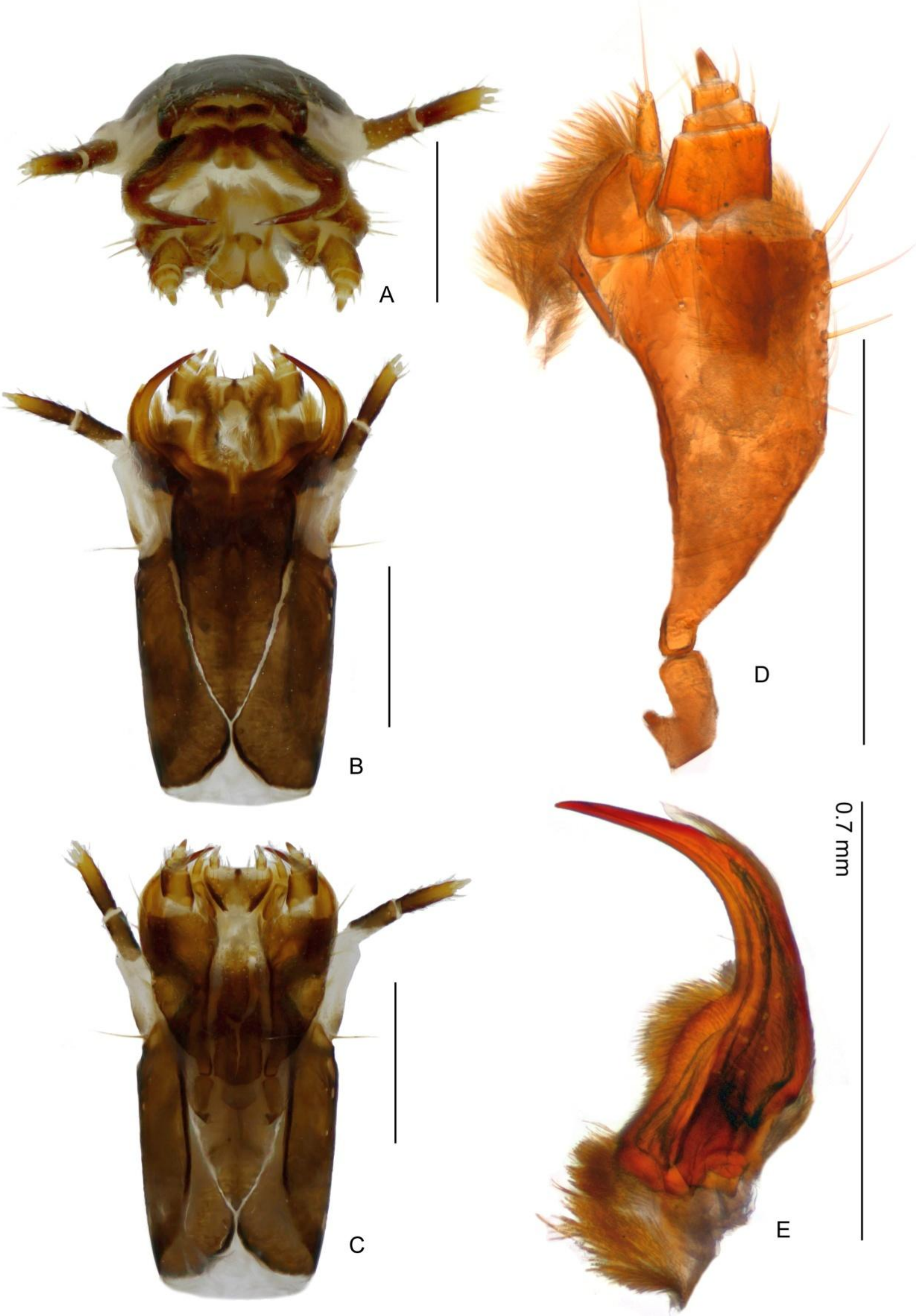


Annex 3: *Lampyris noctiluca*, larva, habitus, dry sample. – A) Ventral view; B) final segments, ventral view; C) lateral view, left; D) lateral view, right; E) dorsal view.

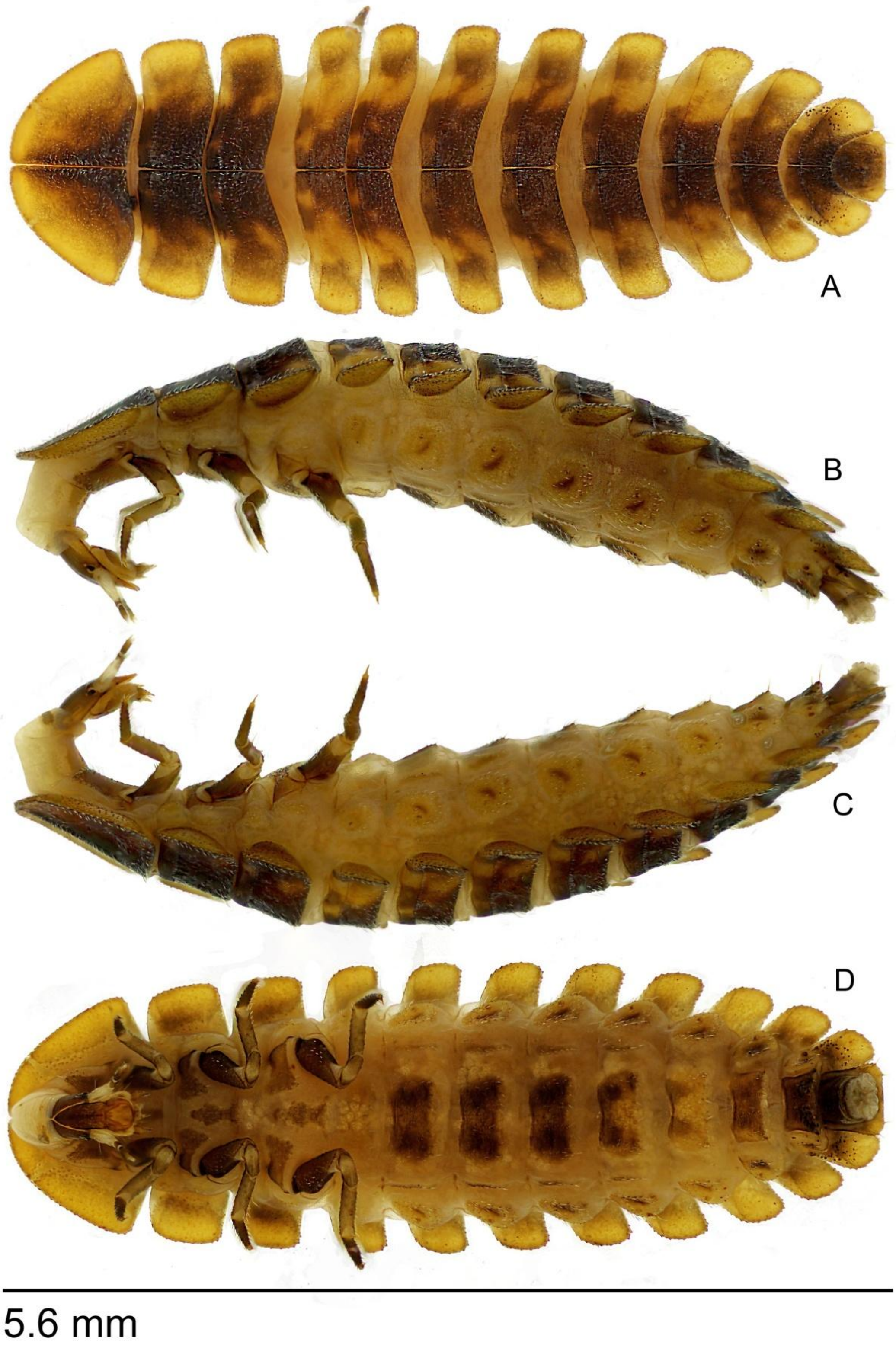
Annex 4: *Lampyris noctiluca*, larva, head, SEM. – A) Dorsal view; B) anterior view; C) ventral view.



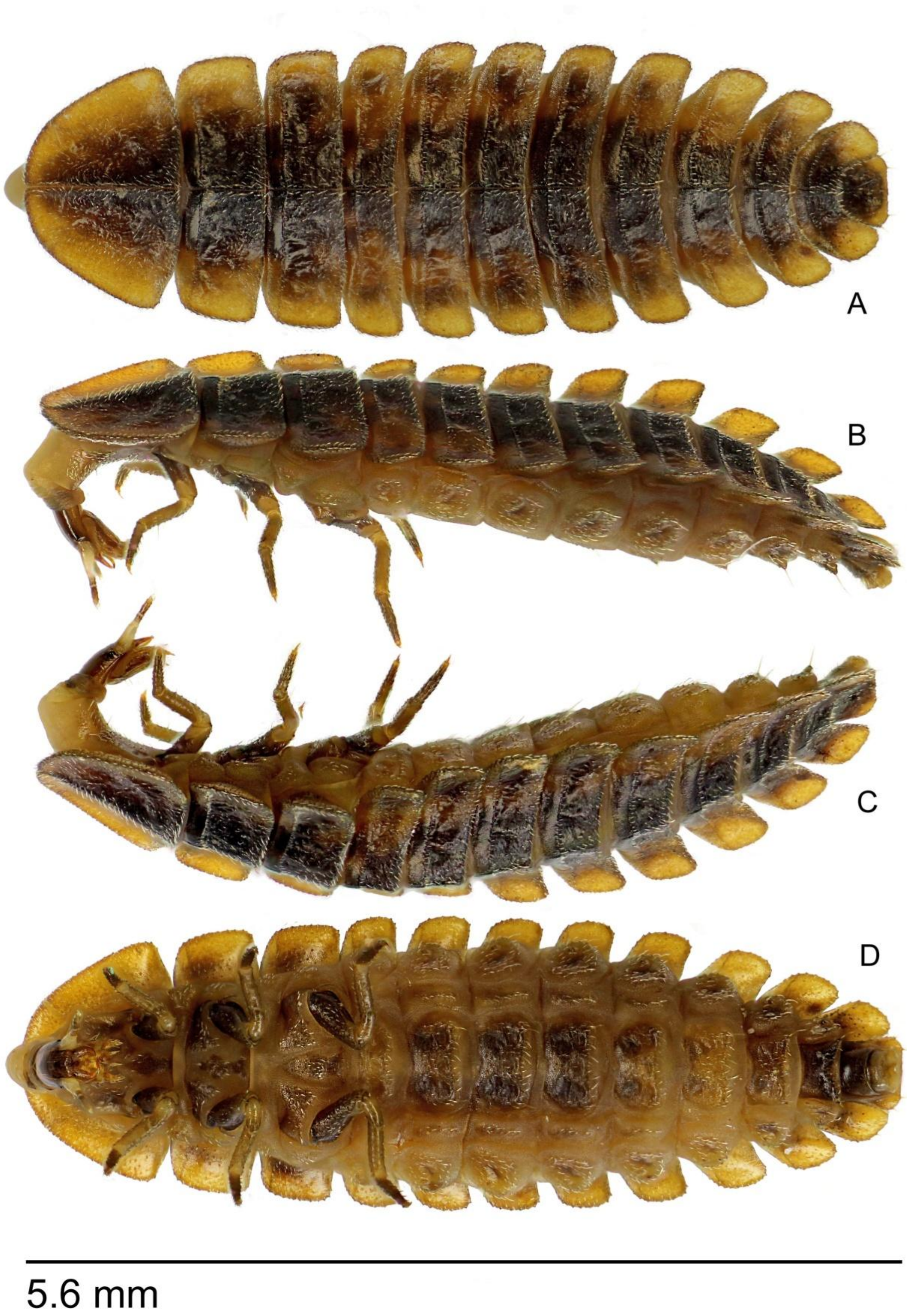
Annex 5: *Lamprohiza splendidula*, larva, head. – A) Anterior view; B) ventral view; C) dorsal view; D) maxilla; E) mandible.



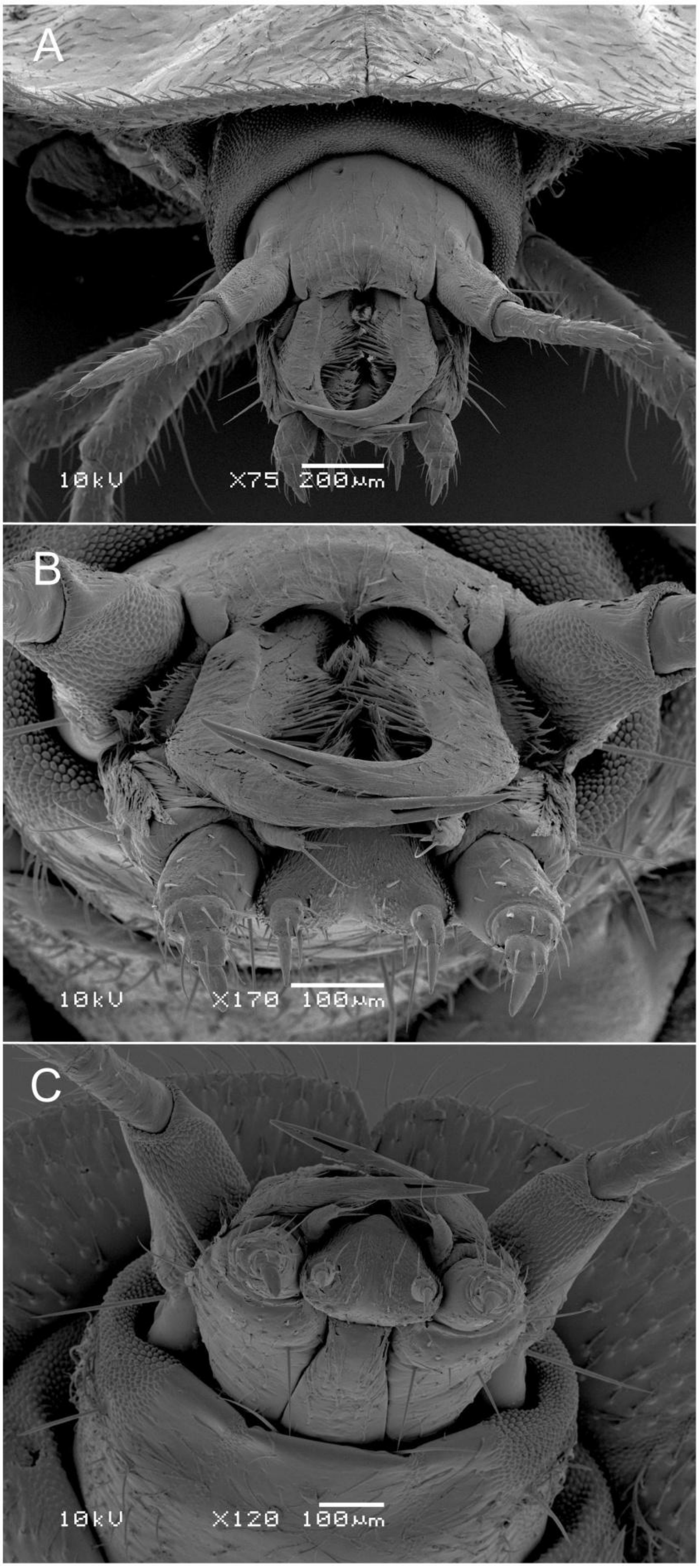
Annex 6: *Lamprohiza splendidula*, larva, habitus. – A) Dorsal view; B) lateral view, left; C) lateral view, right; D) ventral view.



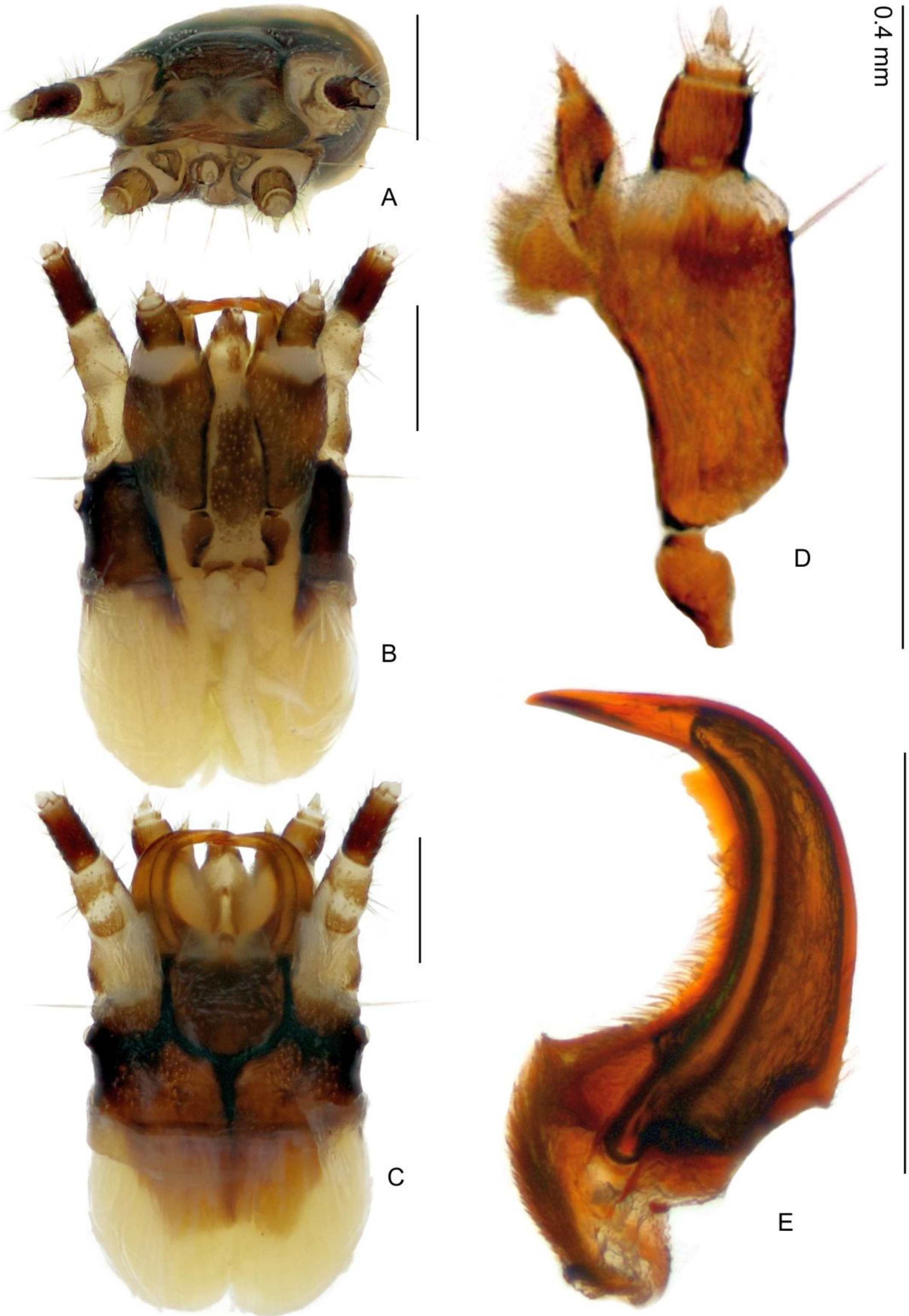
Annex 7: *Lamprohiza splendidula*, larva, habitus, dry sample. – A) Dorsal view; B) lateral view, left; C) lateral view, right; D) ventral view.



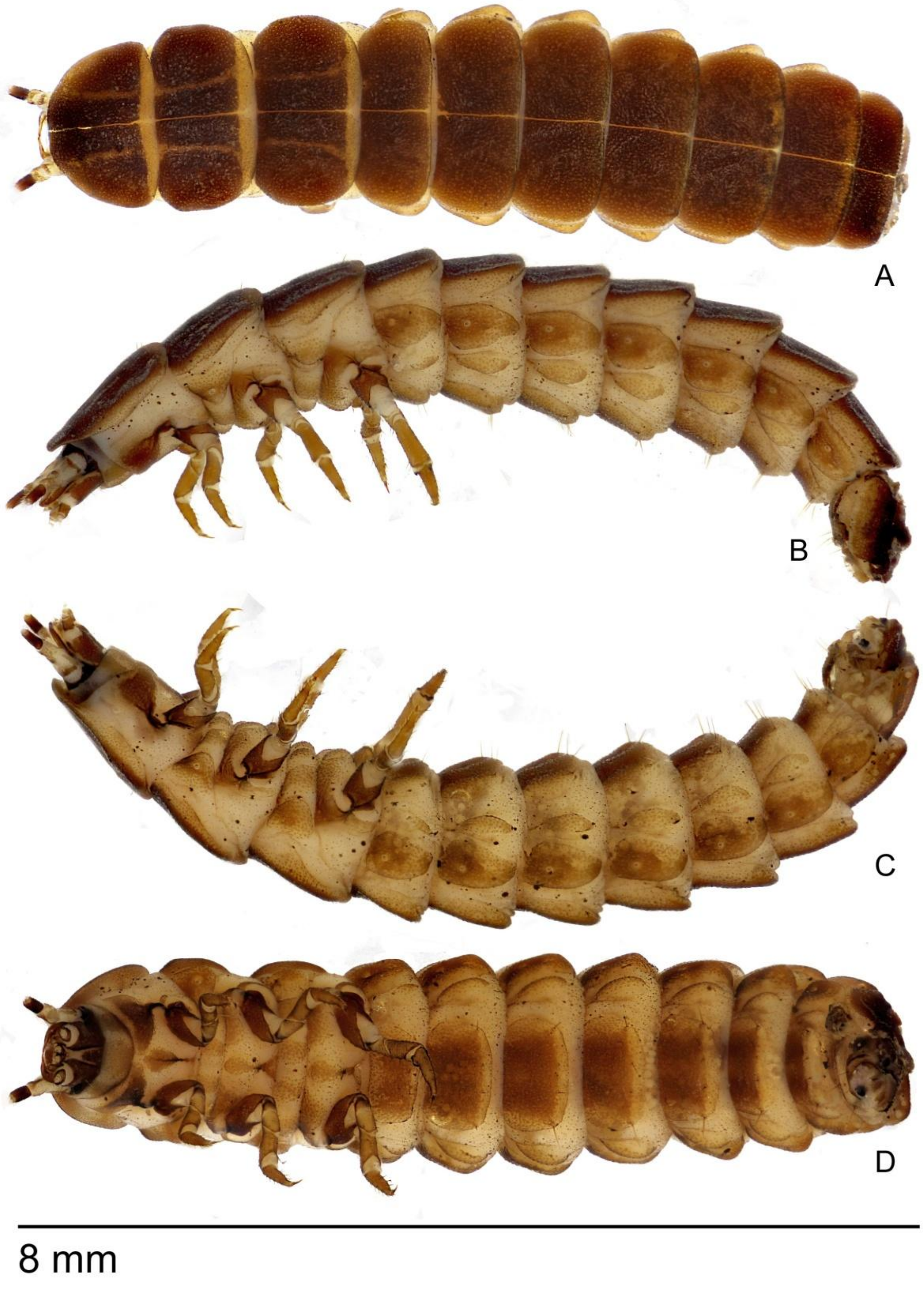
Annex 8: *Lamprohiza splendidula*, larva, head, SEM. – A) Dorsal view; B) anterior view; C) ventral view.



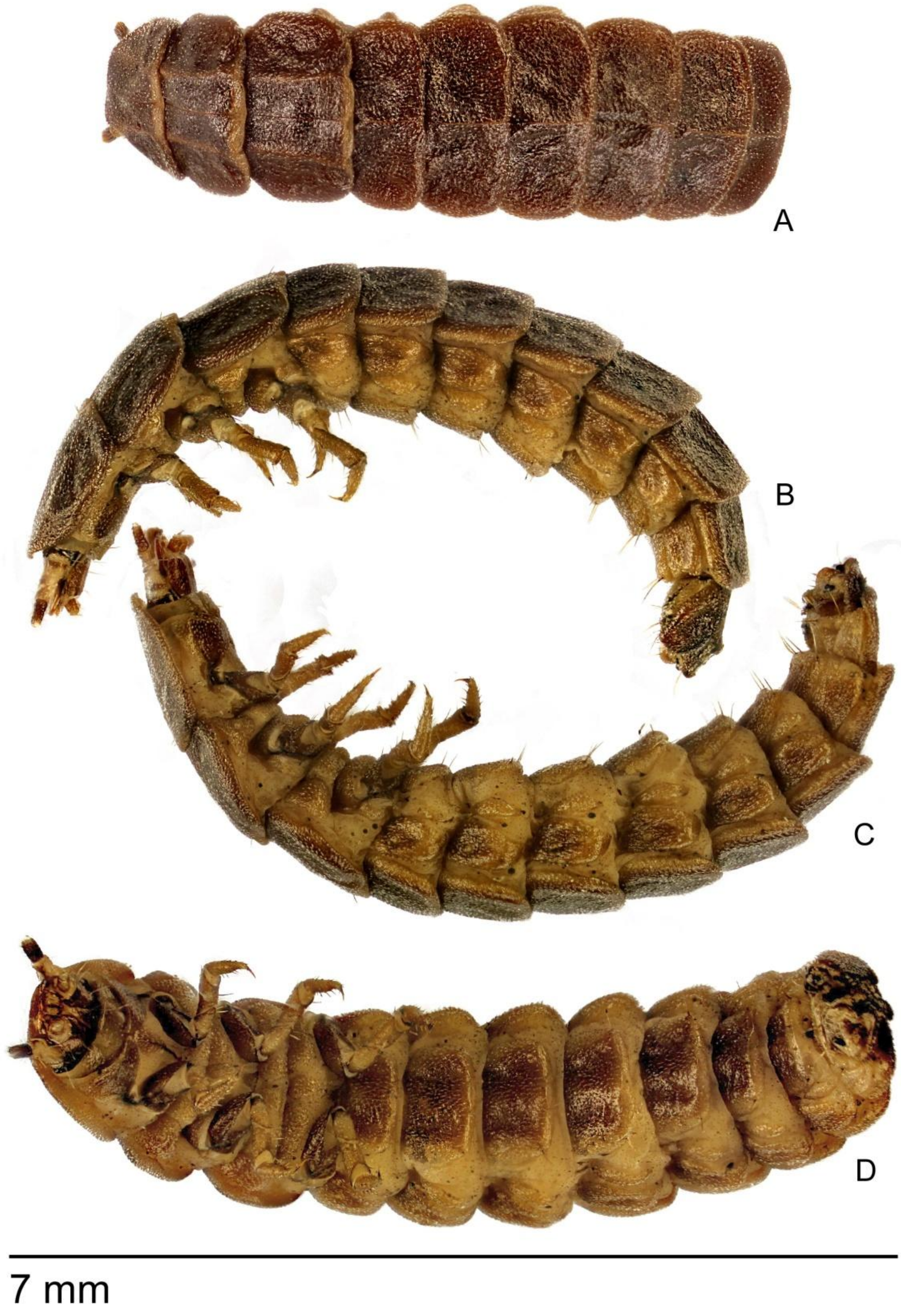
Annex 9: *Phosphaenus hempiterus*, larva, head. – A) Anterior view; B) ventral view; C) dorsal view; D) maxilla; E) mandible.



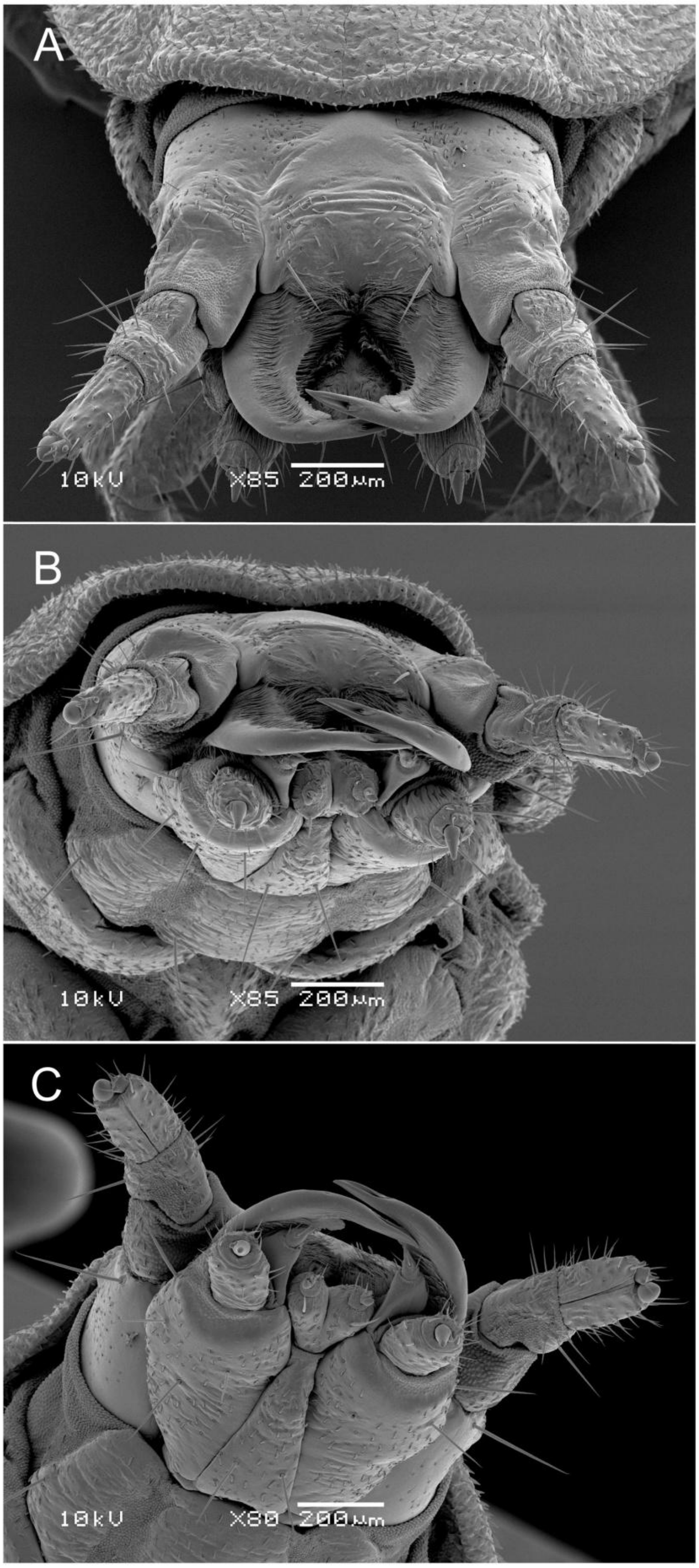
Annex 10: *Phosphaenus hemipterus*, larva, habitus. – A) Dorsal view; B) lateral view, left; C) lateral view, right; D) ventral view.



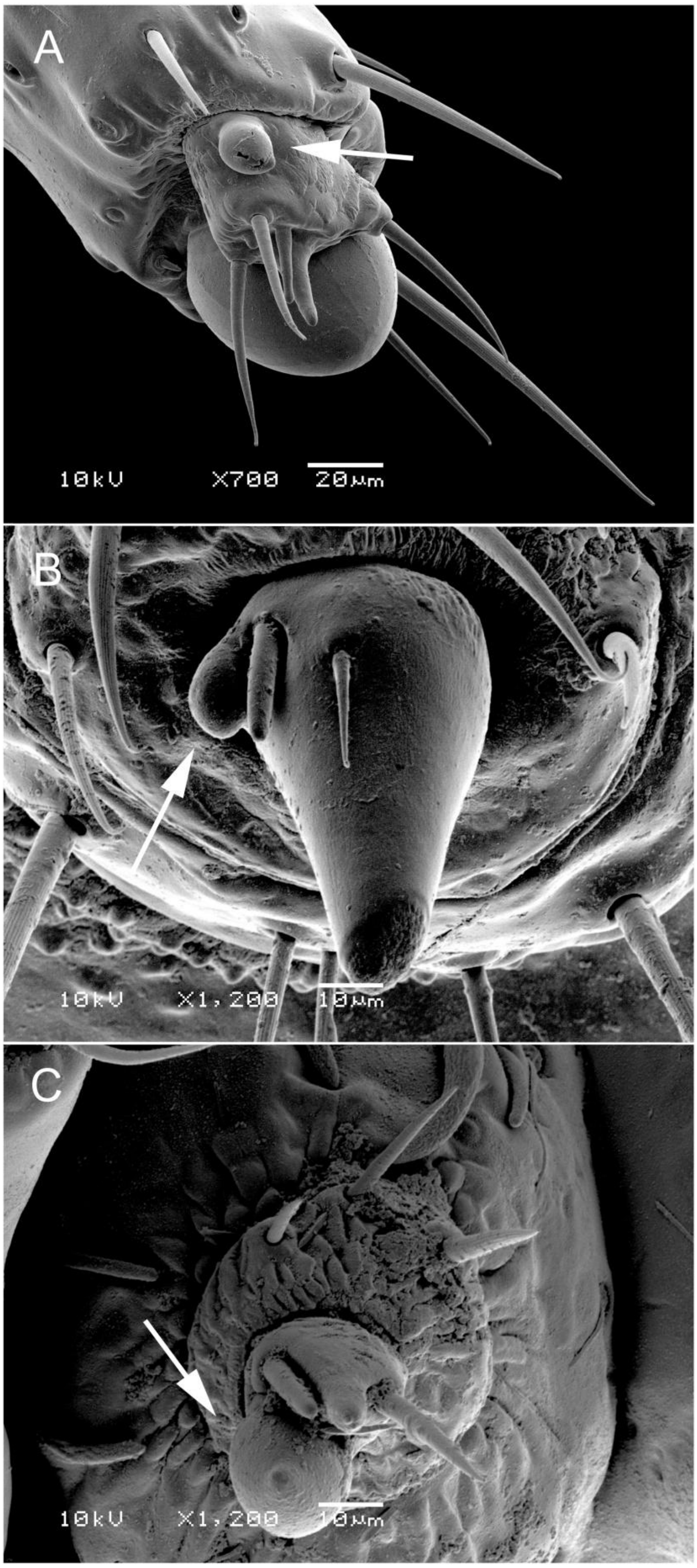
Annex 11: *Phosphaenus hemipterus*, larva, habitus, dry sample. – A) Dorsal view; B) lateral view, left; C) lateral view, right; D) ventral view.



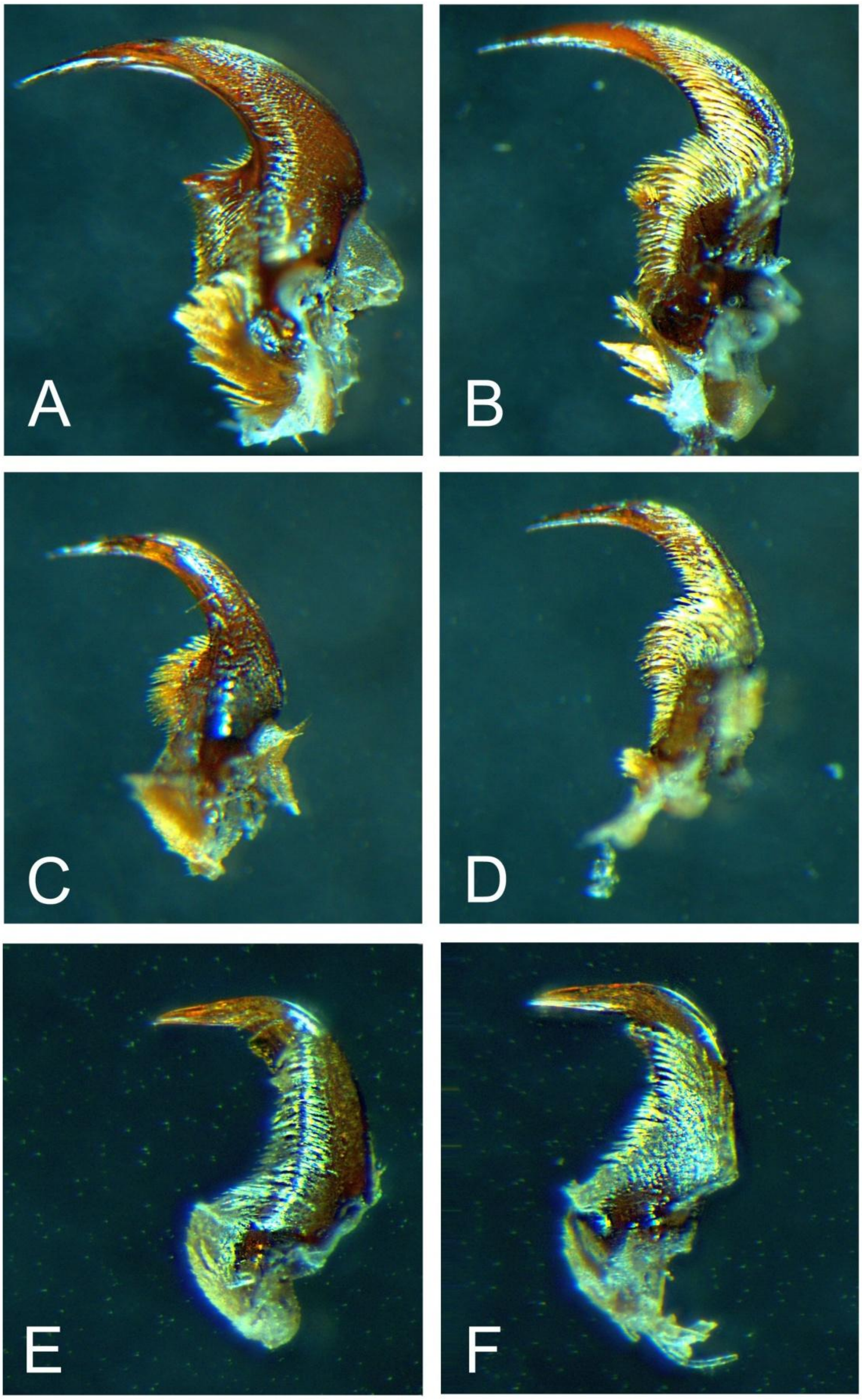
Annex 12: *Phosphaenus hemipterus*, larva, head, SEM. – A) Dorsal view; B) anterior view; C) ventral view.



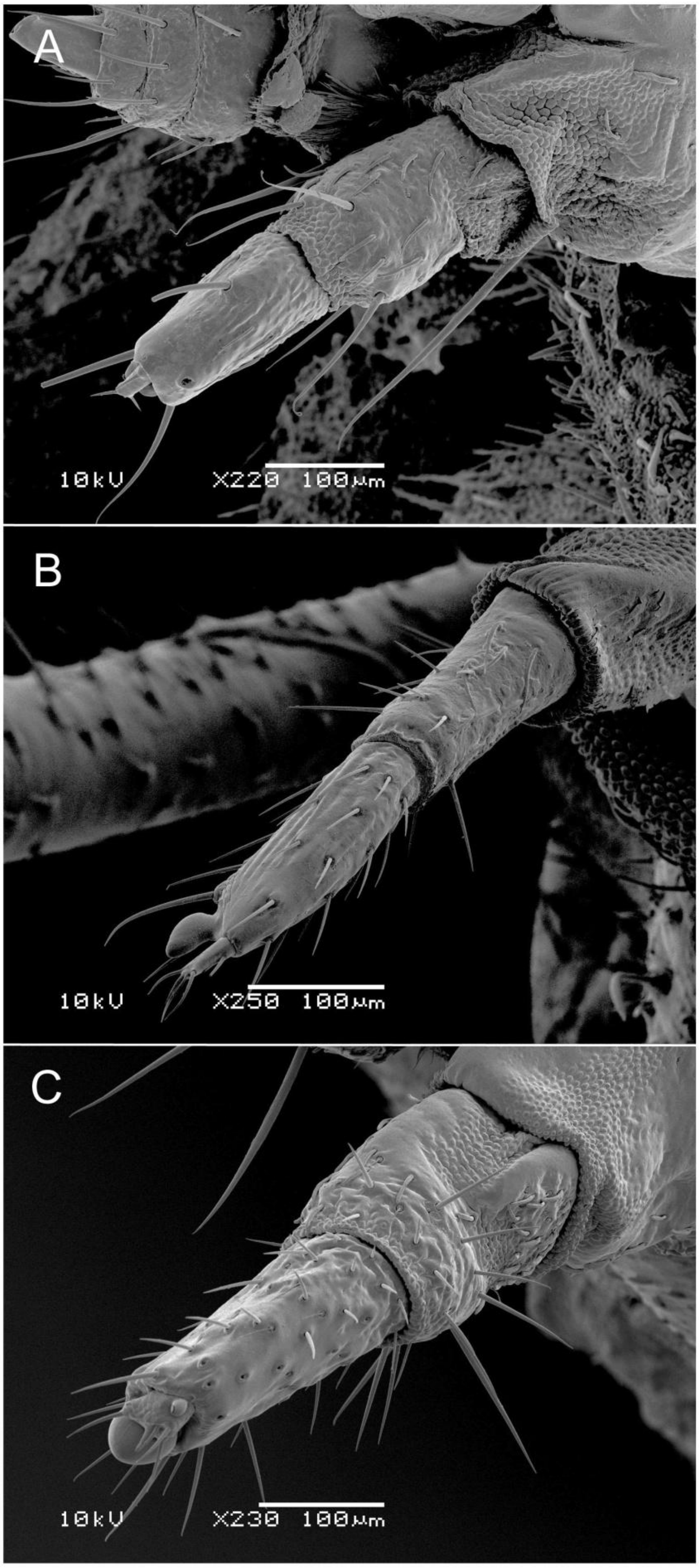
Annex 13: *Phosphaenus hemipterus*, sensoria, SEM. – A) Antenna; B) maxillary palpus; C) labial palpus.



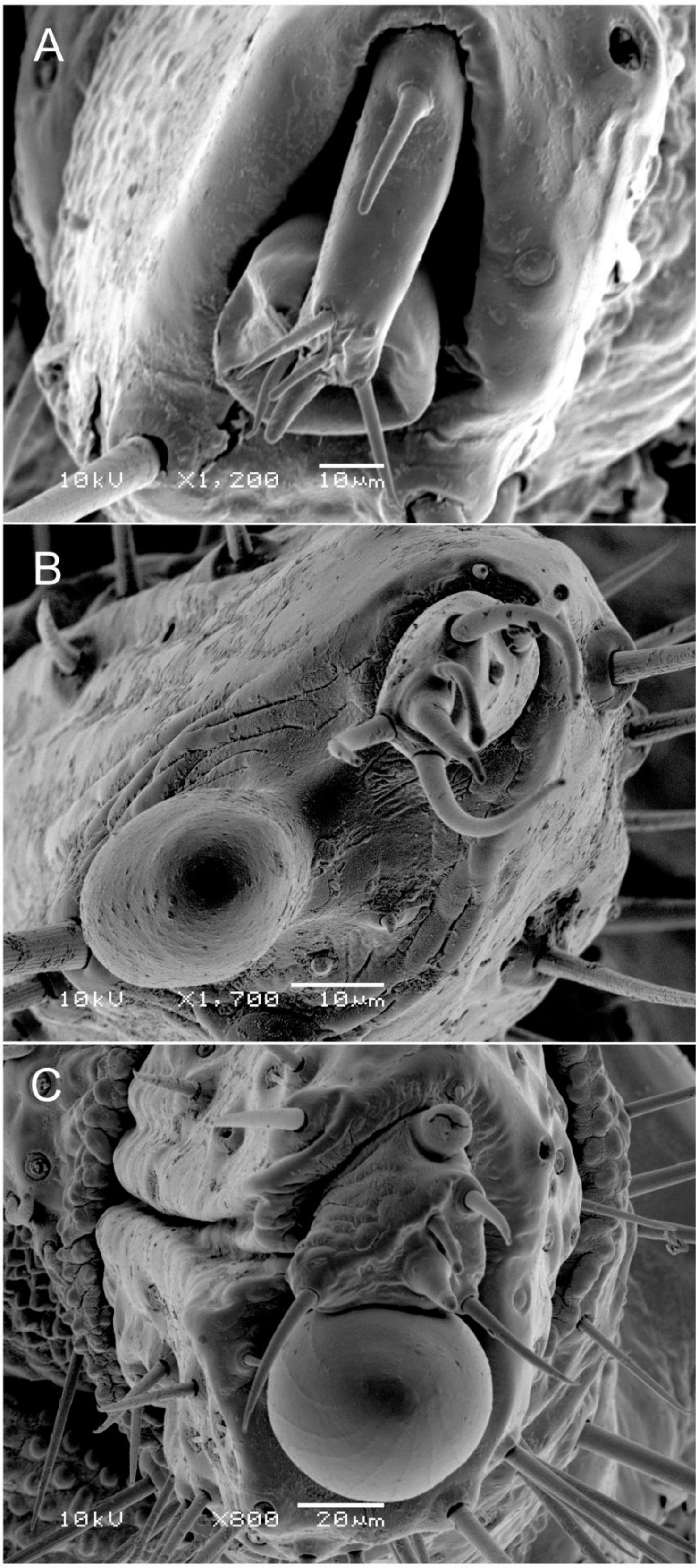
Annex 14: Mandible setation. – A) *Lampyris noctiluca*, dorsal view; B) *L. noctiluca*, ventral view; C) *Lamprohiza splendidula*, dorsal view; D) *L. splendidula*, ventral view; E) *Phosphaenus hemipterus*, dorsal view; F) *P. hemipterus*, ventral view.



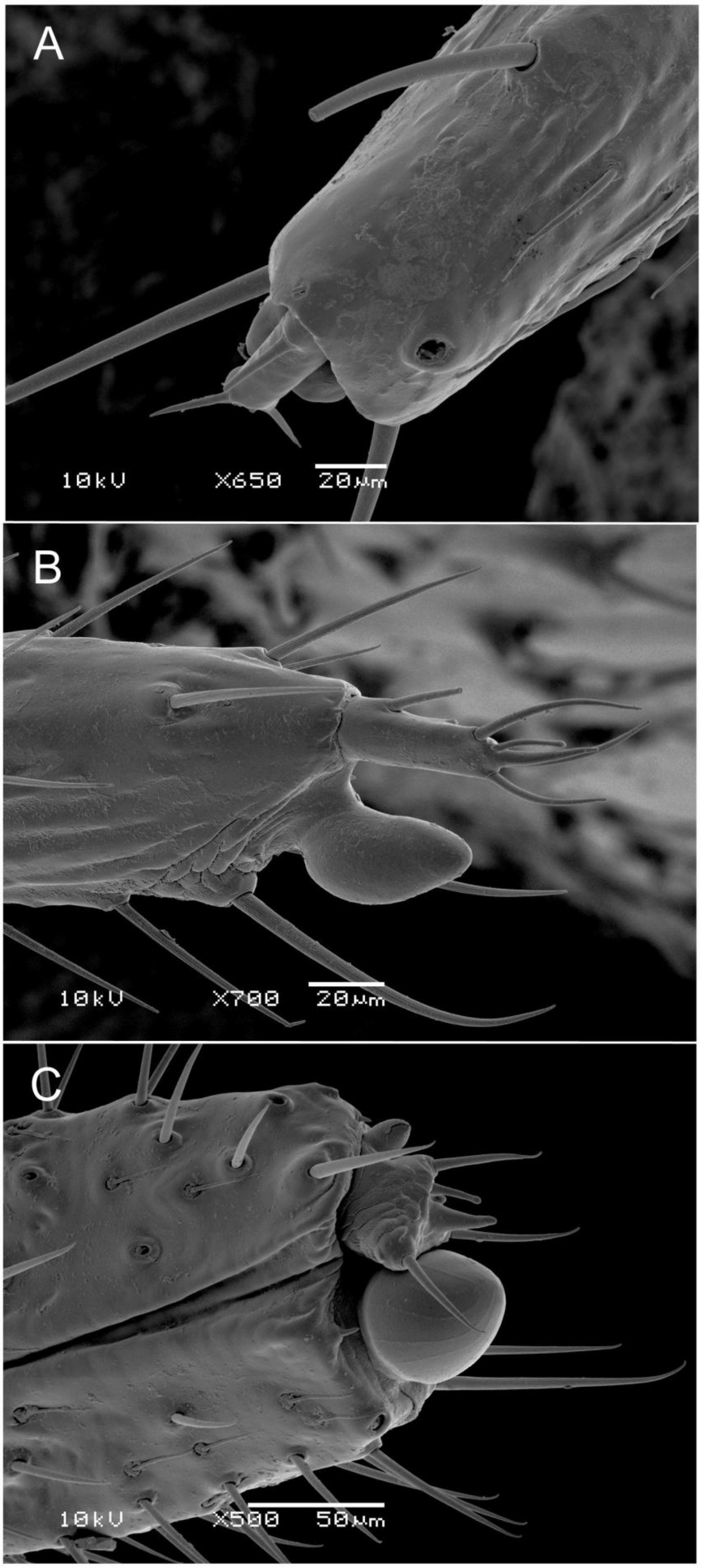
Annex 15: Antenna, SEM. – A) *Lampyris noctiluca*; B) *Lamprohiza splendidula*; C) *Phosphaenus hemipterus*.



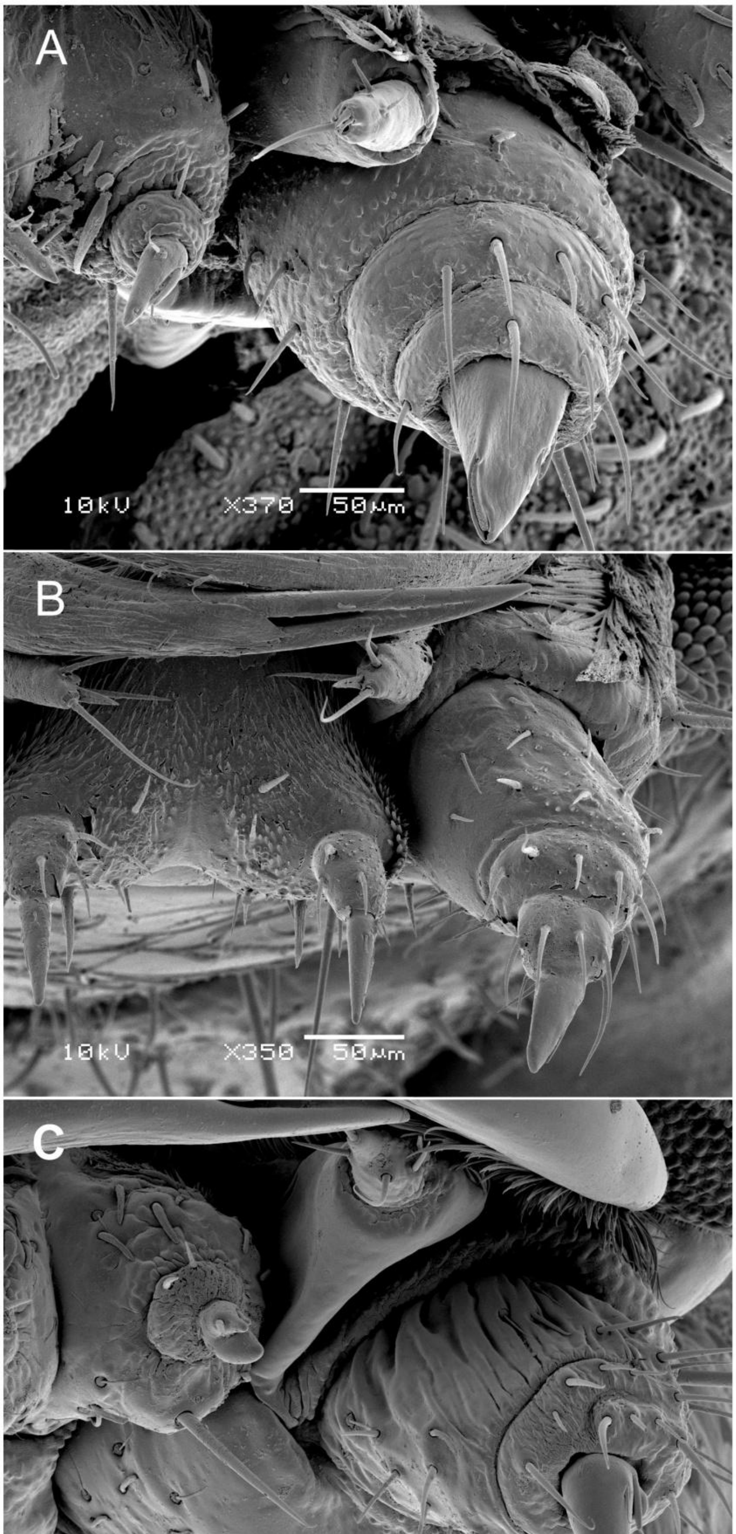
Annex 16: Antenna, distal antennomere and sensorium, anterior view, SEM. – A) *Lampyris noctiluca*; B) *Lamprohiza splendidula*; C) *Phosphaenus hemipterus*.



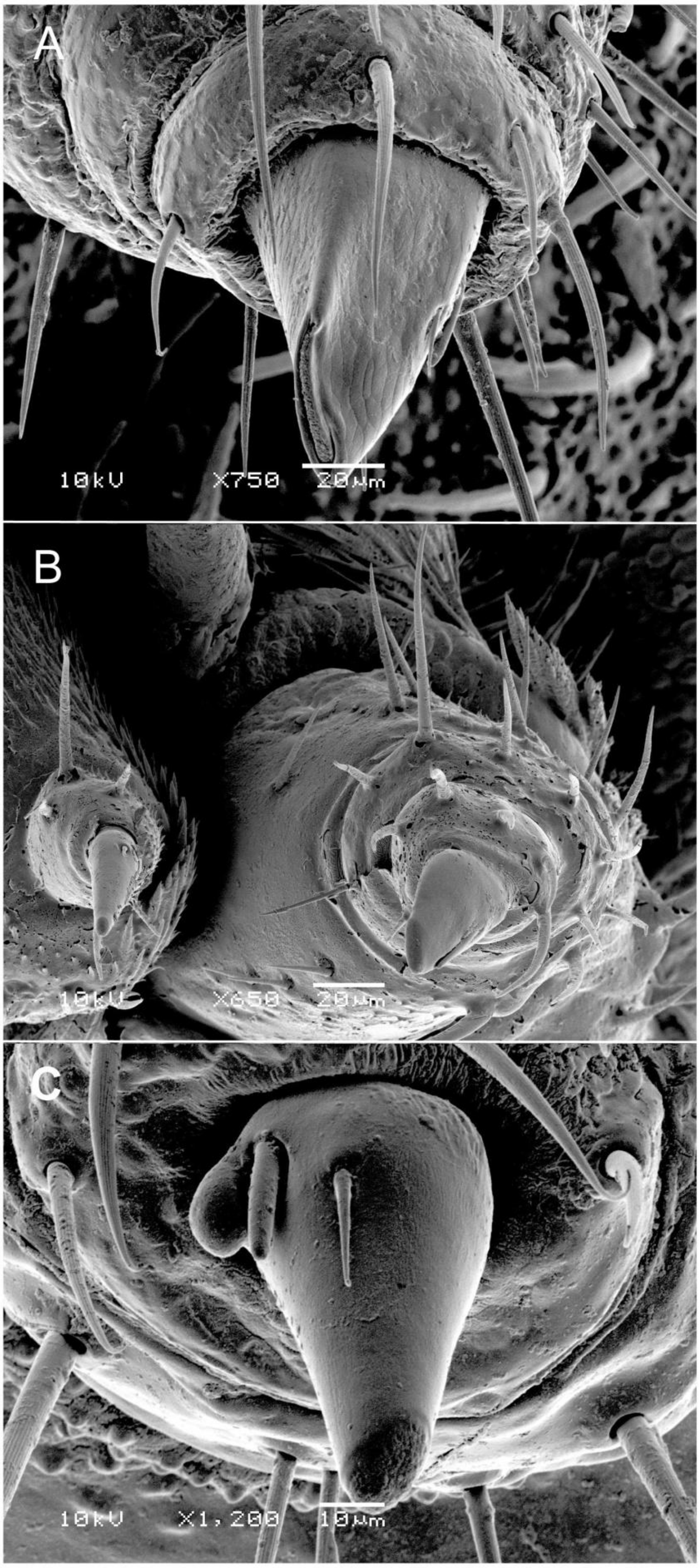
Annex 17: Antenna, distal antennomere and sensorium, lateral view, SEM. – A) *Lampyris noctiluca*; B) *Lamprohiza splendidula*; C) *Phosphaenus hemipterus*.



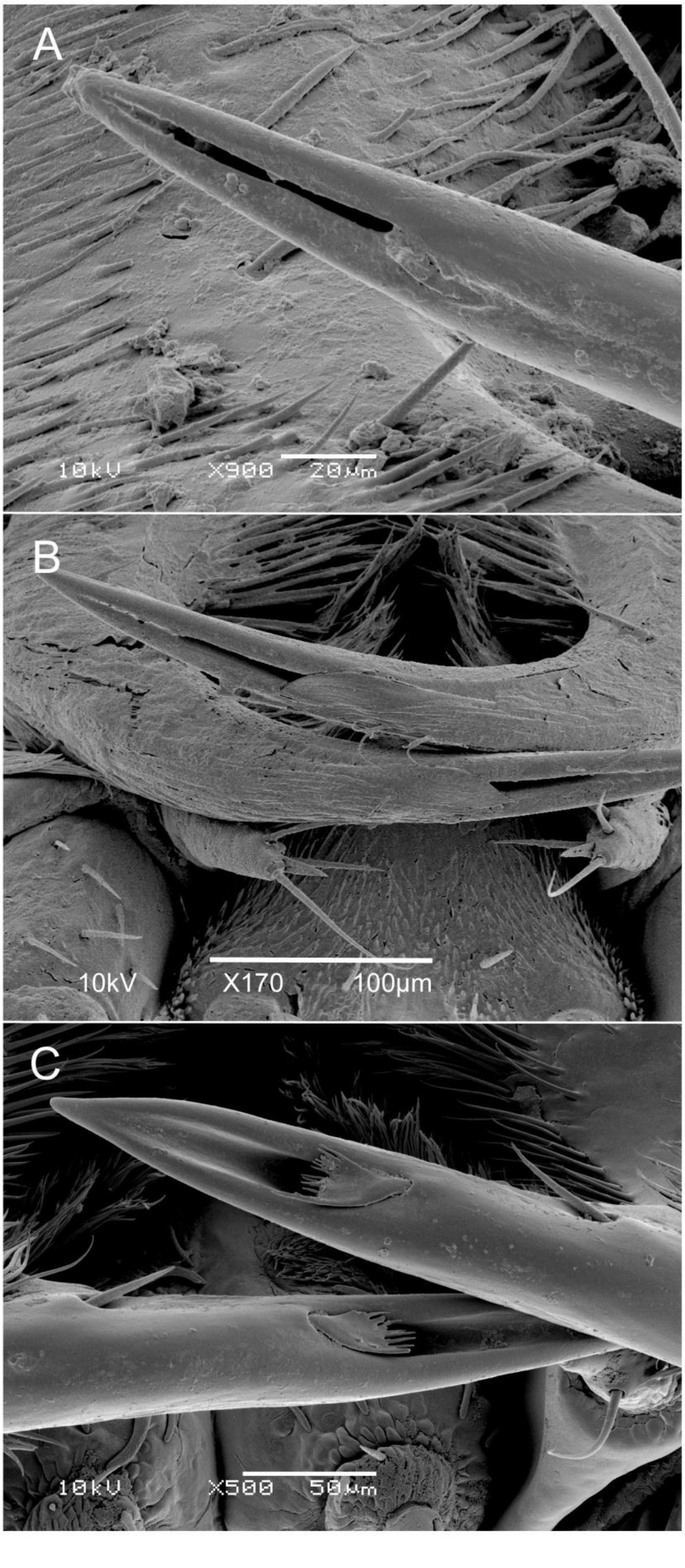
Annex 18: Labiomaxillary complex, detail, anterior view, SEM. – A) *Lampyris noctiluca*; B) *Lamprohiza splendidula*; C) *Phosphaenus hemipterus*.



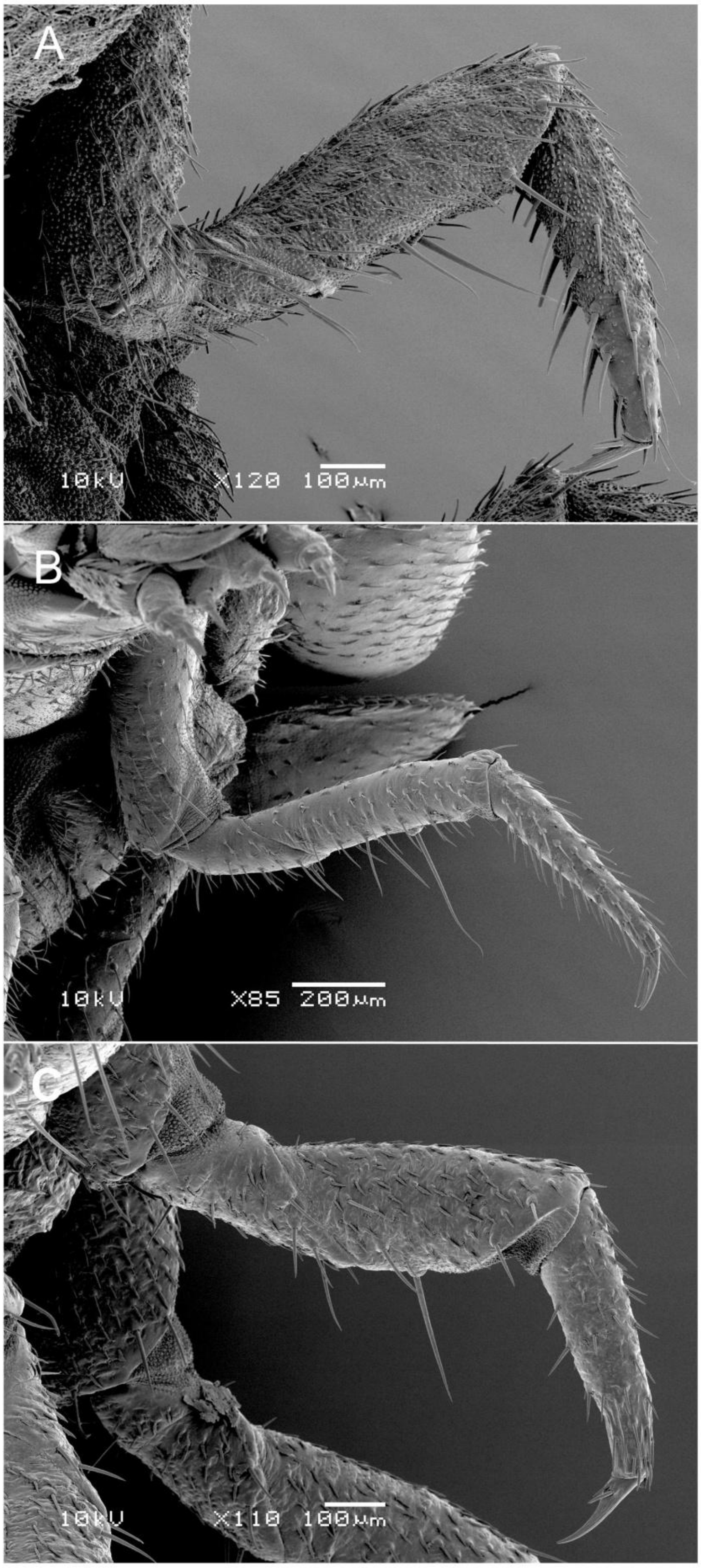
Annex 19: Maxillary palpus, distal palpomere, SEM. – A) *Lampyris noctiluca*; B) *Lamprohiza splendidula*; C) *Phosphaenus hemipterus*.



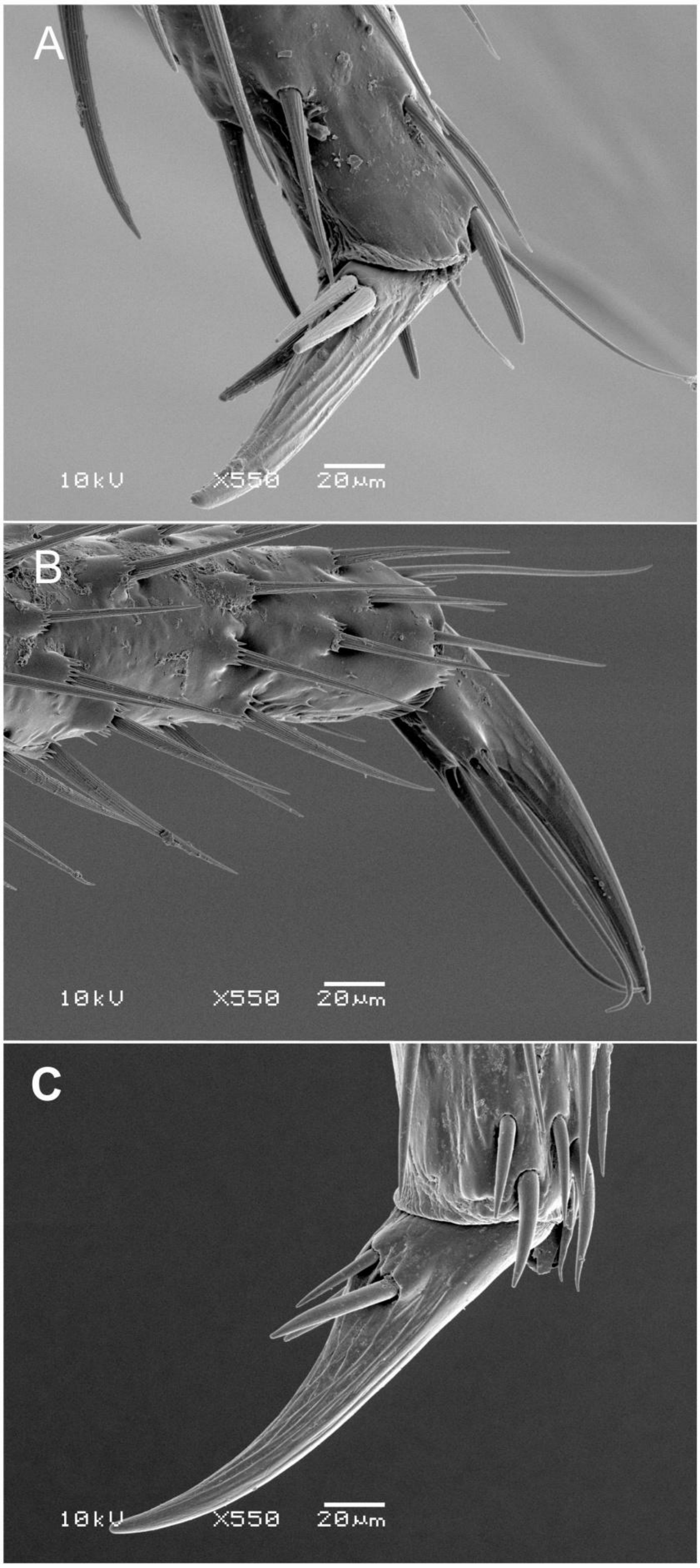
Annex 20: Mandibular channel opening, SEM. – A) *Lampyris noctiluca*; B) *Lamprohiza splendidula*; C) *Phosphaenus hemipterus*.



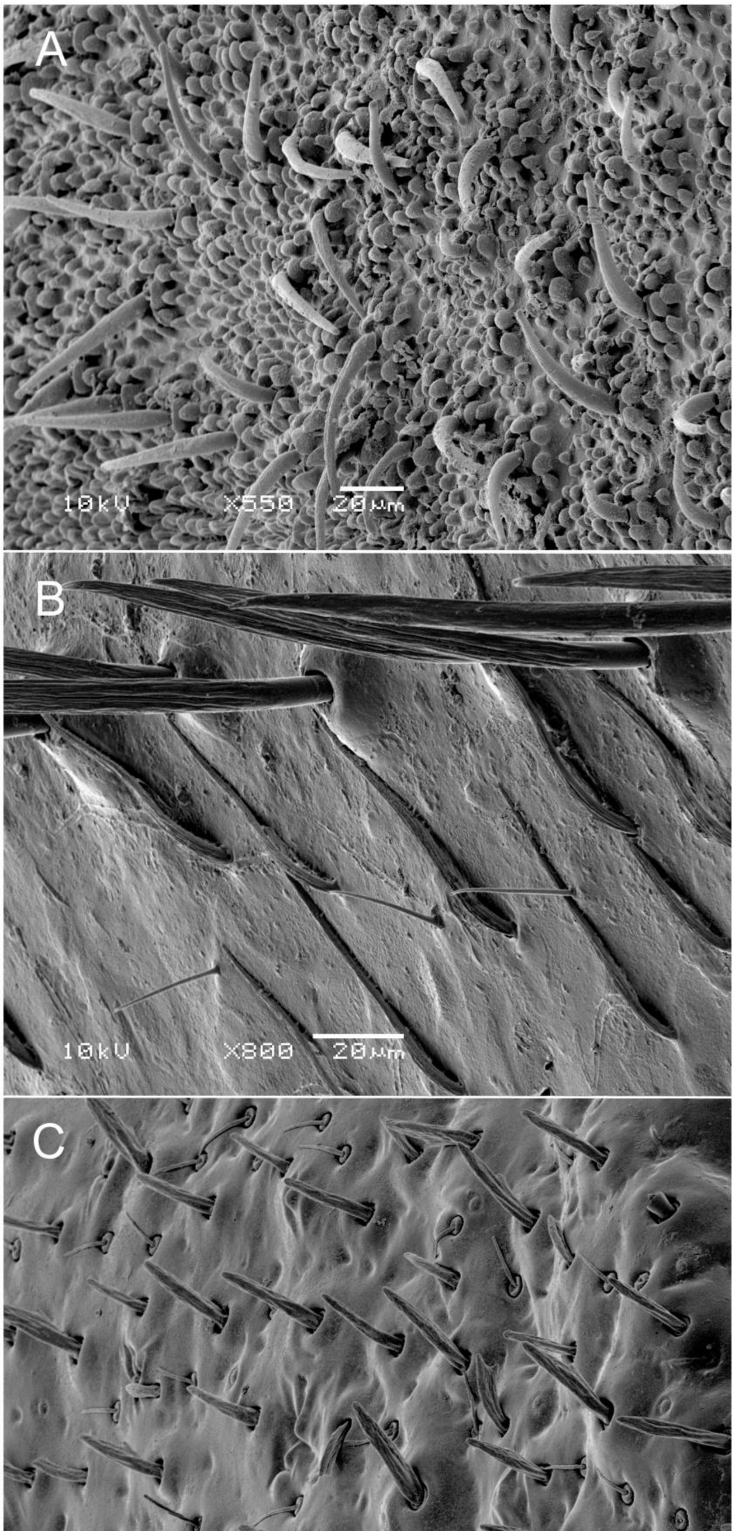
Annex 21: Leg, SEM. – A) *Lampyris noctiluca*; B) *Lamprohiza splendidula*; C) *Phosphaenus hemipterus*.



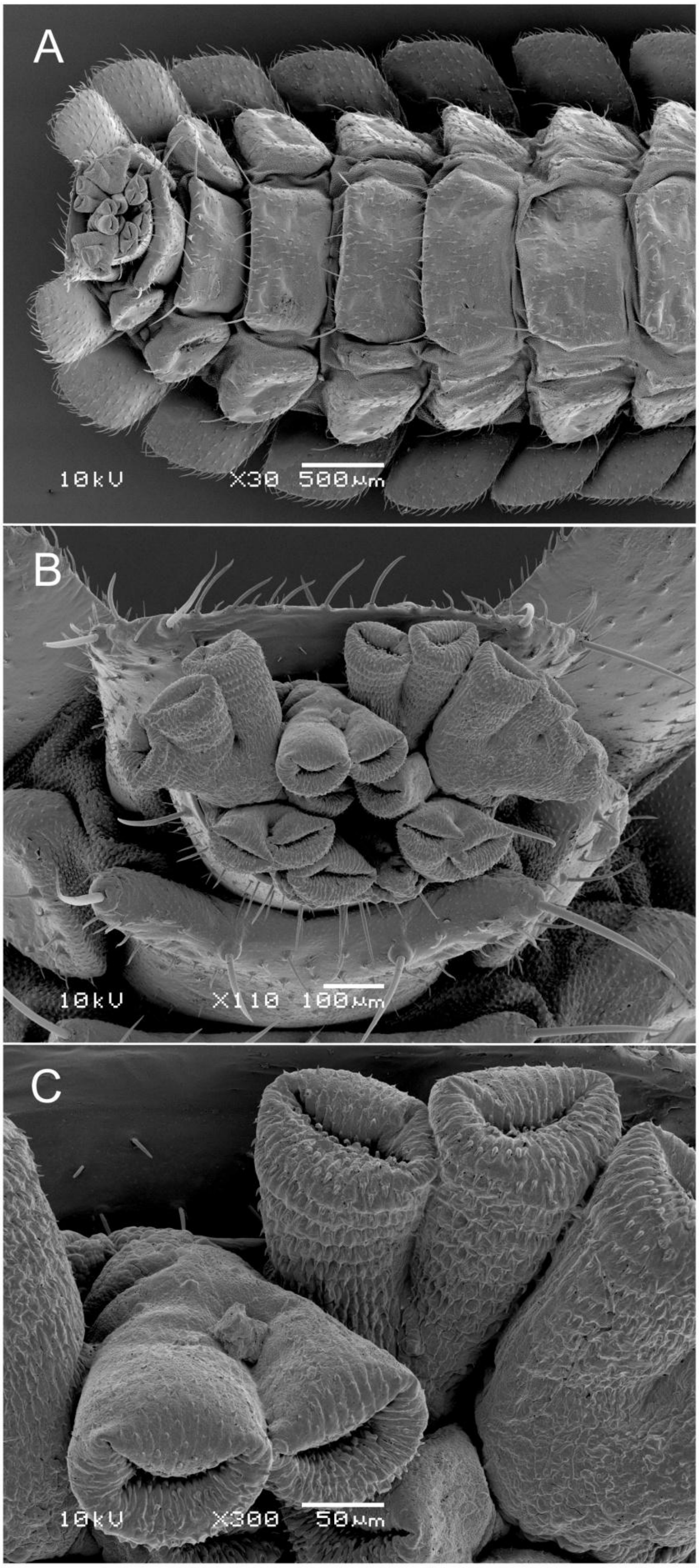
Annex 22: Tarsungulus, SEM. – A) *Lampyris noctiluca*; B) *Lamprohiza splendidula*; C) *Phosphaenus hemipterus*.



Annex 23: Types of cuticular processes on pronotum, SEM. – A) *Lampyris noctiluca*; B) *Lamprohiza splendidula*; C) *Phosphaenus hemipterus*.



Annex 24: *Lamprohiza splendidula*, pygopod, SEM. – A) abdomen, posteroventral view; B) pygopod; C) pygopod, detail.



Annex 25: Sensilla placodea, SEM. – A) *Lampyris noctiluca*, antenna; B) *Phosphaenus hemipterus* antenna; C) *Phosphaenus hemipterus*, mandibles.