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Identity of cavity nesting honey bees of the Indian subcontinent with a description of a new species (Hymenoptera, Apidae, Apinae, Apini, *Apis*)

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ABSTRACT: A new species of cavity nesting honey bees, *Apis karinjodian* **n. sp.,** endemic to the Western Ghats biodiversity hotspot is described and illustrated. *Apis indica* Fabricius, 1798 status restored is resurrected from synonymy with *Apis cerana* Fabricius, 1793. Key to distinguish the three native cavity nesting honey bee species occurring in the Indian subcontinent *viz*., *Apis cerana* Fabricius, 1793, *Apis indica* Fabricius, 1798 and *Apis karinjodian* **n. sp.** is provided. Distribution map is given for the native cavity nesting *Apis* species of the Indian subcontinent. The morphological description of the new species is supplemented with molecular and behavioral information. Radio-Medial Index (RMI), a new measure for species discrimination in *Apis,* is proposed. South India is proposed as the center of origin of the European honeybee, *Apis mellifera* Linnaeus, 1758.

KEY WORDS: *Apis indica, A. cerana, A. karinjodian* **n. sp**., distribution, DNA barcode, Radio-Medial Index

INTRODUCTION

The honey bees of the genus *Apis* Linnaeus, 1758 are far the most famous of all insects owing to their production of honey, pollination of crop plants and advanced eusocial behaviour, which has attracted much attention from biologists. Unfortunately, the systematics of this small and highly visible group is not clearly understood, partly owing to the high levels of intraspecific variation as well as the recent divergence of taxa (Engel, 2002).

Beekeeping has been practiced since time immemorial in India. The honey bees (Apini) occurring in India include the single comb building giant honey bees (subgenus *Megapis* Ashmead, 1904): *A. dorsata* Fabricius, 1793 and *A. laboriosa* Smith, 1871; dwarf honey bees (subgenus *Micrapis* Ashmead, 1904): *A. florea* Fabricius, 1787 and *A. andreniformis* Smith, 1857; multiple parallel comb building cavity-nesting honey bees (subgenus *Apis* Ashmead, 1904): *Apis mellifera* Linnaeus,

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1758, *A. cerana* Fabricius, 1793; *A. indica* Fabricius, 1798 and *A. karinjodian* **n. sp.**

Several species of the stingless honey bee (Meliponini) genera such as *Lepidotrigona* Schwarz, 1939, *Lisotrigona* Moure, 1961, and *Tetragonula* Moure, 1961 also occur in India (Rasmussen, 2013; Shanas and Faseeh, 2019). The European honey bee *A. mellifera* was introduced and successfully established in India during the 1960's (Mishra, 1995).

Two distinct colour morphs, yellow in plains and black in hills have been recognised among cavity nesting honey bees from India by several workers (Smith and Hagen, 1996; Oldroyd *et al.*, 2006; Chalapathy *et al.* 2014a, b; Baskaran, 2016; Gaikwad *et al.*, 2019 and the references therein). Oldroyd *et al.* (2006) provided evidence for the reproductive isolation between the yellow plain and black hill colour morphs in south India and concluded that the yellow plain bees of India could be regarded as a separate species from *A. cerana* based on non-overlap of drone flight times and occurrence of consistently different mitochondrial haplotypes. Lo *et al.* (2010) also supported the recognition of *Apis indica*, the Plains Honey Bee of south India, as a separate species from *A.cerana*.

The natural range of *A. cerana* is spread across temperate and tropical Asia from Afghanistan to Japan, north into the foothills of the Himalayas and south through Indonesia (Koetz, 2013). Radloff *et al.* (2010) revised the taxonomy of *A. cerana* and divided the *Apis cerana* complex into six morphocluster groups based on physiographic and climatic factors, wherein the "Indian Plains cerana" (Morphocluster III) was mentioned to occur across the plains of central and southern India and Sri Lanka as a '*fairly uniform population*' in the Indian subcontinent. They also gave detailed summary of 40 synonymous specific and infra-specific names and pointed out that the former subspecies trinomials such as *Apis cerana indica* no longer have any official, nomenclatural standing in *Apis* classification under the rules of the International Code of Zoological Nomenclature (ICZN, 4th Edition, 1999). However, the subspecific epithet *Apis cerana indica* is still in vogue for want of clarity on the species status of *Apis indica* Fabricius, 1798 (Otis and Smith, 2021).

The present study is an attempt to streamline the taxonomy of native cavity nesting honey bees of the Indian subcontinent.

MATERIALS AND METHODS

The study is based on a collection of native cavity nesting honey bees from different locations in India. Permanent microscope slides of wings were prepared. The wings were separated from dried specimens, passed through ethyl alcohol series (70-100%), dipped in clove oil for 30 minutes and mounted in DPX mountant. Images of wings were processed using Adobe Photoshop and images of body features were processed using Adobe Photoshop and Zerene stacker. Habitus images (Fig. 1) were taken using a Nikon D200 camera and processed using Adobe Photoshop.

The type specimen of *A. indica* is lodged at the Copenhagen collection (NHMD). Zimsen (1964) mentioned one specimen in the "Kiel collection" and two specimens in "Copenhagen collection". The first specimen (NHMD 308727), headless, which carries a label reading "*indica*" is the original Fabrician type in "Kiel collection" which is presently lodged in "Copenhagen collection" (Figs. 2A, B). Among the two non-types of *A. indica*, probably from the "Sehested-Tønder Lund collection" lodged in the Copenhagen collection, the first (NHMD308728) bears a label: *a: indica* / ex ind: or: ed lap / b: fro: Daldorff (Fig. 2C) and the second (NHMD308729) bears no label (Fig. 2D). The Lectotype of *Apis cerana* Fabricius, 1793 from China, designated by Moure, 1958 in Zimsen, 1964 (ZMUC 00241552), is also lodged at the Copenhagen collection (Figs. 2E–F). Images of both the name bearing types were examined.

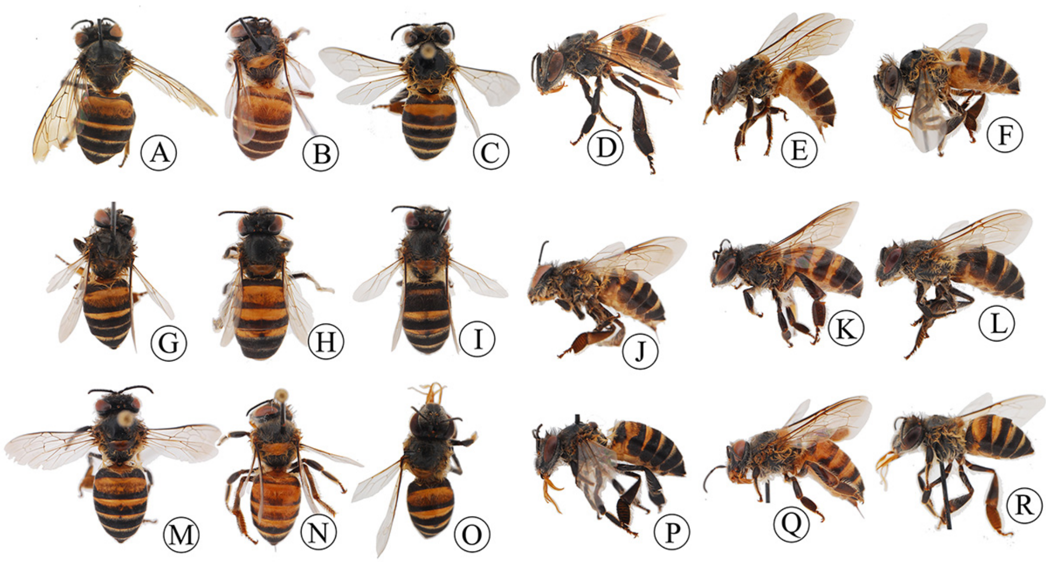

Fig. 1 Workers: A – C dorsal habitus and D – F lateral habitus of *Apis cerana* Fabricius, 1793; G – I dorsal habitus and J – L lateral habitus of *Apis karinjodian* **n. sp**.; M – O dorsal habitus and P – R lateral habitus of *Apis indica* Fabricius, 1798

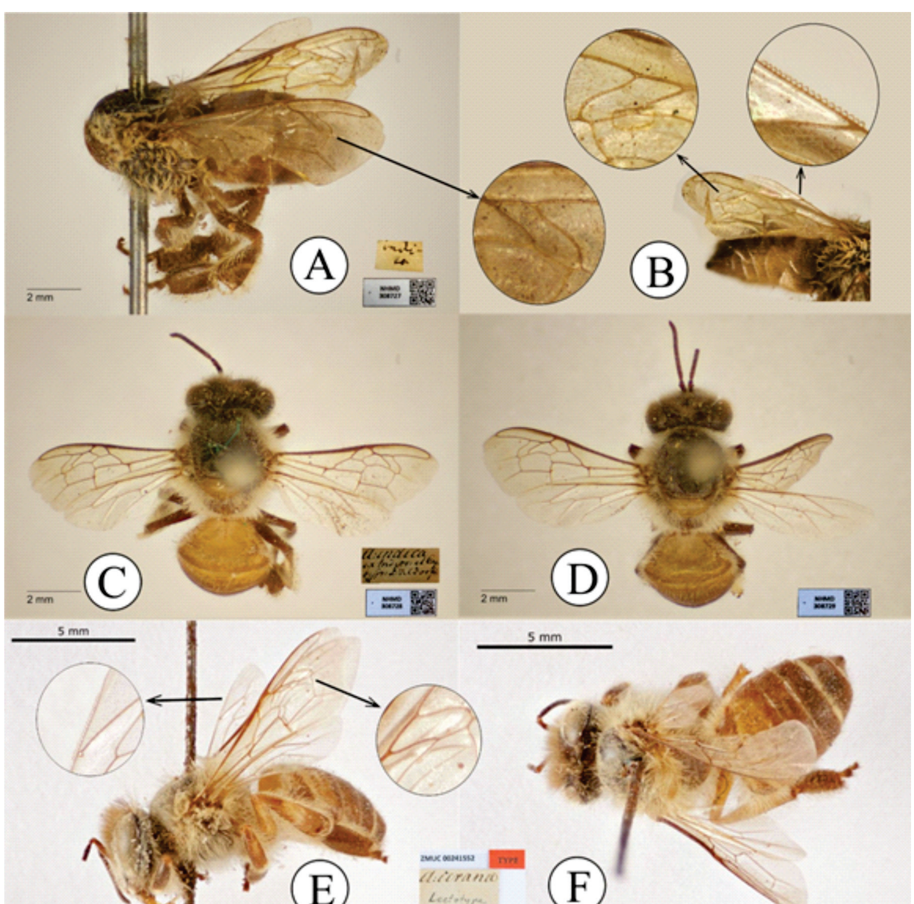

Fig. 2 Worker: A, B. type of *Apis indica* Fabricius, 1798, lateral habitus; C, D. non-types of *A. indica* Fabricius, 1798, dorsal habitus; E, F - lectotype of *A. cerana* Fabricius, 1793 lateral and dorsal habitus; inserts illustrating third submarginal cell and wing hamuli

Uneverted endophallus was dissected and isolated from drones preserved in ethanol (90%). Species identifications were confirmed using morphological characters and molecular genes. Baseline distribution maps were prepared based on collected specimens, published records, DNA barcode sequences from collected specimens as well as NCBI GenBank public database (Table 2).

The images of the type specimens of *A. indica* (Figs. 2A, B) and *A. cerana* (Figs. 2E, F) were studied. The Radio-Medial index (RMI), based on the ratio of veins c/b of the forewing is proposed as a new measure for species discrimination in addition to the widely used Cubital Index a/b (Fig. 3H). The RMI was found robust and foolproof in discriminating populations of *A. indica* and *A. cerana* and the same has been here used to resurrect *Apis indica* Fabricius, 1798 from synonymy with *A. cerana* Fabricius, 1793. The RMI and CI (Table 1) were determined from the type image of *A. indica* and *A. cerana* by superimposing a fine micrometer scale on the image of forewings. It was observed that, although the wing image may get distorted to some extent due to the imaging angle, the values of RMI takes care of such minor distortions [eg: Table 1; 4.0 (RW) and 3.8 (LW) for *A. indica*].

To determine the RMI and CI ratios, forewing of workers of *A. indica* from different states *viz*. Karnataka, Kerala, Nagaland, Odisha, West Bengal

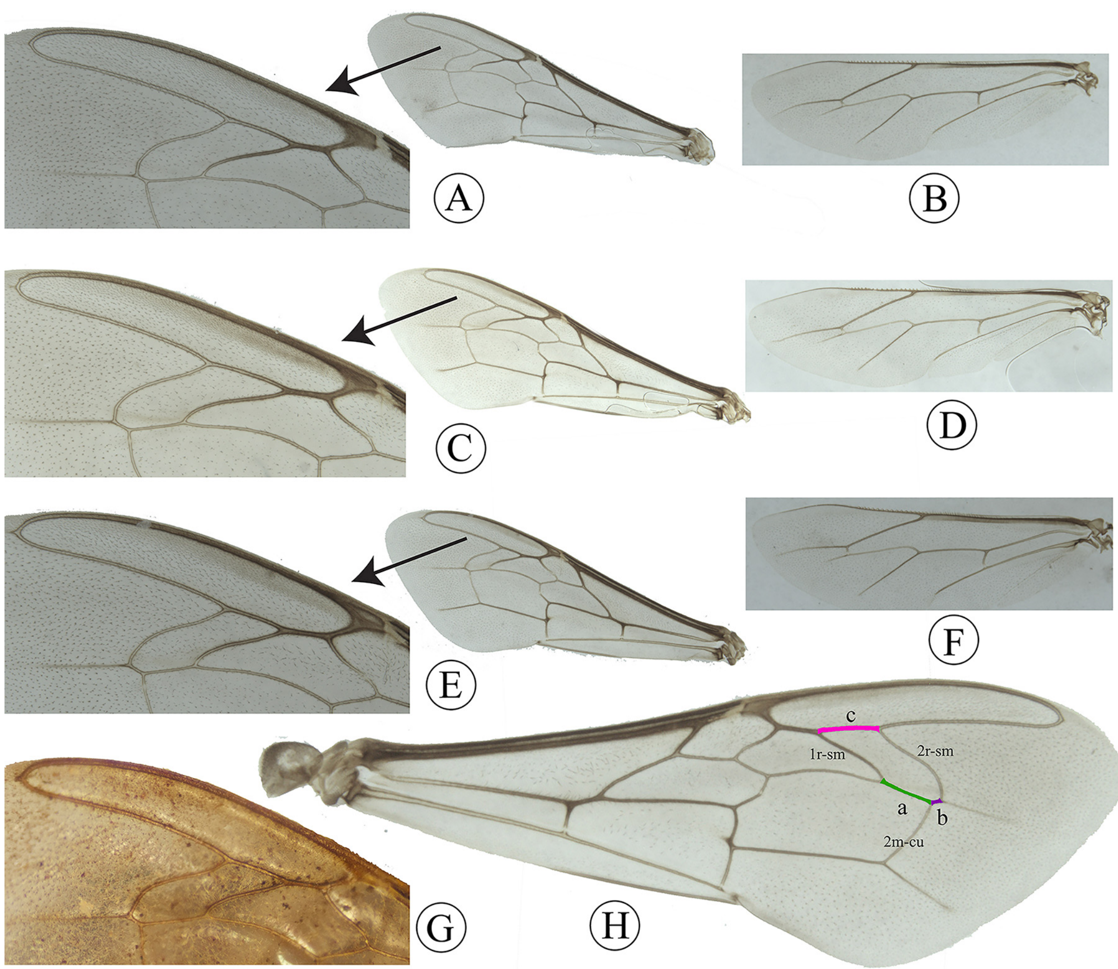

Fig. 3 Wings of worker: 3A forewing and 3B hindwing of *Apis karinjodian* **n. sp.**; 3C forewing and 3D hindwing of *A. cerana* Fabricius, 1793; 3E forewing and 3F hindwing of *A. indica* Fabricius, 1798; 3G forewing of *A. indica* Fabricius, 1798 (type); 3H forewing of *A. karinjodian* **n. sp.**, illustrating veins of RMI and CI forming third submarginal cell

and Tamil Nadu (n=60 individuals from 19 locations) were measured; forewing of workers of *A. cerana* from north India (New Delhi, Maharashtra, Uttarakhand), north-east India (Assam, Nagaland, West Bengal) and south India (Tamil Nadu) were measured (n=41 individuals from eight locations); forewing of workers of *A. karinjodian* **n. sp.** from Tamil Nadu and different parts of Kerala (n=35 individuals from five locations) were measured and forewing of *A. mellifera* (n=5 individuals from two colonies in a single location) were also measured.

The veins of forewing *viz*. a, b and c, defining RMI and CI, are defined as follows: 'a' is defined as the segment of median vein laying between distal end of cross vein 1rs-m and proximal end of cross vein 2m-cu. 'b' is defined as the segment of median vein laying between proximal end of the cross vein 2m-cu and the distal end of the 2rs-m and 'c' is defined as the segment of radial sector laying between proximal end of the cross veins 1rs-m and 2rs-m (Fig. 3H).

The distribution map (Fig. 13) was prepared by correlating the information available from material examined for this study, sequence data (540 nos) available at NCBI-GenBank (Table 2) and by consulting several topographic maps and maps of maximum and minimum temperature limits that helped to demarcate the species boundary. The species are categorised as per annotation provided in the IUCN Red List Categories and Criteria (IUCN, 2022).

Morphological terminology follows Ruttner (1988), Koeniger *et al.* (1991), Engel (2001) and Michener (2007).

Phylogenetic Analysis

The PCR amplification and sequencing of the CO1 genes (Table 3) were performed at the Rajiv Gandhi Centre for Biotechnology (RGCB), Thiruvananthapuram, Kerala, India. In addition to the new sequences, the remaining sequences used in this study (Table 2) were obtained from the GenBank of National Center for Biotechnology Information (NCBI). The sequences were aligned with the CLUSTAL omega to depict the intraspecific conserved sites. The phylogenetic analysis was performed and average nucleotide composition of each species was determined using MEGA11 software (Tamura *et al*., 2021). Phylogenetic trees were constructed using the Neighbor-Joining (Saitou and Nei, 1987; Tamura et al., 2004), Maximum Likelihood, Tamura-Nei model (Tamura and Nei, 1993), Minimum Evolution (Rzhetsky and Nei, 1992) and UPGMA (Sneath and Sokal, 1973) methods. *Apis florea* was chosen as the out-group for the evolutionary studies.

The topology of the Neighbor-Joining (NJ) tree was congruent with that of the tree topology obtained from Maximum Likelihood (MCL), Tamura-Nei model, Minimum Evolution method and UPGMA. Hence, only the NJ tree (Fig. 12A) is presented. The percentage of repeat trees wherein the connected taxa huddled together in the bootstrap test (1000 replicates) are shown near the branches. The phylogenetic tree was generated with the branch lengths expressed in units equivalent to those of the evolutionary distances by which the evolutionary tree is inferred. The evolutionary distances, which are measured in terms of the number of base substitutions per site, were calculated using the MCL approach. Two separate phylogenetic trees for the Western population (Fig. 12B) and the Eastern population (Fig. 12C) were prepared for better interpretation of the phylogenetic affinity.

The holotype (Accession no. NIM/NBAIR/HYM/API/15922-H) and three paratypes (Accession nos NIM/NBAIR/HYM/API/15922-P1/P2–♀; P3–♂) of the new species are deposited in the National Bureau of Agricultural Insect Resources, Bengaluru (NBAIR). Paratypes will be deposited in the National Pusa Collection, Indian Agricultural Research Institute (NPC) and Zoological Survey of India, Kolkata (ZSI).

RESULTS

Systematics

Apis karinjodian **Shanas, Anju & Mashhoor, new species**

LSIDurn:lsid:zoobank.org:act:CE978E58-4219-459D-A452-1317982A8A57

(Figs 1 G–L; 3A, 3B, 3H; 4 A–P; 5 A–F; 6A–B; 7A–D; 8A–D)

Diagnosis: The new species is relatively large in size (10.8–11.6 mm) and darker in general appearance. The female worker is characterized by a prominent 'V' shaped projection on the propodeum (Figs. 4D–F); dark yellow scutellum, with or without a black patch (Figs. 1G–I) and abdominal terga I-IV prominently black-banded (Figs. 4G–I). Rugosely reticulate irregular sculptures were rarely observed on the frons of drones (Fig. 7B).

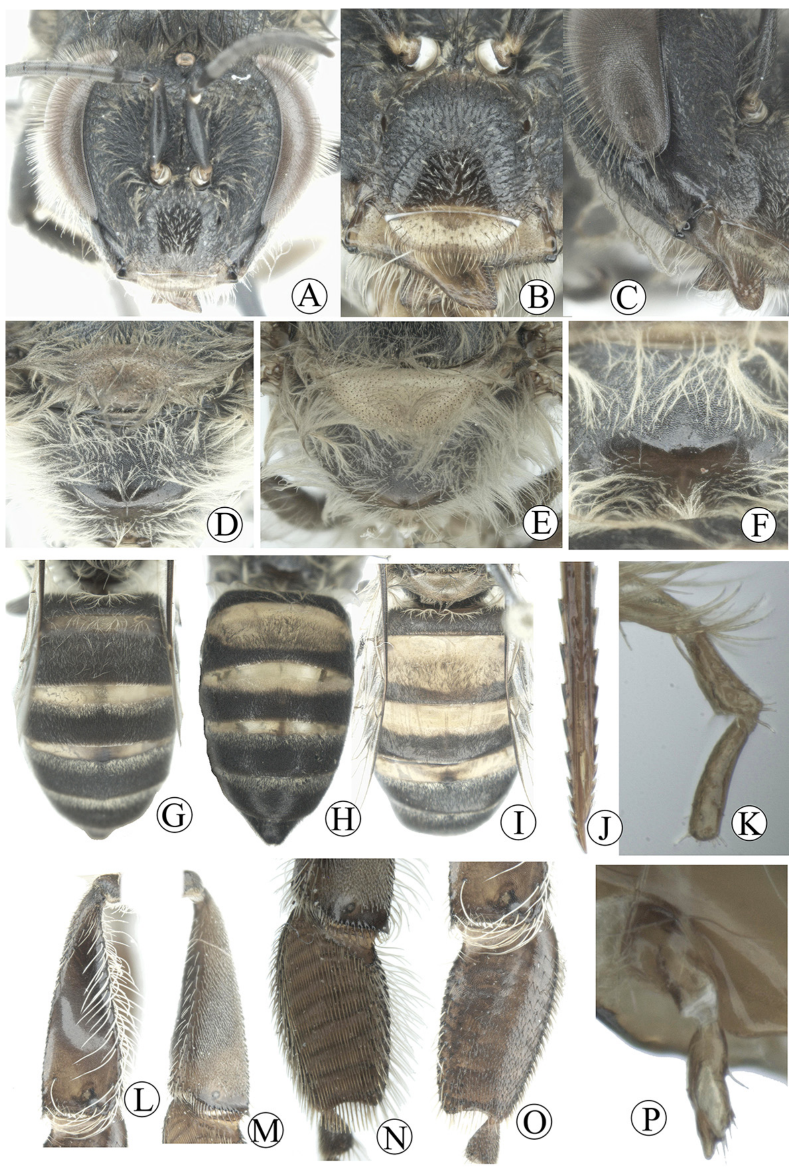

Fig. 4 Workers of *Apis karinjodian* **n. sp.:** A. frontal view of head; B. clypeus and labrum; C. malar area of face; D–F. propodeum; G–I. abdomen; J. sting; K. labial palp; L. outer surface of hind tibia; M. inner surface of hind tibia; N. inner surface of hind basitarsus; O. outer surface of hind basitarsus; P. maxillary palp

Description: Female (worker): Wings clearly hyaline; abdominal terga I with proximal part prominently black-banded (Fig. 4G–I); body length (10.8–11.6 mm); forewing length (7.31– 8.16 mm); 2.8x longer than broad; hind wing length (5.1–5.6 mm); 3.2x longer than broad; head length from anterior margin of clypeus to summit of vertex, in facial view 2.84 mm; head width 3.34 mm; length of compound eye 2.12 mm; inter antennal distance (0.19–0.27 mm); length of scape 1.16 mm; length of 2nd flagellomere 0.13 mm; length of 3rd flagellomere 0.28 mm; length of metatibia 2.82 mm; length of metatarsus 2.82 mm (n=3).

Compound eye 2.41x longer than wide; length of compound eye/length of scape ratio 1.82; interocellar distance/ocellar diameter ratio 1.1; ocelloorbital distance/interocellar distance 1.54; width/length of head ratio 1.18; length/width of scape ratio 5.7; length/width of 3rd tibia ratio 3.14;

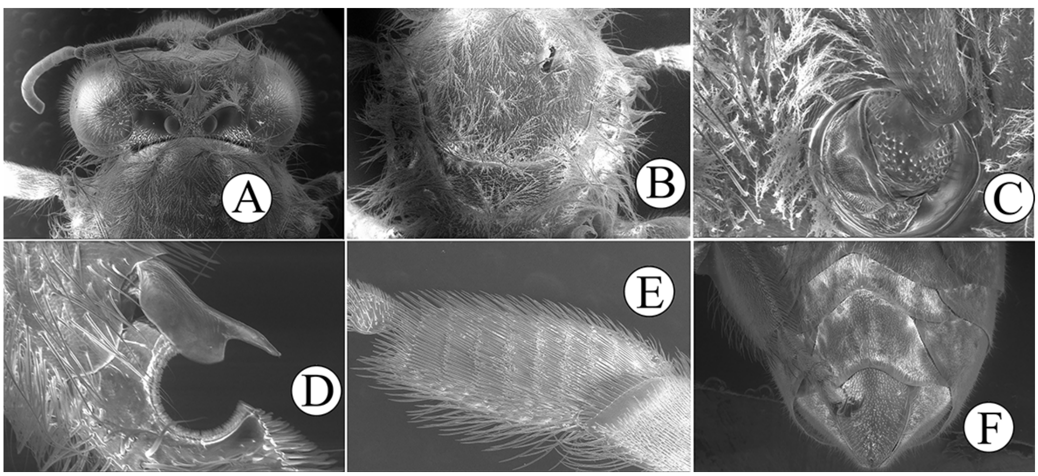

Fig. 5 Workers of *Apis karinjodian* **n. sp.:** A. dorsal view of head; B. mesothorax; C. antennal socket; D. notch and velum of foreleg; E. inner surface of hind basitarsus; F. posrero-ventral view of abdomen

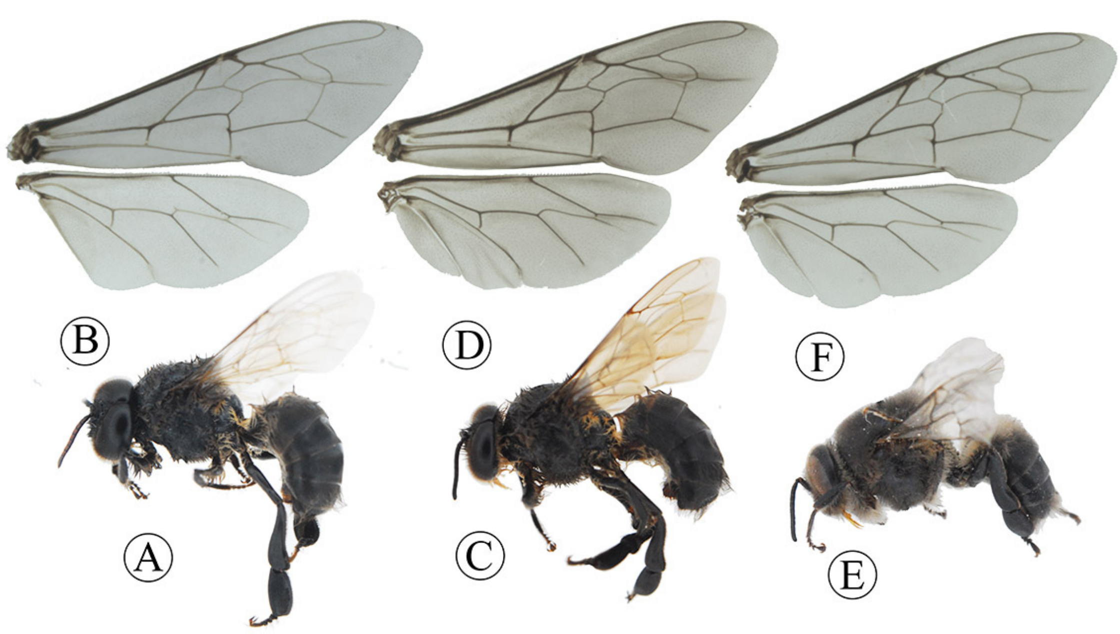

Fig. 6 Drone: A – *Apis karinjodian* **n. sp**., B – forewings and hindwing. C – *Apis indica* Fabricius, 1798, D – forewings and hindwing. E – *Apis cerana* Fabricius, 1793. F–forewing and hindwing

head width/length of metatarsus ratio 1.9; malar space/F3 ratio 2.26; malar length: 0.63–0.65 mm; interalveolar distance/interantennal space (0.19–0.27 mm; maxillary palps sometimes pointed (Fig. 4P). Wings hyaline, forewing with Radio-Medial index 5.4–6.4; Cubital index 4.6–5.4; Hindwing with 17–19 hamuli.

Male (drone): Wings mostly hyaline (Fig 6B), very feebly stained brown (Fig. 6 A–B); scutellum black (Fig. 7C); body black; body length (10.3–11.3 mm); lateral ocellar line (LOL) 0.07mm, posterior ocellar line (POL) 0.3mm, POL/LOL ratio 4.2; forewing (length 9.35 mm); 3.1x longer than broad; hind wing (length 3.1 mm); 3.1x longer than broad; head length from anterior margin of clypeus to summit of vertex, in facial view 1.92 mm; head width 3.52 mm; length of compound eye 2.88 mm; length of metatibia 3.2 mm; length of metatarsus 2.0 mm. Forewing with

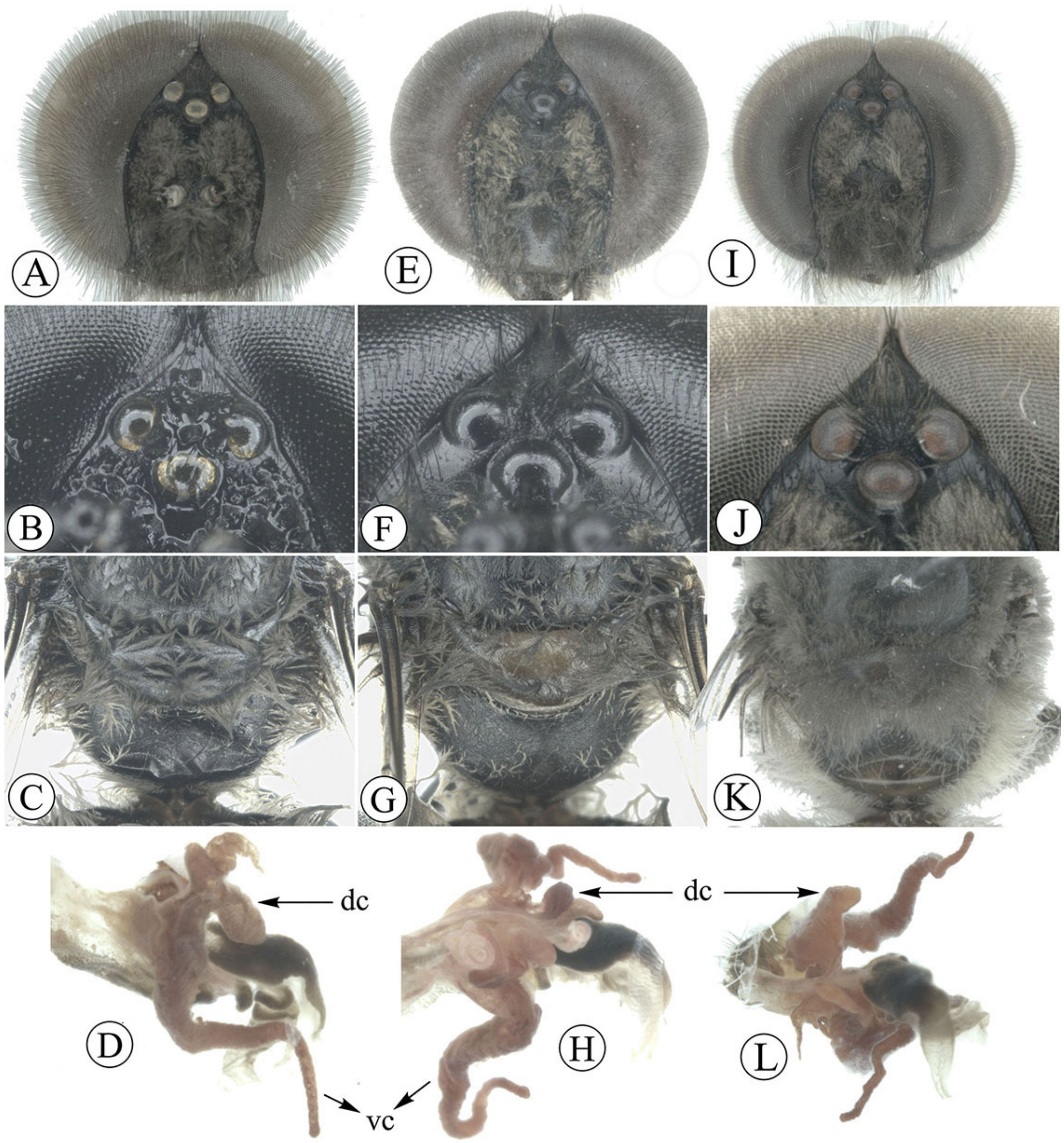

Fig. 7 Drone: A–D. *Apis karinjodian* **n. sp**. A. frontal view of head; B. vertex; C. dorsal view of thorax; D. endophallus. E–H. *Apis indica* Fabricius, 1798. E. frontal view of head; F. vertex; G. dorsal view of thorax; H. endophallus. I–L. *Apis cerana* Fabricius, 1793. I. frontal view of head; J. vertex; K. dorsal view of thorax; L. endophallus. Abbreviations: dc: dorsal cornua; vc: ventral cornua

Radio-Medial index (4.44–5.50); Cubital index (3.22–3.25); Hindwing with 14–20 hamuli (n=2); uneverted endophallus with a prominently large round lobe (0.8mm, Fig. 7D) on the three lobed dorsal cornua.

Material examined: Holotype: ♀ (worker): INDIA, KERALA, Wayanad, Shanas, S. coll, 22-XII-2019 (NBAIR); **Paratypes** [94 nos] : 30 ♀ (worker): Same data as that of Holotype; 10 ♀ (worker): Idukki, Thoppipala, Shanas, S. coll, 14-II-2019; 10♀(worker): Idukki, Marayoor, Shanas, S. coll, 14-II-2019; 30 ♀ (worker): Thiruvananthapuram, Attingal, Shanas, S. coll.25-X-2021; 5 ♀ (worker): TAMIL NADU, Coimbatore, Shanas, S. coll. 20-V-2007; 8 ♂ (drone): Kerala, Thiruvanantha- puram, Maruthankuzhy, Shanas, S. coll. 20-II-2022; 1♂: Kerala, Idukki, Mattupetty, Shanas, S. coll. 14-V-2022: (3 NBAIR).

Distribution: INDIA (Goa, Karnataka, Kerala and Tamil Nadu). An analysis of the sequences (Table2) reveals the presence of *A. karinjodian* **sp. n.** in Goa (KF497586, KF497587); Karnataka (KF497265 to KF497275; KF497293 to KF497296; KF497298), Kerala (KF497550) and Tamil Nadu (KF497510). The distribution ranges from the central Western Ghats and Nilgiris to the southern Western Ghats, covering the states of Goa, Karnataka, Kerala and parts of Tamil Nadu. From its restricted distribution extending from Goa to Kerala, it is inferred that this secluded population is endemic and distributed mainly along the Western Ghats biodiversity hotspot of southern India (Fig.13).

Conservation status: Near threatened (NT) in Kerala: This species is only occasionally encountered in managed as well as feral colonies in Western Ghats region of Kerala. Data Deficient (DD) in remaining places.

Remarks: The workers of the new species resemble *A. cerana* Fabricius, 1793 by most other diagnostic characters. However, they can be distinguished by the narrow size of the median vein

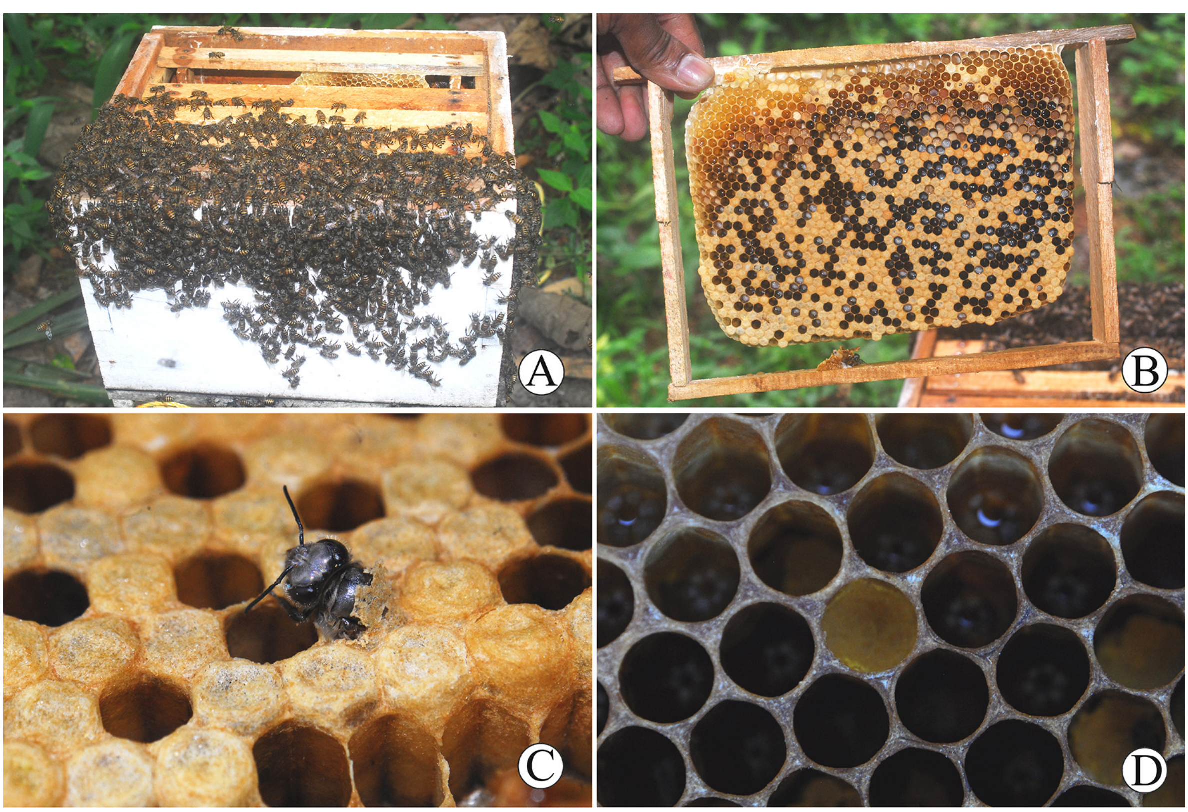

Fig. 8 *Apis karinjodian* **n. sp.** A. hive; B. brood comb; C. emerging worker; D. pollen and honey chamber

segment 'b' (0.1 mm, Figs. 3A, H) and the prominent 'V' shaped projection on propodeum (Figs. 4D–F) which are absent in *A. cerana* (Fig. 10E) and *Apis indica* (Fig. 9H). Rugosely reticulate irregular sculptures are observed rarely on frons of the drone (Fig. 7B).

Note on identity and behaviour: The distinct identity of this species was recognized by the bee-keepers in Kerala who coined the term 'karinjodian' for these visibly dark bees. According to bee-keepers, *A. karinjodian* **n. sp**. gnaws and dismantles the combs of *A. indica* when the combs are exchanged between colonies. The hive cleaning behaviour is superior to that of *A. indica* as the wax debris falling on the bottom of the hive are regularly removed, which prevents wax moth infestation during lean season. It is observed that the *A. karinjodian* **n. sp.** colonies are generally strong even during the monsoon season. They also produce more honey which is thicker in consistency compared to that of *A. indica.* However, they are not preferred for beekeeping as swarming and absconding are more during the honey flow season and they sting more profusely and their stings are more painful than that of *A. indica*. Due to these undesirable traits, bee keepers generally do not utilise this species for beekeeping. The ability of *A. karinjodian* **n. sp.** to produce higher quantities of honey, which is thicker in consistency, has been noted by bee-keepers. This could be due to the ability of *A. karinjodian* **n. sp.** to exploit diverse floral resources and their stronger fanning ability that ripens honey. When a sufficiently strong beehive is opened, the bees get disturbed and their restless movement inside and outside the colony can be easily noticed (Fig. 8A). Since the honey produced seems to be of better quality, the potential of beekeeping with *A. karinjodian* **n. sp.** should be explored.

Etymology: The specific epithet '*karinjodian*' literally means black honey bee in the vernacular local language, Malayalam. The species name is a noun in apposition. The common name, 'Indian black honey bee' is coined for the new species.

Apis indica Fabricius, 1798 Status Restored

(Figs. 1 M–R; 2 A, B; 3 E–G; 6 C, D; 7 E–H; 9 A–L)

Fabricius, 1798: 274.

Lindauer and Kerr (1960) gave systematic priority to *A. cerana* Fabricius, 1793 and treated the Indian bee as *A. cerana indica* which, according to them, is a valid subspecies, along with the conspecific nominotypical subspecies *A. cerana cerana*. The same synonymy as well as subspecific epithet has been followed by most workers (Ruttner, 1988; Engel, 1999; Radloff *et al.,* 2010) for *A. indica* Fabricius, 1798 till date.

Based on the Radio-Medial index (RMI) of 4.0 on the right forewing and 3.8 on the left forewing of the Fabrician type of *A. indica* (Figs. 2 A, B) and the calculated value (1.9–4.2) from the material examined, the name *A. indica* Fabricius, 1798 is here resurrected.

Type material: Type image: ♀(worker): Labels: (1) indi-/ca; (2) NHMD 308727 Figs. 2 A–B (NHMD, Copenhagen).

Type locality: INDIA: Tamil Nadu, Tharangambadi.

Material examined [112 nos] : 3♀(worker) each: Kerala, Ernakulam, Kannur, Kasargod, Kottayam, Kozhikode, Malappuram, Palakkad, Pathanamthitta, Thrissur, Mashhoor, K. coll. 1-III-2022 to 5-III-2022. 9♀(worker): Kerala, Wayanad, Shanas. S. coll, 22-XII-2019; 10♀(worker): Kerala, Idukki, Thoppipala, Shanas, S. coll, 14-II-2019; 10♀ (worker): Kerala, Idukki, Marayoor, Shanas, S. coll, 14-II-2019; 30♀ (worker): Kerala, Thiruvananthapuram, Attingal, Shanas, S. coll. 25-X-2021; 10 ♂: Kerala, Thiruvananthapuram, Attingal, Shanas, S. coll. 20-II-2022; 1♀(worker): Nagaland, Nagaland University, SASRD, Medziphema, Shanas, S. coll. 9-III-2019; 2♀ (worker): Odisha, Bhubaneswar, Shanas, S. coll. 12-III-2019; 1♀ (worker) Type image: INDIA: Tamil Nadu, Tharangambadi: Label: NHMD 308727; 2♀(worker): Tamil Nadu, Madurai, Anju

Krishnan, G coll. 14-IV-2022; 10♀(worker): West Bengal, Jayanagar (Nr. Sundarban), Shanas, S. coll. 11-III-2019.

Distribution: INDIA: Maharashtra, Goa, Karnataka, Kerala, Tamil Nadu, Andhra Pradesh, Telangana, Odisha, West Bengal, Nagaland, Andaman and Nicobar Islands; Sri Lanka.

The distribution range has been estimated based on the material examined, mt.DNA sequences (Table 2) and the estimated range.

Conservation status: Data Deficient (DD)

Remarks: Fabricius (1798) mentions the collector as Daldorff. According to Holthuis (1986), the collector Ingobert Karl Daldorff, a Danish officer, was stationed in Tranquebar (S.E. India, 11°02'N; 79°51'E) from 1790 to 1793. The former Danish colony, "Tranquebar" refers to Tharangambadi in Tamil Nadu, India. Hence, the type locality is here fixed as Tharangambadi in Tamil Nadu.

Apis cerana Fabricius, 1793

(Figs. 1. A–F; 2. E, F; 3. C, D; 6. E–F; 7. I–L; 10. A–L)

Type material: Lectotype image: ♀ (worker): Labels: (1) *a: cerana* / Lectotype/ x *Apis* Moure 58; (2) TYPE; (3) ZMUC00241552 (Fig. 2 E–F) (ZMUC).

Material examined [50 nos]: INDIA: 6♀ (worker): Assam, Digant, K. coll, 22-II-2022; 1♀ (worker): New Delhi, Shanas, S. coll, 25-III-2018; 8♀ (worker), 9♂: Uttarakhand, Tanakpur, Bablu, P. coll, 14-II-2022; 2♀ (worker): Maharashtra, Pune, Shanas, S. coll, 2-II-2019; 12♀ (worker): Maharashtra, Mumbai, Johnson. coll, 18-II-2022; 7♀ (worker): Nagaland, Nagaland University, SASRD, Medziphema, Shanas, S. coll. 9-III-2019; 3♀(worker): Tamil Nadu, Madurai, Anju Krishnan, G coll. 14-IV-2022; 2♀ (worker): West Bengal, Mohanpur, Nadia, Shanas, S. coll. 11-III-2019 (S. Shanas personal collection).

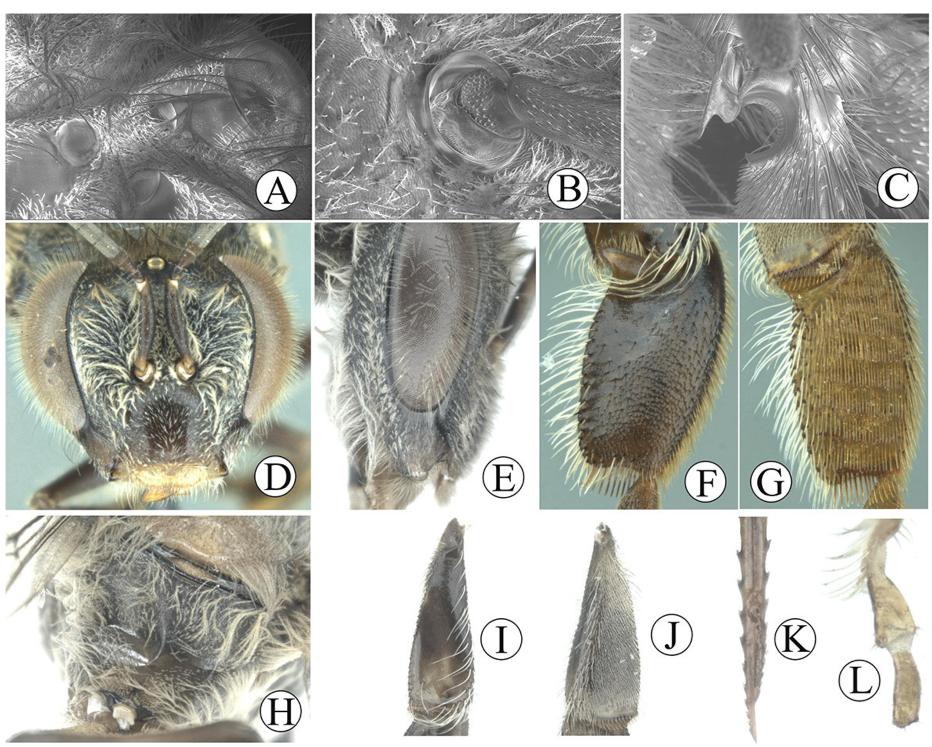

Fig. 9 Workers of *Apis indica* Fabricius, 1798: A. vertex of head; B. antennal socket; C. notch and velum of foreleg; D. frontal view of head; E. malar area of face; F. outer surface of hind basitarsus; G. inner surface of hind basitarsus; H. Propodeum; I. outer surface of hind tibia; J. inner surface of hind tibia; K. sting; L. labial palp

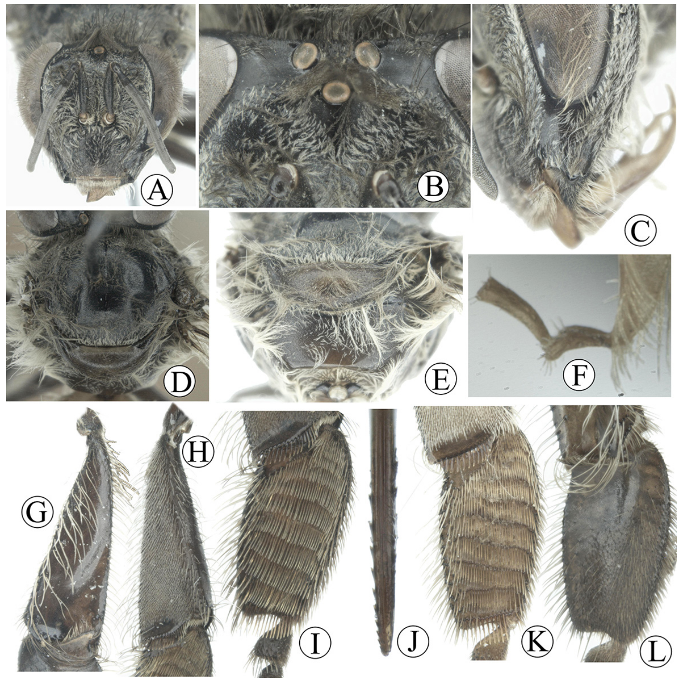

Fig. 10 Workers of *Apis cerana* Fabricius, 1793: A. frontal view of head; B. vertex of head; C. malar area of face; D. dorsal view of mesoscutellum and scutellum; E. Propodeum; F. labial palp; G. outer surface of hind tibia; H. inner surface of hind tibia; I. inner surface of hind basitarsus; J. sting; K. inner surface of hind basitarsus; L. outer surface of hind basitarsus

Distribution: Asia (South-West, South, East, South-East), Russian Far-east, Australia

Conservation status: Least Concern (LC)

Apis mellifera Linnaeus, 1758

(Figs. 11 A–D)

Material examined [5 nos] : 3♀ (worker): Kerala, Trivandrum, Rajan Nadar, K. C coll. 15-III-2022; 2♀ (worker): Nagaland, Nagaland University, SASRD, Medziphema, Shanas, S. coll. 9-III-2019 (S. Shanas Personal Collection).

Key to the species of native cavity nesting honey bees of the Indian Subcontinent

1. RMI of worker forewing = 1.9–4.2 (Figs 3E, 3G); Drone: Scutellum yellowish black (Fig 7G) [COI barcode: GenBank Accession Number. OP168348] ....*Apis indica* Fabricius, 1798.

— RMI of worker forewing = 4.7–6.4 (Figs 3A, 3C, 3H); Drone: Scutellum black (Figs 7C, 7K) 2

2. Drone: POL < 4x LOL; Worker: Propodeum without prominent 'V' shaped projection (Fig 10E) [COI barcode: GenBank Accession Number. OP168351]........... *Apis cerana* Fabricius, 1793.

— Drone: POL > 4x LOL; Worker: Propodeum with prominent 'V' shaped projection (Figs 4D–F) [COI barcode: GenBank Accession Number. OP071087]............... *Apis karinjodian* **sp. n.**

DISCUSSION

The calculated RMI value of the right and left forewings of the Fabrician Type NHMD308727 (Figs. 2A, B) is 4.0 and 3.8 respectively (Table 1), which confirms the specimen as *A. indica* Fabricius, 1798. The calculated RMI value obtained for the right wing of non-type NHMD308728 (Fig. 2C) and the left wing of non-type NHMD308729 (Fig. 2D) is 3.8, which also confirms the non-type specimens as *A. indica* Fabricius, 1798.

Out of the 40 synonyms of *A. cerana* enlisted by Radloff *et al.* (2010), five type localities are from

India (excluding the Himalayan region): *A. indica* Fabricius, 1798 ('Tharangambadi' in Tamil Nadu); *A. socialis* Latreille, 1804 (Bengal), *A. perrottetii* Guérin-Méneville, 1844 ('Neelgherries', Tamil Nadu); and *Apis delessertii* Guérin-Méneville, 1844 (Pondicherry). Among these, only *A. perrottetii* Guérin-Méneville, 1844 has been reported to inhabit the Western Ghats (Nilgiris), which falls inside the geographic range of *A. karinjodian* **n. sp.**

Guérin-Méneville (1844) described *A. perrottetii* and *A. delessertii* without comparing with *A. indica*, Fabricius, 1798. Guérin-Méneville briefly mentioned about *A. indica* Fabricius, seen in Bosc's collection being similar to *A. zonata* (currently treated as a synonym of *A. dorsata*). Smith (1857) considered the specimen from Borneo (Sarawak) though paler, to be *A. perrottetii* Guérin-Méneville, 1844. He later synonymized *A. perrottetii* with *A. indica* Fabricius, 1798 (Smith, 1865). In the description of *A. perrottetii* Guérin-Méneville, 1844, the species is reported to have its entire front of first segment of abdomen yellow and its protruding part entirely brown. However, the proximal part of first segment of the abdomen appears black in all observed specimens of *A. karinjodian* **n. sp.** (Figs. 1G–I, 4G–I); which clearly distinguishes it from *A. perrottetii* Guérin-Méneville, 1844.

Although *A. cerana* Fabricius, 1793 and *Apis indica* Fabricius, 1798 are being treated as synonyms (Lindauer and Kerr, 1960), confusion still persists as the widely accepted name, *Apis indica*/ *Apis cerana indica*/ *Apis cerana* based on which, most publications are authored in India. Engel recognized two subspecies of *Apis cerana* which occur in India, of which, the plains bee taxonomically corresponds to the subspecies *Apis cerana indica* Fabricius, 1798 while the hills bee appears to be *A. cerana cerana* Fabricius, 1793 (Engel, 1999, 2002).

Several indices of wing venation were introduced (Louis 1963; Goetze 1964). All of these indices, except the very important Cubital Index (CI), became obsolete since the introduction of venation angles (Ruttner, 1988).

Cubital Index (Fig. 3H) in this work, is calculated as per Ruttner (Fig. 6.8, 1988). It was observed that, the cubital index was not useful to discriminate between representative populations of *A. indica* (CI=2.1–4.2) and *A. cerana* (CI=3.1–5.2) effectively. However, the c/b ratio (Fig. 3H), defined herein as the Radio-Medial Index (RMI), gave an accurate measure for discriminating populations of *A. indica* and *A. cerana* + *A. karinjodian* **n. sp**. The RMI, to the best of our knowledge, has never been used earlier for morphometric discrimination of *Apis* species. The closest index to RMI ever used as per literature, is the dumb-bell index (Fig. 4.7, Goetze 1964). The calculated dumb-bell index for *A. indica* (0.5–1.2) and *A. cerana* (1.0-1.2) did not help in discriminating these species.

The RMI, in our opinion, provides higher resolution and may be used along with the Cubital Index (CI) for discriminating sufficiently diverged *Apis* species confined to the subcontinental boundaries. The RMI

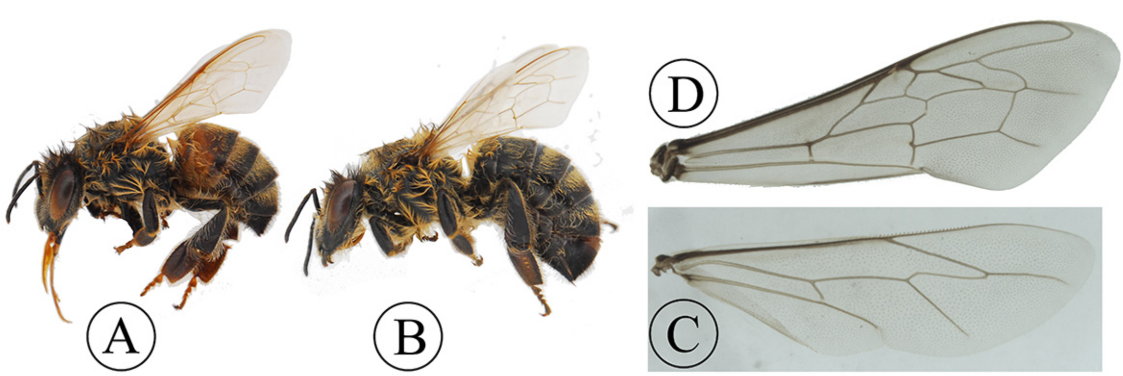

Fig. 11 Worker. A–*Apis mellifera;* B–*A. mellifera carnica,* C– hindwing, D–forewing

Table 1**. Forewing index ratios of cavity nesting honeybees of the Indian subcontinent**

Forewing Index (Fig 3H)	*A. indica* (Type)	*A. cerana* (Lectotype)	*A. indica*	*A. cerana*	*A. karinjodian*	*A. mellifera*
Cubital index CI= a/b	4.2 (RW) 3.4 (LW)	3.4 (LW)	2.1-4.2	3.1-5.2	4.6-5.4	2.3-2.9
Radio-Medial index RMI= c/b	4.0 (RW) 3.8 (LW)	5.0 (LW)	1.9-4.2	4.7-6.4	5.4-6.4	3.5-3.8

RW= Right wing; LW= Left wing (Figs 2A, 2B)

Table. 2. The COI and COII region of the mtDNA examined in this study from NCBI Genbank.

COI			COII		
Apis cerana Fabricius 1793	*Apis indica* Fabricius 1798	*Apis karinjodian* n. sp	*Apis cerana* Fabricius 1793	*Apis indica* Fabricius 1798	*Apis karinjodian* n. sp
KF760518(IN)KA	KC414930(IN)KA	MH588669(IN)KA	KF497250(IN)KA	KF497249(IN)KA	KF497265(IN)KA
KF760521(IN)KA	KF760519(IN)KA	MH588653(IN)KA	KF497251(IN)KA	KF497252(IN)KA	to
KF861941(IN)KA	KF760523(IN)KA	MH588675(IN)KA	KF497261(IN)KA	to	KF497275(IN)KA
KM495732(IN)KA	to	KU963189(IN)KA	KF497277(IN)KA	KF497260(IN)KA	KF497293(IN)KA
KM495733(IN)KA	KF760527(IN)KA	KR010696(IN)KA	to	KF497262(IN)KA	to
KM591907(IN)KA	KJ139456(IN)KA	KF760522(IN)KA	KF497292(IN)KA	to	KF497296(IN)KA
KM591908(IN)KA	KM230116(IN)KL	KF760520(IN)KA	KF497297(IN)KA	KF497264(IN)KA	KF497298(IN)KA
KM591909(IN)KA	KM495728(IN)KA	**7**	KF497299(IN)KA	KF497276(IN)KA	KF497510(IN)TN
KM610315(IN)KA	KM495730(IN)KA	OK465105(IN)HP	KF497300(IN)KA	KF497301(IN)KA	KF497550(IN)KL
KM610318(IN)KA	KM495731(IN)KA	OK483361(IN)HP	KF497306(IN)KA	to	KF497586(IN)GA
KM610319(IN)KA	KM593931(IN)KA	OK602702(IN)HP	KF497307(IN)KA	KF497305(IN)KA	KF497587(IN)GA
KM610320(IN)KA	to	OK626675(IN)HP	KF497309(IN)JK	KF497308(IN)KA	**20**
KP255460(IN)MH	KM593939(IN)KA	OK626676(IN)HP	to	KF497425(IN)AP	
to	KM610316(IN)KA	OK626762(IN)HP	KF497368(IN)JK	to	KF497509(IN)TN
KP255467(IN)MH	KM610317(IN)KA	OK626764(IN)HP	KF497369(IN)AS	KF497432(IN)AP	KF497511(IN)TN
KT960839(IN)PB	KU963191(IN)KA	OK626778(IN)HP	to	KF497434(IN)AP	to
KU212336(IN)MI	KX587509(IN)KL	OK626780(IN)HP	KF497377(IN)AS	KF497436(IN)AP	KF497534(IN)TN
to	MH331013(IN)KL	OK632479(IN)HP	KF497380(IN)AS	KF497442(IN)AP	KF497545(IN)KL
KU212341(IN)MI	MH588650(IN)KA	OL436247(IN)HP	to	to	to
KU963187(IN)KA	to	OL457389(IN)HP	KF497392(IN)AS	KF497478(IN)AP	KF497549(IN)KL
KU963188(IN)KA	MH588652(IN)KA	OL468548(IN)HP	KF497393(IN)ME	KF497480(IN)AP	KF497551(IN)KL
KU963190(IN)KA	MH588654(IN)KA	OL589569(IN)HP	to	KF497485(IN)TN	to
MH588658(IN)KA	to	OL589591(IN)HP	KF497396(IN)ME	to	KF497560(IN)KL
MH588661(IN)KA	MH588657(IN)KA	OL639224(IN)HP	KF497397(IN)AR	KF497496(IN)TN	KF497562(IN)KL
to	MH588659(IN)KA	OM319700(IN)HP	to	KF497500(IN)TN	to
MH588668(IN)KA	MH588660(IN)KA	OM320364(IN)HP	KF497404(IN)AR	KF497505(IN)TN	KF497585(IN)KL
MH588670(IN)KA	MH588671(IN)KA	OM320444(IN)HP	KF497405(IN)AS	to	KF497588(IN)GA
MH588672(IN)KA	MH588673(IN)KA	OM321429(IN)HP	to	**150**	
MH588674(IN)KA	MH682148(IN)KA	OM766175(IN)MI	KF497412(IN)AS	KF497479(IN)AP	KF497544(IN)TN
MK904657(IN)WB	MW093739(IN)TN	OM766178(IN)MI	KF497413(IN)ME	KF497481(IN)AP	KF497561(IN)KL
MK904727(IN)WB	**39**	ON331706(IN)PB	to	to	KF497589(IN)MH
MK904728(IN)WB	MT027905(IN)HP	ON506013(IN)UT	KF497420(IN)ME	KF497484(IN)AP	to
MK904731(IN)WB	MT027915(IN)HP	M6Z558042(BD)	KF497421(IN)AS	KF497497(IN)TN	KF497648(IN)MH
to	to	M7Z558043(BD)	to		
MK904735(IN)WB	MT027917(IN)HP	MZ558037(BD)	KF497424(IN)AS	KF497499(IN)TN	
MK904739(IN)WB	MT027919(IN)HP	MZ558038(BD)	KF497433(IN)AP	KF497501(IN)TN	
MK904756(IN)WB	to	MZ558039(BD)	KF497435(IN)AP	to	
MK904774(IN)WB	MT027922(IN)HP	MZ558040(BD)	KF497437(IN)AP	KF497504(IN)TN	
MN242984(IN)KA	OK287086(IN)HP	MZ558041(BD)	to	KF497535(IN)TN	
MT027904(IN)HP	OK310864(IN)HP	KY834222(PK)	KF497440(IN)AP	to	
97			**227**		
TOTAL: 97+39+7+227+150+20 = **540**					

Country Abbreviations: India (IN); Bangladesh (BD); Pakistan (PK)

India State Codes: AP: Andhra Pradesh, AR: Arunachal Pradesh, AS: Assam, GA: Goa, HP: Himachal Pradesh, JK: Jammu and Kashmir, KA: Karnataka, KL: Kerala, ME: Meghalaya, MH: Maharashtra, MI: Mizoram, PB: Punjab, TN: Tamil Nadu, UT: Uttarakhand, WB: West Bengal.

NCBI Genbank access date: until 20/6/2022 and CI values should always be given as ranges for accuracy rather than a single average value that may lead to misidentifications.

With regard to occurrence of colour morphs of yellow and black bees in south India, especially in Bengaluru, contrary to the popular belief that the occurrence of intermediate colour morphs, suggest the absence of mating barrier among both colours (Viraktamath *et al.,* 2013) and migratory beekeeping being an exclusive reason for the merger of black and yellow strains, which could have led to genetic recombination between the strains (Chalapathy *et al.,* 2014a), it is evident that black as well as yellow colour morphs are present in both *A. cerana* and *A. indica* populations in Karnataka (in Chalapathy *et al.,* 2014a) as well as Tamil Nadu (in Chalapathy *et al.,* 2014b). In a study undertaken by Chalapathy *et al.* (2014a) in Karnataka, ACBLR COIB refers to black *A.cerana* from Bengaluru (Table 2, KF760518) and ACBLR COIY refers to yellow *A. indica* from Bengaluru (Table 2, KF760519). The presence of black *A. indica* (Table 2, KF760523) and yellow *A. indica* (Table 2, KF760524) too is evident elsewhere in Karnataka (Madikeri), which is not too far from Bengaluru, in terms of the species range. Similarly, in a study by Chalapathy *et al.* (2014b) from the Nilgiri Biosphere Reserve, spread over regions of Karnataka, Kerala and Tamil Nadu, presence of black *A. indica* (ACCNRCOI_B) from Tamil Nadu (Coonor) along with the Yellow strains (ACCKHCOI_Y and ACVZTCOI_Y) from Chokkanahalli and Vazhaithottam is evident. Also, presence of yellow *A. cerana* (ACBNGCOI_Y) in Banagudi and Black strains (ACKTGCOI_B and ACOTYCOI_B) in Kotagiri and Ooty in Tamil Nadu are evident.

Light (yellow) and dark (black) colour morphs occur among *A. cerana* and *A. indica* populations in south India (Fig. 1). They appear yellow or black to the unaided human eye due to the light yellow (Figs. 1B, 1N) as well as dark colours (Figs. 1A, 1G, 1M) on their scutellum and abdomen. Hence, we cannot distinguish species only based on body colouration. In the south Indian states of Kerala, Tamil Nadu and Karnataka, the presence of the Indian black bee (*A. karinjodian* **n. sp**.) in the Western Ghats region adds to this conundrum. The black and yellow bees seen in Bengaluru are exclusively a mixture of *A. cerana* and *A. indica* populations. The Indian black honey bee (*A.karinjodian* **n. sp**.) may not be present in Bengaluru. The bees encountered there are mostly *A. cerana* with a dark scutellum (Fig 1A) or light yellow scutellum (Fig. 1B) and *A. indica* with a dark scutellum (Fig. 1M) or light yellow scutellum (Fig. 1A).

Lo *et al.* (2010) had supported recognition of *A. indica*, the plains honey bee of south India, as a separate species from *A. cerana*. Extensive differentiation between two forms of honey bees, not physically separated by any substantial barriers, almost certainly indicates that they are reproductively isolated and consequently distinct species and the only data available to address this question other than morphological differences are the timing of mating flights of drones (Otis, 1996). Oldroyd *et al.* (2006) proved that the yellow plain bees of India could be regarded as a separate species from *A. cerana* based on non-overlap of drone flight times and occurrence of consistently different mitochondrial haplotypes. A similar study by Hadisoesilo and Otis (1996) confirmed the species status of *A. nigrocincta* Smith, 1860, a species distinct from *A. cerana* F., 1793, by drone flight times in Sulawesi, Indonesia.

Hence it is emphasized that *A. cerana* Fabricius, 1793 and *A. indica* Fabricius, 1798 are distinct valid species and they do not interbreed in nature.

Male genitalia

The anatomy of uneverted endophallus of drones were studied. It was observed that the endophallus of *A. karinjodian* **n. sp.** displayed three lobed dorsal cornua with a prominently large round lobe (0.8mm, Fig.7D). *Apis cerana* Fabricius, 1793 seems to possess a comparatively smaller lobe on dorsal cornua (0.5mm, Fig. 7L). It was also observed that the endophallus of *A. indica* Fabricius , 1798 had a comparatively small dorsal cornua (0.3mm, Fig. 7H).

Table 3. Details of *Apis* species and its CO1 partial coding sequence generated in the study

No	Species	Voucher No.	GenBank Accession No.	Collection location
1	*A. karinjodian* **n. sp**.	ACKWD1	OP071087	Wayanad, Kerala, India
2	*A. karinjodian* **n. sp**.	ACKWD2	OP068196	Wayanad, Kerala, India
3	*A. karinjodian* **n. sp**.	ACKID1	OP071086	Idukki, Kerala, India
4	*A. karinjodian* **n. sp**.	ACKID2	OP161981	Idukki, Kerala, India
5	*A. karinjodian* **n. sp**.	ACKID3	OP161980	Idukki, Kerala, India
6	*A. indica* F., 1798	ACIWB1	OP168188	West Bengal, India
7	*A. indica* F., 1798	ACITVM1	OP168315	Trivandrum, Kerala, India
8	*A. indica* F., 1798	ACIID4	OP168348	Idukki, Kerala, India
9	*A. indica* F., 1798	ACIOR1	OP168349	Odisha, India
10	*A. cerana* F., 1793	ACCNG1	OP168351	Nagaland, India
11	*A. cerana* F., 1793	ACCNG2	OP168371	Nagaland, India

Table 4. Nucleotide frequencies of CO1 sequence of *A. karinjodian* **n. sp.**, *A. cerana* and *A. indica*

Name of Species	T%	C%	A%	G%
A. karinjodian **n. sp**.	40.90	15.80	33.70	9.60
A. cerana Fab., 1793	41.70	15.10	33.70	9.50
A. indica Fab., 1798	43.20	13.60	33.70	9.50

Viraktamath (2015) undertook a pioneering study of comparative morphometry of drones of all the three species of honey bees (*Apis cerana*, *A. dorsata* and *A. florea*) known to occur in India from seven states and concluded that, the genitalia of drone of each species of honey bee are distinct but the genitalial structures within the species varied. The results of scatter plot (Fig. 1 in Viraktamath, 2015) indicates that, the cluster 1, 2 and 3 containing drones of *A.cerana* from Jammu & Kashmir, Assam and Karnataka seems to denote *A. cerana* Fabricius, 1793 and the cluster 4 containing drones of *A.cerana* from Andhra Pradesh, Karnataka, Kerala, Maharashtra and Tamil Nadu seems to denote *Apis indica* Fabricius,1798.

Molecular Analysis

The CO1 sequences generated in this study were submitted to the NCBI- GenBank and the accession numbers are given in Table 3.

Table 5. Evaluation of evolutionary divergence between CO1 partial coding sequences of native cavity-nesting honey bees *Apis* spp.

No	Name of Species	1	2	3	4	5	6	7	8	9	10
1.	*A. karinjodian* ACKWD1										
2.	*A. karinjodian* ACKWD2	0.006									
3.	*A. karinjodian* ACKID1	0.016	0.010								
4.	*A. karinjodian* ACKID2	0.006	0.000	0.010							
5.	*A. karinjodian* ACKID3	0.012	0.010	0.012	0.010						
6.	*A. cerana* ACCNG1	0.030	0.028	0.022	0.028	0.028					
7.	*A. cerana* ACCNG2	0.030	0.028	0.022	0.028	0.028	0.004				
8.	*A. indica* ACIWB1	0.056	0.054	0.050	0.054	0.059	0.056	0.057			
9.	*A. indica* ACIID4	0.054	0.052	0.048	0.052	0.056	0.054	0.054	0.006		
10.	*A. indica* ACIOR1	0.065	0.063	0.059	0.063	0.068	0.066	0.066	0.008	0.014	
11.	*A. indica* ACITVM1	0.054	0.052	0.048	0.052	0.056	0.054	0.054	0.006	0.000	0.014

Multiple sequence alignment using CLUSTAL omega revealed the percentage of intraspecific conserved sites in the COI gene of Indian cavity-nesting honey bees *A. karinjodian* **n. sp**., *A. cerana,* and *A. indica*. The COI conserved nucleotide sites observed in *A. karinjodian* **n. sp**., *A. cerana* and *A. indica* are 95.42, 92.37 and 97.53 per cent respectively.

The COI sequence of *A. karinjodian* **n. sp**., *A. cerana,* and *A. indica* exhibited bias to nucleotides A and T (Table 4). *Apis indica* has high AT content (76.90%) followed by *A. cerana* (75.40%) while *A. karinjodian* **n. sp.** has less AT content (74.60%) when compared to the other two species.

Evolutionary divergence estimation (Table 5) clearly depicts the degree of divergence between the Indian cavity-nesting honey bees *A. karinjodian* **n. sp**., *A. cerana,* and *A. indica*. The overall average divergence within populations of *A. karinjodian* **n. sp.** was 0.009. The degree of divergence of the CO1 partial coding sequence of *A. karinjodian* **n. sp.** was high with *A. indica* than with *A. cerana*. The mean divergence of *A. karinjodian* **n. sp.** with *A. cerana* was 0.0275 and with *A. indica* it was recorded at 0.0557. *Apis cerana* exhibited 0.0578 mean divergence with *A. indica*.

The phylogenetic trees (Figs. 12A-C) depict the phylogenetic relationship of the Asian cavity-nesting honey bees and the phylogenetic position of *A. karinjodian* **n. sp**. The out group, *A. florea* was placed at the base of the tree and the Asian cavity-nesting honey bees *A. karinjodian* **n. sp**., *A. cerana, A. indica, A. koschevnikovi, A. nigrocincta and A. nuluensis* formed separate clads in the phylogenetic tree (Fig. 12A). The trees confirm that all cavity nesting honey bees analyzed here are monophyletic and it is also noted that the Indian cavity-nesting honey bees *A. karinjodian* **n. sp**., *A. cerana,* and *A. indica* diverged from a common ancestor. The new species *A. karinjodian* **n. sp.** formed a sister clad to *A. cerana* with a strong support of bootstrap value 99 per cent.

The Indian black bee, *A. karinjodian* **n. sp.** has evolved from *A. cerana* morphotypes which got acclimatized to the hot and humid environments surrounding the Western Ghats. Apparently, the sympatric origin of the species could have been facilitated by the lower temperature and the abundant untapped floral resources in the humid and moderate environments surrounding the Western Ghats range of mountains. The high humidity and moderate temperatures appear to be the limiting factors those confine *A. karinjodian* **n. sp.** to the Western Ghats region, while low humidity seems to be the favoring factor restricting *A. cerana* populations from spreading into the Western Ghats, whereas, extremely low winter temperatures seem to be the limiting factor preventing the spread of *A. indica* towards the central and northern India.

It is interesting to note that the RMI of the European honey bee *A. mellifera* (3.45–3.8), falls within the RMI range of *A. indica* (1.9–4.2), suggesting a possible synapomorphy. This indicates that the ancestral population of *A. mellifera* could possibly have descended from ancestral *A. indica,* rather than ancestral *A. cerana*. Hence, based on this single synapomorphy, phylogenetic affinity (Fig.12B) and the occurrence of *A. indica* populations spread over most of south India, it is proposed that the center of origin of the ancestral clade of the European honey bee *A. mellifera*, could possibly be south India. The origin of *A. mellifera* seems to be from the ancestral

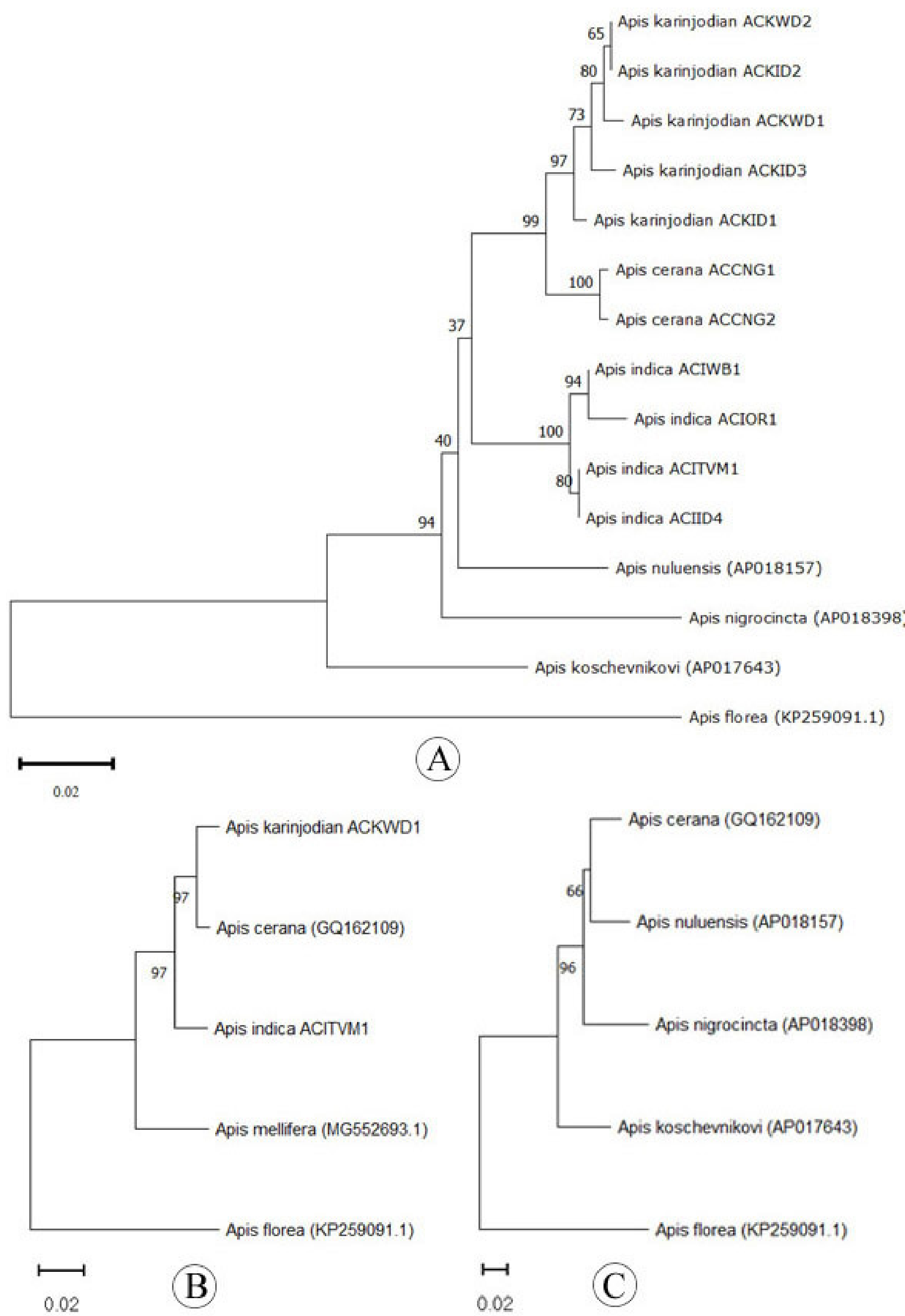

Fig. 12 Neighbor-joining trees (A–C) depicting the evolutionary relationship of cavity nesting honey bees. B: western population, C: eastern population. NJ bootstrap values are shown near the branches

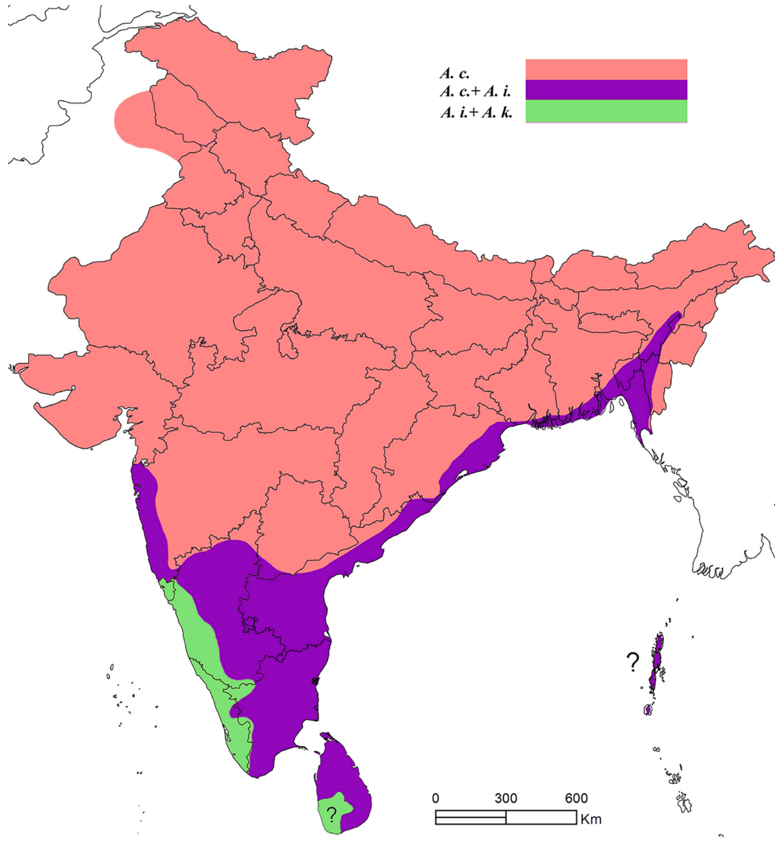

Fig. 13 Distribution of the cavity nesting honey bees of the Indian subcontinent. Abbreviations: *Ac*: *Apis cerana* Fabricius, 1793; *Ai*: *Apis indica* Fabricius, 1798; *Ak*: *Apis karinjodian* **n. sp**

A. indica + *A. mellifera* morphotypes (IM morphotypes) inhabiting the moist evergreen forests of Peninsular India during the late Miocene. The IM morphotypes from peninsular India could have reached the present day Iran and the Arabian Peninsula taking the coastal route along the Arabian Sea. As the ancestral origin of contemporary *A. mellifera* lineage remains unresolved (Dogantzis *et al.*, 2021), the findings narrow down to the hypothesis of an Indian origin of *A.mellifera*.

Ecological segregation has played the major role in the origin of *A. indica* and *A. karinjodian* **n. sp.** in south India whereas allopatric speciation seems to be the dominant factor responsible for the orgin of *A. koschevnikovi* and *A. nigrocincta* in south east Asia.

Distribution

The distribution map (Fig. 13), being the first baseline map of cavity nesting honey bees of India, will serve the purpose of future ground surveys for demarcating the accurate species limits in south, south-east and north east India. Most of the sequence data available through NCBI GenBank (Table 2) only mentions the particular state due to which, the exact species location inside the state could not be plotted. The first confirmed reports of *Apis indica* from Nagaland, Odisha and West Bengal are based on specimens obtained by field collection. Its distribution along the eastern coastal planes could be due to the moderate range of mean temperature prevailing in the coastal region.

Apis cerana, the eastern honey bee, is the most widespread among the cavity nesting honey bees occurring in the Oriental region (Radloff *et al.*, 2010). It is omnipresent in India (Fig. 13) as, out of 324 COI sequences of *Apis cerana* analysed (Table 2), it is inferred that its distribution ranges from Pakistan (Islamabad) in West, to Bangladesh and Assam in the East and Jammu and Kashmir in the North to Kerala and Tamil Nadu in south India. The sequence KF497561 (Table 2) confirms the presence of *Apis cerana* in Kerala. In a study by Baskaran (2016), based on sequence analysis of intergenic region between CO1 and CO II of mitochondrial DNA, the specimens obtained from Perambalur, Pichavaram, Paramakudi and Mayiladuturai in Tamil Nadu appears to be *A. indica*; specimens from Kodaikanal and Mudhumalai belongs to *A. cerana* and a specimen from Udhagai seems to be *A. karinjodian* **n. sp.**, thus confirming the presence of all three species in Tamil Nadu and also the presence of *A. cerana* near to borders of Kerala state.

It is interesting to note that, all 68 sequences available from Maharashtra (Table 2) belong to *Apis cerana* and a study by Gaikwad *et al.* (2019), wherein the sampling was carried out from Bhimashankar in northern Maharashtra to Mahabaleshwar and Wai in the southern part of Maharashtra (Table 2 KP255460 to KP255467), has confirmed *Apis cerana* as the only species present. These results confirm that, *A. cerana* is the only species encountered in Maharashtra state and it is the only species encountered in the northern Western Ghats region beyond Goa as well. The sequence KF497588 confirms the presence of *A. indica* in Goa. Hence, there is all possibility of *A. indica* inhabiting the Northern Western Ghats region of Maharashtra from Goa, along the coastal stretch up to northern limits of Maharashtra bordering Gujarat. The confirmed presence of "western" form in Andaman Islands (Smith and Hagen, 1996), which was probably introduced from Mumbai ("Bombay"), also points to the presence of *A. indica* in Maharashtra. The species complex occurring in the Andaman Islands is presently unknown.

The northern range limit of *A. indica* seems to lay at upper state boundary limits of Karnataka which is inferred from the study undertaken by Chalapathy *et al.* (2014a, Fig. 3) where ACRCH_COIY from Raichur pools together with ACMLR_COIY: KF760525 and ACBLR_COIY: KF760519 which are confirmed as *A. indica* sequences.

Apis indica is the dominant species occurring in Kerala followed by *A. karinjodian* **n. sp.** and *A. cerana*. The three species are present in Tamil Nadu and Karnataka as well. Further, statewide distribution of any species is not being attempted here for the lack of clarity on the specimens collected (managed / feral / field collected) and lack of details on exact place of collected samples in the NCBI GenBank database (Table 2).

Based on non-coding region of mitochondrial DNA sequence data, Smith and Hagen (1996) divided *A.cerana* into "western" form which was found in India, Sri Lanka and the Andaman Islands, and the "eastern" form found in all other localities. The western form refers to *A. indica* Fabricius, 1798, which confirms its presence in Sri Lanka and the Andaman Islands. Lo *et al.* (2010) indicated the possibility of existence of *A. indica* in Sri Lanka as well. The presence of *A. cerana* in Sri Lanka is also evident from Tan *et al.* (2008, Fig. 1), wherein the wing RMI index can be calculated as 4.8 which falls within the range of *A.cerana* (Table 1). It is highly possible that *A.indica* and *A. cerana* occur as sympatric populations in Sri Lanka as well as *A. karinjodian* **n. sp.** present in the south west evergreen forests of Sri Lanka since the two land masses were connected during the Pleistocene.

The mention by Smith and Hagen (1996) about the presence of "eastern" form of *A. cerana* in Nepal and sequences KT174434, KT174435, KT174436 and KT174437 (Tan *et al.,* 2016) confirm the presence of *A. cerana* in Nepal. Nidup and Dorji (2016) reported *A. cerana* to be very common in Bhutan as well. A study in Bangladesh (Riaz *et al.*, 2021) reported only sequences of *A. cerana* and all sequences obtained from West Bengal and the north eastern states of Arunachal Pradesh, Assam, Meghalaya and Mizoram confirm the presence of only one species, *A. cerana* in the north eastern states (Table 2).

Rajkumari *et al.* (2020, Table 4), indicate that sympatric populations of *A. cerana* and *A. indica* could be present in the south eastern hill tract and Barak valley since the reported CI values for the region are 3.46±0.26 and 3.63±0.16 respectively, which fall near CI values for *A. indica* (Table 1).

The range of *A. indica* towards the eastern borders has shown to encompass the eastern coastal plains and the mangrove areas of West Bengal and Bangladesh, since these are the probable areas with moderate temperature fluctuations. Proper collections and study have to be carried out in the eastern coastal states of Andhra Pradesh, Odisha, West Bengal and the Barak valley of Assam, Nagaland, Manipur, Mizoram and Tripura to demarcate species range of *A. indica*.

Bee-keepers select only the less aggressive bees for Apiculture. Hence, the more aggressive and wild populations are left out during routine collection surveys. All the results presently obtained from NCBI GenBank database (Table 2) are probably from specimens collected from managed colonies as these specimens are the most easily obtainable. Only this can explain the stark biased absence of *A. indica* sequences in GenBank from Maharashtra and North Eastern states. This could also be the reason for *A. cerana* as the only species obtained from Maharashtra, especially the sides bordering Western Ghats and Karnataka border. The states of central India and eastern coastal belt are already data deficient.

Hence it is cautioned that, specimen collection during surveys should only be based on field collected material, ideally from honey bees foraging on different flowering plants. This method alone can give the exact species distribution in each locality leading to an authentic distribution mapping for the whole country. Collections based on managed colonies or few feral colonies from anywhere can lead to biased results that may not be useful for accurate distribution mapping and delimiting population ranges thereafter.

It is also emphasized that only reproductively isolated, valid species can coexist as sympatric populations whereas sympatric subspecies can never exist. Hence, designating any valid species as sympatric subspecies is erroneous. Cavity nesting honey bees should ideally be treated as two distinct species groups *viz.* “cerana species group” and “mellifera species group” and sufficiently diverged populations among these species groups which do not display any intermediate haplotypes should be treated as valid species instead of subspecies. Thirty-three distinct honey bee subspecies of *A. mellifera* (Ilyasov *et al.*, 2020) should ideally be reduced to distinct valid species based on this approach or a combination of molecular and morphometric approaches.

The current study has added two more species to the honey bee fauna of the world thus, bringing the total number of valid species to 11 *viz*. the cavity-nesting honey bees: *A. cerana* Fabricius, 1793; *A. indica* Fabricius, 1798; *A. karinjodian* **n. sp**.; *A. koschevnikovi* Enderlein, 1906; *A. mellifera* Linnaeus, 1758; *A. nigrocincta* Smith, 1860; *A. nuluensis* Tingek, Koeniger and Koeniger, 1996; the dwarf honey bees: *A. florea* Fabricius, 1787; *A. andreniformis* Smith, 1857; the giant honey bees: *A. dorsata* Fabricius, 1793 and *A. laboriosa* Smith, 1871.

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