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First Indian DNA barcode record for the moth species

*Pygospila tyries* (Cramer, 1780) (Lepidoptera: Crambidae: Spilomelinae) distributed in Asia and Australia

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**Abstract:** The species *Pygospila tyries* was described from the Coromandel region of India about 240 years ago, accommodating in the family Crambidae having immense importance. The species is morphologically cryptic and is known to have 10 extant species under the genus. Earlier mt DNA Barcodes for the species were available from Pakistan, Korea, and Australia, here we report the first barcode of the species from the country of its type locality. Morphological details for the collections with the male and female genitalia are provided for the taxonomic identification. Identities of the mt COI DNA sequences for the genus in the GenBank are discussed.

**Keywords:** *Holarrhena*, host plant, India, Maharashtra, *Wrightia*.

The members of the superfamily Pyraloidea are known to cause crop yield loss between 10 to 100 per cent across the world (Jotwani & Young 2007). Earlier the family Crambidae was originally a part of the family Pyralidae, but separated from it by Munroe (1972). They are of immense economic importance as they are the pest on many agricultural important cash crops like sugarcane and other crops like maize, brinjal, tomato, cabbage, cotton, oil seed, and bamboo (Solis 1997). Most of the crambid moths are morphologically cryptic (cryptic species is a group of individuals that are morphologically identical to each other but belong to different species) and difficult to study. The moths of the subfamily are characterized by the absence of chaetosemata, presence of bilobed subcostal retinaculum in male, praecinctorium fornx tympani projecting and pointed spinula. Corpus bursae in the female genitalia lack signum and gnathos absent (Minet 1981; Solis & Maes 2003; Solis 2007; Kumar et al. 2013).

The genus *Pygospila* was established by Achille Guenée in 1854 and in 1896 Hampson subsequently designated the type species for this genus as *Pygospila tyries* Cramer, 1780, which was included by Guenée (1854) as *Pygospila tyrellasis* Cramer, 1780. Earlier, Hampson (1896) has recognized four species under the genus namely, *Pygospila octormaculis* Moore, 1867; *Pygospila tyries* Cramer, 1780; *Pygospila cuprealis*, (Swinhoe, 1892); *Pygospila costiflexalis*, Guenée, 1863. Further *Pygospila bivittalis* Walker, [1866]; *Pygospila hyalotyta* Turner, 1908; *Pygospila imperialis* Kenrick, 1907; *Pygospila marginalis* Kenrick, 1907; *Pygospila macogastra* Meyrick, 1936; *Pygospila minoralis* Caradja, 1937; *Pygospila yuenanensis* Caradja, 1937 were...
added. As of now a total of eleven species (Hampson 1896; Kenrick 1907; Turner 1908; Carda & Meyrick 1916; Meyrick 1937; Carda & 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.](image-url)
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 25µL 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 Pryr.: 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. Forewings 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, Japan, Malaysia, Myanmar, 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 clad 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.
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 |
|-----------------------|----------|--------------------------|-------------------------------|
| HQ953036.1            | Australia: Northern Territory | Pygospila tyres | Unpublished |
| HQ953033.1            | Australia: Northern Territory | Pygospila tyres | Unpublished |
| KF392550.1            | Australia: New South Wales, Mt. Lewis | Pygospila tyres | Hebert et al. 2013 |
| MT776312.1            | India: Maharashtra | Pygospila tyres | This study |
| KX862292.1            | Pakistan: Kashmir, Peer Chinassi, Azad Kashmir | Pygospila tyres | Ashfaq et al. 2017 |
| KT888774.1            | Korea | Pygospila tyres | Unpublished |
| HQ990826.1            | Pakistan | Pygospila tyres | Unpublished |
| HQ953034.1            | Australia: Queensland, Keating Gap, 3 km SW of Cooktown | Pygospila tyres | Unpublished |
| HQ990827.1            | Pakistan | Pygospila tyres | Unpublished |
| HQ953035.1            | Australia: Western Australia, 18 km from Fitzroy Crossing | Pygospila tyres | Unpublished |
| HQ990828.1            | Pakistan | Pygospila tyres | Unpublished |
| HQ990824.1            | Pakistan | Pygospila tyres | Unpublished |
| HQ990828.1            | Pakistan | Pygospila tyrisses | Unpublished |
| HQ953031.1            | Australia: Queensland, Gordon’s Mine, Claudie Riv | Pygospila hyalotypa | Unpublished |
| HQ953030.1            | Australia: Queensland, Moses Ck 4km Nby E of Mt. Finigan | Pygospila hyalotypa | Unpublished |
| HQ953032.1            | Australia | Pygospila hyalotypa | Unpublished |
| HQ953029.1            | Australia: Queensland, Gap Creek, rainforest | Pygospila bivittalis | Unpublished |
| HQ953028.1            | Australia: Queensland, Gap Creek, rainforest | Pygospila bivittalis | Unpublished |
| HQ953027.1            | Australia: Northern Territory, Solar Village Humpty Doo | Pygospila bivittalis | Unpublished |
| JX017862.1            | Australia | Pygospila tyrisses | Hains & Rubinoff 2012 |
| GU695393.1            | Papua New Guinea | Pygospila marginals | Unpublished |
| KY370911.1            | Papua New Guinea: Madang, Mis village | Pycnarmon jaguaralis | Unpublished |
| KF394279.1            | Australia: Queensland, Mt. Bartle Frere, east base | Pycnarmon jaguaralis | Hebert et al. 2013 |
| KF391152.1            | Australia: Queensland, Etty Bay | Pycnarmon jaguaralis | Hebert et al. 2013 |
| MK459732.1            | China | Pycnarmon pantherata | Mally et al. 2019 |
| KF492066.1            | Japan: Chubu, Shizuoka-shi, Honkawane, Kaminagao | Pycnarmon pantherata | Unpublished |
| KF390443.1            | Australia: Queensland | Pycnarmon meritalis | 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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