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The First Record of *Calvolia summersi* (Mostafa, 1970) (Acari: Winterschmidtiidae) from the Oriental Region and a new record of host association with *Xylocopa* (*Ctenoxylocopa*) *fenestrata* (Fabricius, 1798) with a review on *Xylocopa*-mite associations in India

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Abstract

Mites have long been associated with bees, often showing a close relationship with particular taxa, probably due to a co-evolutionary process. The present study is the first confirmation on the occurrence of the mite species *Calvolia summersi* (Mostafa, 1970) in India and its association with *Xylocopa fenestrata* (Fabricius, 1798), a large carpenter bee species. The mite species was previously reported from Brazil in association with *Zethus*, a neotropical potter wasp genus. *C. summersi* is isolated and studied from different populations of *X. fenestrata* in Sagar Islands, West Bengal, the biggest island of mangrove Sundarban deltaic complex. A literature-based review of the *Xylocopa*-mite associations from India is provided herewith to understand the diversity and pattern of mite species on *Xylocopa* host selection in the country. Instigating a baseline study of *Xylocopa*-mite association is recommended to demark the status of dynamism for better conservation and protection.

Introduction

Islands are very important systems for harboring unique biodiversity around the globe due to the high amount of species endemism, distinctive functional traits, and spectacular evolutionary patterns, such as adaptive radiations or instances of repeating convergent evolution (Kier et al., 2009; Whittaker & Fernández-Palacios, 2007). In addition, island species are particularly vulnerable with rapid decline in biodiversity and exhibiting an alarming rate of extinction (Simberloff, 2000). The island's biodiversity has under significant stress during the past century from invasive alien species, habitat loss and overuse, and, increasingly, from climate change and pollution (Stein et al., 2013). Bees are known to be very efficient insect pollinator and though Indian Sundarbans constitute a very critical ecosystem, studies on bee diversity from the region have gained little attention. Hence, we aimed to conduct surveys to understand the current status of bee fauna and their floral associations from the region. During the ongoing study mite infestation on a specific large carpenter bee i.e., *Xylocopa* (*Ctenoxylocopa*) *fenestrata* (Fabricius, 1798) was observed from Sagar Island, West Bengal. Large carpenter bees are characterized with the large body size with higher flight range and act as a fundamental resource to the mangrove ecosystem delivering essential pollination services; *X. fenestrata* is known to be an integral component of pollination biology of subtropical crop and non-crop plants as it has a comparatively longer activity period ranging from early March to November covering a wide seasonal variation and also because of its higher foraging rate (Sihag, 1993; Rahman & Deka, 2011; Ali et al., 2017; Chakraborti et al., 2019; Kachhawa et al., 2020).

The genus *Calvolia* Oudemans, 1911 was previously known by a single species from India, i.e., *C. bakeri* Hughes, 1962 collected from jute leaves (Hughes, 1962). Our present study confirms the occurrence of *Calvolia summersi* (Mostafa, 1970) in India (Baker & Wharton, 1952). Historically, the species was found in the Amazonas State of Brazil in association with more specialized subgenera of neotropical predatory potter wasp genus *Zethus* Fabricius, 1804. This, by showing a host change to the bee species *Xylocopa* in Indian Mangroves, mostly implies their ascendancy over conspecific groups.

Most hymenopterans with an established social structure, fundamentally host a diverse spectrum of mite species (Eickwort, 1990). Still, the diversity of mite species associated with large carpenter bees is comparatively lower (Krantz, 1998). Their large size and pattern of keeping food contents with high protein and energy in their nests make them a preferred target for natural enemies. Thus, they have a history of hosting a diverse spectrum of natural enemies (Gerling et al., 1989). At present, no study has evaluated the distribution patterns of phoretic mites on large carpenter bees nor the reasons that might be influencing such association in India. To understand and summarize the host range of large carpenter bees with their associated mite species, a literature-based scrutiny on the history of the *Xylocopa*-mite associations from India is done and augmented herewith.

Materials and methods

Study area, specimen collection, and identification

Sundarban is one of the major mangrove ecosystems with riverine regions comprising different rivers, streams, and creeks. Indian Sundarbans, being the world's largest mangrove delta, harbors a variety of flora and fauna in its significant habitat. Despite being under protection, the Indian Sundarban was tagged as endangered in an assessment done by the IUCN Red List of Ecosystems framework in 2020 (Sievers et al., 2020). The Sundarban forest deltaic complex in the Indian part is stretched over 4260 km^2 situated between 21°56′42″N and 88°53′45″E. Sagar Island, located at 21°39′10″N and 88°04′31″E, is the largest island of the Sundarban Ganges-deltaic complex (Das et al., 2021).

Surveys were conducted once a month at six localities and their adjoining areas in Sagar Island between January and October 2018, covering pre and post-monsoon seasons. Actively targeted sampling for bees was carried out in the field, employing flower insect timed count method (FIT count) (Figure 1; Table 1). A random transect walk for a day (7:00 AM to 5:00 PM) on different habitat types at each of the six sites were carried out once a month for the entire duration of the study to document both the bees and the visited plants (Nielsen et al., 2011). In total, 600 hours of transect walk effort (6 sites x 10 x 10) were commenced. Samplings were conducted on multiple available transects to cover more spatial extent at each site. Individuals were caught on air while foraging on flowers with the help of insect nets and from their respective nest burrows using transparent glass bottles. Net sweeping were only commenced on encountering the *Xylocopa* individuals. After collection, they were scanned, and individuals with confirmed presence of potential mite load were euthanized using a killing jar (ethyl acetate fume). Geo-coordinates were recorded using Garmin Etrex-30 handheld GPS. The specimens were then preserved in 90% ethanol and returned to the laboratory. High-resolution images were taken of the *Xylocopa* specimen using Leica M205 A stereo microscope. Mite specimens were temporarily mounted in Lactic Acid for study, and photographs were taken using a Nikon H600L microscope. The *Xylocopa* specimens were identified following the keys of Maa (1938) and Michener (2007). All collected mite specimens were identified following the references of Krantz (1970) and Mostafa (1970). The identified materials are deposited in the Zoological Survey of India, HQ (Accession code: 6989/17). Descriptive terminology follows Krantz (1970). QGIS v.2.4.0 was used to prepare the study site map (Quantum GIS Development Team 2019). The *Xylocopa*-mite interaction network (non-weighted) plot was prepared using the 'Bipartite' package (Dormann, 2020) in R 4.2.2 statistical data processing packages (R Core Team, 2022).

Results and Discussion

Encountered specimens of Xylocopa at Sagar Island

We encountered two species of large carpenter bees in our study site *viz*. *Xylocopa* (*Koptortosoma*) *pubescens* Spinola, 1838 (on *Calotropis gigantean* (L.) Dryand., *Solanum melongena* L., *Acanthus ilicifolius* L., *Baccharis* sp.) and *Xylocopa* (*Ctenoxylocopa*) *fenestrata* (Fabricius, 1798) (on *Eucalyptus* sp., *Justicia gendarussa* Burm.f., *Solanum melongena* L., *Helianthus annuus* L.) (Fig 3. J, K). In our study site, we found only *X. fenestrata* (comparatively bigger in size than *X. pubescens*) associated with *Calvolia summersi*. *Xylocopa* specimens were collected from different habitats on flowers and from nests on *Eucalyptus* sp. and man-made bamboo structures from which mite specimens were isolated and studied. In total, 21 individuals (17 ♀, 4 ♂) of *Xylocopa* (*Ctenoxylocopa*) *fenestrata* (Fabricius, 1798) were recorded from the six sites in the current study out of which all 17 ♀ specimens were found infested with mites. The heavy infestation of *C. summersi* to different appendages of the whole body of females of *X. fenestrata* from the study site is the first confirmed occurrence of *Xylocopa*-*Calvolia* interaction, but it is subject to future study to verify whether the symbiotic association is of mutualistic or predatory nature.

Taxonomic details of the recorded mite specimens

Family: Winterschmidtiidae Oudemans, 1923

Genus: Calvolia Oudemans, 1911

Calvolia summersi (Mostafa, 1970) (Figure 2. A–C)

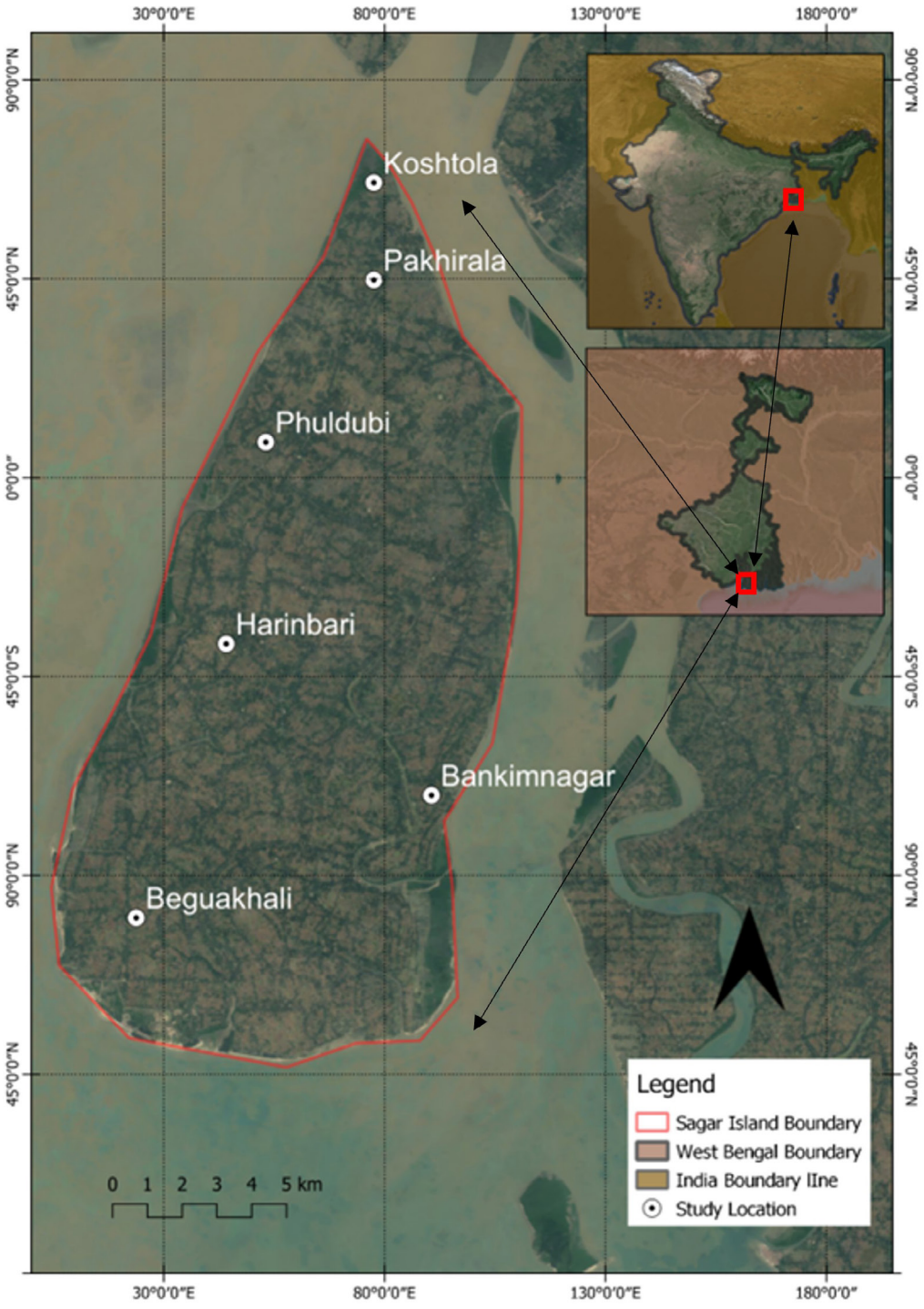

Fig 1. Map of the Sagar Island, West Bengal, India. Locality details of the surveyed sites are summarized in Table 1.

Fig 2. *Calvolia summersi* (Mostafa, 1970). A. Dorsal view, B. Ventral view, C. Hysterosomal shield.

Table 1. Sampling locations in Sagar Island, West Bengal, during 2018-2019.

Locality	Co-ordinates	Elevation	Habitat type
Koshtola	N21.873°, E88.131°	7m	Residential gardens with human settlement.
Beguakhali	N21.669°, E88.065°	3m	Agricultural land and village settlements.
Bankimnagar	N21.703°, E88.147°	4m	Small scale farming.
Harinbari	N21.745°, E88.09°	4m	Sunflower fields, betel leaf farming, gardens with ornamental flowers, human settlement.
Phuldubi	N21.801°, E88.101°	6m	Small-scale agricultural fields, residential gardens, human habitation.
Pakhirala	N21.846°, E88.131°	7m	Small-scale agricultural fields and residential gardens with ornamental flowers, village settlement.

Diagnosis

Opaque, essentially unsclerotized saprophagous mite. Soft-bodied, often with a prodorsal shield, sejugal furrow present; chelicerae chelate-dentate. The hysterosomal shield covers the whole of the dorsal surface and, anteriorly, is reflected onto the ventral side. Tarsi of legs usually with fleshy pulvillus. The tarsi have no digitiform or other spines.

Species identification (Morphological description)

Dorsum – Body orbicular, smoothly rounded posteriorly, gradually narrows anteriorly to form protruding rostrum; eyes are contiguous; the furrows on the hysterosomal shield run freely in a longitudinal direction giving rise to wrinkled rugosities on the hysterosomal plate.

Venter –Two pairs of equally long gnathosomal setae placed in apical position on idiosoma shorter than vertical setae. Pretarsalapodeme swollen at the free end. Each apodeme I has a tiny triangular blade on its free end. Apodemes III slightly arched and joined together in midline without projecting apophyses.

Legs – Leg IV is much shorter than leg III. Tarsi I bears only two leaf-shaped setae near its distal end, tarsi II to IV have no such flattened setae, largest seta on tarsi III arises laterally on the distal end.

Distribution: India (**new record**); elsewhere: Brazil

Hosts*:* Wasp of the genus *Zethus* Fabricius, 1804; large carpenter bees – *Xylocopa* (*Ctenoxylocopa*) *fenestrata* (Fabricius, 1798) association is reported for the first time.

Host association level: The biological perspective of such association is still limited. A mite-bee relationship could show the means to different interactions, ranging from negative impacts on the host bees to the potential benefits to them. Further study can shed light on the extent of their association and assess their possible role in commensalism or as a carrier of parasites. Host associations: Female carpenter bees of the genus *Xylocopa* are well known for having a metasomal acarinarium of various levels of specialization (Eardley, 1983; Klimov & Oconnor, 2008). Here we recorded the mite presence on the different segments of the bee body, specifically in the head (occiput and proboscidial fossa), legs (pro and meso femur), pronotum, thorax (scutum, forewing base, propodeal pit: metathorax acarinarium), uppers surface of both wings, except the face (Fig 3. A–J).

Biodiversity in islands is qualified as of hotspot component and subject to studies on a unique level of organismal diversity, fundamentally categorical of comparatively lower species richness (Whittaker & Fernández-Palacios, 2007; Russell & Kueffer, 2019), and thus implications for monitoring bee population and health assessment is imperative to conserve the pollination services for the native pollinators and flowering plants (Crichton et al., 2018). Studies have shown that wood-boring bees have a relatively higher dispersal rate than other bees based on their functional traits (wood nesting types and body size) among islands (Poulsen & Rasmussen, 2020); carpenter bees are referred as their nesting habits are divided into two sister groups, *Xylocopa* spp. known as large carpenter bees and *Ceratina* spp. as small carpenter bees denoting their body sizes. Several studies have given conclusive evidence on *Ceratina* mite infestations (Klimov et al., 2007a; Klimov & Oconnor, 2007b; Vickruck et al., 2010); albeit during our present study we encountered three *Ceratina* species *viz*. *Ceratina* (*Ceratinidia*) *hieroglyphica* Smith, 1854, *Ceratina* (*Pithitis*) *binghami* Cockerell, 1908 and *Ceratina* (*Ceratinidia*) sp. but none of them showed mite associations. Hence, proving the host specificity of *C. summersi* towards *X. fenestrata* species from the study site. Therefore, further measures to be taken to assess the mite infestation on *Xylocopa* in Sundarban mangroves which has ample opportunity of consequential sporadic distribution of the mite species among mainland bee populations with opportunistic co-evolutionary host shifts in randomized pattern (Klimov et al., 2007b; Haas et al., 2019). *X. fenestrata* is a very important pollinator of both crop and non-crop plants (Sihag, 1993; Dorjay et al., 2017; Kumar et al., 2019; Layek et al., 2021), and mite infestation decreases pollen carrying capacity and hence diminishes potential pollinating efficiency. We recorded fewer pollen grains in heavily-infested female bees (Fig 3. E). This raises concerns regarding the adverse effects and limitations of severe mite infestation on the pollination services of large carpenter bees and on the provisioning efficiency for their forthcoming progeny. *X. fenestarata* are known for their nest guarding nature where multiple females share a common nest space allotted for their offspring and thus while foraging their nests does not remain unguarded (Kapil & Dhaliwal, 1968).

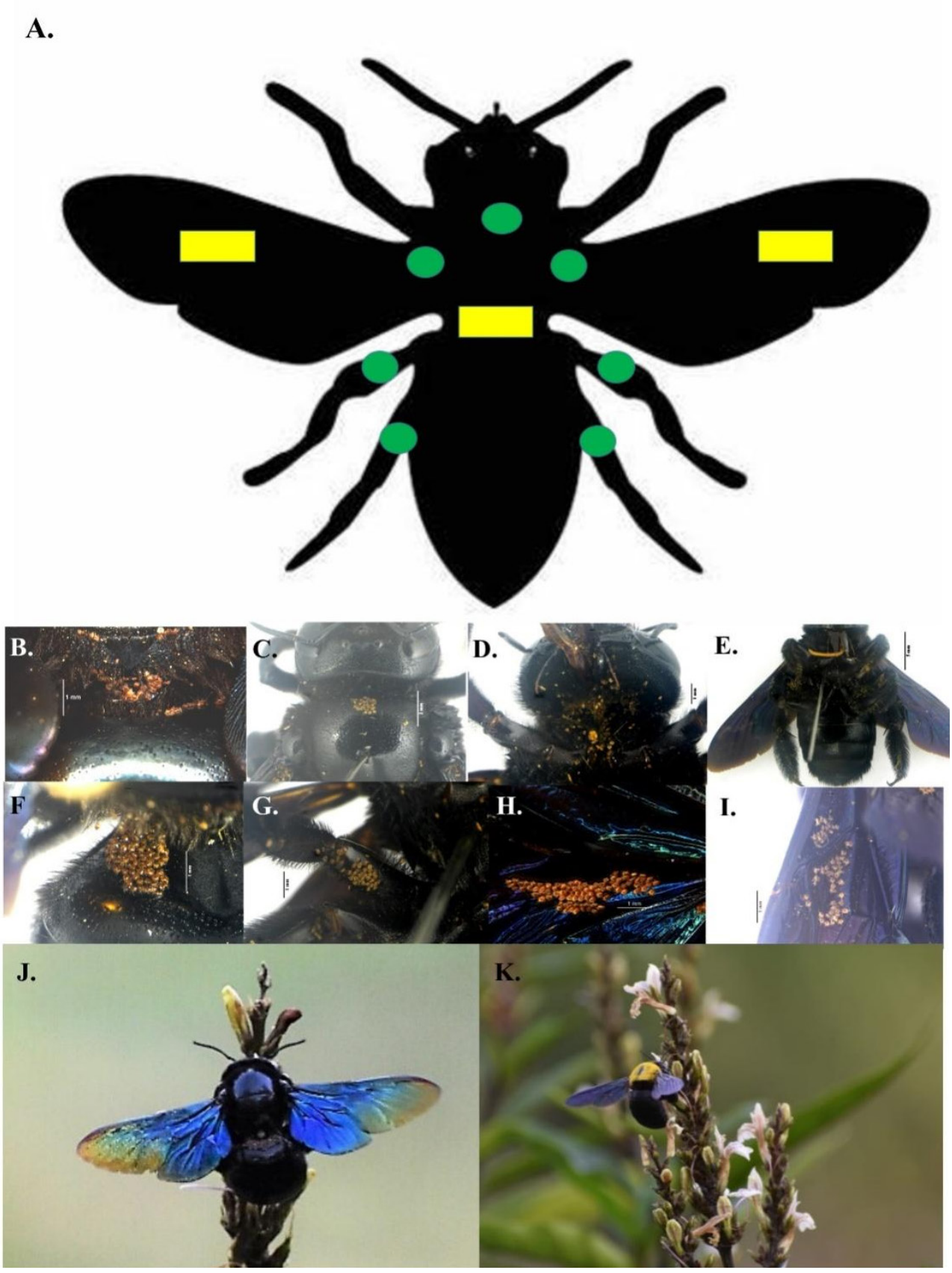

Fig 3. *Calvolia summersi* attachments in *X. fenestrata* body A. Diagrammatic representation of the level of mite attachment in bee body: yellow denotes higher abundance, and green denotes a lower number of individual attachments. B. dorsal view of acarinarium, C. dorsal view of thorax, D. ventral view of the occiput and proboscidial fossa, E. ventral view of sternal segments and legs, F-G. attachments in pro and meso femur, H. dorsal view of the forewing, I. dorsal view of hind wing, J. *X. fenestrata* in a natural habitat with mite load in the metathorax, K. *X. pubescens* in *Justicia gendarussa* in the study site. (Photo courtesy. A–I. D. Ghosh; J–K. Swati Das)

The exclusivity of the *C. summersi* association with female bees give speculative indication of the mite ecology association with nest ecosystem. Pre-monsoon seasons in our study site is characterized with higher temperature (37.18 ± 3.79 °C) than the post monsoon seasons (34.23 ± 2.16 °C). Mite infestations were found on bees collected in pre monsoon seasons which is referred as summer in local climate; hence, it is suggestive that increased temperature might play a pivotal role in sporadic spreading of the mite species.

A review of the literature to explore the diversity of mite species on different large carpenter bees from India revealed nine species of *Xylocopa* from six subgenera associated with 26 species of mites belonging to nine genera from five families. The study shows that five genera and seven mite species are associated with *Xylocopa* (*Platynopoda*) *tenuiscapa* Westwood, 1840 which exhibits the highest host range followed by *Xylocopa* (*Ctenoxylocopa*) *fenestrata* (Fabricius, 1798) with six species belonging to two genera. Interestingly, the genus *Cheletophyes* Oudemans, 1914 (family Cheyletidae) exhibits particular host preference towards the bee species *Xylocopa* (*Ctenoxylocopa*) *fenestrata* with five species followed by *Xylocopa (Koptortosoma) pubescens* Spinola, 1838 with two species (Fig 4; Table 2). The host specificity of *Cheletophyes* is already studied in African large carpenter bees and a positive correlation been established (Klimov et al., 2006) which suffices with our present investigation outcomes from India. Genus *Sennertia* (family Chaetodactylidae) also exhibits a wide range of host selection having association with six *Xylocopa* species from India.

Regardless of rising concerns over the conservation of pollinator bees, recent scrutiny has revealed a dearth of detailed studies regarding the association of mite symbionts, as well if there is any inclination on host ranges of specific or random preferences which gives direction on certain dynamics of shaping the drivers of their community structure. Understanding the mite host selection dynamism in relation to their geography shall open exclusive avenues to relate the underlying ecological perspective and thus helping in future conservational approaches. To the best of our knowledge, the present study is the first to report the bee-mite association from the Indian Sundarban mangrove region. The present detection of bee-mite association is an initial work requiring future studies for detailed exploration and to increase the existing knowledge gap on bee health assessment.

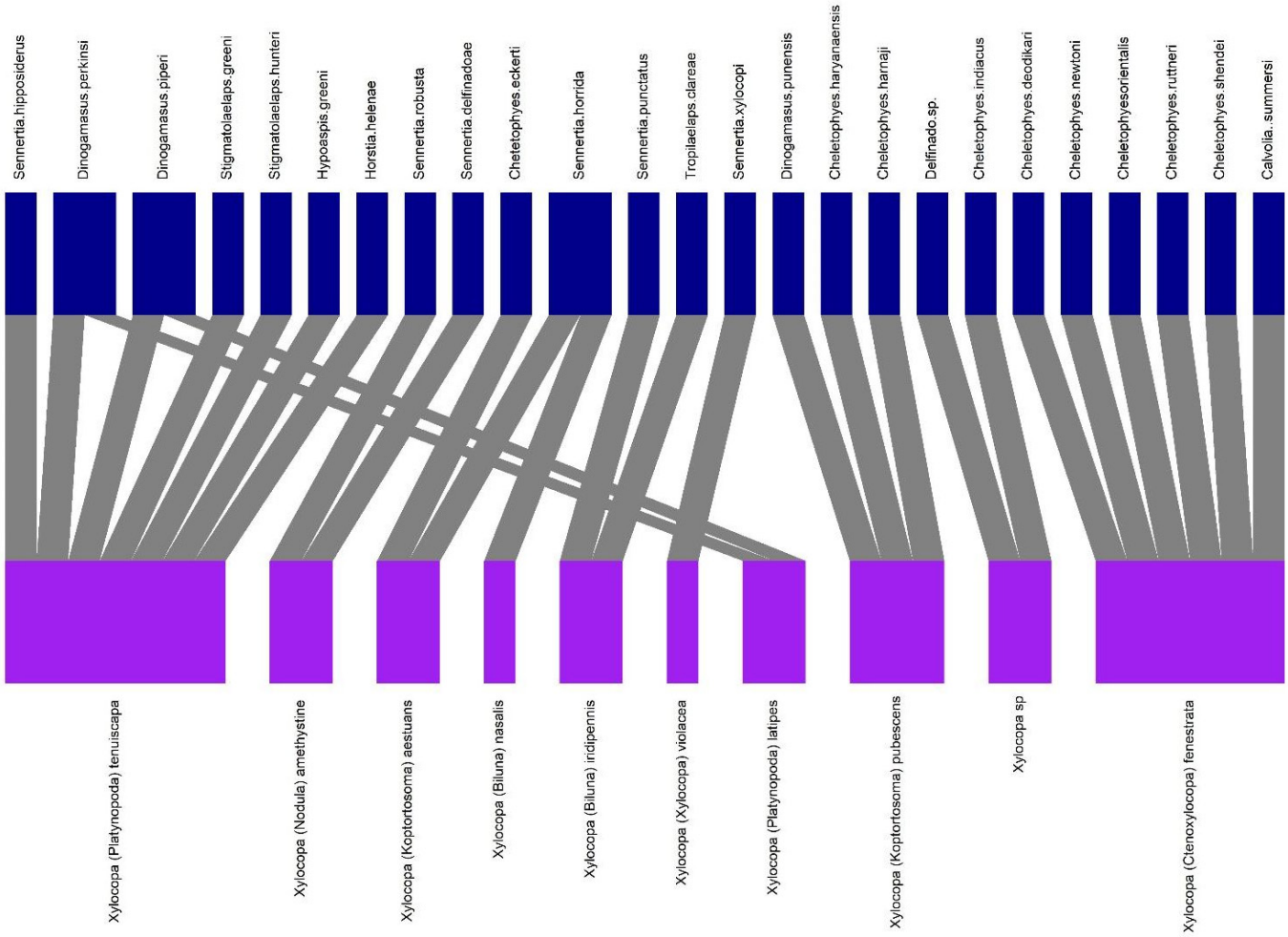

Fig 4. *Xylocopa*-mite interaction network (non-weighted) from historical records obtained from literature review (1900-2022) in India. The lower node denotes bee species and the upper node denotes associated mite species.

Table 2. Overview of Genus *Xylocopa* (Apidae: Xylocpini) and mite association by literature reviews from India.

Sl no.	*Xylocopa* species	Mite species	Family	Location (State, area)	Association type	Special comments	Reference
	Xylocopa (*Platynopoda*) *tenuiscapa* Westwood, 1840	*Sennertia hipposiderus* (Oudemans, 1902)	Chaetodactylidae	India	Phoretic	Association through abdominal acarinarium	Oconnor, 1993
	Xylocopa (*Nodula*) *amethystine* (Fabricius, 1793)	*Sennertia robusta* Delfinado and Baker, 1976 (= *Sennertia carpenteri* Ramaraju and Mohanasundaram, 2001)	Chaetodactylidae	Coimbatore, Tamil Nadu	Phoretic	This small, pale yellow to brownish orange-colored mites were attached individually on the head, in between antennae and compound eyes on the large carpenter bee. Additional examples were observed at the basal pleural joints of the wings and their bases.	Ramaraju & Mohanasundaram (2001)
	Xylocopa (*Nodula*) *amethystine* (Fabricius, 1793)	*Sennertia delfinadoae* Fain, 1981 (= *Sennertia bakeri* Ramaraju and Mohanasundaram, 2001)	Chaetodactylidae	Coimbatore, Tamil Nadu	Phoretic	--	Ramaraju & Mohanasundaram (2001)
	Xylocopa (*Koptortosoma*) *aestuans* (Linnaeus, 1758) [=*Xylocopa leucothorax* (DeGeer, 1773)]	*Sennertia horrida* - Vitzthum, 1912 (= *Sennertia leucothorae* Ramaraju and Mohanasundaram, 2001)	Chaetodactylidae	Coimbator, Tamil Nadu; Idukki, Kerala	Phoretic	--	Ramaraju & Mohanasundaram (2001); Klimov et. al., 2007b
	Xylocopa (*Biluna*) *nasalis* Westwood, 1838	*Sennertia horrida* (Vitzthum, 1912)	Chaetodactylidae	India	--	--	Okabe et al., 2010
	Xylocopa (*Biluna*) *iridipennis* Lepeletier, 1841	*Sennertia punctatus* Sarangi, Gupta & Saha, 2013	Chaetodactylidae	Kolkata, West Bengal	Deutonymph; phoretic	--	Sarangi et al., 2014
	Xylocopa (*Xylocopa*) *violacea* (Linnaeus, 1758)	*Sennertia xylocopi* Sarangi, Gupta & Saha, 2013	Chaetodactylidae	Kolkata, West Bengal	Deutonymph; phoretic	--	Sarangi et al., 2014
	Xylocopa (*Platynopoda*) *latipes* (Drury, 1773); *Xylocopa* (*Platynopoda*) *tenuiscapa* Westwood, 1840	*Dinogamasus piperi* Le Veque, 1930	Laelapidae	India	Association with abdominal pouch	In *X. pubescens Dinogamasus* feed on surface exudates of bee immatures or on fungi associated with these exudates (Skaife, 1952)	Andhale et al., 2020
	Xylocopa (*Platynopoda*) *tenuiscapa* Westwood, 1840; *Xylocopa* (*Platynopoda*) *latipes* (Drury, 1773)	*Dinogamasus perkinsi* (Oudemans 1901)	Laelapidae	India	--	This species was originally described from the abdominal acarinarium of *X. tenuiscapa* from Java and India	Oudemans, 1901; Leveque, 1930; Oconnor, 1998; Lundqvist, 1999; Attasopa et. al., 2021
	Xylocopa (*Biluna*) *iridipennis* Lepeletier, 1841	*Tropilaelaps clareae* Delfinado & Baker, 1961	Laelapidae	Jammu & Kashmir, India	Parasitic association	*A. mellifera* is primarily the host of the mite species, considered a major threat to bee-keeping industry.	Abrol, 1996
	Xylocopa (*Koptortosoma*) *pubescens* Spinola, 1838	*Dinogamasus punensis* Andhale, Pai, Pai, Pandit, 2020	Laelapidae	Pune & Mahrashtra	--	Captured from eggplant flower (*Solanum melongena* L.)	Andhale et al. 2020

Table 2. Overview of Genus *Xylocopa* (Apidae: Xylocpini) and mite association by literature reviews from India. (Continuation)

Sl no.	*Xylocopa* species	Mite species	Family	Location (State, area)	Association type	Special comments	Reference
	Xylocopa (*Platynopoda*) *tenuiscapa* Westwood, 1840	*Stigmatolaelaps greeni* (Oudemans, 1903)	Laelapidae	India	Phoretic	Association through abdominal acarinarium	Krantz 1998
	Xylocopa (*Platynopoda*) *tenuiscapa* Westwood, 1840	*Stigmatolaelaps hunteri* Krantz, 1998	Laelapidae	India	Phoretic	Association through abdominal acarinarium	Krantz 1998
	Xylocopa (*Platynopoda*) *tenuiscapa* Westwood, 1840	*Hypoaspis greeni* Oudemans, 1902	Laelapidae	India	--	Association through abdominal acarinarium	Oconnor 1998
	Xylocopa sp.	*Delfinado* sp.	Delfinadoidae	Jammu and Kashmir	Ectoparasitic	--	Putatunda & Abrol, 2003
	Xylocopa (*Platynopoda*) *tenuiscapa* Westwood, 1840	*Horstia helenae* (Oudemans, 1902)	Acaridae	India	--	--	Oudemans, 1902
	Xylocopa sp.	*Cheletophyes indiacus* Smiley and Whitaker, 1981 (= *Cheletophyes xylocopae* Ramaraju & Mohanasundaram, 1999)	Cheyletidae	Tamil Nadu	Host specific mutualistic association.	Attached in hairs on Leg	Smiley & Whitaker, 1981; Ramaraju & Mohanasundaram, 1999
	Xylocopa (*Ctenoxylocopa*) *fenestrata* (Fabricius, 1798)	*Cheletophyes deodikari* Putatunda & Kapil, 1989	Cheyletidae	Haryana	--	--	
	Xylocopa (*Ctenoxylocopa*) *fenestrata* (Fabricius, 1798)	*Cheletophyes newtoni* Putatunda & Kapil, 1989	Cheyletidae	Haryana	--	--	
	Xylocopa (*Ctenoxylocopa*) *fenestrata* (Fabricius, 1798)	*Cheletophyes orientalis* Putatunda & Kapil, 1989	Cheyletidae	Haryana	--	--	
	Xylocopa (*Ctenoxylocopa*) *fenestrata* (Fabricius, 1798)	*Cheletophyes ruttneri* Putatunda & Kapil, 1989	Cheyletidae	Haryana	--	--	
	Xylocopa (*Ctenoxylocopa*) *fenestrata* (Fabricius, 1798)	*Cheletophyes shendei* Putatunda & Kapil, 1989	Cheyletidae	Haryana	--	--	Putatunda & Kapil, 1989
	Xylocopa (*Koptortosoma*) *pubescens* Spinola, 1838	*Cheletophyes harnaji* Putatunda & Kapil, 1989	Cheyletidae	Haryana	--	--	
	Xylocopa (*Koptortosoma*) *pubescens* Spinola, 1838	*Cheletophyes haryanaensis* Putatunda & Kapil, 1989	Cheyletidae	Haryana	--	--	
	Xylocopa (*Koptortosoma*) *aestuans* Linnaeus, 1758)	*Chetetophyes eckerti* Summers & Price, 1970	Cheyletidae	Ludhiana	--	--	Summers and Price (1970)
	Xylocopa (Ctenoxylocopa) fenestrata (Fabricius, 1798)	*Calvolia summersi* Mostafa, 1970	Winterschmidtiidae	Sundarban,West Bengal	--	--	**Present study**

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