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A new species of *Sacada* Walker, 1862 from Thailand (Lepidoptera, Pyralidae, Pyralinae)

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http://zoobank.org/4EEF9283-29EF-47AE-A1A5-ED43ECF87299

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Abstract

A new species of *Sacada* from northern Thailand is described: *S. chaehomensis* **sp. nov.** Pellinen & Zahiri (Lepidoptera: Pyralidae, Pyralinae). Morphological characters and DNA barcode data are provided for the new species, with a morphological comparison to *S. dzonguensis* and *S. umtasorensis*, and a DNA-barcode comparison to *S. ragonotalis* and *S. albioculalis*, respectively. After this addition, the current number of valid species in the genus *Sacada* is 43.

Key Words

Species description, morphology, DNA barcoding, snout moth, integrative taxonomy

Introduction

The genus *Sacada* Walker, 1862 (Pyralidae, Pyralinae) has recently been the target of intensive systematic studies, and as a result its taxonomy has been considerably improved (e.g. Yamanaka 1995; Yamanaka 1998; Bae et al. 2008; Leraut 2013; Sutton et al. 2015). The latest work on the genus was performed by Singh et al. (2020), who described two new species of *Sacada* from India, described the new genus *Pseudosacada* Singh, Kirti & Ranjan with one species, provided a world checklist, and more importantly for subsequent research they provided good illustrations of adult moths and other morphological features. Currently 42 species are included in *Sacada* and the species are known from the Indo-Malay region, Australasia, the Afrotropics, and a few species from the Palearctic region (Singh et al. 2020; Nuss et al. 2003–2020). Biology, life histories and females of many species are still unknown. The Australasian *Sacada albioculalis* Hampson, 1917 has been reared from *Pometia pinnata* (Sapindales: Sapindaceae), *Intsia bijuga* (Fabales: Fabaceae) and probably *Trema* (Rosales: Cannabaceae) in lowland Papua New Guinea, potentially being polyphagous (Scott Miller, pers. comm.). A distinct diagnostic feature of *Sacada* is the male's resting posture with forelegs stretched out in front, midlegs stretched out below and tip of abdomen pointing upwards. When the legs are stretched out, their massive hair tufts become visible (Fig. 1).

Only two *Sacada* species have been recorded from Thailand: *S. decora* Walker, 1862 and *S. pyraliformis* (Moore, 1879) (Singh et al. 2020, but online websites such as https://www.thaibugs.com provides photos of additional, potentially undescribed species).

A single *Sacada* specimen was collected from Lampang province, Northern Thailand in 2020 using mixed and UV-light. Comparisons with the literature, particularly Singh et al. (2020) revealed that it represents an undescribed species. Hence, we provide the description of the new species, *Sacada chaehomensis* sp. nov., based on morphology and DNA barcode sequence data, and give diagnostic characters distinguishing it from the most similar taxa.

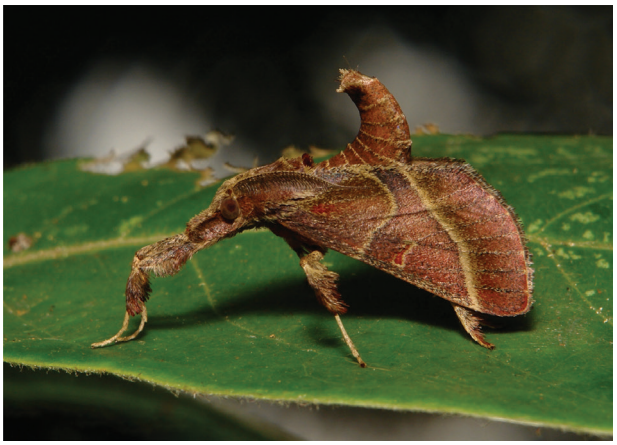


Figure 1. *Sacada* sp., showing the typical resting posture of male, and large hair tufts on fore- and midlegs. China, Yunnan, Pu'er, 25.6.2015, photo by John Horstman/ itchydogimages, used with permission.

Materials and methods

The abdomen and reproductive organs of the specimen (23.05.2020 / Thailand, Lampang/ Chae Hom 340 M / 18°43'19"N, 99°33'11"E) were prepared following standard methods (e.g., Hardwick 1950). The vesica was everted via caecum, which was cut open by placing the aedeagus inside a hypodermic syringe (Sihvonen 2001). Structures were photographed with a Leica DM1000 microscope (Wetzlar, Germany) and an integrated Leica DF295 digital camera. Several structures were photographed in two to six images of different depth of focus and combined into single images using image-stacking as available in Adobe Photoshop 19. The final plates were compiled in CorelDraw (v. 22). Morphological terms follow Klots (1970).

Total genomic DNA was extracted from one leg, using the DNeasy tissue extraction kit (Qiagen, Hilden, Germany) following the manufacturer's instructions. We sequenced the 5' region of the *cytochrome c oxidase subunit I* (COI5P) from the mitochondrial genome, known as DNA barcode (Hebert et al. 2003). PCR amplification and cleanup of the barcode region was performed at the Centrum für Naturkunde (CeNak, Hamburg, Germany) following the protocols of Wahlberg and Wheat (2008). The cleaned PCR products were sent to Macrogen Europe for Sanger Sequencing. The resulting chromatograms were checked and DNA sequences aligned by eye using BIOEDIT v. 7.0.4.1 (Hall 1999). Because CO1 is a protein-coding gene, pseudogenes can be detected through translation of the nucleotide sequence to ensure the absence of stop codons or frameshift mutations (performed in MESQUITE v. 3.61 (Maddison and Maddison 2019). The DNA barcodes of outgroups were taken from the BOLD, BARCODE OF LIFE DATA SYSTEM v4 (Ratnasingham and Hebert 2007). A gene tree was reconstructed using the Neighbor-Joining (NJ) algorithm and Bayesian Inference calculated with the analytical tools of BOLD and MrBayes v.3.2.7 (Ronquist et al. 2012), respectively. The Bayesian analysis was run for 8 million generations, with every 1000th tree sampled. The dataset was divided into three sets by codon positions. The GTR+G model was chosen as the most appropriate model for COI sequence evolution with IQ-TREE (Trifinopoulos et al. 2016). There were only 18 public *Sacada* DNA sequences available on BOLD and GenBank to be applied to our analysis. The molecular data for the voucher specimen is available on BOLD (sampleID ZMH-DNA0199) and via GenBank accession number SUB8441214 (www.ncbi.nlm.nih.gov/Genbank).

Results and discussion

Sacada chaehomensis Pellinen & Zahiri, sp. nov.

http://zoobank.org/D4C3AC48-60D0-4E02-9C37-FF09BA7099EA

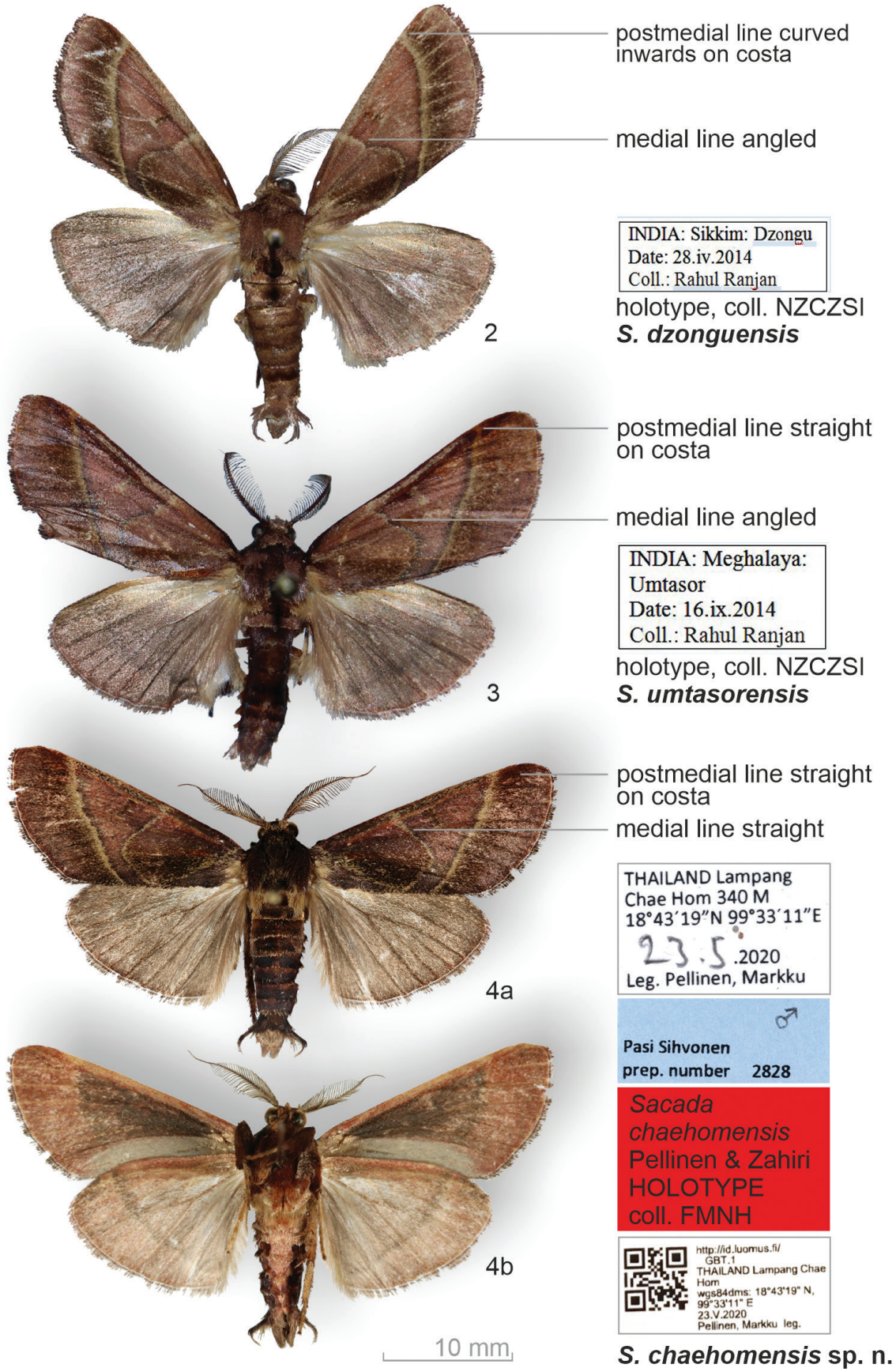
Figs 4, 7, 8

Type material. ***Holotype*** ♂ (label data given verbatim, "/" indicates new line, ";" indicates new label]: Thailand Lampang/ Chae Hom 340 M/ 18°43'19"N, 99°33'11"E/ 23.5.2020/ Leg. Pellinen, Markku [white label]; MJPT0020 [green label, indicates M. J. Pellinen DNA tissue sample]; ZMH-DNA0199 [indicates Universität Hamburg DNA process number]; 0146 [pink label, indicates M. J. Pellinen photograph number]; Pasi Sihvonen,/prep. number 2828 [blue label]; http://id.luomus.fi/GBT.1; *Sacada/ chaehomensis*/ Pellinen & Zahiri/ HOLOTYPE [red label] (in coll. Finnish Museum of Natural History, Helsinki, Finland).

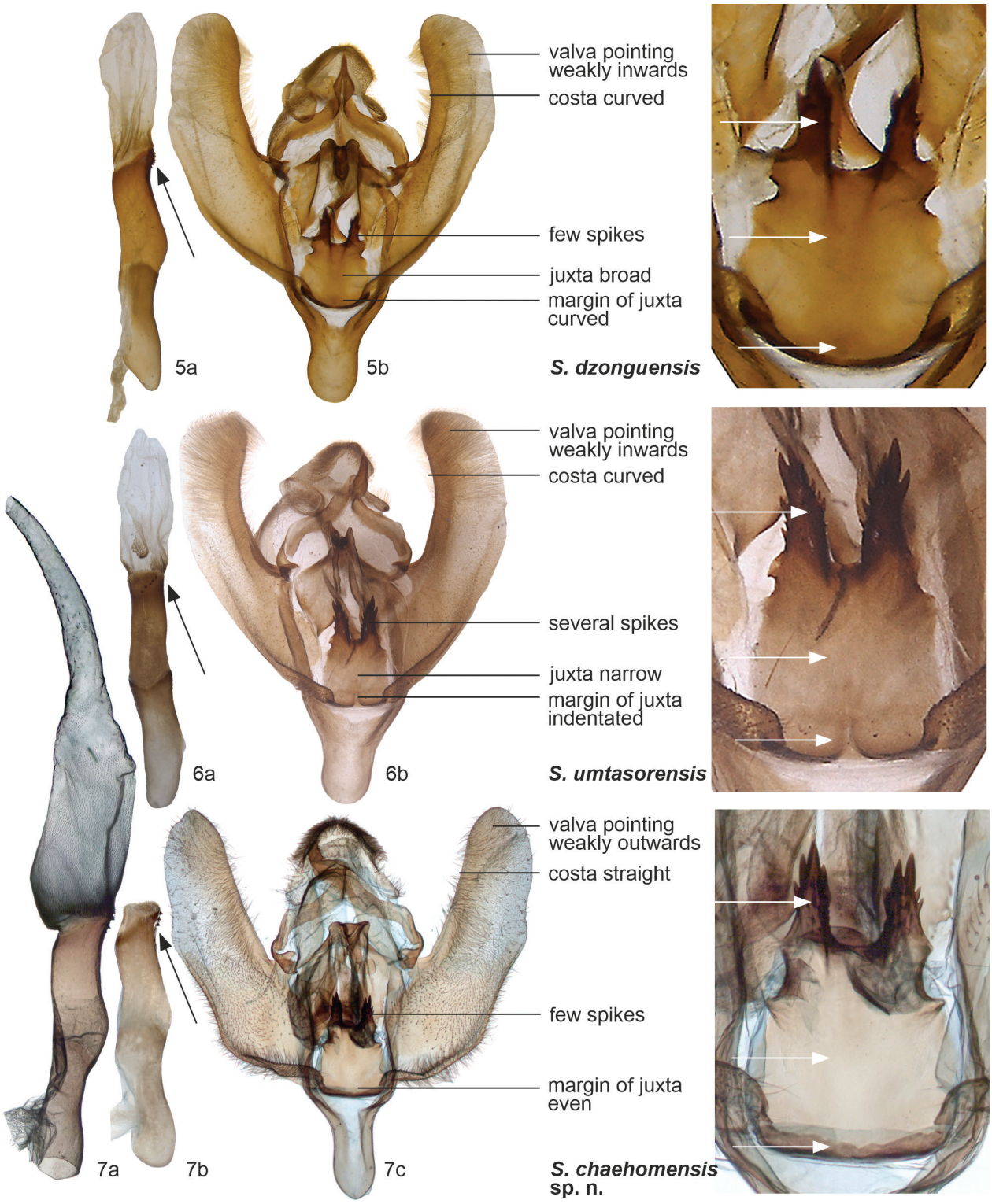
Other material examined. Only holotype male is known.

Diagnosis. *Sacada chaehomensis* sp. nov. is morphologically similar to *S. dzonguensis* Singh, Kirti & Ranjan, 2020 and *S. umtasorensis* Singh, Kirti & Ranjan, 2020. Diagnostic male characters of these three species are indicated on Figs 2–7 (females of these three species are unknown). Forewing postmedial line is straight on costa and medial line is straight in *S. chaehomensis* sp. nov. (postmedial line curved and medial line angled in *S. dzonguensis*; postmedial line straight and medial line angled in *S. umtasorensis*). With regard to diagnostic male genitalia characters, see Figs 5–7.

Description. Description is based on male, female is unknown. Wingspan 40 mm. Antennae with basal two thirds bipectinate, apical third fasciculate. Head, thorax and abdomen rufous. Tegula with iridescent scales of purplish-grey hue. Labial palps short. Base of proboscis covered densely with scales. Fore- and midleg tibia with massive hair tuft, spur formula of legs 0–2–4. Forewing narrow, reddish brown, medial, postmedial and terminal lines dry straw colored (fuscous). Medial line angled, postmedial line straight, forming triangular area on midwing. Terminal line narrow, fringes concolorous with wings. Hind wing wide, grey, weakly rufous near margin, veins darker. Terminal



Figures 2–4. Adults of *Sacada* spp. with diagnostic characters indicated **2** *S. dzonguensis* Singh, Kirti & Ranjan (male) **3** *S. umtasorensis* Singh, Kirti & Ranjan (male) **4a** *S. chaehomensis* sp. nov. (male, dorsal view) **4b** *S. chaehomensis* sp. nov. (male, ventral view). Figs **2, 3** based on Singh et al. (2020), used with permission.



Figures 5–7. Male genitalia of *Sacada* with diagnostic characters indicated **5** *S. dzonguensis* Singh, Kirti & Ranjan (holotype) **5a** aedeagus **5b** genitalia **6** *S. umtasorensis* Singh, Kirti & Ranjan (holotype) **6a** aedeagus **6b** genitalia **7** *S. chaehomensis* sp. nov. (holotype, slide Sihvonen 2828) **7a** aedeagus with vesica everted **7b** aedeagus **7c** genitalia. Figs **5, 6** based on Singh et al. (2020), used with permission.

line fuscous, fringes concolorous with wings. Forewing underside rufous grey-brown, medially darker and inner margin pale. Hindwing underside rufous grey-brown on costa, otherwise paler. Postmedial line weakly visible on both wings. Tympanal organs large, semi-circular, fornix tympani separate but connected via narrow sclerotised ridge (in Fig. 7c metathorax sclerotisations and metafurca are visible between tympanal organs). Abdominal segments weakly sclerotised, 8th tergite with two posterior lobes, 8th sternite with round posterior margin.

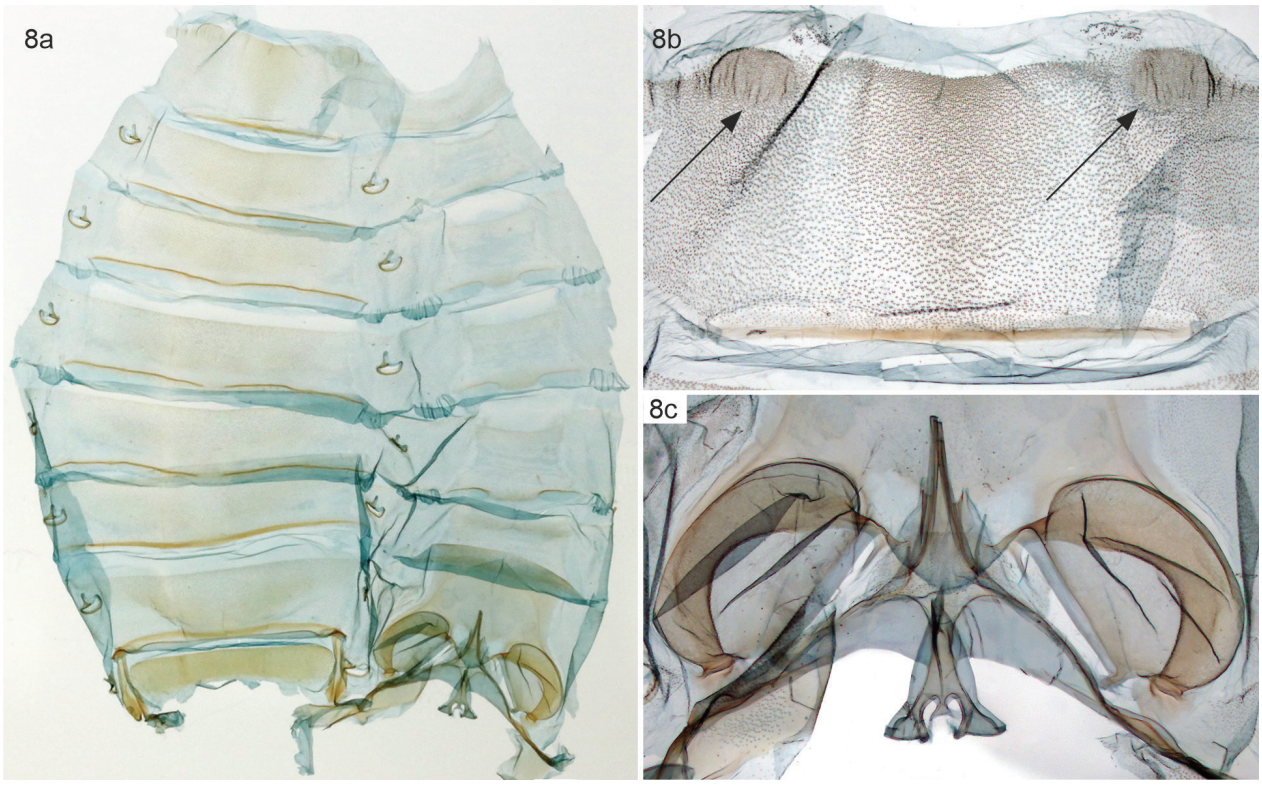


Figure 8. Descaled male abdomen of *Sacada chaehomensis* sp. nov. (holotype) **8a** abdomen **8b** 8th tergite **8c** tympanal organs (slide Sihvonen 2828).

Figure 9. Collecting site of *Sacada chaehomensis* sp. nov. Thailand, Lampang, Chae Hom, 8.9.2013, photo by Markku Pellinen.

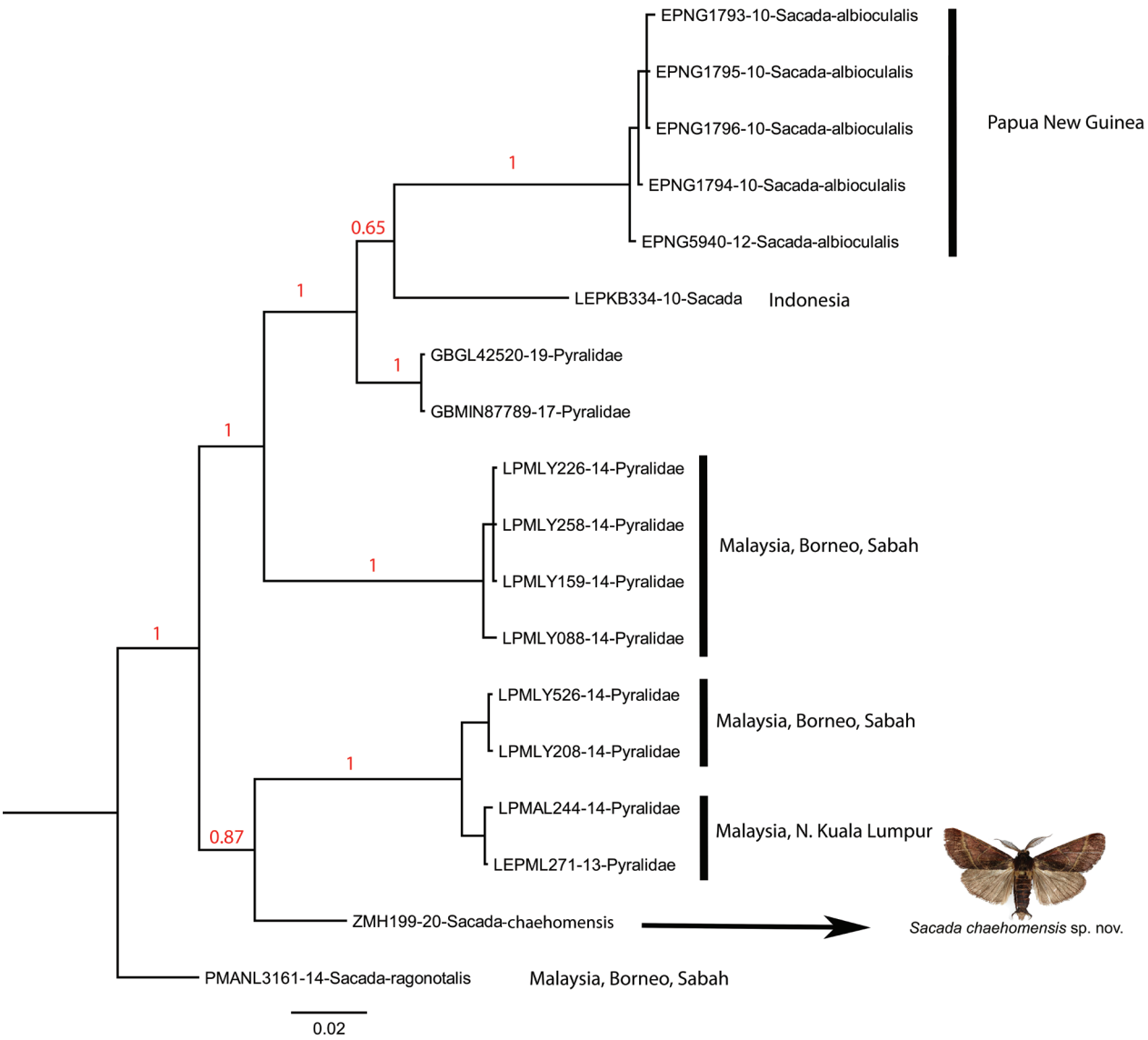


Figure 10. The Bayesian tree from analysis of the COI5P sequences of *Sacada chaehomensis* sp. nov. and 17 public *Sacada* DNA sequences. The nodes are scored by posterior probabilities (PP).

Male genitalia. Uncus hood-shaped, curved, densely setose, with rounded lobes basolaterally. Gnathos sclerotised, arms forming narrow bands, arms fused medially forming upwards directed weakly dentate hook; basolateral parts curved inwards, lobe-shaped. Tegumen formed of two narrow plates. Valva simple, setose, pointing weakly outwards; costa straight. Transtilla large, sclerotised, plate-like, with distinct elongated, upwards directed projection medially, its apex blunt. Juxta broad, weakly constricted laterally, anterior margin even, posterior margin with two heavily sclerotised arms bearing few spikes. Vinculum narrow. Saccus elongated, apex round. Aedeagus stout and weakly undulating, caecum round, apical apodeme ventrally with small group of dentate spikes. Vesica large, simple, without distinct diverticula, basal part wider, apical part narrow and weakly curved; base covered with minute spikes, followed by minute sclerotisations, those become fewer towards vesica apex.

Etymology. The species is named after its type locality, Chae Hom, in Lampang province, Thailand.

Biology. The single known male was collected in May 2020 at 340 m altitude; it was attracted to light in forest with diverse tree species, interspersed with small vegetable plantations (Fig. 9). Immature stages are unknown.

Distribution. The only specimen was collected in Lampang (Chae Hom) in northern Thailand.

Barcode analysis. A 642 bp barcode of *Sacada chaehomensis* sp. nov. was submitted to the identification engine on BOLD. Genetically nearest neighbors are unidentified pyralids from Malaysia, Sabah (North of Borneo) with a minimum divergence of 6.17% and another from Malaysia, North of Kuala Lumpur with 6.50%. The other pre-existing data for *Sacada* on BOLD were *Sacada ragonotalis* (6.54%) and *Sacada albioculalis* (8.93%), thus indicating that the unidentified pyralids may be *Sacada* sp. The species morphologically most similar (i.e., *S. dzonguensis* and *S. umtasorensis*) to the new taxon

lacked representations on the BOLD DNA library. The tree topology generated by the BI analysis (Fig. 10) was very similar to the NJ-tree confirming the association (PP = 0.87) of the new species (ZMH199) with the sister pair of unidentified pyralids from Malaysia, Borneo (LPMLY526-14+ LPMLY208-14) and Malaysia, North of Kuala Lumpur (LPMAL244-14+LEPML271-13). The relationships between analyzed *Sacada* must be treated with caution, because those are based on short DNA barcode sequences only, and are likely to change with addition of more molecular data and expanding taxon sampling.

Acknowledgements

Figs 2, 3, 5, 6 have been published earlier in Singh et al. (2020), and those are used here with permission from the authors. We are grateful to Dr Navneet Singh (India) for the permission and for supporting our study. John Horstman (Australia) is thanked for providing the photo of adult in resting posture (Fig. 1). Scott Miller (National Museum of Natural History, Smithsonian Institution, Washington, DC, USA) is acknowledged for providing the hostplant information. Finally, we wish to thank the reviewers, Richard Mally and Matthias Nüss as well as the subject editor, Martin Husemann for their informative and constructive comments.

References

Bae YS, Byun BK, Paek MK (2008) Pyralid moths of Korea (Lepidoptera, Pyraloidea). Korea National Arboretum, Seoul, 426 pp.

Hall TA (1999) BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. 41. Nucleic Acids Symposium Series, 95–98.

Hardwick DF (1950) Preparation of slide mounts of Lepidopterous genitalia. Canadian Entomologist 82: 231–235. https://doi.org/10.4039/Ent82231-11

Hebert PDN, Cywinska A, Ball SL, deWaard JR (2003) Biological identifications through DNA barcodes. Proceedings of the Royal Society B 270: 313–321. https://doi.org/10.1098/rspb.2002.2218

Klots AB (1970) Lepidoptera. In: Tuxen SL (Ed.) Taxonomists' Glossary of Genitalia in Insects. Munksgaard, Copenhagen, 115–130.

Leraut PJA (2013) Espèces et genres nouveaux de Pyralinae (Lepidoptera, Pyraloidea, Pyralidae). Bulletin de la Société entomologique de France 118: 41–72.

Maddison WP, Maddison DR (2019) Mesquite: a modular system for evolutionary analysis. Version 3.61. http://www.mesquiteproject.org

Nuss M, Landry B, Mally R, Vegliante F, Tränkner A, Bauer F, Hayden J, Segerer A, Schouten R, Li H, Trofimova T, Solis MA, De Prins J, Speidel W (2003–2020) Global Information System on Pyraloidea. www.pyraloidea.org

Ratnasingham S, Hebert PDN (2007) BOLD: The Barcode of Life Data System (www.barcodinglife.org). Molecular Ecology Notes 7(3): 355–364. https://doi.org/10.1111/j.1471-8286.2007.01678.x

Ronquist F, Teslenko M, van der Mark P, Ayres DL, Darling A, Höhna S, Larget B, Liu L, Suchard MA, Huelsenbeck JP (2012) MRBAYES 3.2: Efficient Bayesian phylogenetic inference and model selection across a large model space. Systematic Biology 61(3): 539–542. https://doi.org/10.1093/sysbio/sys029

Sihvonen P (2001) Everted vesicae of the *Timandra griseata* group: methodology and differential features (Geometridae, Sterrhinae). Nota Lepidopterologica 24: 57–63.

Singh N, Kriti JS, Ranjan R, Chandra K, Speidel W (2020) On the taxonomy of the genus *Sacada* Walker, 1862 from India, with descriptions of a new genus and two new species (Pyralinae, Pyralidae, Lepidoptera). ZooKeys 962: 139–163. https://doi.org/10.3897/zookeys.962.51194

Sutton S, Barlow H, Whitaker T (2015) A preliminary guide to pyralid moths of Borneo (Vol. 1). Natural History Publications (Borneo), Kota Kinabalu, in association with Southdene Sendirian Berhad. Kuala Lumpur, 89 pp.

Trifinopoulos J, Nguyen L-T, von Haeseler A, Minh BQ (2016) W-IQ-TREE: a fast online phylogenetic tool for maximum likelihood analysis. Nucleic acids research 44(W1): W232–W235. https://doi.org/10.1093/nar/gkw256

Wahlberg N, Wheat CW (2008) Genomic outposts serve the phylogenomic pioneers: Designing novel nuclear markers for genomic DNA extractions of Lepidoptera. Systematic Biology 57: 231–242. https://doi.org/10.1080/10635150802033006

Yamanaka H (1995) Pyralidae of Nepal, I. In: Haruta T (Ed.) Moths of Nepal, Part 4. Tinea, 14, Supplement 2. Japan Heterocerist's Society, Tokyo, 182–193.

Yamanaka H (1998) Pyralidae of Nepal, II. In: Haruta T (Ed.) Moths of Nepal, Part 5. Tinea, 15, Supplement 1. Japan Heterocerists' Society, Tokyo, 99–193.