BIOSYSTEMATICS AND BARCODING OF PTEROMALIDAE (HYMENOPTERA: CHALCIDOIDEA) OF KERALA

by

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(2017-11-048)

THESIS

Submitted in partial fulfilment of the

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2019

DECLARATION

I, hereby declare that this thesis entitled **"Biosystematics and barcoding of Pteromalidae (Hymenoptera: Chalcidoidea) of Kerala"** is a bonafide record of research work done by me during the course of research and the thesis has not previously formed the basis for the award of any degree, diploma, associateship, fellowship or other similar title, of any other University or Society.

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CERTIFICATE

Certified that this thesis entitled **"Biosystematics and barcoding of Pteromalidae (Hymenoptera: Chalcidoidea) of Kerala"** is a record of research work done independently by Mr. Manu Govind K K (2017-11-048) under my guidance and supervision and that it has not previously formed the basis for the award of any degree, diploma, fellowship or associateship to him.

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Manu Govind K K

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LIST OF ABBREVIATIONS AND SYMBOLS USED

F	Female
F1-F7	Funicular segments 1 to 7
M	Male
MV	Marginal vein
OOL	Ocello-ocular distance
PMV	Post marginal vein
POL	Post ocellar distance
SMV	Submarginal vein
sp. nov.	Species nova.
STV	Stigmal vein
Syn.	Synonym
T1-T6	Tergites 1 to 6
%	Per cent
@	At the rate of
mm	Millimeter
cm	Centimeter
DNA	Deoxyribo nucleic acid
rpm	Rotations per minute
et al.	and other Co workers
Fig.	Figure
g	Gram
i.e.	That is
KAU	Kerala Agricultural University
viz.	Namely
° C	Degree Celsius
µL	Microliter

mg	Milligram
nm	Nanometer
s	Seconds

Introduction

1. INTRODUCTION

Taxonomy opens up the vast world of published information pertaining to any particular species. Taxonomy plays an inevitable role in agricultural research. It helps in the correct identification of both the pest and the natural enemies associated with the pest on a crop and plays an important role in biocontrol programmes. The term alpha taxonomy refers to the discipline of finding, describing and naming a taxa, particularly species. The study based on any organism requires the correct name of the organism which is the functional label of the organism. The functional label or the scientific name helps in retrieving all the pieces of information available, which can be used as a reference. Taxonomic study not only helps in finding out the identity of the organism but also helps the scientists to know the details of the locality, distribution and hosts for designing a proper integrated management programme. It also plays an imperative role in classical biological programme were a natural enemy is imported for the management of any invasive pest. It is also of great ecological importance as it helps to identify the threatened or endangered species. Taxonomic data are also of great significance as it helps to conserve biodiversity.

Hymenoptera is one of the most species-rich orders of the superclass insecta, comprising ants, wasps, bees and sawflies. A restructured classification of the Hymenoptera with the current number of genera and species described so far is given by Aguiar *et al*. (2013). A total of 153,088 extant species have been described so far, in addition to 2,429 extinct species. This order composed of two suborders, 27 superfamilies, 132 families, 8,432 extant genera with an additional 685 extinct genera. The suborders are Symphyta (sawflies) and Apocrita (wasps, bees and ants). Parasitic hymenoptera plays an important role in terrestrial ecosystems in the management of insect pests of various crops. They are very important in maintaining the natural balance and also a good indicator of the ecosystem's state (Dorn *et al*., 2002).

Among the various parasitic groups in Hymenoptera, superfamily Chalcidoidea is the largest, taxonomically most difficult, ecologically perhaps the

most complex and economically important group. It comprises of 23 families, 2,045 genera and 22,784 species worldwide and is omnipresent in biogeographical areas (Aguiar *et al*., 2013). Adults of chalcids are free living insects that hardly feed, but larvae have diverse and mostly specialised feeding habits. Most of the chalcids are entomophagous and some of them rarely feed on arthropods like spiders and mites. Entomophagous parasitoids are of two types, koinobionts and idiobionts. Koinobionts co-exists with the host until the completion of its life cycle allowing the host to grow and develop. Idiobionts immediately kill the host or arrest the development and feeding of the host. Primary parasitism is most prominent in chalcids, but hyperparasitism is also common and most frequently reaching tertiary and quarternary levels. Multiparasitism and superparasitism are not frequent. Chalcids show high degree of variability as they may exhibit monophagy with strict host specificity, oligophagy and in many cases polyphagy.

Pteromalidae is one of the most difficult families of Superfamily Chalcidoidea (Hymenoptera: Parasitica). No single character or set of characters separate all species of Pteromalidae from other families. Most of the Pteromalidae can be separated from closely related families and related groups by 8-13 segmented antennae and five tarsal segments in the fore and hind tibia. Another peculiar character is the prominently curved fore tibial spur. The size of chalcids vary from 1-48 mm and usually metallic. Forewing with postmarginal and stigmal vein well developed and with a distinct speculum.

The significance of the family Pteromalidae is that it is one of the biggest families of the Superfamily Chalcidoidea, members of which are distributed worldwide in all biogeographical regions. Majority of pteromalids are primary and secondary parasitoids attacking a vast range of insects in their various stage for its development and some of them on arachnida. They are economically important, as they play vital role in the biological control of noxious insect pests (Sureshan, 2015). Pteromalidae comprises 3,450 described species under 640 genera and 32 subfamilies worldwide and 279 species under 105 genera and 18 subfamilies from India (Noyes, 2017).

Hebert *et al.* (2003b) proposed the concept of DNA barcoding as a rapid and precise way of species discrimination of a broad range of biological specimens using a selected 658bp fragment of the 5′ end of the mitochondrial cytochrome oxidase-1 (CO-I) gene Researchers have envisioned "DNA taxonomy", a concept of adopting DNA sequencing as a central criterion for taxonomic decisions and descriptions, as a standard method of analysis (Vogler and Monaghan, 2007). In addition to species discovery, DNA barcoding can also be used to identify cryptic species, biotypes, haplotypes, etc. (Boykin *et al.*, 2012).

Although several Indian workers have contributed towards classical studies, none have contributed towards phylogenetics or molecular systematics. Pteromalidae constitutes one of the largest families of Chalcidoidea yet; bulk of the diversity remains undescribed / unknown to science as can be ascertained from recent discoveries of new species (Gupta *et al.* 2016). Keeping this in view the present study on this economically important family is proposed with the following objective.

- Identification, morphological and molecular characterization, documentation of Pteromalidae of Kerala.

Review of Literature

2. REVIEW OF LITERATURE

The literature pertaining to the morphological and molecular characterization of Pteromalidae is reviewed here.

2.1. FAMILY PTEROMALIDAE

The family Pteromalidae is one among the largest families of Chalcidoidea. The earliest studies on Pteromalidae was started by Linnaeus (1758). He described *Ichneumon puparum* and *Sphex colon* from Sweden which was renamed *Pteromalus puparum* and *Dinotiscus colon* respectively, he also described *Habrocytus capreae* under the name *Cynips capreae*. Fabricius (1787) described *Cheiropachus quadrum* under the name *Ichneumon quadrum* which was again rediscribed under the name *Pteromalus bimaculatus* by Swederus (1795). He also put forth the genus *Pteromalus* with *Pteromalus puparum* as the type species. Fabricius (1798) introduced one more species *Ichneumon depressus* later renamed as *Cleonymus laticornis*. He also described *Diplolepis depressa* (Fabricius, 1804).

With the efforts of Spinola (1811 a, b) several new genera were added to the family Pteromalidae like *Chrysolampus*, *Callitula*, *Sphegigaster*, *Halticoptera*, etc. Dalman (1820) isolated the family Pteromalini which is the second earliest group name available in the superfamily Chalcidoidea. A new genus was described by Curtis named *Colas* and *Colas dispar* as type species (Curtis, 1827). Westwood (1932) put forth several genera like *Cercocephala*, *Theocloax*, *Macroglemus* and *Trignoderus*. Gahan (1919), published a report on parasitic hymenoptera of India belonging to superfamily Chalcidoidea and Serphoidea, in which he included many species of Pteromalidae. Gahan and Fagan (1923), published a complete catalogue of known genera of Chalcidoidea in the Bulletin of United States National Museum. Timberlake (1926) described 60 new species of Pteromalidae, including several collected from India.

Burks (1975) reviewed 72 species of Chalcidoidea described by Walker from North America. This work included 24 species of Pteromalidae. Bouček *et al*., (1978) published the first authentic and comprehensive work on Pteromalidae from

India. They reviewed the family which included 82 genera in which 56 with 86 identified species and 26 unidentified species. This taxonomic reclassification resulted in many changes including 21 generic transfer and 30 new specific synonymies. Farooqi and Subba Rao (1985), published a key to the Indian genera of Pteromalidae.

An excellent work of Bouček (1988) on Australasian Chalcidoidea was published. He made a biosystematic revision of 14 subfamilies of Australasian Chalcidoidea which included 28 subfamilies of Pteromalidae with 235 genera. He provided the biology, morphology, distribution of family and a good key to the genera. Mani (1989) published an elaborate work on chalcid fauna from India and adjacent countries in two parts and part one contains information about all the known genera and species of Pteromalidae. Grissel and Schauff (1990) published a handbook of the families of the Nearctic Chalcidoidea with an account of family Pteromalidae.

Bouček and Rasplus (1991) made a noteworthy contribution to the West Palaearctic fauna. They published an illustrated key which included 221 genera and ten subgenera with 491 elaborate drawings and 110 electroscan photographs.

Sureshan and Narendran (2003) published a checklist of Pteromalidae from Indian subcontinent, in which they listed 84 genera with 185 identified species and 12 genera with unidentified species. Sureshan (2007) also carried out taxonomic studies on Pteromalidae of Southeast Asia based on collection of Bohart Museum of Entomology, California, USA. The study included 54 species of Pteromalidae under 8 subfamilies.

Askew and Mifsud (2016) published a preliminary check-list of the Chalcidoidea (Hymenoptera) of the Maltese Islands. They enlisted 147 species of Chalcidoidea including 33 Pteromalidae.

2.2. SUBFAMILY: MISCOGASTERINAE

Walker (1833), made a significant contribution by describing new genera like *Mersmus, Dipara, Miscogaster, Pachyneuron, Syntomopus, Psilocera* etc. in his Monographia Chalciditum. He also added a new family Miscogasteridae. Haliday (1844), established a group called tribus Pirenianii under Pteromalidae which was later classified as tribus Pirenini under subfamily Miscogastrinae by Graham (1969). He also described a new genus *Agamerion* with *Miscogaster gelo* as type species.

Ashmead (1904), published his monumental work in which he keyed out the subfamilies, tribes and genera of Pteromalidae, Cleonymidae and Miscogasteridae the two latter families are now included under Pteromalidae.

Graham (1956 a) erected a new genus *Thinodytes* with *Miscogaster cyzius* Walker as type species. He also described a new genus, *Chlorocytus* (Graham, 1956 b). Delucchi (1956) added two new genera, *Cyrtoptyx* and *Oxysychus*. Hedqvist (1975) published keys to the Swedish species of *Halticoptera* Spinola, *Halticopterina* Erdös, *Schimitschekia* Bouček and *Thinodytes* Graham and described *Halticoptera longipterolus* Burks. Huggert (1976) erected a new genus *Zdenekia* with *Zdenekia plana* as type species and described *Spathopus monotanus*, *Spanopus hedqvisti* and males of *Stichomischus longiventris*.

Mani (1939), reported several species of Pteromalidae under the family Miscogasteridae (now miscogasterinae) and made remarkable contributions towards the study of Indian Pteromalidae. Bhatnagar (1951) published an account on the family Pteromalidae and reported several species from India, under the subfamily Miscogasterinae. Sureshan *et al.*, (2016), described a new species *Halticoptera cavatura* from Tamil Nadu.

2.3. SUBFAMILY: ORMOCERINAE

Walker (1834), again made a contribution by describing a new genus *Systasis* with the type species *Systasis encyrtoides* under the subfamily Ormocerinae.

A new species of *Systasis* named *S. cenchrivora* infesting on seeds of *Cenchrus* sp. a weed, was described from India (Farooqi and Ramdas, 1972). Ahmad and Mani (1939), described a new species of Pteromalidae parasitizing linseed midge *Dasyneura lini* Barnes, named *Systasis dasyneurae* Mani with detailed biology and morphology. Mani (1942), described a new species, *Systasis dalbergiae* parasitic on larvae of Cecidomyiidae, *Contarinia dalbergiae* Mani from Dehra Dun.

Sureshan (2002 b) described three new species of Pteromalidae from Eravikulam National Park, *Stictomischus turneri* Sureshan, *Systasis nigra* Sureshan and *Trichomalus keralensis* Sureshan.

2.4. SUBFAMILY: PANSTENONINAE

Walker (1846 a, b) described a new genus *Miscogaster* with *Miscogaster oxylus* as the type species. Later Walker (1850), described a new new genus *Caudonia* with *Caudonia agylla* as the type species. The genera *Miscogaster* and *Caudonia* was later synonymised by Kerrich and Graham (1957) as the genus *Panstenon* Walker. Bouček (1976) described a new species, *Panstenon collare* Bouček from Africa. Another species *P. impube* Xia and Huang was described from China and was compared to *P. collare* Bouček. Narendran and Girishkumar (2009) described a new species *P. bengalense* from India which was compared to *P. lankaensis* Sureshan.

2.5. SUBFAMILY: PIRENINAE

Westwood (1833), erected a new genus *Gastrancistrus* with *Gastrancistrus vagans* as the type species. Bouček (1986) erected a new subgenus *Mangistrus*

under the genus *Gastrancistrus* with description of a new species *Gastrancistrus* (*Mangistrus*) *cherry* Bouček.

Ferrière (1934), erected a few new genera and species under the subfamily Pireninae with a detailed key to the genera. They are *Platecrizotes* with *Platecrizotes sudanensis* as type species and *Bairamlia nidicola*.

Rao (1981), contributed much to the family Pteromalidae, his efforts yielded five new species from Oriental region which include *Propicroscytus indicus* Subba Rao, *Colotrichnus agromyzae, Mokrzeckia menzeli, Gastrancistrus magniferae and Psilocera ghanii.*

Narendran *et. al.* (2001), studied the genus *Gastrancistrus* Westwood of India and provided a key to the Indian species of *Gastrancistrus*.

2.6. SUBFAMILY: PTEROMALINAE

Ratzeburg (1852) described a new species *Pteromalus seiboldi* under the subfamily Pteromalinae. Howard (1894), described a new genus *Herbertia* with *H. lucens* as the type species. Ashmead (1894) also described a new genus *Paracarotomus* with *P. cephalotes* as the type species.

Eunotinae a controversial group was considered as a tribe under subfamily Pteromalinae by Schmiedeknecht (1909), Muesebeck *et al.*, (1951), Nikolskaya (1952) and Peck *et al.*, (1964). Later, the subfamily status of Eunotinae was retained by Graham (1969).

Crawford (1909) erecting a new genus *Lariophagus* with *L. texanus* as the type species. Girault and Saunders (1910) described the new genus *Muscidifurax*. Several new genera and species to the family were made from India by Crawford (1913) which include *Zacalochlora*, *Trichomalopsis* and *Aplastomorpha* with *Z. milleri*, *T. shirakii* and *A. pratti* as the type species respectively. He also reported the species *Bruchobius colemani* Crawford from Mysore and *Bruchobius laticeps* Ashmead from Bangalore. Girault (1913 a), enriched Pteromalidae by adding

several genera like *Amoturella*, *Isoplatoides*, *Coelocyboides*, *Parurios*, *Amerostenus*, *Pachyneuronella*, *Neapterolelapas*, *Sphegipterosema*, *Eurydinotomorpha* and *Sphegipterosemella* . Waterston (1915) described three new species *Polycystus propinquus*, *Trigonogastra rugose* and *Trigonogastra megacephala* from Sri Lanka. Girault (1915 a, b and c), also contributed several genera to the family like *Miscogasteriella*, *Toxeumorpha*, *Acroclisoides*, *Trigonogastrella*, *Perilampella*, *Tomicobiella*, *Acroclisella*, and *Neopolycystus* belonging to subfamily Pteromalinae.

Girault (1920a and b) included many genera like *Eupelmophotismus*, *Neochalcissia*, *Eurytomomma*, and *Nerotolepsia* thus giving a thrust to the study of Pteromalidae. Ferrière (1930) described two new species under the subfamily Sphegigasterinae from Sri Lanka and Malay Peninsula, viz. *Trigonogastra brunneicornis* and *Agiommatus attaci*.

Mokrzecki (1933) described a new genus *Mokrzeckia* with *Pteromalus pini* Hartig as the type species. Rao (1973 a) described four new species *Norbanus africanus*, *Homoporus aegyptiaeus* (both from Africa), *Mokrzeckia orientalis* from Indonesia and India and *Mokrzeckia* indica from India with a key to *Mokrzeckia* species. Rao (1981) published key to the species of *Propicroscytus* and *Mokrzeckia* and recorded *M. orientalis* for the first time from Thailand.

Mani (1941) reported *Dinarmus sauteri* Masi from India for the first time and synonymized *D. coimbatorensis* Ferrière under *D. sauteri* Masi. Prinsloo (1984) studied the African fauna of Pteromalidae, he transferred the species *Bruchobius magnus* Rohwer to *Dinarmus*. Eventhough *Dinarmus* Thomson was well known senior synonym of *Bruchobius* Ashmead, the species *B. magnus* Rohwer was not transferred earlier.

Rao (1973b) erected new genus *Obtusiclava* with *O. oryzae* as type species, parasitizing *Pachydiplosis oryzae*, a serious pest of rice in India.

Heydon (1988a) reviewed world species of *Notoglyptus* Masi. He added four *Notoglyptus* species, *N. bidentatus*, *N. luteicrus*, *N. nesiotes* and *N. tzeltales*. Another excellent work also came in the same year (1988b), in which he reviewed the Nearctic species of *Cryptoprymna* Förster. Askew (1991) also contributed a new European species *C. paludicola*.

Sureshan (2006a) for the first time from Oriental Region recorded the genus *Coelopisthia* Förster with a new species from Sri Lanka, *Coelopisthia lankana* Sureshan (2006 b) further broadened the Pteromalid fauna of Sri Lanka by erecting a new genus *Neolyubana* Sureshan with *Neolyubana noyesi* as type species. Sureshan (2007), described a new species *Lyubana longigastra* from Sri lanka which was the first record of genus from the country. Sureshan *et al*., (2013) revised Oriental species of *Merismomorpha* Girault with description of a new species *M. tamilnadensis*.

Sureshan and Narendran (1994 a) made a valuable contributions by describing a new species under the little known genus of Pteromalidae, *Oniticellobia longigastra* they also recorded two genera *Trichomalus* Thomson and *Uniclypea* Bouček first time from India with new species *T. kannurensis* and *Uniclypea kumarani* from Kerala (Sureshan and Narendran, 1994 b).

Sureshan and Narendran (1997 a, b) contributed two new species *Uniclypea elongata* and *Inkaka keralensis* from Kerala. They described two species of genera *Sphegigaster* Spinola namely *S. anamudiensis* and *S. reticulata*. They recorded the species *S. brunneicornis* Ferrière and *S. stepicola* Bouček for first time from Kerala.

Suershan (1999), erected two new genera *Kumarella* Suershan with *K. angulus* as type species and *Narendrella* Suershan with *N. nilamburensis* as type species. Sureshan and Narendran (2000) added three new species to the family Pteromalidae. They are *Cryptoprymna elongate*, *Cryptoprymna Indiana* and *Toxeumorpha minuta*.

2.7. MOLECULAR CHARACTERIZATION OF PTEROMALIDAE THROUGH DNA BARCODING

In the absence of obvious morphological characters, independent assessment of insect species distinctions can be done through recent advances in molecular techniques. DNA sequencing of a standard gene region or ''DNA barcoding'' can be helpful in species diagnosis (Hebert *et al.*, 2003 a). DNA barcoding has great implications for taxonomy and one of the greatest promises is in the studies of biological diversity within regional and poorly studied habitat-specific biotas (Smith *et al.*, 2005). To detect genetically discrete units, DNA barcoding provides an independent data set and facilitates the discovery or verification of morphological differences among similar species (Packer *et al.*, 2009).

For species identification and discovery in large assemblages of life, DNA barcoding employs sequence diversity in short, standardized gene regions. By aggregating molecular, morphological and distributional data, it bridges a traditional bioinformatics gap. In animal kingdom 648 bp region of the cytochrome c oxidase I (COI) gene serves as the primary barcode sequence. (Hebert *et al.*, 2003b, Savolainen., 2005).

Hebert *et al.* (2003b) confirmed that in animals, for global bio-identification the mitochondrial gene cytochrome c oxidase I (COI) can serve as the core and it employs DNA sequences as taxon, 'barcodes' as a mean of sustainable species identification tool. Since then, 'Biological identifications through DNA barcodes', is considered a keystone publication and over the last ten years cited more than 2,500 times.

DNA barcoding can also be used for identification of cryptic species, biotypes and haplotypes. in addition to species discovery (Boykin *et al.*, 2012).

Vogler and Monaghan (2007) opined that, for taxonomic decisions and descriptions, and as a standard method of analysis "DNA taxonomy", is a concept

of adopting DNA sequencing as a central criterion. For assessing and understanding the extent of diversity in groups that have proven difficult by classical taxonomic techniques, DNA barcoding has shown a great role (Kohler, 2007). Ratnasingham and Hebert (2007) stated that, Barcode of Life Data System (BOLD) has been developed to manage and provide analytical tools for large amounts of data.

In order to aid the species identification resources, Consortium for the Barcode of Life (CBOL) and an online resource, Barcode of Life Data Systems (BOLD) were established. Later, in 2010 the International Barcode of Life (IBOL) project greatly increased the speed of barcode data generation (IBOL, 2013)

An overview of molecular identification of insect fauna with special emphasis on chalcid wasp was carried out (Rasool *et al.*, 2018) and they concluded that a very meagre number of insect species have DNA barcoding in India out of which species barcode is at the highest in Tichogrammatidae followed by Encirtidae and Eulophidae. According to the data collected from BOLD system, they concluded that even though the specimens of Pteromalidae with sequences are well above ten thousand, DNA barcode was available only for around two hundred species with great amount of repetition.

Materials and Methods

3. MATERIALS AND METHOD

The present study on 'Biosystematics and barcoding of Pteromalidae (Hymenoptera: Chalcidoidea) of Kerala' was carried out in the Department of Agricultural Entomology, College of Agriculture, Vellayani, Thiruvananthapuram, Kerala with the objective of identification, morphological and molecular characterization, documentation of Pteromalidae of Kerala. The materials used, techniques adopted and the observations made for the present study are presented in this chapter.

3.1. COLLECTION OF SPECIMEN

3.1.1. Study area

For biosystematics study of Pteromalidae, specimens were collected from fourteen districts of Kerala, including a variety of ecosystems and habitats (Plate 1). Kerala lies in the southern part of India, bordered by Karnataka up north and northeast and Tamil Nadu to east and south. It lies nearer to the equator, nonetheless is blessed with a pleasing and equable climate throughout the year. This is due to the land's proximity to the Arabian Sea and also the presence of Western Ghats on the east. It occupies 1.18% of total land area of India with an area of 38, 863 km^2. It lies between latitudes 8.32187 and 12.7549 N and longitudes 74.89400 and 77.15012 E.

Geographically Kerala can be divided into three distinct regions, *viz.*:

1. Highlands that are above 76 m altitude. The highlands slope down from the Western Ghats, generally having an average altitude of 900 m with several peaks well over 1800 m.
2. Midlands, the area between 7.6 and 76 m altitude which lie between the mountains low lying coastal areas.
3. Lowlands are regions below 7.6 m altitude, which are formed by deposition of sediments brought down by rivers of Western Ghats and sand deposited by sea.

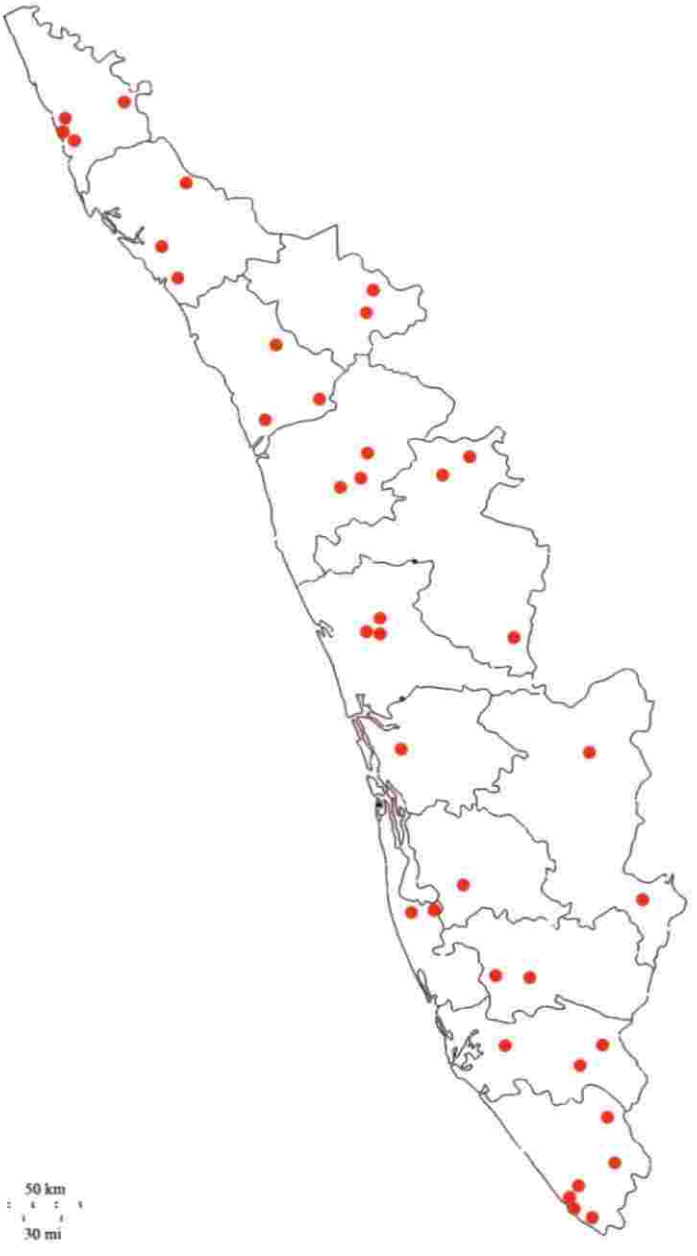


Plate 1: Locations of purposive sampling

3.1.2. Field surveys

Various localities were selected from all districts of Kerala and collections were done randomly. The places surveyed include forests which are part of National parks, sanctuaries and some agroecosystems lying near to forest covers. Hundreds of specimens were collected, sorted and preserved during the period of 2018-2019.

3.1.2.1. Method of collection

Triangular shaped sweep net was used for collecting Pteromalids (Plate 2 A). Early morning hours and evening hours were found to be the golden hours for sweep net collection giving maximum yield.

The net used for collection was manufactured by Hunt Wilde following the design by Boucek (1982). The frame and handle is made with aluminium and the handle measures 120 cm long which is detachable when not in use. The net bag is made of fine durable cotton material which is about 60 cm long. The rim of the bag is reinforced with thick canvas material with helps to withstand any impact during sweeping.

After collecting, the pteromalids were separated from the cloth bag using an aspirator (Plate 2 B) and transferred into absolute alcohol. Labels with details of locality and date of collection were placed in the collection vials (Plate 2 C).

3.2. PROCESSING

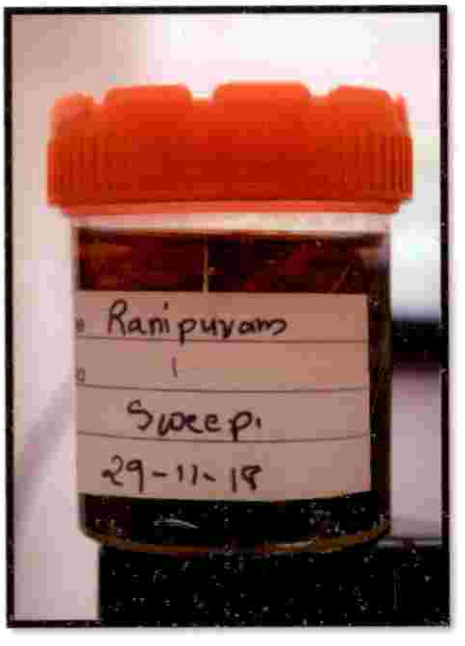
Processing of the specimens include sorting, relaxing, mounting, labelling and preserving the mounted and un-mounted specimens (Plate 3).

3.2.1. Un-mounted material

The un-mounted specimens were kept in air tight vials containing absolute alcohol. The alcohol was changed periodically and the vials were kept in dark place (Plate 4 A).

A.

B.



C.

Plate 2: A. Sweep net according to Dr. Z. Boucek's design B. Aspirator

C. Sample container with label

Plate 3: Sorting of pteromalid wasps from field sweep

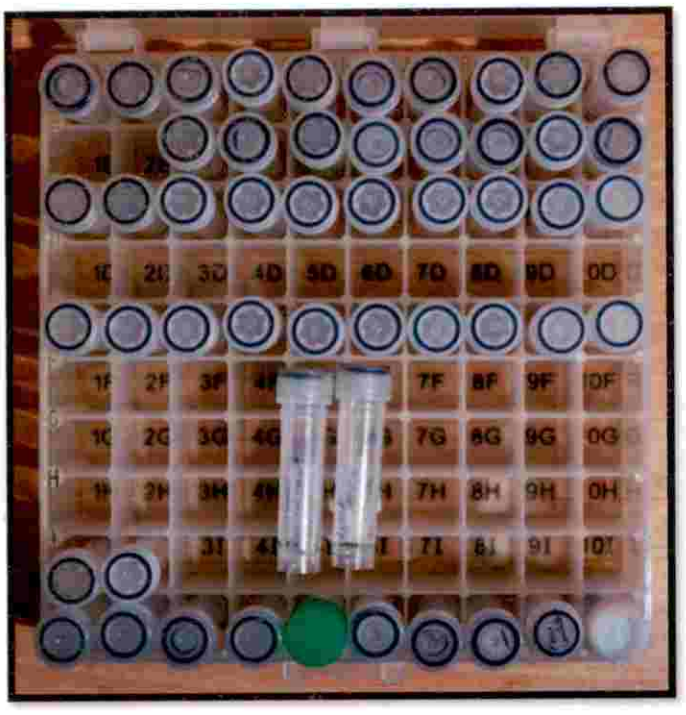
3.2.2. Card mounting

The materials used for mounting are cavity block, blotting paper, HMDS (Hexamethyldisilazane) series, fine zero-point brush, lamp, mounting cards, entomological pins, water soluble gum and stereo zoom microscope. Before mounting the specimen is transferred into 1:2 solution of HMDS to alcohol and kept for about 20 minutes, later transferred to 2:1 solution of HMDS to alcohol and kept for 20 minutes. After this the specimen is transferred to 100% HMDS and kept for 30 to 45 minutes before transferring to blotting paper for proper drying (Heraty, 1998). Hard bodied Pteromalids were not treated with HMDS. A small droplet of water soluble gum is placed at the tip of triangular card, and specimens were glued to the card point on the mesosoma. Specimens were placed in such a way that all the taxonomically relevant characters are correctly visible. The card was pinned on an entomological pin on a pinning block. This was followed by labelling. Rectangular labels were made containing essential information regarding location, name of the collector and date of collection. After proper drying of specimens, it is kept in insect box containing naphthalene balls to avoid damage by fungi and other small insects (Plate 4 B).

3.3. IDENTIFICATION OF PTEROMALİD WASPS

3.3.1. Microphotography (digital imaging) and Measurements

Photographs of specimens both whole and parts were taken under high resolution stereo microscope Leica M165c equipped with Leica DFC295 digital camera mounted on the microscope (Plate 5 A). The images were captured in desktop computer using the software LAS V3.6. images were taken in different focal planes and focus stacked using Zerene stacker into a single in-focus composite image. Editing of the images in a permissive level was done using Adobe Photoshop CC. Measurements of different parts were taken manually by using ocular micrometer (Plate 5 B).



A.

B.

Plate 4: A. Alcohol preservation B. Dry preservation of specimens

A.

B.

Plate 5: A. Macro focus-stacking photography B. Micrometry

3.3.2. Morphological Characterisation

The subfamily level identification was done following Australasian Chalcidoidea (Hymenoptera) by Bouček (1988). The generic level identification was done using the keys of Sureshan and Narendran (2004). Species level identification was done using the keys provided by Narendran (2003), literatures available in Universal Chalcidoidea Database and Natural History Museum, London. New species and genus encountered have been dealt with, in detail and previously known species encountered have been described pictorially in brief.

3.3.3. Terminology

The morphological terms and abbreviations are, as used by Bouček (1988) except thorax and abdomen. The term mesosoma includes propodeum and is equivalent to thorax. The gaster along with petiole is equivalent to abdomen (Plate 6 A). The terms mesosoma and metasoma are as used by Gibson (Gibson *et al.* 1991).

3.3.3.1. Head

Clypeus: The sclerite present in head just above the labrum.

Antennae: The appendage present in head between the eyes and is sensory in function (Plate 6 B).

Toruli: The paired socket present on head in which the antenna is placed.

Scape: The first antennal segment.

Pedicel: The second antennal segment.

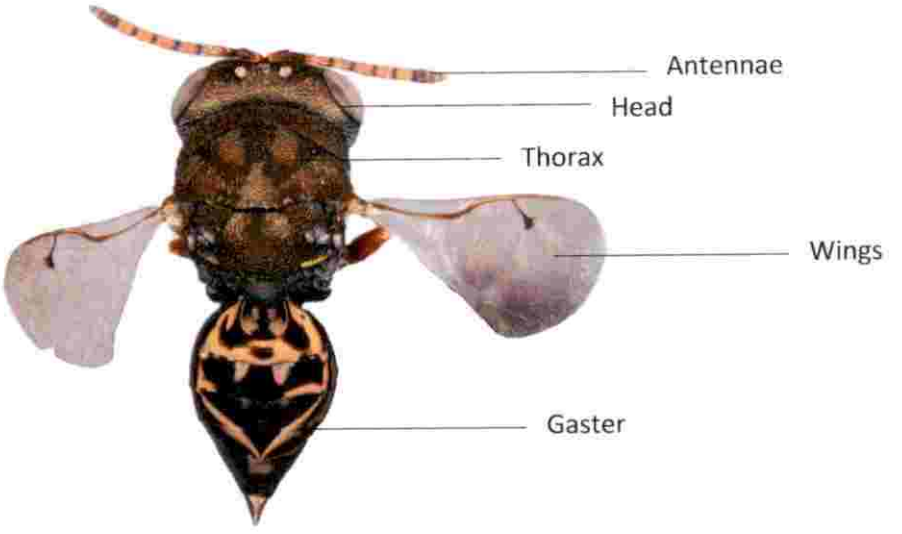
Anelli: Small ring segments between pedicel and funicular segments.

Funicle: Segments between anelli and clava (represented by F1, F2....F7).

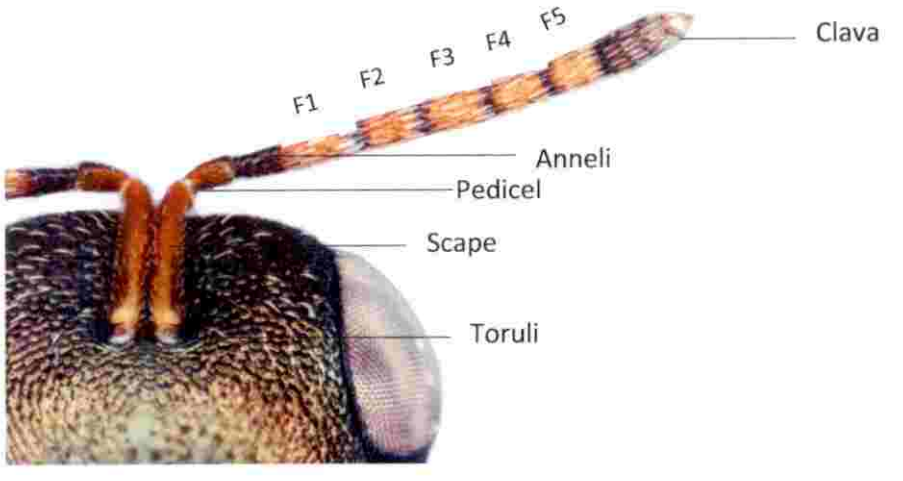
Clava: The last three segments of antenna which looks like a clump.

Scrobe: The groove on head to accommodate the scape.

Frons: The area of head between the front ocellus and the toruli.



A.



B.

Plate 6: A. Body of Pteromalid wasp in dorsal view. B. Antennae and it's parts.

Malar space: The shortest lateral distance between the base of the mandible and the compound eye.

Gena: The lateral part of head below compound eyes.

Malar sulcus: The vertical groove present in malar space.

Carina: Ridge or raised area.

Mandible: Highly sclerotized paired lateral mouth appendage for chewing and contains teeth.

Occiput: The area behind vertex (Plate 7 A).

Ocelli: Simple eyes present on the dorsal part of head, arranged in a triangle shape (Plate 7 B).

Vertex: The area between anterior ocellus and occiput.

3.3.3.2. Mesosoma

Pronotum: First segment of thorax dorsally.

Mesoscutum: Pronotum followed by mesoscutum, it is the second thoracic segment and usually has three lobes.

Notauli: The groove placed longitudinally on mesoscutum.

Mesopleuron: Lateral part of mesothorax.

Metapleuron: Lateral part of metathorax.

Mesepisternum & mesepimeron: The mesopleural suture sub divides mesopleuron into mesepisternum and mesepimeron. The mesepimeron is further divided unto lower and upper messpimeron.

Tegula: Small rounded sclerite which covers the base of the forewing.

Prepectus: The triangular sclerite between lateral sides of mesepisternum and pronotum.

Scutellum: The region between mesoscutum and propodeum. Posteriorly the scutellum sometimes has a subapical region, the frenum, differentiated by frenal groove (Plate 7 C).

Propodeum: propodeum follows scutellum and can be slightly prolonged into a neck like nucha.

3.3.3.3. Metasoma

Petiole: The stalk like structure that connects gaster and propodeum.

Gaster: 7-8 post petiolar segments compose the gaster (Plate 8 A).

Tergites: Dorsal segments of gaster.

Sternites: The ventral segments of gaster.

3.3.3.4. Wings

PMV: Post Marginal Vein.

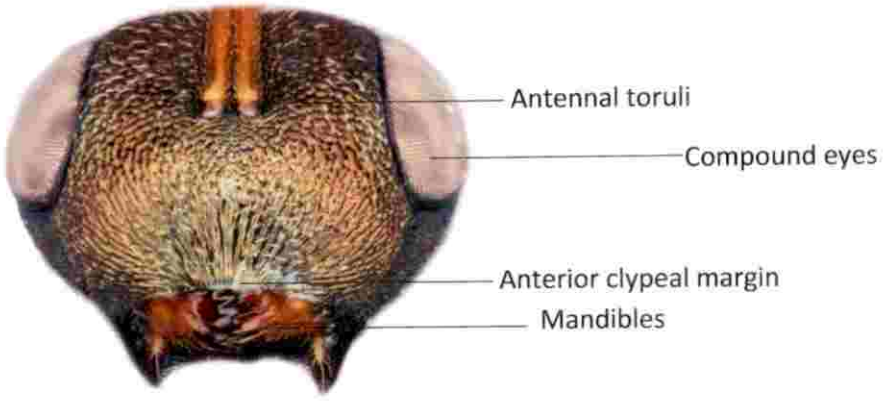
MV: Marginal vein.

SMV: Sub marginal vein.

STV: Stigmal vein. (Plate 8 B)

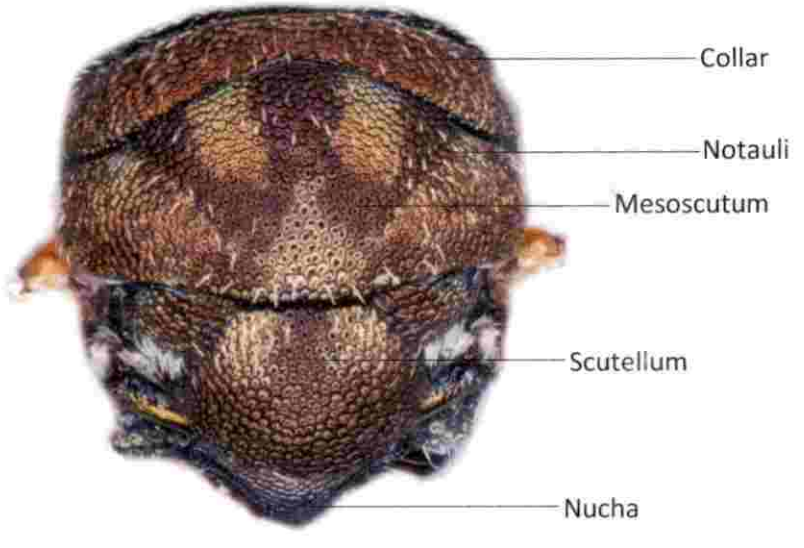
3.4. Molecular characterisation

Selected specimens identified through morphological characterization were subjected to DNA sequencing.



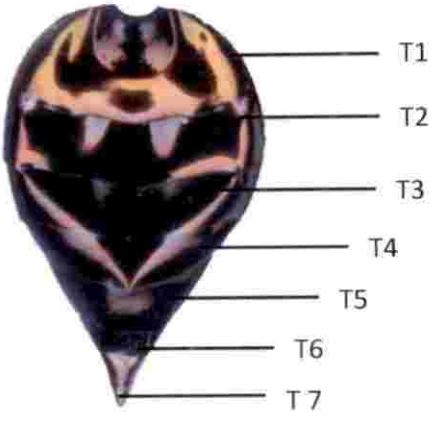
A.

B.

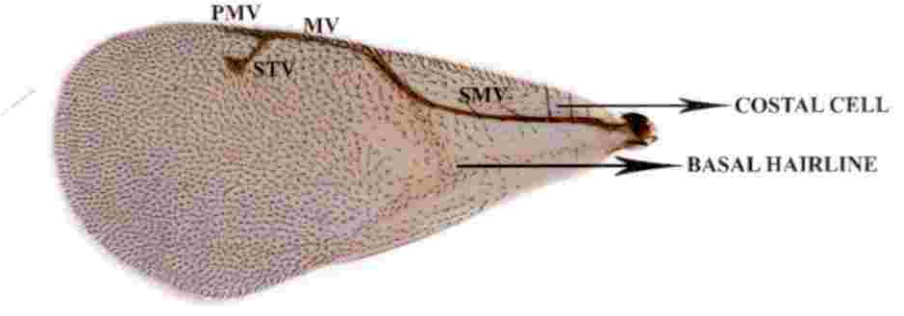


C.

Plate 7: A. Head front view B. Head dorsal view. C. Thorax dorsal view



A.



B.

Plate 8: A. Gaster B. Forewing of Pteromalid wasp

3.4.1. Genomic DNA Isolation

Genomic DNA was isolated from the tissues using NucleoSpin® Tissue Kit (Macherey-Nagel) following manufacturer's instructions. Single or few specimens of selected species based on availability of the specimens were subjected for barcoding. Sex of the specimens used depends on the availability. The specimens were preserved in absolute alcohol and stored in dark place.

Tissues were placed in a 1.5 ml microcentrifuge tube. 180 µl of T1 buffer and 25 µl of proteinase K was added and incubated at 56 °C in a water bath until the tissue was completely lysed. After lysis, 5 µl of RNase A (100 mg/ml) was added and incubated at room temperature for 5 minutes. 200 µl of B3 buffer was added and incubated at 70°C for 10 minutes. 210 µl of 100% ethanol was added and mixed thoroughly by vortexing. The mixture was pipetted into NucleoSpin® Tissue column placed in a 2 ml collection tube and centrifuged at 11000 x g for 1 minute. The NucleoSpin® Tissue column was transferred to a new 2 ml tube and washed with 500 µl of BW buffer. Wash step was repeated using 600 µl of B5 buffer. After washing the NucleoSpin® Tissue column was placed in a clean 1.5 ml tube and DNA was eluted out using 50 µl of BE buffer.

3.4.2. Agarose Gel Electrophoresis for DNA Quality check

The quality of the DNA isolated was checked using agarose gel electrophoresis. 1µl of 6X gel-loading buffer (0.25% bromophenol blue, 30% sucrose in TE buffer pH-8.0) was added to 5µl of DNA. The samples were loaded to 0.8% agarose gel prepared in 0.5X TBE (Tris-Borate-EDTA) buffer containing 0.5 µg/ml ethidium bromide. Electrophoresis was performed with 0.5X TBE as electrophoresis buffer at 75 V until bromophenol dye front has migrated to the bottom of the gel. The gels were visualized in a UV transilluminator (Genei) and the image was captured under UV light using Gel documentation system (Bio-Rad).

3.4.3. PCR Analysis

PCR amplification reactions were carried out in a 20 µl reaction volume which contained 1X Phire PCR buffer (contains 1.5 mM $MgCl_2$), 0.2mM each dNTPs

(dATP, dGTP, dCTP and dTTP), 1 µl DNA, 0.2 µl Phire Hotstart II DNA polymerase enzyme, 0.1 mg/ml BSA and 3% DMSO, 0.5M Betaine, 5pM of forward and reverse primers.

Primers used (Universal Primer)

Target	Primer Name	Direction	Sequence (5' → 3')
COI	LCO	Forward	GGTCAACAAATCATAAAGATATTGG
	HCO	Reverse	TAAACTTCAGGGTGACCAAAAAATCA

The PCR amplification was carried out in a PCR thermal cycler (GeneAmp PCR System 9700, Applied Biosystems).

PCR amplification profile

COI

98 °C	-	30 sec	
98 °C	-	5 sec	10 cycles
45 °C	-	10 sec	
72 °C	-	15 sec	
98 °C	-	5 sec	30 cycles
50 °C	-	10 sec	
72 °C	-	15 sec	
72 °C	-	60 sec	
4 °C	-	∞	

3.4.4. Agarose Gel electrophoresis of PCR products

The PCR products were checked in 1.2% agarose gels prepared in 0.5X TBE buffer containing 0.5 µg/ml ethidium bromide. 1 µl of 6X loading dye was mixed with 5 µl of PCR products and was loaded and electrophoresis was performed at 75V power supply with 0.5X TBE as electrophoresis buffer for about 1-2 hours, until the bromophenol blue front had migrated to almost the bottom of the gel. The molecular standard used was a 2-log DNA ladder (NEB). The gels were visualized in a UV transilluminator (Genei) and the image was captured under UV light using Gel documentation system (Bio-Rad) (Figure 2).

3.4.5. ExoSAP-IT Treatment

ExoSAP-IT (GE Healthcare) consists of two hydrolytic enzymes, Exonuclease I and Shrimp Alkaline Phosphatase (SAP), in a specially formulated buffer for the removal of unwanted primers and dNTPs from a PCR product mixture with no interference in downstream applications.

Five micro litres of PCR product is mixed with 2 µl of ExoSAP-IT and incubated at 37°C for 30 minutes followed by enzyme inactivation at 80°C for 15 minutes.

3.4.6. Sequencing using BigDye Terminator v3.1

Sequencing reaction was done in a PCR thermal cycler (GeneAmp PCR System 9700, Applied Biosystems) using the BigDye Terminator v3.1 Cycle sequencing Kit (Applied Biosystems , USA) following manufactures protocol.

The PCR mix consisted of the following components:

PCR Product (ExoSAP treated)	-	10-20 ng
Primer	-	3.2 pM (Either forward or reverse)
Sequencing Mix	-	0.28 µl
DMSO	-	0.30 µl

5x Reaction buffer	-	1.86 µl
Sterile distilled water	-	make up to 10µl

The sequencing PCR temperature profile consisted of a 1st cycle at 96°C for 2 minutes followed by 30 cycles at 96°C for 30 sec, 50°C for 40 sec and 60°C for 4 minutes.

3.4.7. Post Sequencing PCR Clean up

Master mix I of 10µl milli Q and 2 µl 125mM EDTA per reaction and master mix II of 2 µl of 3M sodium acetate pH 4.6 and 50 µl of ethanol were prepared. 12µl of master mix I was added to each reaction containing 10µl of reaction contents and was properly mixed. 52 µl of master mix II was added to each reaction. Contents were mixed by inverting and incubated at room temperature for 30 minutes. Spun at 14,000 rpm for 30 minutes and decanted the supernatant and added 100 µl of 70% ethanol. It was again spun at 14,000 rpm for 20 minutes, decanted the supernatant and repeated 70% ethanol wash. Decanted the supernatant and air dried the pellet. The cleaned up air dried product was sequenced in ABI 3500 DNA Analyzer (Applied Biosystems).

3.4.8. Sequence Analysis

The sequence quality was checked using Sequence Scanner Software v1 (Applied Biosystems). Sequence alignment and required editing of the obtained sequences were carried out using Geneious Pro v5.1 (Drummond et al., 2010).

Results

4. RESULT

During the present study, 150 specimens belonging to the family Pteromalidae were collected from 38 locations spread across fourteen districts of Kerala including forest ecosystems. Twenty species belonging to 15 genera and 5 subfamilies are treated in this study, of which 6 species are new to the world of science (Table 1). Among the six new species mentioned above, two species belong to two genera which are recorded for the first time ever. All the new taxa with distinct morphological characters are described in detail with appropriate ratios and images and other diagnostic characters are given in detail. Dichotomous key to the new species described are given. DNA barcoding was performed on select species of Pteromalid wasps. A complete checklist to the Pteromalidae of Kerala is provided.

Diagnosis

Pteromalidae is one of the most difficult families of Superfamily Chalcidoidea (Hymenoptera: Parasitica). No single character or set of characters separate all species of Pteromalidae from other families. Most of the Pteromalidae can be separated from closely related families and related groups by 8-13 segmented antennae and five tarsal segments in the fore and hind tibia. Another peculiar character is the prominently curved fore tibial spur. The size of these chalcids vary from 1-48 mm, usually metallic. Forewing with postmarginal and stigmal vein well developed and with a distinct speculum (Plate 9).

Distribution: Cosmopolitan

Table 1. Materials Examined

S. No.	Genus/ Species	Location	Specimens Examined
1.	*Genus* nov.1	Ranipuram	2 X Females
2.	*Systasis nigra* Sureshan	Vellayani, Ranni	4 X Females, 2 X Males
3.	*Panstenon* sp. nov.1	Theerthankara	5 X Females
4.	*Panstenon* sp. nov.2	Veallayani	2 X Females
5.	*Gastrancistrus* sp. Westwood	Theerthankara, Kakadanpoyl, Kodikuthimala, Attapadi, Kavalam, Idukki, Pala, Ranni, Vellayani	12 X Females, 1 X Male
6.	*Callitula peethapada* Narendran & Mohana	Ambalawayal	2 X Females
7.	*Callitula travancorensis* Sureshan	Ranipuram	2 X Females
8.	*Cryptoprymna elongata* Sureshan & Narendran	Kakkadanpoyl	2 X Females, 2 X Males
9.	*Dinarmus maculatus* Masi	Vellanikara, Vellayani	2 X Females
10.	*Dinarmus undans* sp. nov	Pilicode	1 X Female
11.	*Genus* nov.2	Ranipuram	1 X Female
12.	*Kumarella angulus* Sureshan	Ranipuram	1 X Female, 1 X Male
13.	*Merismomorpha elongata* Sureshan	Kakkadanpoyl	2 X Females, 2 X Males
14.	*Mokrzeckia* sp. nov.1	Thenmala	1 X Female
15.	*Notoglyptus scutellaris* Dodd and Girault	Ambalawayal, Periyar	7 X Females
16.	*Propicroscytus oryzae* Subba Rao	Theerthankara. Kalliaseri, Idukki, Mancombu	17 X Females, 3 X Males
17.	*Trichomalopsis apantolectena* Crawford	Karamana	1 X Female

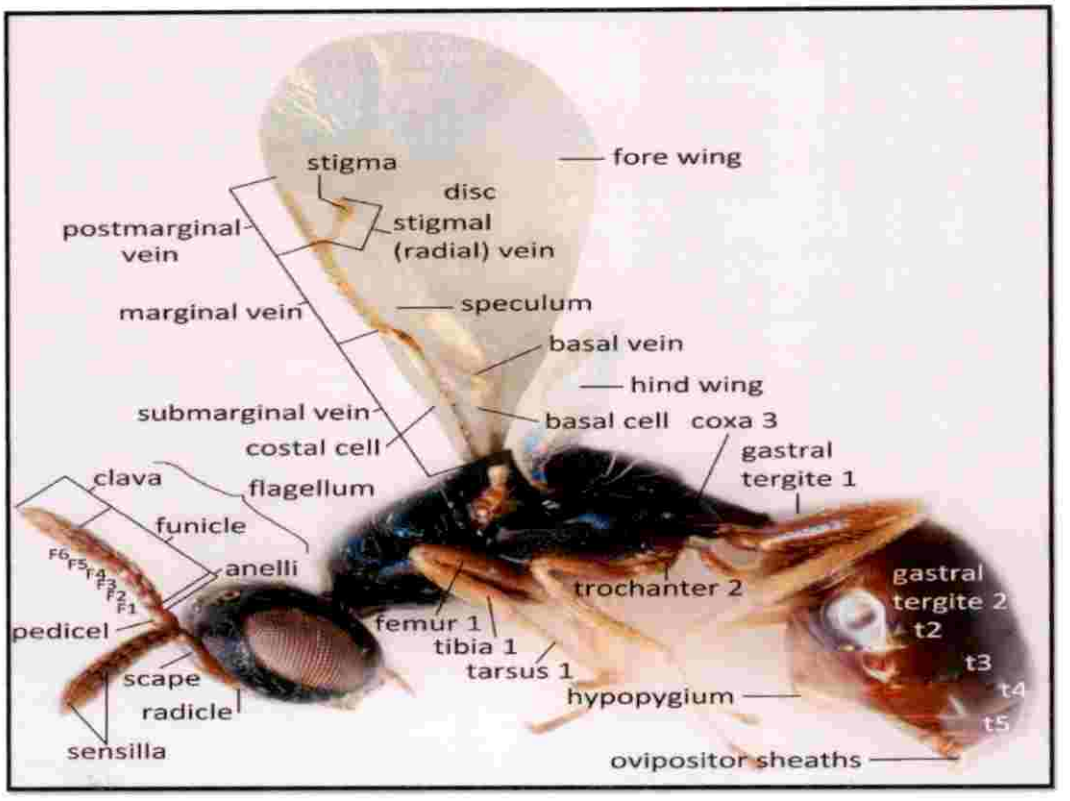


Plate 9: Morphology of Pteromalidae

4.1 SYSTEMATICS

4.1.1 Subfamily: MISCOGASTERINAE

4.1.1.1. Genus nov. 1 (Plate 10)

Diagnosis

Holotype: Female: Body length 2.59 mm. Head and mesosoma bluish-black with green metallic reflections; petiole and gaster brown; antennae with scape and pedicel testaceous, F1 and F2 dark brown F3 and F4 testaceous and F5, F6, F7 and clava dark brown in colour; head, mesosoma with dense dark brown setae; eyes red with pubescence.

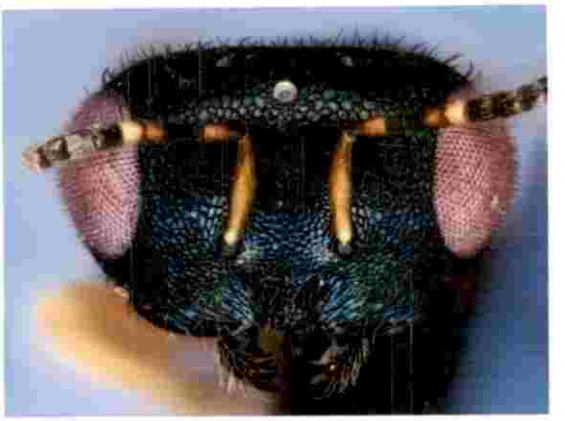
Head: With reticulate striations all over and distinct thick, black setae; head 1.34X wider than thorax; mandibles brown tridentate; anterior clypeal margin with two deep incisions forming a median tooth; antennal toruli placed just above the clypeus at the same level of lower margin of the eyes; head 2.2X wider than long dorsally; POL 1X OOL; eyes separated apart by 0.67mm when viewed from front; malar height 0.23X ocular height which in turn is 1.5X the ocular width; occiput deeply curved inside forming an inverted 'U' when viewed dorsally.

Mesosoma: Pronotum with as distinct carina anteriorly; mesoscutum with dense brown setae, notauli absent; scutellum convex, axillar grooves mild; axillae brown, prepectus small and suquadrate; propodeum with nucha concave, brown and with distinct median carina; spiracles oval; fore and hind leg pale yellow with coxae concolorous to the body, hind leg dark brown with trochanter globose and have two long setae, hind femora highly setaceous; wings hyaline, forewing with dense setae all along, speculum absent, basal hairline represented by a dark line of hair, basal cell and costal cell not bare; stigma with small uncus; SMV 2.4X MV, MV 2.5X PMV, STV 1.1X longer than PMV.

Metasoma: Petiolated abdomen; petiole 3X longer than broad, with a small projection on each side laterally; gaster brown, non-collapsing, laterally flatenned

Materials Examined: Holotype: Female, INDIA, Kerala, Kasaragod, RARS

A.



B.

C.

D.

E.

F.

Plate 10: A. *Genus* nov.1 female B. Head C. Thorax dorsal D. Thorax lateral E. Nucha and petiole F. Gaster lateral

Pilicode, 05-11-2018, coll. Manu Govind K K; Paratype: Female, INDIA, Kerala, Kasaragod, Ranipuram, 05-11-2018, coll. Manu Govind K K.

4.1.2. Subfamily: ORMOCERINAE

***4.1.2.1 Systasis* Walker** *Systasis*

Walker. 1834: 196, 288.

Paruriella Girault. 1913b: 107.

Diagnosis: Body robust; antennae 12 segmented; antennal formula 11253; notauli complete, sharply cut; face with scattered umblicate puntures; wings hyaline with speculum; under surface of forewing bearing a row of erect hairs behind the MV; gaster sessile.

Distribution: North America, Europe, Africa, Asia, Australia.

***4.1.2.1.2. Systasis nigra* Sureshan**

Diagnosis: Body is metallic green; antennae dark brown in colour; legs with tibia and tarsi pale yellow and the rest concolourous to the body; face rugosely punctuate between scape and inner orbital border; both mandibles tridentate; antennae with pedicel much longer than F1; all funicular segments subequal and quadrate; mesosoma with distinct complete notauli; gaster sessile and with a distinct median carina (Plate 11).

Materials Examined: 2 females and 2 males, INDIA, Kerala, Trivandrum, Vellayani, 10-08-2018, coll. Manu Govind K K; 2 females INDIA, Kerala, Pathanamthitta, Ranni, 12-08-2018, coll. Manu Govind K K.

Distribution: India: Kerala

4.1.3. Subfamily: PANSTENONINAE

***4.1.3.1.* Genus: *Panstenon* Walker**

Panstenon Walker. 1846: 29.

Plate 11: *Systasis nigra* female

Caudonia Walker. 1850: 125-126.

Diagnosis: Forewing unusually long and narrow, about 3X as long as broad with MV at least 3X as long as STV; petiole subquadrate, broadening posteriorly with irregular longitudinal rugosity; toruli very high on strongly convex shiny face; head weakly sculptured below toruli; clypeus as high as broad; legs slender and mostly yellow.

Distribution: North America, Europe, South Asia, Australia.

***4.1.3.1.1. Panstenon* sp. nov.1** (Plate 12)

Diagnosis

Holotype: Female: Length of body 2.1mm. Head is black with green and violet refringence. Eyes and ocelli silvery white. Antennae dark brown, scape testaceous, pedicel pale brown, collar pale yellow, mesosoma metallic green, gaster yellow except at the periphery, which is brown with green shiny tinge. Petiole brown. Legs yellow with tarsal tips brown. Tegulae pale yellow, wings hyaline and densely hairy. Veins pale yellow.

Head: with reticulate pattern engraved not deep and sparse white pubescence, in front view head 1.21X wider than height, dorsally 1.67X wider than long; malar groove present, gena smooth, malar space 0.45X ocular height, which in turn is 1.13X its width in profile; mandibles testaceous with tips brown; POL 1.35X OOL; antennae placed above the middle of the face, scape 0.5X the width of head and reaching the middle ocellus. Pedicel 1.14X F1, F1 equal to F6 in length, F2, F3, F4, F5 are of equal length, two anelli present, which together measures 0.5X the length of funicular segments F2 to F5; clava as long as the two preceding segments combined.

Mesosoma: Pronotum engraved reticulate; collar with a distinct ridge at the posterior end; mesoscutum convex; mesosoma 2.16X longer than wide; notauli deep groove like and incomplete; transcutal groove broad; scutellum reticulately

A.

B.

C.

D.

E.

F.

Plate 12: A. *Panstenon* sp. nov.1 female B. Head front view C. Antennae and malar sulcus D. Thorax dorsal E. Thorax lateral F. Gaster dorsal

punctuate; metanotum broad and coarsely punctate; propodeum with median carina absent, medially 2.5X long as broad.

Metasoma: Gaster more than 2X longer than broad, yellow with distinct brown colouration laterally; T1 reaching 1/3rd of the gaster.

Male: Unknown.

Materials Examined: Holotype: Female, INDIA, Kerala, Kasaragod, Theerthankara, 27-11-2018, coll. Manu Govind K K; Paratype: 4 females, INDIA, Kerala, Kasaragod, Theerthankara, 27-11-2018, coll. Manu Govind K K.

Remarks: This species closely resembles *Panstenon collaris* Bouček but differs in having pronotal collar margined; antennae inserted middle of face; petiole brown; gaster yellow; MV shorter than PMV; gastral petiole strongly transverse, not tapering forwards; sides without erect hairs (in *Panstenon collaris* Bouček pronotal collar not margined; antennae inserted very high; MV longer than PMV; gaster petiole as long as broad, tapering forwards, sides with erect hairs).

***4.1.3.1.2. Panstenon* sp. nov.2** (Plate13)

Diagnosis

Holotype: Female: Length of body 2.7mm. head is black with green and violet refringence; eyes and ocelli silvery white; antennae with scape pale yellow and the rest dark brown; mesosoma metallic green except collar which is testaceous; legs yellow except tarsal tip brown; wings hyaline and densly hairy; gaster brown with a median pale yellow patch.

Head: Black with violet refringence, with fine reticulate striations engraved into it; antennae dark brown except scape which is testaceous; antennal toruli placed on the equatorial line of the eyes with a fine depression around the toruli; in front view head 1.21X taller than broad, dorsally 1.57X wider than long; POL 2.15X OOL; malar grove present but not deep; malar space 0.34X ocular height which in turn is 1.18X ocular width; scape 3X pedicel; F2 to F6 subequal and F1 slightly shorter than the rest; clava 2X F6.

A.

B.

C.

D.

E.

F.

Plate 13: A. *Panstenon* sp. nov.2 female B. Head front view C. Antennae and malar sulcus D. Thorax dorsal E. Thorax lateral F. Gaster dorsal

Mesosoma: Mesosoma engraved reticulate with sparse pubescence; mesosoma 2.4X longer than broad; pronotal collar without ridging; mesoscutum convex, notauli forming a deep groove; scutellum with reticulate punctuations; propodeum rugulose-alveolate; petiole without median carina; forewing with SMV equal to PMV; MV 1.1X PMV, MV 4.33X STV.

Metasoma: Gaster 2.35X longer than broad; lateral side with refringence, yellow colour ventrally.

Male: Unknown

Materials Examined: Holotype: Female, INDIA, Kerala, Trivandrum, COA Vellayani, 30-11-2019, coll. Manu Govind K K; Paratype: Female, INDIA, Kerala, Trivandrum, COA Vellayani, 30-11-2019, coll. Manu Govind K K.

Remarks: This species closely resembles *Panstenon* sp. nov.1, but differs greatly in the absence of ridging on the pronotal collar. The position of antennal toruli also shows prominent difference. This species also lacks the median carina on the propodeum which is present in the other species. The ratios of the forewing veins also emphasise the difference of this species.

4.1.4. Subfamily: PIRENINAE

4.1.4.1. Genus: *Gastrancistrus* Westwood

Diagnosis: Antennae 11 segmented with very small ring joints; mandibles tridentate; parapsidial furrows complete; scutellum with transverse suture at the tip; metathorax and propodeum very small, without carinae; abdomen ovate and as long as thorax; second abdominal segment about a third length of the abdomen.

Distribution: Asia, Africa, Europe

4.1.4.1.1. Gastrancistrus sp.

Diagnosis: Body green with bluish reflections; head and mesosoma strongly reticulate; notauli complete; antennae with 5 funicular segments in females and 6

in males, 2 anelli present with 2nd anelli hidden below pedicel; antennae inserted below the centre of the face (Plate 14 A).

Materials Examined: 2 females, INDIA, Kerala, Kasaragod, Theerthankara, 2711-2018, coll. Manu Govind K K; 1 female coll. Manu Govind K K , INDIA, Kerala, Kozhikode, Kakadanpoyl, 05-01-2019, coll. Manu Govind K K; 1 female, INDIA, Kerala, Malappuram, Kodikuthimala, 30-09-2018, coll. Manu Govind K K; 1 female, , INDIA, Kerala, Palakkad, Attapadi, 04-02-2019, coll. Manu Govind K K; 1 female, , INDIA, Kerala, Ernakulam, Kavalam, 19-10-2018, coll. Manu Govind K K; 1 female, , INDIA, Kerala, Idukki, Idukki dam, 12-03-2019, coll. Manu Govind K K; 1 female, INDIA, Kerala, Kottayam, Pala, 16-04-2019, coll. Manu Govind K K; 1 female, , INDIA, Kerala, Pathanamthitta, Ranni 11-11-2018, coll. Manu Govind K K; 3 female and 1 male, INDIA, Kerala, Trivandrum; COA, Vellayani, 10-08-2018, coll. Manu Govind K K.

Distribution: Kerala.

4.1.5. Subfamily: PTEROMALINAE

4.1.5.1. Genus: *Callitula* Spinola

Diagnosis: Body mostly metallic; head wider than mesosoma; antennae with three anelli and five funicular segments; clava acuminate or with a narrow spicule; pronotal collar with a sharp margin; propodeum usually with long convex, reticulate nucha; gaster with often with T1 or both T1 and T2 prominently enlarged; petiole is visibly bordered ventrally on both sides by a flange which is an extension of first gastral sternite.

Distribution: All countries from the northern temperate zone to tropics to southern temperate zone.

4.1.5.1.1. Callitula peethapada Narendran and Mohana

Diagnosis: Head and mesosoma metallic green with bronzy reflection dorsally; gaster brown with a broad yellowish spot dorsally at the base; antennae brown except scape and pedicel testaceous; all legs including coxae brownish yellow; POL

A.

B.

Plate 14: A. *Gastrancistrus* sp. female B. *Callitulla peethapada* female

1.6X OOL; anterior margin of clypeus shallowly emarginate; antennal scape reaching well beyond the level of vertex; Pedicel as long as F1; clava as long as preceeding segments combined; terminal stylus long; pronotal collar anteriorly margined; propodeum with nucha well constricted, almost half of the median length; gaster elongate, ovate; petiole short, finely reticulate (Plate 14 B).

Materials Examined: 2 females, INDIA, Kerala, Wayanad, Ambalawayal, 11-02-2019, coll. Manu Govind K K

Distribution: India, Kerala, Bihar, Gujarat, Tamil Nadu, Telangana; Srilanka.

4.1.5.1.2. Callitula travancorensis Sureshan

Diagnosis: Body black with gaster mostly yellowish brown; antennae dark brown except scape and pedicel; anterior margin of clypeus weakly emarginate; scape reaching above the level of vertex; pronotal collar sharply carinate; propodeum with almost blunt plicae, reaching almost the base of the nucha; basal cell of forewing closed below; speculum very narrow; gaster short and ovate (Plate 15 A).

Materials Examined: 2 females, INDIA, Kerala, Kasargod, Ranipuram, 29-112019, coll. Manu Govind K K.

Distribution: India: Kerala, Arunachal Pradesh, Gujarat, Tamil Nadu, Uttar Pradesh.

4.1.5.2. Genus: *Cryptoprymna* Förster *Prosodes*

Walker. 1833: 371, 374.

Cryptoprymna Förster. 1856: 52, 56.

Polycystelomorpha Girault. 1915a: 340.

Diagnosis: Head, mesosoma, coxae and petiole black; gaster dark brown; head transversely oval in front; anterior clypeal margin truncate; genae with broad concavity extending from mouth margin to orbit; antennal formula 11263; antennae inserted below the middle of the face; clava distinctly wider than F6; pronotal collar

with sharp transverse anterior carina; propodeum as long as scutellum; strongly arched; median carina and plicae sharp; gaster ovate, petiole elongate and sculptured; T1 enlarged; hypopygium extending to the tip of the gaster.

Distribution: Europe, Africa, South Asia to New Guinea and Australia.

4.1.5.2.1. Cryptoprymna elongata Sureshan & Narendran

Diagnosis: Body black; antennae testaceous with clava darker; head finely reticulate; anterior margin of clypeus slightly emarginate; antennal scape reaching the median ocellus; clava little longer than three preceeding segments combined; mesosoma finely reticulate; gaster 1.5X width; petiole 3X longer than wide (Plate 15 B).

Materials Examined: 2 females and 2 males, INDIA, Kerala, Kozhikode, Kakkadanpoyl, 05-01-2019, coll. Manu Govind K K.

Distribution: India: Kerala, Karnataka.

4.1.5.3. Genus: *Dinarmus* Thomson *Dinarmus*

Thomson. 1878: 56.

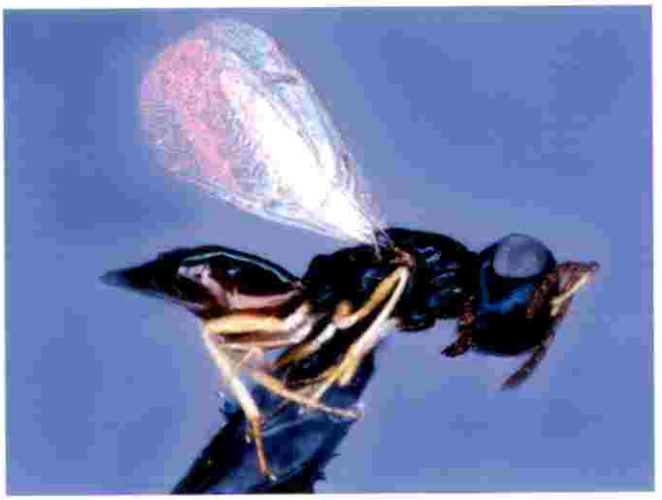
Bruchobius Ashmead. 1904: 314.

Metastenoides Girault. 1915a: 190.

Oedaule Waterston. 1922: 31.

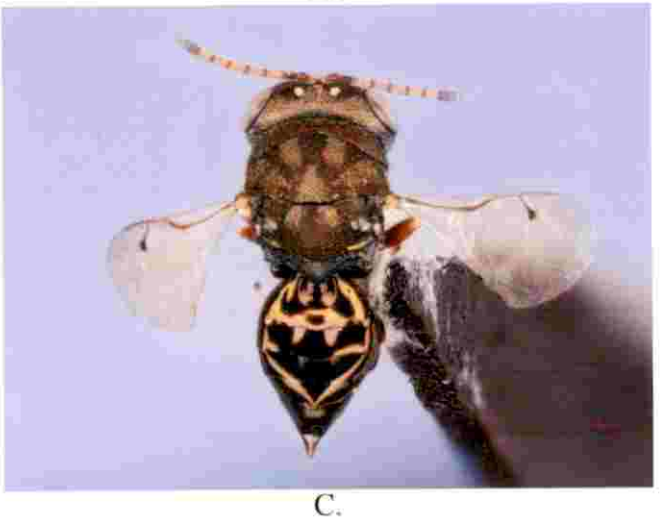
Diagnosis: Head large, not prominent behind eyes; anterior margin of clypeus shallowly emarginate or toothed; female antennae with three anelli and male with two anelli; third anellus sometimes quadrate; mesosoma stout, convex; pronotum as broad as mesoscutum; collar round, bluntly rounded, not carinate; neck hardly visible from above; prepectus small, subquadrate; propodeum short, reticulate, nucha subglobose; forewing with stigma more or less capitate; costal cell enlarged sometimes; gaster short; legs stout; hind tibia with two spurs.

Distribution: All temperate, subtropical and tropical zones reaching Australia.



A.

B.



C.

Plate 15: A. *Callitula travancorensis* female B. *Cryptoprymna elongata* female C. *Dinarmus maculatus* female

4.1.5.3.1. Dinarmus maculatus Masi

Sphaerakis maculatus Masi. 1924: 159.

Dinarmus maculatus (Masi): Bouček *et al.*, 1979: 433-467.

Diagnosis: Body metallic blue with bronzy patch on vertex and mesosoma; gaster darker; antennae testaceous except anelli and clava brown; antennae slender with distinct similar brown bands at the junction of each funicular segments distally; scape reaching median ocellus; propodeum with nucha moderate in length; basal part of gaster with short vertical rugae; PMV little longer than MV; gaster cordiform (Plate 15 C).

Materials Examined: 1 female, INDIA, Kerala, Thrissur, COH Vellanikara, 0602-2019, coll. Manu Govind K K; 1 female, INDIA, Kerala, Trivandrum, COA, Vellayani, 24-01-2019, coll. Manu Govind K K.

Distribution: India: Kerala, Maharashtra, Karnataka, West Bengal; Myanmar.

4.1.5.3.2. Dinarmus sp. nov. 1 (Plate 16)

Diagnosis:

Holotype: Female: Length 3.14mm, in dorsal view length of the body is 3.0X width of the body. Body blue with punctations all over and metallic shine to it except gaster, which is dark brown in colour with bluish shade at the edges.

Head: Antennae 13 segmented with 3 anelli and with the third anelli bigger than the preceding two. Scape and pedicel light yellow colour and rest of the antennae testaceouos except for the similar brown bands at the junction of funicular segments and clava with a dark brown shade. Scape 3.8x times the length of pedicel and F1 4.5X pedicel. Clava 1.16X the preceding two segments combined. Head 1.13X wider than thorax with mild white pubescence all over. Antennae placed well above the lower margin of the eyes with scape reaching the frontocellus. Ocelli dark brown, scape almost reaching the middle ocelli. POL 1.5X OOL, eyes separated by 1.23 times their height. Clypeal margin emarginate with anterior margin weakly

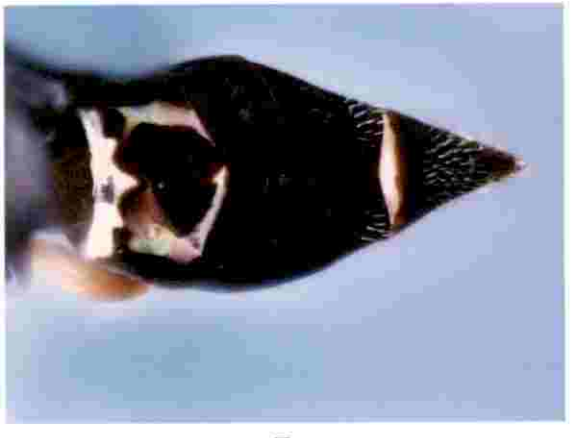
A.

B.

C.

D.

E.



F.

Plate 16: A. *Dinarmus* sp. nov.1 female B. Head front view C. Antenna D. Thorax dorsal E. Thorax lateral F. Gaster dorsal

bidentate and striations almost reaching the toruli. Mandibles dark brown with 4 sharp teeth.

Mesosoma: Mesosoma highly compact with reticulate punctations and white pubescence. Collar narrow, notauli incomplete, deep at the beginning and gradually becoming shallow and eventually disappearing. Scutellum large. Tegula testaceous and bigger than prepectus. Frenal groove not prominent. Nucha small and convex distally. Legs with coxa and femur concolorous testaceous on all the three pairs, hind leg with two tibial spur. Forewing with PMV 1.15X MV, SMV 2X MV. Costal cell margin slightly curved out with two rows of setae in the costal cell. Basal hairline distinct.

Metasoma: Metasoma brown with metallic green shine at the periphery, abdomen 1.3X head plus mesosoma combined. Gastral tergite T1 with distinct white wavy pattern at the base. Gaster ovate and non-collapsing.

Materials Examined: Holotype: Female, INDIA, Kerala, Kasaragod, RARS Pilicode, 05-11-2018, coll. Manu Govind K K.

***4.1.5.4. Genus* nov. 2** (Plate 17)

Diagnosis

Holotype: Female: Body length 1.9mm. Entire body black with metallic shine except antennae and legs, which are brown and ovipositor yellow; head and mesosoma with reticulate punctuations engraved in; body sparsely hairy.

Head: Head widely obovate with reticulate striations; mandible brown with four tooth; anterior clypeal margin emarginate, bidentate; antennal toruli placed in the middle of the face; scrobe present; antennae testaceous, with three anelli and five funicular segments; scape 4X as long as pedicel, the F1 slightly longer than the other funicular segments which are sub equal, clava as long as the previous three funicular segments combined; eyes are silvery white; malar space 0.32X ocular height, which in turn is 1.45X its width; head is 2.21X wider than long dorsally; POL 1.8X OOL; occipital carina absent.

A.

B.

C.

D.

E.

F.

Plate 17: A. *Genus* nov.2 female B. Head front view C. Thorax dorsal D. Thorax lateral E. Nucha and petiole F. Gaster lateral

Mesosoma: Collar narrow with sharp carina anteriorly; mesoscutum and scutellum with alveolate striations and weak white pubescence, notauli incomplete; tegulae brown, prepectus small; scutellum convex; wings hyaline; forewing with stigma prominently capitate; basal hairline absent, base of the wing bare; costal cell with single row of setae; SMV 2X MV; MV 1.25X PMV; PMV 1.33X STV; nucha convex with a median carina which extends side wards forming a cordiform shape; mesosoma 1.22X longer than broad.

Metasoma: Gaster sub sessile; ovate lanceolate; T2 medially incised dorsally, forming a bilobed structure; T3 occupying 2/3rd of the gaster; ovipositor long; metasoma 3.5X longer than broad.

Materials Examined: Holotype: Female, INDIA, Kerala, Kasaragod, Ranipuram, 09-11-2018, coll. Manu Govind K K.

*4.1.5.5.*Genus: *Kumarella* Sureshan

Diagnosis: Body with metallic blue reflections; head wider than mesosoma; clypeus broad, clearly demarcated anteriorly with a broad median tooth; scrobe deep; antennae with five funicular segments and three anelli in females and six funicular segments and 2 anelli in males; pronotum anteriorly weakly carinate; collar with a median notch thus forming two blunt tooth like structures laterally; propodeum with median carina and costula; forewing with PMV longer than MV; hind tibia with one spur; sessile gaster.

Distribution: India, Malaysia, Taiwan, Thailand, Vietnam.

4.1.5.5.1. Kumarella angulus Sureshan

Diagnosis: Length 4 mm; body dark metallic blue with gaster brown; antennal toruli above the lower margin of the eyes; antennal scape reaching the median ocellus; pedicel as long as F2; third anellus little longer than first and second; clava shorter than the preceding two segments combined; propodeum medially with four depressions; forewing with MV 1.5X PMV (Plate 18 A).

Materials Examined: 1 male and 1 female, INDIA, Kerala, 14-02-2019, coll. Manu Govind K K.

Distribution: India: Kerala, Karnataka, Maharashtra; Malaya, Taiwan, Thailand, Vietnam.

4.1.5.6. Genus: *Merismomorpha* Girault *Merismomorpha*

Girault. 1913a: 82-83.

Neopolycystella Girault, A. A. 1915a: 336.

Epipolycystus Girault. 1915b: 336.

Diagnosis: Head little wider than mesosoma; lower face little convex along the median line; clypeus produced; lower margin of clypeus often rounded, angulate, rounded, blunt, truncate or even slightly emarginate; antennal formula 11353 or 11263; arched mesosoma; notauli complete or incomplete; propodeum with distinct nucha and with converging submedian channels; forewing with MV longer than PMV; hind tibia with single spur; gaster ovate, lanceolate.

Distribution: India, Australia.

4.1.5.6.1. Merismomorpha elongata Sureshan

Diagnosis: Length around 2-2.5mm. Headand mesosoma bluish black; gaster dark metallic blue, brown ventrally; head moderately reticulate, almost shiny on convex median line of lower face; pedicel as long as F1; third anellus as long as first and second combined; propodeum with median carina indicated; gaster elongate, ovate, petiole as long as hind coxa, broadened before middle, embraced by a very short extension of first gastral sternite; gaster slightly longer than head and mesosoma combined (Plate 18 B).

Materials Examined: 2 female and 2 male, INDIA, Kerala, Kozhikode, Kakkadanpoyl, 05-1-2019, coll. Manu Govind K K.

Distribution: India (KL).

A.

B.

Plate 18: A. *Kumarella angulus* female B. *Merismomorpha elongata* female

4.1.5.7. Genus: *Mokrzeckia* Mokrzecki

Mokrzeckia Mokrzecki. 1934: 143.

Beierina Delucchi. 1958: 271.

Diagnosis: Head with clypeus large, its lower margin produced, with a sharp median incision; antennae with two anelli and five funicular segments, each flagellar segments with two rows of sensilla; pronotum produced dorsally, very short but sharply carinate; mesoscutum densely pubescent; propodeum with adpetiolar region raised an deeply curved inside medially; gaster ovate, about as long as mesosoma.

4.1.5.7.1. Mockrzeckia sp. nov.1 (Plate 19)

Diagnosis:

Holotype: Female: Body length 2.79mm. Head and mesosoma green in colour with metallic texture; gaster sessile, brown with white patch both dorsally and ventrally; head and mesosoma with long white pubescence all around.

Head: Globose, with reticulate striations deep; eyes red in colour; clypeal margin with a clear median incision, forming a bidentation; clypeal margin with short striations, clypeus not clearly demarcated; mandibles with four sharp teeth; antennal toruli on the upper margin of the head; head 1.9X wider than long dorsally; POL 1.45X OOL; malar sulcus not prominent, malar space 0.42X ocular height, which in turn is 1.33X ocular width; antennae brown, except F4 which is yellow, acuminate clava; antennae with two anelli and six funicular segments and with each funiculae segments having two rows of sensillae; occipital carina absent.

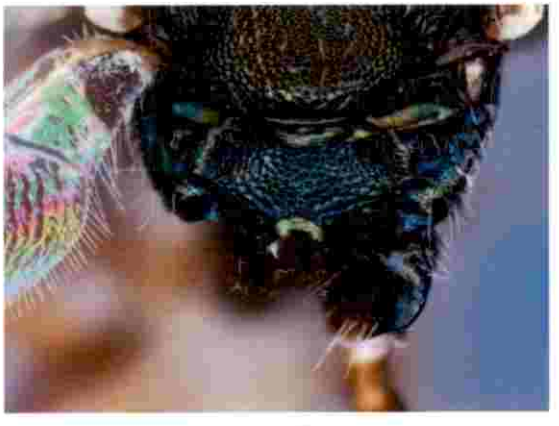
Mesosoma: Collar short with a sharp carina anteriorly; mesoscutum with alveolate striations, notauli incomplete; scutellum convex with white pubescence and concentric alveolate striations; tegula testaceous, smaller than prepectus which is subquadrate; propodeum with adpetiolar region raised and deeply curved inside; all legs pale yellow except coxae which is concolorous to the body; forewings hyaline with distinct speculum, basal hairline; basal cell and costal cell not bare; SMV 1.6X

A.

B.

C.

D.



E.

F.

Plate 19: A. *Mokrzeckia* sp. nov.1 female B. Head front view C. Antennae D. Thorax dorsal E. Nucha F. Gaster dorsal

MV, MV 1.26X PMV, PMV 1.76X STV.

Metasoma: Gaster ovate, dorso-ventrally flattened; brown with white round patch over T2, T2, T3 and T4 dorsally and ventrally; gater 1.68X longer than broad.

Materials Examined: 1 female, INDIA, Kollam, Thenmala, 15-01-2019, coll. Manu Govind K K.

Remarks: This species was compared to *M. pini* Hartig; the prominent differences shown are acumainate antennae with F4 yellow in colour which uniformly brown colour in later; this species also has prominent white patch on the gaster, which is absent in the later and has a uniform bluish black gaster.

4.1.5.8. Genus: *Notoglyptus* Masi

Diagnosis: Colour black to metallic green; head ovate to triangular in anterior view; clypeus with anterior margin straight or produced and sometimes bidentate; genal concavities well developed; antennae with two anelli and six funicular segments; pronotum reduced much narrower and depressed; collar with weak to strong carina anteriorly; notauli complete; scutellum with scuto- scutellar sulcus foveolate in the middle; frenal grove distinct; propodeum with plicae and median carina; forewing with MV longer than PMV; gaster petiolate, ovate, petiole quadrate to elongate; T1 nearly concealing succeeding tergites.

Distribution: India, rom central Europe to South Africa, throughout southern Asia to Japan and Queensland America.

4.1.5.8.1. Notoglyptus scutellaris Dodd & Girault

Merismus scutellaris Girault. 1915a: 328.

Notoglyptus niger Masi. 1917: 181.

Diagnosis: Length 1.2-1.5mm. Head, mesosoma and petiole dark green; gaster brown; antennae with scape yellow, remainder brown; anterior margin of clypeus almost straight; head lightly alveolate; scutellum with distinct discal fovea; petiole alveolate; length 1.2X width with weak median carina; gaster smooth (Plate 20 A).

Materials Examined: 4 females, INDIA, Kerala, Wayanad, Ambalawayal, 11-02-2019, coll. Manu Govind K K; 3 females, INDIA, Kerala, Idukki, Periyar, 13-03-2019, coll. Manu Govind K K.

4.1.5.9. Genus: *Propicroscytus* Szelenyi

Propicroscytus Szelényi. 1941: 121.

Obtusiclava Subba Rao. 1973a: 627.

Diagnosis: Head wider than mesosoma; occiput without margin; lower margin of clypeus emarginate; scrobes virtually absent; antennae inserted distinctly above the lower margin of the eyes, slender with two anelli and six funicular segments; pronotum short and collar carinate; notauli incomplete, present only anteriorly; nucha short without carina; plicae only indicated anteriorly by small depression; forewing with very long MV and PMV; hind tibia with single spur; gaster longer than head and mesosoma combined, mostly yellow with dark longitudinal markings.

Distribution: Africa, Southeast Asia to Australia, Zimbabwe.

4.1.5.9.1. Propicroscytus oryzae Subba Rao

Obtusiclava oryzae Subba Rao. 1973b: 627-629.

Diagnosis: Head and mesosoma dark green; gaster yellow with longitudinal brown bands dorsally; clypeus with distinct striations; clypeal margin slightly emarginate; antennae inserted middle of the face; POL 085X OOL; pronotal collar short and margined; forewing densly hairy outside speculum; basal vein irregularly setose; gaster sessile, 3X longer than broad (Plate 20 B).

Materials Examined: 1 female, INDIA, Kerala, Kasaragod, Theerthankara, 27-11-2018, coll. Manu Govind K K; 2 females amd 1 male, INDIA, Kerala, Kannur, Kalliaseri, 30-01-2019, coll. Manu Govind K K; 4 females, INDIA, Kerala, Idukki, Idukki dam, 12-03-2019, coll. Manu Govind K K; 10 females and 2 males, INDIA, Kerala, Alapuzha, Mancombu, 18-04-2018, coll. Shanas S.

Distribution: India: Kerala, Arunachal Pradesh, Andhra Pradesh, Maharashtra, Orissa; Indonesia; Peoples Republic of China; Sri Lanka; Thailand.

4.1.5.10. Genus: *Trichomalopsis* Crawford

Trichomalopsis Crawford. 1913: 251.

Metadicylus Girault. 1926: 71.

Diagnosis: Occiput with a strong '∩' shaped carina placed about half way down to the foramen; eyes superficially bare; antennae 13 segmented with two anelli and six funicular segments in both the sexes; slender flagellum, clavate in females and less clavate in males; pronotal collar weakly emarginate; notauli complete posteriorly; petiole highly transverse and supported ventrally by flange like extension of the first gastral sternum; basal cell usually bare, marginal vein uniformly slender and marginal fringe present.

Distribution: Known from all continents.

4.1.5.10.1. Trichomalopsis apanteloctena Crawford

Trichomalopsis apantolectenus Crawford. 1911: 618.

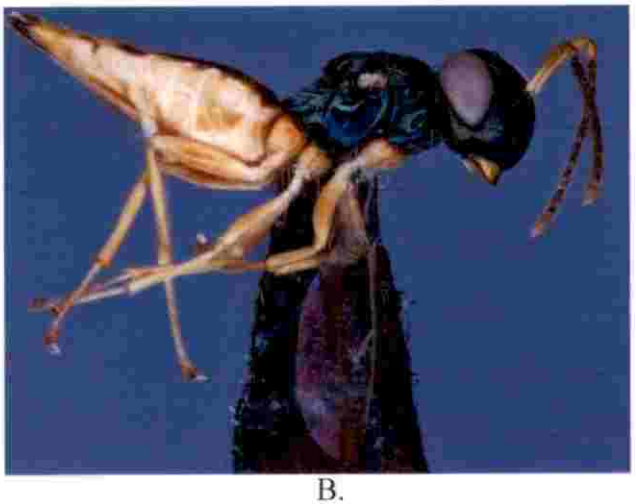
Eupteromalus parnarae Gahan. 1919: 513-524.

Diagnosis: Length 1.6-2.5mm. Lower margin of clypeus rather deeply incised medially; striations of clypeus extending to lower margin of eyes and to malar sulcus; head thick, occipital carina sharp in postero-dorsal view, strongly curved medially; pronotal collar indistinctly margined; scutellum with frenal furrow usually distinct; forewing with MV 1.55- 2.1X as long as STV; gaster almost 2X as long as broad (Plate 20 C).

Materials Examined: 1 female, INDIA, Kerala, Trivandrum, Karamana, 12-02-2018, coll. Manu Govind K.

Distribution: India: Kerala, Andra Pradesh, Bihar, Karnataka, Meghalaya, Orissa,

A.



B.

C.

Plate 20: A. *Notoglyptus scutellaris* female B. *Propicroscytus oryzae* female C. *Trichomalopsis apantolectena* female

Tamil Nadu, West Bengal; Bangladesh, Japan; Korea; Malaysia; People's Republic of China; Philippines; Russia; Taiwan; Vietnam.

4.2 MOLECULAR CHARACTERIZATION

Molecular characterization was attempted for 10 species. However, DNA barcode was obtained for three species using COI specific primers.

The DNA barcode obtained are as follows:

4.2.1. *Pteromalus* sp.

TTAATTGGTAATGATCAAATTTATAATTTTATTGTTACTACTCATGCAT
TTACAATAATTTTTTTTTTTGTTATACCTGTAATAATAGGGGGATTTGG
TAATTTTTTAATTCCTATAATTTTAGGAGCTCCAGATATATCATTTCCTC
GAATAAATAATATAAGATTTTGACTATTACCGCCAAGATTAATATTAT
TAATATCTAGGATATTTATTGGGTCAGGAACTGGTACTGGATGAACAG
TTTATCCTCCTTTATCTTCTAATCTTTCTCATAGAGGTCCATCAGTAGAC
TTATCAATTTTTTCTTTACATATTGCCGGTCTTTCATCTATTATAGGATC
AATTAATTTTATTACTACAATCATTAATATAAAAATTTATAAAATTGAT
AATGTTCCATTATTAGCTTGAGCAATATTATTAACAGCAATTTTATTAT
TATTATCATTACCAGTTTTAGCAGGAGCTATTACAATATTATTATTTGA
TCGTAATTTAAATACATCATTTTTTGATCCAGCAGGAGGGGGG

4.2.2. *Scutellista* sp.

TTAGTTCCAATAATGTTAGGAGCTCCAGATATAGCTTTTCCTCGAATGA
ATAATATAAGATTTTGAATATTACCCCCAAGTTTAATATTATTAATGGG
AAGAATATTTATTGGGGAAGGTACAGGTACTGGTTGAACTGTTTATCC
TCCATTATCTTCCATTATTGGGCATAATTCTCCTTCTGTAGATTTATCTA
TTTTTTCTCTGCATATTGCTGGTATCTCTTCAATTATAGGGTCTATTAAT
TTTATTAGGACTATTTTAAATATAAAAATTTTTAAAATAGAATTAATCT

CTTTATTTTCTTGGTCTATATTATTAACAACGATTTTATTACTTCTTAGG
CTCCCTGTTTTAGCAGGGGCTATTACTATATTGTTATTTGATCGAAATT
TTAACTCTTCTTTTTTTGATCCTGCAGGA

4.2.3. *Callitula* sp.

ACAGGTAAAGATAAAAGTAATAAAATCGCAGTTAATAATATTGATCAT
GCAAATAAAGAAATATTTTCTATTTTATAGATTTTTATATTAATAATAG
TTGTAATAAAATTAATTGATCCTATAATTGAGGAAATTCCAGCAATAT
GTAATGAAAAAATTGAGAGATCTACTGATGGTCTTCTATGAGATATAT
TAGAGGATAATGGAGGATAAACTGTTCATCCTGTTCCAGTACCCCTAC
CAATAAATATTCTAGAAATTAATAGTATTAATCTTGGGGGTAATAATC
AAAATCTTATATTATTTATCCGTGGAAATGCTATATCTGGTCTACCTAA
TATTATAGGAATTAGATAGTTACCAAATCCTCCTATTATTACTGGTATA
ACGAAAAAAAAAATTATAGTAAAGGCATGAGTAGTAACAATAGAATT
ATAAATTTGATCATTACCAATTAAAGAGCCTGGATTTCCTAATTCTAAA
CGAATAATTATTCTTATAGATAAATCCTATTACTCCTGCTCATATTTCC
AAAAAATAAAATATAAAATTCCAATAATCTTTTATGATTTGTTTGACC
A

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Discussion

5. DISCUSSION

Results of the study 'conducted during 2017-2019' with an objective of identification, morphological and molecular characterization and documentation of Pteromalidae of Kerala are discussed in this chapter.

5.1 IDENTIFICATION AND MORPHOLOGICAL CHARACTERIZATION

The family Pteromalidae is one of the most difficult among other Chalcidoidea because of their small size and huge diversity (Boucek, 1988). Twenty species of pteromalid wasps were identified and documented from the state. The study brought to light six new species of pteromalid wasps under five genera, of which, two are new to science. During the study, three species of pteromalid wasps were subjected to DNA extraction and DNA barcoding namely *Pteromalus* sp., *Scutellista* sp. and *Callitula* sp.

The family currently includes 31 subfamilies globally, of which 18 are known from Kerala (Gupta, 2016). Five subfamilies are treated here Miscogasterinae, Ormocerinae, Panstenoninae, Pireninae and Pteromalinae.

The subfamily Miscogasterinae is distributed in Paleartic, Neartic, Oriental and Australian Regions. With this wide distribution comes the fairly high diversity of the genera coming under this subfamily. A total of 36 genera has been reported so far from this subfamily, of which 5 has been reported from India. One species was collected from Ranipuram, Kasaragod which displayed all the subfamily characters, mainly a prominent petiolated gaster, digitate labrum and the number of antennal segments, but did not fit into any of the genera described so far. Some of the deviant characters shown by this species are, the colouration of the antennae with distinct alternate black and yellow segments and eyes with dense yellow pubescence. The ratios of the species examined also showed a significant deviation from all the described genera which helped to confirm that specimen was coming under a new genus and is depicted as *Genus nov.*1. Biology of the genera belonging to the subfamily is little known although most of them are pupal parasitoids of leaf

miners (Askew, 1968), thus making them an economically important group and opening up the possibility of their deployment in biological control programmes.

The subfamily Ormocerinae was earlier considered as a separate family and was later merged with Pteromalidae as a subfamily (Walker, 1833). Currently this subfamily includes 42 genera. Out of which only 2 are reported from India. Of these two genera only one species *Systasis nigra* Sureshan. was studied here. This genus is distributed only in Spain and India. In India it is confined to Kerala, Tamil Nadu, Uttar Pradesh and Rajasthan (Sureshan, 2007a). The species coming under the genus are usually parasitoids of Cecidomyiidae (Mani, 1939). Some of the most reliable characters which are common to this genus are, complete notauli and hidden annelar segment below the pedicel. However, exhibit great variation in colour and size between the two Indian species.

Panstenoninae is the smallest subfamily under Pteromalidae with only one genus namely *Panstenon* Walker. The genus was earlier classified under Pteromalinae and was placed in a separate monotypic subfamily by Boucek (1988). Most of the 14 described species of *Panstenon* Walker are distributed in India, Sri Lanka and Australia, except for one species *Panstenon collare* Boucek. which is distributed in Africa, Zimbabwe, Srilanka, Australia and India and shares many characters with most of the congenerics. This study revealed two new species of *Panstenon* Walker. These two new species were denoted as *Panstenon* sp. nov.1. collected from Theerthankara, Kasaragod and *Panstenon* sp. nov.2. collected from Vellayani, Thiruvananthapuram. The holotype of *Panstenon.* sp. nov.1 is a female and can be distinguished by a prominent pronotal ridge which is absent in all other species under this genus which was found to be the most deviant character of the species. This species was compared with *P. collare* Walker, with which it closely resembles. However, they differed significantly with respect to antennal segment ratios and wing vein ratios. The most important distinguishing character is a unique ridge on the pronotal collar and also abdominal colouration. *P.* sp. nov.2 was compared to *P. collare* and *P.* sp. nov.1 and the prominent difference that distinguished this species is the placement of antennal toruli, which is situated in

the middle of the face for *P.* sp. nov.2, but placed very high near to the fronto-ocellus in the case of *P.*sp. nov.1 under this genus. This species lacks prominent malar sulcus and propodeal carina which is present in other species of this genera. *P.* sp. nov.2 also showed differences in ratios of length of wing vein by around 0.25X in ratios of length to breadth of gaster and also ratio between PMV and MV, which clearly confirms the identity of this new species. Species belonging to this genus are mostly grass dwellers and parasitizes eggs of Hemiptera (Boucek 1988).

Gastrancistrus Westwood is one of the 18 genera described under the subfamily Pireninae. It is the largest and globally distributed genus under Pireninae. The genus comprises of 135 species. This is the only genus under Pireninae known from India so far. This genus is known to parasitize cecidomyiids (Westwood, 1833). The widespread and naturally high population of this genus thus plays an important role in checking the population of gall midges in the nature.

The next subfamily treated here is Pteromalinae, which is the largest and the most diverse subfamily of Pteromalidae. The subfamily characters of Pteromalinae were originally marked out by Howard (Howard, 1886). The present study involves 12 species under 10 genera of Pteromalinae. Out of the twelve species, three are new to science. They fall under the genera *Dinarmus* Thomson, *Mokrzeckia* Mokrzecki and a new genus *Genus nov.*2. The species of Pteromalinae treated here are *Callitula peethapada* Narendran and Mohana, *Callitula travancorensis* Sureshan, *Cryptoprymna elongata* Sureshan and Narendran, *Dinarmus maculatus* Masi, *Kumarella angulus* Sureshan, *Merismomorpha elongata* Sureshan, *Notoglyptus scutellaris* Dodd and Girault, *Propicroscytus oryzae* Subbarao, *Trichomalopsis apantolectena* Crawford.

Callitula Spinola is one of the most common genera under the family Pteromalidae and is worldwide in distribution. The present study includes two species of *Callitula* Spinola, namely *Callitula peethapada* Narendran and Mohana. and *Callitula travancorensis* Sureshan. All species of *Callitula* parasitizes Diptera especially Agromyzidae and Cecidomyiidae in their larval stage.

One species of *Cryptoprymna* Forster *C. elongata* Sureshan and Narendran was examined during the study. Only 14 species have been reported worldwide under this genus and two species is reported from India. The genus is currently distributed in Egypt, Kenya and India. The biology and life cycle of the genus is little known, which is suspected to be parasitic on syrphids (Heydon, 1988).

Two species of *Dinarmus* Thomson, *Dinarmus maculatus* Masi and *Dinarmus* sp. nov.1 was examined during the study. The genus *Dinarmus* is distributed worldwide with 23 species reported globally. Seven species has been reported so far from India under this genus. One specimen was obtained from RARS Pilicode by sweepnet collection and was compared to all other species of *Dinarmus* reported from the Oriental Region and India. From India, a total of seven species of *Dianrmus* has been reported. An attempt was made to key out this new species based on the revised key of Indian *Dinarmus* (Gupta, 2007), however, it did not fit into the key. This species was compared to the nearest species *viz*., *Dinarmus maculatus* Masi with which it shared some common characters like scape reaching the median ocelli or slightly above the median ocelli, anterior margin of clypeus not projecting, PMV as long as or slightly longer than MV, funicular segments with similar brown rings on the junction. However, this species was designated new, based on the deviating characters like anterior margin of the clypeus with weak bidentation, antennae robust and reaching the median ocellus, F1 4.5X pedicel, POL 1.5X OOL, gaster long and ovate and as long as head and mesosoma combined and T1 having a distinct wavy white marking at the base. The species was described and was denoted as *Dinarmus* sp. nov.1 and a revised key to the Indian species of *Dinarmus* was prepared.

Kumarella Sureshan endemic to India, was originally described from Kerala with a type species *Kumarella angulus* Sureshan as the type species. Globally only two species have been described so far. The other species is *Kumarella sandroi* Sureshan is also endemic to Kerala.

The genus *Merismomorpha* Girault currently includes 14 species worldwide of which six species are known from India (Sureshan, 2003). This study includes

one species i.e. *Merismomorpha elongata* Sureshan which is endemic to Kerala and closely resembles another Indian species *M. minuta* Sureshan. Biology and life history of this species is not known.

The genus *Notoglyptus* Dodd and Girault includes only five species recorded worldwide. The only species known from our region is *N. scutellaris* Dodd and Girault. This is the most widespread species under this genus and is present in all the zoogeographical regions of the world. Little is known about the biology of the genus and are suspected to be pupal parasitoids of Diptera in grassy vegetation (Sureshan, 2003).

One species of *Propicroscytus* Szelenyi was examined. Only 3 species are reported worldwide, and two are reported from India. The distribution of the species is limited to rice growing belts of the world as these are parasitoids of rice gall midge *Orseolia oryzae* L (Rao, 1973). The species studied here is *P. oryzae* Subbarao known from India, Sri Lanka, China, and Thailand.

Trichomalopsis Crawford is known from all regions of the world with the highest diversity in Europe and N. America. One species was examined during the study *viz., T. apantolectena* Crawford. This species is distributed in India, Oriental and Palearctic Regions. This group is known to parasitise *Cnaphalocrocis medinalis* Guenee and *Pelopidas mathias* Fabricius. A total of 8 species has been described from India and is demarcated mainly by the variations in the occipital carina.

Mokrzeckia Mokrzecki is one of the rarest genera in Pteromalidae and only six species are so far known worldwide, out of which two are from India. Except for one species, *Mokrzeckia pini* Hartig which is distributed worldwide, rest of them are confined to Oriental and Australian Region. One specimen was collected from Thenmala, Kollam. The holotype is a female and was compared with all the six species under this genus. It closely resembled *Mokrzeckia pini* Hartig which was originally described from Italy (Hartig, 1838). The new species when compared to *M. pini* displayed deviating characters like acuminate antennae which is capitate in

the latter; abdomen with a distinct white patch over T1 to T4 which is absent in the latter. Different ratios were also worked out and compared based on the original description and it was confirmed that the specimen in question is a new species and is denoted as *Mokrzeckia.* sp. nov.1. The genus *Mokrzeckia* Hartig are generally hyperparasitoids of *Apanteles sp.* (Boucek, 1988).

The third new species that was obtained under the subfamily Pteromalinae falls under a new genus and was depicted as *Genus* nov.1. Pteromlinae includes well over 160 genera and is the most diverse group under Pteromalidae. The boundaries of Pteromalinae are not well defined and it is one of the most heterogeneous groups (Sureshan, 2003). The specimen in question was collected from Ranipuram, Kasaragod. This specimen shared most of the characters of the subfamily Pteromalinae such as incomplete notauli, antennae with five funicles and three anelli in females and other morphological characters such bidentate anterior clypeal margin. It also shared a few characters with the genus *Halticopterella* Girault & Dodd based on the taxonomic revision of *Halticopterella* (Sureshan, 2001). The species differed in a few characters like a prominent capitate stigma in the forewing, a peculiar bilobed structure in the gastral dorsum which is formed by medially incised T2 segment and a prominently long ovipositor. All these characters implied that the specimen in question does not belong to any genus described so far and is thus denoted as *Genus* nov.1.

5.2 MOLECULAR CHARACTERIZATION

In the absence of obvious morphological characters, independent insect identifications can be done through molecular techniques. DNA sequencing of a standard gene region or ''DNA barcoding'' can be helpful in species diagnosis (Hebert *et al.,* 2003 a).

A total of 10 different species of Pteromalid wasps were subjected to DNA extraction and molecular characterization and DNA barcode was obtained for three species namely *Pteromalus* sp., *Scutellista* sp. and *Callitula* sp. DNA barcoding could not be done in the other attempted species due to their very small size and

hence a very small DNA content. The DNA barcode of the obtained species can be added to the very meagre molecular data available for future reference. The obtained DNA barcode was blasted to the data available, this showed a match up to generic level in two species and up to family level in one species.

5.3. LIST OF PTEROMALIDAE OF KERALA

1. *Acroclisoides indicus* Ferrière, 1931
2. *Acroclisoides maculatus* Sureshan & Narendran, 2002
3. *Anisopteromalus calandrae* (Howard, 1881)
4. *Asaphes vulgaris* Walker, 1834
5. *Callitula anguloclypea* Sureshan, 2002
6. *Callitula bambusae* Narendran & Jobiraj, 2001
7. *Callitula keralensis* Sureshan, 2002
8. *Callitula peethapada* Narendran & Mohana, 2001
9. *Callitula robusta* Sureshan, 2002
10. *Callitula rugosa* (Waterston, 1915)
11. *Callitula travancorensis* Sureshan, 2002
12. *Calyconotiscus frontofasciatus* Narendran & Saleem, 2012
13. *Cephaleta autraliensis* (Howard,1896)
14. *Cephaleta brunniventris* Motschulsky, 1859
15. *Cephaleta elongata* Sureshan, Dhanya, Bijoy and Ramesh Kumar, 2011
16. *Cerocephala dinoderi* Gahan, 1925
17. *Chlorocytus indicus* Sureshan, 2000
18. *Cleonymus indicus* Sureshan, 2015
19. *Cleonymus kamijoi* Sureshan & Balan, 2013
20. *Cleonymus keralicus* Narendran & Rajmohana, 2008
21. *Coelopisthia indica* Sureshan, 2015
22. *Cryptoprymna elongata* Sureshan & Narendran, 2000
23. *Cryptoprymna indiana* Sureshan & Narendran, 2000
24. *Cyclogastrella nigra* Sureshan, 2000
25. *Cyrtogaster clavicornis* Walker, 1833
26. *Cyrtoptyx wayanadensis* Sureshan, 2012
27. *Delislea rahimani* Narendran & Anil, 1992

28. *Dinarmus acutus* (Thomson, 1878)

29. *Dinarmus basalis* (Rondani, 1877)

30. *Dinarmus colemani* (Crawford, 1913)

31. *Dinarmus maculatus* (Masi, 1924)

32. *Dinarmus vagabundus* (Timberlake, 1926)

33. *Dipara angulata* Sureshan & Nikhil, 2015

34. *Dipara bouceki* (Narendran, 2006)

35. *Dipara eukeralensis* özdikmen, 2011

36. *Dipara gastra* (Sureshan & Narendran, 2004)

37. *Dipara hayati* Sureshan, 2013

38. *Dipara intermedia* Sureshan & Narendran, 2005

39. *Dipara kannurensis* Sureshan & Raseena, 2015

40. *Dipara keralensis* (Narendran, 2000)

41. *Dipara malabarensis* (Narendran & Mini, 2000)

42. *Dipara miniae* Narendran & Sureshan, 2001

43. *Dipara mohanae* Narendran & Sureshan, 2001

44. *Dipara nigra* Sureshan, 2013

45. *Dipara nigriscuta* Sureshan 2013

46. *Dipara ponmudiensis* Sureshan & Farsana, 2015

47. *Dipara yercaudensis* Sureshan, 2014

48. *Erotolepsiella indica* Narendran, 2001

49. *Eurydinotomorpha indica* Sureshan, 2016

50. *Eurydinotomorpha malabarensis* Sureshan & Narendran, 1990

51. *Halticoptera agaliensis* Sureshan, 2003

52. *Halticoptera propinqua* (Waterston, 1915)

53. *Halticopterella burwelli* Sureshan, 2001

54. *Halticopterella longiflagellum* Sureshan, 2001

55. *Halticopterella rampurensis* Sureshan, 2001

56. *Halticopterella robusta* Sureshan, 2001

57. *Herbertia indica* Burks,1959

58. *Herbertia malabarica* Narendran, 2006

59. *Heydenia tuberculata* Sureshan, 1990

60. *Homoporus acuminatus* Sureshan & Narendran, 2000

61. *Homoporus gladiatus* Sureshan & Narendran, 2000

62. *Inkaka keralensis* Sureshan & Narendran, 1997

63. *Kumarella angulus* Sureshan, 1999

64. *Kumarella sandroi* Narendran & Mohana, 2001

65. *Lariophagus distinguendus* (Förster, 1841)

66. *Macroglenes sivani* Narendran & Sureshan, 2004

67. *Merismomorpha elongata* Sureshan, 2000

68. *Merismomorpha tamilnadensis* Sureshan et al, 2012

69. *Merismomorpha truncata* Sureshan, 2000

70. *Merosmomorpha minuta* Sureshan, 2000

71. *Mesopolobus harithus* Sureshan & Narendran, 2002

72. *Mesopolobus keralensis* Sureshan & Narendran, 2002

73. *Mesopolobus minutus* Sureshan & Narendran, 2002

74. *Metastenus concinnus* Walker, 1834

75. *Metastenus indicus* Sureshan & Narendran, 2002

76. *Miscogasteriella bijoyi* Sureshan & Nikhil, 2013

77. *Miscogasteriella jayasreeae* Sureshan, 1999

78. *Mokrzeckia menzeli* Subba Rao, 1981

79. *Mokrzekia orientalis* Subba Rao, 1973

80. *Moranila californica* (Howard, 1881)

81. *Narendrella nilamburensis* Sureshan, 1999

82. *Netomocera calicutensis* Sureshan & Raseena, 2017

83. *Netomocera maculata* Raseena & Sureshan, 1990

84. *Netomocera minuta* Sureshan & Nikhil, 2015

85. *Netomocera nigra* Sureshan & Narendran, 1990

86. *Norbanus acuminatus* Dutt & Ferrière, 1961

87. *Norbanus equs* Sureshan, 2003

88. *Norbanus malabarensis* Sureshan, 2003

89. *Norbanus scrobatus* Sureshan, 2003

90. *Norbanus thekkadiensis* Sureshan, 2003

91. *Notanisus indicus* Sureshan, 2015

92. *Notoglyptus scutellaris* (Dodd & Girault, 1915)

93. *Oniticellobia longigastra* Sureshan & Narendran, 1994

94. *Oxysychus coimbatorensis* (Ferrière, 1939)

95. *Oxysychus macregaster* Sureshan & Narendran 2002

96. *Oxysychus nupserhae* (Dutt & Ferrière, 1961)

97. *Pachycrepoideus veerannai* Narendran & Anil, 1992

98. *Pachycrepoideus vindemmiae* (Rondani, 1875)

99. *Pachyneuron groenlandicum* (Holmgren, 1872)

100. *Pachyneuron leucopiscida* Mani, 1939

101. *Pachyneuron solitarium* (Hartig, 1838)

102. *Panstenon collaris* Bouček 1976

103. *Papuopsia striata* Sureshan, 2005

104. *Paraiemea convexa* Sureshan & Narendran, 1998

105. *Paraiemea vishnuae* Sureshan & Narenadran, 1998

106. *Platecrizotes keralensis* Sureshan & Raseena 2015

107. *Propicroscytus mirificus* (Girault, 1915)

108. *Propicroscytus oryzae* (Subba Rao, 1973)

109. *Psilocera clavata* Sureshan & Narendran, 1995

110. *Psilocera heydoni* Sureshan, 2001

111. *Psilocera keralensis* Sureshan, 2014

112. *Psilocera scutellata* Sureshan, 2001

113. *Psilocera vinayaki* Sureshan & Narendran, 1995

114. *Pteromalus keralensis* Sureshan, 2001

115. *Pteromalus metallicus* Sureshan, 2001

116. *Pteromalus nigrus* Sureshan, 2001

117. *Pteromalus puparum* (Linnaeus, 1758)

118. *Pteromalus semotus* (Walker, 1834)

119. *Pycnetron keralaensis* Raseena & Sureshan, 2017

120. *Solenura ania* (Walker, 1846)

121. *Solenura keralensis* (Narendran, 1992)

122. *Spalangia gemina* Bouček, 1963

123. *Spalangia impunctata* Howard, 1897

124. *Spalangia parfuscipes* Ahmad, 1998

125. *Spalangia simplex* Perkins, 1910

126. *Sphegigaster anamudiensis* Sureshan & Narendran, 1997

127. *Sphegigaster brunneicornis* (Ferrière, 1930)

128. *Sphegigaster indica* Sureshan & Narendran, 2001

129. *Sphegigaster karnatakaensis* Sureshan, 2007

130. *Sphegigaster reticulata* Sureshan & Narendran, 1997

131. *Sphegigaster stepicola* Bouček, 1965

132. *Stictomischus turneri* Sureshan, 2002

133. *Storeya minuta* Sureshan, 1999

134. *Syntomopus carinatus* Sureshan & Narendran, 1999

135. *Syntomopus nigrus* Sureshan & Narendran, 1999

136. *Syntomopus rajamalaiensis* Sureshan & Narendran, 1999

137. *Systasis dalbergiae* Mani 1942

138. *Systasis dasyneurae* Mani 1939

139. *Systasis nigra* Sureshan 2002

140. *Theocolax elegans* (Westwood, 1874)

141. *Toxeumorpha minuta* Sureshan & Narendran, 2000

142. *Trichomalopsis acarinata* Sureshan & Narendran, 2001

143. *Trichomalopsis apanteloctena* (Crawford, 1911)

144. *Trichomalopsis deplanata* Kamijo & Grissell, 1982

145. *Trichomalopsis neelagastra* Sureshan & Narendran, 2001

146. *Trichomalopsis nigra* Sureshan & Narendran, 2001

147. *Trichomalopsis ovigastra* Sureshan & Narendran, 2001

148. *Trichomalopsis thekkadiensis* Sureshan & Narendran, 2001

149. *Trichomalopsis travancorensis* Sureshan & Narendran, 2001

150. *Trichomalus kannurensis* Sureshan & Narendran, 1994

151. *Trichomalus keralensis* Sureshan 2002

152. *Trigonoderus pulcher* Walker, 1836

153. *Uniclypea elongata* Sureshan & Narendran, 1997

154. *Uniclypea kumarani* Sureshan & Narendran, 1994

Summary

6. SUMMARY

The investigation on "Biosystematics and barcoding of Pteromalidae (Hymenoptera: Chalcidoidea) of Kerala" was implemented in three stages- collection of pteromalid wasp from various parts of Kerala; identification of specimens through morphological characterization, micrometry; molecular characterization. The project was carried out in College of Agriculture, Vellayani with an objective of identification, morphological, molecular characterization and documentation of Pteromalidae of Kerala.

A total of 20 species under 15 genera and 5 subfamilies of Pteromalidae collected from 38 locations spread across 14 districts of Kerala are treated in this study. Among them, six species are new to science. In the present work, Pteromalid wasps were collected from different ecosystems including agricultural ecosystems. Out of the six new species, two are from new genera. Detailed descriptions of new species, diagnosis of known species, key to species for genera in which new discoveries are made, and checklist of Pteromalidae of Kerala are provided. DNA has been isolated from select species and subjected to barcoding and DNA barcode was obtained for 3 species.

Collections were made using sweep net in the field, which were later transferred to alcohol and later sorted out in lab under high zoom stereo microscope. Well over 100 specimens were examined and based on requirement the specimens were card mounted for morphological characterization and remaining specimens preserved in absolute alcohol in vials.

Eleven known species are identified and morphologically characterized and three species are molecularly characterized. These are *Callitula peethapada, Callitula travancorensis, Cryptoprymna elongate, Dinarmus maculatus, Kumarella angulus, Merismomorpha elongate, Notoglyptus scutellaris, Propicroscytus oryzae, Systasis tenuicornis, Trichomalopsis apantolectena,* and *Gastrancistrus* sp. The already known species were identified using taxonomic keys and original description. Photographs and diagnostic characters for this species are

provided. Based on the collection made from each districts it was found out that *Gastrancistrus* is the most widespread genus in Kerala followed by *Callitula.*

Six new species are identified, morphologically characterized and documented in this study. Four species are identified from already known genera *viz*., *Dinarmus* sp. nov. 1, *Mokrzeckia* sp nov.1, *Panstenon* sp. nov1 and *Panstenon* sp. nov. 2. Two species from new genera are denoted as *Genus* nov.1 (Miscogasterinae) and *Genus* nov.2 (Pteromalinae). Each of the newly identified species has been described in detail with the help of micrometry and photographs. Key to the species is also provided wherever possible.

Two new genera has been identified, which are denoted as *Genus* nov.1 (Miscogasterinae) and *Genus* nov.2 (Pteromalinae). The type species for each of the genera has been described in detail with distinguishing characters and morphometric ratios.

A total of 10 different species of pteromalid wasps were subjected to DNA extraction and molecular characterization. DNA barcode was obtained for three species namely *Pteromalus* sp., *Scutellista* sp., and *Callitula* sp. DNA sequence was not obtained in the other attempted species due to their very small size and hence a very small DNA content. The DNA barcode obtained was blasted to existing database to identify the nearest species data available.

References

REFERENCES

Aguiar., Alexandre, P., Deans., Andrew R., Engel., Michael S., Forshage., Mattias., Huber., and John T. 2013. Order Hymenoptera. *In*: Zhang, Z.-Q. (Ed.) Animal Biodiversity: An Outline of Higher-level Classification and Survey of Taxonomic Richness (Addenda 2013). *Zootaxa*. 3703 (1): 51.

Ahmad, T. and Mani, M. S. 1939. Two new chalcidoid parasites of the linseed midge, *Dasyneura lini* Barnes. *Indian J. Agric. Sci.* 9(3): 531-539.

Ashmead, W. H. 1894. Descriptions of new parasitic Hymenoptera. *Trans. Amer. Entomological Soc.* 21: 318-344.

Askew, R. R. 1968. A survey of leaf-miners and their parasites on laburnum. *Trans. R. Entomological Soc. of Lond.* 120(1): 34.

Askew, R. R. 1991. A second European species of *Cryptoprymna* Förster (Hymenoptera: Pteromalidae) described from Norfolk. *Entomological Mon. Mag.* 127: 205-208.

Askew, R. R. and Mifsud, D. 2016, A preliminary check-list of the Chalcidoidea (Hymenoptera) of the Maltese Islands. *Bull. Entomological Soc. of Malta* 8: 47-72.

Barron, M. 2002. Impact of an introduced parasitoid (*Pteromalus puparum*) on the abundance and dynamics of the red admiral butterfly (*Bassaris gonerilla*), a preliminary report. *Proceedings of the 1st International Symposium on Biological Control of Arthropods*, Honolulu, Hawaii, 429.

Baur, H., Muller, F. J., Gibson G. A. P., Mason, P. G., and Kuhlman, U. 2007. A review of the species of *Mesopolobus* (Chalcidoidea: Pteromalidae) associated with *Ceutorhynchus* (Coleoptera: Curculionidae) host- species of European origin. *Bull. Entomological Res.* 97(4): 389

Bhatnagar, S. P. 1951. Descriptions of new and records of known Chalcidoidea (Parasitic Hymenoptera) from India. *Indian J. Agric. Sci.* 21: 155-178.

BOLD [The Barcode of Life Data Systems]. 2019. Available: http://www.boldsystems.org. [May 15 2019].

Bouček, Z. 1954a. Chalcidologicke Poznamky I, Pteromalidae, Torymidae, Eurytomidae, Chalcididae (Hymenoptera). *Acta Entomological Mus. Nat.* 29: 49-80.

Bouček, Z. 1954b. Hymenopterous parasites of *Pityophthorus polonicus* Karp. (In Czech and English). *Roczan Naukles* 11: 83-92.

Bouček, Z. 1955. Chalcidologicke Poznamky 111, Torymidae, Pteromalidae, Perilampidae and Eucharitidae (in English and Czech). *Sb. Entomological Odd. Nar. Praze.* 30(462): 305-330.

Bouček, Z. 1958. Eine Cleonyminen- Studie; Bestimmungstabelle der Gattungen mit Beschreibungen und Notizen, eingeschloseen einige Eupelmidae (Hymenoptera: Chalcidoidea). *Acta. Entomological Museum Nat.* 32: 353-404.

Bouček, Z. 1972a. On European Pteromalidae (Hymenoptera): A revision of *Cleonymus, Eunotus* and *Spaniopus* with descriptions of new genera and species. *Bull. Br. Museum Nat. Hist. Entomol.* 27(5).

Bouček, Z. 1972b. A new genus and species of Pteromalidae (Hymenoptera) parasitic on Sphecids in South America. *Bull. De la Soc. Entomol. Sulsse* 45: 1-3.

Boucek, Z. 1976. African Pteromalidae (Hymenoptera); new taxa synonymies and combinations. *J. Entomological Soc. of South. Afr.* 39(1): 17-18.

Bouček, Z. 1976. African Pteromalidae (Hymenoptera): new taxa, synonymies and combinations. *J. Entomol. Soc. S. Afr.* 39(1): 9-31.

Bouček, Z. 1978. *Oricoruna* and *Manineura*, new Pteromalid genera (Hymenoptera) from the Oriental region. *Oriental Insects* 12(4): 469-472.

Bouček, Z. 1986. Taxonomic study of Chalcid wasps (Hymenoptera) associated with gall midges (Diptera: Cecidomyiidae) on mango trees. *Bull. Entomological Res.* 76: 393-407.

Bouček, Z. 1988. *Australasian Chalcidoidea (Hymenoptera). A biosystematic revision of genera of fourteen families, with a reclassification of species.* CAB International, Wallingford, U. K. 832p.

Bouček, Z. and Rasplus, J. Y. 1991. Illustrated key to West- Palearctic genera of Pteromalidae (Hymenoptera: Chalcidoidea). *Inst. Natl. Res. Agron.* 1: 140.

Bouček, Z. Subba Rao, B. R. and Farooqi, S. I. 1978. A preliminary review of Pteromalidae (Hymenoptera) of India and adjacent countries. *Oriental Insects.* 12(4): 433–467.

Bouček, Z., Subba Rao, B. R., and Farooqi, S. I. 1979. A preliminary review of Pteromalidae (Hymenoptera) of India and adjacent countries. *Oriental Insects*. 12(4): 433–467.

Boykin, L. M., De Barro, P., Hall, D. G., Hunter, W. B., and McKenzie, C. L. 2012. Overview of worldwide diversity of *Diaphorina citri* Kuwayama mitochondrial cytochrome oxidase I haplotypes: Two old world lineages and a new world invasion. *Bull. Entomol. Res.* 102: 573-582.

Burks, B. D. 1975. The species of Chalcidoidea described from North America North of Mexico by Francis Walker (Hymenoptera). *Bull. Br. Mus. Nat. Hist. Entomol.* 32: 139-170.

Crawford, J. C. 1909. New Chalcidoidea (Hymenoptera). *Proc. Enomological Soc. Wash.* 11: 51-52

Crawford, J. C. 1913. Descriptions of new Hymenoptera No. 6. *Proc.U.S. Natl. Mus.* 45: 241-260.

Curtis, J. 1827. *British entomology: being illustrations and descriptions of the genera of insects found in Great Britain and Ireland.* London, pp.147-194.

Dalman, J. W. 1820. Forsok till uppstallning af insect-famijen Pteromalini. 1 Synnerhet. medafseende pade isverige funne arter. *K. Ventenskakad. Handl.* 41. 123-174, 177-182, 340-385.

Darling, D. C. 1991a. Revision of the world species of *Spalangiopelta* (Hymenoptera: Chalcidoidea: Pteromalidae: Ceinae). *Royal Ont. Mus. (ROM) Life Sci. Cont.* 155: 1-43.

Darling, D. C. 1991b. *Bopha rnaculata,* a new genus and species of Ceinae from South Africa (Hymenoptera: Chalcidoidea: Pteromalidae). *Proc. Entomological* Soc. *Wash.* 93: 622-629.

Delucchi, V. 1956. Beitrage zur kenntnis der Pteromaliden (Hymenoptera: Chalcidoidea). *Z. Angew. Entomol.* 39: 229-259.

Delucchi, V. 1958. *Pteromalus pini* Hartig, specie tipo di Beierina gen. nov. (Hymenoptera: Chalcidoidea). *Entomophaga* 3(3): 271.

Dorn, S., Schmale, I., Schärer, D., Wäckers, F., and Cardona, C. 2002, Parasitic wasps for on-farm control of a coleopteran pest feeding within stored grains. [abstract].In: *Abstracts, Eighth European Congress of Entomology*; 13, July, 2012, Thessaloniki, Greece, p.318. Abstract No. 206.

Erdös, J. 1946. Genera nova et species novae Chalcidoidarum (Hym.). *Annis. Hist. Nat. Mus. Natn. Hung.* 39: 131-165.

Fabricius, J. C. 1787. *Mantissa Insectorum Sistens eorum species detctas.* Copenhagen 1: 20, 348.

Fabricius, J. C. 1798. *Supplementum Entomologiae Systematicae*. Copenhagen. pp. 25, 72.

Fabricius, J. C. 1804. *Systema Piezatorum*. Brunswick. 439p.

Farooqi, S. I. and Menon, M. G. R. 1972. A new phytophagus species of *Systasis* Walker (Hymenoptera: Pteromalidae) infesting seeds of *Cenchrus* species. *Muhsi.* 46: 111-114.

Farooqi, S. I. and Menon, M. G. R. 1973. Two new genera and three new species of Brachyscelidiphagini (Hymenoptera: Pteromalidae) associated with fruits of *Ficus infectoria* at *Delhi. Ind. J. Zool.* 1: 139- 144.

Farooqi, S. I. and Rao, S. B. R. 1985. Family: Pteromalidae. In: Subba Rao, B. R. and Hayat, M. (ed.). The Chalcidoidea (Insecta: Hymenoptera) of India and the adjacent countries Part. I. Review of families and keys to families and genera. *Oriental Insects.* 19: 161-310.

Farooqi, S. I. and Rao, S. B. R. 1986. Family: Pteromalidae. In: Subba Rao, B.R. & Hayat, M. (ed.). The Chalcidoidea (Insecta: Hymenoptera) of India and the Adjacent countries Part. II. A catalogue. *Oriental Insects*. 20: 1- 430.

Ferrière, C. 1930. Notes on Asiatic Chalcidoidea. *Bull. Entomological Res.* 21: 353-360.

Ferrière, C. 1934. Note sur les pireninae avec descriptions de deux nouvelles especes. *Mitt. Schweiz. Entomological Ges.* 16: 83-93.

Flinn, P. W. and Hagstrum, D. W. 2001. Augmentative releases of parasitoid wasps in stored wheat reduces insect fragments in flour. *J. Stored Products Res.* (37) 179-186.

Förster, A. 1856. Hymenopterologische pp. Studien 2. Heft. Chalcididae and Proctotrupii. *Aachen.* 1: 152.

Gahan, A. B. 1919. Report on a small collection of Indian parasitic Hymenoptera. *Proc. U.S. Natl. Mus.* 56: 513-524.

Gahan, A. B. and Fagan, M. M. 1923. The type species of the genera of Chalcidoidea or Chalcid flies. *Bull. U.S. Natl. Mus.* 83: 481-486.

Gibson, G. A. P., Huber, J. T., and Woolley, J. B. 1997. *Annotated keys to the genera of Nearctic Chalcidoidea (Hymenoptera)*. NRC Research Press, Canada, 794p.

Girault, A. A. 1913a. Some Chalcidoid Hymenoptera from North Queensland. *Arch. Naturgesch.* 79(6): 70-90.

Girault, A. A. 1913b. New genera and species of Chalcidoid Hymenoptera in the South Australian Museum. *Trans. R. Soc. S. Aust*. 37: 67-115.

Girault, A. A. 1915a. Australian Hymenoptera Chalcidoidea. VI. Supplement. *Mem. Qd. Mus.* 3: 313-346.

Girault, A. A. 1915b. Some Chalcidoid Hymenoptera from North Queensland (continued). *Can. Entomol*. 47: 42-48.

Girault, A. A. 1915c. Australian Hymenoptera Chalcidoidea. VIII. The family Miscogasteridae with descriptions of new genera and species. *Mem. Qd. Mus*. 4: 185-202.

Girault, A. A. 1920a. New genera and species of Chalcid flies from Australia (Hymenoptera). *Insecutor Inscitiae Menstr.* 8: 37-50.

Girault, A. A. 1920b. New genera of Chalcid flies from Australia (Hymenoptera). *Insecutor Inscitiae Menstr*. 8: 142-146.

Girault, A. A. and Saunders, G. E. 1910. The Chalcidoid parasites of the common house or typhoid fly *(Musca domestica* Linn.) and its allies. Descriptions of a new North American genus and species of the family Pteromalidae from Illinois. Parasitic on *M. domestica* Linn. with biological notes. *Psyche* 17: 145-160.

Gordh, G. 1976. A new genus of Pteromalidae from Missouri, the type species of which parasitises *Uloborus octonarus* Muma (Hym.:Chalcidoidea: Araneidae: Uloboridae). *I. Kansas. Entomological Soc. 49:* 100-104.

Graham, M. W .R. deV. 1992. Second revision of Western European *Psilocera* (Hymenoptera: Pterornalidae) with descriptions of three new species. *Entomologist's Mon. Mag.* 128: 15-21.

Graham, M. W. R. deV 1969. The Pteromalidae of North-Western Europe (Hymenoptera: Chalcidoidea). *Bull. Br. Mus.* 16: 1-908.

Graham, M. W. R. deV. 1956a. A revision of the Walker types of Pteromalidae (Hym: Chalcidoidea) part I (including descriptions of new genera and species). *Entomologist's Mon. Mag.* 92: 76-98.

Graham, M. W. R. deV. 1956b. A revision of the Walker types of Pteromalidae (Hym: Chalcidoidea) Part II (including descriptions of new genera and species). *Entomologist's Mon. Mag.* 92 246-263.

Graham, M. W. R. deV. 1992a. A new species of *Zdenekiana* Huggert (Hymenoptera: Pteromalidae) from France. *Entomologist's Mon. Mag.* 128: 113- 114.

Graham, M. W. R. deV. 1992c. A new species of *Synedrus* (Hymenoptera: Pteromalidae) from France. *Entomologist's Mon. Mag.* 128: 23-24.

Grissel, E. E. and Schauff, M. E. 1990. *A handbook of the families of Nearctic Chalcidoidea (Hymenoptera).* Entomological Society, Washington, 85p.

Gupta, A. 2007. A new species of *Dinarmus* Thomson (Hymenoptera: Chalcidoidea: Pteromalidae) from India. *Entomon* 32(3): 162-165.

Gupta, A. 2016. Checklist of Indian Pteromalidae (Hymenoptera: Chalcidoidea) [on-line]. Available: http://www.nbair.res.in/Pteromalidae/checklist.php [05-June 2019]

Gupta, A. and Sureshan, P. M. 2014. A new Pteromalid species of the genus *Anisopteromalus* Ruschka (Hymenoptera) from India. *Oriental insects* 48 (1-2): 68-70.

Haliday, A. H. 1844. Contributions towards the classification of the Chalcididae. *Trans. Ent.Soc. Lond.* 3: 295-401

Haliday, A. H. 1844. Contributions towards the classification of the Chalcididae. *Trans. Ent.Soc. Lond.* 3: 295.

Hartig, T. 1838. Uber den Raupenfrass im Koniglichen Charlottenburger Forste unfern Berlin, wahrend des Sommers. Jahresberichte über die Fortschritte der Forstwissenschaft und Forstlichen Naturkundede im Jahre 1836 und 1837 nebst *Original-Abhandlungen aus dem Gebiete und Cameralisten* 1(2): 253

Hebert, P. D. N., Cywinska, A., Ball, S. L., and deWaard, J. R. 2003a. Biological identifications through DNA barcodes. *Proc. R. Soc. Biol. Sci.* 270: 313–321.

Hebert, P. D. N., Ratnasignham, S., and deWaard, J. R. 2003b. Barcoding animal life: cytochrome c oxidase subunit 1 divergences among closely related species. *Proc. R. Soc. Biol. Sci.* 270: 96-99.

Hedqvist, K. J. 1972. Notes on Chalcidoidea (Hymenoptera) The genus *Syntomopus* Walker (Pteromalidae, Miscogasterinae: Sphegigasterini). *Entomol. Tidskr.* 93: 210-215.

Hedqvist, K. J. 1975. A key to the Swedish species of the genus *Halticoptera* Spinola and three related genera (Hymenoptera: Pteromalidae). *Entomol. Scand.* 6: 167-181.

Heraty, J. M. 1998. Hexamethyldisilazane: A chemical alternative for drying insects. *Entomological News* 109: 369-374.

Heydon, S. L. 1988a. A review of the world species of *Notoglyptus* Masi (Hymenoptera: Pteromalidae). *Proc. Entomol. of Wash.* 91: 112-123.

Heydon, S. L. 1988b. A review of Nearctic species of *Cryptoprymna* Förster with the description of a new genus *Polstonia* (Hymenoptera: Pteromalidae). *Proc. Entomol. of Wash.* 90: 1-11.

Howard, L. O. 1886. A generic synopsis of the hymenopterous family Chalcididae. *Entomol. Am.* 1: 197-199.

Howard, L. O. 1894. In: Riley, Ashmead & Howard. Report on the Chalcididae of the subfamilies Chalcidinae, Eucharitinae, Perilampinae, Encytrinae, Aphelinae, Pireninae, Elasminae and Elachistinae. *J. Linn. Soc. of Lond.* 25: 79-108.

Huggert, L. 1976. Description of a previously unknown male of a new genus and three new species of Pteromalidae (Hymenoptera: Chalcidoidea) from Northern Sweden. *Entomol. Tidskr.* 97: 1-2.

IBOL [International Barcode of Life]. 2013. Ten years of DNA barcoding. *Barcode Bull.* 4 (2): 1-20. Available: http://www.ibol.org [24 June 2019].

Kamijo, K. 1977. A new genus and three new species of Ormocerini (Hymenoptera: Pteromalidae) from Japan. *Kontyu* 45: 531-537.

Kamijo, K. 1981a. Three new species of *Callitula* (Hymenoptera: Pteromalidae) from Japan. *Akitu* 40: 1-8.

Kerrich, G. J. and Graham, M. W. R. de V. 1957. Systematic notes on British and Swedish Cleonymidae, with description of a new genus (Hymenoptera: Chalcidoidea). *Trans. of the Soc. for Br. Entomol.* 12: 265-311.

Kohler, F. 2007. From DNA taxonomy barcoding how a vague idea evolved into a biosystematic tool. *Zoologische Reihe,* 83: 44-51.

Kryger, J. P. 1934. *Bestemmelsetabel over de Danske Chalcidiesi-aegter (Chalcidoidea)*. Copenhagen. 30p.

Latreille, P. A. 1805. *Histoire naturelle generate et particuliere des crustaces et des insectes*. 13: 432.

Latreille, P. A. 1809. *Genera crustaceorurn et insedorum*. Paris. 4: 399.

Linnaeus, C. V. 1758. *Systema naturae vol. 1*. 335p.

Mani, M. S. 1939. Descriptions of new and records of some known Chalcidoid and other hymenopterous parasites from India. *Indian J. Entomol.* 1: 69-99.

Mani, M. S. 1939. Two new chalcidoid parasites of the linseed midge, *Dasyneura lini* Barnes. II - Description of the parasites. *Indian J. Agric. Sci.* 9: 535.

Mani, M. S. 1941. Studies on Indian parasitic Hymenoptera. I. *Indian J.Ent.* 3: 25-36.

Mani, M. S. 1942. Studies on Indian parasitic Hymenoptera. II. *Indian J. Entomol.* 4: 153-162.

Mani, M. S. 1989. *The fauna of India and the adjacent countries. Chalcidoidea (Hymenoptera) Part I & 11.* Zoological Survey of India, Madras. 1067p .

Mani, M. S. and Kurian, C. 1953. Descriptions and records of Chalcids (parasitic Hymenoptera) from India. *Indian J. Entomol.* 15: 1-21.

Mani, M. S., Dubey, O. P., Kaul, O. K. and Saraswat, G. G. 1973. On some Chalcidoidea from India. *Mem. Sch. Entomol. St. John's Coll.* 2: 1-127.

Masi. L. 1917. Chalcididae of the Seychelles Islands. *Novit. Zool.* 24: 121- 230.

Masi. L. 1924. Chalcidid del Giglio Quarata serie: Pteromalinae (Seguito). *Ann. Mus. Civ. Stor. Nat. Giacoma Doria.* 50: 213-235.

Masi. L. 1926. H. Sauter's Formosa-Ausbeute. Chalcididae (Hym.) I. Teil. *Konowia* 5: 325-381.

Mokrzecki, Z. 1933. (Die in den Forstschadlingen lebenden parasiten des I, Und 2. Grades aus der Gruppe dur Chalcidoidea) (in Polish). *Polskie Pismo Entomol.* 12: 143-144.

Nakamura, S., Konishi, K., Nakatani, Y., Ogata, K., Visarathanonth, P., Kengkarnpanich, R., and Uraichuen, J. 2004 [abstract]. In: *XXII International Congress of Entomology,* 15-21, August, 2004, Brisbane, Australia, pp. 1-1156.

Narendran, T. C. and GirishKumar, P. 2009. Three new species of Pteromalidae (Hymenoptera: Chalcidoidea) from Sunderbans, West Bengal, India. *J. Environ. and Sociobiology* 6(2): 124-126.

Narendran, T. C., Das, B. K., and Rajmohana, K. 2001. A study of *Gastrancistrus* Westwood (Hymenoptera: Pteromalidae) of India. *J. Ecobiology* 13(2): 149-155.

Narendran, T. C., Rajmohana, K., and Jobiraj, T. 2001. Three new species of Pteromalidae (Hymenoptera) from Kerala (India). *Uttar Pradesh J. Zoology* 21(1):31-33

Nauman, I. D. 1991. Revision of the Australian genus *Enoggera* Girault (Hymenoptera: Pteromalidae: Asaphinae). *J. Aust. Entomological Soc.* 30: 1-17.

Nikolskaya, M. N. 1952. The chalcid fauna of the USSR (Chalcidoidea). Moscow and Leningrad, Moscow, 574p.

Noyes, J. S. 2017. Universal Chalcidoidea database [on-line]. Available: http://www.nhm.ac.uk/ jdsml/research-curation/projects/ chalcidoids. [July 2018].

Packer, L., Gibbs, J., Sheffield, C., and Hanner, R. 2009. DNA barcoding and the mediocrity of morphology. *Mol. Ecological. Resour.* 9 (Suppl. 1): 42–50.

Peck, O., Bouček, Z., and Hoffer, G. 1964. Keys to the Chalcidoidea of Czechoslovakia (Insecta: Hymenoptera). *Mem. Entomological Soc. of Can.* 34:170.

Prinsloo, G. L. 1980. *An illustrated guide to the families of African Chalcidoidea (Insecta: Hymenoptera)*. Department of Agriculture and Fisheries, South Africa, 500p.

Rao, S. B. R. 1973a. Description of four new species of Pteromalidae (Hymenoptera). *Oriental Insects* 7**:** 335-362.

Rao, S. B. R. 1973b. Description of a new species and genus of Pteromalidae (Hymenoptera) parasitic on *Pachydiplosis* oryzae (Wood-Mason) (Diptera: Ceccidomydae). *Bull. Entomological Res.* 62: 627- 629

Rao, S. B. R. 1981. Descriptions of new species of Pteromalidae from the orient (Hymenoptera: Chalcidoidea). *Proc. Ind. Acad. Sci*. 90: 473- 482.

Rasool, A., Ahmad, T., Ganai, B. A. and Shaziya, G. U. L. L. 2018. An overview of molecular identification of insect fauna with special emphasis on chalcid wasps (Hymenoptera: Chalcidoidea) of India. *Acta agriculturae Slovenica*, 111(1): 229-239.

Ratnasingham, S. and Hebert, P. D. N. 2007. BOLD: [The Barcode of Life Data System] (http://www.barcodinglife.org). *Mol. Ecol. Notes*, 7: 355– 364.

Ratzeburg, J. T. C. 1844. Die Ichneumonen der Forstinsecten in Entomologischer und Forstlicher. *Beziehung* 2: 238.

Ratzeburg, J. T. C. 1852. Die Ichneumonen der Forstinsecten in entomologischer und forstlicher. *Beziehung* 3: 272.

Riley, C. V. 1890. An Australian Hymenopterous parasite of the fluted scale. *Insect life* 2: 248-250.

Roomi, M. W., Khan, Z. I., and Khan, S. A. 1972. *Pteromalus schwenkei* (Hymenoptera: Pteromalidae) a new species as a primary parasite of the bean-weevil *Bruchus chinensis* L. from Pakistan. *Z. Angew. Entomol.* 72: 395-400.

Savolainen, V., Cowan, R. S., Vogler, A. P., Roderick, G. K., and Lane, R. 2005. Towards writing the encyclopedia of life: an introduction to DNA barcoding. *Philos. Trans. R. Soc. London Biol.* 360: 1805-1811.

Schmiedeknecht, O. 1909. Hymenoptera fam. Chalcididae. *Genera Insectorum* 97: 550.

Smith, M. A., Fisher, B. L., Hebert, P. D. N. 2005. DNA barcoding for effective biodiversity assessment of a hyper diverse arthropod group: The ants of Madagascar. *Trans. R. Soc. Biol. Sci.* 360: 1825– 1834.

Spinola, M. 1811a. Essai d' une nouvelle classification der Diplolepaires. *Ann. Mus. Hist. Natl. Paris* 17: 138-152.

Spinola, M. 1811b. Essai d'une nouvelle classification générale des Diplolépaires. *Ann. Mus. Natl. Hist. Nat.0* 17:151

Sureshan, P. M. & Nikhil, K. 2015. A new species of *Netomocera* Bouček (Hymenoptera: Chalcidoidea: Pteromalidae) from the southern Western Ghats, Karnataka, with a key to world species. *J. Threatened Taxa* 7(2):6904-6906.

Sureshan, P. M. 1999. Two new genera and three new species of Pteromalidae (Hymenoptera: Chalcidoidea) from India. *Oriental Insects* 33: 101-102.

Sureshan, P. M. 2000c. Taxonomic studies of *Merismomorpha* with the description of three new species from India (Hymenoptera: Chalcidoidea: Pteromalidae). *Rec. Zool. Surv. India* 98(3): 105-107.

Sureshan, P. M. 2001. A taxonomic revision of the genus *Halticopterella* (Hymenoptera: Chalcidoidea: Pteromalidae). *Oriental Insects,* 35: 32, 35.

Sureshan, P. M. 2001a. A taxonomic revision of the genus *Halticopterella* (Hymenoptera: Chalcidoidea: Pteromalidae). *Oriental Insects* 35: 31, 33-34.

Sureshan, P. M. 2001b. Studies on *Pteromalus* Swederus (Hymenoptera: Chalcidoidea: Pteromalidae) of the Indian subcontinent with the description of three new species. *Rec. Zool. Surv. India* 99(1-4): 6, 12- 13.

Sureshan, P. M. 2002a. Taxonomic studies on *Cyclogastrella* Bukowski and *Psilocera* Walker (Hymenoptera: Chalcidoidea: Pteromalidae) with the description of a new species from the Oriental region. *Hexapoda* 12(1 & 2): 36.

Sureshan, P. M. 2002b. Insecta: Hymenoptera: Chalcidoidea. *Fauna of Eravikulam National Park, Conservation Area Series*. No.13: 28-30.

Sureshan, P. M. 2002c. Taxonomic studies on *Callitula* Spinola with the description of four new species from Indian subcontinent (Hymenoptera: Chalcidoidea: Pteromalidae), 100 (12): 22-26.

Sureshan, P. M. 2003. Pteromalinae (Pteromalidae: Chalcidoidea: Hymenoptera) of Indian subcontinent. *Rec. Zool. Surv. India* 205: 1-170

Sureshan, P. M. 2006a. First record of *Coelopisthia* Förster (Chalcidoidea: Pteromalidae) from the Oriental region with description of a new species from Sri Lanka. *Zoos' Print J.* 21(3): 2187-2188.

Sureshan, P. M. 2006b. A new genus of Pteromalidae from Sri Lanka with a note on synonymy (Hymenoptera: Chalcidoidea) *Rec. Zool. Surv. India* 106 (1): 64-66.

Sureshan, P. M. 2007a. Taxonomic studies on Pteromalidae (Hymenoptera: Chalcidoidea) of Southeast Asia based on collections of Bohart Museum of Entomology, University of California, Davis, USA. *Rec. Zool. Surv. India. Occational Paper* 268: 36-37.

Sureshan, P. M. 2015. On the fauna of Pteromalidae (Hymenoptera: Chalcidoidea) of South Western Ghats. *Rec. Zool. Surv. India. Occasional Paper* 359p.

Sureshan, P. M. and Narendran, T. C. 1994b. New species and new record of Pteromalidae (Hymenoptera: Chalcidoidea) from India. *Hexapoda* 6(2): 59-64.

Sureshan, P. M. and Narendran, T. C. 1997a. Two new species of Pteromalidae (Hymenoptera: Chalcidoidea) from India. *Hexapoda* 9(1-2): 27-29.

Sureshan, P. M. and Narendran, T. C. 1997b. Studies on *Sphegigaster* Spinola (Hymenoptera: Chalcidoidea: Pteromalidae) from India. *Entomon* 23(3&4): 194-195.

Sureshan, P. M. and Narendran, T. C. 2000. Three new species of Pteromalidae (Hymenoptera: Chalcidoidea) from India. *J. Bombay Natural History Society* 97(3): 403-405.

Sureshan, P. M. and Narendran, T. C. 2003. A checklist of Pteromalidae (Hymenoptera: Chalcidoidea) from the Indian subcontinent. *Zoos' print J.* 18(5): 1099-1110.

Sureshan, P. M. and Narendran, T. C., 1994a. A new species of a little known genus of Pteromalidae (Hymenoptera: Chalcidoidea) from India, Kerala. *Rec. Zool. Surv. India.* 94(1): 113-117.

Sureshan, P. M., 2007b. First record of *Lyubana* Bouček from Indian Subcontinent with description of a new species from Sri Lanka (Hymenoptera: Chalcidoidea: Pteromalidae). *Rec. Zool. Surv. India* 107(3): 2-4.

Sureshan, P. M., Kumar, J. B. N., Nikhil, K., and Kumar, V. 2014a. A new species of *Trichomalopsis* Crawford (Hymenoptera: Pteromalidae) hyperparasitoid of silkworm uzi fly *Exorista bombycis* (Louis) (Diptera: Tachinidae) from Karnataka, India.. *J.Biol. control* 28(4): 180-184.

Sureshan, P. M., Manickavasagam, S., and Dhanya, B. 2013. A review of Oriental species of *Merismomorpha* Girault (Hymenoptera: Pteromalidae) with description of a new species parasitising *Cercococcus* sp. (Hemiptera: Sternorrhyncha: Cerococcidae) from Tamil Nadu, India. *Hexapoda* 19(1): 18.

Sureshan, P. M., Nikhil, K., Girish Kumar, P., and Sheeja, U. M. 2016. Description of a new species and records of *Halticoptera* Spinola from India and taxonomic notes on species from the Indian subcontinent (Hymenoptera : Chalcidoidea: Pteromaldiae). *J. Insect Syst.* 1&2: 01-10.

Swederus, N. S. 1795. Beskrifning pa et nytt genus *Pteromalus* ibland insecterna hoer ande til hymenoptera. *K. sveenska vetensk. Akad. Handl.* 16: 201-222.

Szelènyi, G. von. 1941. Uber die Chalcididen- Gattungen Arthrolysis Förster und Picroscytus Thomson, (Hymenoptera). *Annales Historico- Naturales Musei Nationalis Hungarici (Zoologici)* 34: 123.

Thomson. C. G. 1878. *Hymenoptera Scandinaviae.* Tom. V *Pteromalus* (Swederus) Lundae, 307p.

Timberlake, P. H. 1926. New species of Hawaiian Chalcid flies (Hymenoptera). *Proc. Hawaii. Entomological Soc.* 6: 305-320.

Vogler, A. P. and Monaghan, M. T. 2007. Recent advances in DNA taxonomy. *J. Zool. Syst. Evol. Res.* 45(1):1-10.

Walker, F. 1833. Monographia Chalciditum. *Entomological Mag.* 1(4): 367-384.

Walker, F. 1834. Monographia Chalciditum. *Entomological Mag.* 2: 148-301.

Walker, F. 1837. Monographia Chalciditum. *Entomological Mag.* 4: 349-358.

Walker, F. 1846a. List of the specimen of hymenopterous insects in the collection of the British Museum. Part I. *Chalcidites* 100p.

Walker, F. 1846b. List of the specimens of hymenopterous insects in the collection of the British Museum, Part 1. *Chalcidites* 26p.

Walker, F. 1848. *List of the specimens of hymenopterous insects in the collection of the British museum. Part II.* Chalcidites, London, 237p.

Walker, F. 1850. Notes on Chalcidites, and descriptions of various new species. *An. and Mag. of Nat. Hist.* 5(2): 125-126.

Waterston, J. 1922. On Chalcidoidea. (Mainly bred at Dehra Dun, U.P., from pests of Sal, Toon, Chir and Sundri). *Indian Forest Rec.* 9(2): 31.

Waterston, J. C. 1915. New species of Chalcidoidea from Ceylon. *Bull. Entomological Res.* 5: 325-342.

Westwood, J. O. 1832b. Descriptions of several new British forms amongst the parasitic hymenopterous insects. *Lond. Edinb. Dubl. Phil.Mag.* 3: 127-129.

Xiao, H. and Huang, D. W. 2000. A taxonomic study of *Panstenon* (Hymenoptera: Pteromalidae: Panstenoninae) from China. *Oriental Insects* 34: 301-307.

Abstract

BIOSYSTEMATICS AND BARCODING OF PTEROMALIDAE (HYMENOPTERA: CHALCIDOIDEA) OF KERALA

by

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ABSTRACT

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ABSTRACT

The study entitled " Biosystematics and barcoding of Pteromalidae (Hymenoptera: Chalcidoidea) of Kerala" was conducted during the 2017-19 at the Department of Agricultural Entomology, College of Agriculture, Vellayani with the objective of identification, morphological and molecular characterization and documentation of parasitoid wasps (Pteromalidae) of Kerala. The base material for study was field collected specimens. Purposive sampling was carried out in 38 locations spread across 14 districts of Kerala. The specimens were collected by random sweep net method.

The collections were made in the morning and afternoon when the insect activity was at the peak. The sweep contents were immediately transferred into absolute alcohol and the location and date of collection were recorded on the containers. The field collected specimens were examined under high power stereo microscope and initially all the specimens coming under Chalcidoidea were sorted out based on superfamily characters and stored in labelled vials in absolute alcohol. Pteomalid wasps were separated out from these vials and stored in alcohol in another set of vials according to location. The specimens were card mounted for morphometric analysis and characterization.

The specimens were keyed out using regional taxonomic keys and original descriptions. During the morphological characterisation, the specimens which did not fit into any generic or species keys were compared with all existing generic descriptions.

The study revealed 2 genera and 4 species of Pteromalids which are new to the World of science. The two new genus fall under the subfamily Miscogasterinae and Pteromalinae respectively. The four new speicies discovered are, *Dinarmus* sp. nov. 1, *Mokrzeckia* sp nov. 1, *Panstenon* sp. nov. 1 and *Panstenon* sp. nov. 2. The *Dinarmus* sp. nov. 1 was compared to *Dinarmus maculatus*, *Mokrzeckia* sp. nov. 1 was compared to *M. pini* and *Panstenon* sp. nov. 1 and *P.* nov. 2 was compared to *P. collare*

The required measurements of the specimens were taken with ocular micrometer, appropriate ratios were worked out based on the genus and descriptions of the new species were prepared. Taxonomic keys to the Indian species of *Dinarmus* Thomson was prepared.

In addition to the new species, 14 previously reported species were also morphologically characterized and documented through photographs. The species documented are *Callitula peethapada* Narendran and Mohana, *Callitula travancorensis* Sureshan, *Cryptoprymna elongate* Sureshan and Narendran, *Dinarmus maculatus* Masi, *Kumarella angulus* Sureshan, *Merismomorpha elongata* Sureshan, *Notoglyptus scutellaris* Dodd and Girault, *Propicroscytus oryzae* Subbarao, *Systasis nigra* Sureshan, *Trichomalopsis apantolectena* Crawford, *Gastrancistrus* sp. A complete checklist of all the reported species of Pteromalids from Kerala was prepared which includes 154 species.

The DNA was extracted from 10 specimens which were collected in multiple numbers. The cytochrome c oxidase subunit I mitochondrial gene (COX1 or COI) was used as the barcode sequence. Folmer's "universal" primers were used to amplify the COI sequences. Three barcodes were obtained, adding to the very sparse documentation of genetic data of Pteromalidae from India for future reference. The obtained barcodes were blasted to confirm the identity of the species up to generic level.