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Focusing on *Keroplatus nipponicus* Okada, 1938 (Diptera: Mycetophiloidea: Keroplatidae) and Its Adults

Im Zentrum des Interesses: *Keroplatus nipponicus* Okada, 1938 (Diptera: Mycetophiloidea: Keroplatidae) und seine Imagines

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Summary: Of the 27 *Keroplatus* species described worldwide only five are known to possess luminescent larvae. The role of their blue lights, the biochemistry of the reaction and its control as well as the phylogeny of keroplatids are all aspects that are still largely incompletely understood, a consequence no doubt of the rarity of those species with luminescent larvae. Some larvae of the luminescent fungus gnat *Keroplatus nipponicus* were collected on the Japanese Pacific island Hachijojima and at a length of around 17 mm three turned into pupae. Physical damage to the cocoon during its construction was not repaired by the larva. At an environmental temperature of around 28 °C two adults eclosed after a pupal phase that lasted only 3 days. The male was slightly shorter and slimmer than the female (7.06 versus 7.36 mm), had larger eyes and significantly more plumose antennae. It was also noticeably more active than the female that it was attempting to copulate with. Both male and female died three days after eclosion, but the female laid 306 black spherical eggs of a diameter of 0.4 mm on her last day.

Keywords: Bioluminescence, fungus gnats, glowworms, Hachijojima

Zusammenfassung: Von den weltweit 27 beschriebenen Arten der Gattung *Keroplatus* haben nur funf lumineszierende Larven. Die Rolle des blauen Lichts, Biochemie und Kontrolle der Reaktion sowie die phylogenetische Position der Keroplatiden sind noch weitgehend unbekannt, zweifelsohne eine Konsequenz der Seltenheit jener Arten mit lumineszierenden Larven. Einige *Keroplatus nipponicus*-Larven wurden auf der japanischen Pazifikinsel Hachijojima gesammelt. Zum Zeitpunkt der Verpuppung hatten die lumineszierenden Larven dieser Pilzmücke eine Länge von ca. 17 mm. Ein Schaden des Kokons wurde während der Konstruktion nicht von einer Larve repariert. Die Puppenphase zweier beobachteter Tiere dauerte bei einer Temperatur von 28 °C nur drei Tage. Das Männchen war mit 7,06 mm Länge etwas kürzer als das 7,36 mm messende Weibchen und hatte außerdem größere Augen und stärker gefiederte Antennen; ferner war es bei Kopulationsversuchen sehr viel aktiver. Beide Adulte lebten nur drei Tage, doch legte das Weibchen am letzten Tag noch 306 schwarze, kugelige Eier von 0,4 mm Durchmesser.

Schlüsselwörter: Biolumineszenz, Pilzmücken, Glühwürmchen, Hachijojima

1. Introduction

There were mainly two reasons that prompted us to write this short article. One dealt with the fact that knowledge on any member of the keroplatid genus *Keroplatus* irrespective as to where in the world it was occurring is extremely sparse and any new piece of information could be of importance. The second reason was the availability of some excellent photographs of *K. nipponicus*, especially those of adult males and females. Okada (1938) named this species *Ceroplatus nipponicus* on the basis of a male holotype and described the species' adults well, but failed to provide any illustrations.

The dipteran family Keroplatidae of the superfamily Mycetophiloidea contains species of an astonishingly variety of ecological specialists and different feeding methods. Termed 'fungus gnats' in English, the majority of the species are indeed feeding on fungi and their spores and are not known to be bioluminescent. However, there are some species like the weakly blue-luminescent *K. nipponicus* that in order to collect spores construct slime webs on the underside of the fungal bodies of certain mushrooms, e.g. *Grammothele fuligo*, that grow on trees (Osawa et al. 2014). Yet others like the somewhat brighter but also blue-luminescent *Orfelia fultoni*, which also secretes spore-trapping webs, will, however, not hesitate to accept collembolans or other small arthropods as food in addition to its main diet of fungal spores (Sivinski 1998). Both *K. nipponicus* and *O. fultoni* do not occur in caves, but the Australian and New Zealand *Arachnocampa* species often do. They go a step further and do not feed on fungal spores but only accept other arthropods as food, mainly small insects capable of flight that are attracted to the bluegreen photic lures of larval *Arachnocampa* spp. and then become ensnared by the larvae's sticky 'fishing lines' to be subsequently consumed (Meyer-Rochow 2007).

Although at least ten species of fungivorous mycetophiloids were considered from the Holarctic region to possess weakly luminescent larvae by Matile (1990), only five keroplatids of the 27 species in the genus *Keroplatus* recognized by Myers et al. (2016), namely *K. nipponicus* and *K. biformis* in Japan and *K. tipuloides, K. testaceus*, and *K. reaumurii* in Europe, are amongst them (Oba et al. 2011). None of these species is common and most are regarded as vulnerable; in fact some seem exceedingly rare and knowledge of their presence is sometimes based on just one trapped and identified specimen kept in a museum collection. For instance, a single male *K. testaceus* is known from southern Norway, found by Økland & Søli (1992) in association with carpophores of the tinder fungus *Fomes fomentarius*. Another single male of the same species was identified by Kurina & Hedmark (2004) in Latvia from sweep net collections and more recently a male *Keroplatus tuvensis* of the *testaceus* group has been reported by Polevoi et al. (2006) from Finland.

Stammer (1932) collected specimens from trees suffering from butt rot (*Polyporus = Placodes ungulates*) in the Heuscheuergebirge (today Poland) and places near Breslau (today Wroclaw, Poland) to study the larval anatomy and luminescent behaviour of *Ceroplatus testaceus* (the spelling used for the genus at that time). Scherf (1970) provided some information on *K. testaceus* larvae that he found on trees attacked by *Trametes gibbosa* growing on the Vogelsberg (Hessonia, Germany) and Kato (1953) and Haneda (1957) occupied themselves with the Japanese species *K. nipponicus*. Despite these studies and the most recent observations by Osawa et al. (2014) on the general biology of *K. nipponicus* and Meyer-Rochow & Yamahama (2017) on this species' larval eye ultrastructure (wrongly described by Kato 1953 as being a ''colorless compound eye''), numerous open questions remain. The dearth of adequate photographs of larvae, pupae and in particular living adults has not helped either, which is why our observations accompanied by photographic evidence are a small additional step in understanding some aspects of the biology of this most enigmatic of luminescent insects.

2. Materials and methods

Larvae of *Keroplatus nipponicus* were collected on two occasions during the summer of 2017 on the Japanese Pacific island of Hachijojima from the undersides of various tree fungi that were growing on palm trees (*Livistona chinensis*) attacking the remains of the bases of discarded palmfronds still part of the tree (Figs 1, 2). Some larvae were taken to the lab, pupated thereafter and then were taken to Taiwan and maintained at temperatures of around 28 °C and a humidity not less than 90%.

With the exception of Figure 4, for which a Canon EOS-1D Mark IV mounted on a tripod and equipped with a Canon EF 100 mm f2.8L Macro IS USM lens and no flash was used (f/2.8, 30 sec, ISO 10000) and then via photoshop combined with another clear image taken with flash (f/5.6, 2 sec, ISO 200), all other photos were taken with an Olympus E-5 equipped with an Olympus 35 mm f3.5 Zuiko Digital Macro lens. The settings for most photos were f/16, 1/125 sec, ISO 200 or 250 + flash (1/64). Altogether approximately 700 photographs were taken of *K. nipponicus* larvae, pupae, adults and eggs.

Adult specimens, currently in a tube without any liquid in the freezer, will be stored in the National Museum of Natural Sciences in Taiwan as dried, pinned specimens.

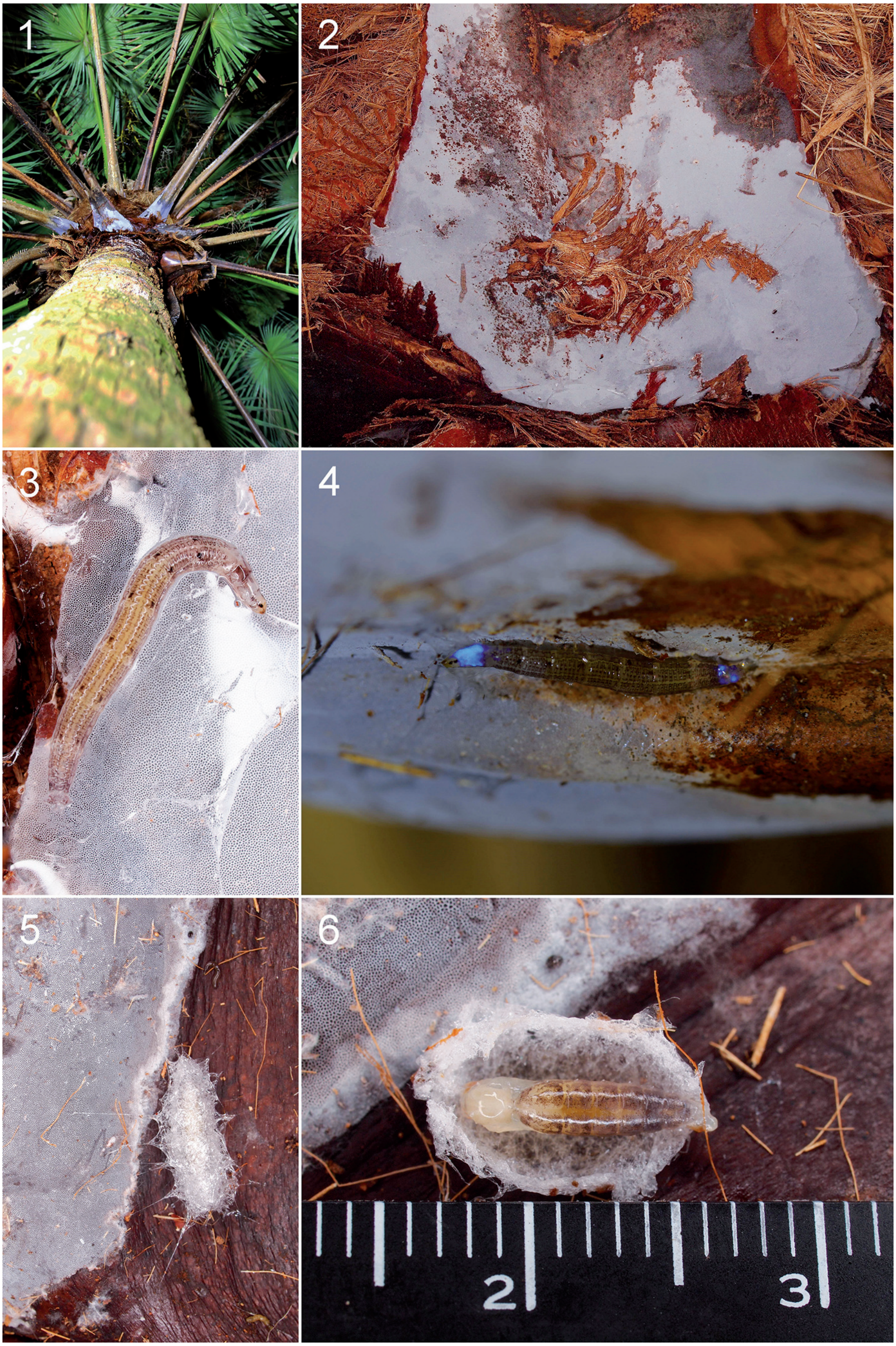
3. Results and discussion

The larvae collected (Figs 3,4) varied in total length and were almost certainly not all of the same developmental stage. In some larvae the entire body emitted light, but anterior and posterior regions usually exhibited the brightest glow with the anterior usually 'outshining' the posterior light as shown in Figure 4. Larvae ready to pupate, in agreement with observations made by Kato (1953), can be assumed to be at least 15-20 mm long. Due to the larvae's ability to wriggle and contract it is, however, not easy to accurately measure dimensions of actively moving specimens. When during the construction of the white pupal casing (Fig. 5), made of coarse, white silk threads and released from the mouth, part of the construction was deliberately physically removed, no attempt by the larva to repair the damage was made and the pupa became thus freely exposed (Fig. 6). Numerous hymenopteran parasites are known to attack mycetophilids (Kolarov & Bechev 1995) and an exposed pupa can be assumed to be in danger of being parasitized (although so far only one case of a parasitoid *Megastylus* sp. having ever been reported to have emerged from a pupa of *Keroplatus nipponicus* by Oba et al. 2011).

The times when pupations and eclosions occurred were noted and both male and female needed only 3 days to complete their development (see Tab. 1). This differs from observations made by Kato (1953), who reported a pupal phase of 7 days, and Scherf (1970), who stated that the pupal stage in *Keroplatus testaceus* lasted approximately 14 days. Environmental temperature that insect pupae generally are exposed to will affect the length of the pupal phase and our observations, being done close to

Tab. 1: Data based on the observations made on the collected larvae of *Keroplatus. nipponicus.*
Tab. 1: Zusammengefasste Daten der gesammelten Larven von *Keroplatus nipponicus.*

Collection date	Pupation	Eclosion	Date of death	Larval total length (mm)	Larval pronotum width (mm)	Adult total length (mm)	Adult pronotum width (mm)
2017.7.26				15.74	1.01		
dto				13.59	0.86		
dto				16.01	1.05		
2017.9.11	9.13	9.17	9.20	17.11		Female 7.36	1.35
2017.9.12	9.14	9.18	9.21			Male 7.06	1.11



Figs 1-6: 1, 2 *Keroplatus nipponicus* larvae occur on the underside of tree fungi, whose spores they consume. **3** Larva during daylight. **4** Photograph of a larva showing bright anterior but considerably dimmer posterior luminescence. **5** Cocoon of white silk close to the tree fungus. **6** Physically opened cocoon showing the pupa inside.

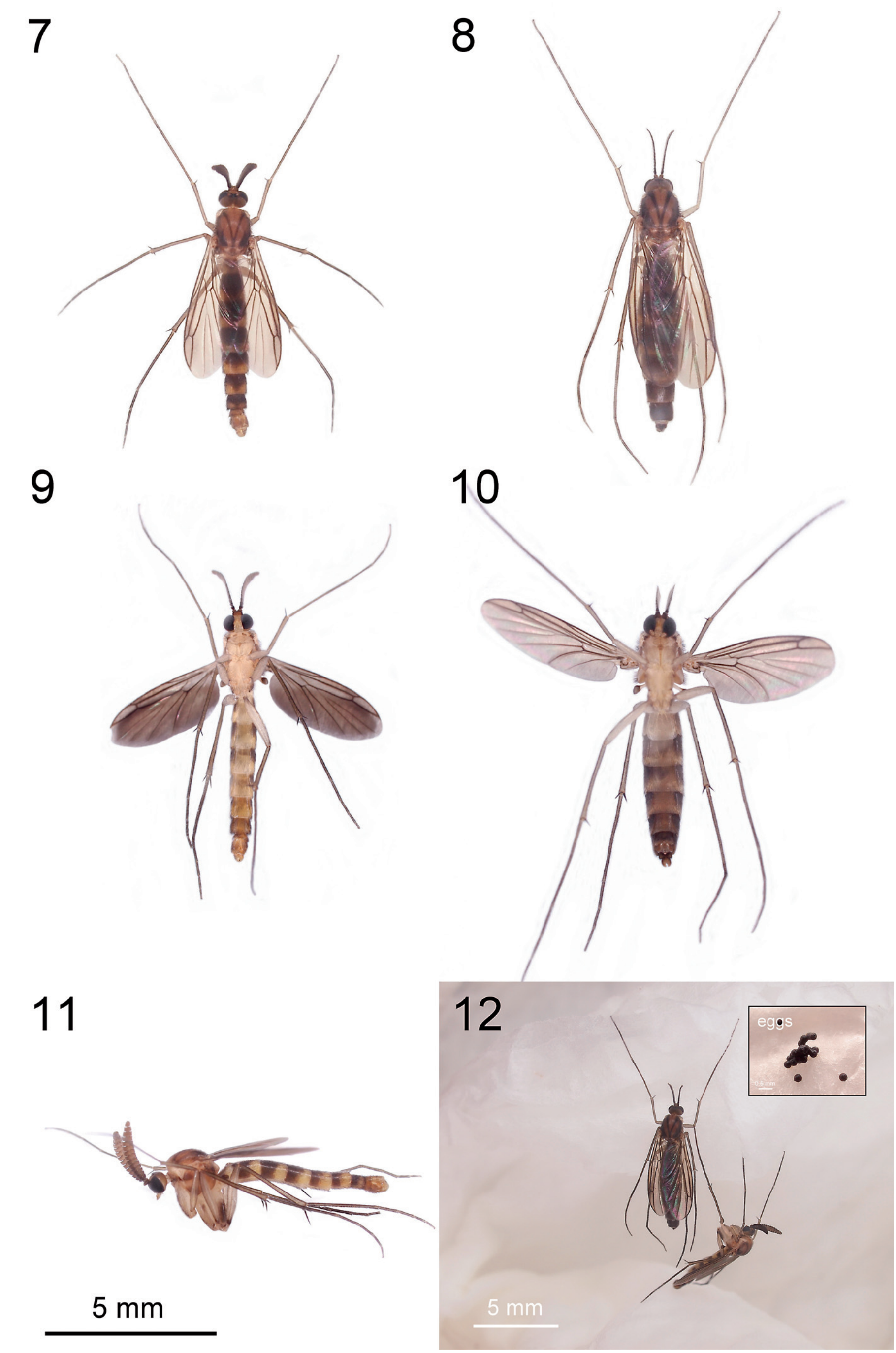
30 °C, almost certainly would have been responsible for the shorter pupal durations seen in our *K. nipponicus* than those reported by Kato (1953) and Scherf (1970). That humidity levels as well as other factors like, for instance, quantity and quality of larval food uptake, etc., could also affect pupal duration is likely, but has never been investigated in any keroplatid species. Regrettably only two adults were available for further study, but in view of the scarcity of the species (and in particular its adults) we feel our observations are worthwhile to report.

In agreement with Kato (1953) no obvious morphological differences between male and female larvae were noticed. However, our female adult was slightly longer than the male and also had longer legs, a thicker abdomen and a wider pronotum than the latter; furthermore it made only very few attempts to fly following eclosion despite the presence of apparently functional wings (they could be spread and flap). The male differed from the female mainly with regard to its slightly smaller size and appearance of its longer and more plumose antennae compared with those of the female (Figs 7-11). This feature suggests that the single drawing provided of an adult by Kato (1953), but not sexed, was based on a female specimen. Scherf (1970) incidentally features a line drawing of a male *K. testaceus* in his Figure 3, which resembles our male *K. nipponicus*, but lacks the latter's dark brown dorsal markings on the abdominal segments and Oba et al. (2011) in their Figure 7a,b show photographs of male *K. nipponicus* and *Keroplatus biformis*. The article by Sivinski (1998) contains the drawing of a male *Orfelia fultoni*.

Given the slight difference between the two genders' body sizes, the wings of the male in *K. nipponicus* can be considered to be somewhat broader and longer than those of the female; they also seemed slightly darker in colour especially when viewed from below. The male moreover possessed somewhat bigger compound eyes than the female with a border between the posterior and forward looking eye regions clearly delineated through short bristles and different coloration and it was significantly more active attempting numerous times to get under the latter to copulate (Fig. 12), most likely seeking to engage in a back-to-back position typical of other fungus gnats (cf. Murray's photo of mating fungus gnats: https://bugguide.net/node/view/1235971/bgimage), mosquitoes or tipulid dipterans. Short physical attachment was observed, but whether sperm transfer occurred is doubtful. Flight in the circular plastic container of 6 cm height and 10 cm in diameter, supplied with moist filter paper for the insects to drink from, was restricted, but it allowed the insects to be observed in.

The more sedentary behaviour of the female and greater activity of the male would provide an explanation for the fact that the few adult keroplatids caught in the field in Europe were exclusively males (Økland & Søli 1992; Kurina & Hedmark 2004; Polevoi et al. 2006) and their somewhat bigger eyes and significantly more plumose antennae collectively suggest that the males search for their females during the adults' short active lifespan of just a few days (our individuals both died three days after eclosion). Our sole female started to lay all of her 306 blackish, spherical eggs of ca. 0.4 mm in diameter on the third day of her adult life shortly before she died (Fig. 12, inset). Her longer legs could be an indica-

Abb. 1-6: 1, 2 Die Larven von *Keroplatus nipponicus* leben von Pilzsporen an der Unterseite von Baumschwämmen. **3** Foto einer Larve bei Tageslicht. **4** Foto einer Larve, deren Lumineszenz am Vorderende sehr viel heller ist als am Hinterende. **5** Ein Kokon aus weißer, grober Seide nahe des Baumpilzes. **6** Physisch eröffneter Kokon, die Puppe zeigend.



Figs 7-12: 7, 9, 11 *Keroplatus nipponicus* male in dorsal, ventral and lateral view. **8, 10** *K. nipponicus* female in dorsal and ventral view. **12** Male (on the right) attempting to copulate with the female. Inset: tiny black eggs. All photographs were of the one pair that was available for observations.

tion that hanging on to a substrate would be of greater importance to females than it is for males. Given the short lifespans of the adults, obviously male and female eclosion times need to be synchronized, but how that is achieved is another open question to be resolved.

Since awareness of mycetophiloid luminescence generally and that of *Keroplatus* spp. in particular is not widespread we feel it is useful to briefly highlight the uniqueness of this group's bioluminescence with peak emissions of around 460 nm in *K. nipponicus* (Oba et al. 2011) and *O. fultoni* (Sivinski 1998) being the bluest light of all bioluminescent terrestrial arthropods. Together with *O. fultoni* from North America (Sivinski 1998) and various species of *Arachnocampa* in Australia and New Zealand (Baker 2009) keroplatids are the only Diptera known to be able to produce biological light not based on bacterial contributions. What is interesting with regard to the light production, however, is that the species do not employ a common method to produce their lights. Species of the genus *Arachnocampa* use their Malpighian tubules to metabolize waste products in a process during which light is produced and emitted from the rear end of the abdomen (Green 1979). *O. fultoni* larvae apparently possess light-emitting structures only in their five anterior segments (in the form of cells with granules derived from mitochondria) and in a region near the abdominal tip (Bassot 1978). In *Keroplatus tipuloides* and *Keratoplus reaumuri pentophthalmus* specialized proteinaceous granules of the fat body have been described as being responsible for the glow that can light up the entire larval body, but primarily involves the anterior region (Baccetti et al. 1987).

Biochemically light production in mycetophiloids is typically based on luciferins as substrates and luciferases as catalyzing enzymes, but the chemical reactions that produce the biological light vary and even taxonomically closely related genera employ different reactions (Viviani et al. 2002). Contrary to that of *O. fultoni* (and presumably *Keroplatus* spp.), in *Arachnocampa* spp. the emission is activated by Mg-ATP and not by ascorbic acid, dithiothreitol, CoA or $FMNH_2$ (the latter two exhibiting weaker effects than the former two compounds), while inhibitory signals come from the brain (Mills et al. 2016). *Arachnocampa flava* luciferase, in contrast to the 140 kDa heterodimer of *O. fultoni*, is a relatively small enzyme of 36 kDa that acts on the luciferin by catalysing a chemical reaction which, in the presence of oxygen, leads to the emission of almost 'cold light' (Shimomura et al. 1966), but how exactly ATP activates the *Arachnocampa* system (but not that of *Orfelia)* is not yet known. The cytological differences in the light-emitting structures and the sizes of the luciferases of the three genera may provide a clue (Bassot 1978). The weight of any *Keroplatus* spp. luciferase is still unknown, but it may be similar to that of *O. fultoni* (the highest known for any luminescent terrestrial arthropod: Viviani 2002), because light emission mechanisms, emission peaks in the blue near 460 nm wavelength and certain aspects of their biologies are shared by both of them. A recent molecular study by Ševčik et al. (2016) also suggested a closer phylogenetic relationship of the genus *Keroplatus* with *Orfelia* rather than *Arachnocampa*.

With the exception of *Arachnocampa* spp. larvae, where the light possesses a well-studied function as a lure to attract prey

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Abb. 7-12: 7, 9,11 *Keroplatus nipponicus*-Männchen in dorsaler, ventraler und lateraler Ansicht. **8, 10** *K. nipponicus*-Weibchen in dorsaler und ventraler Ansicht. **12** Männchen (rechts) beim Versuch der Kopulation mit dem Weibchen. Einsatz: kleine schwarze Eier. Alle Fotos stammen von dem einzigen zur Verfügung stehenden Paar.

to be consumed by the former (BROADLEY & STRINGER 2001), the purpose of the light in *O. fultoni* is dubious and that of all *Keroplatus* spp. is outright unknown, although some speculation by OSAWA et al. (2014) suggested that it could possibly be aimed at the fungus to provide more spores rather than act aposematically to deter predators. Both KATO (1953) and HANEDA (1955) were unable to see light emissions in adult *K. nipponicus* and concluded that the adults were non-luminescent and that the larvae would not change their luminescence upon physical stimulation and no longer luminesced when dried. We too could not detect any bioluminescence coming or stemming from the adult individuals as well as their eggs, but we had earlier been able to show that physical and other forms of stimulation did affect the larval light's intensity and that dried extracts could be made to luminesce (OSAWA et al. 2014).

In conclusion: what is new in this paper are observations on pupating larvae and their inability to repair physical damage to their cocoon as well as photographic evidence of male/female differences. The eggs of *K. nipponicus* are described and illustrated for the first time. Regrettably, no larvae ever hatched from them. Furthermore the information on pupal duration and adult longevity supplements the scant data available in the literature on this aspect of the species' biology and the photographs of the adults, to the best of the authors' knowledge, are the first published images of both genders of *K. nipponicus*.

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