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Discovery of *Eubasilissa signata* Wiggins, 1998 (Trichoptera, Phryganeidae) from Taiwan

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Abstract

The phryganeid caddisfly *Eubasilissa signata* Wiggins, 1998 was originally described based on a single female specimen from Korea, and no other information on the species was available. Here we record an additional five male and five female specimens of *E. signata* from Taiwan and describe the male morphology for the first time. Based on morphological differences with other *Eubasilissa* species, *E. signata* should be regarded as a separate species and also not a member of the "*tibetana*" species group. A 658-bp fragment of the mitochondrial cytochrome c oxidase subunit I (COI) gene of the new specimens of *E. signata* is provided as a DNA barcode.

Key words: redescription, endophallus, COI, type specimen, species group

Introduction

The phryganeid caddisfly *Eubasilissa signata* Wiggins, 1998 was originally described based on a single female specimen deposited in the Humboldt State University Natural History Museum in Berlin. According to the original description, the word "Korea" was the only information on the label of the type specimen, and the exact locality, date, and collector remain unknown. A recent survey in Korea by the senior author and a Korean colleague failed to collect any additional specimen of this species. The senior author found a female specimen of *E. signata* collected from Taiwan at the Kitakyushyu Museum of Natural History and Human History. Moreover, the second author collected male and female specimens of *Eubasilissa* species from the central mountainous area in Taiwan, which was confirmed as *E. signata* by the senior author.

In this paper, we describe the morphology of the male *E. signata* for the first time, provide the COI gene fragment as a "barcode" marker for the species, discuss the phylogenetic relationships between *E. signata* and congeners, and corroborate Wiggins' (1998) prediction that the male *E. signata* has uniquely shaped inferior appendages.

Material and methods

Before removing the abdomen, pinned specimens were placed in a humidifier for overnight to rehydrate. Abdomens were cut off by scissors at the base, put in 10 ml glass vial with 5% KOH solution, held overnight at room temperature, then heated at 70 ℃ in the same solution for 4-6 hours. Abdomens were then washed with distilled water and placed in lactic acid and heated at 70 ℃ for 2 hours. Finally, the abdomen was washed with distilled water in a watch glass, flushed with water from a small syringe to remove macerated tissue and to inflate the endophallus of male. The cleared abdomen was transferred to 80% ethyl alcohol. K-Y Jelly (Johnson & Johnson) was used to hold the genitalia in a stable position for photography.

Photographs were taken with an Olympus OM-D E-M1 and DP20 digital microscope camera mounted on

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an Olympus SZX16 stereoscopic microscope. Partially focused serial images were combined with Helicon Focus software (Helicon Soft Ltd.) to produce completely focused photographs.

Total genomic DNA was extracted from ethanol-preserved tissue specimens and purified using the DNeasy Blood and Tissue Kit (QIAGEN, Hilden). The mitochondrial COI gene was amplified by a polymerase chain reaction (PCR) method using the primer HCO2198 (5'- TAA ACT TCA GGG TGA CCA AAA AAT CA-3') and LCO1490 (5'-GGT CAA CAA ATC ATA AAG ATA TTG G-3') (Folmer *et al.* 1994). PCR products were purified using the ExoSAP-IT (GE Healthcare UK, Buckinghamshire). The purified DNA was sequenced directly by an automated method using the DYEnamic ET Terminator Cycle Sequencing Kit (GE Healthcare UK, Buckinghamshire) and BigDye Terminator v1.1 Cycle Sequencing Kit (Applied Biosystems, CA, USA) on an automated sequencer ABI 3130*xl* DNA Analyzer (Perkin Elmer/Applied Biosystems, CA, USA). The sequence chromatographs were assembled using the CLC Workbench software (CLC bio, Aarhus). The COI sequence data of mitochondrial DNA have been submitted to the DNA data bank of JAPAN (DDBJ database).

Specimens examined in this study are deposited in the Natural History Museum and Institute, Chiba (CBM), Kitakyushyu Museum of Natural History and Human History (KMNH), Leibniz-Institut für Evolutions- und Biodiversitätsforschung an der Humboldt-Universität zu Berlin Museum für Naturkunde (ZMHB), National Museum of Nature and Science, Tsukuba (NSMT).

Results

Eubasilissa signata Wiggins, 1998

Figure 1

Eubasilissa signata Wiggins, 1998: 237-238, female, (Korea).

Description

Adult (Figs.1A-D): Length of forewing, male 28.0-32.8 mm (n = 5), female 30.5-35.0 (n = 6). Dorsum of head dark brown; vertex around compound eye and warts light brown; antennae dark brown, approximately half length of forewing. Dorsum of thorax and all of abdomen almost dark brown, thorax below attachment of wings light brown, mesopleuron and coxae light brown, tibiae and tarsi dark brown. Forewing with dark brown reticulations on yellow background over basal 2/3rds, brown marking of anterior area in female but lighter color in male; whitish-yellow patches of fine small setae prominent on cell M2 and thyridial cell, crossvein sc-r absent. Hind wing brown, a yellow band extending from costa to Cua.

Male genitalia (Figs.1F, G): Segment IX narrow dorsally; tergal area with black tuft of long setae along posterior edge; sternal area with raw of setae on posterior one-forth laterally, more widely covered with strong setae on ventral area; posteroventral edge simple, not extended as median lobe. Segment X almost trapezoidal in dorsal view; posterolateral processes well develop and weakly convergent; median incision weak; posteromesal process small; transversely oblong concave below preanal appendage, without pigment.

Preanal appendages long and clavate, with thin setae coarsely distributed on its surface. Inferior appendages evenly tapered basal two-third, remaining part almost paralell sided and rounded at tip; basal and terminal segment almost fused, mesal surface with indistinct line of fusion between segments marked by membranous area; basal segment with broad base, covered with dense setae, terminal segment bare laterally with two or three strong setae on dorsal edge, with small dense setae on its inside wall. Area prior to phallotheca a smooth sclerotized ring, as long as phallotheca; phallotheca smooth, ventromesal process with long, stout point at apex, endophallus with pair of large pointed sclerites, each sclerite with apical part broad, upturned in lateral view, groove on outer surface, with very small setae on inside of wall.

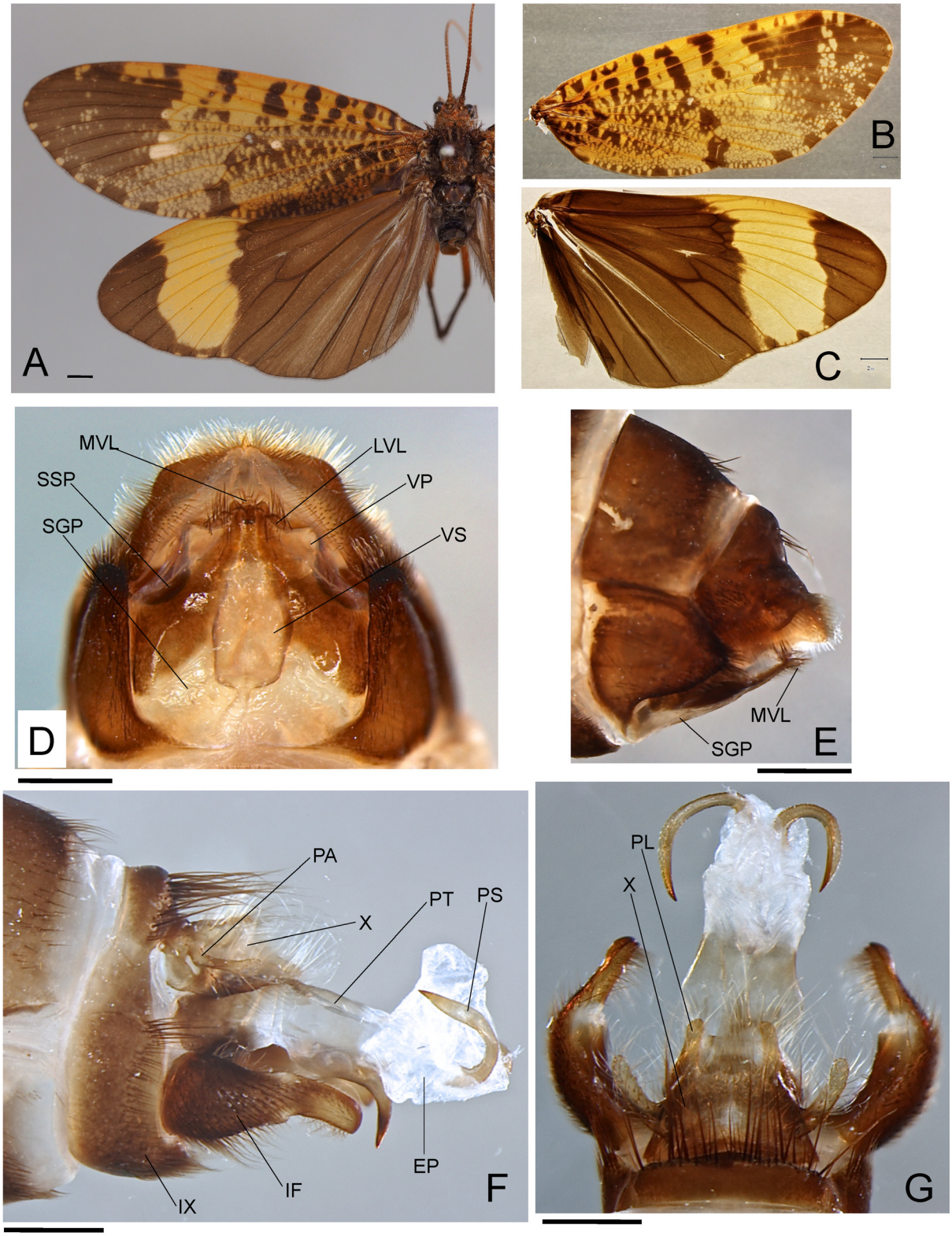

FIGURE1. Adult. A: Female left. B: Male forewing. C: Male hind wing. D: Female genitalia, ventral. E: Female genitalia, lateral. F: male genitalia, lateral. G: male genitalia, dorsal. Scale bar. A, B, C: 2 mm; D, E, F, G: 1mm. LVL: lateral valval lobe, MVL: median valval lobe, SGP: subgenital plate, SSP: sclerotized spherical pocket, VP: vaginal pouch, VS: vaginal sclerite, EP: endophallus, IF: inferior appendage, IX: segment IX, PA: preanal appendage, PL: posterolateral process, PS: pointed sclerite, PT: phallotheca, X: segment X.

Female genitalia (Figs.1D, E): Subgenital plate with large sclerotized rhombic shaped area on both sides, tapered posteriorly and terminating in median valval and lateral valval lobes; median valval lobe short, broadly pointed, without pigment and with medium setae over ventral surface; lateral valval lobes not conspicuous, with sclerotized low ridge with pigmented edges and bearing 2-3 setal tufts on its posterior margin; pair of heavily sclerotized spherical pockets, deeply concaved internally just below posterior corner of Xth tergum, posterior edge of each pocket continuous with scletotized lip at entrance to genital passage, terminating in obtuse triangular lobe. Vaginal pouch short, double-walled, highly membraneous, tranversely oblong in ventral view but ventral surface with weakly sclerotized area and with its anterior margin truncate and bearing pair of small lobes. Vaginal sclerite with its body almost exposed under vaginal pouch, tongue shaped and narrowing to posterior half, continuous to genital passage, with sesame-seed shaped terminal duct in its anterior position.

DNA barcode. A 658-bp and 640-bp fragment of the *COI* gene collected from a specimen CBM ZI-155556 and CBM ZI-155555, respectively. There is only 3-bp sequence difference in the 640-bp region of their *COI*, it gives authenticity to identify same species. The sequence data has been deposited as a DNA barcode of *E. signata* in the DDBJ Nucleotide Sequence Database under accession number LC107779, LC 107780.

Specimens examined. Holotype: female of *Eubasilissa signata* Wiggins, 1998; type locality: **Korea**, (ZMHB). **Taiwan**. **Chiayi County**: 1 female, Mt. Alishan, 9.iv.1965, S. Ueno, (NSMT); 1 female, ibid., 9.vii.1981. Light trap, K. Ueda, (KMNH). **Hualien County**: 1 male (CBM-ZI 158857), 1 female (CBM-ZI 158858), Guanhsin bridge, Guanyan, Xiulin Township, Alt.2350 m, 14.vii.2012, U. Jinbo, (CBM). **Nantou County**: 1 male, 1 female, Meifeng, Alt.2150 m, 27- 30.iv.1999, Mey and Ebert, (ZMHB). **Taichung County**: 1 male (CBM-ZI 158855), 1 female (CBM-ZI 158856), Hoping Co. Sheishan, Alt.2950 m, 4.vii.2011,L.Hsu leg, (CBM). 2 males, Mt. Nanhutashan, Yunlieng-shan-chang, Alt.2500 m, 4-5.viii.1990, M. Owada, (NSMT).

Discussion

The lack of collection data for the holotype of *E. signata* made it difficult to rediscover new specimens. The present examination established that all the voucher specimens of *Eubasilissa* species from Taiwan were ascribed to *E. signata*, and it is highly probable that former records of Taiwanese *E. regina* (McLachlan,1871) correspond to *E. signata*. Moreover, the habitus of *E. signata* and *E. regina* are similar. In addition, these two species are easily misidentified, as in both male specimens examined the inferior appendages and Xth segment strongly curved inward to hide its characteristics, particularly in dry specimens. In female specimens, it is impossible to distinguish between the two species without preparation of the genitalia in hot KOH. The large body size and very similar wing markings between *E. signata* and *E. regina* may also lead to misidentification. Wiggins (1998) mentioned that Martynov (1930) also misidentified *E. signata* as variety of *E. regina*. Repeated studies by Korean and Japanese entomologists have failed to collect *E. signata* in Korea, and it is therefore highly probable that *E. signata* is not distributed in Korea. Furthermore, it is probable that the locality data placed on the type specimen was incorrect.

Wiggins (1998) noted that the female genital segment of *E. signata* had unique characteristics, such as invaginated pockets in the subvaginal plate. The present examination revealed that male *E. signata* also have uniquely shaped inferior appendages compared with other *Eubasilissa* species, which correspond with the female invaginated pockets.

Wiggins (1998) classified *E. signata* in the *tibetana* species group, which consists of *E. tibetana* Martynov, 1930; *E. alaknanda* Schmid, 1962; and *E. rahtkirani* Schmid, 1965 a along with *E. signata*. However, *E. signata* differs from other members of the *tibetana* species group by the presence of a simple ventral edge on the male inferior appendages (a ventral lobe is present in all another *tibetana* species) and segment X with conspicuous preanal appendages and posterolateral processes (without or indiscernible in all another *tibetana* group species). Female genitalia of *E. signata* have a sclerotized median lobe (semi membranous in all other *tibetana* species), a transverse oblong shaped vaginal pouch (with bilobate shaped vaginal pouch in all other tibetana group species), and a pair of heavily sclerotized spherical pockets deeply concaved internally just below the posterior corner of the Xth tergum (with simple subgenital plate without sclerotized spherical pockets in all other *tibetana* group species). Therefore, it is reasonable to regard *E. signata* as a separate species and to remove it from the *tibetana* species group.

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