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RESEARCH

Identification of Larvae of Endangered *Cucujus cinnaberinus* and *C. haematodes* (Coleoptera: Cucujidae)

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ABSTRACT. The red flat bark beetle *Cucujus cinnaberinus* (Scopoli, 1763) is included as a protected species in the Berne Convention and the European Habitat Directive—Annex II and IV (92/43 EU of 21 May 1992) which requires the establishment of special areas of conservation in the European Union, and listing the species in the International Union for Conservation of Nature Red List of Threatened Species under the near-threatened category. *Cucujus haematodes* Erichson, 1845 is considered to be one of the most threatened saproxylic species, a relic of primeval forests, in many European countries (including Poland) under protection. Morphology of the larvae of two rare European species of the genus *Cucujus* (*cinnaberinus* and *haematodes*) is compared here. We point out differences which allow the two species to be properly distinguished and which, until now, have been omitted or misinterpreted in literature. The best characteristics seem to be the arrangement of minor spines on the top of basal tooth, the shape of frontal suture, size of stemmata, shape of I antennal joint, localization of basal tooth, and morphology of VIII abdominal tergites.

Key Words: morphology, larva, Cucujidae, *Cucujus*, identification

Species of the genus *Cucujus* Fabricius, 1775 (Coleoptera: Cucujidae) are one of the most impressive red flat bark beetles living in forests of the northern hemisphere. Currently, this small genus is composed of 12 species (Lee and Satô 2007, Horák and Chobot 2009, Bonacci et al. 2012), of which 4 species have been reported in Europe: *C. cinnaberinus* (Scopoli, 1763), *C. haematodes* Erichson, 1845, *C. tulliae* Bonacci, Mazzei, Horák and Brandmayer, 2012, and *C. clavipes* Fabricius, 1781. The first three are indigenous to Europe; the latter is a species originating from North America (Lee and Thomas 2011), dragged to Europe and reported in Sicily and near Venice (Ratti 2000).

Of these four species, *C. cinnaberinus* has become the flagship species in protection of saproxylic organisms in the European Union. This species is included in Annexes II and IV of the Habitats Directive, and its occurrence was, in many cases, the basis for the creation of areas within the Natura 2000 network, which is the largest international initiative against observed decline in biodiversity. The interest in this species has led to increase in knowledge about its bionomy, ecology, and biogeographical distribution (Horák et al. 2010, Nieto et al. 2010, Mazzei et al. 2011).

C. cinnaberinus is an European species, with the most well-known distributions mainly focused in several Central European countries such as Hungary, Czech Republic, Slovakia, Slovenia, Poland, Austria, and Germany. In other parts of the continent, knowledge about its occurrence is very little. In part, this seems to be related to the difficulty of locating imagines, whose season is short (limited to 2–3 mo per year), and has a secretive lifestyle. In contrast to imagines, larvae are easy to find throughout the whole year in their breeding sites: freshly dead trees (lying or standing) of various species (Horák and Chobot 2011). Descriptions of the morphology of larvae of this species may be found in the studies of Rosenhauer (1882), Ganglbauer (1899), Palm (1941), Mamaev et al. (1977), Klausnitzer (2001), and a recent one from Bonacci et al. (2012).

C. haematodes is the most widespread species in the genus, populating a large part of the Palaearctic (Ślipiński 1982, Horák et al. 2009). Within this taxon, researchers distinguish three subspecies: the nominal *C. h. haematodes* living in forest areas from central and southern Europe to the Russian Far East, *C. h. opacus* Lewis, 1888 known from Taiwan and Japan and *C. h. caucasicus* Motschulsky, 1845 reported from the Caucasus. Imagines of the given forms show a number of differences in coloration, size, structure, and proportions of various parts of the body including the genitals, prompting some authors to regard them essentially as rightful and closely related species (Bonacci et al. 2012). Descriptions of the morphology of larvae of this species may be found in the studies of Erichson (1845), Assmann (1851), Rosenhauer (1882), Palm (1941), Mamaev et al. (1977), Klausnitzer (2001), and Bonacci et al. (2012).

Despite its huge range, *C. haematodes* is considered to be one of the most threatened saproxylic species, a relic of primeval forests, in many European countries under protection and found almost exclusively in areas of extensive and well-preserved forest complexes (Burakowski et al. 1986, Gutowski et al. 2006). However, as in the case of *C. cinnaberinus*, because of the rarity of observations of imagines, the distribution data are very scarce and often outdated. Moreover, the ranges of both species overlap, which does not rule out the erroneous identifications and the findings are based solely on observation of larvae.

Studies on the rich larval material from Poland convinced that descriptions available in the literature do not allow one to correctly distinguish *C. cinnaberinus* from the often co-occurring *C. haematodes*. This is partly because the characteristics provided by the authors are either highly variable or difficult to see or incorrectly/misleadingly stated.

The purpose of this article is to discuss and present features allowing for correct identification of larvae of *C. cinnaberinus* and *C. haematodes*, which will allow the determination of these species not only on the basis of the rare imagines but also of the more ubiquitous larvae.

Materials and Methods

Abbreviations to the depository collections are as follows: DIBEC, Department of Invertebrate Biology, Evolution and Conservation, Faculty of Biology, Evolution and Ecology, University of Wroclaw, Poland.

FRI, Forest Research Institute, Department of Natural Forests, Białowieża, Poland.

Specimens were collected from the Białowieża Forest, northeastern Poland (∼100 exx. of *Cucujus cinnaberinus*, and ∼100 exx. of *C. haematodes*—collected by Jerzy M. Gutowski in 2008–2012, deposited in FRI), from the Knyszyńska Forest, NE Poland (25 exx. of *C. cinnaberinus*, and 10 exx. of *C. haematodes*—collected by J. M. Gutowski in 2010–2011, deposited in FRI), from the Barycz Valley, south-western Poland (10 exx. of *C. cinnaberinus*—collected by Marcin Kadej in 2011, deposited in DIBEC, see Smolis et al. 2012), and from south-eastern Poland (10 exx. of *C. cinnaberinus*—collected by Dariusz Tarnawski in 2011, deposited in DIBEC). Total number of studied larvae: 145 specimens of *C. cinnaberinus* and 110 specimens of *C. haematodes.*

Several freshly transformed imagines of both species were also collected—in pupal cells with the last larval exuvium, allowing for confirmation of species determination. Adult larvae of *C. cinnaberinus* and *C. haematodes* were bred in a laboratory until they reached adulthood, which also served to confirm correct identification of the species. Several freshly transformed imagines of both species were also collected—in pupal cells with the last larval exuvium, allowing for confirmation of species determination. Larvae of the last stadium were inserted into large jars (2.5 liters) separately; they were placed between pieces of cork (whose internal sides were strung together) gathered from their feeding grounds. Humidity of the substrate was maintained by periodically injecting several drops of water into the jar. Breeding was conducted in room temperature until obtaining imagines. Larvae were determined up to species before breeding using a stereoscopic microscope.

Larvae were preserved in alcohol. Before examination larvae were boiled for 3–10 min in 10% KOH, rinsed with distilled water, and placed in distilled water for about 1 h to clean and soften the cuticle. All structures were placed on glycerin mounts. Larvae were examined with a Nikon Eclipse E600 (Nikon Instruments Inc., Amsterdam, The Netherlands) phase contrast microscope and a Nikon SMZ–800 (Nikon Instruments Inc., Amsterdam, The Netherlands) binocular microscope. Photographs were taken with a Canon 500D (Canon Inc., Tokyo, Japan) and a Nikon Coolpix 4500 (Nikon Instruments Inc., Amsterdam, The Netherlands) camera under a Nikon Eclipse 80i (Nikon Instruments Inc., Amsterdam, The Netherlands) or a Nikon SMZ–800 (Nikon Instruments Inc.). Image stacks were processed using Combine ZM (Hadley 2010). Plates with pictures of selected structures were prepared from larvae. The terminology used in this article follows that of Bonacci et al. (2012).

Results

The frequent co-occurrence of *C. cinnaberinus* and *C. haematodes* in the same habitats justifies the need to develop a set of characteristics, allowing for their correct identification in the field. This is because (as evidenced by published data) in both cases it is easier to ascertain the presence of the species in the field by looking at larvae rather than imagines (Horák and Chobot 2011, Smolis et al. 2012). The analysis of characteristics has been carried out on a relatively large quantity of larval material, allowing the determination of variation in their morphology. Accordingly, we believe that the characteristics included in Table 1 are eminently suitable for effective use during field observation. From the proposed set of characteristics, we excluded variable features and those difficult to observe or occurring in both species simultaneously. In Table 2, we provide characteristics which, due to their nature, should only be considered complementary or supporting in the identification process. Features in both tables are ordered by usability.

Table 1. Comparison of larval characters between *C. cinnaberinus* and *C. haematodes*

Species character	*C. cinnaberinus* (Scopoli)	*C. haematodes* Erichson
Arrangement of minor spines on the top of basal tooth	In dorsal view not directed outward. Line between the minor spines is more or less parallel to the median body line (Fig. 3C)	In dorsal view directed outward. Line between the minor spines is more or less perpendicular to the median body line (Fig. 3D)
Shape of frontal suture	Bell-like, a line running alongside the base of the bell intersects the body axis at an obtuse angle (>90°) (Figs. 2A and C); reaching over hypothetical lines parallel to body axis, intersecting the chaetopor of long dorsal setae of frontoclypeal region (Fig. 2E)	Fluke-like, a line running alongside the base of the bell is orthogonal to the body axis (=90°) (Figs. 2B and D); contained within hypothetical lines parallel to body axis, intersecting the chaetopor of long dorsal setae of frontoclypeal region (Fig. 2F)
Shape of I antennal joint	Relatively thin, ratio of length to width around 2.37:1 (Figs. 1C and 2A)	Relatively wide, ratio of length to width around 2.10:1 (Figs. 1D and 2B)
Shape of apical antennomere	Apical joint thin, five times longer than wide at the base (Fig. 1C)	Apical antennomere four times as wide as at the base (Fig. 1D)
Size of stemmata	Relatively large, distance between posterior pair of stemmata less than ½ diameter of stemma (Fig. 1E)	Relatively small, distance between posterior pair of stemmata more than ½ diameter of stemma (Fig. 1F)
Localization of basal tooth	Located high on urogomphi, the hypothetical line between their basis situated below the base of urogomphi (Fig. 3A)	Located low on urogomphi, the hypothetical line between their basis crossed the base of urogomphi (Fig. 3B)
Morphology of VIII abdominal tergites	One tubercle on lateral margin (Fig. 3A)	Two (sometimes even three) tubercles on lateral margin (Fig. 3B)

Table 2. Additional features helpful in identification of *C. cinnaberinus* and *C. haematodes*

Species character	*C. cinnaberinus* (Scopoli)	*C. haematodes* Erichson
Shape of head (dorsal view)	The sides of the head become narrower toward the front in a mostly linear fashion, which makes it look more triangular (Figs. 1A and 2A)	The sides of the head are swollen near the eyes, which makes the head look more square and angular (Figs. 1B and 2B)
Shape of urogomphi	Urogomphi (major, hooked spines) at the end of abdomen usually more narrowly spaced (Fig. 1A). Ratio of width of VIII:IX (maximum spacing of urogomphi) around 1.97:1	Urogomphi at the end of abdomen usually more widely spaced (Fig. 1B). Ratio of width of VIII:IX around 1.66:1
Ratio of length of VII:VIII abdominal segments	0.94:1 (Fig. 1A)	0.84:1 (Fig. 1B)
Length of two last visible abdominal segments	Nearly equal, ratio of length of VIII:IX like 1.01:1	VIII abdominal segment longer than IX, ratio of length of VIII:IX like 1.20:1
Colour and sclerotization	Larva usually brighter, less sclerotization	Larva usually darker, more sclerotization

Fig. 1. Larva of *C. cinnaberinus*. (A) Habitus (dorsal view). (C) Antenna. (E) Head with stemmata (lateral view). Larva of *C. haematodes*. (B) Habitus (dorsal view). (D) Antenna. (F) Head with stemmata (lateral view). st stemmata.

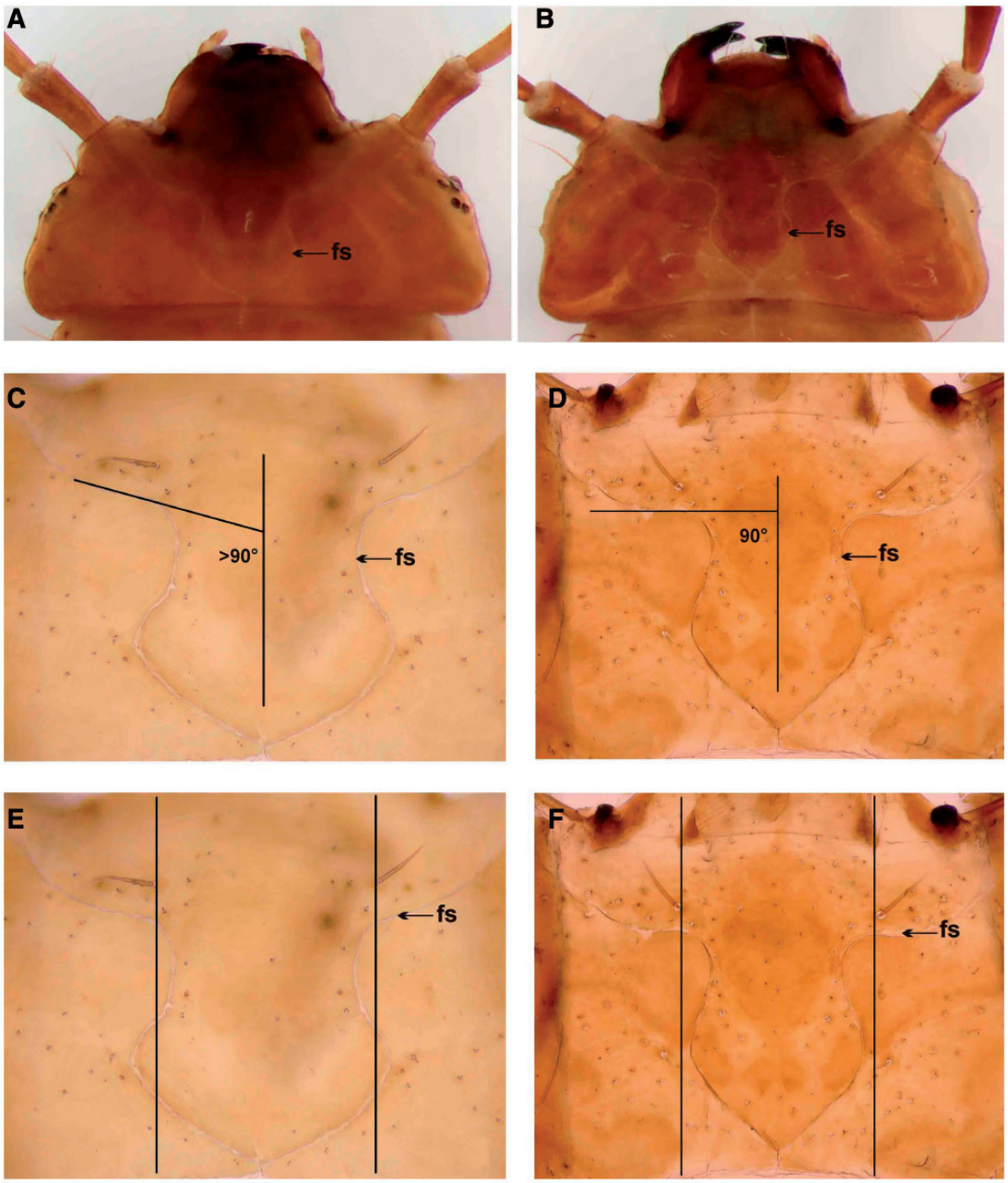

Fig. 2. Larva of *C. cinnaberinus*. (A) Head (dorsal view). (C and E) Frontal suture. Larva of *C. haematodes*. (B) Head (dorsal view). (D and F) Frontal suture. fs frontal suture.

Discussion

Determination (especially in field conditions) of both larvae and imagines requires a set of features which are constant, easily observable (without special equipment and specific methods), and described so as to fully ensure proper interpretation by the users. Otherwise, the determination can be difficult, fraught with error or even impossible. Proper identification is of particular importance for rare, endangered, or protected species. It is the basis for reporting the species in its environment, which further allows taking appropriate protective measures. *C. haematodes*, in contrast to *C. cinnaberinus*, while currently protected in Poland, is not protected by EU law (it is absent from Annexes II, IV of the Habitats Directive). In our opinion (Smolis et al. 2012), it is as deserving of such protection as its close relative. This is justified by its habitat requirements, as well as current data on its distribution in Europe (Horák et al. 2009). Moreover, according to the European Red List of Saproxylic Beetles, it is far more endangered on our continent than *C. cinnaberinus*, as it has been included in the endangered category, and in the EU—even critically endangered (Nieto and Alexander 2010).

Descriptions of larvae of *C. cinnaberinus* and *C. haematodes* appeared in the literature (Erichson 1845, Assmann 1851, Thomson 1863, Ganglbauer 1899), but only a few authors pointed out the features differentiating the two taxa. In the available literature, Rosenhauer (1882) is the first to do so, pointing to the position of the minor spines on the top of basal tooth (in *C. cinnaberinus* located parallel to the urogomphi; in *C. haematodes* between the urogomphi). He based his findings on a confirmed larva of *C. cinnaberinus* (publishing its description) and drawings of a larva of *C. haematodes* presented by Assmann (1851). However, this crucial feature has not been recognized by subsequent researchers and was forgotten. Then Palm (1941) gave a difference in morphology expressed by the ratio of the length of the eighth abdominal segment to the seventh—in *C. haematodes* the eighth segment is almost 1.5× the length of the seventh, and in *C. cinnaberinus* it is only

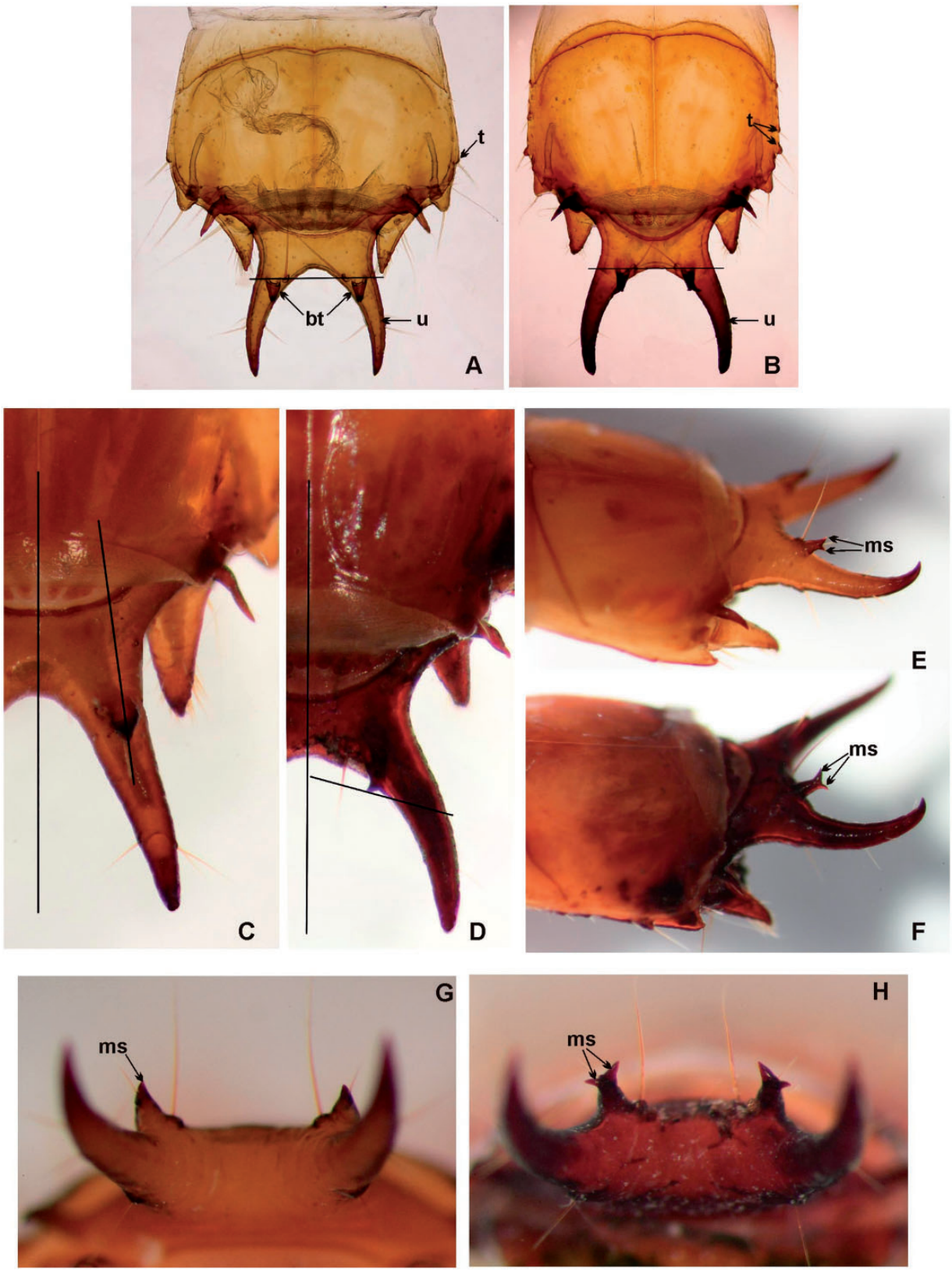

Fig. 3. Larva of *C. cinnaberinus*. (A) VIII and IX abdominal segments (dorsal view). (C) Urogomphi with basal tooth and minor spines (dorsal view). (E) Urogomphi with basal teeth and minor spines (lateral view). (G) Minor spines (back view). Larva of *C. haematodes*. (B) VIII and IX abdominal segments (dorsal view). (D) Urogomphi with basal teeth and minor spines (dorsal view). (F) Urogomphi with basal teeth and minor spines (lateral view). (H) Minor spines (back view). bt basal tooth, ms minor spines, t tubercle(s), u urogomphi.

slightly longer. In reality, these differences are not very clear (see Table 2), but statistically significant. In addition, the mobile segments of the abdomen may be more or less extended and this impacts upon the ratio. This can be a supporting character for determination, especially when larger series of both species are being compared. The author also mentions that the head of *C. haematodes* is slightly wider than in *C. cinnaberinus*, and the first antennomere is somewhat thicker. Mamaev et al. (1977) pointed out that the larvae of *C. cinnaberinus* have a much longer first antennomere than second, unlike *C. haematodes*, where both are similar in length. The researchers also suggest that indentation between the urogomphi in *C. cinnaberinus* is rounded, whereas in *C. haematodes* it is more or less triangular. Recently Bonacci et al. (2012) in a key to the European species of *Cucujus* differentiated the two species as follows: in case of *C. cinnaberinus*: 'antennae slender, second joint distinctly longer than the first one, apical joint thin five times longer than wide at the basis. Lateral border of parietale a little swollen in correspondence of the stemmata. Epistomal front margin moderately oblique toward the mandibular basis. Urogomphi well curved and apically a little converging to the median plane. Basal tooth with minor spine directed outward and far from the apex. Spiracular process small and little pronounced. Conical appendage very large at the basis, distinctly setose around the apical part, chitinous apex short,' while in *C. haematodes*: 'antennae very short, second joint longer like the first one. Apical antennomere is four times as wide as at the basis. Head robust and not swollen in the stemmata area. Front epistomal margin straight from antennal basis to mandibular insertion. Maximum head width behind the stemmata. Urogomphi well curved but apically not converging to the median plane. Basal tooth with less distant apical spines. Conical appendage slender and of different shapes. Spiracular process inconspicuous.'

However, our morphological analysis of Polish larvae of both species found the characteristics given by aforementioned authors to be unreliable, which is due to high intraspecific variety or misleadingly stated features. Moreover, the given characteristics do not allow for proper identification of larvae of both species.

One of the most frequently cited characteristics is difference in the ratio of lengths between the first and second antennomeres. Our measurements of 30 larvae of each species prove that the situation is exactly the opposite to that described by Mamaev et al. (1977) and Bonacci et al. (2012). The second antennomere is inconspicuously longer than the first, and relatively longer in *C. haematodes* than in *C. cinnaberinus* (Figs. 1C and D). This feature is however unreliable and therefore unsuitable for practical application. On the other hand, another characteristic based on antenna structure, namely the ratio of length of the apical joint to length of its base, is correct (Bonacci et al. 2012) and is one of the key distinguishing features (Figs. 1C and D).

Another characteristic which is, in our opinion, erroneously stated by earlier researchers is one pertaining to maximum head width, because, as our studies show, it is largest behind the stemmata, again in both species (Figs. 2A and B). Hence, this feature is unreliable and in consequence useless. Regarding head shape, Bonacci et al. (2012) stated that the head in *C. haematodes* is massive and not swollen near the stemmata. Our observations show differently. In *C. haematodes*, the sides of the head are swollen near the eyes, which makes the head look more square and angular. Meanwhile in *C. cinnaberinus*, the sides of the head become narrower toward the front in a mostly linear fashion, which makes it look more triangular (Figs. 2A and B). However, this characteristic can be only used as supplementary. The characteristic regarding the epistomal front margin turned out to be very variable; therefore, it should not be provided as a key feature at all. Very good head-located distinguishing features are the shape of frontal suturae and the size of stemmata (see Table 1 and Figs. 1E and F, 2).

Further features described by earlier authors are located on two last segments of abdomen. The characteristics regarding the morphology of the spiracular process and the conical appendage (including setae and chitinous apex) given by Bonacci et al. (2012) are very variable and look similar in series of specimens of both compared species. Also, the chitinous apex does not vary between the two species—it is variable, and sometimes even undeveloped. Therefore, they surely should not be treated as key identification features. Furthermore, the shape of urogomphi as suggested by Mamaev et al. (1977) and Bonacci et al. (2012) has limited value, as there are specimens that have the spacing and rounding of the opposite species. It may be helpful when comparing series of species. As our measurements of 30 larvae of each species show, the ratio of width of eighth and ninth segments is conspicuously larger in *C. cinnaberinus* than in *C. haematodes* (however, the last abdominal segments are generally hard to observe in alive larvae due to their mobility); therefore, it should be treated only as a complementary characteristic (see Table 2). With regard to the basal tooth, the important feature is not the size of the spikes (as suggested by Bonacci et al. 2012), but their location relative to the body axis. It is constant and we find its diagnostic potential to be high (see Table 1, Figs. 3A and B). Also, in *C. cinnaberinus* the basal tooth is located high on the urogomphi (Fig. 3A), whereas in *C. haematodes* it is at their base (Fig. 3B). Our analysis finds that a constant and good characteristic is the number of tubercles on the sides of the eighth abdominal tergites (see Table 1; Figs. 3A and B). We think that the ratio of length of the two last abdominal segments (see Table 1) is a reliable feature in laboratory conditions, provided that both segments are positioned horizontally relative to each other (the ninth segment is often slanted upward, which causes it to look shorter under the microscope than in reality). In field conditions (which is what we should assume; we identify the larvae and release them), it is difficult to position the specimen so as to scrutinize the ratio. Experienced observers can additionally look at the coloration of larvae and their degree of sclerotization (see Table 2).

In our opinion, one of the best diagnostic features (and yet the easiest to verify) is the arrangement of the minor spines on the top of basal tooth (Figs. 3C and D, G and H). This has been marginalized by all previous authors, except by Buchholz (2012), with whom we shared a draft of our key to identification for both species, to be used in a publication presenting the methodology for monitoring Habitat Directive species (applied only in Poland). We think that the other features of Table 1 should be considered together, as minor deviations are sometimes seen. Therefore, the determination of species should be ultimately based on a set of complementary features rather than one chosen characteristic. After acquiring some experience, identification does not cause major problems. In the field it is possible with as low as 30× magnification. It is worth noting that the features in Tables 1 and 2 are visible not only in adult larvae, but also in younger stages (except for the degree of sclerotization).

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