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Cover: *Geodorum laxiflorum* Griff.—inflorescence (Orchidaceae) © Ashish Ravindra Bhojar.



added. As of now a total of eleven species (Hampson 1896; Kenrick 1907; Turner 1908; Caradja 1916; Meyrick 1937; Caradja & Meyrick 1937; Kitching et al. 2020) are considered extant in the genus of which, five species are reported from India (Kitching et al. 2020). Hampson (1896) mentioned the distribution of *P. tyres* as throughout India (having type locality in the Coromandel region of southern India).

For easy identification of the morphological cryptic species, mt DNA barcoding are being used as an alternative tool for insect species identification and documentation of new species (Hebert et al. 2003). Although DNA barcode-based species identification works are in infancy in the developing countries, the technique provides robust and rapid approach for biodiversity analysis (Ashfaq et al. 2017), exploiting low conspecific and high interspecific genetic variation principle (Hebert et al. 2003). DNA barcodes have been constructively utilized for diverse aims in addition to serving as an aid to conventional slow-paced taxonomic delimitation approaches (Ashfaq et al. 2017). DNA barcodes having effectively applied to unpin species identity for numerous animal taxa, the order Lepidoptera has seen particularly intensive barcode analysis (Ashfaq et al. 2017). The identification using DNA barcoding approach exclusively depends on the quality of reference library, which is strengthened if the barcodes are linked

to registered voucher specimens. Identification of moths using mt DNA barcode has been introduced in the moth groups of *Olepa* (Kalawate et al. 2020a,b). Despite its widespread distribution there are no genetic data available for the species from India. Hence, during one of our exercise of generation of mt DNA barcodes for the moth species, here we report the first mt DNA barcode for the species *P. tyres* from India, having a wide range of distribution.

MATERIALS AND METHODS

The specimens were collected by installing light trap during night, and were euthanized by ethyl acetate vapours. The specimens were transferred to the laboratory in insect packets under dry conditions. They were stretched, pinned, labelled, and dry-preserved in fumigated entomological boxes for further study. For morphological studies the specimens were studied under Leica EZ4E stereomicroscope. The map of the collection locality was prepared using open free QGIS software. The details of collection locality are given under material examined and also shown in Figure 1. The identification was done with the help of Hampson (1896). The genitalia of male and female were studied following Robinson (1976). The identified materials are deposited at the National Zoological Collections of the Zoological Survey of India, Western Regional Centre,

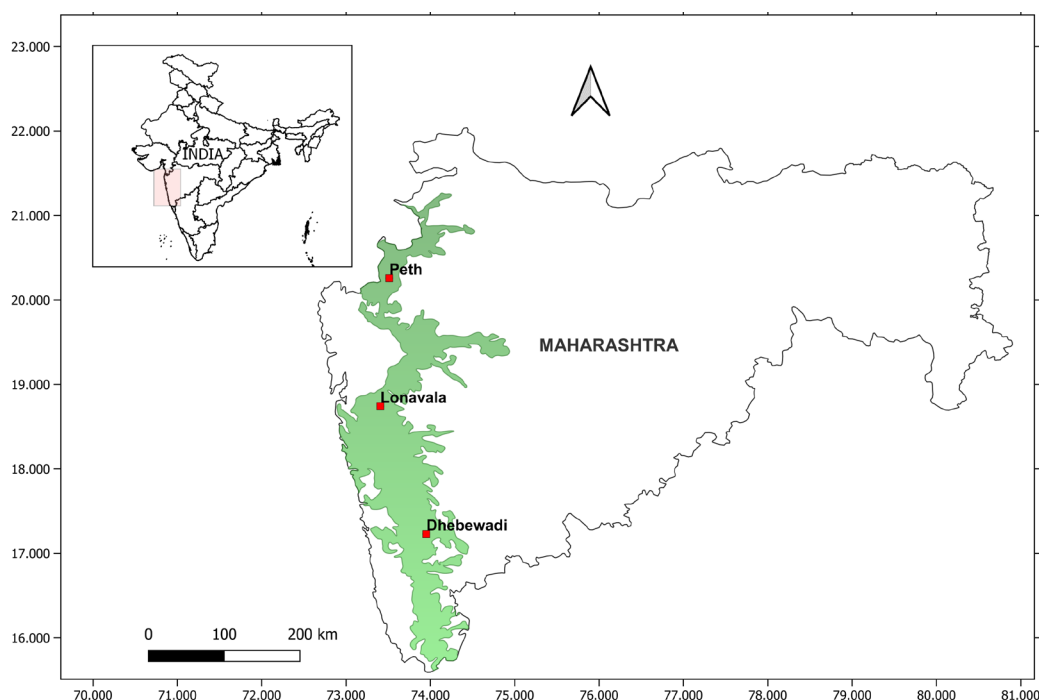


Figure 1. Collection localities of *Pygospila tyres* from northern Western Ghats, India.

Pune, Maharashtra, India (ZSI, WRC).

DNA extraction and purification were performed using leg and thoracic muscle from dried specimen, followed by quantitation utilizing HS dsDNA assay kit on Qubit 2.0 fluorometer. Amplification of mt COI gene was attempted using universal primer (Folmer et al. 1994), LCO1490 and HCO2198 in 25 µL reaction volume constituted by 12.5 µL of Master Mix (Promega), 10 pmol of each forward and reverse primer along with Nuclease free water up to Q.S. thermal cycling profile as per Kalawate et al. (2020a). Amplified PCR product was confirmed by gel electrophoresis stained by SYBR safe DNA gel stain (Invitrogen), visualized under UV by gel documentation system, followed by purification of amplified product by Invitrogen's Pure Link PCR Purification Kit. Purified PCR product was sequenced bidirectionally by Sanger's method on ABI 377 (Applied Biosciences) sequencer.

Both the forward and reverse sequences generated in the current study were verified manually for corrections. From the GenBank 21 mt COI gene sequences available for the *Pygospila* were downloaded (Table 1) and were aligned with Clustal W algorithm in MEGA 5.2 software (Tamura et al. 2011). For phylogenetic reconstruction, Maximum Likelihood tree was built with RaxML (Silvestro & Michalak 2012) for thorough bootstrap 1,000 replicates under the GTR+GAMMA+I model and the final consensus tree was visualized by Fig Tree v1.4.0 treating species *Pycnarmon* as out groups (Figure 2).

RESULT AND DISCUSSIONS

Morphologically the collected samples were identified as *Pygospila tyres* (Cramer, 1780) (Image 1).

Taxonomic account

Superfamily Pyraloidea Latreille, 1809
Family Crambidae Latreille, 1810
Subfamily Spilomelinae Guenée, 1854
Genus *Pygospila* Guenée, 1854
1854. *Pygospila* Guenée, Delt. and Pyr.: 312.
Type species: *Pygospila tyres* (Cramer, 1780)
Species *Pygospila tyres* (Cramer, 1780) (Image 1A–D)
1780. *Phalaena tyres* Cramer, *Pap. Exot.*, 3: 263.
Type Locality. Coromandel, southern India.

Morphological description Adult (Image 1A): Wing expanse: 40–45 mm. Olive-brown with purple tinge reflects in light; palpi white underside; frons with lateral white lines; white line on thorax and patagia; abdomen slender, long with paired white spots placed dorsal and lateral. Forewing olive brown with several nacreous

spots, these spots shine with a purple tinge in light. Hindwing with nacreous streaks in and below the cell. A pair of spots present between origin of vein 3 and 5, three submarginal spots and a spot present below vein 2; cilia brown and white towards anal angle. Underside exactly same pattern on both fore and hindwings. Hind wing of male with vein 8 widely separated from 7, 6 bent downward, the veins beyond the cell roughly scaled.

Male genitalia (Image 1B): Uncus thin, bulbous with hairs; tegumen well developed with a process resembles feather of peacock; valvae broad, laterally surrounded by long hairs, ampulla thin, sclerotized and hooked; saccus relatively well developed, broad u-shaped, with two curved process. Aedeagus (Image 1C) very long, thin, whip-like, with swollen apex.

Female genitalia (Image 1D): Apophyses thin, both anterior and posterior are of equal length; Corpus bursae membranous, elongated, devoid of signum; papillae anales large, setosed.

Material examined: 01 #, Peth, Nashik (N 20.259; E 73.513, altitude 593 m), 28 viii 2013, Coll. P.S. Bhatnagar & Party (L-1465); 04 #, Dhebewadi, Satara (N 17.229; E 73.952, altitude 731 m), 17 vii 2017, Coll. A.S. Kalawate & Party (L-1630); 01 #, Dhebewadi, Satara (N 17.229; E 73.952, altitude 731 m), 12 vii 2017, Coll. A.S. Kalawate & Party (L-1706); 01 #, Dhebewadi, Satara (N 17.229; E 73.952, altitude 731 m), 13 vii 2017, Coll. A.S. Kalawate & Party (L-1716); 04 #, Dhebewadi, Satara (N 17.229; E 73.952; altitude 731 m), 15 vii 2017, Coll. A.S. Kalawate & Party (L-1759); 01 #, Lonavala, Pune (N 18.742; E 73.405, altitude 622 m), 23 viii 2017, A.S. Kalawate & party (L-1583).

Distribution in India: Bihar, Chhattisgarh, Himachal Pradesh, Jharkhand, Madhya Pradesh, Maharashtra, Punjab, Rajasthan, Sikkim, Tamil Nadu, and West Bengal.

Outside India: Africa, Australia, Borneo, China, Indonesia, Java, Japan, Malaysia, Myanmar, Nepal, New Guinea, Pakistan, Philippines, Sri Lanka, Thailand, and Vietnam.

Host plants: *Wrightia tinctoria*, *Wrightia arborea*, *Holarrhena antidysentrica*, *Tabernaemontana heyneana* (Apocynaceae) (ICAR-NBAIR 2020).

DNA Barcode diagnosis

The genetic sequence of sample of *P. tyres* from Pune, Maharashtra matches completely with the *P. tyres* sequences from Pakistan, Korea, and Australia. The clade composing the *P. tyres* is homologous without any genetic distance variation. One of the sequences (JX017862.1) from Australia is labelled as *P. tyres*, where the identity should be rechecked with the voucher

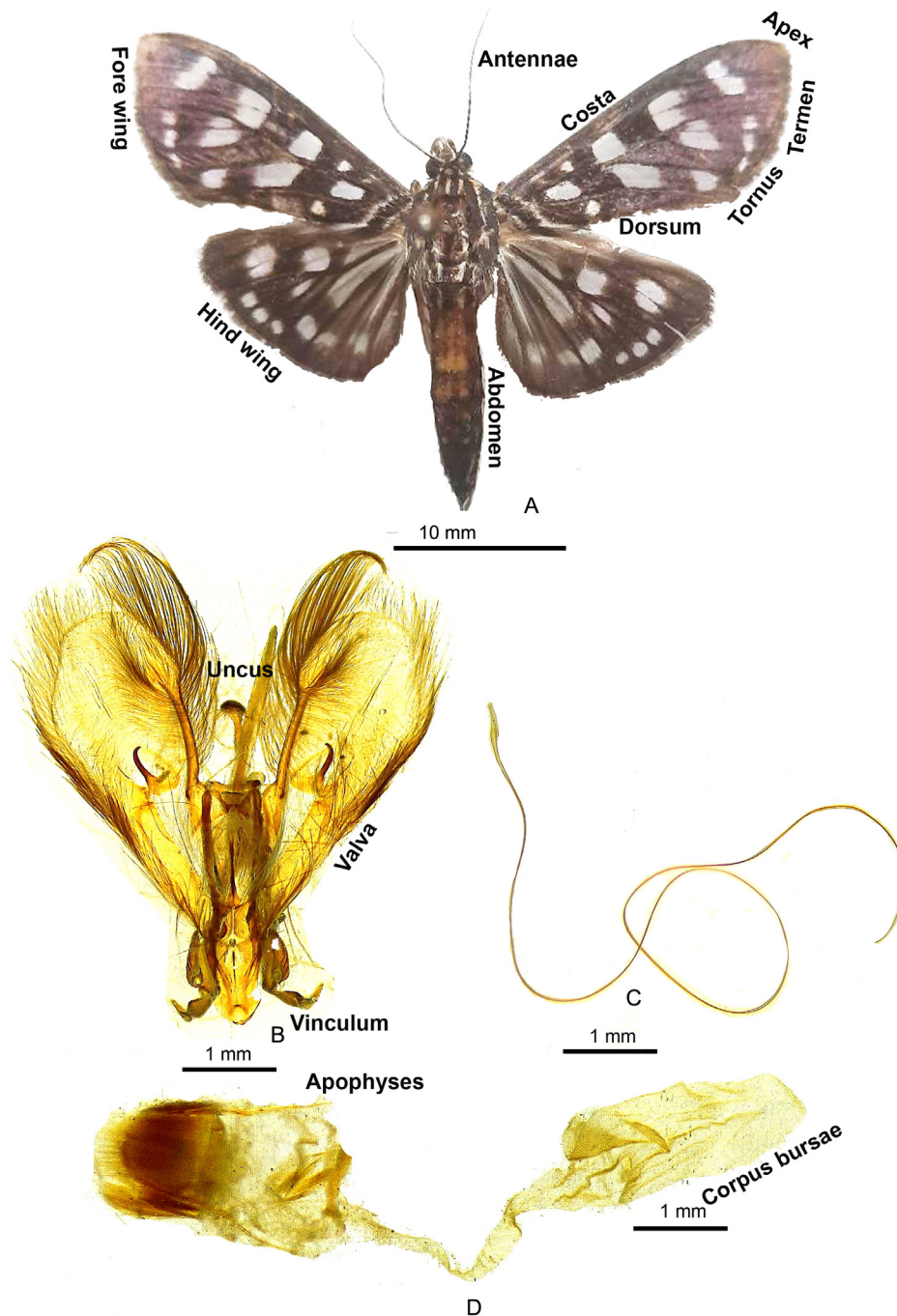


Image 1. *Pygospila tyres*: A—Adult | B—Male genitalia | C—Aedeagus | D—Female genitalia.

specimens as the sequence is forming monophyletic clade with the members of *P. bivittalis* from Australia. Although there are limitations with the phylogenetic inferences of mt COI DNA barcode trees, our studies could discern three clear clades for the species *P. tyres*, *P. bivittalis* and *P. hyalotypa*. Of the extant eleven species of *Pygospila*, we could include data of three species in the phylogenetic studies including our sequences from

India for *P. tyres*.

Since the species *P. tyres* is of economic importance, the present mt DNA Barcode data generated is expected to be helpful in building a reliable DNA barcode library for the country intimated with a voucher specimen and helpful in addressing the taxonomic problems as the morphological characters are cryptic. Interestingly *P. tyres* was described almost 240 years ago from India

Table 1. GenBank details for the mt DNA COI sequences utilized in the construction of the phylogenetic tree.

	GenBank Accession No.	Locality	Species name as per NCBI	Publication details as per NCBI
1	HQ953036.1	Australia: Northern Territory	<i>Pygospila tyres</i>	Unpublished
2	HQ953033.1	Australia: Northern Territory	<i>Pygospila tyres</i>	Unpublished
3	KF392550.1	Australia: New South Wales, Mt. Lewis	<i>Pygospila tyres</i>	Hebert et al. 2013
4	MT776312.1	India: Maharashtra	<i>Pygospila tyres</i>	This study
5	KX862292.1	Pakistan: Kashmir, Peer Chinassi, Azad Kashmir	<i>Pygospila tyres</i>	Ashfaq et al. 2017
6	KT988774.1	Korea	<i>Pygospila tyres</i>	Unpublished
7	HQ990826.1	Pakistan	<i>Pygospila tyres</i>	Unpublished
8	HQ953034.1	Australia: Queensland, Keating Gap, 3 km SW of Cooktown	<i>Pygospila tyres</i>	Unpublished
9	HQ990827.1	Pakistan	<i>Pygospila tyres</i>	Unpublished
10	HQ953035.1	Australia: Western Australia, 18 km from Fitzroy Crossing	<i>Pygospila tyres</i>	Unpublished
11	HQ990825.1	Pakistan	<i>Pygospila tyres</i>	Unpublished
12	HQ990824.1	Pakistan	<i>Pygospila tyres</i>	Unpublished
13	HQ990828.1	Pakistan	<i>Pygospila tyres</i>	Unpublished
14	HQ953031.1	Australia: Queensland, Gordon's Mine, Claudie Riv	<i>Pygospila hyalotypa</i>	Unpublished
15	HQ953030.1	Australia: Queensland, Moses Ck 4km Nby E of Mt. Finnigan	<i>Pygospila hyalotypa</i>	Unpublished
16	HQ953032.1	Australia	<i>Pygospila hyalotypa</i>	Unpublished
17	HQ953029.1	Australia: Queensland, Gap Creek, rainforest	<i>Pygospila bivittalis</i>	Unpublished
18	HQ953028.1	Australia: Queensland, Gap Creek, rainforest	<i>Pygospila bivittalis</i>	Unpublished
19	HQ953027.1	Australia: Northern Territory, Solar Village Humpty Doo	<i>Pygospila bivittalis</i>	Unpublished
20	JX017862.1	Australia	<i>Pygospila tyres</i>	Hains & Rubinoff 2012
21	GU695393.1	Papua New Guinea	<i>Pygospila marginalis</i>	Unpublished
22	KY370911.1	Papua New Guinea: Madang, Mis village	<i>Pycnarmon jaguaralis</i>	Unpublished
23	KF394279.1	Australia: Queensland, Mt. Bartle Frere, east base	<i>Pycnarmon jaguaralis</i>	Hebert et al. 2013
24	KF391152.1	Australia: Queensland, Eddy Bay	<i>Pycnarmon jaguaralis</i>	Hebert et al. 2013
25	MK459732.1	China	<i>Pycnarmon pantherata</i>	Mally et al. 2019
26	KF492066.1	Japan: Chubu, Shizuoka-shi, Honkawane, Kaminagao	<i>Pycnarmon pantherata</i>	Unpublished
27	KF390443.1	Australia: Queensland	<i>Pycnarmon meritalis</i>	Hebert et al. 2013

and now the species is known to have a wide range of distribution in Asia and Australia. Original description of the species *P. tyres* from Coromandel region and our multiple collections from the parts of Deccan plateau and the northern Western Ghats are similar in morphological characters. Genetic homogeneity with mt COI DNA gene studies across the two continents (Asia and Australia) reestablishes the wide distribution across these landscapes.

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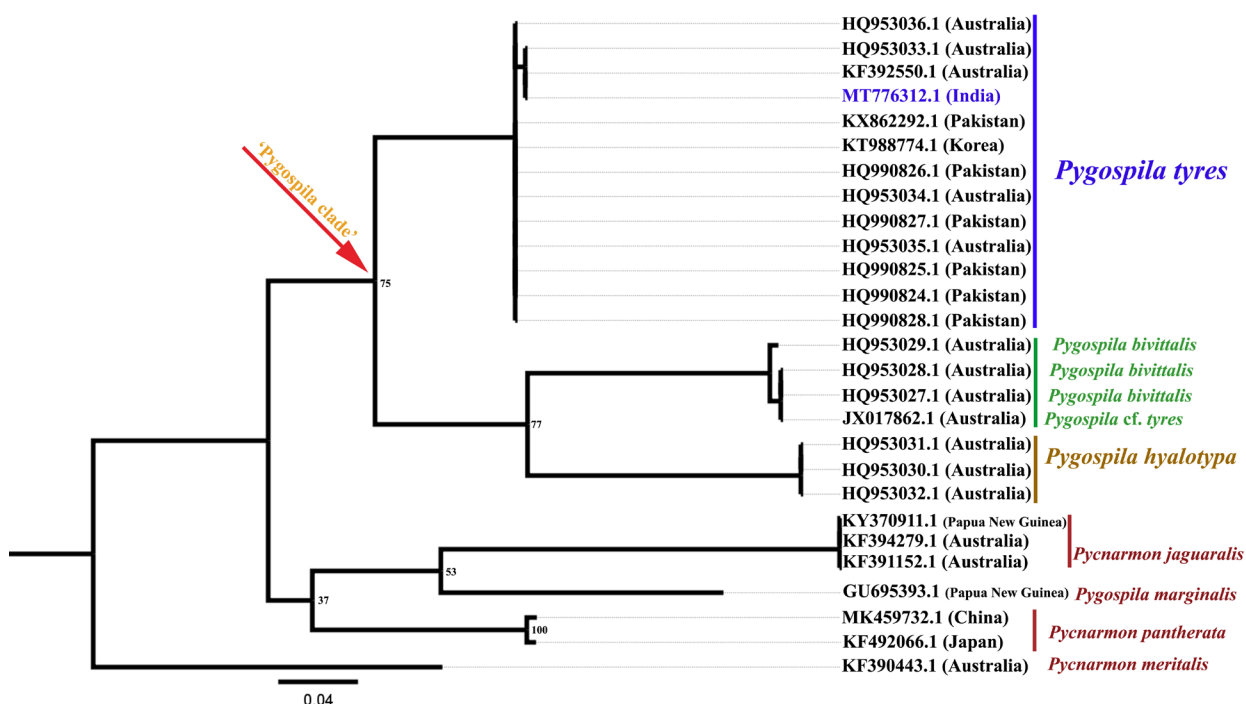


Figure 2. Maximum likelihood (ML) tree for the species of *Pygospila* based on 599 bp of mt COI DNA.

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