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The Picasso stick insect. A striking new species of *Calvisia* from Vietnam with notes on captive breeding and new methods for incubation of eggs (Phasmida: Diapheromeridae: Necrosciinae)

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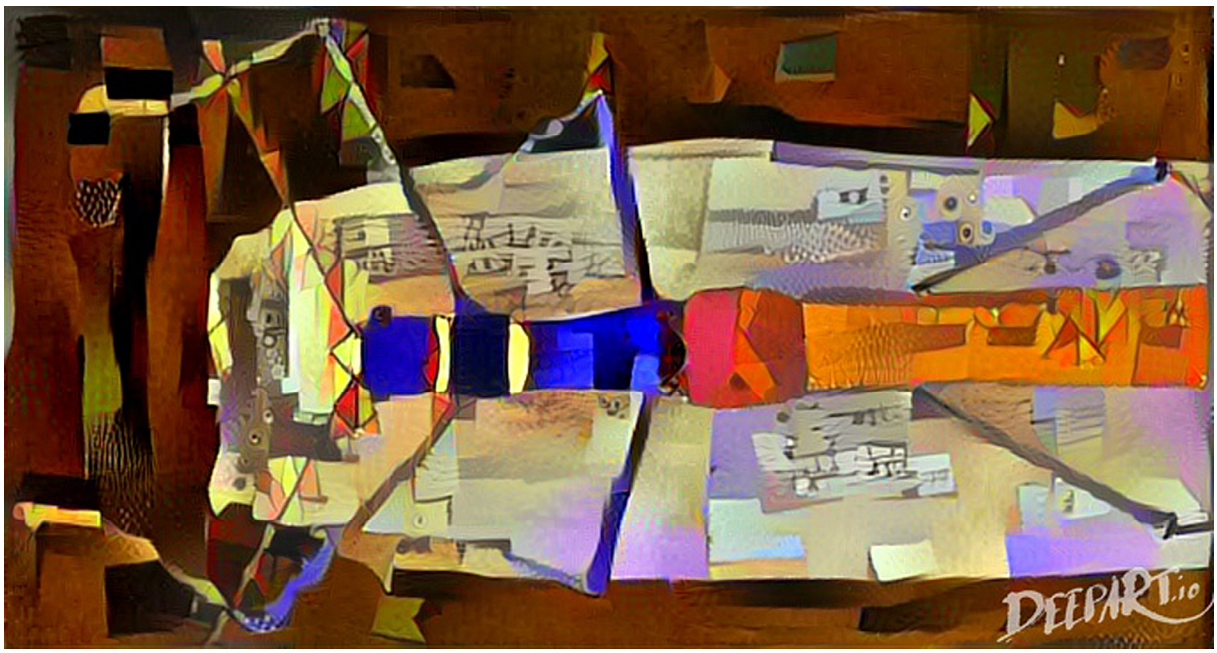

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Front cover: artistic representation of a female of the "Picasso stick insect" *Calvisia kneubuehleri* sp. nov. generated by deepart.io from a photograph of a captive reared female by B. Kneubühler and the cubist painting "Trois musiciens" by Pablo Picasso (1921).

The Picasso stick insect. A striking new species of *Calvisia* from Vietnam with notes on captive breeding and new methods for incubation of eggs (Phasmida: Diapheromeridae: Necrosciinae)

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Abstract

A colourful new species of *Calvisia* Stål, 1875, *C.* (*Calvisia*) *kneubuehleri* sp. nov., is described from South Vietnam based on the male, female, nymphs and eggs. The new species is placed in the subgenus *C.* (*Calvisia*) Stål, 1875 and compared to the closely related *C.* (*Calvisia*) *torquata* Bates, 1865. Both species are illustrated and new records of *C.* (*Calvisia*) *torquata* from Thailand are provided. A distribution map and details on biology and development are given for *C.* (*Calvisia*) *kneubuehleri* sp. nov. New techniques developed to breed the species in captivity are explained, illustrated and discussed: the low temperature-induced dormancy method (LTD) and the glued eggs technique method (GET).

Keywords: stick insect; Phasmatodea; Dong Nai Biosphere Reserve; Thailand; Cambodia

Introduction

The genus *Calvisia* was erected by STÅL (1875) to include 4 species: *Necroscia sangarius* Westwood, 1859, *N. medora* Westwood, 1859, *N. virbius* Westwood, 1859 and *N. hemus* Westwood, 1859. REHN (1904) later designated *N. sangarius* as the type-species. The Bornean species of the genus were recently revised by SEOW-CHOEN (2016) who introduced six subgenera.

The genus is widely distributed over South-East Asia, from India in the west to Bali and Sulawesi in the southeast, and currently contains 35 species (BROCK *et al*., 2016). *Calvisia* species are winged and often very colourful phasmids living in tropical rainforests.

Recent fieldwork in South Vietnam revealed a brightly coloured new species which extends the distribution of the genus to that country. However, this was no great surprise as another species, *C.* (*Calvisia*) *torquata* (Bates, 1865) was described from Thailand, close to Cambodian border.

The present paper aims to describe the new species and give information on its breeding, biology and development observed in captivity, and to provide new data for *C.* (*Calvisia*) *torquata*.

Material and methods

A female was kept alive in a plastic box for producing eggs during a fieldtrip to Dong Nai Biosphere Reserve in 2012. The wild caught specimens were euthanized with etylacetate fumes. The specimens were then stored in airtight plastic "zip"-bags in wood chips (used in rodent cages) and sprinkled with etylacetate (EtOAc) to prevent rotting, mould and to keep the specimens flexible. The bags were frozen on arrival and the specimens mounted and labelled later on.

Eggs were hatched and the species was reared to adulthood by Dr. Bruno Kneubühler (Switzerland).

Photographs of the collection specimens were taken with a Canon 700D camera equipped with a Tamron 90 mm Macro lens and staked with CombineZ software. They were optimized with Adobe Photoshop CS3. Observations were done with a Leica MZ8 stereo- microscope. Measurements were done with an electronic calliper. Description of colouration is based on live specimens.

The nomenclature for the egg morphology follows that of CLARCK-SELLICK (1997; 1998). The description of the colouration is based on live specimens.

Acronyms used for the collections:

BMNH	=	Natural History Museum, London, U.K. (formerly British Museum, Natural History)
CAU	=	Entomological Museum, China Agricultural University, Beijing, China
OC	=	Oskar V. Conle private collection, Bolsterlang, Germany
OUMNH	=	Oxford University Museum of Natural History, Oxford, U.K.
RBINS	=	Royal Belgian Institute of Natural Sciences, Brussels, Belgium
VNMN	=	Vietnam National Museum of Nature, Hanoi, Vietnam

Taxonomy

Family **Diapheromeridae** Kirby, 1904
Subfamily **Necrosciinae** Brunner von Wattenwyl, 1893
Tribe **Necrosciini** Brunner von Wattenwyl, 1893

Genus ***Calvisia*** Stål, 1875

Calvisia STÅL, 1875: 42, 87. Type species: *Necroscia sangarius* Westwood, 1859 by subsequent designation by REHN (1904).

Subgenus ***Calvisia* (*Calvisia*)** Stål, 1875

The subgenus *Calvisia* (*Calvisia*) can be recognized by the combination of the following set of characters (adapted from BRAGG, 2001 and SEOW-CHOEN's 2016 key to the subgenera):

1. Macropterous. Wings at least reaching tergum VIII, sometimes projecting over apex of abdomen.
2. Tegmina humped anterolaterally.
3. Head not conical.

4. Colouration of body mainly black.
5. Scutellum not visible when wings closed.
6. Antennae long and filiform, surpassing the apex of body.
7. Bases of profemora almost completely straight, not strongly incurved.
8. Mesonotum with a pair of humps or posterior portion clearly higher than the anterior one; posterior portion swollen.
9. Eggs more or less rectangular, with the operculum on the dorsal part of the capsule; eggs glued in a continuous row, attached to each other by the anterior and polar ends.

***Calvisia* (*Calvisia*) *kneubuehleri* sp. nov.**

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Figs 1-5

DIAGNOSIS. Fairly slender for the genus. Closely related to *C.* (*Calvisia*) *torquata* (Bates, 1865), but easily distinguishable by its body coloration. Both species share the transverse yellow markings along the anterior and posterior margins of the pronotum (Fig. 2 D; Fig. 6 D) but can be easily separated by the tegmina and the costal area of the hind wings which is red in *C.* (*Calvisia*) *kneubuehleri* sp. nov. (Figs 1 A, 2 A); brown with bluish dots in *C.* (*Calvisia*) *torquata* (dried specimens) (Fig. 6 A); and the colour of the anal area of the posterior wings: whitish in *C.* (*Calvisia*) *kneubuehleri* (Fig. 2 A), infuscate in *C.* (*Calvisia*) *torquata* (Fig. 6 A).

ETYMOLOGY. The species is named after Dr. Bruno Kneubühler (Lucerne, Switzerland) in acknowledgement for breeding this and many other species that we brought back from Southeast Asia.

TYPE MATERIAL. Holotype ♂: Vietnam, Dong Nai Biosphere Res., 11°18'N 107°06', 25.VI-6.VII.2012, Leg. J. Constant & J. Bresseel, I.G. 32.161 (RBINS).
Paratypes (6♂♂, 8♀♀) 1♀: Vietnam, Dong Nai Biosphere Res., 11°18'N 107°06', 25.VI-6.VII.2012, day collecting, Leg. J. Constant & J. Bresseel, I.G. 32.161 (RBINS); 5♂♂, 6♀♀: ex breeding Bruno Kneubühler 2016, origin: Vietnam, Dong Nai Biosphere Res., 11°18'N 107°06', 25.VI-6.VII.2012, Leg. J. Constant & J. Bresseel, I.G. 32.161 (3♂♂, 3♀♀: RBINS; 2♂♂, 2♀♀: VNMN; 1♀: BMNH); 1♂: Ex Zucht B. Kneubühler 2013, Vietnam: Dong Nai N.P., Gen F1, OC-0326-17 (OC); 1♀: same data but OC-0326-3 (OC); 1♀: Dong Nai prov., Tan Phu district, Cat Tien National Park, Bau Sau Station, 21.IX.2011, Xingyue Liu (CAU).

DESCRIPTION.

Table 1. Measurements [mm] of *Calvisia* (*Calvisia*) *kneubuehleri* sp. nov.

	HT ♂	PT ♂♂	PT ♀♀
Body:	48.3	49.2–49.5	61.7–64.7
Head:	2.4	2.6–2.9	5.3–5.5
Pronotum:	2.4	2.6–2.7	3.5–3.8
Mesonotum:	7.4	6.8–7.0	6.8–6.9
Profemora:	13.8	12.6–15.4	14.4–16.5
Mesofemora:	8.9	9.6–10.3	9.8–10.3
Metafemora:	14.1	14.5–15.3	14.1–16.2
Protibiae:	12.8	13.0–13.9	13.9–15.1
Mesotibiae:	8.7	8.9–9.3	9.3–10.0
Metatibiae:	12.7	13.9–15.1	13.6–15.2
Tegmina:	3.8	3.7–3.8	6.7
Alae:	30.3	28.9–30.0	42.7–46.0

Fig. 1. *Calvisia* (*Calvisia*) *kneubuehleri* sp. nov. holotype ♂. A, habitus, dorsal view. B, habitus, lateral view. C, habitus, ventral view. D, head and thorax, dorsal view. E, apex of abdomen, dorsal view. F, apex of abdomen, ventral view. G, apex of abdomen, lateral view. H, head and thorax, lateral view.

Fig. 2. *Calvisia* (*Calvisia*) *kneubuehleri* sp. nov. paratype ♀**.** A, habitus, dorsal view. B, habitus, lateral view. C, habitus, ventral view. D, head and thorax, dorsal view. E, apex of abdomen, dorsal view. F, apex of abdomen, ventral view. G, apex of abdomen, lateral view. H, head and thorax, lateral view. I, egg, dorsal view. J, operculum of egg. K, egg, polar area.

MALE (Figs 1, 3 C, 4 A-E, 5 A).

Head: (Fig. 1 D, H) globose, black and smooth. Impressed between bases of antennae, between eyes a small oval raised area, followed by a mediolongitudinal groove reaching vertex. Eyes strongly projecting hemispherically. Antennae strongly projecting over apex of abdomen, black with several pale portions. Scapus slightly flattened dorsoventrally; pedicellus cylindrical, round in cross section. Antennomeres filiform, variable in length towards apex.

Thorax: (Fig. 1 D, H) pronotum smooth with anterior margin concave and yellow, slightly shorter than head. Behind anterior margin, a transverse groove and a median longitudinal groove. Just before centre, a second transverse groove, not reaching lateral margin of pronotum, but instead laterally joining S-shaped, longitudinal grooves. Longitudinal S-shaped grooves not reaching apex of pronotum. Two transverse grooves joined by longitudinal groove. Median longitudinal groove just reaching over half of pronotum. Posterior margin convex and with a broad yellow band. Other portions mainly black, central portion with a bluish hue. Mesonotum blackish, some portions with a bluish hue; longer than head and thorax combined, slightly widening towards the posterior. Anterior margin concave and slightly raised. Posterior margin with a small tubercle posteromedially.

Wings: (Fig. 1 A, C, D, H) tegmina small; base with a black spot, other portions red. Shape more or less rectangular with interior margin slightly rounded. A small but definite hump anterolaterally. Alae well developed; costal area coloured as tegmina; veins black; anal area with outer rim slightly greyish, rest of anal fan translucent white. Alae projecting over apex of tergum VI but not reaching anterior margin of tergum VII.

Legs: (Fig. 1 A-B, D, H) slightly setose, slender. Base of femora greenish, following portion banded black and white. Tibiae and tarsomeres banded black and white. Profemora only very slightly laterally compressed basally. All carinae of femora indefinite, hence femora almost circular in cross section. Tibiae as femora. Basitarisi longer than the following tarsomeres combined. Tarsomeres with a dorsal triangular tooth posteromedially. Claws small but acute.

Abdomen: (Fig. 1 E, F, G) black, sometimes dark brownish. Median segment well developed, rounded anteriorly, widening towards the posterior. Terga II-VI smooth, more or less uniform in length; VII shorter. Segments II-VII cylindrical. Tergum VIII almost half the length of VII, slightly tectifrom, but rounded dorsally and widening towards the posterior; IX slightly longer than VIII, with a small longitudinal carina dorsomedially, and acute postero-laterally. Medially, a pair of small transverse impressions. Anal segment almost half the length of tergum IX, rounded posteriorly. Ventrally armed with several small black denticles. Vomer well developed, shaped as an elongated, triangular, spinose plate. Poculum keel shaped, rounded posteriorly, not reaching posterior margin of anal segment. Cerci incurved, black with apex whitish and abruptly hooked.

FEMALE (Figs 2, 3 C, 4 F-J, 5 B).

Head: (Fig. 2 D, H) globose, black and smooth. Indistinctly impressed between bases of antennae, between eyes a small round raised area; vertex slightly swollen. Eyes strongly projecting hemispherically. Antennae reaching apex of abdomen, black with several pale portions. Scapus slightly flattened dorsoventrally; pedicellus cylindrical. Antennomeres filiform, variable in length towards apex.

Thorax: (Fig. 2 D, H) pronotum smooth, slightly shorter than head with anterior margin raised, concave and yellow. Behind anterior margin, a transverse groove and a median longitudinal groove. Just before centre, a second transverse groove, not reaching lateral

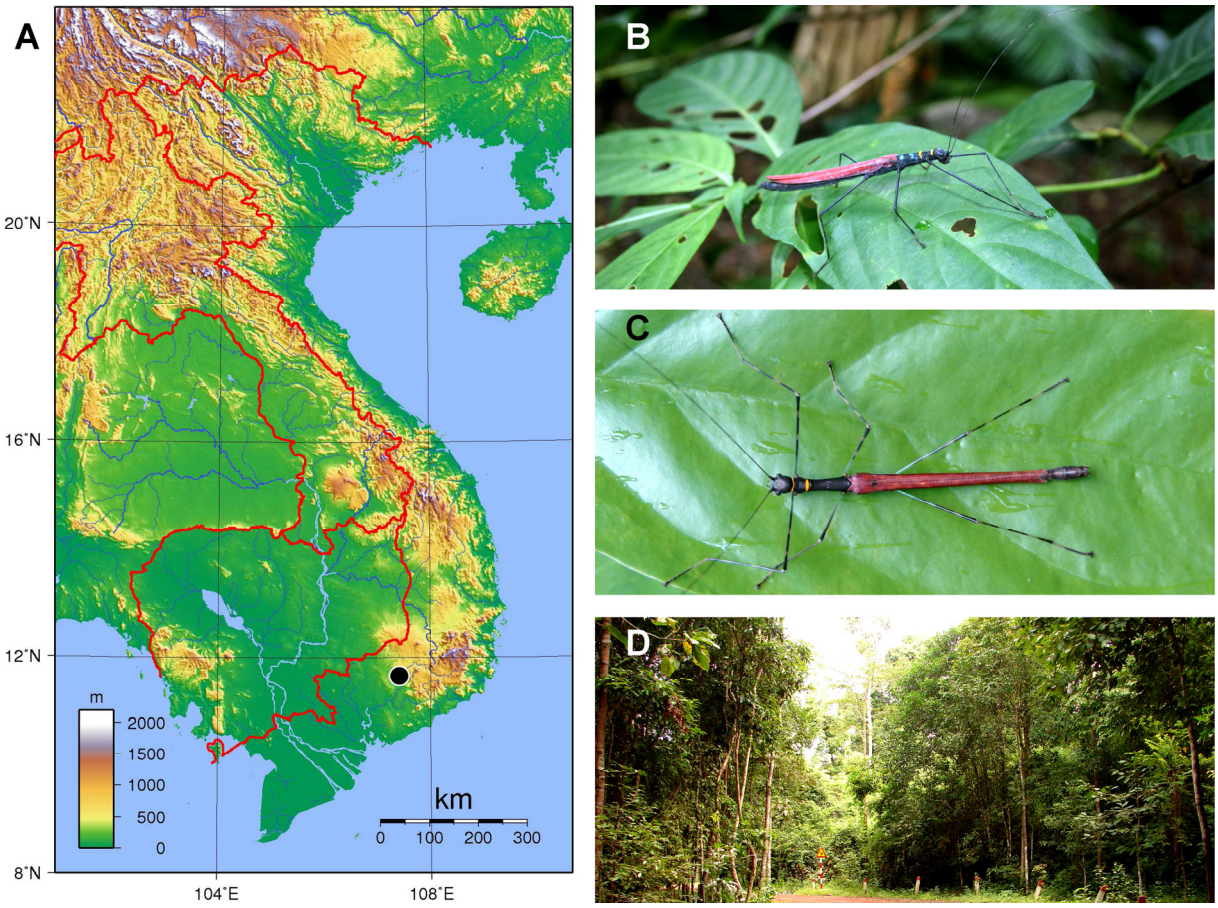

Fig. 3. *Calvisia* (*Calvisia*) *kneubuehleri* sp. nov. A, distribution map. B, female in nature, Dong Nai Biosphere Reserve 30.VI.2012. C, male in nature, Dong Nai Biosphere Reserve 4.VII.2012. E, habitat in Dong Nai Biosphere Reserve. Photographs by J. Constant.

margin of pronotum, but instead laterally joining S-shaped, longitudinal grooves. Longitudinal S-shaped grooves not reaching apex of pronotum. Two transverse grooves joined by longitudinal groove. Median longitudinal groove just reaching beyond centre of pronotum. Posterior margin convex and with a broad yellow band. Other portions mainly black, but with two faint bluish dots in the anterior part. Mesonotum black with two pairs of bright blue markings. Anterior pair of markings slightly elongated, deeply emarginated at posterolateral angle. Posterior pair of markings subcircular and smaller than anterior pair. Mesonotum slightly shorter than head and thorax combined, widening towards the posterior and slightly swollen in its posterior portion. Anterior margin slightly concave. Posterior margin with a small hump posteromedially.

Wings: (Fig. 2 A, C, H) tegmina red, more or less rectangular with interior edge slightly rounded. A definite hump anterolaterally. Alae well developed; costal area coloured as tegmina; veins black; anal area translucent whitish. Alae slightly projecting over apex of abdominal segment VII.

Legs: (Fig. 2 A, B, D, H) slightly setose, slender, with femora and tibiae completely black. Tarsomeres with some whitish markings. Profemora only very slightly laterally compressed basally. All carinae of femora and tibiae indefinite. Claws small but acute.

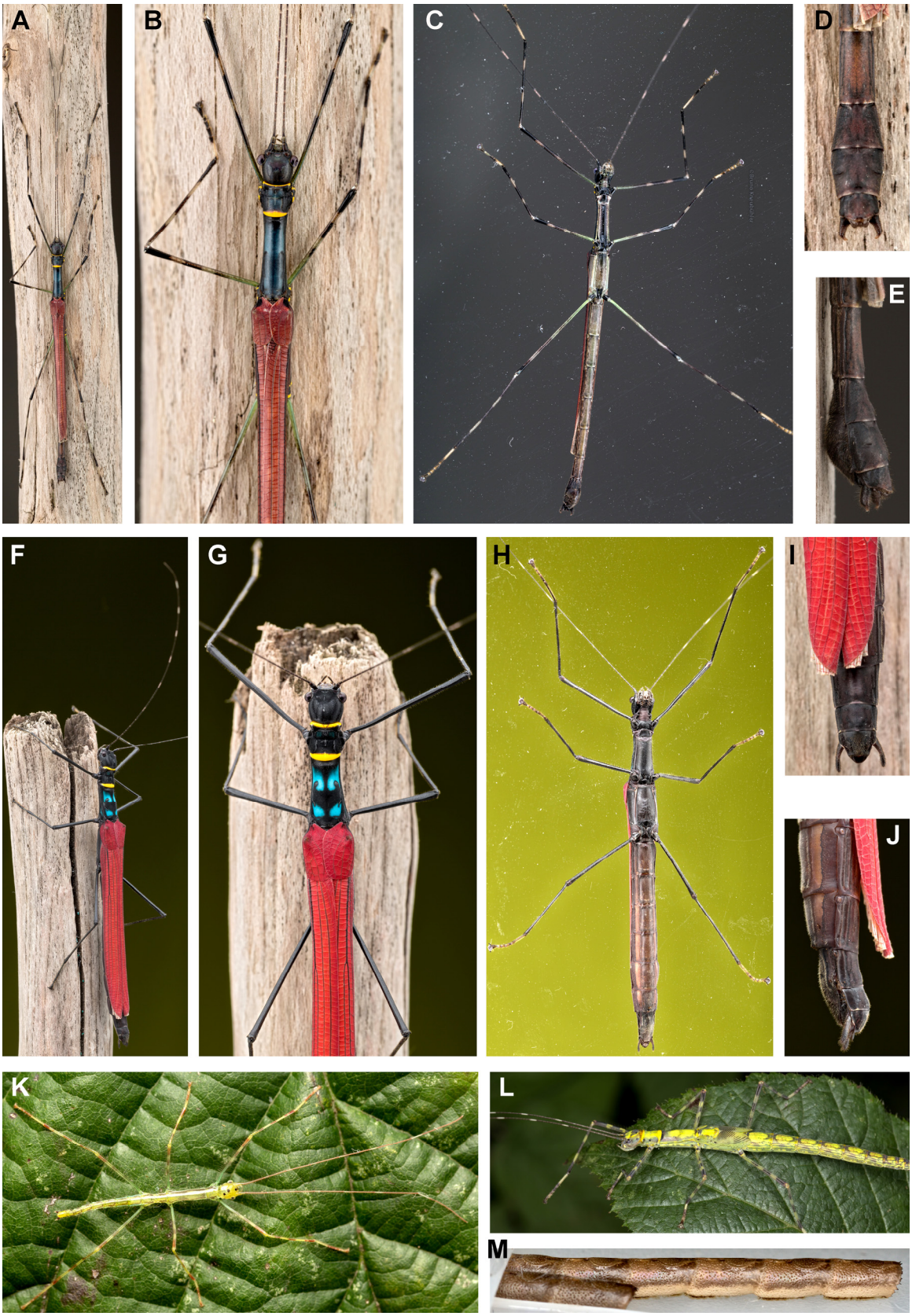

Fig. 4. *Calvisia* (*Calvisia*) *kneubuehleri* sp. nov. A-E, male. A, habitus, dorsal view. B, head and thorax, dorsal view. C, habitus, ventral view. D, apex of abdomen, dorsal view. E, apex of abdomen, lateral view. F-J, female. F, habitus, dorsal view. G, head and thorax, dorsal view. H, habitus, ventral view. I, apex of abdomen, dorsal view. J, apex of abdomen, lateral view. K, newly hatched nymph. L, subadult female nymph. M, eggs laid in a row. Photographs by B. Kneubühler.

Fig. 5. *Calvisia* (*Calvisia*) *kneubuehleri* sp. nov., colour change after final ecdysis. A, male. B, female. Photographs by B. Kneubühler.

Abdomen: (Fig. 2 A, C, E, F, G) black, sometimes dark brownish. Median segment well developed, rounded anteriorly, widening towards the posterior. Terga II-VI smooth, more or less uniform in length; tergum VII shorter. Segments II-VII cylindrical. Tergum VIII 2/3 the length of VII; IX slightly longer than half the length of VIII. Anal segment longer than tergum IX; basal half tectiform and rounded dorsally for about ¾ of its length; apical portion strongly flattened and rounded apically; lateral margins strongly emarginated after middle, creating a pseudoforamen. Subgenital plate sparsely setose, slightly compressed laterally and keeled; apex notched and not reaching apex of abdomen.

EGG (Figs 2 I, J, K, 4 M).

Measurements [mm]: length: 3.0; width: 1.5; height: 1.5; length of micropylar plate including median line: 1.2.

General colour light to dark brown, more or less paler laterally and slightly glossy. Capsule roundly rectangular in shape and roundly rectangular in cross-section; operculum on dorsal surface of capsule. Surface of capsule and operculum with small pits irregularly scattered. Anterior portion of capsule with a crown-like rim. Polar area flattened. Micropylar plate lanceolate, just posteriorly of operculum, not reaching polar area; micropylar cup placed centrally.

NYMPH (Fig. 4 K, L).

Newly hatched nymphs measure 15–17 mm. Body, legs and antennae slightly setose. Older nymphs yellowish with more contrasted dark markings until final ecdysis.

Head: greenish yellow with three black dots on vertex, genae with a black dot behind eye. Frons with black dot. Eyes semiglobular with a bluish hue. Antennae filiform and longer than body; scapus and pedicellus coloured as head. Antennomeres pinkish brown with pale markings; markings more definite towards the posterior.

Thorax and abdomen: pro-, meso-, metanotum and abdomen greenish yellow with darker markings laterally. Darker markings becoming more definite in older nymphs. Cerci short with base brown and apex white.

Legs: femora green basally, with remaining part banded brown and yellowish. Tibiae coloured as posterior portion of femora.

DISTRIBUTION

Vietnam: Dong Nai Province: Dong Nai Biosphere Reserve and Cat Tien National Park (Fig. 3 A).

BIOLOGY.

The specimens were collected during daytime, sitting on leaves of small trees some 2 metres above the ground, in tropical lowland rainforest (Fig. 3 D). Both sexes can fly away quickly.
Eggs are laid attached to each other by the anterior and polar ends, in chains of 2–8 in a continuous row, and glued to different surfaces by their ventral side (Fig. 4 M).
After the final ecdysis to adulthood, it takes up to 14 days for the males to develop their final, characteristic coloration (Fig. 5 A), while it takes only 4-5 days for females (Fig. 5 B) (B. Kneubühler pers. comm. 2016).

***Calvisia* (*Calvisia*) *torquata* (Bates, 1865)**

Fig. 6

Necroscia torquata BATES, 1865: 359 [described], pl. 45 fig. 3 [habitus illustrated] (type in OUMNH).

Calvisia torquata – KIRBY, 1904: 370 [transferred to *Calvisia*]. — REDTENBACHER, 1908: 566 [keyed]; 568 [described]. — OTTE & BROCK, 2005: 79 [catalogued].
Calvisia (*Calvisia*) *torquata* – SEOW-CHOEN, 2016: 49 [placed in subgenus *Calvisia* (*Calvisia*)].

TYPE MATERIAL EXAMINED. Holotype ♀ (examined from photographs – BROCK *et al*., 2016): Chantaboun, Mouhot (OUMNH).

NOTE: Chantaboun is the former name of Chanthaburi (Chanthaburi Province, 12°36′31″N, 102°6′14″ E), and is located in Thailand near the Cambodian border rather than in Cambodia as stated in BATES (1865).

ADDITIONAL MATERIAL. Thailand: 1♀: Siam: Me Song Forest, April 1919, E.J. Godfrey, 1920—244 (RBINS); 2♀♀: S.E. Siam: Chantaboon, Khao Sabap.?, III. 1932, J. Macbeth, B.M. 1933—241 (BMNH).

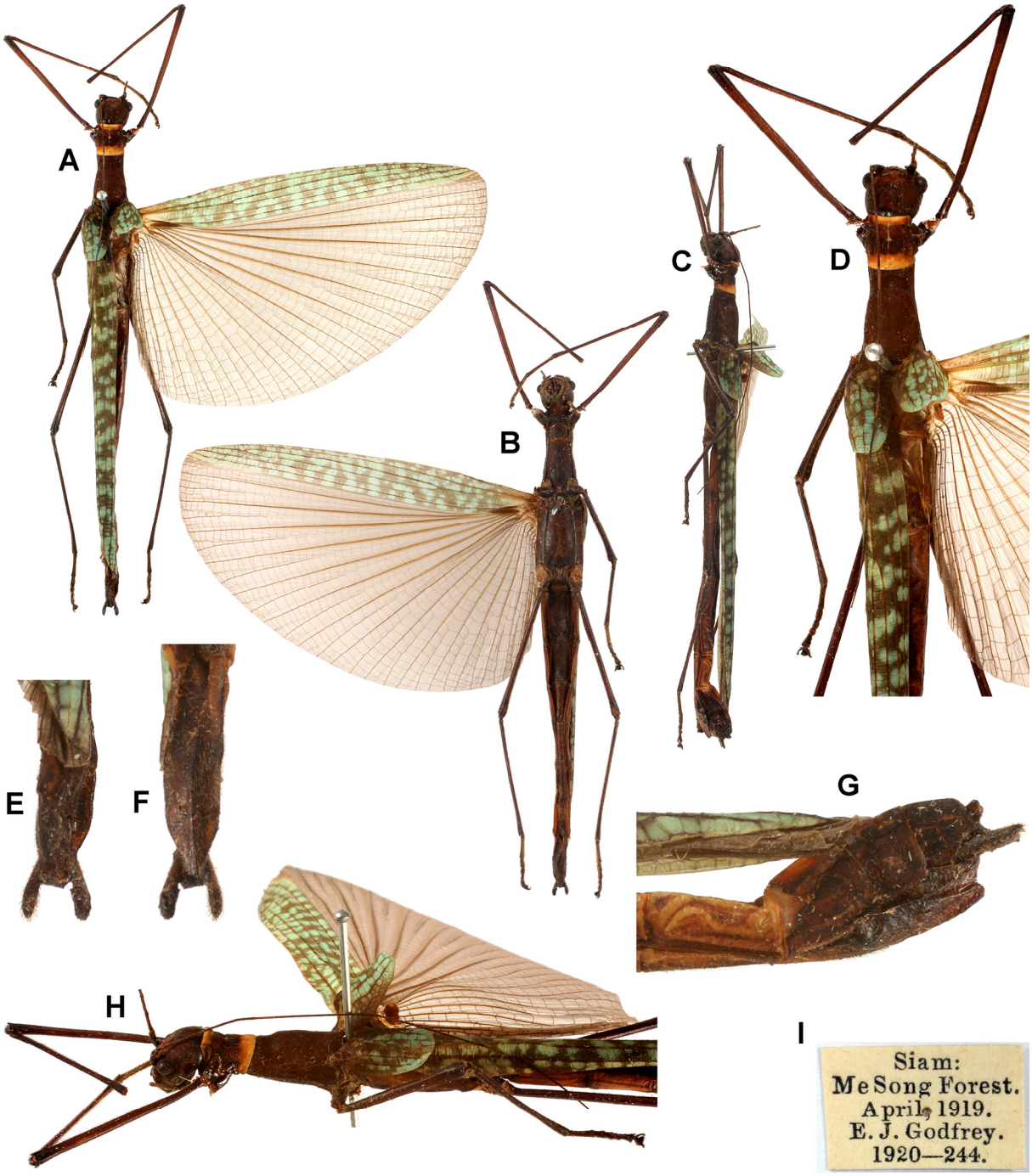

Fig. 6. *Calvisia* (*Calvisia*) *torquata* (Bates, 1865) ♀. A, habitus, dorsal view. B, habitus, ventral view. C, habitus, lateral view. D, head and thorax, dorsal view. E, apex of abdomen, dorsal view. F, apex of abdomen, ventral view. G, apex of abdomen, lateral view. H, head and thorax, lateral view. I, label.

NOTE: *C.* (*Calvisia*) *torquata* (Bates, 1865) was described from a single female. We provide here additional data and a detailed illustration of the species (Fig. 6) to allow comparison with *C.* (*Calvisia*) *kneubuehleri* sp. nov.

Notes on captive breeding

(based on B. Kneubühler, pers. comm., 2016)

A small series of eggs obtained from the female paratype collected in Dong Nai Biosphere Reserve were kept for captive breeding and sent to Dr Bruno Kneubühler. Incubation on

slightly damp sand at 20–23°C lasted about 8–10 weeks. Some nymphs however hatched a few to several months after the first nymphs hatched. To facilitate hatching and to a certain extent prevent mould, moss was spread over the eggs.
The following foodplants were accepted (for first instar nymphs, the edges of the leaves were cut away to facilitate feeding): (1) *Prunus laurocerasus* L. (Rosaceae): young leaves were well accepted by freshly hatched nymphs, while older leaves were well accepted by older nymphs and adults; (2) *Salix* sp. close to *S. triandra* L. (Salicaceae) was well accepted by older nymphs and adults; (3) *Ficus lyrata* Warb., *F. benjamina* L. and *F. elastica* Hornem. (Moraceae) were moderately well accepted by nymphs and adults, however they did not really thrive on the latter species, maybe because of the milky white latex produced by the plant; (4) *Fagus sylvatica* L. (Fagaceae) was moderately well accepted by nymphs and adults.
In winter when only old leaves of *P. laurocerasus* were available, newly hatched nymphs were provided with slices of apple every other night to keep them hydrated and prevent starvation. Slices were attached at the ceiling of the cage as the nymphs use to stay high in the cage. After some time the nymphs fed nicely on leaves and apple was no more necessary.
The duration of the nymphal development can vary according to the foodplant: on *P. laurocerasus*, males were adult after 3–4 months (at 20–23°C), females after 4–5 months, while males were adult after 2.5 months when fed on *Salix* sp.
Once they are adult, females start laying eggs after 3–4 weeks at a rate of about 10 eggs per female and week. Adults live for several months. Nymphs and adults were kept in well ventilated cages with a high level of humidity (70% relative humidity for adults, 80% for nymphs), maintained by a constantly wet paper towel covering the bottom of the cage rather than by spraying water. Cages for adults were at least 30x30x30 cm for 3–4 pairs, nymphs were kept in smaller cages, separated from adult to avoid disturbance and facilitate monitoring of their feeding and growth. At all stages, the species is mainly nocturnal, though feeding and mating was also observed during daytime. When mating, the male does not stay with the same female for a long time. If disturbed, the specimens react frantically, drop down, wriggle and freeze again after a few steps. The females laid their chains of eggs glued to different substrates near the ground; the eggs could be easily detached from their substrate after a short time when moistened with water.

Low Temperature-induced Dormancy (LTD) method for incubation of eggs
(based on B. Kneubühler, pers. comm., 2016)

This method allowing an extension of the incubation period was designed by Bruno Kneubühler during the 2012–2013 winter especially for the breeding of *Calvisia* (*Calvisia*) *kneubuehleri* sp. nov. which was known at the time to accept a single plant as food, a deciduous species of *Salix* L.
The idea of inducing a diapause by exposing the eggs to lower than normal temperatures arose in order to avoid the eggs hatching in winter when no food is available for the nymphs. Tentatively, freshly laid eggs (7 days old) were kept in a fridge at 8–10°C at very high relative humidity level (90+%). After 4 months they were taken out of the fridge and put in normal incubation conditions. The eggs then hatched after a normal 6–8 weeks period, proving the efficiency of the method. For *C.* (*Calvisia*) *kneubuehleri* sp. nov., the LTD method allowed to triple the normal incubation time. Tests on other species (Table 2) gave similar results, with the LTD duration extended up to 6 months and extending the normal incubation period up to 5.5 times. Hatching rate for all tested species is >50% at 8–10°C. Tests conducted at 4–5°C gave very poor results, with no or nearly no hatching.

Table 2. Results of the LTD tests on different species. *LTD temperature* = temperature at which eggs were preserved in the fridge; *LTD duration* = duration of the stay in the fridge; *prolongation* = how much the natural incubation time was prolonged by the LTD method.

Species	LTD Temperature	LTD Duration	Prolongation	Hatching rate
Acanthoxyla gesovii (Kaup, 1866) (from Scilly Islands, U.K.)	8–10 °C	2 months	0.5 x	> 50%
Asceles sp. (from Cat Tien, Vietnam)	8–10 °C	2.5 months	2 x	> 50%
Asceles sp. (from Cat Tien, Vietnam)	8–10 °C	6 months	4 x	> 50%
Aschiphasma annulipes Westwood, 1838 (from Tapah Hills, Malaysia)	8–10 °C	3 months	2.5 x	> 50%
Aschiphasma annulipes Westwood, 1838 (from Tapah Hills, Malaysia)	4–5 °C	3 months	2.5 x	~ 0%
Calvisia (*Calvisia*) *kneubuehleri* sp. nov.	8–10 °C	4 months	3 x	> 50%
Calvisia (*Calvisia*) *kneubuehleri* sp. nov.	4–5 °C	2 months	1.5 x	~ 0%
Calvisia (*Conocalvisia*) *leopoldi* Werner, 1934 (from Pakung Jae, Bali Island)	4–5 °C	2 months	1.5 x	~ 0%
Extatosoma tiaratum (Macleay, 1826) (from Innisfail, Australia)	8–10 °C	4 months	1 x	> 50%
Eurycnema versirubra (Serville, 1838) (from Timor Island)	8–10 °C	4 months	0.75 x	> 50%
Lobofemora scheirei Bresseel & Constant, 2015 (from Cat Tien, Vietnam)	8–10 °C	2.5 months	3 x	> 50%
Lobofemora scheirei Bresseel & Constant, 2015 (from Cat Tien, Vietnam)	8–10 °C	6 months	5.5 x	> 50%

NOTE. Eggs of *E. versirubra* from an old parthenogenetic stock from Malaysia even hatched after being kept for almost one week in a freezer at around -10°C. The hatching rate was however very low.

Instructions for LTD method (Fig. 7)

- Use freshly laid eggs, these yield a higher hatching ratio than older eggs.
- Put the eggs on a dry substrate (e.g. cotton wool) in a small, low inner container to prevent mould (Fig. 7 B).
- Put that small container in a bigger clear plastic cup with wet paper towel (or something similar) on the bottom (Fig. 7 A–B).
- Keep the paper towel at the bottom of the outer cup moist at all time; check regularly (Fig. 7 A).
- Close the bigger container with a tightly closing lid, to keep the relative humidity level very high (Fig. 7 A-B).
- Make some (pin) holes in the lid of the outer, bigger container to avoid condensation which would facilitate mould growth (Fig. 7 A–B).
- A variation of this LTD unit is illustrated (Fig. 7 C–D).
- Put it in the fridge at around 10°C (the outer compartments in the fridge door are good places).
- Make sure that the temperatures stay within a range of 9–11°C, monitor regularly with a digital thermometer.
- Incubate the eggs normally after the LTD-period.

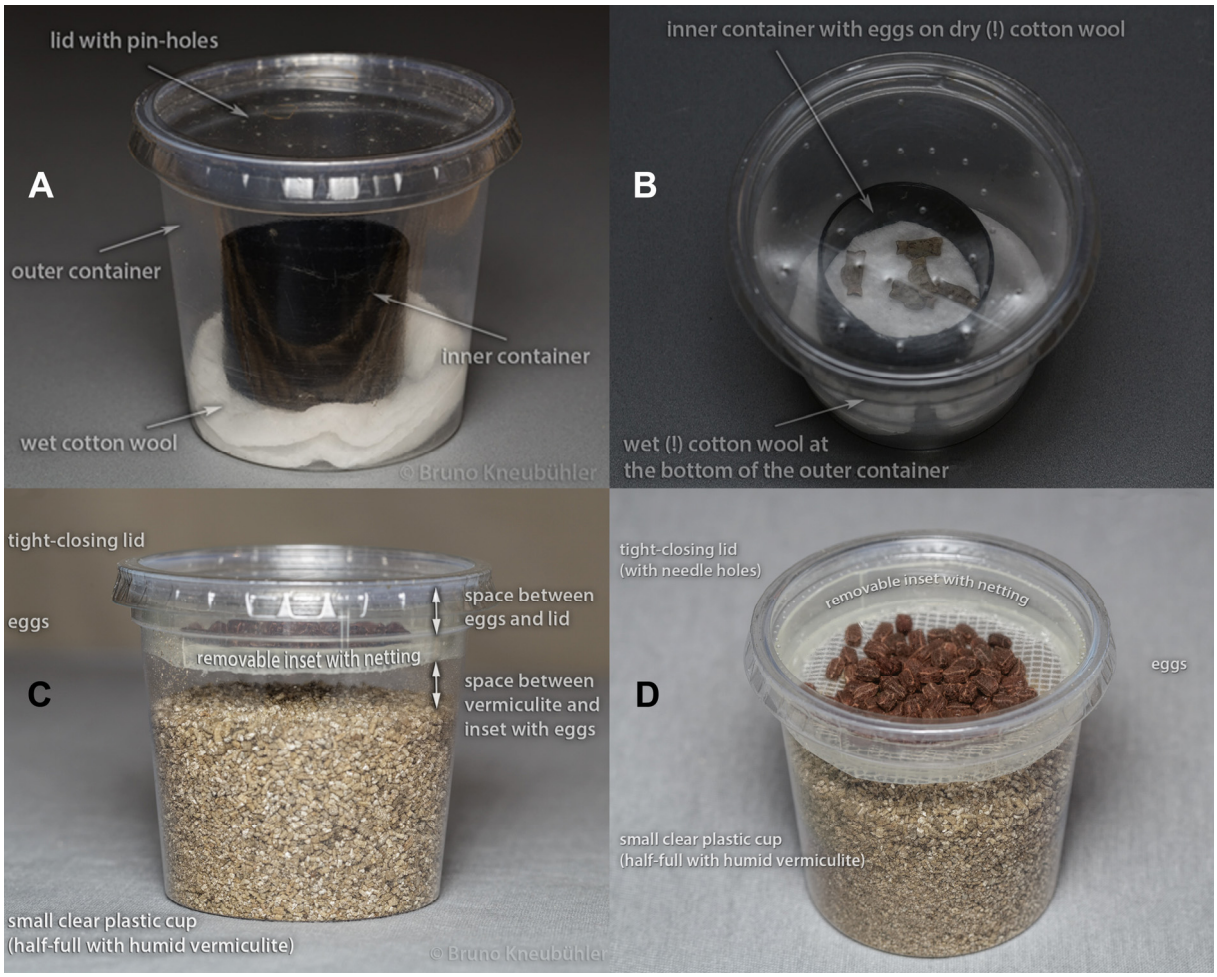

Fig. 7. Low Temperature-Induced Dormancy Method. A-B, incubation container with cotton wool. C-D, incubation container with humid vermiculite. Photographs by B. Kneubühler.

GLUED EGGS TECHNIQUE (GET) FOR INCUBATION OF EGGS
(based on B. Kneubühler, pers. comm., 2016)

Several phasmid species, including those of the genus *Calvisia*, glue their eggs to different parts of their host-plants. In captivity, rooted plants are seldom used, and foodplants have to be changed regularly. Therefore eggs sometimes have to be removed for incubation purpose. Nymphs from such dislodged eggs easily get stuck in their egg shell upon hatching, which is usually fatal. As a solution, a simple "Glued Eggs Technique" was developed by Olivier Salord and improved by Bruno Kneubühler. This technique tends to mimic the natural incubation conditions of the eggs. The original method used double-sided adhesive tape, while another uses xanthan gum as glue.

Instructions for GET method (Fig. 8)

- Spread the xanthan gum paste or adhesive tape onto a pre-cut piece of plastic (Fig. 8 A–B).
- Place the dislodged eggs carefully on the adhesive material on their ventral side (Fig. 8 C–D).
- All eggs must be oriented so that the polar areas will be directed upwards when the piece of plastic will be placed vertically in the incubation container (Fig. 8 D).

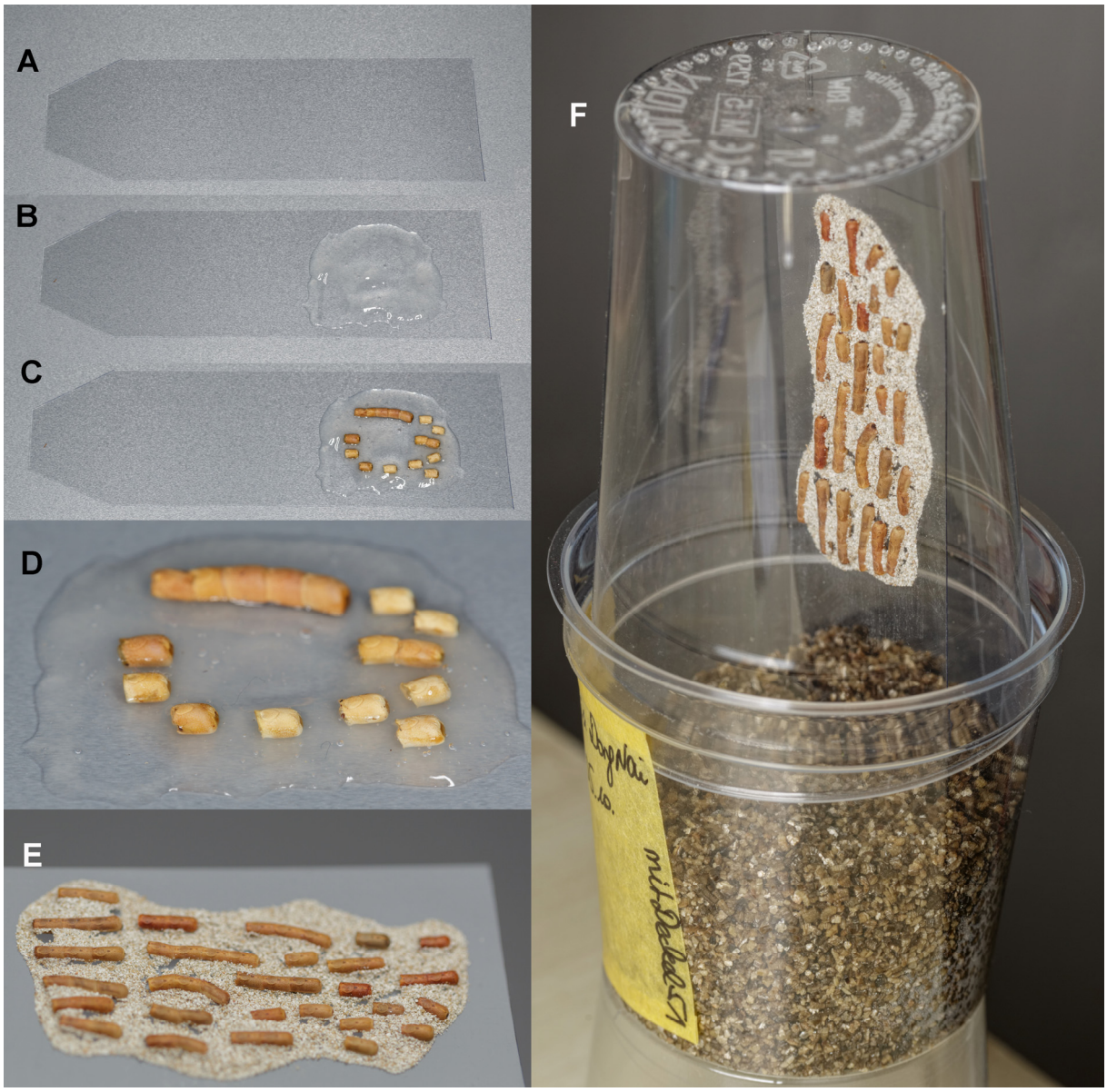

Fig. 8. Glued Eggs Technique. A, piece of plastic. B, xanthan gum on plastic piece. C, eggs glued on xanthan gum. D, idem, detail. E, xanthan gum covered with fine sand. F, incubation container. Photographs by B. Kneubühler.

- Spread very fine sand over the eggs and adhesive material so that the sand completely covers the adhesive surface. Very gently shake the sand in excess, if necessary, use a fine brush (Fig. 8 E).
- Place the re-glued eggs in an incubation container (Fig. 8 F).
- Provide a constant high humidity in the incubation container (moist vermiculite, paper, peat etc.) (Fig. 8 F).
- Avoid condensation and do not spray reglued eggs otherwise they might break loose; if necessary, add some ventilation.

Discussion

The genus *Calvisia* Bates, 1865 was recently treated by SEOW-CHOEN (2016) who introduced six subgenera based on a study of the species from Borneo and Malaysia. However, the genus is widespread in Asia, from India over China (Tibet) and Vietnam, and southwards to Bali

and Sulawesi (BROCK *et al*., 2016). It is obvious that more species await discovery and description, especially in continental Southeast Asia (Bresseel & Constant, unpublished data), hence the subgenera proposed by SEOW-CHOEN (2016) will need to be validated or refined. Molecular studies could also provide very interesting data in this scope.

The present study also shows the benefits of collaboration with enthusiast citizen-scientists. In this case, the difficulty to breed the species combined with its spectacular coloration resulted in the design of new breeding methods. Those innovations will help to successfully breed similarly difficult species in the future.

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