Larvae of Three Japanese Species of Myrmeleontidae (Neuroptera)

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Abstract Larvae of three Japanese species of Myrmeleontidae, Epacanthaclisis moiwana (Okamoto), Dendroleon pupillaris (Gerstaecker) and Pseuformicaleo nubecula (Gerstaecker) are described with notes on their habitats. The larva which had been identified as D. pupillaris turned out to be that of E. moiwana. The larvae of E. moiwana were found in silt piled at the bottom of a small excavation on the side of a trail; larvae of D. pupillaris were found hiding itself in thin silt or dust piled in a small depression on a stone and dried clay, or inner part of a wood hollow; those of P. nubecula were found in silt or fine gravel collecting in a small depression of windswept rocks. These three antlions are not pitfall builders but ambush predators.

Introduction

Myrmeleontids are well-known insects because whose larvae are distinctive in building a conical pit to capture small arthropod preys. However, the larger part of antlion species do not build a pit but ambush in the soil to capture a passing prey. Seventeen species of the Myrmeleontidae are known in Japan (Sekimoto 2014), of which immatures of 6 species had been unknown. These larva-unknown species are supposed not to build a pit because pits are highly obvious constructions to detect the larva. Each myrmeleontid species shows narrow preference in habitat selection, for example Synclisis japonica (MacLachlan, 1875) and Myrmeleon solers Walker, 1853 are both restricted to natural coastal dune, and some species have been at risk of extinction or decrease of local population due to the deterioration of habitats. Recently we found antlion larvae which do not agree with ones of any known species, so the identities of these antlions were considered by rearing adults and DNA barcoding. Here we give descriptions of larval morphology with notes on their habitat and behavior observed in the sampling and rearing process for each newly discovered species. These information seem helpful in identification of larvae, survey of distribution and fundamental in conservation of the group.

Materials and Methods

Collecting antlion larva is conducted by shifting soil. Blowing off or brushing away the shallow pile of silt in concavities was effective in search of Dendroleon pupillaris (Gerstaecker) and Pseuformicaleo nubecula (Gerstaecker). The obtaind larvae were brought to the laboratory, some of them were kept in a plastic containers (30 x 20 x 20 to 120 x 120 x 50 mm) with soil (5–20 mm in depth) took from their habitat, at room temperature to obtain adults and others were fixed in 95% ethanol. The antlions were fed with woodlouses, Porcellio spp., or small caterpillars once to twice a week. For the first instar larvae, adults of Dorsophilidae (wingless strain) were cultured and provided. When cocooned, they were transferred to a cage whose inner walls are covered with sheet of facial tissue to hold legs of emerged adults.

The images of larvae were taken using an Olympus E-M5 Mark II digital camera and 60 mm macro lens. Several partially focused images were captured using Focus bracketing function and they were combined using Combine ZP. Terminology and measurement follows Badano & Pantaleoni (2014).

Partial sequences (570 bp) of cytochrome c oxidase I (COI) obtained from adults and larvae were used for DNA barcoding. Total genomic DNA was extracted from right middle legs using DNeasy Blood and Tissue Kit (Qiagen, Netherlands) according to the manufacturer’s protocol for animal tissues. The primer set LCO1490 and HC02198 for COI designed by Folmer et al. (1994) was used in PCR. The reaction program had an initial 5-min denaturation at 94°C, followed by 40 cycles at 94°C for 15 s, 46°C for 15 s and 72°C for 15 s and ending with a 10-min extension period at 72°C. Gene regions were sequenced with the same primers as in the PCR using the BigDyeTM Terminator ver. 3.1 Cycle Sequencing Kit (Applied Biosystems). Cycle sequencing reactions were run on an ABI Prism 310 Genetic Analyzer (Applied Biosystems). Voucher specimens and their extracted genomic DNA are deposited in Osaka Museum of Natural History (OMNH) unless otherwise noted. The sequences obtained are registered in DDBJ/GenBank. The accession numbers and specimen IDs are indicated in Table 1 and list of specimens examined. We choose Ascalohybris subjacens (Walker, 1853) (Ascalaphidae) as out group. The molecular dataset was analyzed using maximum-likelihood (ML) analysis. We used PAUP* (Swofford, 2002) with TBR branch swapping and a NJ starting tree. The best fit substitution model was estimated using hierarchical likelihood ratio tests (hLRT) as implemented in Modeltest 3.7 (Posada & Crandall, 1998) and the GTR+I model was selected. We also performed 100 ML bootstrap pseudoreplicates in PAUP* using the same search conditions.

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Table 1. INSD accession number and Specimen IDs for DNA barcoding. (*: reared from larva)

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Results

Descriptions

Genus _Epacanthaclisis_ Okamoto, 1910

(Fig. 1a–h)

Diagnosis. Head capsule slightly dilated posteriorly (Fig. 1a, b); mandibles (Fig. 1a) very long, distal portion weakly bent outward, armed with three small teeth, of which distal one is the longest; basal tooth positioned at the middle of mandible; clypeo-labrum around base of mandible densely covered with dolicohasters with inflated tip (Fig. 1f); ocellar tubercles prominent, as long as wide; meso- and metathoracic lateral processes well developed (Fig. 1g), bearing both dolicohasters and pale hair-like setae; sternite VIII with a pair of small odontoid process near posterior margin (Fig. 1e); sternite IX with rastra weakly sclerotised, positioned near apical margin (Fig. 1e).

Comments. _Epacanthaclisis_ is a palaearctic genus composed of 13 species, most of which are known from China (Stange, 2004; Ao et al., 2010). No description had been available for larvae of this genus.

_Epacanthaclisis moiwana_ (Okamoto, 1905)

(Fig. 1a–h, Fig. 4a)

Diagnosis of 3rd instar larva. Size: BL 14.0–16.2 mm; HL 2.32–2.70 mm, HW 2.14–2.32 mm, ML 3.20–3.61 mm, HW/HL 0.86–0.92, ML/HL 1.34–1.38. General coloring pale ochre, with brown markings and spots (Fig. 1a); clypeo-labrum reddish brown; dorsal side of head capsule reddish ochre with brown markings; mandibles reddish brown, darkened apically; mesothoracic tubercle apically black (Fig. 1a); legs pale ochre; abdomen dorsally with median and pair of submedian swaying longitudinal dark brown streaks (Fig. 1a); small dark brown spots scattered all over; ventral surface of abdomen (Fig. 1b) whitish along midline, with many small dark brown spots; head about as long as wide (Fig. 1a, b), with deep postero-dorsal depression (Fig. 1f); head capsule with median paired row of dolicohasters on blackish markings behind posterior margin of clypeo-labrum (Fig. 1f); interdental mandibular setae (Fig. 1a, b): (5–8) (1–3) (1–2) (0); some (about 10) short setae are disposed on lateral to ventral surface of the mandibles; dorsal and ventral surface of abdomen moderately covered with short dolicohasters; mesothoracic process (Fig. 1a, g) larger than metathoracic process, with anterior portion bent backward and posterior portion weakly bent forward; Sternite IX (Fig. 1e) equipped with pair of postero-median and three pairs of digging setae, each rastrum with four digging setae.


Distribution. Hokkaido, Honshu, Shikoku, Kyushu; China.

Remarks. Matsura (1989) illustrated this antlion for the first time but misidentified it as _D. pupillaris_, see discussion. This species shows several unique morphological characters in larval stage viz. elongate mandibles and posteriorly slightly dilated head, which make the larva somewhat look like that of...
Fig. 1. *Epacanthaclisis moiwana* (Okamoto), 3rd instar larva. a: dorsal aspect, b: ventral aspect, c: lateral aspect, d: IX and VIII abdominal sternite, dorsal aspect, e: ditto, ventral aspect, f: head capsule, dorsal aspect, g: mesothoracic process, dorsal aspect, h: live larva covered with silt.
Fig. 2. *Dendroleon pupillaris* (Gerstaecker), 3rd instar larva. a: dorsal aspect, b: ventral aspect, c: lateral aspect, d: IX and VIII abdominal sternite, dorsal aspect, e: ditto, ventral aspect, f: mesothoracic process (MP), dorsal aspect, g: live larva covered with silt and debris.
Larvae of three Japanese myrmeleontids

Ascalaphidae as Matsura (2002) pointed out.

**Genus Dendroleon** Brauer, 1866

(Fig. 2a–g)


**Diagnosis.** Anterior margin of the clypeo-labrum slightly concave; mandibles weakly bent upward, equipped with 3 equally distant pairs of teeth (Fig. 2a, b); ocular tubercles prominent but small (Fig. 2a, b); mesothoracic and abdominal spiracles not prominent; thoracic setiferous processes pedunculated (Fig. 2a, b, f); VIII abdominal sternite without odontoid processes; Abdominal sternite IX (Fig. 2e) noticeably longer than wide, without rastra or fossoria.

**Comments.** Dendroleon is a widely distributed genus, composed of about 20 species (Stange, 2004). According to Badano and Pantaleoni (2014) the larva is known for four species viz. European *D. pantherinus*, Nearctic *D. asboletus* (Say, 1839) and *D. speciosus* Banks, 1905 and Taiwanese *D. esbenpeterseni* Miller & Stange, 2000 (Stange et al., 2003; Stange, 2004, 2008).

**Dendroleon pupillaris** (Gerstaecker, 1893)

(Figs. 2a–g, 4b)

**Diagnosis of 3rd instar larva.** Size: BL 9.52–10.60 mm; HL 2.70–2.83 mm, HW 1.95–2.10 mm, ML 2.55–2.85 mm, HW/HL 0.72–0.74, ML/HL 0.94–1.01 General coloring whitish ochre, whitish pink (Fig. 2a, b, c); ocular tubercles darker, with median pair of obsolete dark posteriorly (Fig. 2a); lateral sides of the head slightly darker posteriorly; ventral side paler; mandibles reddish brown; legs pale; abdomen with median and pair of broader sub-median longitudinal dark lines dorsally; body covered with black dolichasters and thinner pale bristles. Head longer than wide (Fig. 2a); mandibles almost as long as head capsule and armed with long teeth (Fig. 2a, b); interdental mandibular setae: (4–5) (1–2) (0–2) (0); some (about 18) setae are disposed on lateral to ventral surface of the mandibles, these setae longer near base of mandible. Pronotum covered with short black dolichasters (Fig. 2f), with long bristles anterolaterally (Fig. 2a); mesonotum lacking median tuft of black hair-like setae, mesothoracic processes well developed (Fig. 2a, b, f), setiferous, with anterior process longest, about 6 times as long as its width, metathoracic processes setiferous, relatively short (Fig. 2a), with posterior process 2 times as long as its width; these processes bear dense long hair-like setae distally (Fig. 2f). Abdominal segment IX subcomical in shape (Fig. 2d, e); sternite IX provided with long setae (Fig. 2e). The body of live individuals is usually covered with debris dorsally (Fig. 2g).


**Distribution.** Hokkaido, Honshu, Sado Is., Shikoku, Kyushu. (Sekimoto, 2014)

**Habitat.** The larval habitat of this species is dry, almost always in the shade and unexposed to rainfall (such as Fig. 4b). The larvae are not pitfall builders but ambush predators often found in shallowly piled silt in a small depression on stone foundation of a shrine building in woods or on hard clay beside it. They were found not only at the surface of substratum simply, but also under an object, such as a garbage can left there, or even in gaps at some height above the ground level, i.e. gaps between piled roofing tiles besides an old shrine building, and in gaps of stone arrangement at the base of a small shrine. These observations indicate that the larva of this species can move vertically unlike most other species of antlions. The larvae of european congener, *D. pantherinus* are known to be collected on trees, in tree holes filled with dry detritus and under barks (Badano and Pantaleoni, 2014). Gepp & Hölzel (1989) and Gepp (2010) noted this species is remarkably able to colonize human buildings beside woods, where they hide in sheltered corners. Badano and Pantaleoni (2014) pointed out the presence of *D. pantherinus* larva in artificial structures suggests that, at least potentially, it is able to colonize different kinds of cavities in forested habitats. In fact antlions of *D. pupillaris* were also found at inner part of cavity on the side of a trail and in the tree hollow. While larvae of Taiwanese *D. esbenpeterseni* inhabit under lock and latches in the mouth of caves of moderate size, which contain fine dust (Stange et al., 2002).

**Remarks.** The larva of this species was erroneously recorded by Matsura (1989) based on that of *E. moiwana* (see discussion) and no information of larval stage had been available. Larval morphology of *D. pupillaris* well agrees with diagnosis of the genus by Badano and Pantaleoni (2014) except for that *D. pupillaris* lacks median tuft of black hair-like setae on mesonotum.

**Genus Pseudoformicaleo** van der Weele, 1909

(Figs. 3a–e, 4C)


**Diagnosis.** Mandibles rather short and robust (Fig. 3a, b), slightly bent upward, equipped with 3 pairs of teeth, with middle tooth closer to the basal one (Fig. 3a, b); ocular tubercles prominent; mesothoracic processes weakly developed; VIII abdominal sternite (Fig. 3e) with pair of moderately developed odontoid processes near posterior margin; Abdominal sternite IX (Fig. 3e) wider than long,
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Fig. 4. Habitats. a–c: larval habitat (arrows indicate points where larvae were found), a: *E. moiwana* (Honzanji, Takatsuki, Osaka), b: *D. pupillaris* (Nagano Park, Kawachinagano, Osaka), c: *P. nubecula* (Amidacho-Kitayama, Takasago, Hyogo), d: female of *P. nubecula* laying eggs into gap of granite gravel.

Fig. 3. *Pseudoformicaleo nubecula* (Gerstaecker), 3rd instar larva. a: dorsal aspect, b: ventral aspect, c: lateral aspect, d: IX and VIII abdominal sternite, dorsal aspect, e: ditto, ventral aspect (DS: digging setae, R: rastrum, OP: odontoid process).

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with pair of moderately sclerotized rastra equipped with four subequal digging setae.

Comments. The genus *Pseudoformicaleo* is composed of 9 species distributed in Africa, Madagascar, Asia and Australia (Stange, 2004). The antlion of any species of this genus had been unknown.

**Pseudoformicaleo nubecula** (Gerstaecker, 1885)
(Figs. 3a–e, 4c)

See Sekimoto (2014) for synonyms.

*Diagnosis of 3rd instar larva.* Size: BL 8.16–8.42 mm; HL 2.09–2.14 mm, HW 1.81–1.84 mm, ML 1.68–1.74 mm, HW/HL 0.86–0.87, ML/HL 0.79–0.83. Body dark chocolate with contrasting pale markings on meso- and metathoracic processes (Fig. 3a, c); dorsal face of head capsule brown (Fig. 3a), with posterior portion pale with pair of sub-median dark spots; ocular tubercles paler, postero-lateral sides of head darker; ventral side of head capsule (Fig. 3b) anteriorly and laterally darker, posteriorly pale; mandibles dark brown with inner teeth reddish; legs pale (Fig. 3b, c) except dark brown hind coxa; abdominal tergite VIII laterally and medially pale (Fig. 3d); tergite IX pale; ventral surface of abdomen pale along midline (Fig. 3b); dolichasters on body blunt or tapered, not inflated apically; Head longer than wide (Fig. 3a, b), with rather distinct ocular tubercles (Fig. 8b), ventrally sparsely covered with short setae; mandibles (Fig. 3a, b) comparatively robust, shorter than head capsule and armed with teeth of moderate length; distance between base of mandible and basal tooth almost same as or a little shorter than that between basal and apical teeth; median tooth slightly closer to basal tooth; interdental mandibular setae: (3–4) (0–1) (1) (0); several short setae are disposed on lateral margin of mandibles. Pronotum covered by black setae; thoracic processes short (Fig. 3a). Abdominal sternite VIII lacking digging setae; IX sternite (Fig. 3e) with median pair and lateral three pairs of digging setae in addition to rastra.


*Distribution.* Japan (Honsyu, Shikoku, Kyushu, Ishigaki-jima Is.); China, Taiwan, Malaysia, Java, Sri Lanka, Palau, Western Caroline Island, Australia. (Sekimoto 2014)
**Habitat.** Restricted to barren land such as rocky hills and slopes (Fig. 4c). This species is locally common in Setouchi district (especially southern part of Okayama and Hyogo Prefectures) where many rocky hills of granite or welded tuff are distributed. The habitat of this species is dry, open to sunlight and rainwater. The larva is not a pitfall builder but an ambush predator found in silt or fine gravel collecting in a small depression of windswept rocks.

**Remarks.** The larva of this species has distinct pale markings on meso- and metathoracic processes (Fig. 3a). These markings are seen also in the first and second instars and useful characters to identify this species. In many cases carcasses of ants were found around the depression where larvae of this species inhabited. Such possible prey includes *Pheidole noda* F. Smith, *Tetramorium tsushimae* Emery, *Camponotus japonicus* Mayr and *Paepychoydia chinensis* (Emery) in Amidacho-Kitayama, Hyogo Prefecture. Adults fly during the summer season (from July to September). Matsumoto observed females laying eggs into gap in granite gravel (Fig. 4d) in Okayama Prefecture.

**Discussion**

Sequences of DNA barcode region of larvae in all three species are almost invariably congruent with those of adults respectively and each species clustered as a single clade (Fig.5). Furthermore the reared adults confirmed the identification in *E. moiwana* and *D. pupillaris*. Matsura (1989, 2000, 2002) illustrated an antlion found in somewhat damp silt and noted that it grew up to be an adult of *D. pupillaris* subsequently. Though, according to his illustration and description of morphology and habitat, it is most probable that the antlion he treated as *D. pupillaris* was that of *E. moiwana* in actuality. This is the first record of a larva of the genus *Epacanthaclisis*.

The true *Dendroleon pupillaris* larva newly discovered in this study lives on the dry hard clay or stone substrate in concealed situations and in dry wood hollow, where a thin layer of fine silt and debris is piled. In such microhabitats, long and dense hairlike setae of the body are adaptive to hold silt and debris, so that the larvae can camouflage themselves. They seem avoiding deep silt or dust where they cannot anchor to the substrate as mentioned for *D. esbenpeterseni* (Stange et al., 2002). They are possibly avoiding competition with other sympatric antlions such as *Baliga micans* (McLachlan) and *Myrmeleon formicarius* Linnaeus, which form pits in the silt or debris of adequate depth.

*Dendroleon pupillaris* seems closely related to *Gatczara jezoensis* (Okamoto) concerning the larval and adult morphology. Larvae of *G. jezoensis* lurk usually vertically on the surface of rocks covered with lichens with head directed downwards and rarely move to other places. On the other hand, antlion larva of other species, viz. *Distoleon contubernalis* (McLachlan) and *Synclisis japonica* that do not form pits are ambushing somewhat deeper in the sand and can crawl both forward and backward depending on the situation. Larvae of *D. pupillaris* hide themselves in a shallow depression with silt and debris and are sometimes observed to crawl forwards even on vertical surface of the wall. Concerning the larval stage, *D. pupillaris* seems at the intermediate stage in transition from antlions ambushing in the sand or silt to those lurk on the rocks like *G. jezoensis*. When disturbed and left on the surface of debris the antlion of *D. pupillaris* and *P. nubecula* lift debris around their body onto head and thorax using front legs rather than digging through by recoiling. This is a characteristic behaviour for antlion larvae which do not form pits and hide themselves in shallow depressions.

These three species treated in this paper seem semivoltine because larvae of different instars were found in one place and at the same time of the year in several localities in each species.

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**References**


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