SAPIENZA
UNIVERSITÀ EDITRICE

E-ISSN 2284-4880
ISSN 0429-288X

Fragmenta entomologica, 54 (2): 233–246 (2022)

Review article
Submitted: January 22nd, 2022 – *Accepted:* October 9th, 2022 – *Published:* December 15th, 2022
DOI: 10.13133/2284-4880/703

An overview on non-*Apis* bees *vis*-à-*vis* the exploration of integrated taxonomic approach (Hymenoptera: Apoidea)

Jyoti FALSWAL[1], Debjani DEY[2], Sarita KUMAR[1,*]

[1]*Department of Zoology, Acharya Narendra Dev College, University of Delhi, New Delhi-110019* – saritakumar@andc.du.ac.in
[2]*National Pusa Collection, Division of Entomology, ICAR-Indian Agricultural Research Institute, New Delhi-110012*
*Corresponding author

Abstract
Easy and proficient identification of species or organisms is important for various users, such as conservationists, physiologists and ecologists, etc. Taxonomy is a significant branch of biological sciences to classify the different species and understand their relationships. Currently, taxonomy's existence is under crisis and its future protection is required in coming times. Majority of taxonomists are using phylogenetic approach for classifying different species. However, scientists believe that taxonomy should be integrative based on a comprehensive framework for delimiting and describing taxa through integrated information using various data and methodologies. This novel approach does not aim to replace the traditional taxonomy but stresses upon the delineation of species over naming new species. Integrative taxonomy defines the units of species diversity employing multiple approaches; like population genetics, phylogeography, ecology, comparative morphology, development and behaviour, etc. Disagreements among disciplines over the number and demarcation of species can be resolved by using molecular data explaining the evolutionary relationships among species. We present a comprehensive review to explore and identify various non-*Apis* bees and their relationships using integrated taxonomical approaches. We believe that the phylogenies and supportive data can collectively provide a comparative framework for understanding the evolutionary relationships among bee families.

Key words: Integrative taxonomy, non-*Apis* bees, phylogenetic relationships, review, species delineation.

Introduction

Taxonomy is a highly significant branch of biological sciences which mainly aims to classify the different species and understand their relationships between them. Currently, scientists believe that taxonomy' existence is under crisis and its future protection is required in coming times (Wheeler et al. 2004). It is estimated that around 10 million more species exist in nature which still remain to be discovered and in order to do this, we must clarify the nature of primarily two taxonomic tasks, namely delineating and classifying species (Wheeler et al. 2004). These two scientific tasks are currently performed by taxonomists in different ways.

Earlier species diversity and identification studies completely relied on the traditional taxonomy based on morphological characters. Currently, phylogenetic study is a major approach of taxonomists for classification of different species an to reconstruct innate relationships among living organisms; though, in early 1970s, phylogenetic method was a challenge to the taxonomists (Felsenstein 1979). Traditional taxonomists, however were not only in consonant with population biologists and phylogeographers but they also disagreed methodology developed by them for delimiting species (Sites & Marshall 2003). The communication gap between different disciplines involved in delimiting species resulted in 'taxonomy crises'. In order to solve this, scientists believed on integrative taxonomy proposing that this amalgamation will decide the future of taxonomy in coming times.

The term 'Integrative taxonomy' came into existence in 2005 implying a comprehensive framework for delimiting and describing taxa through integrated information using various data and methodologies (Will et al. 2005). The diversity and phylogenetic relationships studies on the families of various major insect groups, such as Coleoptera (Bhardwaj & Jyoti 2018), Hymenoptera (Sharkey et al. 2011), Diptera (Cameron et al. 2007) and Hemiptera (Medeiros et al. 2013) have been carried out by many researchers worldwide. However, the suspect of many systematists that majority of species would remain undescribed using any sole approach (Costello et al. 2013) and there is chance that some will become extinct before getting described (Barnosky et al. 2011), led to integration of approaches.

The taxonomists have realized that study of speciation, origin of species and their evolutionary trajectories, could be a better aid to understand the diversification of species. Moreover, the propositions that the using molecular tools, specific molecular barcoding (Hebert et al. 2003) will ease this "taxonomic impediment" were welcomed by the taxonomists. In addition, synergistic integration of taxonomy with update knowledge and phylogenetics, methods of the populated biology, and all other evolutionary disciplines involving morphological taxonomists, naturalists, field ecologists, and statisticians; possesses the potential to provide a toolkit for proficient taxonomy. Thus, it is now widely known that integrating several paths of evidences, instead of relying on one, is productive and theoretical way to determine new species (Yeates et al. 2011).

Traditional versus Integrative Taxonomy

The traditional morphology-based taxonomy relied on the discrimination of 'morpho-species' by studying the 'morpho-diversity' of living organisms bearing in mind their multiple facets (Cain 1954). However, lack of contemplation that morpho-species are never valid species led them to hypothesize about diversity which needs testification *via* various biological approaches with multiple kinds of data set. According to new hypothesis, the sole morphological approach to discover and identify the species is not enough. Instead, the multidisciplinary approach of integrative taxonomy is necessitated for the validation of species which is based on the initial analysis of the variable morphological features. It has been advocated to thoroughly address intraspecific, interspecific as well as individual variation in morphology before proposition of morpho-species (Steppan et al. 2003). The proposed hypothetical morpho-species are filtered by employing other approaches like molecular, behavioral, developmental, ecological, etc. supplementing the information (Will & Rubinoff 2004).

Since last three decades, various novel methods for delineating species have been developed for testing species hypotheses (Marshall 2003). Use of these new approaches has not only helped to discover the cryptic species but also cleared the doubt about interspecific and intraspecific morphological variations. Researchers have already started using an integrated approach instead of sole traditional taxonomy for studying species diversity (Malhotra & Thorpe 2004). They have realized the need of integrative approach in taxonomy due to the requirement of multiple and complementary perspectives in the complexity of species biology. Moreover, species identification through multiple data raises the level of confidence in comparison to the single method of identification. More efficient results are expected through collaboration with disciplines; like comparative anatomy, phylogeography, ecology, population genetics and behavioral biology.

Relationships of bees and other families

Worldwide, bees comprise more than 20,000 extant species and their relationship with all angiosperms represents the most successful and efficacious co-evolutionary alliance on the Earth (Soltis et al. 2018). The occurrence of co-evolution in bees and angiosperms has demanded an adequate understanding of bee biodiversity, diversification and phylogeny. The availability of efficient molecular data and phylogenetic analysis methods, like maximum-likelihood and Bayesian methods, has dramatically improved the understanding of bee phylogeny and evolution (Huelsenbeck & Ronquist 2001). Additionally, fossil data combined with molecular phylogenies provide supportive evidence for better understanding of phylogenetic relationships of the bees (Drummond & Rambaut 2009).

Earlier studies have confirmed that bees arose within a paraphyletic group of hunting wasps *viz*., sphecoid wasps (Prentice 1998), apoid wasps (Melo 1999), and spheciform wasps (Michener 2007). Though, bees are placed in Superfamily Apoidea, their origin with apoid wasps is not clear yet. The monophyly of the long-tongued bees (Apidae and Megachilidae) has been contradicted by the paraphyly of short-tongued bees (Halictidae, Colletidae, Melittidae, Andrenidae and Stenotritidae) (Danforth et al. 2006). Thus, integrative taxonomy comprising multiple analyses can provide new insight into bee family-level phylogeny.

Utility of integrative taxonomy approaches for non-*Apis* bees

The integrative taxonomy approaches for validating morphological and molecular data, and correctly identifying the species and their interspecific/intraspecific phylogenetic relationships can provide conclusive answers for the controversies in bee phylogeny. A few such approaches are discussed below.

1. Comparative morphology

Comparative morphology is one of the important branches of morphology that is closely related to evolutionary biology and phylogeny. It primarily analyses the structural patterns within the body plan of an organism and forms the basis of taxonomical categorization (Gaucher et al. 2010). Morphological comparison in Hymenoptera, especially bees and their relatives, has been studied extensively. The antenna cleaner (strigilis), a distinctive character of the aculeate Hymenoptera, shows signifcantly derived morphology in some groups such as, Apterogynini, Bradynobaeninae and Scoliidae (Brothers 1975). The antennae cleaners of 175 species of bees belonging to more than 50 analyzed genera revealed the presence of ancestral kind of antenna cleaners in most of the short-tongued bees as well as in Ceratini, Nomadinae and some Megachilidae (except Oxaeidae and Ctenoplectridae) (Schönitzer 1986).

The subsequent studies were primarily focused on the body fluid and its chemical composition (Williams et al. 1987). Later, 46 species of the male carpenter bees belonging to three genera were investigated for the comparative morphological characters of the mesosomal gland (Minckley 1994). Except 6 species of *Xylocopa*, males of rest of the species exhibit an invagination lined by cuticle. The arrangement of tubules and size of reservoir were observed to be consistent within subgenera (with a single exception in *Nodula*).

Comparative morphology in bees and their related genera has also been supported by phylogenetic relationships. Nevertheless, phylogenetic relationships of corbiculate bees are still controversial since the past three decades. Previously, morphological characters explored mouth parts (Plant & Paulus 1987), legs (Michener 1981; Schönitzer 1986) and wings (Roig-Alsina & Michener 1993). Similarly, internal exoskeleton structures (like tentorium, apodemes, internal ridges, phragmata and furcae) were also studied extensively in other hymenopterans groups (Karlsson & Ronquist 2012). The investigations on the morphology of internal head capsule structure, analysing phylogenetic relationships in corbiculate and other bees effectively, could solve the corbiculate controversy to some extent (Porto et al. 2016). The last-mentioned study proposed and optimized 33 characters and resulted in 7-derived character states advocating the monophyly of the Meliponina, Apina, Bombina, while 9-characterstates backed the Apina + Meliponina clades. In addition, two previously undescribed character states on sitophore and hypostoma supported the Bombina and Meliponina clades (Porto et al. 2016). The investigations concluded that inclusion of new character states helped to solve the corbiculate controversy in bees. Similar comparative morphology studies were conducted on the bee mesosoma and its internal structures (e.g., propleuron, prosternum, mesophragma and mesofurca/metafurca). The study proposed a total of 28 characters and demonstrated that prosternum has the largest number of distinct characters, showing changes with nine transformations (Porto et al. 2016).

2. Correct identification

Morphometric methodology is a foremost approach in taxonomy for identification of bee species. The "traditional morphometrics" analyses several variables, like height, ratios, counts, width, angles and length through multivariate statistics (Marcus 1990), while "geometric morphometrics" includes captured size and shape of analysed organ employing outline or landmark-based methods. Landmark technique using multivariate statistical approach was used for distinguishing populations and subspecies of *Bombus* (Bombinae) (Aytekin et al. 2007), stingless bees and others (Villemant et al. 2007). Researchers consider geometric morphometric approaches superior to traditional morphometrics. It is considered a powerful, alternative and useful tool to analyse shapes, and refine molecular characters of species or populations (Adams et al. 2004; Smith et al. 2008).

A few quantifiable wing characters have been used to recognize euglossine (male) bees. Landmark-based method and outline-based method were applied together to identify five species of the genus *Euglossa* (subgenus: *Glossura, piliventris* group). Landmark-based method could classify 84% samples and 77%, respectively. In contrast, utilization of integrated analysis could classify 91% samples (Francoy et al. 2012). On the other hand, when wing cell was only under contour consideration, some insect species showed significantly high re-classification percentages. In addition, all the specimens who had damaged wing have also been correctly identified by using this methodology (Francoy et al. 2012). Subsequently, the outline-based morphometrics was used to assess the phenotypic variation in similar euglossine bees (Roubik 2004), followed by investigations on morphological information gained through combination of outline-based methods and landmark.

Utilization of quantitative characters for the phylogenetic studies is still under deliberation (Klingenberg & Gidaszewski 2010). It is suggested that phylogenetic signal could be detected by wing morphology, but did not capture any phylogenetic information further. However, the biggest problem faced by all researchers is the limitation of specialized taxonomists who can identify specimens (Silveira et al. 2006). Ironically, the biology and taxonomy of Euglossine bees, which play immense role in ecology and conservation, is still less known (Roubik & Hanson 2004). Non-specialists try to identify species by using keys and photographs developed by specialists (Nemesio 2010) along with molecular techniques describing the species specific DNA sequences (Ramírez et al. 2010).

Currently, integrative approach, a blend of morphological features, ecological data as well as genetic data, has successfully solved many uncertainties in taxonomic cases (Gibbs 2009; Monaghan et al. 2009). DNA barcoding of mitochondrial gene sequencing has been definitely useful to limit the evolutionary unit boundaries (Hebert et al. 2003; Murray et al. 2008), followed by species delimitation algorithms development (Williams et al. 2012).

3. Cryptic species identification

Integrative taxonomy is also considered as a reliable tool for identifying and classifying pseudo-cryptic species. In non-*Apis* bees, species identification based on morphological criteria was apparent and subtle, once supported by other methods of identification (Kress et al. 2015; Lajus et al. 2015; Struck et al. 2017). Molecular data is combined with ecological, geographical and morphological data, to provide powerful support for cryptic species identification, and set down species boundary with estimation of bee diversity (Bossert et al. 2016; Gibbs 2018).

Among halictid bees, *Lasioglossum villosulum* (Kirby 1802) species complex shows highest variability with worldwide distribution. Other species complexes like *Bombus lucorum* (Bossert et al. 2016) or *Andrena bicolor* (Praz et al. 2019) reveal combination of some morphological characters. Genetic analysis information of *L. villosulum* complex revealed exclusive species patterns providing conclusive information for correct identification. Pauly and co-workers (2019) analysed morphological and allozymes data for incidence in the *L. villosulum* species complex (Kirby 1802). Morphological examination and DNA analysis provided supplementary information resulting in recognition of two species, *L. berberum* and *L. medinai,* different from all of the other specimens of *L. villosulum* studied. The tree-based species delineation approaches, such as Bayesian Poisson Tree Process (bPTP) and Generalized Mixed Yule Coalescent (GMYC) model, recognized 5 to 10 species within the *L. villosulum* species group, with *L. berberum* and *L. medinai* separated from other species. The study concluded that both morphological and genetic analysis support the extant of *L. berberum* and *L. medinai*, which differs from *L. villosulum* (Pauly et al. 2019).

Utilization of molecular tools during ecological studies on cryptic species of bumble bees has revealed differences in the ecology, abundance and distribution of these species. In Middle Europe, the *Bombus* subgenus includes four distinct species, *B. terrestris*, *B. lucorum*, *B. cryptarum* and *B. magnus.* Murray et al. (2008) investigated this widespread bumble bee complex and studied the cryptic species diversity by using mitochondrial DNA RFLP. Earlier, the taxonomic status of the latter three species, forming the *B. lucorum* complex, was uncertain for a long period until notable methods like nucleotide sequences were used for their correct identification. Similar studies were carried out in Austria by Bossert et al. (2016) who investigated the *B. lucorum* group of cryptic species with the help of DNA-based identification methods. They used CO1 sequence data for investigating the distribution of the *B. lucorum* complex and exact species composition. The species status of some taxa was confirmed with the use of CO1 barcoding which was then considered a productive tool for the species identification within the same group. Earlier, Ellis et al. (2006) were able to separate the morphologically indistinguishable *Bombus ruderatus* and *B. hortorum,* based on the restriction enzyme digestion of cytochrome B region of mtDNA.

Use of state-of-the-art techniques, such as generation of DNA barcodes can be easily used for identification of cryptic species. In *Apis*, the artificial bee colony (ABC) algorithm has been studied to solve the single and multi-objective optimization problems (Mishra et al. 2013). DNA based phylogeny clearly indicates the success of modern approach in Halictidae family (Gibbus et al. 2012; Pauly et al. 2019). In North America, quantitative morphometric approach was utilized to separate queens and workers of three *Bombus* species based on the measurement of the cheek length and width, and antennal segments which were validated utilizing DNA barcoding (Milam et al. 2020).

4. Phylogeographical studies

Comparative phylogeography studies include role of geographic shifts on the distribution of genetic diversity across sympatric species (Schneider et al. 1998). By studying biogeographic histories, comparative phylogeography gives information on historical assemblage of ecological communities which not only helps in understanding the origin, derivation and preservation of local bee species diversity but also provides supportive information on the bee species composition variation across the landscapes (Ricklefs 2004).

Dick et al. (2004) studied the comparative phylogeography in 14 Euglossine bee species based on mitochondrial DNA (mtDNA) analysis across the Amazon basin and/or Andes and reported low divergences of mtDNA within the species. In two *Eulaema* species uniform cross Andean conspecific CO1 haplotypes were observed. The gene flow across the cross-Amazon haplotypes also differed from the Amazon basin found in five species (Dick et al. 2004). The phylogeographic patterns investigated in five species of Eurasian bumblebees with the help of mitochondrial and two nuclear DNA fragments (~2380 bp) established association of stronger species fragmentation with the stronger geographic differentiation (Dellicour et al. 2015). They proposed the role of specialized diet which apparently increased the population structure at the landscape level.

Diversity studies on Neotropical bee biome have discovered a compounded evolutionary history impacted by tectonic and paleoclimatic events (Carnaval et al. 2014). Phylogeographic studies confirmed influence of the Pleistocene climatic changes on the Neotropical biome richness of species, spatial distribution, endemism, historical demography, biogeographic patterns, and genetic diversity (Carnaval et al. 2008). An integrative approach has been utilized to re-establish the evolutionary history of an endemic stingless bee *Partamona rustica*, from the Brazilian forests. Miranda et al. (2016) sequenced eight microsatellite loci and four mitochondrial genes, and recognized two population groups; namely east and west of São Francisco River Valley (SFRV), assumed to break in the late Pleistocene. The Approximate Bayesian Computation (ABC) access and the phylogenetic reconstruction showed that *P. rustica* originated in the west SFRV, afterwards colonising the eastern region. They proposed the compatibility of both genetic data analyses and spatial distribution modelling with historical demographic stability in this bee species (Miranda et al. 2016).

5. Behavioural studies

Evolutionary studies have shown differential behaviour in bees. Repeated evolutionary transitions in bees might have association with their escape from enemies or competitors. Evolution in nocturnal bee's foraging on the night-blooming flowers unlocked a new niche for them, though these all-evolutionary transformations are not enough to generate succeeding radiations. In ancestors of solitary bee the origin of eusociality is considered a big evolutionary transition (Bourke 2011a), and has been a matter of longstanding biological interests (Dugatkin 2006).

Evolutionary inconsistency in organisms has been explained by appropriate ecological conditions and inclusive fitness theory which advocates that individuals gain fitness by reproducing collaterally with those with which they share their genes (Bourke 2011b). However, the underlying mechanism that drives the transition to eusociality has received low attention till date (Field et al. 2012). It has been observed that multiple mating and nest usurpation or worker drifting in some species can reduce relatedness between bee workers (Brand & Chapuisat 2016). Moreover, dead queen replacement by workers in their social system can express that all these workers are not sisters to all the reproductive brood females (Brand & Chapuisat 2016).

Eusocial behaviour in *Augochlora* s. str. was explained by investigating nests of *A. isthmii* Schwarz and *A. amphitrite* (Schrottky) in the fields (Wcislo et al. 2003; Dalmazzo & Roig-Alsina 2012, 2015). They suggested that this subgenus may exhibit more frequent eusocial behaviour, and that solitary forms could be derived from eusocial ancestral behaviour. A nest of the genus *Patellapis* (s. str.) in the western South African winter rainfall area, was inhabited by 8 females indicating a communal nesting behaviour in this group. Analyses of all nest cells with scopal pollen loads of females decoded the polylectic (generalist) behaviour collecting pollen from large number of flower species, namely Asteraceae, Oxalidaceae and Zygophyllaceae (Ritchie et al. 2016).

Most *Xylocopa* species make nests in decaying woods or deadwoods, with an exception of *Proxylocopa hedicke*, which makes nest in soil (Gottlieb et al. 2005). Colletid bees are known for the specific brood cell lining, designated as "cellophane-like" by Michener (2000). It is suggested that the cell lining (polyester) present with the ancestor of colletid bees modified subsequently into the clade formed by all colletid bees except Diphaglossinae. Cocoon-spinning lost and evolutionary change from semi-liquid to firm provisions took place while members of subfamily Hylaeinae added silk to their cell lining. It was believed that there was a possibility of sole shift from soil-nesting to wood-nesting, followed by many soil nesting; but this hypothesis it is not very clear yet (Eduardo & Almeida 2008).

6. Phylogenetic analysis

Phylogenetic relationship study in corbiculate bee tribes was first explored by Michener (1944) who studied inclusive phylogeny of bees primarily based on their external morphology. He found that the bees belonging to Euglossini were the first lineage to diverge and they were followed by Bombini, Meliponini and Apini. Engel (2001) used morphological data including data regarding fossil corbiculate tribes and supported the Michener's phylogeny. Studies conducted by Fernandes-Salomão et al. (2005) investigated the phylogenetic relationships of *Melipona* bees. They analysed 8 species of *Melipona* using Internal transcribed spacer 1 (ITS-1) sequences (394 to 496 bp) of the nuclear rDNA, while 3 species were studied with complete ITS-1 along with flanking regions. Variability among these species was compared using PCR amplification method. Studies revealed only low levels of variability between *M. mandacaia* and *M. quadrifasciata* sequences indicating their recent divergence. Constructed phylogenetic tree demonstrated monophyly of the *Melipona* genus with 4 well-defined clades. The ITS-1 sequences-based phylogenetic relationships validated the taxonomic classification of *Melipona* along with morphological characters (Fernandes-Salomão et al. 2005).

Kawakita et al. (2008) analysed phylogenetics of corbiculate bee tribes by using 12 sets of nuclear protein coding genes. Similar studies were performed by Almeida & Danforth (2009) who analysed four nuclear gene loci (wingless, opsin, 28S rRNA, and elongation factor-1a (F2 copy)) for 122 species of Colletid bees. They employed Bayesian, maximum likelihood, and parsimony methods for studying phylogenetic relationships within Colletidae and obtained consistent phylogenetic trees. Their results demonstrated monophyly in Colletidae as well as in all subfamilies (excepting Paracolletinae) recognized conventionally. In earlier studies, cellophane-like cell lining character was considered as the supreme evidence of monophyly of the Colletidae bee family because of its uniqueness and unreversed nature, found only among colletid bees (Almeida 2008). Molecular phylogenetic analysis of relationships up to family-level in bees was conducted using various nuclear genes and placed the Halictidae as a sister group to Colletidae + Stenotritidae (Danforth et al. 2006; Brady et al. 2011). The relationships and monophyly of family Halictidae have been intensely supported by the molecular sequencing (Danforth et al. 2008) and morphological synapomorphies (Rozen 2008). Cameron et al. (2007) performed comprehensive phylogenetic studies on 250 species of bumble bees, belonging to 38 genera. They analysed 4 nuclear genes (opsin, EF-1α, PEPCK- Phosphoenolpyruvate carboxykinase and arginine kinase) and mitochondrial (16S) sequences to study species phylogeny. Analysis of results using Bayesian statistical paradigm revealed monophyly of almost all traditional morphology-based subgenera supporting phylogeny from base to top. Most of the subgenera belonged to two distinct clades (long-faced and short-faced) indicating variation in head morphology. The short-faced clade comprised almost 25% of the currently recognized subgenera and was considered as diverse New World clade. This inclusive phylogenetic study provided the basis for evaluation of character evolution and re-classification of bumble bees.

Similar molecular phylogenetic studies were carried out on *Xylocopa,* the large carpenter bees (Leys et al. 2000). A total of 22 subgenera of *Xylocopa* were investigated based on the sequencing of mitochondrial genes with cytochrome b and cytochrome oxidase 1. Results determined three clades, a South American group containing *Xylocopa* s. str. and *Ctenoxylocopa*, and an Ethiopian group. They also demonstrated divergence of Ethiopian group from African and the Oriental taxa. Gondwana vicariance studies explained the present-day distribution of subgenera confirming the occurrence of recent divergences between geographic groups.

Limitations of DNA barcoding in taxonomy to tackle the problematic cases of species delimitation in bees has been addressed with the use of nuclear genomic marker, such as Ultraconserved Elements (UCEs). Utilizing this robust approach, Gueuning et al. (2020) assessed six different bee species complexes with cryptic diversity, and mitochondrial introgression and paraphyly. The explicit species delimitation was demonstrated by sequencing of UCEs recovered between 686 and 1,860 homologous nuclear loci. Shimodaira-Hasegawa (SH) test has been used as a modern approach in phylogenetic studies of bees and wasps. Branstetter et al. (2017) used UCE phylogenomics and analysed the taxon-rich 187-taxon dataset and a taxonomically balanced 100-taxon dataset. Based on the analyses, such as the rejection of alternative topologies ($p < 0.01$) and their current level of taxon sampling, they evidently concluded ants as the sister group to the Superfamily Apoidea, and nesting of bees with a paraphyletic crabronid wasps. Bossert et al. (2018) used a blended approach of UCEs and transcriptomes in analysing the phylogenetic relationship between 79 bees. Using concatenation- and coalescent-based methods, they constructed phylogenetic trees and clarified the relationships among the genera of orchid bees (Euglossini) and the monophyly of the Centridini.

7. Molecular biology studies

The molecular techniques can efficiently address the taxonomic problems that could hamper bee ecological research and monitoring efforts. These techniques provide complementary approach to identify natural bees and allow novel visions into the bee population statistics, habitat preferences and inclinations for foraging areas (Vaudo et al. 2018). Use of genetic information for studying population structure, genetic variability, gene flow, and the past events can help in the placement of bees at correct taxonomic position (Lozier & Zayed 2017).

Microsatellite markers are extensively practiced in the research on social evolution. Parsons et al. (2017) conducted molecular investigations on Palaearctic sweat bee, *Lasioglossum malachurum.* They identified and successfully optimized a total of 24 new highly polymorphic microsatellite loci. On amplification these loci across 23–40 unrelated females, they obtained 3 to 17 alleles/locus and 0.45 to 0.95 heterozygosity. These studies implicate that significant deviation from Hardy–Weinberg equilibrium could help researchers in understanding the intraspecific colony relationships and some aspects of the eusociality origin. Similar studies conducted on *Lasioglossum calceatum* resulted in successful amplification of 22 loci, assisting in examining the ecology and evolution of sweat bees.

It is well known that DNA methylation plays an important role in gene imprinting, gene silencing, inactivation of X-chromosome and other important biological processes (Hellman & Chess 2007). Nowadays, DNA cytosine methylation has been recommended in understanding the caste differentiation in social insects and behavioural plasticity in bumblebees (Elango et al. 2009). Li et al. (2018) recognized six genes facilitating the alteration in DNA methylation and established their expression in *Bombus terrestris.* Many genes were highly expressed in the fat bodies and gonads; while in adult antennae and brains, low expression of genes was noticed. They observed that in queens there was higher transcription levels than in the drones and workers and proposed that these genes mainly govern bumble bees' caste-specific expression patterns (Li et al. 2018). These investigations concluded that in bumble bees' caste differentiation, DNA methylation-related genes have significant role.

8. Population genetics

Significant genetic differentiations can occur between populations at small geographic scales due to distance isolation among populations. The genetic differentiation due to isolation by distance was studied with the help of multi-locus genotype assignment tests. Studies showed that migrants of 11 first-generation *Lasioglossum oenotherae* sub-populations have experienced gene flow (Zayed & Packer 2007). It was also revealed that southern population of *L. oenotherae* diverged from Hardy–Weinberg equilibrium and also from genotypic equilibrium significantly. They suggested the involvement of regional difference in the gene flow and inbreeding of these bees (Zayed & Packer 2007).

Population genetic studies can examine the relevance of bee conservation to the population genetic parameters (Packer et al. 2005), ecological speciation (Funk et al. 2002) and patterns of bee diversity. Population genetics investigations on the sweat bee *L. oenotherae* (Oligolectic), revealed eight species, all known for pollen collection on evening primroses (McGinley 2003). The females were found to have a specialized scopa (pollen collecting hairs) adapted for pollen collection, held together by viscin threads. Furthermore, female foraging activity was recorded at night, which is rare amongst bees (McGinley 2003).

A study by Oppenheimer et al. (2018) used eight microsatellite loci as markers for illustration of the genetic constitution of three different populations of *Ceratina australensis* at Murray–Darling River basin. A total of 57 female bees were genotyped, exhibiting geographically significant variation in allelic diversity and heterozygosity. This study also analyzed the chances of dispersal of male-biased and less interpopulation migration in the species. These authors also established the spread of *C. australensis* into dry regions created due to severe anthropogenic disturbances. Their hypothesis provided evidences that diffusion of *C. australensis* into Australia might be due to the invasive pithy stemmed plant species (Oppenheimer et al. 2018).

Nowadays, whole-genome sequencing is very popular for population genetic studies. It provides maximal data but the approach has limited applications as it usually assesses only a few samples. In contrast, sufficient data can be provided by sequencing a pool of samples (pool-seq). Chen et al. (2022) studied 14 populations of bee workers and concluded that while pool-seq offers genome-wide polymorphism data at relatively much lower costs, individual sequences (ind-seq) can also provide supplementary information. They proposed that the though pool-seq is a cost-efficient approach, the ind-seq population genetic structure can provide alike advantage with additional insight into individual-based admixture. Population genetic studies supplemented with Next Generation sequencing has allowed researchers to identify potential genes under selection, putatively perilous to species' survival in their rapidly changing environment. Kelemen & Rehan (2021) proposed that while genetic studies provide perceptions about bee biology, additional studies focusing on a bigger phylogenetic and life history extent of species are also necessary.

Future perspectives

Integrative taxonomy mainly relies on evolutionary biological and can help the evolutionary biologists to address species-level problems (Padial et al. 2010). During past years, taxonomic impediment has reduced and confusions about different species concepts were minimized by consent on the view of species as lineages (Wiens 2007). Now, DNA sequencing data is considered the stronger basis for identification of cryptic and candidate species (Lahaye et al. 2008). It is believed that these advances will help to surge the species description annual rates at 14,000-25,000 in spite of decline in the number of taxonomists (Lords 2008).

Improving taxonomic protocols can assist in providing stable names and accelerating the pace of taxonomic descriptions (De Carvalho et al. 2008). Nowadays, efficient tools have been designed by population geneticists to estimate linkage disequilibrium which occurs if only combinations of the alleles occur more regularly than expected (Slatkin 2008). These methods can be more efficiently used for the identification of cryptic species in bees (Lukhtanov & Shapoval 2008). Novel approach like CONCATERPILLAR (Leigh et al. 2008) considers differential evolutionary rates of dissimilar loci for identification. Extending these approaches to non-molecular characters proves more stable protocols of integrative taxonomy.

Improvement of phylogenetic software, like Mesquite (Maddison & Maddison 2010) provides statistical modules for morphological data analysis that can extract characters evidence from the bi- and tridimensional imagery (La Salle et al. 2009) and also from sequence data for geographical and ecological modelling and mapping. This kind of software can be used for bee species delimitation, description and publishing. Another latest approach in taxonomy includes genomic analysis in taxonomy (Geno Taxonomy) which uses automatic identification of regions in insect genome as diagnostic characters and accelerate species discovery, through modular taxonomic software (Butlin 2010). Latest next-generation sequencing of bee transcriptomes and genomic data can provide concrete evidences for bee and wasp phylogeny. Future studies with wider axon testing across the apoid wasps could help in recognizing the sister group to the bees.

Conclusions

Future of the taxonomy is integrative approach in all branches of biology. Indeed, there is a need to study species diversity using multiple approaches. Thus, taxonomists believe to integrate the recent approaches as a synthetic approach in order to delimit the species boundaries. Though, it is high time that discipline evolves as an entire unit and becomes integrative and inclusive. Using multiple disciplines to solve taxonomic problems can ensure increased rigor in bee species delimitation. Nevertheless, employing integrative taxonomy approach to recognize bees is not an easy task and requires efforts and inputs from all the sides. Earlier conflicts and difference in opinions between some morphologists and some molecular biologists on bee phylogeny should end now. More discussions shall be held on different approaches and concepts of phylogeographic, population genetics and phylogenetic analyses rather on simply assimilating different characters.

Conflicts of interest – The authors declare no conflicts of interests.

Authors' contributions – JF did the literature search, compiled and wrote the manuscript. DD and SK edited and critically revised the work.

Acknowledgments – The authors gratefully acknowledge the financial support for this study by the Area Department of Biotechnology (DBT), Government of India, New Delhi-110003. Authors also acknowledge Director, ICAR-IARI and Head, Division of Entomology, ICAR-IARI, New Delhi – 110012 and Department of Zoology, Acharya Narendra Dev College, Delhi University, New Delhi-110019 for all the facilities required for the study.

References

Adams D.C., Rohlf F.J., Slice D.E. 2004. Geometric morphometrics: ten years of progress following the 'revolution'. Italian Journal of Zoology, 71(1): 5–16.

Almeida E.A. 2008. Colletidae nesting biology (Hymenoptera: Apoidea). Apidologie, 39(1): 16–29.

Almeida E.A., Danforth B.N. 2009. Phylogeny of colletid bees (Hymenoptera: Colletidae) inferred from four nuclear genes. Molecular Phylogenetics and Evolution, 50(2): 290–309.

Almeida E.A.B. 2008. Colletidae nesting biology (Hymenoptera: Apoidea). Apidologie*,* 39: 16–29.

Arneson L., Wcislo W.T. 2003. Dominant-subordinate relationships in a facultatively social, nocturnal bee, *Megalopta genalis* (Hymenoptera: Halictidae). Journal of the Kansas Entomological Society, 183–193.

Ascher J.S., Pickering J. 2016. Discover Life′s bee species guide and world checklist. http://www.discoverlife.org

Aytekin M.A., Terzo M., Rasmont P., Çağatay N. 2007, January. Landmark based geometric morphometric analysis of wing shape in *Sibiricobombus* Vogt (Hymenoptera: Apidae: *Bombus* Latreille). In Annales de la Société Entomologique de France (Vol. 43, No. 1, pp. 95–102). Taylor & Francis Group.

Barnosky A.D., Matzke N., Tomiya S., Wogan G.O., Swartz B., Quental T.B., Mersey B. 2011. Has the Earth's sixth mass extinction already arrived? Nature, 471(7336): 51–57.

Bhardwaj D.K., Falswal J. 2018. The Coccinellids (Coleoptera: Coccinellidae) fauna in Manduwala region, Dehradun, India. Journal of Experimental Zoology, 21(1): 177–183.

Bingham C.T. 1897. The Fauna of British India, Including Ceylon and Burma. Hymenoptera-Vol. 1. Wasps and Bees. London: Taylor & Francis.

Boontop Y., Malaipan S., Chareansom K. 2008. Large carpenter bees in Thailand and biology of *Xylocopa nasalis* (Westwood). Thailand Natural History Museum Journal, 3(1): 5–15.

Bossert S., Gereben-Krenn B.A., Neumayer J., Schneller B., Krenn H.W. 2016. The cryptic *Bombus lucorum* complex (Hymenoptera: Apidae) in Austria: Phylogeny, distribution, habitat usage and a climatic characterization based on COI sequence data. Zoological Studies, 55: 1–15.

Bossert S., Murray E.A., Almeida E.A.B., Brady S.G., Blaimer B.B., Danforth N. 2018. Combining transcriptomes and ultraconserved elements to illuminate the phylogeny of Apidae. Molecular Phylogenetics and Evolution, 130: 121–131.

Bourke A.F. 2011a. Principles of social evolution. Oxford University Press.

Bourke A.F. 2011b. The validity and value of inclusive fitness theory. Proceedings of the Royal Society B: Biological Sciences, 278(1723): 3313–3320.

Brady S.G., Litman J.R., Danforth B.N. 2011. Rooting phylogenies using gene duplications: an empirical example from the bees (Apoidea). Molecular Phylogenetics and Evolution, 60(3): 295–304.

Brand N., Chapuisat M. 2014. Impact of helpers on colony productivity in a primitively eusocial bee. Behavioral Ecology and Sociobiology, 68(2): 291–298.

Brand N., Chapuisat M. 2016. Low relatedness and frequent inter-nest movements in a eusocial sweat bee. Insectes Sociaux, 63(2): 249–256.

Branstetter M.G., Danforth B.N., Pitts J.P., Ward P.S., Buffington M.L., Gates M.W., Kula R.R and Brady S.G. 2017. Phylogenomic Insights into the Evolution of Stinging Wasps and the Origins of Ants and Bees. Current Biology 27, 1019–1025.

Brothers D.J. 1975. Phylogeny and classification of the aculeate Hymenoptera, with special reference to Mutillidae. University of Kansas Science Bulletin, 50: 483–648.

Burgett D.M., Sukumalanand P. 2000. Flight activity of *Xylocopa (Nyctomelitta) tranquebarica*: a night flying carpenter bee (Hymenoptera: Apidae). Journal of Apicultural Research, 39(1-2): 75–83.

Butlin R.K. 2010. Population genomics and speciation. Genetica, 138(4): 409–418.

Cain A.J. 1954. Animal Species and Their Evolution*.* Princeton University Press.

Cameron S.L., Lambkin C.L., Barker S.C., Whiting M.F. 2007. A mitochondrial genome phylogeny of Diptera: Whole genome sequence data accurately resolve relationships over broad timescales with high precision. Systematic Entomology, 32(1): 40–59.

Carnaval A.C., Moritz C. 2008. Historical climate modelling predicts patterns of current biodiversity in the Brazilian Atlantic forest. Journal of Biogeography, 35(7): 1187–1201.

Carnaval A.C., Waltari E., Rodrigues M.T., Rosauer D., VanDerWal J., Damasceno R., Pie M. R. 2014. Prediction of phylogeographic endemism in an environmentally complex biome. Proceedings of the Royal Society B: Biological Sciences, 281(1792): 20141461.

Chen C., Parejo M., Momeni J., Langa J., Nielsen R.O., Shi, W., Vingborg R., Kryger P. and Bouga, M. 2022. Population

Structure and Diversity in European Honey Bees (*Apis mellifera* L.)—An Empirical Comparison of Pool and Individual Whole-Genome Sequencing. Genes 13(2):182.

Costello M.J., May R.M., Stork N.E. 2013. Can we name Earth's species before they go extinct? Science, 339(6118): 413–416.

Dalmazzo M., Alsina A.R. 2012. Nest structure and notes on the social behaviour of *Augochlora amphitrite* (Schrottky) (Hymenoptera, Halictidae). Journal of Hymenoptera Research, 26: 17–29.

Dalmazzo M., Roig-Alsina A. 2015. Social biology of *Augochlora (Augochlora) phoemonoe* (Hymenoptera, Halictidae) reared in laboratory nests. Insectes Sociaux, 62(3): 315–323.

Dalmazzo M., Roig-Alsina A. 2018. Primitively eusocial behavior observed in colonies of *Augochlora amphitrite* (Hymenoptera: Halictidae) reared in laboratory. Sociobiology, 65(4): 773–776.

Danforth B. N., Brady S. G., Sipes S. D., Pearson A. 2004. Single-copy nuclear genes recover Cretaceous-age divergences in bees. Systematic Biology, 309–326.

Danforth B.N., Eardley C., Packer L., Walker K., Pauly A., Randrianambinintsoa F.J. 2008. Phylogeny of Halictidae with an emphasis on endemic African Halictinae. Apidologie, 39(1): 86–101.

Danforth B.N., Fang J., Sipes S. 2006. Analysis of family-level relationships in bees (Hymenoptera: Apiformes) using 28S and two previously unexplored nuclear genes: CAD and RNA polymerase II. Molecular Phylogenetics and Evolution, 39(2): 358–372.

Danforth B.N., Ji S., Ballard L.J. (2003). Gene flow and population structure in an oligolectic desert bee, *Macrotera (Macroteropsis) portalis* (Hymenoptera: Andrenidae). Journal of the Kansas Entomological Society, 221–235.

Davison P.J. 2016. Social polymorphism and social behaviour in sweat bees (Hymenoptera: Halictidae) (Doctoral dissertation, University of Sussex).

De Carvalho M.R., Bockmann F.A., Amorim D.S., Brandao, C.R.F. (2008). Systematics must embrace comparative biology and evolution, not speed and automation. Evolutionary Biology, 35(2): 150–157.

Dellicour S., Michez D., Mardulyn P. 2015. Comparative phylogeography of five bumblebees: Impact of range fragmentation, range size and diet specialization. Biological Journal of the Linnean Society, 116(4): 926–939.

Dick C.W., Roubik D.W., Gruber K.F., Bermingham E. 2004. Long-distance gene flow and cross-Andean dispersal of lowland rainforest bees (Apidae: Euglossini) revealed by comparative mitochondrial DNA phylogeography. Molecular Ecology, 13(12): 3775–3785.

Drummond A.J., Rambaut A. 2009. Bayesian evolutionary analysis by sampling trees. Evolutionary Biology, 7: 214.

Dugatkin L.A. 2006. The altruism equation: Seven scientists search for the origins of goodness. Princeton University Press.

Elango N., Hunt B.G., Goodisman M.A., Soojin V.Y. 2009. DNA methylation is widespread and associated with differential gene expression in castes of the honeybee, *Apis mellifera*. Proceedings of the National Academy of Sciences, 106(27): 11206–11211.

Ellis J., Knight M.E., Carvell C., Goulson D. 2006. Cryptic species identification: a simple diagnostic tool for discriminating between two problematic bumblebee species. Molecular Ecology Notes, 6(2): 540–542.

Engel M.S. 2000. Classification of the bee tribe Augochlorini (Hymenoptera: Halictidae). Bulletin of the American Museum of Natural History, 2000(250): 1–89.

Engel M.S. 2001. Monophyly and extensive extinction of advanced eusocial bees: Insights from an unexpected Eocene diversity. Proceedings of the National Academy of Sciences, 98(4): 1661–1664.

Estoup A., Solignac M., Cornuet J.M., Goudet J., Scholl A. 1996. Genetic differentiation of continental and island populations of *Bombus terrestris* (Hymenoptera: Apidae) in Europe. Molecular Ecology, 5(1): 19–31.

Felsenstein J. 1979. Alternative methods of phylogenetic inference and their interrelationship. Systematic Biology, 28(1): 49–62.

Fernandes-Salomão T.M., Rocha R.B., Campos L.A.O., Araújo E.F. 2005. The first internal transcribed spacer (ITS-1) of *Melipona* species (Hymenoptera, Apidae, Meliponini): characterization and phylogenetic analysis. Insectes Sociaux, 52(1): 11–18.

Field J., Paxton R., Soro A., Craze P., Bridge C. 2012. Body size, demography and foraging in a socially plastic sweat bee: a common garden experiment. Behavioral Ecology and Sociobiology, 66(5): 743–756.

Francoy T.M., de Faria Franco F., Roubik D.W. 2012. Integrated landmark and outline-based morphometric methods efficiently distinguish species of *Euglossa* (Hymenoptera, Apidae, Euglossini). Apidologie, 43(6): 609–617.

Friese H. 1898. Die Bienen Europa's (Apidae europaeae) nachihrenGattungen, Arten und Varietäten auf vergleichendmorphologisch-biologischerGrundlage. Theil IV: SolitäreApiden: Genus *Eriades*. Genus *Trachusa*. Genus *Anthidium*. Asher, Innsbruck.

Friese H. 1917. Neue Arten der Bienengattung *Anthidium* (Hym.) (Paläarktische Region und von Formosa. Deutsche Entomologische Zeitschrift, 49–60.

Funk D.J., Filchak K.E., Feder, J. L. 2002. Herbivorous insects: model systems for the comparative study of speciation ecology. In Genetics of Mate Choice: From Sexual Selection to Sexual Isolation. Springer, Dordrecht, pp. 251–267.

García-París M., Alcobendas M., Buckley D., Wake D.B. 2003. Dispersal of viviparity across contact zones in Iberian populations of fire salamanders (*Salamandra*) inferred from discordance of genetic and morphological traits. Evolution, 57(1): 129–143.

Gaucher E.A., Kratzer J. T., Randall R.N. 2010. Deep phylogeny—how a tree can help characterize early life on Earth. Cold Spring Harbor Perspectives in Biology, 2(1): a002238.

Gibbs J. 2009. Integrative taxonomy identifies new (and old) species in the *Lasioglossum (Dialictus) tegulare* (Robertson) species group (Hymenoptera, Halictidae). Zootaxa, 2032(1): 1–38.

Gibbs J. 2018. DNA barcoding a nightmare taxon: assessing barcode index numbers and barcode gaps for sweat bees. Genome, 61(1): 21–31.

Gibbs J., Brady S.G., Kanda K., Danforth B.N. 2012. Phylogeny of halictine bees supports a shared origin of eusociality for *Halictus* and *Lasioglossum* (Apoidea: Anthophila: Halictidae). Molecular Phylogenetics and Evolution, 65(3): 926–939.

Gottlieb D., Keasar T., Shmida A., Motro U. 2005. Possible foraging benefits of bimodal daily activity in *Proxylocopa olivieri* (Lepeletier) (Hymenoptera: Anthophoridae). Environmental Entomology, 34(2): 417–424.

Gueuning M., Frey J.E., Praz C. 2020. Ultraconserved yet informative for species delimitation: Ultraconserved elements resolve long-standing systematic enigma in Central European bees. Molecular Ecology, 29(21): 4203–4220.

Hammond P. 1992. Species inventory. Pp. 17–39. In: B. Groombridge (ed.) Global Biodiversity, Status of the Earth's Living Resources. Chapman & Hall.

Hebert P.D., Cywinska A., Ball S.L., Dewaard J.R. 2003. Biological identifications through DNA barcodes. Proceedings of the Royal Society of London. Series B: Biological Sciences, 270(1512): 313–321.

Hellman A., Chess A. 2007. Gene body-specific methylation on the active X chromosome. Science, 315(5815): 1141–1143.

Hongjamrassilp W., Warrit N. 2014. Nesting biology of an Oriental carpenter bee, *Xylocopa (Biluna) nasalis* Westwood, 1838, in Thailand (Hymenoptera, Apidae, Xylocopinae). Journal of Hymenoptera Research, 41: 75.

Huelsenbeck J.P., Ronquist F. 2001. MRBAYES: Bayesian inference of phylogenetic trees. Bioinformatics, 17(8): 754–755.

Hunt J.H., Jeanne R.L., Keeping M.G. 1995. Observations on *Apoicapallens*, a nocturnal neotropical social wasp (Hymenoptera: Vespidae, Polistinae, Epiponini). Insectes Sociaux, 42(3): 223–236.

ITIS. 2012. World bee checklist. Compiled by MA Ruggiero et al. 2012. Integrated Taxonomic Information System (ITIS). www.itis.gov.

Karlsson D. Ronquist F. 2012 Skeletal morphology of *Opiusdissitus* and *Biosteres carbonarius* (Hymenoptera: Braconidae), with a discussion of terminology. PLoS One, 7: e32573.

Kelemen E.P., Rehan S.M. 2021. Conservation insights from wild bee genetic studies: Geographic differences, susceptibility to inbreeding, and signs of local adaptation. Evolutionary Applications, 14(6): 1485–1496.

Kirby W. 1802. Monographia Apum Angliae. Vol. 2. J. Raw, Ipswich, 387 pp.

Klingenberg C.P., Gidaszewski N.A. 2010. Testing and quantifying phylogenetic signals and homoplasy in morphometric data. Systematic Biology, 59(3): 245–261.

Knapp S., Lughadha E.N., Paton A. 2005. Taxonomic inflation, species concepts and global species lists. Trends in Ecology & Evolution, 20(1): 7.

Kress W.J., García-Robledo C., Uriarte M., Erickson D.L. 2015. DNA barcodes for ecology, evolution, and conservation. Trends in Ecology & Evolution, 30(1): 25–35.

Kumari P., Kumar N.R., Sidhu A.K., Chandra K. 2018. Taxonomical and behavioural studies on *Megachile conjuncta* (Fabricius) (Hymenoptera: Megachilidae: *Cressoniella*). Journal of Entomology and Zoology Studies, 6(5): 2198–2201.

La Salle J., Wheeler Q., Jackway P., Winterton S., Hobern D., Lovell D. (2009). Accelerating taxonomic discovery through automated character extraction. Zootaxa, 2217(1): 43–55.

Lahaye R., Van der Bank M., Bogarin D., Warner J., Pupulin F., Gigot G., Savolainen V. 2008. DNA barcoding the floras of biodiversity hotspots. Proceedings of the National Academy of Sciences, 105(8): 2923–2928.

Lajus D., Sukhikh N., Alekseev V. 2015. Cryptic or pseudocryptic: can morphological methods inform copepod taxonomy? An analysis of publications and a case study of the *Eurytemora affinis* species complex. Ecology and Evolution, 5(12): 2374–2385.

Leigh J.W., Susko E., Baumgartner M., Roger A.J. 2008. Testing congruence in phylogenomic analysis. Systematic Biology, 57(1): 104–115.

Leys R., Cooper S.J., Schwarz M.P. 2000. Molecular phylogeny of the large carpenter bees, genus *Xylocopa* (Hymenoptera: Apidae), based on mitochondrial DNA sequences. Molecular Phylogenetics and Evolution, 17(3): 407–418.

Li B., Hou L., Zhu D., Xu X., An S., Wang X. 2018. Identification and caste-dependent expression patterns of DNA methylation associated genes in *Bombus terrestris*. Scientific Reports, 8(1): 1–10.

Lords H.O. 2008. Systematics and Taxonomy: Follow-up report with Evidence**.** The House of Lords, UK.

Lozier J.D., Zayed A. 2017. Bee conservation in the age of genomics. Conservation Genetics, 18(3): 713–729.

Lukhtanov V.A., Shapoval N.A. 2008. Detection of cryptic species in sympatry using population analysis of unlinked genetic markers a study of the *Agrodiaetus kendevani* species complex (Lepidoptera: Lycaenidae). Doklady Biological Sciences, 423(1): 432–436.

Maddison W.P., Maddison D.R. 2010. Mesquite: a modular system for evolutionary analysis. Version 2.6. 2009. Available at mesquiteproject.org.

Malhotra A., Thorpe R.S. 2004. Maximizing information in systematic revisions: a combined molecular and morphological analysis of a cryptic green pitviper complex (*Trimeresurus stejnegeri*). Biological Journal of the Linnean Society, 82(2): 219–235.

Mallet J., Willmott K. 2003. Taxonomy: renaissance or Tower of Babel? Trends in Ecology & Evolution, 18(2): 57–59.

Marcus L.F. 1990 Traditional morphometrics. In: Rohlf, F.J., Bookstein, F.L. (eds) Proceedings of the Michigan morphometrics workshop. Special Publication Number 2, p. 77–122. University of Michigan Museum of Zoology, Ann Arbor.

Martins A.C., Melo G.A., Renner S.S. 2014. The corbiculate bees arose from New World oil-collecting bees: Implications for the origin of pollen baskets. Molecular Phylogenetics and evolution, 80: 88–94.

McGinley R.J. 2003. Studies of Halictinae (Apoidea: Halictidae) II: Revision of *Sphecodogastra*, floral specialists of Onagraceae. Smithsonian Contributions to Zoology, 168(2): 221–425.

McKinney M.I., Park Y.L. 2012. Nesting activity and behavior of *Osmia cornifrons* (Hymenoptera: Megachilidae) elucidated using videography. Psyche, 2012.

Medeiros M.J., Eiben J.A., Haines W.P., Kaholoaa R., King C., Krushelnycky P. D., Starr K. 2013. The importance of insect monitoring to conservation actions in Hawaii. Hawaiian Entomological Society, 45: 149–166.

Michener C.D. 1944. Comparative external morphology, phylogeny, and a classification of the bees (Hymenoptera). Bulletin of the AMNH, 82(6).

Michener C.D. 1981. Comparative morphology of the middle coxae of Apoidea. Journal of the Kansas Entomological Society, 319–326.

Michener C.D. 2007. The Bees of the World. 2nd Edition. Johns Hopkins University Press, Baltimore, 953 pp.

Michener C.D. 2000. The Bees of the World. The Johns Hopkins Press, Baltimore.

Milam J., Johnson D.E., Andersen J.C., Fassler A.B., Narango D.L., Elinkton J.S. 2020. Validating morphometrics with DNA barcoding to reliably separate three cryptic species of *Bombus cresson* (Hymenoptera: Apidae). Insects, 11: 669, Doi: https://doi.org/10.3390/insects11100669

Minckley R.L. 1994. Comparative morphology of the mesosomal 'gland' in male large carpenter bees (Apidae: Xylocopini), Biological Journal of the Linnean Society*,* 53(3): 291–308, Doi: https://doi.org/10.1111/j.1095-8312.1994.tb01014.x

Minckley R.L., Roulston T.H. 2006. Incidental mutualisms and pollen specialization among bees. *Plant-pollinator interactions: from specialization to generalization*, University of Chicago Press: Chicago, USA, 69–98.

Miranda E.A., Batalha-Filho H., Congrains C., Carvalho A. F., Ferreira K. M., Del Lama M. A. 2016. Phylogeography of *Partamona rustica* (Hymenoptera, Apidae), an endemic stingless bee from the Neotropical dry forest diagonal. PloSOne, 11(10): e0164441.

Mishra B.S.P., Dehuri S. and Wang G.N. 2013. A State-of-the-Art Reviewof Artificial Bee Colony inthe Optimization of Singleand Multiple Criteria. International Journal of Applied Metaheuristic Computing, 4(4), 23–45.

Monaghan M.T., Wild R., Elliot M., Fujisawa T., Balke M., Inward D.J., Vogler A. P. 2009. Accelerated species inventory on Madagascar using coalescent-based models of species delineation. Systematic Biology, 58(3): 298–311.

Moretto G., Arias M.C. 2005. Detection of mitochondrial DNA restriction site differences between the subspecies of *Melipona quadrifasciata* Lepeletier (Hymenoptera: Apidae: Meliponini). Neotropical Entomology, 34(3): 381–385.

Moroń D., Szentgyörgyi H., Skórka P., Potts S.G., Woyciechowski M. 2014. Survival, reproduction and population growth of the bee pollinator, *Osmia rufa* (Hymenoptera: Megachilidae), along gradients of heavy metal pollution. Insect Conservation and Diversity, 7(2): 113–121.

Muñoz I., Dall′ Olio R., Lodesani M., De la Rúa P. 2014. Estimating introgression in *Apis mellifera siciliana* populations: are the conservation islands really effective? Insect Conservation and Diversity, 7(6): 563–571.

Murray T.E., Fitzpatrick U., Brown M.J., Paxton R.J. 2008. Cryptic species diversity in a widespread bumble bee complex revealed using mitochondrial DNA RFLPs. Conservation Genetics, 9(3): 653–666.

Nemesio A. 2010. The orchid-bee fauna (Hymenoptera: Apidae) of a forest remnant in northeastern Brazil, with new geographic records and an identification key to the known species of the Atlantic Forest of north-eastern Brazil. Zootaxa, 2656: 55–66.

Oppenheimer R.L., Shell W.A., Rehan S.M. 2018. Phylogeography and population genetics of the Australian small carpenter bee, *Ceratina australensis*. Biological Journal of the Linnean Society, 124(4): 747–755.

Packer L., Owen, R. 2001. Population genetic aspects of pollinator decline. Conservation Ecology, 5(1): 4.

Packer L., Zayed, A., Grixti, J. C., Ruz, L., Owen, R. E., Vivallo, F., Toro, H. 2005. Conservation genetics of potentially endangered mutualisms: reduced levels of genetic variation in specialist versus generalist bees. Conservation Biology, 19(1): 195–202.

Padial J. M., Miralles A., De la Riva, I., Vences M. 2010. The integrative future of taxonomy. Frontiers in Zoology, 7(1): 1–14.

Parsons P.J., Couchoux C., Horsburgh G.J., Dawson D.A., Field J. 2017. Identification of 24 new microsatellite loci in the sweat bee *Lasioglossum malachurum* (Hymenoptera: Halictidae). BMC Research Notes, 10(1): 753.

Pauly A., Noël G., Sonet G., Notton, D.G., Boevé J.L. 2019. Integrative taxonomy resuscitates two species in the *Lasioglossum villosulum* complex (Kirby, 1802) (Hymenoptera: Apoidea: Halictidae). European Journal of Taxonomy, 541: 1–43.

Plant J.D., Paulus H.F. 1987. Comparative morphology of the postmentum of bees (Hymenoptera: Apoidea) with special remarks on the evolution of the lorum. Journal of Zoological Systematics and Evolutionary Research, 25(2): 81–103.

Porto D.S., Almeida E.A., Vilhelmsen L. 2017. Comparative morphology of internal structures of the mesosoma of bees with an emphasis on the corbiculate clade (Apidae: Apini). Zoological Journal of the Linnean Society, 179(2): 303–337.

Porto D.S., Vilhelmsen L., Almeida E.A. 2016. Comparative morphology of the mandibles and head structures of corbiculate bees (Hymenoptera: Apidae: Apini). Systematic Entomology, 41(2): 339–368.

Praz C., Müller A., Genoud D. 2019. Hidden diversity in European bees: *Andrena amieti* sp. n., a new Alpine bee species related to *Andrena bicolor* (Fabricius, 1775) (Hymenoptera, Apoidea, Andrenidae). Alpine Entomology, 3: 11–38.

Prentice M.A. 1998. The comparative morphology and phylogeny of Apoid wasps (Hymenoptera: Apoidea). PhD thesis.

University of California, Berkeley. 1439 pp.

Ramirez S.R., Roubik D.W., Skov C., Pierce N.E. 2010. Phylogeny, diversification patterns and historical biogeography of euglossine orchid bees (Hymenoptera: Apidae). Biological Journal of the Linnean Society, 100(3): 552–572.

Reino L., Ferreira M., Martínez-Solano Í., Segurado P., Xu C., Márcia Barbosa A. 2017. Favourable areas for co-occurrence of parapatric species: Niche conservatism and niche divergence in Iberian tree frogs and midwife toads. Journal of Biogeography, 44(1): 88–98.

Richards M.H., French D., Paxton R.J. 2005. It's good to be queen: classically eusocial colony structure and low worker fitness in an obligately social sweat bee. Molecular Ecology, 14(13): 4123–4133.

Ricklefs R.E. 2004. A comprehensive framework for global patterns in biodiversity. Ecology Letters, 7(1): 1–15.

Ritchie A.D., Ruppel R., Jha S. 2016. Generalist behavior describes pollen foraging for perceived oligolectic and polylectic bees. Environmental Biology, 45(4): 909–919.

Roig-Alsina A., Michener C.D. 1993. Studies of the phylogeny and classification of long-tongued bees (Hymenoptera: Apoidea). University of Kansas Science Bulletin, 55: 123–160.

Roubik D.W. (2004). Sibling species of *Glossura* and *Glossuropoda* in the Amazon region (Hymenoptera: Apidae: Euglossini). Journal of the Kansas Entomological Society, 77(3): 235–253.

Roubik D.W., Hanson P.E. 2004. Orchid bees of tropical America: Biology and field guide. Instituto Nacional de Biodiversidad (INBio), Heredia, Costa Rica.

Rozen J.G. 2008. Biology and immature stages of the bee *Nomioides patruelis* (Halictidae: Halictinae: Nomioidini) and of its cleptoparasite, *Chiasmognathus pashupati* (Apidae: Nomadinae: Ammobatini), with a preliminary phylogeny of the Halictidae based on mature larvae (Apoidea). American Museum Novitates, 2008(3604): 1–23.

Schneider C.J., Cunningham M., Moritz C. 1998. Comparative phylogeography and the history of endemic vertebrates in the Wet Tropics rainforests of Australia. Molecular Ecology, 7(4): 487–498.

Schönitzer B.K. 1986. Comparative morphology of the antenna cleaner in bees (Apoidea). Journal of Zoological Systematics and Evolutionary Research, 24(1): 35–51.

Sharkey M.J., Carpenter J.M., Vilhelmsen L., Heraty J., Liljeblad J., Dowling A.P., Krogmann L. 2012. Phylogenetic relationships among super families of Hymenoptera. Cladistics, 28(1): 80–112.

Sihag R.C. 1986. Reproduction in alfalfa pollinating sub-tropical megachilid bees. I: Functional anatomy and histology of the organs of reproduction. Zoologischer Anzeiger, 216(3-4): 191–203.

Sites Jr J.W., Marshall J.C. 2003. Delimiting species: a Renaissance issue in systematic biology. Trends in Ecology & Evolution, 18(9): 462–470.

Slatkin M. 2008. Linkage disequilibrium—understanding the evolutionary past and mapping the medical future. Nature Reviews Genetics, 9(6): 477–485.

Smith A.R., Wcislo W.T., O'donnell S. 2003. Assured fitness returns favor sociality in a mass-provisioning sweat bee, *Megalopta genalis* (Hymenoptera: Halictidae). Behavioral Ecology and Sociobiology, 54(1): 14–21.

Smith J.M., Szathmary E. 1997. The major transitions in evolution. Oxford University Press.

Smith M.A., Rodriguez J.J., Whitfield J.B., Deans A.R., Janzen D.H., Hallwachs W., Hebert, P.D. 2008. Extreme diversity of tropical parasitoid wasps exposed by iterative integration of natural history, DNA barcoding, morphology, and collections. Proceedings of the National Academy of Sciences, 105(34): 12359–12364.

Smitinand T. 2001. Thai Plant Names, Revised Edition. Royal Forestry Department, Bangkok, 810 pp.

Soltis D., Soltis P., Endress P., Chase M.W., Manchester S., Judd W., Mavrodiev, E. 2018. Phylogeny and evolution of the angiosperms: Revised and updated edition. University of Chicago Press.

Steppan S.J., Zawadzki C., Heaney L.R. 2003. Molecular phylogeny of the endemic Philippine rodent *Apomys* (Muridae) and the dynamics of diversification in an oceanic archipelago. Biological Journal of the Linnean Society, 80(4): 699–715.

Struck T.H., Feder J.L., Bendiksby M., Birkeland S., Cerca J., Gusarov V. I., Stedje, B. 2018. Finding evolutionary processes hidden in cryptic species. Trends in Ecology & Evolution, 33(3): 153–163.

Timmermann K. 2005. Bee communities (Hymenoptera: Apoidea) of different vegetation types in the semiarid part of western South Africa–structure and pollen specialisation. University of Münster.

Timmermann K., Kuhlmann M. 2008. The biology of a *Patellapis* (s. str.) species (Hymenoptera: Apoidea: Halictidae): sociality described for the first time in this bee genus. Apidologie, 39(1): 189–197.

Tofilski A. 2008. Using geometric morphometrics and standard morphometry to discriminate three honeybee subspecies. Apidologie, 39(5): 558–563.

Udayakumar A., Shivalingaswamy T.M. 2019. Nest architecture and life cycle of Small Carpenter bee, *Ceratina binghami* Cockerell (Xylocopinae: Apidae: Hymenoptera). Sociobiology, 66(1): 61–65.

Vaudo A.D., Fritz M.L., López-Uribe M.M. 2018. Opening the door to the past: Accessing phylogenetic, pathogen, and population data from museum curated bees. Insect Systematics and Diversity, 2(5): 4.

Villemant C., Simbolotti G., Kenis M. 2007. Discrimination of *Eubazus* (Hymenoptera, Braconidae) sibling species using geometric morphometrics analysis of wing venation. Systematic Entomology, 32(4): 625–634.

Wcislo W.T., Cane J.H. 1996. Floral resource utilization by solitary bees (Hymenoptera: Apoidea) and exploitation of their stored foods by natural enemies. Annual review of entomology, 41(1): 257–286.

Wcislo W.T., Arneson L., Roesch K., Gonzalez V., Smith A., Fernández H. 2004. The evolution of nocturnal behaviour in sweat bees, *Megaloptagenalis* and *M. ecuadoria* (Hymenoptera: Halictidae): an escape from competitors and enemies? Biological Journal of the Linnean Society, 83(3): 377–387.

Wcislo W.T., Gonzalez V.H., Engel M.S. 2003. Nesting and social behaviour of a wood-dwelling neotropical bee, *Augochlora isthmii* (Schwarz), and notes on a new species, *A. alexanderi* Engel (Hymenoptera: Halictidae). Journal of the Kansas Entomological Society, 76: 588–602.

Wheeler Q.D., Raven P.H., Wilson E.O. 2004. Taxonomy: impediment or expedient? Science, 303: 285.

Wiens J.J. 2007. Species delimitation: new approaches for discovering diversity. Systematic Biology, 56(6): 875–878.

Will K.W., Rubinoff D. 2004. Myth of the molecule: DNA barcodes for species cannot replace morphology for identification and classification. Cladistics, 20(1): 47–55.

Will K.W., Mishler B.D., Wheeler Q.D. 2005. The perils of DNA barcoding and the need for integrative taxonomy. Systematic Biology, 54(5): 844–851.

Williams H.J., Vinson S.B., Frankie G.W. 1987. Chemical content of the dorsal mesosomal gland of two *Xylocopa* species (Hymenoptera: Anthophoridae) from Costa Rica. Comparative Biochemistry and Physiology. B. Comparative Biochemistry, 86(2): 311–312.

Williams P.H., Brown M.J., Carolan J.C., An J., Goulson D., Aytekin A.M., Huang J. 2012. Unveiling cryptic species of the bumblebee subgenus *Bombus s. str.* worldwide with COI barcodes (Hymenoptera: Apidae). Systematics and Biodiversity, 10(1): 21–56.

Yeates D.K., Seago A., Nelson L., Cameron S.L., Joseph L.E.O., Trueman J.W. 2011. Integrative taxonomy, or iterative taxonomy? Systematic Entomology, 36(2): 209–217.

Zayed A., Packer L. 2007. The population genetics of a solitary oligolectic sweat bee, *Lasioglossum (Sphecodogastra) oenotherae* (Hymenoptera: Halictidae). Heredity, 99(4): 397–405.

Zillikens A., Steiner J., Mihalkó Z. 2001. Nests of *Augochlora (A.) esox* in bromeliads, a previously unknown site for sweat bees (Hymenoptera: Halictidae). Studies on Neotropical Fauna and Environment, 36(2): 137–142.

Zimmermann Y., Roubik D.W., Eltz T. 2006. Species-specific attraction to pheromonal analogues in orchid bees. Behavioral Ecology and Sociobiology, 60(6): 833.