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# MORPHOLOGICAL AND MOLECULAR CHARACTERIZATION OF MEGACHILE BICOLOR (FABRICIUS, 1781) (HYMENOPTERA: MEGACHILIDAE) - A NEW RECORD FOR ANDHRA PRADESH AND TELANGANA

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**ABSTRACT**
This study records the subgenus Amegachile Friese, 1909 with species Megachile bicolor (Fabricius, 1781) for the first time from Andhra Pradesh and Telangana, India, expanding its known distribution in India. Identification of the species was done based on detailed morphological examination and further confirmed with the mitochondrial cytochrome C oxidase subunit I (mtCOI) sequencing and phylogenetic analysis. A detailed morphological description, illustrations, distribution and floral host associations are provided.

**KEYWORDS:** Amegachile, Taxonomy, DNA barcoding, Leafcutter bees, Andhra Pradesh, Telangana, India, New record.

## 1. INTRODUCTION

Bees belonging to the genus Megachile Latreille, 1802 are commonly known as leafcutter bees and resin bees because of their distinctive nesting behaviour, in which they use plant leaves or resin to construct their nests (Michener 2007). These bees are the most efficient pollinators of many wild and cultivated crops and play a key role in maintaining ecosystem stability (Batra 1977; Michener 2007; Fleming and Etcheverry 2017). The genus Megachile Latreille, 1802 is among the most diverse bee genera with 1495 species (the third most species-rich bee genus) distributed across more than 70 subgenera around the world (Ascher et al. 2016; Chatthanabun et al. 2020; Ascher and Pickering 2026). Hitherto, 152 South Asian species and 96 Indian species of Megachile in 15 subgenera are recognised as valid (Ascher and Pickering 2026).

The subgenus Amegachile Friese, 1909 consists of 30 species worldwide and has a broad distribution in Africa (from Senegal and Ethiopia to Namibia and South Africa), including Madagascar. It is also distributed across India, Southeast Asia, New Guinea, the Solomon Islands and northern Australia (Michener 2007; Ascher and Pickering 2026). According to Ascher and Pickering (2026), the Indian Amegachile fauna comprises 06 species viz., Megachile bicolor (Fabricius, 1781), Megachile buddhae Dalla Torre, 1896, Megachile devadatta Cockerell, 1907, Megachile penetrata Smith, 1879, Megachile saigonensis Cockerell, 1920 and Megachile sikkimi Radoszkowski, 1882, where five of these species reported in north India, except M. bicolor. The present study documents the occurrence of the subgenus Amegachile Friese, 1909 with species Megachile bicolor (Fabricius, 1781) from Andhra Pradesh and Telangana and provides diagnostic illustrations along with male genitalia and molecular characteristics to facilitate accurate species identification.

## 2. MATERIAL AND METHODS

Surveys were carried out in selected regions of south India, covering Karnataka, Kerala, Tamil Nadu, Andhra Pradesh, Telangana and Puducherry between 2022 and April 2026. The morphological identification of the collected specimens was done using a Nikon SMZ 800 stereo-binocular microscope. Genus and subgenus level identification were carried out using the keys provided by Michener (2007) and Gupta (1992, 1993). Species-level identification was based on the taxonomic keys and descriptions of Bingham (1897) and Gupta (1992, 1993). Digital images of type specimens from the Natural History Museum, United Kingdom (NHMUK) were also examined for accurate species identity. All the specimens collected and examined under the study were deposited in the Department of Agricultural Entomology, University of Agricultural Sciences, Gandhi Krishi Vigyana Kendra, Bangalore, Karnataka, India.

Photographs of diagnostic characters were captured using a Leica SAPO stereomicroscope fitted with a Flexacam C3 digital camera. Habitus photographs were taken using a Canon EOS 90D digital camera equipped with a Canon EF 100 mm ultrasonic macro lens mounted on a WeMacro (100 mm) focus stacking rail. Multiple images were

combined using Helicon Focus software (Version 8.3.8) to obtain fully focused photographs and the final images were processed using Adobe Photoshop CS (Version 6.0).
DNA barcoding was carried out using a partial fragment of the mitochondrial cytochrome c oxidase subunit I (mtCOI) gene. Specimens selected for DNA barcoding were preserved in 95% molecular biology-grade ethanol (DNase- and RNase-free) and stored at −20°C until further processing. Genomic DNA was extracted from the leg and thoracic muscle tissues of morphologically identified specimens using a modified cetyltrimethylammonium bromide (CTAB) method. The quality and integrity of the extracted DNA were assessed by agarose gel electrophoresis and the DNA samples were stored at −20°C until further use. A partial fragment of the mtCOI gene was amplified by polymerase chain reaction (PCR) using the universal primers LCO1490 and HCO2198 described by Folmer et al. (1994). The PCR amplification was performed with an initial denaturation at 95°C for 5 min, followed by 40 cycles of denaturation at 95°C for 1 min, annealing at 45°C for 1 min, extension at 72°C for 1 min 30 s, and a final extension at 72°C for 7 min. The amplified PCR products were purified and outsourced for bidirectional Sanger sequencing.
The obtained mtCOI sequences were examined and edited using BioEdit software (version 7.0.5.3) to remove ambiguous bases and to check for insertions, deletions and premature stop codons. The forward and reverse reads were assembled to generate consensus sequences. The final consensus sequence was submitted to the National Centre for Biotechnology Information, GenBank, to obtain NCBI accession records and for public access. Phylogenetic relationships were analysed using the Maximum-Likelihood (ML) method present in MEGA12. Genetic distances were estimated using the HKY (Hasegawa-Kishino-Yano) model. The reliability of the inferred tree topology was assessed by 1,000 bootstrap replicates.

## 3. RESULTS AND DISCUSSION

**Taxonomy**

**Family** Megachilidae Latreille, 1802
**Genus** Megachile Latreille, 1802
**Subgenus** Amegachile Friese, 1909

**Diagnosis of the subgenus Amegachile**: Medium to large-sized bees, body length 9 to 20 mm; integument black, sometimes with reddish colouration on parts; pubescence variable, consisting of tan, red, white or black hairs on different regions of the body. **Female:** scopa present; abdomen usually without fasciae beneath scopa; clypeus with apical margin shallowly emarginate medially, edges of the emargination slightly raised; mandible four-toothed, second interspace appears without a cutting edge (cutting edge fused with the third tooth, giving the third tooth a truncate appearance); third interspace with a large and complete cutting edge. **Male**: Mandible three- to four-toothed, with inferior basal projection; T6 with large preapical carina, crenulate and medially emarginate; apical margin of T6 bearing small lateral teeth.

**Megachile bicolor (Fabricius, 1781)**

= Apis bicolor Fabricius, 1781; Apis albiventris, Christ, 1791; Anthophora bicolor Fabricius, 1804; Megachile bicolor Lepeletier 1841; Megachile (Amegachile) bicolor kagiana Cockerell, 1911; Megachile (Amegachile) bicolor hönei Hedicke, 1940.

**Material examined: INDIA**: **Karnataka**: Bangalore, GKVK - 1♀, 10.xii.2021, coll. Muddasar; 1♀, 23.xii.2021, coll. Manjunath, K. L; 7♀, 23.ix.2025, coll. Pampareddy. Chintamani: 1♀, 5.x.2017, Hand net, coll. Shwetha, T. N; 1♀, 04.iii.2018, Hand net, coll. Shirisha, S; 1♀, 15.v.2018, Hand net, coll. Shreedevi, R; 1♀, 20.iii.2020, Hand net; 1♂, 15.vi.2022, Hand net, coll. Manjunath, K. L; Chintamani (COS): 1♀, 17.iv.2023; 2♀, 21.iv.2023, coll. Pampareddy. Madanamangala: 1♂, 20.x.2017, Hand net, coll. Nanjunda. Kaivara, Forest: 1♀, 24.ii.2023, Sweep net, coll. Pampareddy. Dharwad: 3♀, 30.iv.2023, 1♂, 30.iv.2023, Sweep net, coll. Pampareddy. Hassan, Avverahalli: 1♀, 29.v.2023, 1♂, 29.v.2023, Sweep net, coll. Pampareddy. Haveri: 1♀, 28.iv.2023, Sweep net, coll. Arati Pannure. **Kerala:** Moyolam, Periya, 8.i.2025, 3♀, coll. Pampareddy. **Andhra Pradesh:** Kurnool, 12.iv.2025, 2♀, coll. Pampareddy; Tirupati, Yerpedu (IIT Campus), 26.ix.2025, 1♂, coll. Pampareddy; **Telangana:** Chilla Sagar (FCRI), 08.iv.2026, 2♀, coll. Pampareddy. **Tamil Nadu:** Coimbatore, TNAU, 21.xii.2025, 5♀; 22.xii.2025, 12♀, 4♂, coll. Pampareddy; Kayyunni, 25.i.2026, 1♂, coll. Pampareddy. **Puducherry:** Karaikal, 1♂, 20.v.2026, coll. Pampareddy.

**Diagnosis:**

**Female** (Fig. 1A & 1E)**:** Body length 15.10 ± 0.25 mm; integument black; metasomal terga T1 to T3 (sometimes T4) and sternum are reddish (Fig. 1H & 1I); tegulae dark brown. White dense pubescence on frontal face (Fig. 1B), gena, hypostoma, anterior portion of scutum and lateral sides of propodeum (Fig. 1G); mixture of black and white pubescence on anterolateral margin of scutum. Clypeus finely and sparsely punctate, with depressed median longitudinal impunctate line (Fig. 1F); apical margin incurved and shallowly emarginate medially (Fig. 1F); supraclypeus nearly flat with sparse punctures. Mandible four-dentate (Fig. 1C & D), cutting edges present in the second and third interspaces; cutting edge of second interspace indistinguishably fused with the third tooth, giving the third tooth a broadened appearance. Anterior tentorial pit located at intersection of the subantennal and

epistomal sulci. Scutum and scutellum convex, with slightly coarse, uniform punctures (Fig. 1G); scutellum rounded, slightly hanging over the metanotum; wings fusco-hyaline (Fig. 1A); claws simple. Metanotum, upper part of propodeum and tergal discs T1 to T5 with red-fulvous pubescence (Fig. 1H). Fore and mid tarsi with black bristles mixed with few white hairs; hind legs with pale white small dense hairs on inner side of tibia and fulvous bristles on inner side tarsi. T6 disc covered with suberect dark greyish pubescence (Fig. 1H); scopa on S2 to S5 white (Fig. 1I and J); S6 sparsely covered with black hairs (Fig. 1I and J).

**Male** (Fig. 2A & 2J)**:** Body length 11.25 ± 0.68 mm. Integument black; femora, tibia and tarsi of all legs reddish-brown; tegulae dark brown; sternum reddish-brown (Fig. 2I). Face, hypostoma and gena with pale white pubescence (Fig. 2B). Punctures on clypeus fine, with dense pale white pubescence, apical margin produced slightly, medially incurved (Fig. 2B & C). Mandible with four teeth, lacking cutting edges (Fig. 2D). Pubescence on mesosoma and metasoma similar to that of females. Scutum with coarse, sparse and uniform punctures (Fig. 2E); wings subfuscous at base, dark fuscous apically, veins black (Fig. 2A); first recurrent vein close to base than second recurrent vein, second recurrent vein slightly farther from apex of second submarginal cell. Fore coxae with elongate, blunt spine (Fig. 2F); short apico-dorsal spine on fore-tibiae; claws cleft with subapical tooth; fore-leg tarsi with ferruginous spot baso-ventrally (Fig. 2G) with laterally produced elongated hairs; mid tibia with short apico-dorsal spine. T2 to T4 with shallow and narrow groove along graduli (Fig. 2H); T6 carina with unevenly distributed spines, median spines stronger (Fig. 2L).

**Male genitalia** (Fig. 2K)**:** Gonostylus hammer-shaped, with sparse, elongated hairs on the outer surface; inner margin of the penis valve slightly curved from the subapical region towards the base.

**Floral Association:** Cajanus cajan, Crotalaria pallida, Crotalaria juncea, Pongamia pinnata, Ocimum tenuiflorum, Ocimum americanum, Hyptis suaveolens, Lablab purpureus, Salvia hispanica, Macroptilium atropurpureum.

**Distribution: India (Fig. 3)**: Andhra Pradesh (new record); Bihar (Ascher and Pickering 2026), Chhattisgarh (Sahu et al. 2023), Gujarat, Haryana, Himachal Pradesh (Ascher and Pickering 2026), Jammu and Kashmir (Abrol et al. 2012), Jharkhand, Karnataka (Suma 2006; Veereshkumar 2015; Veereshkumar and Kumaranag 2018; Pampareddy 2023), Kerala, Madhya Pradesh, Maharashtra, Odisha, Pondicherry, Punjab, Rajasthan, Sikkim, Tamil Nadu (Ascher and Pickering 2026), Telangana (new record), Uttar Pradesh (Ascher and Pickering 2026), Uttarakhand (Kunjwal et al. 2016). **Elsewhere**: Australia, Burma, Cambodia, Cameroon, China, Hong Kong, Indonesia, Nigeria, Pakistan, Sri Lanka, Taiwan, Thailand, Zambia (Ascher and Pickering 2026).

**Pairwise genetic divergence and Phylogenetic analysis**

Pairwise genetic-distance analysis based on the mitochondrial COI sequences using the Kimura 2-parameter model (K2P model) revealed low genetic divergence between the newly generated Megachile bicolor sequences from Karnataka (PZ538054) and Telangana (PZ790424), with a divergence of 1.04%. Both sequences also showed relatively low divergence from previously available M. bicolor sequences from Punjab (1.16% for Karnataka and 2.07% for Telangana). Relatively higher divergence was observed between the Indian and the Chinese M. bicolor sequence, with values of 3.45% for Karnataka and 2.89% for Telangana. Our M. bicolor sequences showed substantially higher divergence from other Megachile species, ranging from 15.87-16.38% with M. lanata and 18.09-19.59% with M. disjuncta (Table 1).

The Maximum Likelihood phylogenetic tree, constructed using mitochondrial COI sequences and rooted with Apis cerana indica and Bombus haemorrhoidalis as outgroups, placed the two newly generated Megachile bicolor sequences from the present study (PZ790424 and PZ538054) within a well-supported clade comprising previously published M. bicolor sequences, thereby confirming the species identity. Within the genus Megachile, all the species belonging to the subgenus Amegachile formed a separate clade that is clearly segregated from the other subgenera such as Pseudomegachile (M. lanata) and Callomegachile (M. disjuncta). The clustering according to the current subgeneric classification of the genus Megachile highlights that the mtCOI region contains enough phylogenetic information to differentiate different subgenera (Fig. 4).

The sequences PZ790424 and PZ538054 grouped with previously reported Megachile (Amegachile) bicolor sequences from Punjab (ON381720.1 and ON398437.1), forming a single M. bicolor clade. The Punjab sequences formed a strongly supported subclade (bootstrap = 94%). M. bicolor sequence from Karnataka (PZ538054) clustered with this subclade (bootstrap = 66%), whereas M. bicolor sequence from Telangana (PZ790424) occupied the next sister position (bootstrap = 43%). The Chinese M. bicolour sequence (PZ339449.1) formed a separate lineage within the M. bicolour clade, with the node separating the Chinese and Indian lineages showing strong bootstrap support (98%), which confirms the close affinity within the Indian population (Fig. 4).

## 4. CONCLUSION

The present study documents the subgenus Amegachile with the species Megachile bicolor as new records for Andhra Pradesh and Telangana states and provides a comprehensive morphological description, diagnostic illustrations including male genitalia, along with information on species distribution and floral associations. Molecular characterization based on the mitochondrial cytochrome c oxidase subunit I (mtCOI) gene and phylogenetic analysis further confirmed the identity of M. bicolor, demonstrating its close genetic affinity with previously reported Megachile bicolor populations from India and China. These findings extend the known distribution of M. bicolor in southern India and contribute to the taxonomic knowledge of the regional megachilid

fauna. Furthermore, the study establishes a valuable baseline for future taxonomic, molecular, ecological and biogeographic investigations of Megachile bees in the Indian subcontinent.

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**Authors' contributions**
All authors contributed to the study conception, design and manuscript preparation. **PR** conducted the survey, taxonomic studies and molecular analysis and wrote the manuscript. **AP** structured and supervised the experiments and edited the manuscript. **KG, FDM** and **PCG** critically reviewed and edited the manuscript. **FDM** - Barcoding of M. bicolor collected from Karnataka. All authors provided feedback on earlier versions of the manuscript and have read and approved the final version.

**Ethics approval and consent to participate**
This article does not contain any studies with human or animal participants performed by any of the authors.

**Conflict of interests**
The authors declare that they have no conflicts of interest.

**Availability of data**
All data relevant to the experiments are included in the paper.

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**Table 1. Pairwise genetic divergence (Kimura 2-parameter model) of the newly generated Megachile bicolor sequences from Karnataka and Telangana with conspecific and related Megachile species.**

| Species | NCBI Accession ID | Locality | Karnataka PZ538054 (% divergence) | Telangana PZ790424 (% divergence) |
|---|---|---|---|---|
| M. bicolor | ON381720.1 | Punjab | 1.159 | 2.070 |
| M. bicolor | ON398437.1 | Punjab | 1.159 | 2.070 |
| M. bicolor | PZ339449.1 | China | 3.454 | 2.885 |
| M. bicolor | PZ790424 | Telangana | 1.039 | - |
| M. bicolor | PZ538054 | Karnataka | - | 1.039 |
| M. lanata | PZ543511 | Karnataka | 16.382 | 15.869 |
| M. disjuncta | PZ543469 | Karnataka | 19.592 | 18.088 |

(**Note:** Values are expressed as percentage K2P (Kimura 2-parameter model) genetic distance (distance × 100).


**Figure 1. Megachile bicolor (Fabricius, 1781), Female. A- Habitus, dorsal view; B- Head, frontal view; C&D- Mandibles; E- Habitus, lateral view; F- Clypeus; G-Thorax, dorsal view; H- Metasoma, dorsal view; I- Metasoma (Scopa), ventral view; J- Metasoma, lateral view.**

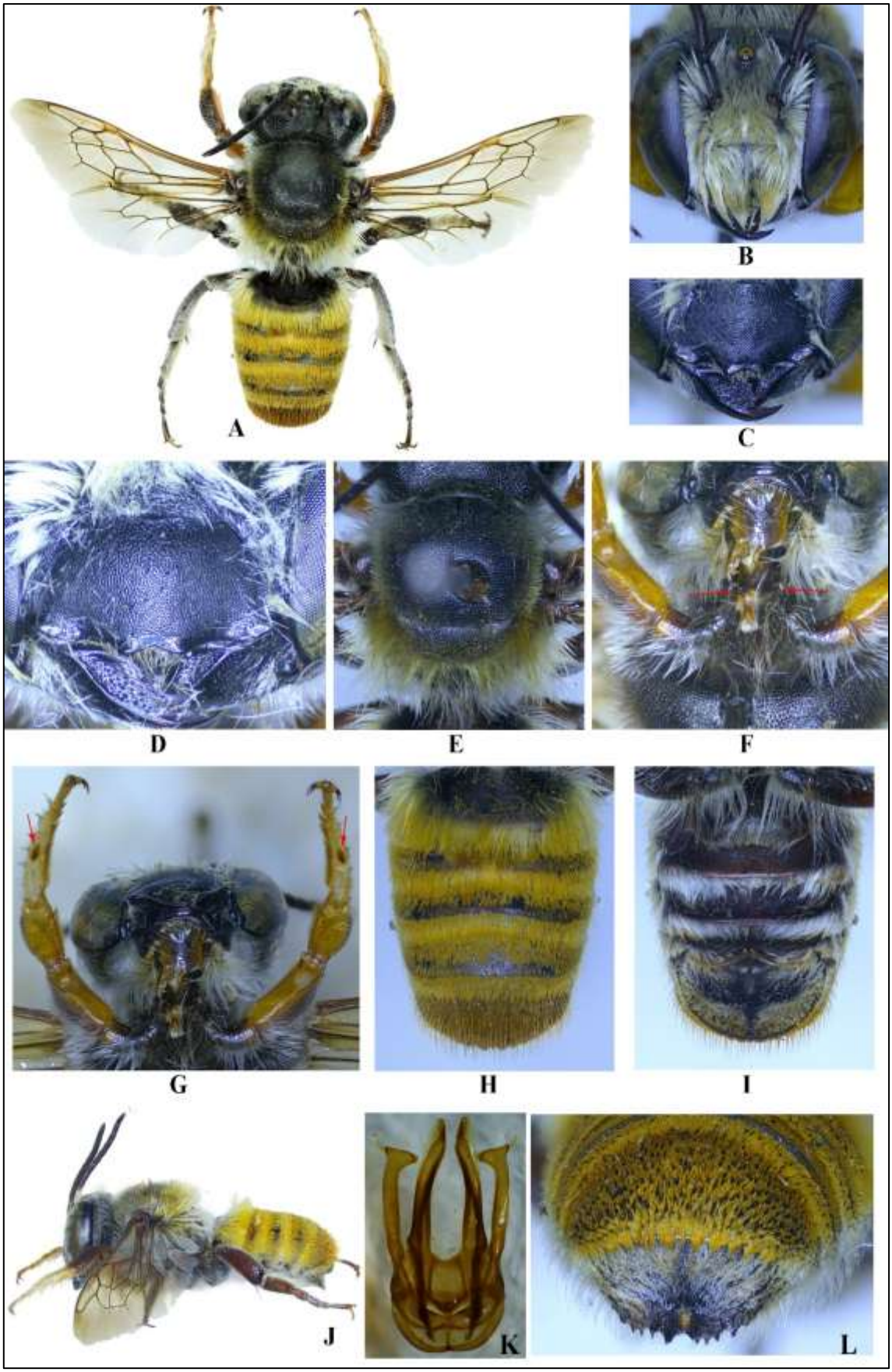


**Figure 2. Megachile bicolor (Fabricius, 1781), Male. A- Habitus, dorsal view; B- Head, frontal view; C- Clypeus; D- Mandibles; E- Thorax, dorsal view; F- Fore tibial spines; G-Fore legs, ventral view; H- Metasoma, dorsal view; I- Metasoma, ventral view; J- Habitus, lateral view; K- Genitalia, dorsal View; L- T6 carina.**

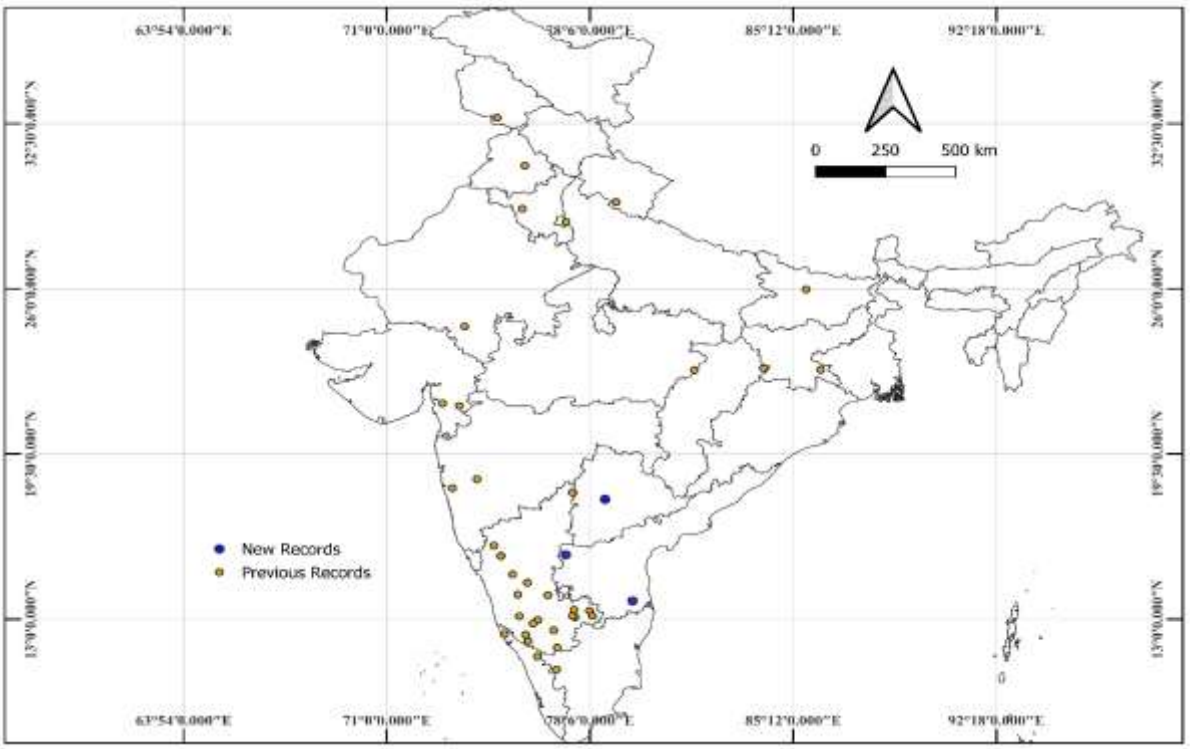


**Figure 3. Distribution of Megachile bicolor (Fabricius, 1781) in India.**

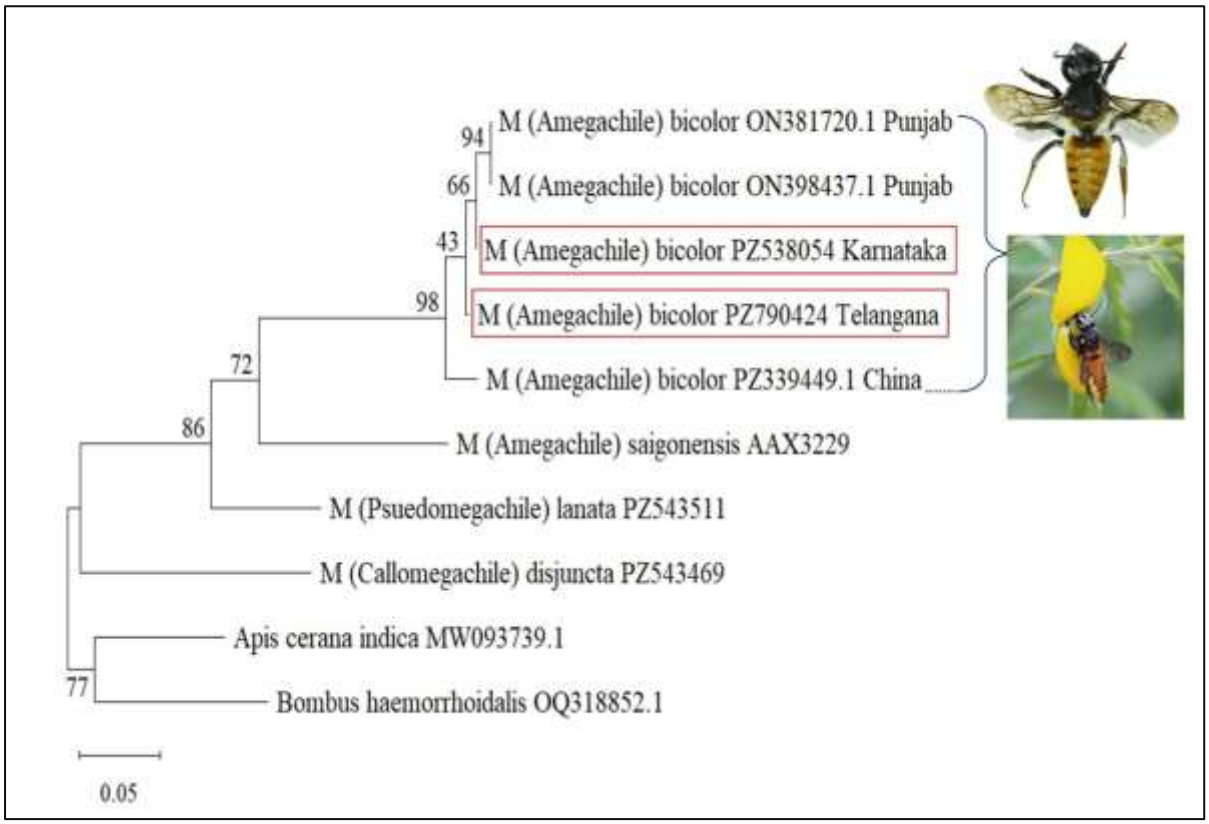


**Figure 4. Maximum Likelihood (ML) phylogenetic tree based on partial mitochondrial cytochrome c oxidase subunit I (mtCOI) gene sequences, showing the phylogenetic placement of Megachile (Amegachile) bicolor from Telangana and Karnataka in relation to other Megachile species. Numbers at the nodes represent bootstrap support values based on 1,000 replicates. Species names are followed by GenBank accession numbers and locality information. Sequences of M. bicolor generated in the present study are highlighted in red. Apis cerana indica and Bombus haemorrhoidalis were included as outgroup taxa.**