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DIAGNOSTICS OF *PERINA NUDA* (F) (LEPIDOPTERA: EREBIDAE)

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ABSTRACT

Clearwing tussock moths were collected from different parts of Tamil Nadu, India. The occurrence of *Perina nuda* (F.) has been documented with morphological studies. Male moths bear no resemblance to females, with almost hyaline forewing and hind wings drab grey to pale purplish grey with a hyaline patch at the apical angle of the hind wing. Females with capucine buff-coloured wings. Males have asymmetrical genitalia with pointed-finger shaped uncus. The species identity was confirmed through DNA barcodes submitted to GenBank (RKB004-21 and MZ540881). The molecular phylogeny revealed that it formed separate clade from the available *P. nuda* barcodes with a meagre 2% variation.

Key words: Clearwing tussock moth, *Perina nuda*, redescription, morphology, genitalia, diagnosis, DNA barcoding, phylogeny

Genus *Perina* was established by Walker (1855) with type species *basalis* described from Nepal and with distribution records from North India and Hong Kong under family Psychidae (Walker, 1855). Earlier, *Bombyx nuda* was described from India (Fabricius, 1787) and was revised under Liparidae (Kirby, 1892) and now classified under *Perina* of family Lymantriidae (Watson et al., 1980) while *Perina basalis* is considered to be the junior subjective synonym of *P. nuda* (Beccaloni et al., 2003). At present, Lymantriidae has been subordinated as a subfamily of Erebidae (Zahiri et al., 2011) and hence, genus *Perina* is now classified under Leucomini (Holloway, 1999) of Lymantriinae of family Erebidae. Genus *Perina* comprises of six species viz*., Perina nuda* (Fabricius, 1787) and *P. pura* Walker, 1869 with distribution records from India (Hampson, 1893; Kaleka, 2010; Smetacek, 2008) and *P. kalisi* Collenette, 1949*, P. lodra* Moore, 1859*, P. psamma* Collenette, 1933 and *P. sunda* Holloway, 1999 from elsewhere (Holloway, 1999). Except for a brief description of Hampson (1893) and distributions records of *Perina* spp. across India, the information is scanty. Perusal of literature indicated that genitalia and molecular details from India are wanting. Owing to morphological similarity and sympatry among various *Perina* sp., proper identification of species is imminent. This study characterizes *Perina nuda* from Tamil Nadu as the occurrence of *P. pura* from peninsular part of India remains to be documented.

MATERIALS AND METHODS

Light trap collections of moths from different parts of Tamil Nadu was undertaken viz*.,* Kodaikanal (10°22'N, 77°45'E, 2133 masl), Thadiyankudisai (10°30'N, 77°69'E, 1100 masl), Thandikudi (10°27'N, 77°60'E, 1500 masl) of Lower Pulney hills of the Western Ghats, Jawadi hills (12°58'N, 78°83'E, 857 masl) of the Eastern Ghats and from the plains of Yethapur (11°65'N, 78°47'E, 282 masl) (Salem district), Aiyyur (10°27'N, 77°71'E, 1060 masl) (Krishnagiri district), Coimbatore (11°01'N, 76°94'E, 425 masl) and Pollachi (10°64'N, 76°88'E, 293 masl) (Coimbatore district). The collected specimens were deposited in the TNAU Insect Museum, Coimbatore. Specimens collected under Network Project on Insect Biosystematics (NPIB), TNAU Insect Museum (IM) Project and students' collection of Tamil Nadu Agricultural University, Coimbatore were also examined. Taxonomic characters viz*.,* antennae, labial palp, fore wing (FW), hind wing (HW), legs and female (♀), male (♂) genitalia were examined. Colours of adult moths were described as per Ridgway (1912). For the study of wing venation, slides of wings were prepared as per Zimmerman (1978); wing venation description follows Comstock and Needham (1898). Genetalia study follows Robinson (1976) and terminology of Klots (1970). Specimens were examined and photographed (genitalia) under stereozoom microscope (Leica: M205A, Software LAS

V4.12) and adult moths were photographed with Digital Single Lens Reflex camera (DSLR) (Nikon D3100).

The DNA barcoding and phylogeny study of *P.nuda* adults collected from Coimbatore were undertaken. Genomic DNA was extracted from 3 legs of the fresh specimen using CTAB method (Gawel and Jarret, 1991). DNA concentration was measured using nanodrop spectrophotometer (Nanodrop One, Thermo Scientific, Madison, USA). The PCR amplification was performed in 30 µl reactions containing 15 µl of master-mix, 2.5 µl of each primer, 6 µl of DNA and 4 µl DNase free water. The amplification cycle of Hebert et al. (2003) was followed. The results were visually verified by electrophoresis on 1.5% agarose gel stained with ethidium bromide. The PCR product was sequenced through Eurofins Genomics India Pvt Ltd., Bangalore, India. Two sequences were submitted to GenBank and accession numbers were obtained (RKB004-21 and MZ540881). These sequences were compared with 12 sequences from GenBank, using Blastn in GenBank to check genetic similarity. Sequences were aligned using CLUSTALW (Thompson et al., 1997) implemented in MEGA version X (Kumar et al., 2018), with default parameters. Model-based phylogenetic analyses were performed using Neighbour-Joining (NJ) method and Bayesian inference in MEGA version X (Kumar et al., 2018) with the branch support values evaluated by 1000 bootstrap replicates under Tamura 3-parameter model with gaps/ missing data treatment as partial deletion and 95% cut-off. The sequence of *Bombyx mori* from GenBank was used as outgroup.

RESULTS AND DISCUSSION

Genus *Perina* Walker, 1855

Perina Walker, 1855: 966 (Type species: *nuda* Fabricius). *P. basalis* is a junior subjective synonym of *Bombyx nuda* Fabricius, 1787, **2**: 119.

Perina nuda (Fabricius, 1787)

Redescription: Medium sized moths with globular compound eyes; antennae bipectinate, bipectinate comb sickle shaped, with pectin long at the base and tapering towards the tip; labial palpi curved upward, second segment longest of the three; proboscis tip without any spines. Fore tibia with epiphysis; mid tibia and hind tibia with two tibial spurs. Forewing (FW) sub triangular, apical margin slightly convex, and hind wing (HW) sub triangular to squarish with apical margin moderately convex. Sexual dimorphism very prominent. The male moths bears no resemblance to female moths. Male: Head, thorax and abdomen densely clothed with drab gray to pale purplish gray to slate black scales; tip of abdomen (scales covering the genital area) clothed with orange to pinkish orange scales. Wings: Upper side and underside: FW hyaline with drab gray to pale purplish gray to slate black scales only near the humeral angle spreading towards anal margin along A1+2; HW with drab gray to pale purplish gray to slate black scales and a hyaline patch at the apical angle revealing Sc+R1 and Rs terminating at the margin. Wingspan: 38 mm. Female: Head, thorax and abdomen densely clothed with capucine buff scales. Fore- and hind wings covered by capucine buff scales. Wingspan**:** 40-42 mm (Figs. 1, 2). Wing venation: FW: Sc ends two third of FW. R1 arises from the anterior part of discal cell and ends near costal angle. Following R1, R2 arises from the corner of discal cell and ends at costal angle. Further R3 and R5 branch from a common stem while R4 branches from R3 and ends prior to R5. HW: Sc+R1 and Rs form a basal areole before diverging and terminating at the apical margin; Sc+R1 ends at costal angle, followed by Rs. Male: M1 absent. M2 and M3 stalked. Cu1 and Cu2 branch nearer M3. A1+2 and A3 arise separately from base of wing. Female: M1 branches from Rs. M2 and M3 branches from discal cell. Cu1 and Cu2 arise from mid-point of discal cell. A1+2 arises from base of wing. A3 either inconspicuous or absent.

Male genitalia: Uncus slender, narrow, pointed finger-like and slightly left oriented; tegumen setose, shoulder on the right more prominent while the shoulder on the left less conspicuous and appear asymmetrical; saccus cylindrical small, setose on plate- like vinculum; valva linear, corona part of the valve appear slightly folded and flap-like, non-setose in the coronal area. Flap more prominent on the right side and almost indistinct on the left side, hence asymmetrical; gnathos beak-like and prominent. Aedeagus twisted and almost ‘z’ shaped. Cornutii absent. (Fig. 3 - 5). Female genitalia: Anal papillae clasp-like and setose, sub-genital plate heavily setose along the ventral side, posterior and anterior apophyses slender and equal in length, lamella post-vaginalis heavily sclerotized and forms an interior ring-like structure connecting to the apophyses, ostium almost circular and sclerotized, ductus bursae slightly sclerotized and short with ridge like longitudinal patterns, corpus bursae ovoid and bulged in the middle, signum line like, two appendix bursae globular at either lateral sides of anterior corpus bursae (Fig. 6).

Through molecular analysis it is confirmed that the collected moths are *Perina nuda* (Fig. 7). *Perina*

Perina nuda (Fabricius, 1787)

Fig. 1. Dorsal view of male adult

Fig. 2. Dorsal view of female adult

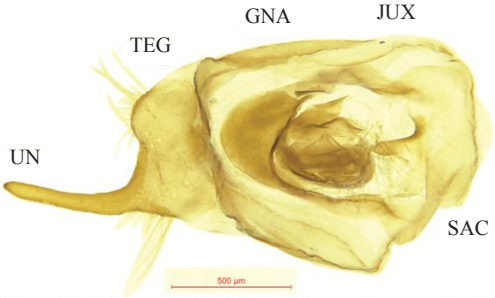

Fig. 3. Ventral view of male genitalia

Fig. 5. Aedeagus

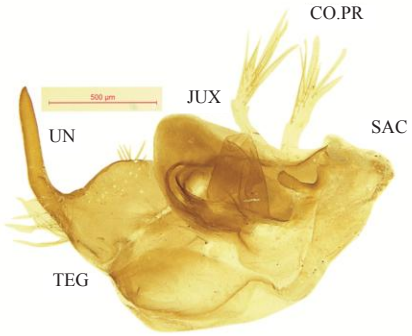

Fig. 4. Lateral view of male genitalia

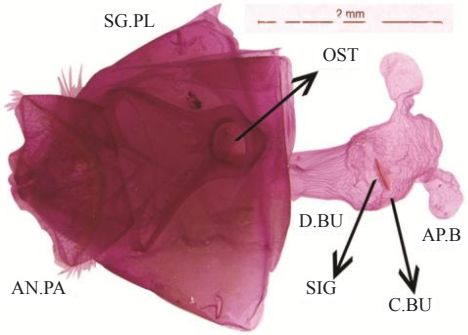

Fig. 6. Dorsal view of female genitalia

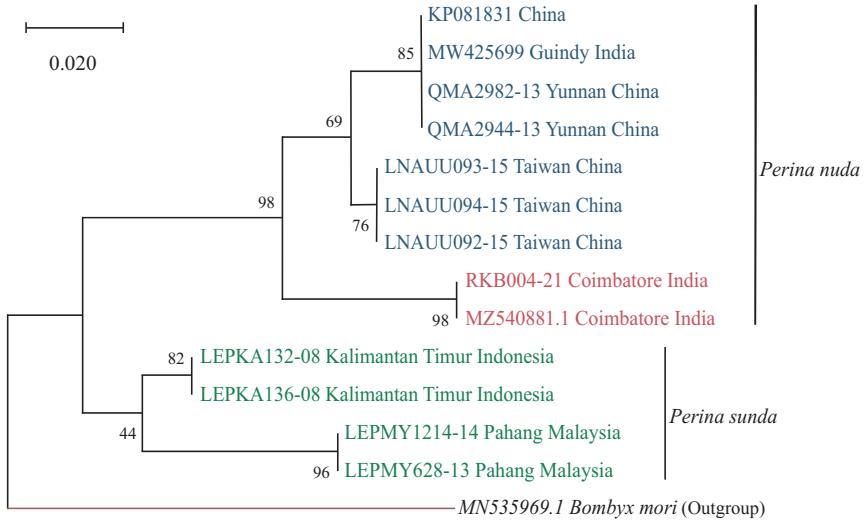

Fig. 7. Phylogenetic tree of *Perina nuda* and *P. sunda*

nuda forms a separate clade / branch from that of *P. sunda*. Within the clades the species group together on geographical basis with the exception of MW425699 Guindy population (Sayers et al., 2021; Ratnasingham and Hebert, 2013).

Remarks: The most comprehensive study focused on phylogenetic relationships of Lymantriinae (Wang et al., 2015) included only *P. nuda* in their analyses. We find that the branch length of Coimbatore population to be longer than the rest which means they have high molecular evolution. Even among *P. sunda*, the Pahang, Malaysia population has a longer branch than that of Kalimantan Timur, Indonesia population. The Guindy population branch is on par with that of Yunnan, China population but this deviation needs confirmation through further sampling and analyses (Sayers, 2021). Sexual dimorphism is common among the species of *Perina*. It is evident that sexual dimorphism is very prominent in other members of Lymantriinae. For instance, it has been observed in *Euproctis dimorphissima* (Holloway, 1979) and *Numenes* spp. (Inoue, 1975). Female moths of *Perina* spp. look akin to moths of tribe Nygmiini (Holloway, 1999). Male moths of *P. kalisi, P. lodra* and *P. psamma* are almost identical to *Perina nuda* (Collenette, 1949; Holloway, 1999). Generally, it is observed that Lymantriinae are pleisomorphic with quadrifid hindwing venation (Ferguson, 1978). In addition, the absence of M1in the HW of male moths as in the present study is a characteristic feature for the genus *Perina* (Holloway, 1999; Kishida, 2011).

In Leucomini, the male genitalia are asymmetrical in all three genera viz*., Perina, Leucoma* and *Dendrophleps* (Holloway, 1999). *Perina nuda* (=*subtincta* Walker) collected in East Indies is speculated to be either from India or Sundaland. The valves have been described to be slender with bulbous rod at the apex (Holloway, 1999). However, in present study, valves are linear with flaps and asymmetrical. In male genitalia, valves of *P. kalisi* and *P. nuda* resemble each other having prominent long right valve and short left valve with pointed right valve in *P. kalisi* and blunt flap-like in *P. nuda*. The female genitalia of *P. nuda* identical as described by Kishida (2011).

Host records: *Artocarpus heterophyllus* (Butani and Jotwani, 1984; Rajkumar et al., 2018; Sharma et al., 2008), *A. integrifolia* (Lefroy, 1909), *Ficus altisimma* (Cheanban et al., 2017), *F. carica* (Fletcher, 1917), *F. benghalensis* (Cheanban et al., 2017), *F. benjamina* (Cheanban et al., 2017; Meena et al., 2018), *F. microcarpa* (Easton and Pun, 1996), *F. racemosa*, *F. religiosa*, *F. retusa*, *F. rumphii* (Cheanban et al., 2017), *Ficus* sp. (Cheanban et al., 2017; Fletcher, 1919), *Mangifera indica* (Fletcher, 1919) and *Sapindus trifoliatus* (Ghorpade and Patil, 1991).

Distribution: Madhya Pradesh (Chandra and Nema, 2006; Khan et al., 1988; Meena et al., 2018), Maharashtra (Ghorpade and Patil, 1991; Gurule and Nikam, 2013), Assam (Arandhara et al., 2017), Chhattisgarh (Chandra and Nema, 2006), Andaman (Chandra, 1994; Chandra and Rajan, 1995), Himachal Pradesh, Jammu, Kashmir, Uttarakhand (Kaleka, 2012), Tamil Nadu (Kathirvelu et al., 2019), Karnataka (Mishra et al., 2016), throughout India (Butani and Jotwani, 1984; Cherian and Israel, 1939; Hampson, 1893; Kaleka, 2010; Lefroy, 1909; Rajkumar et al., 2018; Sharma et al., 2008), Nepal (Kishida, 1993), Vietnam (Schintlmeister, 1987), Bangkok (Cheanban et al., 2017), Japan (Matsumura, 1933), China (Easton and Pun, 1996; Kendrick, 2004; Kwok and Tai, 2016), South East Asia (Wallner and McManus, 1989), Tropical Asia (Muniappan et al., 2012), Asia (Prakash et al., 2009) and Pacific (Elevitch and Manner, 2006).

Material examined: INDIA. Tamil Nadu, Coimbatore, TNAU, 3♂ 4♀; NPIB leg; 29.xii.2006, PNFCBEF05, PNMCBEM08; 24.xi.2009, PNFCBEF02, PNMCBEM04; 03.iii.2011, PNFCBEF01, PNMCBEM03; 10.xi.2010, PNFCBEF03; 1♀; Orchard, 10.vi.2021; Balaji, RK leg; PNFCBEF07; 1♀; Pollachi, VIA, 18.xii.2019; Students coll. leg; PNFPOLF01; Dindigul, 1♀; Thandikudi, 10.xi.2010; NPIB leg; PNFTNKF01; 2♂ 2♀; HRS Thadiyankudisai, NPIB leg; 27.vi.2008, PNFTKDF06; 04.ii.2014, PNMTKDM09; Balaji, RK leg; 21.xii.2017, PNFTKDF03; 09.i.2018, PNMTKDM23; 1♀; Kodaikanal, 09.vii.2009; NPIB leg; PNFKKLF01; 1♂; Vellore, Jawadi hills, 30.x.2014; Ganeshkumar, M leg; PNMJWDM01; 1♂; Salem, Yethapur, 28.i.2009; NPIB leg; PNMYTPM01; 2♂; Krishnagiri, Aiyyur, 09.i.2007; NPIB leg; PNMAYRM01, 02. Abbreviations- (PNM-*Perina nuda* male; PNF-*Perina nuda* female; CBE-Coimbatore; TNK-Thandikudi; TKD-Thadiyankudisai; KKL-Kodaikanal; JWD-Jawadi hills; YTP-Yethapur; AYR-Aiyyur; M-male; F-female).

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AUTHOR CONTRIBUTION STATEMENT

All authors equally contributed.

CONFLICT OF INTEREST

No conflict of interest.

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