Acta Tropica 169 (2017) 133–141

Contents lists available at ScienceDirect

Acta Tropica

journal homepage: www.elsevier.com/locate/actatropica

Uncovering the mask of the *Simulium feuerborni* complex (Diptera: Simuliidae): Description of a new pseudocryptic species *Simulium pairoti* from Malaysia

Zubaidah Ya'cob[a,*], Hiroyuki Takaoka[a], Van Lun Low[b,*], Mohd Sofian-Azirun[a,*]

[a] Institute of Biological Sciences, Faculty of Science, University of Malaya, Kuala Lumpur, 50603, Malaysia
[b] Tropical Infectious Diseases Research and Education Centre (TIDREC), University of Malaya, Kuala Lumpur, 50603, Malaysia

ARTICLE INFO

Article history:
Received 21 December 2016
Received in revised form 24 January 2017
Accepted 6 February 2017
Available online 7 February 2017

Keywords:
Aquatic insect
New species
Pseudocryptic species
COI gene

ABSTRACT

A black fly species of the *Simulium feuerborni* species-group of *Simulium* (*Nevermannia*) from Cameron Highland, Peninsular Malaysia, previously regarded as *S. feuerborni* Edwards, originally described from East Java, is described as *Simulium pairoti* sp. nov. based on complete life stages. High intraspecific variations in the arrangement of the six pupal gill filaments, length of the stalk of the ventral paired filaments, and length of the anterodorsal projection of the cocoon, are noted in this species. This new species is readily distinguished from its congeners by having the characters of male genitalia with simple lamellate ventral plate, short inwardly-twisted styles, several parameral hooks, and a simple narrow median sclerite. Morphological data reported herein plus the chromosomal and molecular data presented elsewhere support *S. pairoti* as a novel pseudocryptic species.

1. Introduction

To date, a total of 90 black fly species belonging to the genus *Simulium* were recorded in Malaysia (Adler and Crosskey, 2016), comprising 47 species of the subgenus *Gomphostilbia* Enderlein, 35 species of the subgenus *Simulium* Latreille s. str., seven species of the subgenus *Nevermannia* Enderlein and one species of the subgenus *Daviesellum* Takaoka & Adler.

The *Simulium feuerborni* species-group of the subgenus *Nevermannia*, redefined by Takaoka (2003), is represented by four species from Malaysia: *S. borneoense* Takaoka and *S. fuscinervis* Edwards from Sabah, East Malaysia (Edwards, 1928; Takaoka, 2001), *S. ledangense* Ya'cob, Takaoka & Sofian-Azirun from southern Peninsular Malaysia (Ya'cob et al., 2014), and *S. feuerborni* Edwards from the central Peninsular Malaysia (Takaoka and Davies, 1995).

Simulium feuerborni was originally described from East Java and Bali (Edwards, 1934) and later recorded from Peninsular Malaysia (Takaoka and Davies, 1995) and Thailand (Kuvangkadilok et al., 1999). It is one of the most geographically widespread species in Southeast Asia (Pramual et al., 2015). Recent cytogenetic investigation showed that this species is in fact a species complex comprising at least four chromosomally distinct lineages: two of which were reported in Thailand (cytoforms A and B), one in Malaysia (cytoform C) and one in Indonesia (cytoform D) (Pramual and Wongpakam, 2013; Pramual et al., 2015). Further, the distinctiveness of *S. feuerborni* between Malaysia and Thailand has also been supported by molecular evidence (Pramual et al., 2015).

We re-examined the morphological characters of *S. feuerborni* (cytoform C) from Cameron Highlands, Peninsular Malaysia and found several characters differing from the previous re-description of *S. feuerborni* from East Java (Takaoka and Davies, 1996). Based on these morphological differences, coupled with the molecular and chromosomal evidence from a previous study (Pramual et al., 2015), we are describing this species as a new pseudocryptic species—a taxon that is readily distinguished morphologically once other diagnostic techniques such as molecular and chromosomal tools are applied (Knowlton, 1993).

2. Materials and methods

2.1. Morphological description and illustration of new species

The terminology of morphological characters was applied as described by Takaoka (2003). The type specimens of this novel species are kept in the Institute of Biological Sciences, Faculty of Science, University of Malaya, Kuala Lumpur, Malaysia.

* Corresponding authors.
E-mail addresses: xuehy_perdana@yahoo.com (Z. Ya'cob), lucaslow24@gmail.com (V.L. Low), sofian@um.edu.my (M. Sofian-Azirun).

http://dx.doi.org/10.1016/j.actatropica.2017.02.005
0001-706X/

2.2. Taxa used for DNA analyses

All life stages of this new species (larvae, pupae, males and females) were used for molecular analyses. For comparison purposes, we included other members of the *S. feuerborni* species-group: *Simulium ledangense* and *S. borneoense* from Malaysia, *S. feuerborni* from East Java (representing the type population) and West Java. Previously published sequences of *S. feuerborni* cytoforms A and B from Thailand (Pramual et al., 2015) were also included for analyses.

2.3. DNA isolation and polymerase chain reaction (PCR)

The genomic DNA was isolated follow those of Low et al. (2016a). The cytochrome c oxidase I (COI) barcode region was amplified by using the universal primers developed by Folmer et al. (1994). The PCR reaction mixture comprised 50–100 ng of genomic DNA, 25 μL of NEXpro e PCR 2x Master Mix (Genes Labs Inc., Gyeonggi-do, South Korea), and 10 pmol of each primer.

2.4. DNA sequence analyses

Forward and reverse COI sequences were assembled and edited using ChromasPro Version 1.7.6 (Technelysium Pty Ltd., Australia) and BioEdit 7.0.9.0 (Hall, 1999). All new COI sequences obtained from the present study were deposited in the NCBI GenBank database under accession numbers KX525217-KX525233. Neighbor-joining (NJ) and maximum parsimony (MP) methods were used to construct phylogenetic tree of the *S. feuerborni* species group. NJ analysis was carried out on the T-REX web server (Boc et al., 2012) by using the Kimura's two-parameter substitution model with 1000 bootstrap replicates. MP analysis was performed in MEGA6 (Tamura et al., 2013) using the bisection reconnection (TBR) branch swapping with 1000 bootstrap replicates. *Simulium nodosum* Puri (KP661450) was used as a candidate outgroup taxon for the construction of phylogenetic tree.

For species delimitation analysis, the Generalised Mixed Yule Coalescent (GMYC) analysis was performed using ultrametric tree generated from BEAST 1.8.2 (Drummond et al., 2012), under the assumptions of a relaxed lognormal clock, constant population size coalescent and GTR + I + G model of DNA substitution. The analysis was run for 20 million generations and sampling every 100 generation. The consensus tree was analyzed in TreeAnnotator 1.8.2 with a 10% burn-in. The GMYC analysis was run on the consensus tree under the single-threshold model, using the SPLITS package (Ezard et al., 2009) in R 3.2.1. The uncorrected *p* genetic distances among the operational taxonomic units (OTUs) were estimated using PAUP 4.0B10 (Swofford, 2002).

3. Results

3.1. Simulium (Nevermannia) pairoti *Ya'cob, Takaoka & Sofian-Azirun sp. nov*

"**Female (n = 3)**. Body length 2.8–3.1 mm. ***Head***. Slightly narrower than thorax. Frons brownish black, whitish-gray pruinose, densely covered with whitish-yellow hairs and several dark longer hairs along each lateral margin, shiny when illuminated at certain angles. Clypeus brownish-black, whitish-gray pruinose and covered with whitish-yellow hairs and several dark longer hairs on lower half and bare in middle. Frontal ratio1.8:1.0:2.7–2.8. Frons-head ratio 1.0:4.9–5.6. Labrum 1.0–1.2 length of clypeus. Antenna composed of scape, pedicel and 9 flagellomeres, brown except scape, pedicel and base of first flagellomere yellow; first flagellomere 1.7–2.1 times length of second flagellomere. Maxillary palp brownish black consisting of 5 segments, proportional lengths of 3rd, 4th, and 5th segments 1.0:0.7–0.9:1.3–1.6; third segment much enlarged, sensory vesicle elongate (Fig. 1A), 0.6–0.7 times length of third segment, with medium-sized opening. Maxillary lacinia with 7 or 9 inner teeth and 12 or 14 outer teeth. Mandible with 22 inner teeth and lacking outer teeth. Cibarium with 22 dark minute spines with pointed apices as well as numerous minute spinous processes near lower margin. ***Thorax***. Scutum light to medium brown except anterolateral calli yellow, two submedian longitudinal vittae and narrow portion along lateral margin and prescutellar area dark brown, white pruinose except 3 longitudinal vittae (1 narrow median, 2 little wider submedian); scutum covered with yellow short hairs and several dark brown longer hairs on prescutellar area, and slightly shiny when illuminated at certain angles. Scutellum light brown, whitish-gray pruinose, slightly shiny when illuminated at certain angles, with whitish-yellow hairs and dark longer hairs. Postnotum brown, whitish-gray pruinose, bare and slightly shiny when illuminated at certain angles. Pleural membrane and ketepisternum bare; ***Halter***. White except grayish near apex. ***Legs***. *Foreleg*: coxa and trochanter yellow; femur yellow with apical cap tip brown; tibia brown except median large portion of outer surface dark yellow to light brown and basal tip yellow; tarsus dark brown. Basitarsus slender, cylindrical, 7.5–8.3 times as long as its greatest width. *Midleg*: coxa brown except posterolateral surface dark brown; trochanter yellow; femur yellow except apical cap brown; tibia brown except medium large portion dark yellow to light brown; tarsus dark brown. *Hind leg*: coxa and trochanter yellow; femur yellow except apical cap dark brown; tibia (Fig. 1B) brown except median large portion of outer surface dark yellow to light brown; tarsus dark brown except basal two-thirds of basitarsus (Fig. 1C) grayish though extreme base dark brown and basal half of second tarsomere yellow; femur slightly wider than tibia; basitarsus parallel-sided, 6.3–7.0 times as long as it greatest width and 0.7–0.8 and 0.6–0.7 times as wide as greatest width of tibia and femur, respectively. Calcipala 0.7 times as long as width at base, and 0.5 as wide as greatest width of basitarsus; pedisulcus moderately developed. Claws each with large basal tooth, 0.46 times length of claw. ***Wing***. Length 2.8–3.2 mm. Costa with two parallel rows of short spines as well as hairs. Subcosta and basal portion of radius fully haired; R1 with dark brown spinules and hairs; R2 with dark-brown hairs; hair tuft on base of radial vein dark brown. Basal medial cell absent. ***Abdomen***. Basal scale light brown with fringe of whitish-yellow long hairs. Dorsal surface of abdomen brown except segment 2 pale; tergites of segments 6–8 shiny. Abdomen with whitish-yellow and brownish-black short hairs. ***Terminalia***. Sternite 8 wide (Fig. 1D), bare medially and furnished with 17–21 medium to long hairs and few short hairs on each side. Ovipositor valves (Fig. 1D) triangular (through posteromedial corner rounded), thin, membranous except inner margin narrowly sclerotized, densely covered with microsetae interspersed with 4 or 5 short setae; inner margins sinuous, narrowly separated from each other. Genital fork (Fig. 1E) of inverted Y-form, with well sclerotized stem and relatively wide arms; each arm with lateral plate bearing triangular lobe-like projection directed posteromedially and short narrow stout projection directed anterodorsally. Paraproct in ventral view (Fig. 1F) subquadrate, as long as its greatest width; anteromedial surface nearly transparent, with 5–9 sensilla; paraproct in lateral view (Fig. 1G) somewhat protruded ventrally beyond ventral margin of cercus, and with 21–23 medium to long hairs on ventral and lateral surfaces. Cercus in lateral view (Fig. 1G) rounded posteriorly, short, 0.5 times as long as basal width. Spermatheca (Fig. 1H) nearly ovoidal, strongly sclerotized except small area around juncture with duct and duct itself unsclerotized, with distinct reticulate surface pattern and without internal setae; main spermathecal duct narrow, somewhat narrower than both accessory ducts."