Description of the larvae of *Dicronocephalus wallichi bourgoini* (Coleoptera: Scarabaeidae: Cetoniinae) with observations on nesting behavior and life cycle of two *Dicronocephalus* species under laboratory conditions

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Abstract. Larva of the fruit beetle *Dicronocephalus wallichi bourgoini* Pouillaude 1914 is described and illustrated. The following unique morphological characters within Cetoniinae are discussed: shape of claw, spindle-shaped last antennomera, general body shape. The presence of metathoracic egg-bursters in the first instar larva has been found. Its significance for distinguishing the first instar larvae of Cetoniidae is discussed. The nesting behaviours of *D. wallichi bourgoini* and *D. adamsi* (Pascoe 1863) are described. The course of the life cycle of both species under laboratory condition is presented and discussed.

Résumé. Description des larves de *Dicronocephalus wallichi bourgoini* (Coleoptera : Scarabaeidae : Cetoniinae) avec des observations sur les la nidification et sur le cycle biologique de deux espèces de *Dicronocephalus* en conditions de laboratoire. La larve de *Dicronocephalus wallichi bourgoini* Pouillaude 1914 est décrite et illustrée. Les caractères suivants sont uniques parmi les Cetoniinae et sont discutés : forme des griffes, dernier antènème fusiforme, forme générale du corps. On note la présence de dents d’éclosion sur le métathorax. La signification de ce caractère pour distinguer le premier stade larvaire des Cetoniidae est discutée. Les comportements de nidification de *D. wallichi bourgoini* et *D. adamsi* (Pascoe 1863) sont décrits. Le déroulement du cycle de vie de ces deux espèces en conditions de laboratoire est présenté et discuté.

Keywords: Coleoptera, larval morphology, nesting behaviour, parental care.


Immature stages of several species of the subtribe Coryphocerina have been already described e.g. Oberholzer (1959), Donaldson (1987), Carl & Kühbander (1993), Nogueira et al. (2004). Larval morphology of only one species from the subtribes Goliathina - *Hypselogenia geotrupina* (Bilberg 1817) and Ichnestomina - *Ichnestoma pringlei* Perissinotto et al 1999 is described (Oberholzer 1959; Perissinotto et al. 1999) so far. No information has been previously published on the Dicronocephalina immature stages.

The presence of metathoracic egg-bursters (“oviruptors” or “hatching spines” by various authors) in the first instar larvae of Cetoniinae has been reported only for a few species; *Heterorrhina elegans* (Fabricius 1871) and *Neoscelis dohrni* (Westwood 1855) from the tribe Goliathini (Gardner 1935, Nogueira et al. 2004), *Gametis histro* (Olivier 1789) and *Cetonia aurata* (L. 1758) from the tribe Cetoniini (Gardner 1935, van Emden 1941). Van Emden (1941) observed similar structures also in *Osmoderma eremita* (Scopoli 1775) (subfamily Trichinae) and in many other species from the family Scarabaeidae and concluded that the presence of egg-bursters may be a good identification character for first instar larvae.

Some aspects of the biology and behavior of the Neotropical species *Neoscelis dohrni* were properly described by Nogueira et al. (2004). A brief description of breeding habits and rearing methods of *Dicronocephalus wallichi bourgoini* was given by Nespolous (2003).

The genus *Dicronocephalus* Hope 1831 involves seven currently recognized species distributed from the Himalayan foothills of Nepal to peninsular Malaysia and Vietnam in the south; Tibet, China and Korea in the north and east, as well as in Taiwan and Hainan (Krajčík 1998; Mikšić 1977). The members of the genus are medium to large sized beetles (20–35 mm)

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of a relatively broad flattened body and distinct sexual dimorphism. Males are larger than females, posses antler-like clypeal horns and distinctly prolonged tarsomers (e.g. Mikić 1977). Due to the general body form and unique appearance among Cetoniinae representatives many authors consider the genus as a primitive representative of Goliathini (e.g. Rataj 1998). This opinion has been also supported in a recently published phylogenetic study of Cetoniinae (Micó et al. 2008). We studied two species of the genus: D. adamsi, distributed in Tibet, China, Vietnam and Korea, as well as D. wallichi Hope 1831 with three described subspecies. The nominotypic subspecies is distributed on Himalayan foothills, Myanmar, Malaysia and Vietnam, D. w. bowringi (Pascoe 1863) is known from mainland China and the Hainan island, and the subspecies and D. wallichi bourgoini Pouillaude 1914 restricted to Taiwan and considered a separate species by some authors (e.g. Smetana 2006).

Material and methods

Three pairs of Dicronocephalus adamsi and ten pairs of D. wallichi have been used in the study. Adults of D. adamsi were collected in the end of May and beginning of June 2006 in the surroundings of Seoul in South Korea by a local collector. Adults of D. wallichi were collected repeatedly in May 2001 and 2002 in Taiwan (Betou) by a local collector and provided to the junior author (O. J.).

The rearing method was similar for both species: three pairs of each species were kept in a glass terrarium (30×30×30 cm), filled up with 10–12 cm of rich organic soil, composed of dry crushed oak (Quercus) and beech (Fagus) leaves and rotten wood of various deciduous trees. The terrarium was kept in a constant temperature of 27 °C (+1 °C) and moistened regularly in order to keep constant high humidity. The photoperiod was 14:10 (L: D) for adults and 12:12 for larvae. Adults were fed by ripe fruits and provided with fresh green leaves of various fruit trees (e.g., Malus, Prunus). Only larvae of D. wallichi were obtained, the breeding of D. adamsi was not successful. The larvae were kept under similar condition as adults, till the construction of the cocoon, when they were kept at lower temperature 18 °C (+1 °C), and the substrate was allowed to dry out.

Based on Švácha & Danilevsky (1986) the larvae used for morphological studies were killed by immersion in boiling water for 20 seconds, than the cuticle was perforated with a slender sharp pin before fixing in Pampel’s fluid. Mouthparts were dissected and mounted on slides in Liquide de Swan. Morphological analysis and measurements were carried out using an Olympus SZX9 and Olympus BX 40 light microscope both equipped with digital camera Olympus Camedia 5060. Drawings were made on the base of a photograph or using camera lucida. Structures examined by the scanning electron microscope JEOL 6380 were mounted on aluminium plates and dried in a heating chamber.

The behavior of adults was observed continuously during the breeds. The behavior of D. adamsi was recorded by digital camera. Photographs from video-recordings have been made using Pinnacle Studio 8 sofware. The material is deposited in the

collections of Charles University in Prague. The morphological terminology used in the present study is adopted from Hayes (1929), Böving (1936), Ritcher (1966), Sawada (1991) and Micó et al (2001). Hair-like setae were classified according to their relative size into four groups: long, medium long, short, minute (figs 12a–d).

Results

Morphology of the third instar larva of Dicronocephalus wallichi bourgoini (figs 1–11)

The following description is based on five specimens of third instar larvae.

Diagnosis. Body C-shaped, thick and fleshy, with numerous long setae (fig. 1). Length of full grown larva 52 mm. Cranium width 5.50–5.75 mm. Epicranium with 1 long and 3–8 minute dorsoepicranial setae; frons with 1 minute anterior and 1 or 2 exterior frontal setae; stemmata present (fig. 2). Ultimate antennomera fusiform with 4–8 dorsal and 2–9 ventral sensory spots; sense cone broad, conical (figs 8a, b). Epipharynx with plate shaped sclerome of haptolachus (fig. 3). Left mandible with 4 scissorial teeth, right mandible with 3 scissorial teeth (figs 9a–d); mandibular stridulatory area with 7–13 ridges (fig. 7). Maxilla with 7–9 apically curved, conical and blunty pointed stridulatory teeth (fig. 4). Thoracic spiracle with 41–44 holes across diameter. Tarsungulus narrow, conical, apex pointed, slightly bent, with 2 macrotrichia in apical third (fig. 10). Palida absent.

Description. Head capsule (fig. 2). Maximum width 5.5–5.75 mm. Cranium smooth, brown to red-brown; mandibles and proximal parts of antennomeres brown; apex of mandibles, basal part of antennifer black. Chaetotaxy of head capsule summarized in the tab. 1. Frontal sutures lyriform; posterior angle with shallow depression. Stemmata present. Clypeus subtrapezoidal, preclypeus weakly sclerotised. Labrum trilobed angle with shallow depression. Stemmata present. Clypeus pointed stridulatory teeth (fig. 6). Antennae with antennomere 1 longest, but shorter than antennomeres 2 and 3 together. Ultimate antennomere prolonged, fusiform, with 4–8 dorsal and 2–9 ventral sensory spots, and small apical spot bearing minute setae (figs 8a, 6). Epipharynx (fig. 7). Clithra present. Corypha distinct. Haptoneral region without heli or haptoneral process. Zy gum transverse, slightly convex, with an arcuate row of 17–22 stout spinelike setae and 8–11 haptoneral sensilla. Posterior to zygum group of 7–9 irregularly distributed spinelike setae. Pregnatum and proplegmata absent. Acanthoparia with 5–8 almost subequal setae, surrounded by distinct sheath at base. Posterior 1–2 setae of acanthoparia often smaller than the remaining ones. Gymnoparia absent. Chaetoaria asymmetrical, right part with 94–126 hair-like to stout setae, left part with 59–84 hair-like setae. Dexiotorma prolonged, right pternotorma absent. Laeotorma narrow, shorter than dexiotorma, left pternotorma well developed. Haplotlachus with conical sense cone with 4–5 apical sensilla. Anteriorly to sense cone distinct plate-shaped sclerome (sensu Böving (1936)). Four sensilla of haplotlachus posteriorly to cone. Crepis absent.

Mandibles (figs 9a–d). Asymmetrical, scrobis with deep longitudinal furrow and 5–7 long setae. External margin of mandible without teeth. Stridulation area small, with of 7–13 transverse ridges on pale oval field. Dorosomal and ventromolar setae present, consisting of 2–7 setae. Small field of asperities
Figures 1–7
Third instar larva of *Dicrocoelium wallichi bourgoini*. 1 habitus; 2 cranium; 3 epipharynx; 4 labio-maxillar complex; 5 last abdominal segment, ventral aspect; 6 hypopharynx; 7 stridulation teeth of right mandible. Scale bars: 1 – 5 mm; 2, 4 – 1 mm; 3, 6, 7 – 0.5 mm; 5 – 2 mm. AAS = anterior frontal angle setae; ACS = anterior clypeal setae; AES = anterior epicranial setae; AFS = anterior frontal setae; DES = dorsoepicranial setae; ECS = exterior clypeal setae; EES = exterior epicranial setae; EFS = exterior frontal setae; ELS = exterior labral setae; LLS = setae of lateral labral lobe; MLS = setae of medial labral lobe; PES = posterior epicranial setae; PFS = posterior frontal setae; PLS = posterior labral setae; PMS = paramedial labral setae.


Figures 8–12
Third instar larva of *Dicronocephalus wallichi bourgoini*. 8 ultimate antennomera of left antenna, a – dorsal aspect, b – ventral aspect. 9 mandibles: a, b – right mandible; c, d – left mandible; a, d – ventral aspect; b, c – dorsal aspect. 10 claw of right metathoracic leg. 11 unci of left maxilla, ventral aspect. 12 relative size of hair-like setae (not to scale): a – long, b – medium long, c – short, d – minute. Scale bars: 8, 10, 11 – 0.5 mm; 9 – 1 mm.
Labium and hypopharynx (figs 4, 6). Hypopharyngeal sclerome asymmetrical with large truncate process. Right lateral lobe of hypopharynx sclerotized, with 6–12 setae, left lateral lobe less sclerotized with 10–14 setae. Three groups of setae without distinct rim (= phoba like process sensu Sawada (1991)) present; 8–11 setae at base of truncate process (occasionally damaged or absent), 5–7 setae proximal to basal margin of hypopharyngeal sclerome and 9–16 setae in medial part of left lateral lobe. Glossa with two longitudinal sclerotized areas, two longitudinal series of 18–33 hair-like setae and a transverse row of 14–15 conical setae in proximal third of glossa.

Thorax (fig. 1). Dorsum of each sublobe of thoracic segments with 2–4 rows of short setae and few long hair-like setae, venter of each sublobe with 2–3 rows of long setae. Lateral sclerite of pronotum brown with longitudinal sulcus and numerous setae. Thoracic spiracle smaller than spiracles on abdominal segments III–VIII (0.854–0.91/0.63–0.7 mm high/wide). Respiratory plate C-shaped, with 41–44 holes across diameter. Legs unequal in size, anterior pair smallest (4.00–4.63 mm), posterior pair longest (5.13–5.48 mm). Unguli (claws, fig. 10) sclerotised, conical, with 2 hair-like setae in apical third. Tip of each ungulus slightly bent and pointed.

Abdomen (fig. 1). Segments IX and X fused, larger than remaining body segments. Spiracles of abdominal segments I–VII gradually increasing in size, spiracle on abdominal segment VIII smaller than on preceding segments, but slightly larger than thoracic spiracle (abd. spiracle I: 0.714–0.812 /0.644–0.714 mm, abd. spiracle VI and VII: 0.994–1.036/0.812–0.938 mm). Chaetotaxy of abdominal segments as in thoracic segments, except of last abdominal segment. Raster (fig. 5) without palida, venter of last abdominal segment almost bald with only 16–20 short setae on each side; hammate setae absent. Anal slit transverse emarginated with a dense row of 73–83 short or medium long setae. Lower anal lip with 35–43 medium long or long hair-like setae and 6–15 short, stout setae.

Figures 13–14
First instar larva of *Dicronocephalus wallichi bourgoini*. 13 egg-burster. 14 stridulatory area of right mandible. Scale bars: 50 μm.

<p>| Table 1. Head chaetotaxy of the third and first instar larvae of <em>Dicronocephalus wallichi bourgoini</em> |
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| Abbreviations: AAS = anterior frontal angle setae; ACS = anterior clypeal setae; AES = anterior epicranial setae; AFS = anterior frontal setae; DES = dorsoepicranial setae; ECS = exterior clypeal setae; EES = exterior epicranial setae; EFS = exterior frontal setae; ELS = exterior labral setae; LLS = setae of lateral labral lobe; MLS = setae of medial labral lobe; PES = posterior epicranial setae; PFS = posterior frontal setae; PLS = posterior labral setae; PMS = paramedial labral setae. |</p>
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Morphology of the first instar larva of *Dicronocephalus wallichi bourgoini*

The following description is based on three first instar larvae (figs 13, 14).

**Description.**
Maximum length of full grown first instar larva 21 mm. Cranium pale yellow, maximal width of head capsule 2.01–2.44 mm. Stemmata present. Ultimate antennomere longest, conical, with 4–6 dorsal and 6–7 ventral sensory spots.


**Mandibles.** Asymmetrical, scrobis with 6 hair-like macrotrichia, and tiny blunt external tooth at lateral margin. Stridulation area (fig. 14) with 11–13 transverse ridges. Dorsomolar and ventromolar setae present. Right mandible: scissorial area with 4 scissorial teeth, 2 prior to scissorial notch, 2 posterior to the notch. Left mandible: seasonal area with 4 teeth.

**Maxillar stridulatory area** with 7 stridulatory teeth and a blunt tubercle. Glossa with 25–26 medium long setae situated in two longitudinal series, and transverse row of 18 conical setae.

**Metathorax with scolosed dorsolateral spinelike egg-burster (fig. 13) bearing subapical seta. Unguli conical with 2 hair-like setae in apical third and sharp pointed tip. Dorsum of each abdominal sublobe with 1 or 2 rows of short and a few long setae, venter of each sublobe with single row of long hair-like setae. Spiracles without bula and ecdysial scar. Raster without palida, venter of last abdominal segment almost bald with only a dense row of 55–75 short or medium long setae. Anal slit transverse emarginated with a dense row of 55–75 short or medium long setae. Lover anal lip with 20 medium long or long hair-like setae and 32 short setae.

**Observation on bionomy of *Dicronocephalus adamsi* and *D. w. bourgoini* under laboratory conditions**

**Male combat behavior**
An encountering of two males in the breeding cage often resulted in a direct combat. At first, the males displayed themselves by erecting their body and stretching out their long forelegs, subsequently they started fencing with their forelegs in order to get as near as possible to the rival. The fight often ended at this point by the withdrawal of the inferior male. In other cases, males fought with their horns and tried to elevate the rival from the substrata and throwing away. Although males fought almost at every encounter, no case of injury or dead has been observed.

**Nesting behavior** (figs 15a–f)
Before the oviposition females were observed to construct a provision burrow. The burrow was filled with a clutch of green leaves and other fresh or dead plant parts. Venter of last abdominal segment nearly bald with only a dense row of 55–75 short or medium long setae. Lover anal lip with 20 medium long or long hair-like setae and 32 short setae.
organic material. The clutch was sized 3 × 2 cm, and was buried in a depth of about three centimetres below the surface. Each burrow contained only a single egg. The construction of each provision burrow lasted for several hours. First of all, the female dug in the soil to gain some hollow space. After that the female crawled to the surface and began to drag organic matter (e.g. green leaves, stalks and petals, dry leaves and organic debris including pieces of filtration paper) to the burrow. The females preferred green leaves fallen to the ground. If no green leaves were available on the burrow. The females handled the leaves with all pairs of legs and clypeus. Unsuitably large leaves were cut by the female to small pieces with the use of the sharp anterior clypeal margin. After depositing the leaf under the surface of soil, smaller parts of humus and decayed organic matter were collected and drug to the burrow, so that the final clutch was organized into layers of leaves and humus. In the laboratory the females used all the types of provided leaves (genera Betula, Crategus, Malus, Prunus and Sambucus) for the provision burrow. The fact indicates that the species is not strictly specialized on a distinct plant species.

**Life cycle**

During our breeds we have observed a complete life cycle of *Dicronocephalus wallichi*. The eggs laid by *D. adamsi* females did not hatch for an unknown reason.

The larvae hatched about ten days after oviposition in the beginning of June and left the provision burrow few days after eclosion. The entire larval development was very fast, the first instar took approximately two weeks, the second about three weeks and the third instar another 7–10 weeks. The first cocoons occurred about 3–4 months after oviposition by the end of August and in September. The larvae used only sand and clay for the construction of their pupating chamber. If no suitable material was available the larvae were not been able to pupate and died. The cocoon phase was extremely long and took about 7–8 months. Adults were found in the cocoons in February, but did not hatch until April.

**Discussion**

**Larval morphology**

Comparing larvae of *Dicronocephalus wallichi* with other described species of Goliathini, we can find the following morphological differences: (1) body shape of full grown *D. wallichi* larvae is rather short, with distinctly thickened three last abdominal segments and numerous long slender setae. The shape of the other Goliathini larvae is usually distinctly prolonged, three last abdominal segments are not distinctly thickened, and the body is usually covered with short stout setae (cf. Carl & Kühbander 1993; Oberholzer 1959; P. Šípek pers. observ.). (2) Shape of claw, the number and position of setae of ungulars are other distinguishing characters. *Dicronocephalus* claw is prolonged conical, slightly bent at apex and pointed, with two setae in the apical third. Similar shape of claw is reported only for *Hypselogenia geotrupina* (Oberholzer 1959). Further known larvae of Goliathini posses different types of claw: falcate claw with four setae (*Anisorrhina flavomaculata* (Fabricius 1798)); claw with cylindrical base and falcate apical half with several setae in the middle of the claw (*Amaurodes passerini* Westwood 1844); cylindrical claw with or without small tip and about 8–10 setae organized in two rows (larvae of other genera) (Donaldson 1987; Kühbander & Carl 1994; Nogueira et al 2004). The other distinctive characters are (3) the spindle-shaped ultimate antennomera, (4) the small stridulatory area of mandibles, consisting of 7–12 transverse ridges or (5) the absence of an external tooth on the lateral face of mandibles.

**Egg-bursters**

Metathoracic egg-bursters in the first instar larvae of Cetoniinae have been reported for a few species so far (see introduction). In the *Dicronocephalus* larvae, we have found the same structure as well. This led us to a brief revision of first instar larvae in our collection. We have found egg-bursters in the first instar larva of all studied species (over 25 genera) including genera *Gnorimus*, *Trichius* (*Trichiine*) and *Valgus* (*Valginae*). This results support van Emden’s (1941) hypothesis that egg-bursters may be used for identification of first instar scarabaeoid larvae.

**Biology and life cycle**

Parental care was described in several families of Scarabaeoidea. The family Passalidae are known for their subsocial way of life, Bolboceratidae, Geotrupidae and Scarabeinae construct simple provision burrows (Scholtz & Grebennikov 2005). The nesting behavior of *Dicronocephalus* is probably the first observation of provision burrows in Cetoniinae. Ritcher (1958) reported a similar strategy in the Dynastinae species *Strategus antaeus* (Drury 1773). The females construct provision burrows with plant debris such as pine needles. However more than one larva has been found in one burrow.

Except for amateur-breeders literature, only few notes have been published on the bionomy of
Goliathini. Virtually no data are available concerning the length of life cycle in nature. During our breeds of many Goliathini species we have noticed an average egg-adult life cycle of a *Dicronocephalus*-sized species about 9–12 months. With almost no exception the longest stage of development was the third larval instar. The cocoon stage lasted usually no longer than two months. The observed life cycle of *D. wallachi* with only 3–4 months of larval development and eight months of combined pupal development and inactive adult (in cocoon) seems to be very extraordinary among Goliathini. In general, relevant life cycle data are scarce in Cetoniidae, but a similar life cycle (under laboratory conditions) has been observed in two small sized Palearctic species of the tribe Cetoniini *Ocythryea funesta* (Poda 1761) and *Tropinota squalida* (Scopoli 1763) (Micó & Galante 2003a). The larval development of these species took 8–9 weeks (6–7 weeks respectively) and the cocoon phase took another six months. It is assumed that the species over-winters only as adults in the cocoon. The life cycle of *Dicronocephalus*-sized species from Palearctic region *Netocia morio* (Fabricius 1781) or *Potosia cuprea* (Fabricius 1775) seems to be more variable. Although the larval development under laboratory conditions takes about 13 weeks, adults may emerge after only 6–8 weeks from pupating or they can rest in the cocoon (Micó & Galante 2003b). Field and laboratory observation also indicate these species often overwinter in the stage of larvae (Balthasar 1956, Micó & Galante 2003b; Šípek unpubl. data). Such life-cycle variability was not observed in *Dicronocephalus*.

The climatic conditions in the distribution area of the genus *Dicronocephalus* are of seasonal character. It means with dry and relatively cold winters and humid and warm summers. It is possible that the prolonged cocoon-phase of *Dicronocephalus* life cycle represents an adaptation for surviving during dry and cold winters. We assume that larvae of *Dicronocephalus* are not able to stand the conditions of winter, thus the larval development must be completed fast. This means less time for growth and preparation of metamorphosis, which in turn can be compensated by boosting the development by utilizing better food source. It is possible that deposing of organic matter along with eggs is a strategy to accelerate larval development, as the clutches can serve as provision burrows and can increase the local temperature in soil due to the microbial process of fermentation of green leaves.

The observed combat behavior of males is in direct contrast to Arrow’s (1910) note, that the males do not appear to fight and that the clypeal horns are rather inconvenient for the males.

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References


Larvae and biology of *Dicronocephalus*


