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# An overview of molecular identification of insect fauna with special emphasis on chalcid wasps (Hymenoptera: Chalcidoidea) of India

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**ABSTRACT**

Identifying organisms has grown in importance as we monitor the biological effects of global climate change and attempt to preserve species diversity in the face of accelerating habitat destruction. Classical taxonomy falls short in this race to catalogue biological diversity before it disappears. Differentiating subtle anatomical differences between closely related species requires the subjective judgment of highly trained specialists – and few are being trained in institutes today. DNA barcodes allow non-experts to objectively identify species – from small, damaged, or even industrially processed material. The aim of DNA barcoding is to establish a shared community resource of DNA sequences commonly used for identification, discrimination or taxonomic classification of organisms. It is a method that uses a short genetic marker in an organism's DNA to identify and distinguish its belonging from particular species, varieties or inter varieties. This simple technique has attracted attention from taxonomists, ecologists, conservation biologists, agriculturists, plant-quarantine officers and studies using the DNA barcode has rapidly increased. The extreme diversity of insects and their economical, epidemiological and agricultural importance have made them a major target of DNA barcoding. In this review, we present an overview of DNA barcoding of insects with emphasis on Chalcid wasps of India.

**Key words:** biological diversity; catalogue; chalcid wasps; classical taxonomy, DNA barcode; DNA sequence, genetic marker

**IZVLEČEK**

**PREGLED MOLEKULARNEGA DOLOČANJA ŽUŽELK V INDIJI S POUDARKOM NA OSICAH NAJEZDNICAH (Hymenoptera: Chalcidoidea)**

Določanje organizmov pridobiva na pomenu pri spremljanju globalnih podnebnih sprememb in pri poskusih ohranjanja biodiverzitete v procesu hitrega uničevanja habitatov. Klasična taksonomija v teh procesih ne uspe določiti vse biodiverzitete pred njenim propadom. Prepoznavanje majhnih anatomskih razlik med ozko sorodnimi vrstami zahteva presojo visoko usposobljenih specialistov, ki jih je danes vedno manj. Vrednotenje DNK zaporedij omogoča tudi nestrokovnjakom objektivno prepoznavanje vrst kot tudi njihovih malih ali poškodovanih ostankov ali celo industrijsko predelanih materialov. Namen te metode je ustvariti nabor DNK zaporedij za vzajemno rabo pri določanju in taksonomskem razvrščanju organizmov poznano tudi pod imenom DNK črtne kode. Pri tej metodi omogoča kratek genetski marker v DNK organizma njegovo določitev in razlikovanje od drugih vrst, različic. Ta preprosta tehnika je pritegnila pozornost taksonomov, ekologov, konzervatorskih biologov, agronomov, fitokarantenskih uradnikov in preučevanje na osnovi sekvenciranja DNK je hitro poraslo. Izjemna raznolikost žuželk in njihov ekonomski, epidemiološki in kmetijski pomen so jih naredile za tarčno skupino preučevanj na osnovi DNK črtnih kod. V tem sestavku predstavljamo pregled analiz z DNK črtnimi kodami žuželk s poudarkom na osicah najezdnicah iz Indije.

**Ključne besede:** biodiverziteta; seznam; osice najezdnice; klasična taksonomija; genetska koda; DNK zaporedje; genetski marker

## 1 INTRODUCTION

Chalcid wasps are one of the most diverse groups of insects numerically, structurally, and biologically belonging to the superfamily Chalcidoidea and order Hymenoptera. With about 150,000 described species, the Hymenoptera is the fourth largest insect order after Coleoptera, Lepidoptera, and Diptera (Grimaldi & Engel, 2005; Beutel & Pohl, 2006). With an estimated total diversity of some 22,500 known species and more than 500,000 morphologically distinct species (Munro et al., 2011) and an even larger number of cryptic

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species possible, the Chalcidoidea superfamily is likely the most diverse group of insects in order Hymenoptera. Most Chalcid wasps are parasitoids attacking immature and adult stages of virtually all insect orders, mostly Hemiptera and Holometabola and hence are used as biological control agents of agricultural and ornamental pests thus having tremendous importance in both natural and managed ecosystems both economically and ecologically (Preethi et al., 2016).

Species identification is a fundamental part of recognizing and describing biodiversity in an ecosystem. Traditionally, identification has been based on morphological diagnoses provided by taxonomic studies. Only experts such as taxonomists and trained technicians can identify taxa accurately, because it requires special skills acquired through extensive experience. As interest in biodiversity has increased in the fields of ecology, evolutionary biology, agriculture and economics, among others, it has become increasingly important to precisely identify species. However, the number of taxonomists and other identification experts has drastically decreased. The characterization based on morphometric characters is not well suited for phylogeographical studies because both phenotypic plasticity and genetic variability in the characters employed for species recognition can lead to incorrect identifications (Pires & Marinoni, 2010). It overlooks morphologically cryptic taxa, which are common in many groups (Jarman & Elliott, 2000) and the use of keys often demands such a high level of expertise that misdiagnoses are common. Faunal and floral studies are besieged by specimens in immature stages that lack the characters necessary for identification, or sexes that cannot be matched, especially if they are dimorphic such as some insects in which the sexes vary dramatically in size or colour (Pinzón-Navarro et al., 2010).Consequently, alternative and accurate identification methods that non-experts can use are required.

One of the most promising approaches to revitalize traditional taxonomy and help it rise above the taxonomic crisis is the use of molecular data for identifying taxa, which has long been a fundamental idea of many biologists (Busse et al., 1996; Blaxter, 2004). This method has received increased acceptance because it is simple and affordable (Padial & De La Riva, 2007). DNA barcoding promises the ability to automate the identification of specimens by determining the sequence of the barcode region, avoiding the complexities inherent in morphological identifications, and prompting advocates arguing for the establishment of a system that ultimately might be applied to all life (Tautz et al., 2003; Blaxter, 2004; Savolainen et al., 2005). Advances in DNA-sequencing technologies have enabled researchers studying biodiversity to conduct simple, cost-effective and rapid DNA analyses. This progress in biotechnology, and the taxonomy crisis itself, played a large role in the creation of DNA barcoding. DNA barcoding, in particular, was formally introduced more than a decade ago as an alternative way to assign species names to specimens, addressing concerns and limitations with traditional morphological identifications (Hebert et al., 2003). The use of DNA sequences to gain information about the taxonomic affinities of an unknown specimen saw its earliest adoption in the least morphologically amenable groups such as viruses and bacteria (Theron & Cloete, 2000). More recently, it has been applied to plants (Chase et al., 2005), to simple metazoan animals such as nematode worms (Floyd et al., 2002) and even to fascinating mega fauna such as birds, fish, and mammals (Ward et al., 2005; Clare et al., 2007; Kerr et al., 2007). This approach relies on the use of algorithms enabling DNA-sequence comparison, such as Basic Local Alignment Search Tool (BLAST) (Altschul et al., 1990), in conjunction with DNA databases such as GenBank.

### 1.1 DNA barcoding and taxonomy

India is one of the mega biodiversity rich countries, home to hotspots like the Western Ghats and the Himalayas (ENVIS, 2011). In spite of this rich biodiversity heritage, well documented in the Fauna of British India volumes and having many endemics in all groups, much still remains to be understood about it. Many species are difficult to identify and are poorly known. Insects are the most abundant of all life forms on earth. India with about 2 % of the global land area is among the top 20 mega biodiversity nations in the world accounting for 7.10 % of the world insect fauna. It is estimated that over 900,000 species of insects are known across the globe with over 60,000 species described from India with nearly as many species yet to be named. However, the number of barcodes generated from India is 4.6 % of known species, while the corresponding global scenario is about 16 % of described species, and hence requires a lot of emphasis to catch up with the world scenario (Jalali et al., 2015).The first initiative in DNA barcoding was led by the Department of Biotechnology (DBT), India, to barcode species of butterflies and amphibians from the Western Ghats of India (Gaikward, 2014). To speed up taxonomic identification, DNA barcoding is now being considered as an alternative tool for insect biodiversity identification in India and the world.

Chalcid wasp species in India have been described and illustrated mostly at morphological level. Keeping in view various drawbacks of morphological taxonomy like lack of taxonomic experts, overlooking cryptic taxa, difficulty in using keys and due to phenotypic plasticity

and genetic variability with changing environmental conditions as has been found in other animal species, alternative and complementary approach (molecular taxonomy) has been used in identification of specimens. Molecular approach to Chalcid identification provides a grim scenario from India with very little work done so far at molecular level. Recently Kumar et al., (2009), Jalali et al., (2015) and Venkatesan et al., (2016) have carried some research work at molecular level in Chalcid fauna and came out with some interesting results. The rDNA internal transcribed spacers region 2 (ITS-2) (Kumar et al., 2009), cytochrome c oxidase subunit 1 (COI), NADH dehydrogenase subunit 1 (nadh1), and cytochrome b (cytb) markers used in recent molecular analysis have significantly increased our understanding of the phylogenetic relationships between insect species. Kumar et al. (2009) used Internal transcribed spacer-2 restriction fragment length polymorphism (ITS-2-RFLP) tool to differentiate some exotic and indigenous Trichogrammatid egg parasitoids from India whereas Venkatesan et al. (2011) studied characterization and identification of *Acerophagus papayae* Noyes & Schauff, 2003 (Hymenoptera: Encyrtidae), an introduced parasitoid of papaya mealybug, *Paracoccus marginatus* Williams & Granara de Willink, 1992 through DNA barcoding. The study was undertaken for the DNA barcoding of *A. papayae*, using CO1 region in order to boost and confirm that the introduced and native populations in Pune belonged to the same species. In addition DNA Barcoding for Identification of Agriculturally Important Insects of India was recently carried out by Jalali et al., (2015). Different parasitoids, predators and other insects were collected from various cities of India and were used for DNA barcoding studies. The specimens, thus collected and morphologically identified, were used for COI barcoding at the National Bureau of Agriculturally Important Insects (NBAII) Bangalore, India. Venkatesan et al. (2016) carried out study to unravel the discrimination success in the two molecular marker loci cytochrome oxidase I (COI) and internal transcribed spacer-2 (ITS-2) region of Trichogrammatids.

### 1.2 DNA barcoding of insect fauna

DNA barcoding, a taxonomic method that uses a short, standardized DNA sequence to identify species, has gained increased attention and acceptance from members of the scientific community interested in documenting the Earths' biodiversity (Hebert et al., 2003; Savolainen et al., 2005; Hajibabaei et al., 2007; Borisenko et al., 2009; Ivanova et al., 2009). One of the advantages of DNA barcoding with respect to traditional taxonomy is the speed and low costs involved in assemblage and analyzing data (Borisenko et al., 2009; Strutzenberger et al., 2010). The creation of the CBOL's online database (The Barcode of Life Data System – BOLD: www.barcodinglife.org) has provided an impetus for numerous researchers to join the barcode initiative. It is easy to access and provides free storage and retrieval of molecular, morphological and geographical data, besides a built-in, integrated analysis tools such as tree reconstructions on the basis of genetic similarity (Ratnasingham & Hebert, 2007; Frézal & Leblois, 2008). DNA barcoding relies on the premises that the genetic variation among species is greater than the variation within species (Hajibabaei et al., 2007). Mitochondrial genes as universal markers were mostly driven by the fact that the mitochondria is maternally inherited, avoiding problems with recombination. Also, the mitochondrial genome has a high mutation rate when compared with the nuclear genome, which results in high degrees of intra-specific polymorphism and divergence, important in evolutionary studies (Williams & Knowlton, 2001; Wheat & Watt, 2008; Hlaing et al., 2009). Taxonomy and systematics of insects using DNA barcoding has been enriched with several contributions from various authors. Molecular studies in the order Hemiptera were carried out by Foottit et al. (2009), Lee et al. (2010) and Shufran & Puterka (2011), whereas Smith et al. (2006), Ekrem et al. (2007) and Rivera & Currie (2009) barcoded Diptera. Hymenoptera was enriched by contributions of Smith et al. (2005), Sheffield et al. (2009) and Smith et al. (2009) while Yoshitake et al. (2008), Raupach et al. (2010) and Greenstone et al. (2011) carried out studies in Coleoptera. Molecular studies in Trichoptera were performed by Salokannel et al. (2010), Geraci et al. (2011) and Zhou et al. (2011). Characteristics intrinsic to insects, such as their diversity, biological control and the economic and epidemiological relevance of some groups, have made them the main target of DNA barcoding studies. This standard database can be used in studies on the taxonomy, phylogeny, ecology, agriculture and conservation of various groups of organisms (Jinbo et al., 2011).Several contributions focusing on identification using the mitochondrial COI have proved useful in the detection of cryptic insect species. Some of those cryptic species which were initially almost impossible to separate using morphological characters alone, have had their identities corroborated by other characters in their natural history and even characters in their morphology (Hebert et al., 2004; Smith et al., 2006; Pfenninger et al., 2007; Decaëns & Rougerie, 2008; Vaglia et al., 2008; Wheat & Watt, 2008; Dasmahapatra et al., 2010; Hausmann et al., 2011. Morphological differences, cases of sexual dimorphism, different castes, or different stages of development have made barcode sequences applicative (Miller et al., 2005; Geraci et al., 2011); Jinbo et al., 2011). Other applications include : identification of host plants by sequencing the stomach contents or plant tissues left on the outside of an insect's body (Jurado-Rivera et al., 2009); identification of the stomach contents of predators in biological control studies

(Greenstone et al., 2005); Greenstone (2006); additional data uncovering trophic relationships (Clare et al., 2009; Hrcek et al., 2011); and finally, population genetics, community ecology and biodiversity inventories (Hajibabaei et al., 2006; Lukhtanov et al., 2009; Craft et al., 2010).

### 1.3 Limitations of DNA barcoding

DNA barcoding has its pitfalls too. Its success is dependent on the strength of the pretension that interspecific variation exceeds intraspecific variation by one order of magnitude, thus establishing a "barcoding gap", or on the reciprocal monophyly of species (Wiemers & Fiedler (2007). The presence of multiple mitochondrial gene haplotypes, such as nuclear pseudogenes of the mitochondria genome (NUMT) or heteroplasmy also reduces the validity of DNA barcoding. This problem has been reported for many insects (Gellissen & Michaelis, 1987; Zhang & Hewitt, 1996; Bensasson et al., 2000; Brower, 2006; Rubinoff et al., 2006) and can also affect the barcoding results (Song et al., 2008).

### 1.4 Summary from barcode of life data system

Barcode of Life Data Systems (commonly known as BOLD) is a sequence database specifically devoted to DNA barcoding. It provides an online platform for analyzing DNA sequences. BOLD is populated with nearly 163617 insect species barcodes out of which India has only 3694 barcodes. There are about 5448764 records of specimens of insects in BOLD statistics with 4404476 specimens with sequences and 4092095 specimens with Barcodes. It represents 218968 species in which 170452 have been barcoded (Fig. 1). As far as hymenoptera are concerned there are 907902 specimen records with 666323 specimens with sequences. 563353 specimens are with barcodes representing 35907 species with 26017 species barcoded (BOLD v4) (Fig. 1).

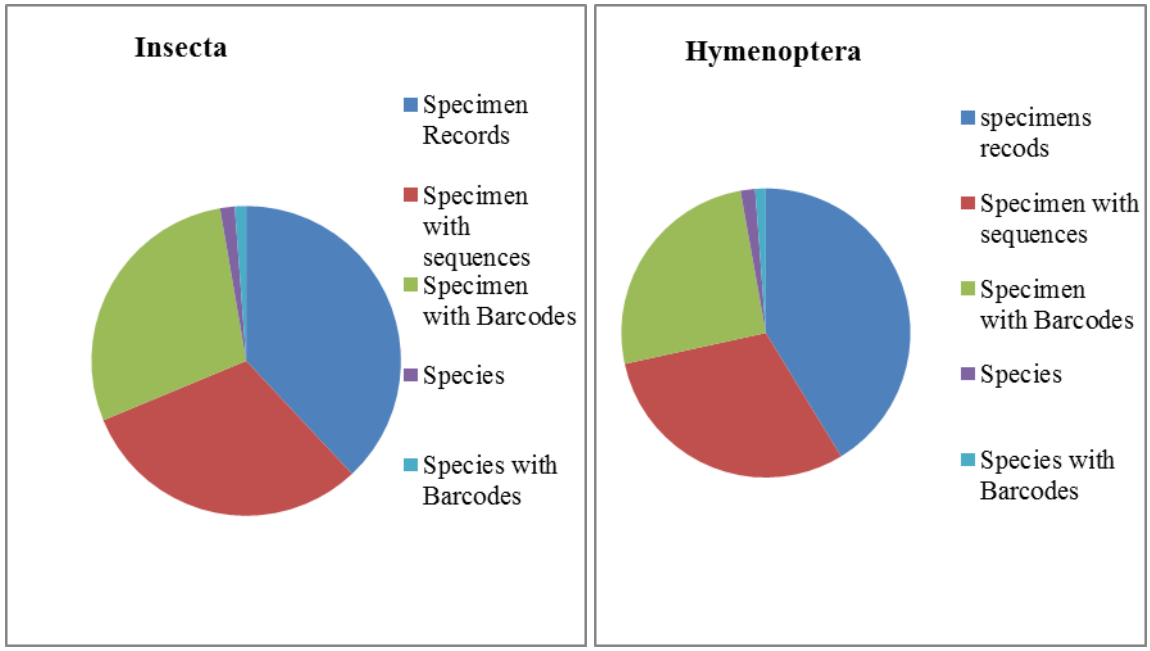


**Figure 1:** Barcoding status of Insecta (left) and Hymenoptera (right) in BOLD**. (**Data accessed on 30 July 2017)

### 1.5 DNA barcoding status of Chalcidoidea in India

An estimated 150000 Hymenopteran species of insects are reported worldwide of which 25169 species have been barcoded (Axel et al., 2013) (Fig. 2). In India, little work has been done so far at molecular level. With an estimated 10000 species, only 167 species of Hymenoptera have been subjected to barcoding in the Insect Barcode Informatica (IBIn): a platform to assist and manage acquisition, storage, analysis and to explore DNA barcode records for species identification and genetic analysis of status data of Indian insects (Fig. 2). Out of 167 hymenopteran species barcoded, 58 belong to superfamily Chalcidoidea including 44 Trichogrammatidae, 5 Eulophidae, 2 Torymidae and 7 Encyrtidae species (Fig. 3).

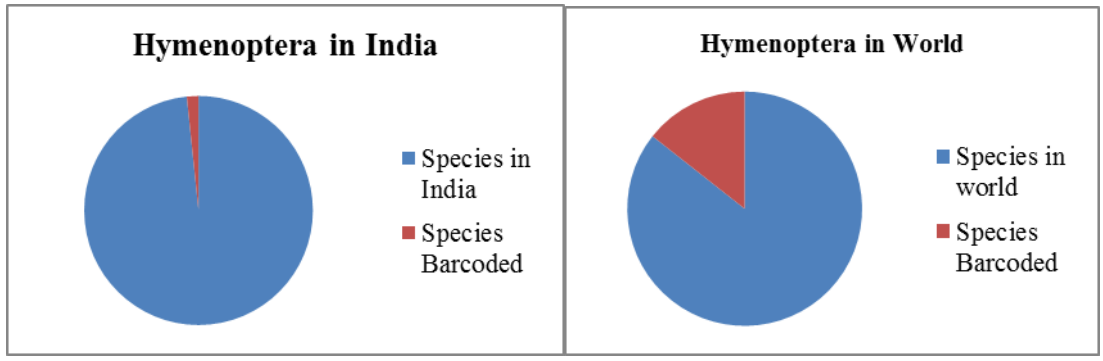


**Figure 2:** Hymenoptera: species and barcodes in the India (left) and in World (right) (Data accessed on 30 July 2017)

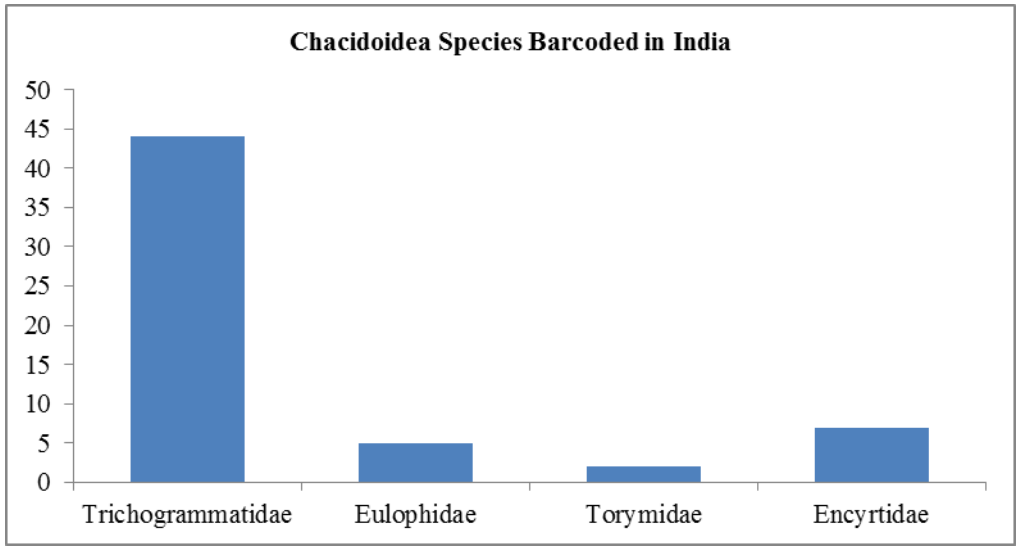


**Figure 3:** Number of chalcid wasps barcoded in India (Data accessed on 30 July 2017)

At global level an estimated 25169 Hymenopteran species have been barcoded so far among 150000 described species (Axel et al., 2013). Species of Chalcidoidea are richly represented among the species barcoded so far in the order Hymenoptera. Out of 38287 Specimens of Chalcid wasps with barcodes, only 3.6 % (1382) of species with barcodes are represented in Barcode of Life Data system (BOLD) with Eulophidae most represented (357) and Mymarommatidae and Rotoitidae least represented with just one species. Records of individual families of superfamily Chalcidoidea in BOLD are shown in Table 1.

**Table 1:** Current summary of DNA barcoding library of Chalcidoidea in the BOLD system

| Sr. No. | Family | Specimen records | Specimen with sequences | Specimen With Barcodes | Species | Species with Barcodes |
|---|---|---|---|---|---|---|
| 1 | Agonidae | 2410 | 2324 | 1569 | 372 | 271 |
| 2 | Aphelinidae | 7892 | 6803s | 2875 | 56 | 47 |
| 3 | Chalcididae | 2493 | 1163 | 611 | 175 | 88 |
| 4 | Encyrtidae | 4622 | 4139 | 1799 | 92 | 66 |
| 5 | Eulophidae | 21321 | 18444 | 11574 | 642 | 357 |
| 6 | Eucharitidae | 241 | 147 | 81 | 44 | 29 |
| 7 | Eupelmidae | 1749 | 1190 | 700 | 126 | 58 |
| 8 | Eurytomidae | 3029 | 2474 | 1287 | 90 | 45 |
| 9 | Leucospidae | 80 | 34 | 09 | 14 | 06 |
| 10 | Mymaridae | 20379 | 18629 | 6637 | 61 | 27 |
| 11 | Mymarommatidae | 56 | 07 | 04 | 01 | 01 |
| 12 | Ormyridae | 278 | 223 | 159 | 22 | 12 |
| 13 | Perilampidae | 1211 | 784 | 543 | 87 | 58 |
| 14 | Pteromalidae | 13114 | 10946 | 6830 | 547 | 227 |
| 15 | Rotoitidae | 01 | 01 | 01 | 01 | 01 |
| 16 | Signiphoridae | 168 | 163 | 85 | 01 | 00 |
| 17 | Tanaostigmatidae | 36 | 15 | 05 | 07 | 04 |
| 18 | Tetracampidae | 21 | 17 | 14 | 07 | 05 |
| 19 | Torymidae | 2420 | 1999 | 1056 | 97 | 53 |
| 20 | Trichogrammatidae | 5707 | 5356 | 2448 | 31 | 27 |

(Data Accessed on 30 July 2017)

## 2 CONCLUSIONS

Species identification is a fundamental part of recognizing and describing biodiversity. Traditionally, identification has been based on morphological diagnoses provided by taxonomic studies. The classical use of morphological trait for species identification has several limitations and requires a high level of expertise for correct identification of species. The DNA barcoding approach might correctly present the best solution for identifying species when their morphology is of limited use (Hebert et al., 2003). DNA barcoding has recently picked up pace in India and helped in the unambiguous identification of insect species of India including Chalcid wasps. This latest method of species identification through DNA barcoding of mitochondrial cytochrome oxidase gene I (COI) (Hebert et al., 2003) clearly gives support to improve classifications and examine the precision of morphological traits commonly used in taxonomy critically.

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