MORPHOLOGICAL AND MOLECULAR TAXONOMY OF FRESHWATER PRAWNS AND SHRIMPS OF FAMILIES PALAEMONIDAE AND ATYIDAE IN THE UPPER STRETCHES OF NEYYAR RIVER SYSTEM, KERALA, INDIA

Dissertation submitted in partial fulfillment
of the requirements
for the degree of

M. F. Sc. (Fisheries Resource Management)

by

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Dedicated

To

My

Beloved Family

केन्द्रीय मात्स्यिकी शिक्षा संस्थान
भारतीय कृषि अनुसंधान परिषद,
CENTRAL INSTITUTE OF FISHERIES EDUCATION
(A university Established Under Sec.3 of UGC Act 1956)
Indian Council of Agricultural Research,
Ministry of Agriculture Govt. of India

Dated: 30th June 2016.

CERTIFICATE

Certified that the dissertation entitled "**MORPHOLOGICAL AND MOLECULAR TAXONOMY OF FRESHWATER PRAWNS AND SHRIMPS OF FAMILIES PALAEMONIDAE AND ATYIDAE IN THE UPPER STRETCHES OF NEYYAR RIVER SYSTEM, KERALA, INDIA**" is a record of independent bonafide research work carried out by **Ms. Ajina S.M.** during the period of study from August 2015 to June 2016 under our supervision and guidance for the degree of **Master of Fisheries Science (Fisheries Resource Management)** and that the dissertation has not previously formed the basis for the award of any degree, diploma, associateship, fellowship or any other similar title.

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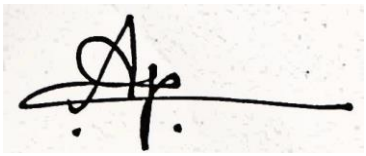

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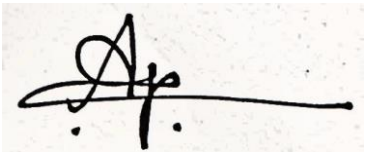

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सारांश

मीठे पानी के झींगे और समुद्री झींगे के परिवार पालिमोनिडे और एटिडे का वर्गीकरण करने हेतु एक खोजपूर्ण सर्वेक्षण केरल की नेय्यार नदी, जिसका उद्भव स्थल दक्षिण - पश्चिमी घाट है, इसके तीन नमूने स्टेशनों पर अगस्त 2015 से मई 2016 तक किया गया था । नमूने एकत्र किये गए और मैक्रोब्रकियम की नौ प्रजातियाँ और करिडिना की तीन प्रजातीयों के रूप में पहचान की गई, जो की पालिमोनिडे और एटिडे परिवारों से संबंधित है । *मैक्रोब्रकियम अब्रहमी*, *एम. एमुलम केरलउन्नी, एम. इंडिकम, एम. ईडेल्ला ईडेल्ला, एम. ईडेल्ला जिओर्गी, मैक्रोब्रकियम कनारे, एम. प्रभाकर्णी, एम. स्कोब्रिकुलम, करिडिना ग्रसिलिरोस्ट्रिस, सी. मथिसि, सी. नटराजनी* के रूप में प्रजातियों की पहचान और पारिस्थितिकी तंत्र से वर्णित की गयी । इन प्रजातियों में सी. ओ. आई. बारकोड अनुक्रम करके वंशावली वृक्ष विकसित किए गए । सभी नौ प्रजातियों की बारकोड अनुक्रम एन. सी. बी. आई. में जमा किया गया, उनमें से ज्यादातर पहली बार उपस्थित है । इस नदी से, मीठे पानी के झींगे और समुद्री झींगे की 15 प्रजातियों की एक चेकलिस्ट वर्तमान संग्रह और पहले के साहित्यों की अध्ययन करके बनाया गया है । मीठे पानी के झींगे और समुद्री झींगे के स्थानिकता के विश्लेषण से पता चला है कि 15 प्रजातियों में से, *एम. एमुलम केरलउन्नी* इसी नदी (नेय्यार) में पाई जाती है, और 5 प्रजातियाँ पश्चिमी घाट की विशेष सीमा में पाई जाती हैं । इन प्रजातियों के निवास स्थान की वरीयता और पारिस्थितिकी तंत्र के लिए प्रमुख खतरों को देखा और वर्णित किया गया हैं । जैव विविधता की क्षति आधुनिक दुनिया में बड़ी समस्या है, संरक्षण नीति के कार्यान्वयन के लिए, प्रलेखन और जीव-विज्ञान एवं जैव विविधता का ज्ञान होना आवश्यक है, जिसकी हमारे यहाँ कमी हैं । इस संदर्भ में वर्तमान अध्ययन नेय्यार नदी के स्थानिक झींगे और समुद्री झींगे की जैव विविधता पर, आगे की संरक्षण और प्रबंधन के उपायों के लिए एक आधार प्रदान कर सकते हैं ।

ABSTRACT

An exploratory survey was carried out at three sampling stations of Neyyar River, originating from southern Western Ghats, Kerala from August 2015 to May 2016 to the taxonomy of fresh water prawns and shrimps of family Palaeomonidae and Atyidae. The samples were collected and identified as nine species of *Macrobrachium* and three species of *Caridina* belonging to the families Palaemonidae and Atyidae*. M. abrahami, M.aemulum keralauni, M. indicum, M. Idella idella, M. Idella georgii, M. canarae, M. prabhakarani, M. scabriculum*, *Caridina gracilirostris, C. mathiassi, C. natarajani* were the species identified and described from the ecosystem. The COI barcode sequences of these species were done and developed phylogenetic tree. The bar code sequences of all the nine species going to submit NCBI, most of them are first time reported. A checklist of 15 species of prawns and shrimps in the river was made from the present collection and earlier literatures. An analysis of endemism of prawns and shrimps revealed that out of 15 species, *M. aemulum keralauni* is endemic to the particular river, and 5 are endemic to the particular range of Western Ghats. The habitat preference of these species and major threats to the ecosystem are also observed and reported. As the loss of biodiversity is major problem in modern world, the documentation and knowledge on biology and diversity of organism required for the implementation of conservation policy is lacking. In this context the present study can provide a reference on the biodiversity of endemic prawns and shrimps in Neyyar river for further conservation and management measures.

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Introduction

1. INTRODUCTION

The freshwater prawns and shrimps belong to the families Palaemonidae, Rafinesque, 1815 and Atyidae De Hann, 1849, (Order: Decapoda, suborder: Pleocyemata, Infraorder: Caridea), distributed all over the world with the species diversity of 981 species (De Grave and Fransen, 2011). They have high commercial value in both domestic and international market as food organisms and ornamental varieties in trade. Ecologically these organisms play an important role in the ecosystem functioning and maintaining integrity. In India, around 127 species of prawns (Palaemonidae) and 28 species of shrimps (Atyidae) were reported from various aquatic ecosystems. From Kerala about 20 species of prawns and 17 species of shrimps were described and reported. (Jayachandran, 2001; Jayachandran *et al.,* 2008; Jayachandran, 2010; Radhakrishnan *et al.,* 2012).

Macrobrachium, one of the genus of family Palaemonidae have high demand in international market contribute 409,771 tonnes with a value of over US$ 1.65 billion in trade (Pillai, 2010). *Macrobrachium rosenbergi* forms a major contribution to the trade followed by *M. nipponesis,* and *M. gangeticum*. India has the second position in trade after China, with *Macrobrachium rosenbegii*, *Macrobrachium malcolmsonii* and Macrobrachium *gangeticum*. Due to the Increase in human population, scarcity of prawns in wild, the capture fisheries is not sufficient meet the demand of them in trade. As considering these things the aquaculture industry start to develop with new culture technologies and seed production systems. In India a group of species categorized as having high aquaculture potential, but are unutilized (Jayachandran and Indira, 2010). The introduction of such candidate species to the aquaculture industry with efficient production procedure is good for our national sustainability. Taxonomy and biology of organism is required for this elaborated procedure.

The freshwater prawns and shrimps belong to the families Palaemonidae, and Atyidae are the astonishing beauty in the aquarium, create a warm feeling in aquarium hobbyists. The lists of such organism started from *Caridina gracilirostris* to

the *Macrobrachium scabriculum* and are non-replaceable candidates of the industry. The prawns *Macrobrachium canarae, M. latimanus, M. gurudeve*, juveniles of *M. rosenbergii, M. ornatus* and the shrimps *Caridina jalihali, C. natrajani* are the recently introduced seven species of prawns and shrimps to the aquarium industry (Jayachandran, 2006; Jayachandran and Raji, 2004). Most of the cases the aquarium industry exploits the organism from wild and export to foreign which is going to be over exploited. Considering biodiversity conservation and promotion of our national sustainability, the captive breeding of indigenous fishes and shellfishes is required. According to CITES (Convention on International trade of endangered flora and fauna), the 10^{th} generation of captive bred organisms only can export with its scientific name. Here also, taxonomy has a significant role from the collection of broods of such organism from wild to export.

The Freshwater prawns and shrimp play a primary role in ecosystem functioning and positioned in various trophic levels in the food chain, and transfer energy from one level to another. The Palaemonids are usually carnivorous to omnivorous in nature, occupied in the higher trophic level, while the Atyids are scavengers on detritus. Both groups are common forage organism for the large commercially and ecologically important aquatic animals inhabited in a particular ecosystem. The food and feeding studies of *Labeo fimbriatus* and *Platinista gangetica* (Gangetic dolphin) proved the role of these organisms as food in ecosystem. (Bhatnagar, and Karamchandani, 1970; Jayachandran *et al.,* 2007)

As a developing country, with a huge population, India has a large gap in between the supply and demand of food and another commodity. As an ancient agriculture country, with about 60 to 70 percentage rural population, goes for the new entity other than agriculture which helps to overcome scarcity in their needs, sustain their lives and livelihood. People living in the inland area may prefer the inland aquatic resources for their livelihood. The ornamental shrimps and prawn species one of the alternative of the rural population going to depend on their sustainability (Jayachandran and Indira, 2010). For the utilization of such resources from the natural environment and maintain the sustainability, the scientific study is required. Scientific study of an organism starts from its nomenclature and classification. In India some Carcinologists (Henderson, and Matthai ,1910;

Natarajan 1942; Chopra and Tiwari, 1947; Tiwari, 1947,1949, 1952, 1955a, 1955b, 1955c, 1956, 1958, 1961, 1963; Pillai, 1964; Tiwari and Pillai, 1973; Chinnayya,1971; Jalihal and Sankolli, 1975a;1975b Ravindranath, 1977; Anantharaman *et al.,* 1978; Jalial, 1978; Jalihal *et al*., 1988, 1993; Jayachandran and Joseph, 1985a, 1985b, 1985c, 1986; and Mariappan and Richard 2006) contributes their life to the study of taxonomy, diversity and biology of Indian freshwater prawns and shrimps.

India with its four global biodiversity hotspots (Western Ghats, North East region, the Himalayas and Nicobar islands) contributes a big share in global fish diversity with 11.72% in share. Among the four of the hotspots, Western Ghats, started from Maharashtra extending up to up to Sri Lanka, act as the origin of numerous peninsular rivers contributing about 69% of total endemic fish and shellfish species of India (Lakra *et al*., 2010). Among the 41 species of prawns and shrimps, present in such ecosystem are and under threats because of illegal activities of human and requires conservation. Taxonomy is the one of the lacking factor of the Crustacean conservation policy making in Western Ghats (Raghavan *et al.,* 2015).

The Neyyar is a small river of Kerala originates from 2nd highest peak (1866 m) of the Western Ghats of length 56 Km and covers a watershed of 483.45 sq. Km flowing Westwards to finally into Lakshadweep sea via a sand bar built estuary at Poovar. A survey conducted by ZSI during the period of 1997-1998 in the rivers of Trivandrum (including Neyyar) district identified a huge number of fish diversity in relation to endemism. Similar studies on the icthyofauna and macrobenthos of such river system showed such an interesting results (Abraham *et al.,* 2011). A taxonomic survey conducted in 2013 reported a new sub species of *Macrobrachium* endemic to the particular river system and also reported the presence of other six *Macrobrachium* species (Pillai and Unnikrishnan, 2013b, 2013c). These reports lead to a conclusion that the river might contribute a good percentage of species diversity regarding of freshwater prawns and shrimps. However, characterization /documentation of crustaceans from the Neyyar River are limited, a good taxonomy study with modern tool is important for documenting them.

Most of riverine ecosystems in the world are under huge threats due to industrialization, human settlement, and tourism (Collen *et al.,* 2008; Vorosmarty *et al.,* 2010). In India, the pollution, sand mining, barriers and anicuts, alien/ exotic species are the major issues in riverine biodiversity (Vass and Moza, 2010). In Kerala sand quarrying and river bank agriculture and land cover change, degradation of water resources by city sewage, land reclamation, construction activities, hospital waste, industrial waste are the major threats to the riverine ecosystem and the organism inhabiting in it (Harikumar and Kokkal, 2010; Abraham *et al.*, 2011). The severe anthropogenic disturbances caused by rapid urbanization, industrialization and large-scale agricultural activities) are threatening the Neyyar river (Sheeja *et al*., 2010). This situation will undoubtfully affect the precious aquatic biota, especially fin fishes, and shell fishes existing there. The identity of existing organism in the ecosystem helps the conservationist to bring out new conservation policies, will protect them from the threats.

The anthropogenic activities and its pressure affect the aquatic ecosystem and its precious biodiversity in worldwide, locally and regionally (Allan and Flecker, 1993; Dudgeon *et al.,* 2005, Vörösmarty *et al.,* 2010). One of the biological methods for the conservation of biodiversity is the declaration of protected areas (PAs), were the organisms preserved in their own environment and threats to the diversity in protected areas are comparatively less than unprotected areas (Abraham et al., 2011). The origin of river Neyyar, Agastyamalai, popularly known as Ashambu hills is one of the protected areas of India, and which is in the Southern part of Western Ghats. The establishment of such practices to other region requires the knowledge of diversity; and the existence of diversity is the taxonomy. The lack of taxonomic decapod crustacean in the Western Ghats is a challenge of making proper conservation plan (Raghavan *et al.,* 2015).

At present taxonomic study is based on two views classical taxonomy and more advanced molecular taxonomy. Under classical taxonomy, the organisms are studied based on their morphological characters and is even now a reliable method. But there are numerous instances in which the morphological analysis alone may not be sufficient to separate species or groups of individuals. The more advanced

taxonomic technique involves the molecular tools. However classical morphological studies are inseparable as the molecular taxonomy cannot be preceded further.

In molecular taxonomy the COI region of mitochondria helps to differentiate the species individually. The genetic composition of this region varies between the species and it is exist as same in within species and can identify the organism at species level.

Among the 17635 decapod Crustaceans in the world only 5.4% have COI barcode region sequences. Challenge is remaining in to compile regional databases that identify and analyse the extent and patterns of decapod diversity. Cryptic species, ambiguity in the morphology, lack of insufficient data are more in the taxonomic context of Crustacea hence the barcoding is important (da Silva *et al*., 2011). Similarly morphologically identified species barcode helps to the upcoming ecologist and conservationist to identify the organism without killing them. (The appendages of crustacean have regenerating power; it will reform in original size within three months). Hence the database (Barcode) is important to identify the organism diversity existing in an ecosystem.

In view importance of prawns and their diversity along Western Ghats, study on taxonomy of prawns and shrimps of upper stretches of the river Neyyar (originates from Southern Western Ghats) by employing morphological and molecular tool is planned with the following objectives.

- To identify the selected prawns (family: Palaemonidae) and shrimps (family: Atyidae) from upper stretches of the Neyyar river system based on morphological characters.
- To generate the species specific molecular markers for selected prawns (family: Palaemonidae) and shrimp (family: Atyidae)
- To examine the endemism of prawn species in the ecosystem.

Literature Review

2. LITERATURE REVIEW

2.1 Global Freshwater Prawns and Shrimps Diversity

Globally the freshwater invertebrate distributed all over the geographical region, its diversity composes of large group of organisms occupies in about 570 families belongs to 17 phyla (Strayer *et al.,* 2006). Among this 570 families of freshwater invertebrate, the families Palaemonidae Rafinesque, 1815 refers freshwater prawns and Atyidae De Hann, 1849, refers freshwater shrimps contribute a major portion from the Crustacean infra order Caridea (De Grave *et al.,* 2008).

In total, the family Palaemonidae composed of 93 valid genera with around 716 species and occupied in two subfamilies Palaemoninae and Pontoninae (Jayachandran, 2001). The family Atyidae comprises of about eight genera with 359 species or subspecies (De Grave *et al.,* 2008 and De Grave *et al.,* 2009). The recently published identification guide line and check list of Crustacea showing the existence of 3047 species of shrimps and prawns in the world to date, and in this 981 are under subfamily Palaemoninae and family Atyidae (De Grave and Frensen, 2011).

2.2 Diversity of Freshwater Prawns and Shrimps in India

India world's richest endemic biodiversity nation, blessed with about 29000 Km of rivers 3.15 million ha of reservoirs, 0.72 million ha of upland lakes, 0.2 million ha of flood plain wet lands (Lakra *et al.,* 2010). Instead of these ecosystems paddy field, hill stream, ditches, ponds occupy the water seasonally or perennially. These ecosystems are rich source of aquatic biodiversity.

In India about 75 species of Palaemonid prawns occupying in 8 genera and 27 species of Atyid shrimps belongs to single genera are distributed various ecosystems (Jayachandran 2010; Jayachandran and Indira, 2010).

Recently published checklist of Indian Decapod and Stomatopod showed the presence of 28 species of Atyid shrimps from a single genera *Caridina* and 127 species of Palaemonid prawns of 28 genera from two t subfamilies (Radhakrishnan *et al.,* 2012).

2.3 Diversity of Freshwater Prawn and Shrimp of Southern India and Western Ghats

The Western Ghats mountain range and its associated streams in peninsular India and Sri Lanka one among the 34 global biodiversity hot spots harbour the huge endemic fish biodiversity of India (Lakra *et al*., 2010). The Western Ghats region is unique from the other biodiversity rich region due to its endemism in fishes, molluscs, odonates, and aquatic plants (Molur *et al.,* 2011).

Thomas and Jayachandran (2007); Jayachandran *et al*. (2008); Valarmathi (2009); Arumugam (2011); Radhakrishnan *et al.* (2012); Jayachandran and Thomas (2012); Thomas (2012); Pillai and Unnikrishnan (2012; 2013a; 2013 b; 2013 c); Pillai *et al.* (2014) and Raghavan *et al.* (2015) studied and explored the diversity of Crustacean Decapods of Western Ghats during last decade. The total 49 species of prawns and shrimps occurs in the Western Ghats ecosystem, in that about 69% are endemic, the limitation in the taxonomic study and data deficiency are the major barrage for the proper biodiversity assessment in the ecosystem (Raghavan *et al.,* 2015).

2.4 Taxonomic Study of Freshwater Prawns and Shrimps in Global

The taxonomy and biogeography of freshwater prawns started during the 2nd half of 19th century. De Man (1879,1881,1892, 1904, 1905,1908a, 1908b, 1911, 1915,1924); Henderson (1893); Borradaile (1898, 1915,1916, 1917); Lanchester (1900,1901,1906); Kemp (1913, 1915, 1917, 1918, 1922, 1924, 1925); Cowles (1914); Roux (1935, 1936); Yu (1931,1936); Kubo (1936,1940, 1949); Armstrong (1940,1941, 1949); Holthius (1948,1949a, 1949b, 1949c, 1950a, 1950b,

1951a, 1951b, 1952, 1955, 1959, 1965, 1978, 1980,1981, 1984a, 1984b, 1986); Johnson (1962, 1973); Baily and Crichton, (1971); Gore (1981); Anger and Moreira (1998); Cai and Ng (2001, 2007); Jayachandran (2001); Cai and Anker (2004); Komai and Fujita (2005); Cai and Shokita (2006); De Grave and Al-Maslamani (2006); De Grave *et al.* (2008); De Grave *et al.* (2009); Holthuis and Ng (2009); Mossolin *et al*., (2010) classified and described the genus and species of Palaemonidae which is known to the world.

The studies on the group Atyidae started parallel to the taxonomic study of Palaemonidae, because both these Caridean family members are distributed in freshwater habitat all over the world. The name of family Atyidae derived from the major genus comes under the family Atya. De Haan (1849) was the first to use the term 'Atyadea' later Dana (1852) changed the name as 'Atyidae. This family distinguished from the other families of Caridean due the spoon shaped first and second cheleped coved with stiff setae. Globally Kemp (1913, 1915); De Man (1879, 1881) and Holthuis (1948, 1986) are the scientist made the taxonomic studies of Atyids. Calman (1906); Holthuis (1978); Choy (1983, 1991), collected the Atyid prawn with Palaemonid and studied the taxonomy and diversity.

2.5 History of Taxonomic Studies of Freshwater Prawns and Shrimps in India

The freshwater prawn taxonomy and Carcinology in India started in the late 19th century. In British India Henderson (1893), Henderson and Mathai (1910); Kemp (1913, 1915, 1917, 1918, 1922, 1924, and 1925) made pioneer studies on the Palaemonid prawns. Among them Henderson (1893); Henderson and Mathai (1910), more concentrated their work on the species found in southern India, and Kemp done his work in north eastern states. After a long period in Independent India Tiwari (1947, 1949, 1952, 1955a, 1955b, 1955c, 1956, 1961, 1963, 1964) made the study of Palaemonid prawns inhabited in the Indian mainland, Islands and Burma and made an evolutionary hypothesis of freshwater prawns occurs in the nation by comparing the species distributed in nearby nation

Burma. During that period he described many of the freshwater prawn species present in Indian waters.

After a small duration (1965 to 1982) the papers and description of species confined and limited. Tiwari and Pillai (1968, 1971, and 1973) studied in the Island ecosystem of India, Andaman and Nicobar and described the species present there. Jalihal and Shenoy (1975a, 1975b), the two eminent scientist of Karnataka they did studies on the *Macrobrachium* species and described species found in the Canara waters. Ravindranath (1979), Tiwari and Holthuis (1996) made studies on prawns during this period. Raman *et al.* (1986) surveyed freshwater habitats around the Bangalore city, Karnataka, India, and studied the abundancy of freshwater decapod crustaceans.

After Tiwari's period the Indian freshwater prawn taxonomy highlighted during the work period of Jayachandran (1982, 1984, 1987, 1991, 1992, 1998, 2006, and 2010). In that period most of the freshwater Palaemonid prawns present in the southern India described and published. The combined work of Jayachandran and Joseph (1982, 1985a, 1985b, 1985c, 1986, 1988a, 1988b, 1989, 1992) made the taxonomy of freshwater prawns of India up to the level of global taxonomic research. After a decade Unnikrishnan *et al.* (2011) Pillai and Unnikrishnan (2012, 2013a, 2013b) Pillai *et al*., (2014) done studies on southern Kerala described the new species with the modern technology DNA barcode along with its morphology.

In the case of Atyid prawns the pioneer taxonomic studies carried out by Henderson 1893, along with the taxonomy of Palaemonid prawns. The taxonomic studies on the family Atyidae is very limited with the literatures of the few scientists (Pillai, 1958; Babu, 1963; Tiwari & Pillai, 1968; Thomas *et al.,* 1973; Jalihal *et al.,* 1984; Richard and Chandran, 1994; Mariappan and Richard, 2006 and Jayachandran *et al.,* 2008). Thomas (2012) made the study of the Atyid shrimps in Kerala.

2.6 Taxonomic Surveys and Reports of Palaemonid Prawns from Indian Waters

Henderson (1893); Henderson and Mathai (1910) described the species of Palaemonidae and Atyidae from British India and surveyed Madras residency and collected some species of *Macrobrachium* along with some *Caridina* species and they described *Macobrachium nobili*, *Macrobrachium sulcatus* from there. Kemp (1913, 1915, 1917, 1918, 1922, 1924, and 1925) surveyed the north eastern states (Chilka Lake, Inle Lake, Siju cave, Bengal) of British India collected and described the species of *Macrobrachium* such as *Macrobrachium altifrons* and *Macrobrachium dayanum*. Among this *M. dayanum,* one of the small verities of *Macrobrachium* distributed all along North Eastern state of India and bengladesh. Later Chopra and Tiwari (1947) collected the samples of same species from the Varuna river and made the study of region wise variation of the species in India. Tiwari 1947 surveyed for the species of family Palaemonidae in Bengal and described three new species such as *Macrobrachium choprai*, *Macrobrachium villosimanus,* and *Macrobrachium kempi*.

Tiwari (1949) described a new species of genus *Macrobrachium* from the Ganga river at Banaras, and compared the species with *Macrobrachium lanchesteri*. Tiwari (1952) described new species and subspecies of freshwater prawns *Macrobrachium kistensis, Macrobrachium manipurensis, Macrobrachium siwalikensis Macrobrachium andamanicum, Macrobrachium hirsutimanus Macrobrachium rogersi, Macrobrachium hendersodayanum*, *Macrobrachium hendersoni cacharense Macrobrachium hendersoni platyrostris, Macrobrachium lamarrei lamarroides* from various rivers and rivulets of India.

Tiwari (1955a) made a study on the distribution of Indo-Burman prawn and strengthen the Satpura hypothesis based on the distribution pattern of these organisms, during this period he collected the species from the different locations of India and Burma described the subspecies *Macrobrachium assamense assamense*. Tiwari (1956) described the major distinguishing characteristics appendix musculina, which is used for the differentiation of two subspecies *Macrobrachium, Macrobrachium lamarrei lamarrei* and *Macrobrachium lamarrei lamarroides*. Tiwari

(1958) collected the *Macrobrachium* species from central India and described the new species *Macrobrachium* species and named as *M. banjarae* hence type locality of the species is Banjar river Madhya Pradesh and described another the new species *Macrobrachium canarae* from Karnataka water and named as *canarae*.

Tiwari (1961) reported the occurrences of *Macrobrachium latimanus* in Indian and Ceylon water. Tiwari (1963) made notes on the freshwater prawn *M. altifrons* (Henderson, 1893) and described a new subspecies *Macrobrachium altifrons ranjhai* from the tributaries Indus river. Tiwari and Pillai (1973) surveyed Andaman and Nicobar Island and Identified and reported the presence *Macrbrachium latidactylus* in Island waters.

Ravindranath (1979) described a new species of Macrobrachium from Andra Pradesh and named as *Macrobrachium johnsoni.* Jalihal *et al*. (1979a, 1979b) described three new species of *Macrobrachium*, *Macrobrachium sankollii* and *Macrobrachium tiwarii*, *Macrobrachium unikaranatake* from Karnataka state of India.

Jayachandran (1984) did the Ph. D work on the Biology of Palaemonid prawns of south West coast of India. During this period he collected and described the species occurs in south west coast of India. Jayachandran and Joseph (1985, 1986) described a new subspecies of *Macrobrachium* from the waters of central Kerala, and a new species *Macrobrachium indicum* from southern Kerala. Jayachandran (1987) described a new species *Macrobrachium elatum* from Quilon district of Kerala the species is only known to the type locality. Jayachandran described the new species of *Macrobrachium* from Veli Lake of Kerala and named that as *Macrobrachium josephi* and he described *Macrobrachium veliensis*.

Jalihal *et al.* (1988) described and reported the *Macrobrachium* (Crustacea, Decapoda, Palaemonidae) species present in Karnataka waters. Jalihal *et al.*, (1993), describe the evolutionary development of larval stage of freshwater prawns belongs to the genera *Macrobrachium* (Decapoda, Palaemonidae).

Jayachandran (2001) published a book on the Taxonomy and Biodiversity of Palaemonid prawns of world. He described the entire genus and

species comes under the family Palaemonidae which has been described up to 1999.

Jayachandran and Raji (2004) described *Macrobrachium gurudeve, Macrobrachium jayasreei, Macrobrachium Kunjuramani* as new from Western Ghats. Jayachandran *et al.,* (2007) described a new species of *Macrobrachium* from the dolphin trenches of Kulsi river and the species is *Macrobrachium kulsiensis.*

Unnikrishnan *et al.* (2011) described a new species *Macrobrachium madhusoodani* from Kerala water it is closely related to other two species such as *Macrobrachium scabriculum* and *Macrobrachium aemulum.*

Pillai and Unnikrishnan (2012) described a new species of *Macrobrachium* from Vamanapuram river of Kerala that species was closer to the known species *Macrobrachium scabriculum* and he named as *Macrobrachium prabhakarani.*

Pillai and Unnikrishnan (2013a) described new species of *Macrobrachium* closer to the *Macrobrachium idella* from the Karamana river of Kerala and the species is *M. snpuri*. *Macrobrachium aemulum madhusoodani* a new subspecies described from Neyyar river Kerala, later replaced the name as *Macrobrachium aemulum keraluni* (Pillai and Unnikrishnan (2013b, 2013c). Pillai *et al*. (2014) described a new species of *Macrobrachium, Macrobrachium abrahami* from the Kerala river system which similar to *Macrobrachium scabriculum.*

2.7 Taxonomic Reports of Family Atyidae from Indian Waters

The first taxonomic report of *Caridina* species started by Henderson (1893) from Madras water, and reported *C*. *wyckki* along with Palaemonid prawns. Kemp (1913) described two new species of *Caridina, Caridina excavata* and *C*. *hodgarti* from Brahmaputra valley.

Natarajan (1942) reported the occurrence *C*. *gracilirostris*, *C*. *laevis*, *C*. *nilotica* var. *gracilipes* and *C*. *weberi* var. *sumatrensis* in Travancore waters. Pillai

(1964) described the new variety of *Caridina* from Travancore namely *C*. *nilotica* (Roux) var. *veliensis*.

Tiwari and Pillai (1968) described a new species *Caridina natarajani* from Museum tank in Trivandrum which shows close affinity with *Caridina laevis*. Tiwari & Pillai (1971) surveyed Andaman Islands and reported 5 species of *Caridina* including one new species, *C*. *prashadi.*

From Cochin backwaters Thomas *et al*. (1973) described a new species namely, *C*. *pseudogracilirostris* and differentiate the species with *Caridina gracilirostris*. Dutt and Ravindranath (1975) recorded *Caridina brachydactyla peninsularis* first time from India.

Richard (1983) reported the presence *Caridina gurneyi lonavalensis* in Madras city. Jalihal *et al.* (1984) described five new species of Atyid shrimps from the Karnataka water bodies the species are *C williamsoni, C. panikkari, C. kempi, C. shenoyi* and *C. gurneyi*.

Raman *et al.* (1986) surveyed freshwater habitats around the Bangalore city, Karnataka, India, for studying the abundancy of freshwater decapod Crustaceans. The study reported the presence of one Palaemonid species *Macrobrachium lanchesteri* and three species of *Caridina* such as *Caridina nilotica* var. *bengalensis*, *Caridina rajadhari,* and *Caridina weberi* var. *sumatrensis* from that particular region.

Richard and Chandran (1994) made a survey for the Atyid shrimps belongs to the genera *Caridina* on Chetput pond, Chembarambakkam reservoir, nursery pond of Fisheries Research Station (FRS) at Chembarambakkam, Porur and Kunnathur ponds of Tamil Nadu state reported the presence of four species of *Caridina* in these waters.

Ebenezer and Richard (1999) reported the occurrence of *Caridina typus* in Tamil Nadu; it is first time from the Indian mainland. Dutta (2001) reported the occurrence of *Caridina weberi in Assam.* Mariappan and Richard (2006) described a new species *Caridina jalihali* from Tamil nadu.

Thomas & Jayachandran (2007) reported *Caridina jalihali* outside the type locality Tamil Nadu and become first report in Kerala. Jayachandran *et al.* (2008) discussed about the Caridean shrimps of Kerala which is collected from the 41 different research stations all along Kerala including rivers and streams. During this research about 11 species of freshwater shrimps collected and identified. This also provided the key for the identification of *Caridina* species inhabited in Kerala waters. During this work about three species were newly described.

2.8 The Major Threats and Conservation status of Prawns and Shrimps in Western Ghats and Taxonomic Need

Biodiversity loss, the environmental crisis faced by the modern society (Krishnamurthy and Francis, 2012). As a scarce aquatic resource the freshwater ecosystems are more affected due to the human development and pollution (Dudgeon *et al.,* 2006, Collen *et al.,* 2008, Vorosmarty *et al.,* 2010). In India Western Ghats ecosystem faces similar threats due to the human activities. (Bhat 2003, 2004; Subashchandran *et al.,* 2007; Raghavan *et al*., 2008; Johnson and Arunachalam 2009; Abraham *et al.,* 2011; Raghavan *et al.,* 2015). Dahanukar *et al.* (2004) reported the threat status of fishes found in Western Ghats argued that at least 41% of fish fauna is threatened by either being VU, EN or CR. Implementation of potent conservation measures is necessary to conserve the fish fauna of Western Ghats.

Cumberlidge *et al.* (2009) conducted a survey in Sri Lanka showed the 80% of data deficient organism are threatened. Molur *et al.* (2011) made the biodiversity assessment on four group of organism such as fishes, odonates, mollusca and aquatic plants and found that 16% the total occurring taxa of 1146 are threatened with extinction.

Harikumar and Kokkal (2010) gave a report to the government of Kerala about the water quality of Kerala rivers and major threats which degrade the quality of water. The report showed that the upstream waters are more pure and

pollution impact is less than that of the downstream. It shows the impact of anthropogenic activity in river water system.

Raghavan *et al.* (2015) reported the conservation status of Decapod crustacean in Western Ghats. According to him among the 41 species (26 endemic) of freshwater prawns of family Palaemonidae present in Western Ghats 15 are data deficient 22 are least concern and one is vulnerable. *Macrobrachium gurudeve* is the vulnerable Palaemonid reported there because of its single ecosystem Bhavanisagar river and concluded with his paper by giving some recommendation to the new researches to take up the conservation of prawns and shrimps of Western Ghats by conducting efficient taxonomic surveys.

2.9 DNA Barcoding and Phylogeny of Prawns and shrimps

It is difficult to find the cryptic and intraspecific variation within the species morphologically. DNA barcoding, the technique which solves the problem by using the gene sequences of conserved area of mitochondria. The COI area act as conserved for each species and organism and this variation of the species and helps and strengthens the taxonomy and helps in the conservation biology. However, a single gene cytochrome c oxidase I (COI) of about 600 to 700 base pairs sequenced and use for the barcode. (BOLI 2012)

Murphy and Austin (2002) did a preliminary molecular work for studying the systematic relationship among the Palaemonid shrimp using 16S rRNA mitochondrial gene sequenced and analysed about five species of Australia such as *Macrobrachium intermedium*, *M. australiense*, *M. atactum*, *M. rosenbergii* and *Palaemon serenus* by using this method.

Murphy and Austin (2003) carried out molecular study on taxonomy and phylogeny of some Palaemonid prawns of Australia. The presence of major anomalies in the current classification of Australian Palaemonidae indicated in phylogeny study. They inferred that the three species of different genera, *Macrobrachium intermedium*, *Palaemon serenus*, and *Palaemonetes australis*, are closely related, with genetic differences more characteristic with that of congeneric

species. This also found that the Australian *Palaemonids* both *Palaemonetes* and *Macrobrachium* are non-monophyly.

Phylogenetic analysis in 18 wild populations of the giant freshwater prawn, *Macrobrachium rosenbergii* by De Bruyn *et al.* (2004), utilising a fragment of the 16S rRNA mitochondrial gene, identified two major reciprocally monophyletic clades either side of a well-known biogeographic barrier, Huxley's line. The divergence level of the two clade found is maximum of 6.2% which exceeds the divergence level within either clade of 0.9% and it does not conceded with geographical distance among sites.

Murphy and Austin (2005), made a phylogenetic study on genus *Macrobrachium* from different locality of Asia, Central/South America and Australia. And they selected 30 *Macrobrachium* species and analysed based on 16S rRNA sequences. And they gave some inference that these species are not share a common ancestry and have a large regional diversification. And they suggested that the large scale dispersal had been the major feature of the evolution and that may due to the transitional life cycle estuarine into purely freshwater.

Udaysuryan (2005) studied for the most suited primer combination among the universal crustacean primers. He conducted the experiment with 4 *Macrobrachium* and one *Caridina* species and universal primers LCO1490 & HCO2198, COIa & COIf, CrustF1 & HCO2198 and CrustF2 & HCO2198 Among this primer sets LCO1490 & HCO2198 and COIa & COIf was worked well in all subjected species. The primer set COIa & COIf showed more significant results in non-synonymous substitution, saturations, transvertional type substitution and divergence rates when compared with LCO1490 & HCO2198.

Costa *et al.* (2007) check the ability of COI to provide identifications crustacean group examined two disparate levels in the taxonomic hierarchy — orders and species. The studies revealed that levels of nucleotide sequence divergence were from 19 to 48 times greater between congeneric species than between individuals of a species and sequence variation in the COI barcode region will be very effective for discriminating species of Crustacea.

A phylogenetic study of the freshwater prawn genus *Macrobrachium* of Taiwan, by Chen *et al*. (2009) from the nuclear 28S rDNA sequences made a hypothesis their biogeographical origin and dispersion routes. From the 16 species *Macrobrachium* of Taiwan 13 are amphidromous and their biological origin were: 1) *M. australe, M. lar, M. latimanus, M. gracilirostre, M. jaroense, M. esculentum, M. lepidactyloides, and M. placidulum* were dispersed to Thailand from the Eastern region of tropical Southeast Asia islands through the Philippines islands 2) *M. equidens, M. latidactylus,* and *M. mammillodactylus* were dispersed to Taiwan from the western region of tropical Southeast Asia islands, through the Philippines and through mainland China for *M. formosense* and *M. japonicum* dispersed through the islands on Taiwan itself. The other species reported from there are *M. asperulum*, a land-locked prawn, and *M. nipponense*, a non-obligatory amphidromous and both are originated from the China mainland.

A molecular phylogenetic analysis of genus *Macrobrachium* by Wowor *et al.* (2009) from Southeast and East Asian fauna based on >3 kb sequence data with three mitochondrial markers construct a phylogenic tree and made a track of evolution. They concluded that the amphidromous (have both marine and estuarine phase), small egg laying species of *Macrobrachium* have a common ancestor. But the organism which is purely freshwater with larger eggs has two type of origin (two origins and three origins.

da Silva *et al.* (2011) made the phylogenetic tree from DNA barcode of some commercially important decapod crustaceans of the world and found out the relationship between the group. Pillai and Unnikrishnan (2013a) barcoded the species of *Macrobrachium*, *Macrobrachium snpuri* and justified as the species is very close to the *Macrobrachium idella idella* in both morphological and molecular characters.

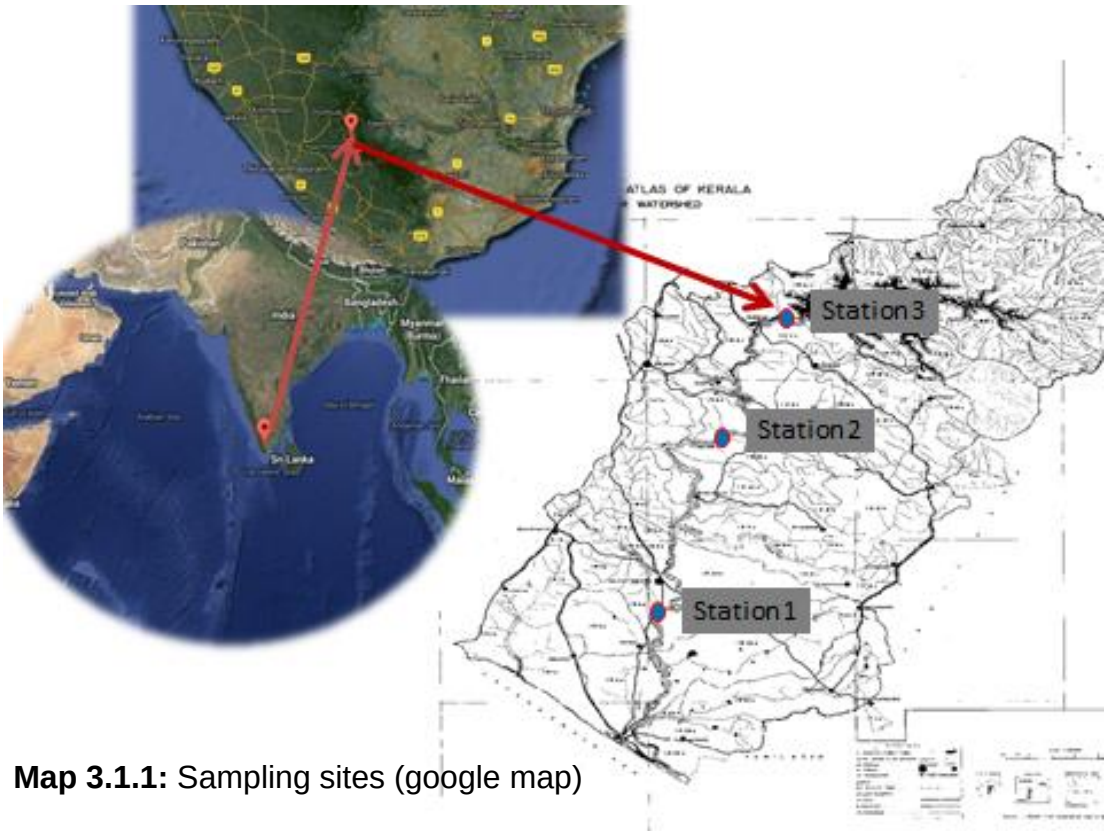
Jose and Harikrishnan (2016) explained issue related to the ‘‘Folmer’’ and ‘‘Palumbi’’ regions of DNA sequence which is common in the sequence of Decapod Crustaceans. These are the limited overlap region of the DNA, and affect the DNA barcoding like specimen identification, population analysis, and phylogeny in decapod crustaceans using COI sequences. The described the situation by choosing *Macrobrachium rosenbergii* as reference species.

Materials and Methods

3. MATERIALS AND METHODS

3.1 Description of Study Area and Geographical Location

The Neyyar river, south most river of Kerala state of India originates from the southern tip of Western Ghats, Agastyamala (Ashambu hills). The river has a total length of 56 km, flows through a small city Neyyattinkara in capital of Kerala and finally joins to the Lakshadweep Sea at Poovar. There are three tributaries Kallar river, Mullayar and Karavaliyar meet to the river at different location. For the present study the sample are collected from the main river of three locations, above the middle stretches of river. The sites includes Amarvila Bridge (8°38'66" N, 77°09'23" E), Pzhamala Devi Temple, Perunkadavila (8°44'87", 77°10'15") and from Near Neyyar dam, Kallikkad (8°53'26" N, 77°13'91" 77.139192). The location is shown in map 3.1.1



Map 3.1.1: Sampling sites (google map)

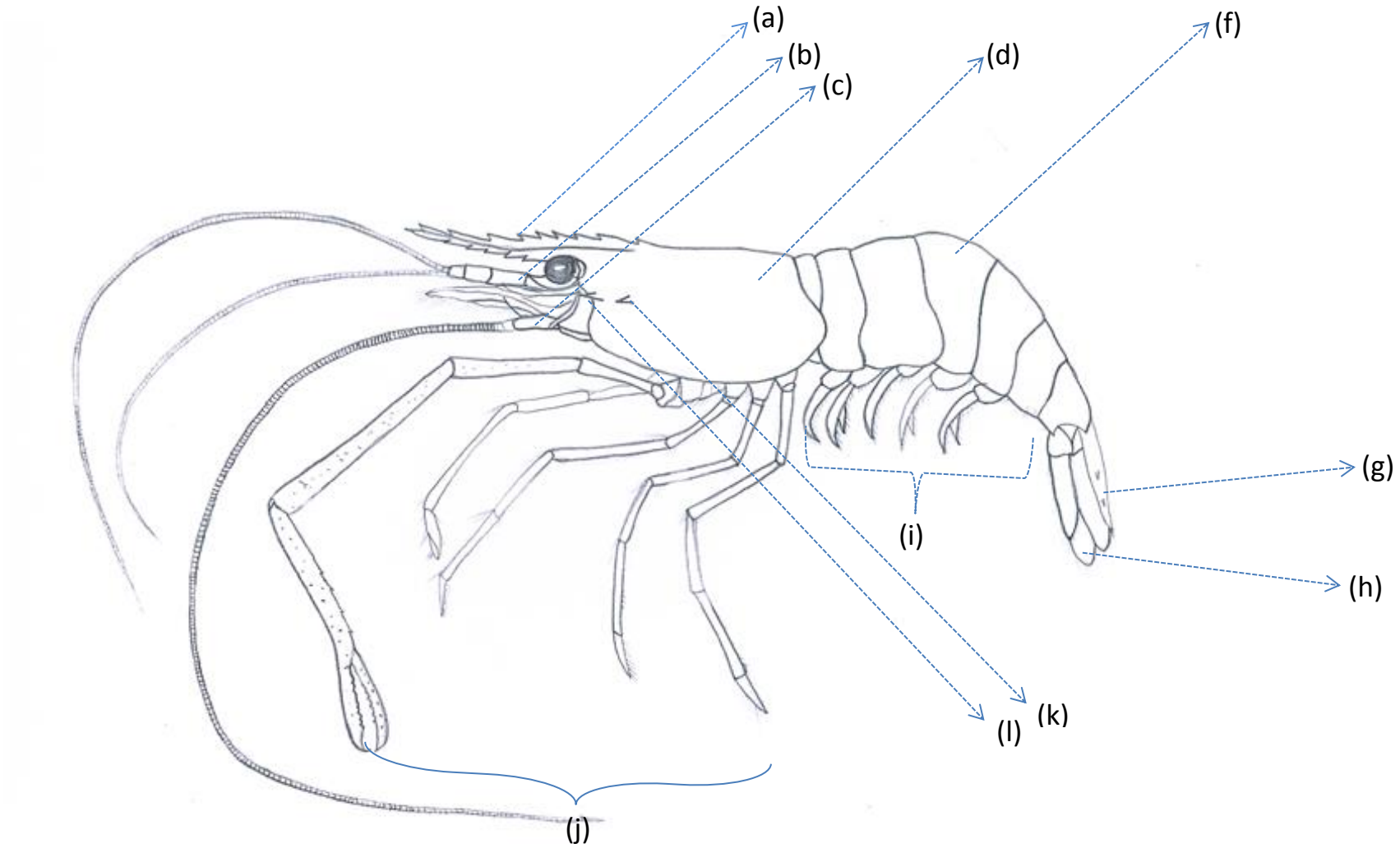


Figure 3.3.1.1: General morphological characters of taxonomic importance present in *Macrobrachium* spp. (a) Rostrum, (b) Antennule, (c) Antenna, (d) Carapace, (f) Abdomen, (g) Telson, (h) Uropod, (i) Pleopods, (j) Periopods, (k) Hepatic spine, (l) Antennal spine

3.2 Sample Collection Period and preservation

Collection of samples from the three locations above the middle stretch of river (map 3.1.1) is carried out from July 2015 to April 2016 by using rectangular mosquito net (of mesh size 1mm). The species they hide under the laterite rocks and bamboo spaith are handpicked. Colour pattern of the specimens noted during collection time itself and photographs taken with the help of Nikon Coolpix L340, specimens are preserved in absolute alcohol. The date of collection and site of collection are recorded.

3.3 Morphological Identification of the Species

3.3.1 Morphology of genus *Macrobrachium* Bate, 1864 of family Palaemonidae Rafinesque, 1815

The relevant literature has been referred for the identification of the Palaemonid to the species level (Chace and Bruce, 1993; Jayachandran 2001; Pillai and Unnikrishnan 2012, 2013a, 2013b, 2013c, 2014). The marked characteristic difference of the specimens collected from the Neyyar river site is compared with description given in the literature. The general morphological characters observed for the study are specified in figure. 3.3.1.1, figure. 3.3.1.2, figure. 3.3.1.3, figure. 3.3.1.4, figure. 3.3.1.5. The minute characteristic features such as egg, telson spinules, denticles in the chelate legs, rostral teeth, type spines, setae, were observed by using dissection microscope, compound microscope, stereo microscope and pictures of the morphological characters were taken by using motic software version. The major morphological characters observed for the study are rostral teeth pattern, number of teeth in both ventral and dorsal region, teeth in post-orbital region, extension of rostrum, colour of the specimen, pattern of setae in the pereiopod, telson length compared to the outer spine of uropod, antennal and hepatic spines location.

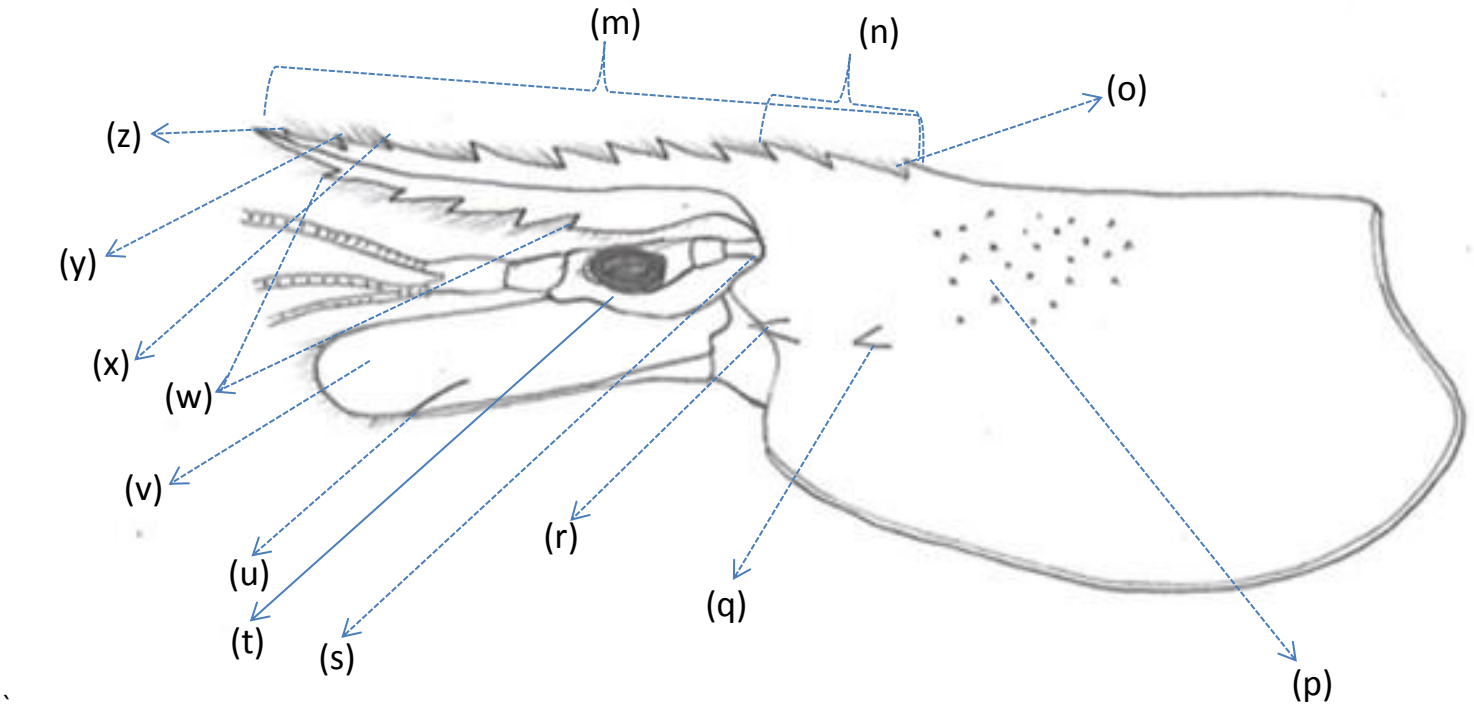


Figure 3.3.1.2: General morphological characters of taxonomic importance present in *Macrobrachium spp*. (Carapace) (m) Dorsal teeth,(n) Post-orbital teeth, (o) Setae present in between the teeth, (p) Spinules present ion carapace, (q) Hepatic spine, (r) Antennal spine (s) Orbit, (t) Antennular peduncle, (u) Outer spine of scaphocerite of antenna, (v) Scaphocerite (w) Ventral teeth, (x) Antepenultimate teeth, (y) Penultimate teeth, (z) Ultimate teeth

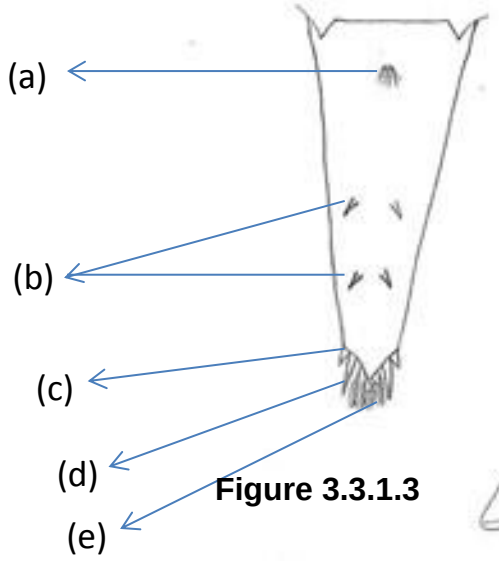


Figure 3.3.1.3

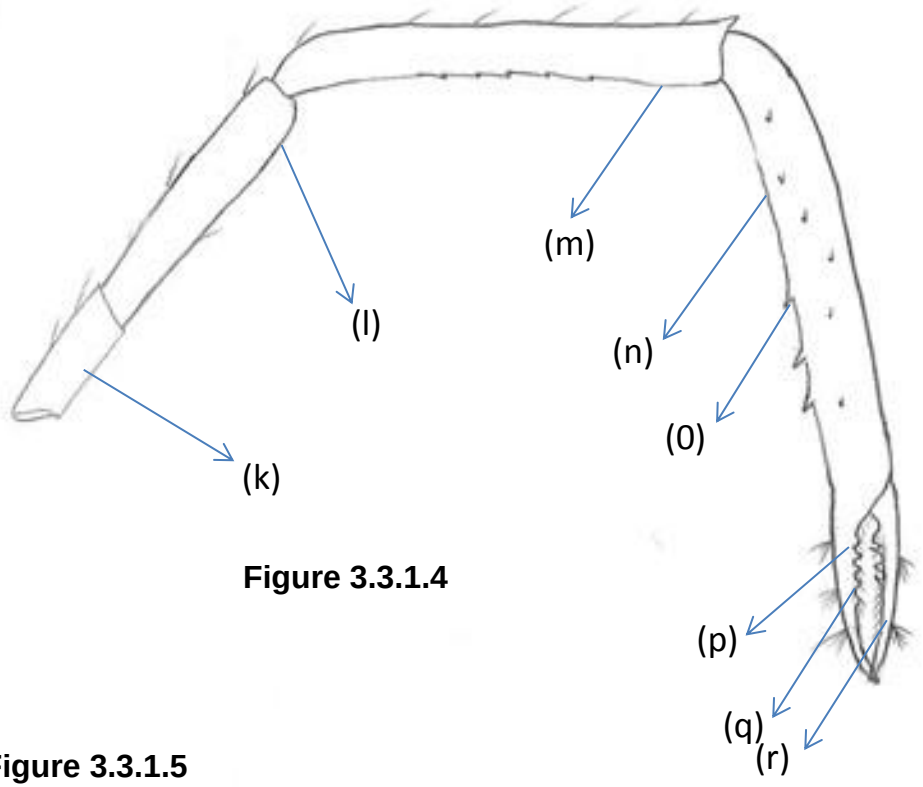


Figure 3.3.1.4

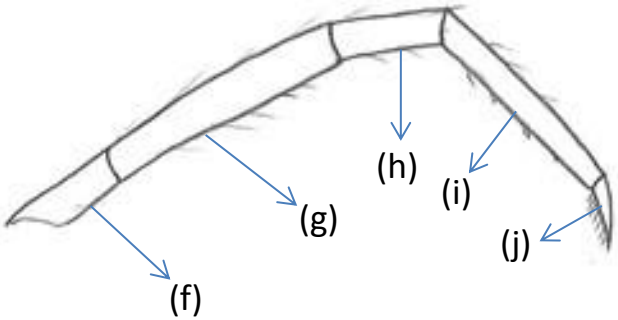


Figure 3.3.1.5

Figures 3.3.1.3- 3.3.1.5: General morphology of chelate and Non- chelate legs of Macrobrachium spp.(a) Tuft of setae present in proximal part of telson, (b) Paired dorsal spines of telson, (c) Outer pair of distal spines of telson, (d) Inner distal spine of telson, (e) Plumose setae (f) Ichium of non-chelate legs, (g) Merus of non-chelate leg, (h) Carpus of non-chelate leg, (i) Propodus of non-chelate leg, (j) Dactylus of non-chelate leg, (k) Ischium of chelate leg, (l) Merus of chelate leg, (m) Carpus of chelate leg, (n) Palm of chelate leg, (o) Spinules in the leg, (p) Denticles, (q) fixed fingers, (r) movable finger

3.3.2 Meristic counts taken for the taxonomic purpose *Macrobrachium* species of Neyyar river

Table 3.3.2.1 Meristic characters taken for the taxonomic analysis *Macrobrachium* spp. Of Family Palaemonidae

Sl.no:	Code	Meristic character
1	DT	Number of Dorsal teeth
2	VT	Number of Ventral teeth
3	POT	Number of Post orbital teeth
4	URO	Number of accessory spines in uropodal exopod
5	DMFL	Number of Denticles in movable finger of large 2nd pereiopod
6	DIFL	number of Denticles in immovable finger of large 2nd pereiopod
7	DMFS	Number of Denticles in movable finger of small 2nd pereiopod
8	DIFS	Number of Denticles in immovable finger of small 2nd periopod
9	PLU	Number of Plumose setae

3.3.3 Morphometric measurements used for the caparison of species of genus *Macrobrachium* Bate, 1864 of the family Palaemonidae Rafinesque, 1815

Twenty four morphometric characters have been measured with the help of Vernier calliper and expressed in millimetres. The way taken for the measurements are shown in figure 3.3.3.1, figure 3.3.3.2, and figure 3.3.3.3 the measurements and its complete abbreviation expressed is given in table 3.4.1 this measures are used for the further statistical analysis for describing the variations between the species.

Table 3.3.3.1: Morphometric measures of *Macrobrachium* spp. have been measured for the analysis

Sl. No	code	Code refers to	Distance of body parts covered for the parameter
1	TL	Total length	The tip of rostrum to the tip of telson
2	CL	Carapace length	From the tip of the rostrum to posterior margin of carapace
3	RL	Rostral length	Orbit to the tip of rostrum
4	FPL	First pereiopod length	Proximal part of ischium to tip of finger

5	SLPL	2^{ND} Large pereiopod	Proximal part of ischium to tip of finger
6	SSPL	2^{nd} small periopod length	Proximal part of ischium to tip of finger
7	TEL	Telson length	Proximal margin of telson to the posterior tip of telson
8	FSTEL	Telson length up to 1^{st} pair of dorsal spine	Proximal margin of telson to proximal pair of dorsal spine
9	SSTEL	Telson length up to 2^{nd} pair of dorsal spine	Proximal margin of telson to distal pair of dorsal spine
10	FPIL	Ischium length of 1^{st} pereiopod	Total length of ischium
11	FPML	Merus length of 1^{st} pereiopod	Total length of merus
12	FPCL	Carpus length of 1^{st} pereiopod	Total length of carpus
13	FPPL	Palm length of 1^{st} pereiopod	Total length of palm
14	FPFL	Finger length of 1^{st} pereiopod	Total length of finger
15	SLPIL	Ischium length of large 2^{nd} pereiopod	Total length of ischium
16	SLPML	Merus length of large 2^{nd} pereiopod	Total length of merus
17	SLPCL	Carpus length of large 2^{nd} pereiopod	Total length of carpus
18	SLPPL	Palm length of large 2^{nd} periopod	Total length of palm
19	SLPFL	Finger length of large 2^{nd} pereiopod	Total length of finger
20	SSPIL	Ischium length of small 2^{nd} pereiopod	Total length of ischium
21	SSPML	Merus length of small 2^{nd} pereiopod	Total length of merus
22	SSPCL	Carpus length of small 2^{nd} pereiopod	Total length of carpus
23	SSPPL	Palm length of small 2^{nd} pereiopod	Total length of palm
24.	SSPFL	Finger length of small 2^{nd} pereiopod	Total length of finger

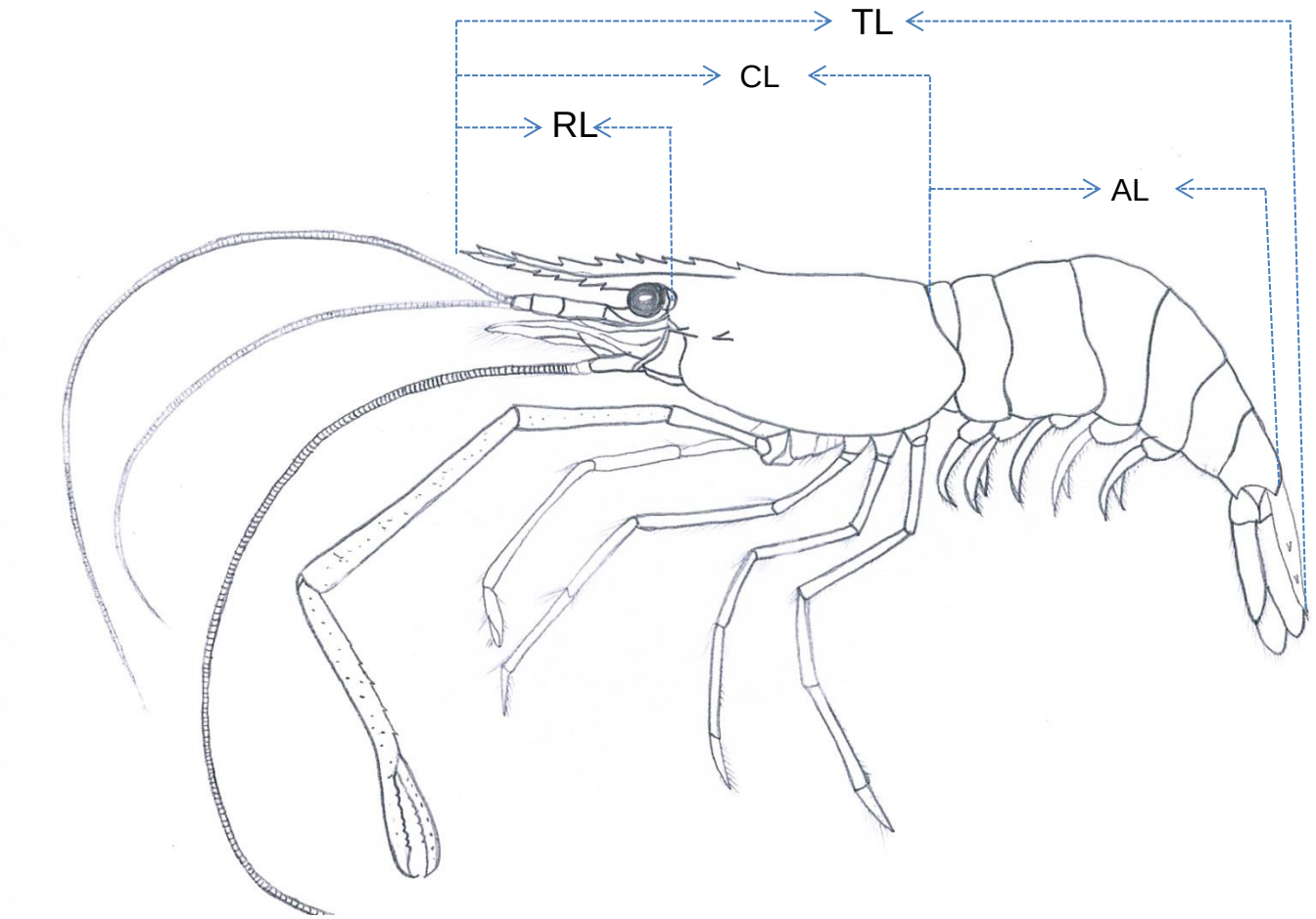


Figure 3.3.3.1: Pattern of morphometric measurements of *Macrobrachium* spp. TL – Total length, RL – Rostral length, CL – Carapace length, AL – Abdominal length

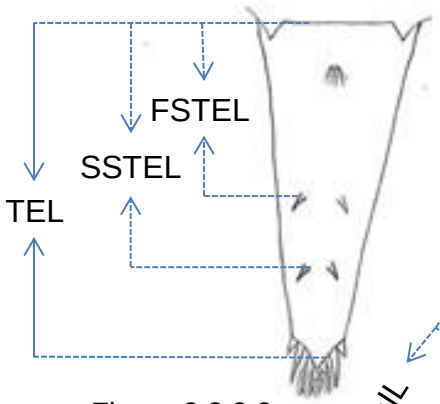


Figure 3.3.3.2

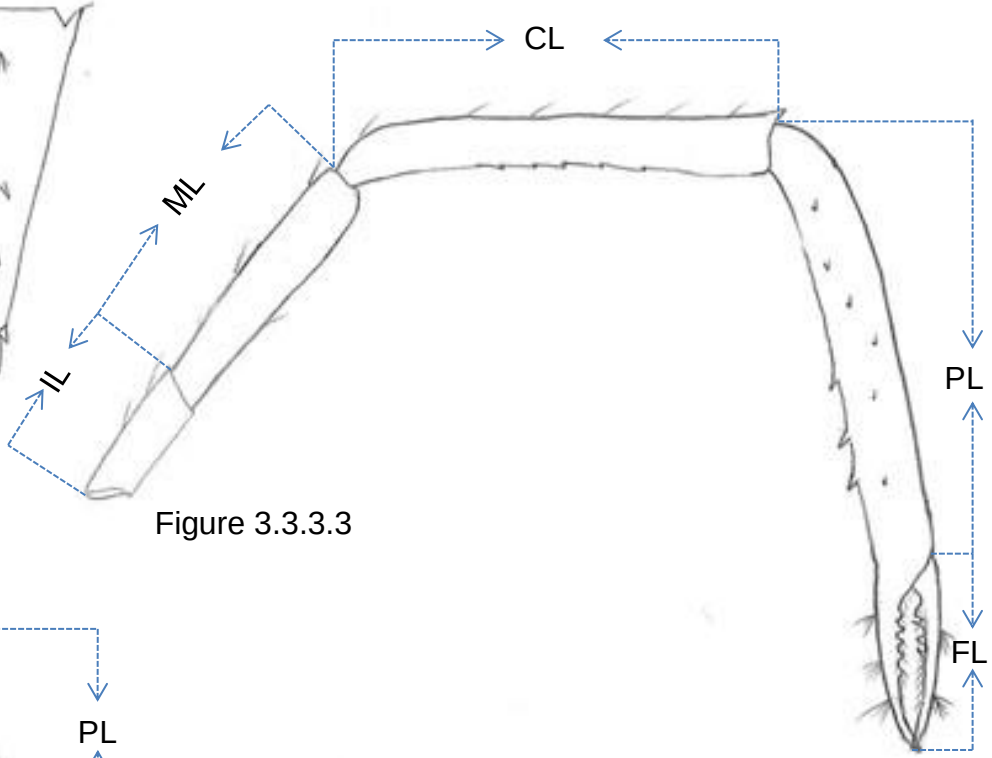


Figure 3.3.3.3

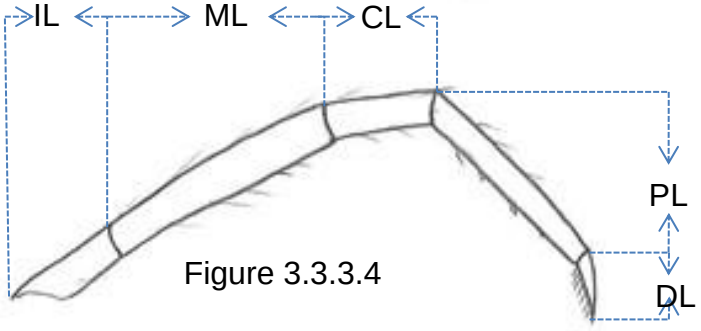


Figure 3.3.3.4

Figures 3.3.3.2- 3.3.3.5: Pattern of morphometric measurements of telson, chelate leg and non-chelate legs of *Macrobrachium* spp. (a) TEL – length of telson, FSTEL - length of telson up to first pair of dorsal spine, SSTEL – Length of telson up to the second pair of dorsal spine, IL – Length of Ichium, ML – Length of merus, CL – Length of carpus, PL – Palm length of chelate leg, FL – Length of finger, PL – Length of Propodus in non-chelate leg, DL – Dactylus length

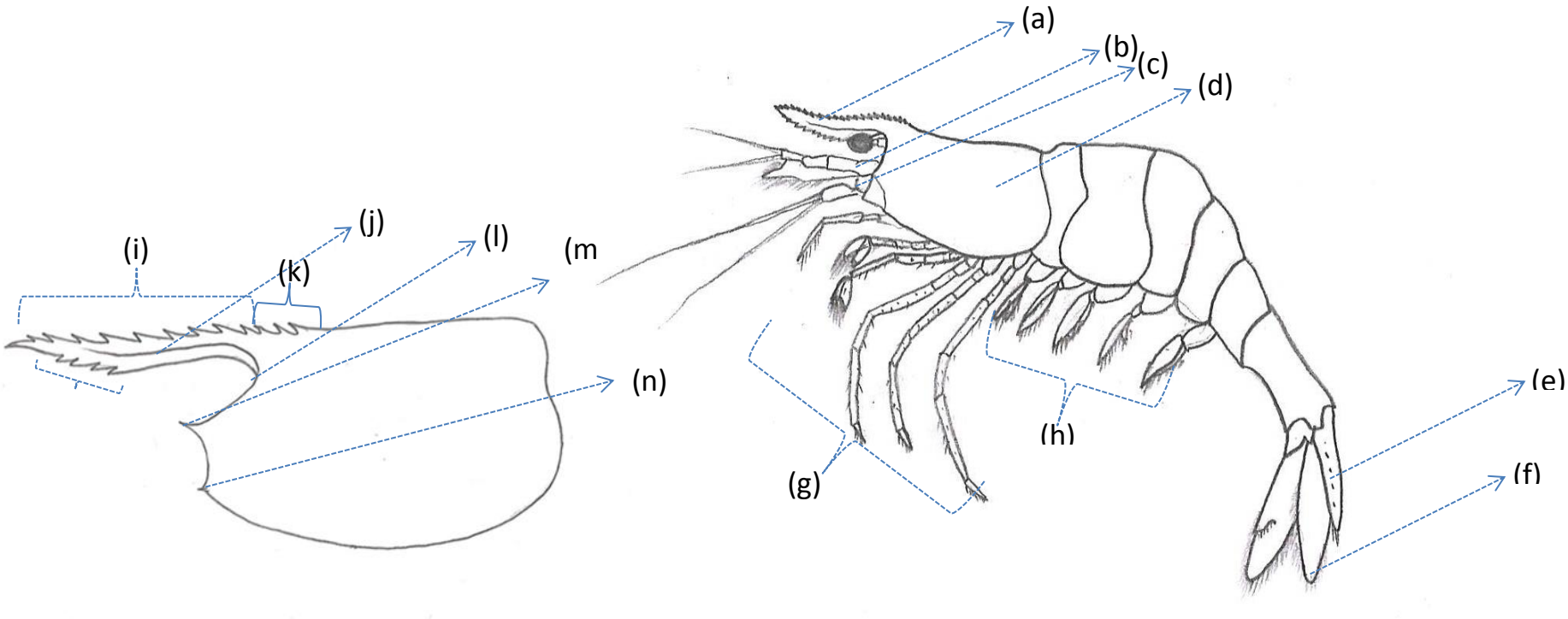


Figure 3.3.4.1: General morphology of *Caridina* spp. (a)Rostrum, (b) Antennule, (c) Antenna, (d) carapace, (e) Telson, (f) Uropod, (g) periopods, (h) Pleopods, (i) Dorsal teeth, (j) Adrostral carina, (k) Post orbital teeth, (l) Orbit, (m) Orbital angle, (n) ptergiostomean spine, (o) venral teeth

3.3.4 Morphology of genus *Caridina* H. Milne Edwards, 1837 of family Atyidae De Hann, 1849

The relevant literature has been consulted for the identification of the genus *Caridina* H. Milne Edwards, 1837 of family Atyidae De Hann, 1849, (De Man 1908a; Kemp, 1913; Tiwari & Pillai, 1968, 1971; Thomas *et al*., 1973; Jalihal *et al*., 1984; Richard & Chandran, 1994; Mariappan & Richard, 2006 and Cai & Ng, 2007, Jayachandran and Thomas, 2007, Thomas, 2012). The features observed during the identification given in the figure 3.3.4.1, figure 3.3.4.2, figure 3.3.4.3 and figure 3.3.4.4 The features observed by using dissection microscope, compound microscope, stereo microscope the pictures of the morphological characters are took by using motic software version.

3.3.5 Meristic measurement taken for the analysis of *Caridina* spp.

There are six meristic counts are taken for the analysis of discriminating the species one another

Table 3.3.5.1: Meristic counts in *Caridina* species chosen for analysis and observation

Sl.no:	Code	Meristic character
1	DT	Number of dorsal teeth
2	VT	Number of ventral teeth
3	POT	Number of post orbital teeth
4	URD	Number of spines Uropod diaeresis
5	TLD	Number of spines in dorsal surface
6	TLS	Number of spinules in distal part of telson

3.3.6 Morphometric characters chosen for the analysis of genus *Caridina* H. Milne Edwards, 1837 of family Atyidae De Hann, 1849

There are about five measurements are used for the morphometric study of *Caridina* species collected from the Neyyar river system. These measurements are shown in figure 3.3.6, table 3.3.6 and taken by using stereoscope.

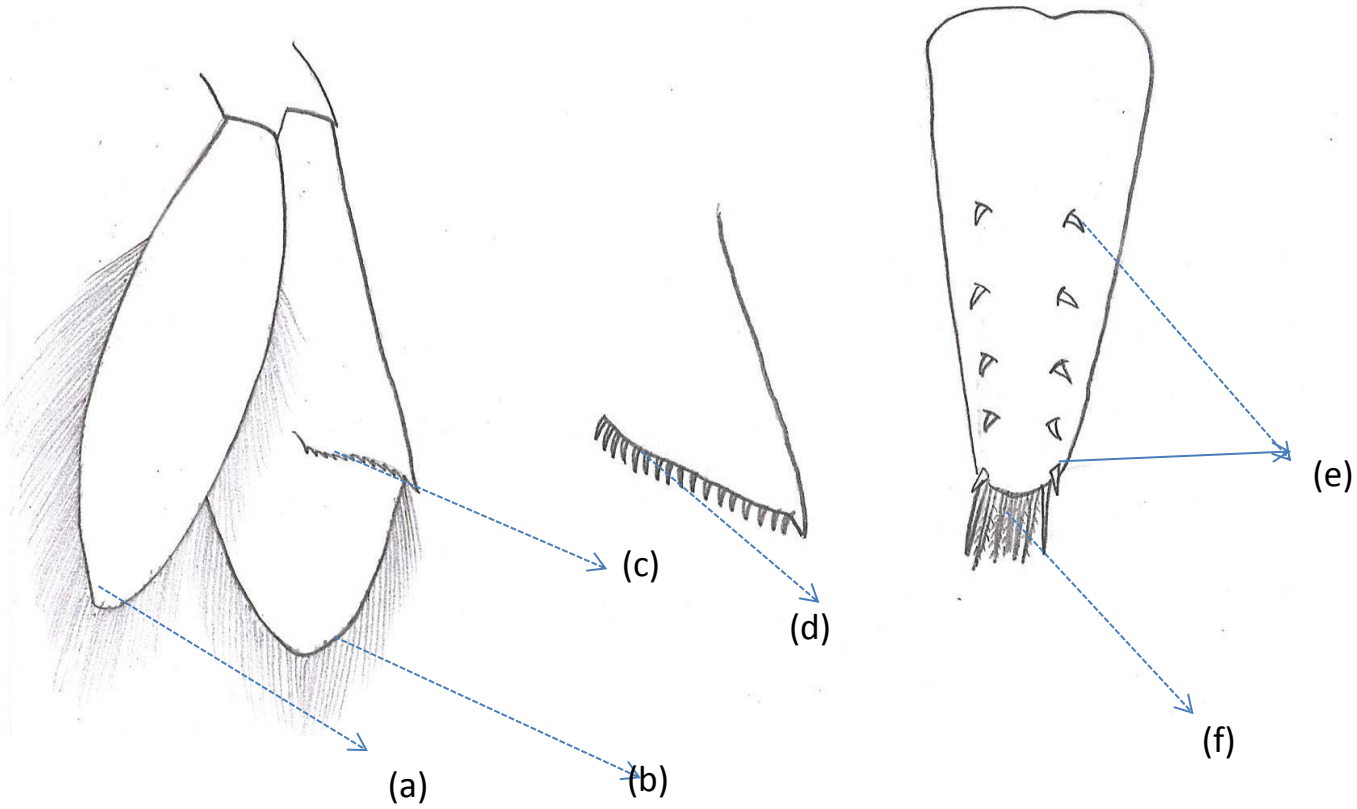


Figure 3.3.4.2: General morphological features used for the taxonomy of *Caridina* spp. **(**a) endopod of uropod, (b) exopod of uropod, (c) uropod diaresis, (d) spines on uropodal diaresis, (e) dorsal spines of telson, (f) distal spines of telson

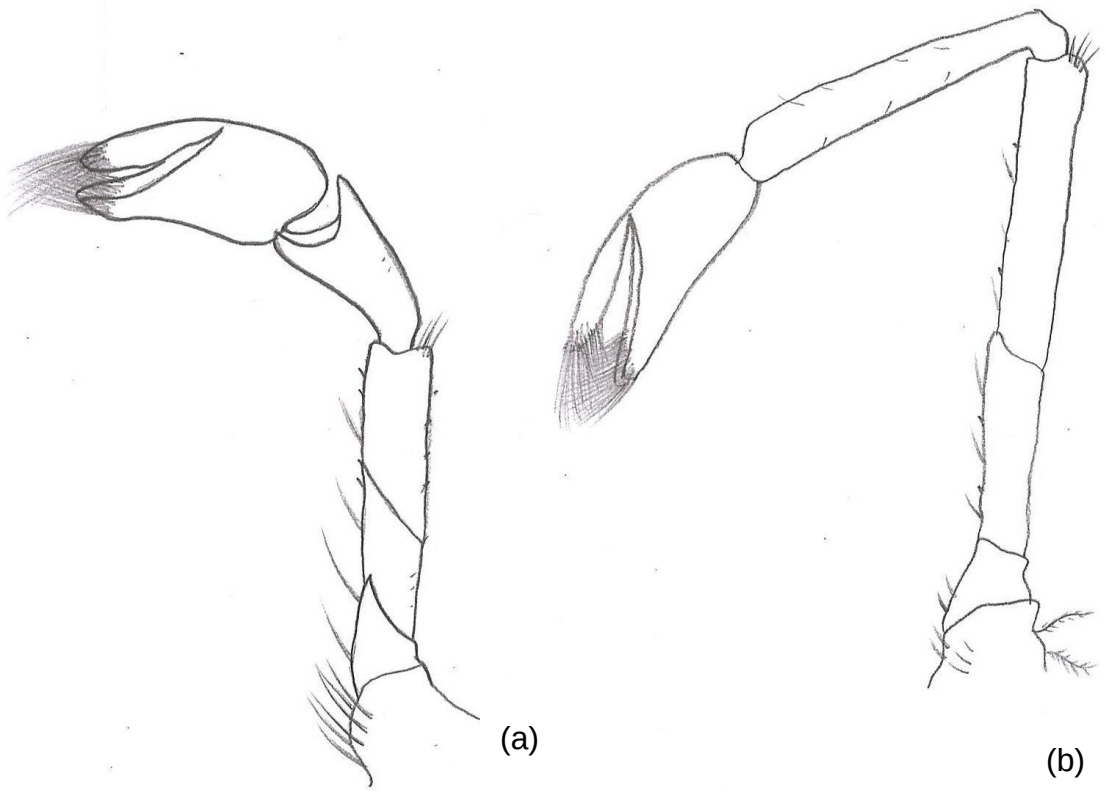


Figure 3.3.4.3: General morphological features used for the taxonomy of *Caridina* spp. (a) first periopod, (b) second periopod

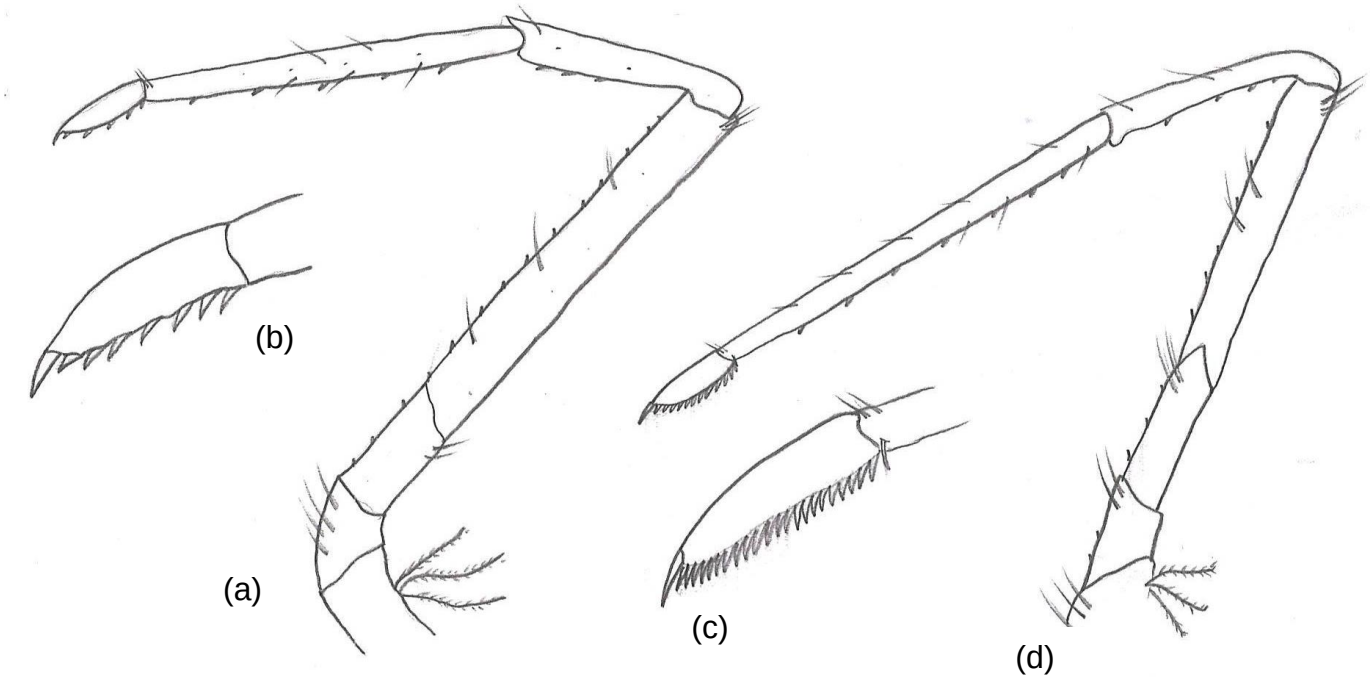


Figure 3.3.4.4: General morphological features used for the taxonomy of *Caridina* spp. (a) 3rd pereiopod, (b) dactylus of 3rd periopod, (c) 5th pereiopod, (d) Dactylus of 5th pereiopod

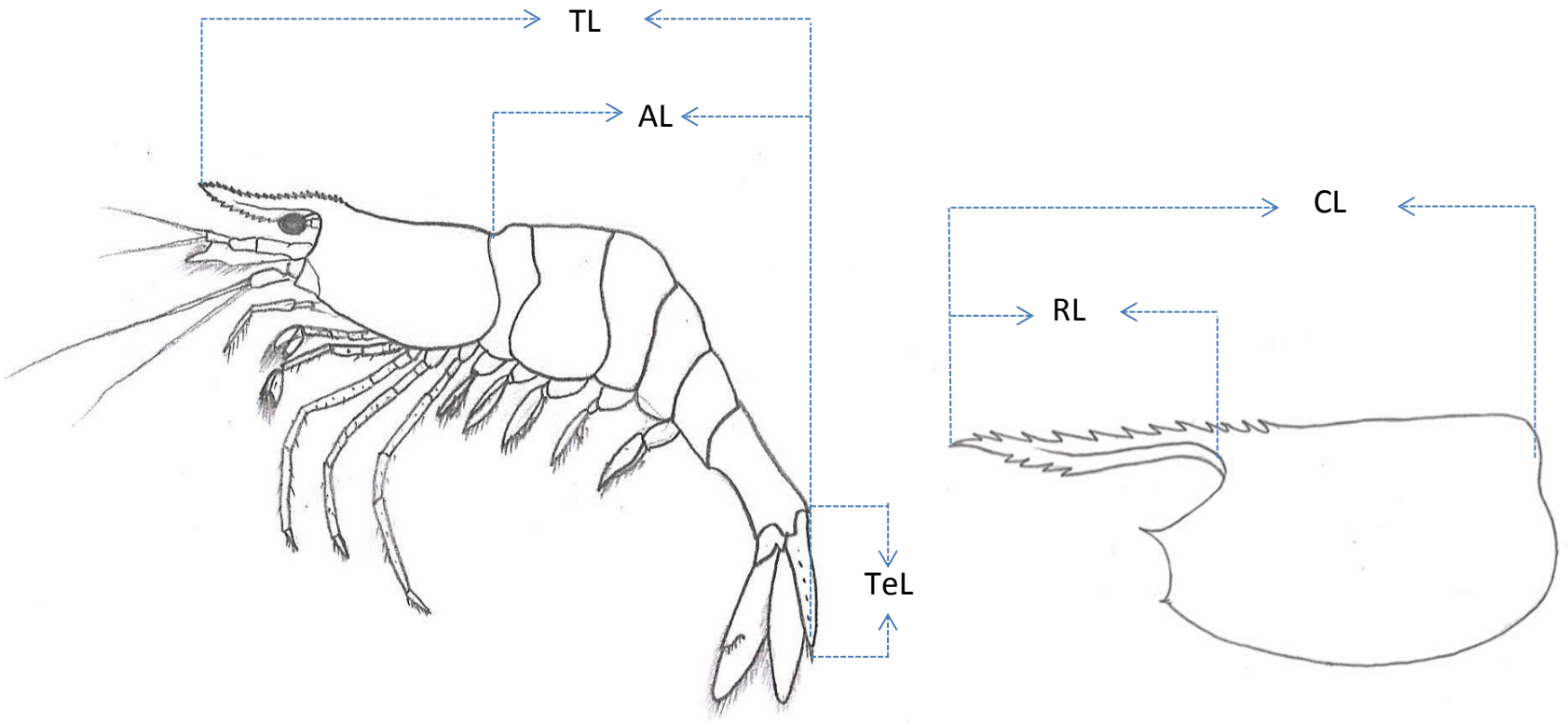


Figure 3.3.6: Pattern of morphometric measurements taken from *Caridina* spp. TL- Total length, AL- Abdominal Length, TeL- Telson Length, RL- Rostral Length, CL- Carapace Length.

Table 3.3.6.1: Morphometric measurements of *Caridina* spp. have been taken for analysis

Sl.no:	Code	Morphometric character
1	TL	Total length
2	AL	Abdominal length
3	CL	Carapace length
4	RL	Rostral length
5	TEL	Telson length

3.4 Barcoding Procedure

3.4.1 Material used for Barcoding

Five specimens of each species selected for Barcoding (if the total number of specimens is less than five all the specimens are used). The Pleopods and muscles are selected and preserved in absolute alcohol and stored in -20° C.

3.4.2 DNA isolation procedure

Simple salting out method (Miller *et al.,* 1988)

Steps

- Wash about 20mg of tissue in Millipore water and dry it in a tissue paper Homogenize the tissue in 700µl solution Ⅰ (Annexure II) in 2 ml tube
- Add 10µl of proteinase K in the vial
- Mix it properly with the help of a stirrer and cover the lid with the help of paraffin film
- Incubate the mixture in a water bath of temperature 55° C overnight (18-20 hrs)
- Remove the covering after incubation and keep the vial in 4 ° C about 10 minutes
- Add 200 µl of solution Ⅱ (Annexure II) and mix the aliquot by inverting the vial up and down
- Keep it in ice (4 ° C) for 5 minutes.
- Centrifuge the mixture at 8000 rpm for 15 minutes 4° C
- Take 1ml of transparent supernatant in 1.5 ml tube

- Add 500 µl molecular grade absolute alcohol and keep it in -20° C for 1 hour
- Centrifuge at 10000 rpm for 10 minutes at ° C
- Take the pellet and washed with 70% alcohol at 10000 rpm for 10 minutes
- Repeat the procedure two times
- Take the pellet and dried it under the fan of laminar flow
- Add 20 µl TAE (10X) buffer and dissolve the pellet
- Add 1 µl RNase enzyme for removing the RNA and incubate one hour at 37° C (if necessary)
- Load it in 1% agarose gel for checking the DNA

3.4.3 Concentration calculation and dilution of DNA

The concentration of DNA is estimated with the help of nanodrop. The concentration of DNA make up to 100 nano gram using nuclease free water.

3.4.4 Agarose Gel electrophoresis

Reagents for electrophoresis

1. Agarose
2. Ethidium Bromide: 10mg/ml
3. DNA sample
4. Electrophoresis buffer, 0.5X TAE (Diluted from 50X stock solution)
5. 6x DNA loading dye (0.25% bromophenol blue 0.25% xylene Cyanol and 30% glycerol)
6. DNA marker (100 plus)

3.4.5 Protocol for Agarose gel electrophoresis

The extracted DNA diluted up to the concentration of 100 umol. Agarose 1% was prepared in 0.5X TAE buffer by heating in microwave oven till the agarose completely dissolved. Appropriate amount of Ethidium Bromide was added and mixed properly before casting in tray. Set the tray by arranging the comb and pour the melted agarose in to the tray, within 20-25 minutes the gel will solidify. The comb is removed after solidification and keeps the tray in to the buffer tank unit of electrophoresis unit. DNA sample (1 µl) was mixed with 0.5X TAE buffer (5 µl) and 6X loading dye (2 µl), and loaded in to the wells. Electrophoresis was carried at a voltage of 80V till the dye migrated up to sufficient distance. The photograph of the gel is taken by using the Biorad gel documentation unit. For PCR product 2% gel is used for electrophoresis.

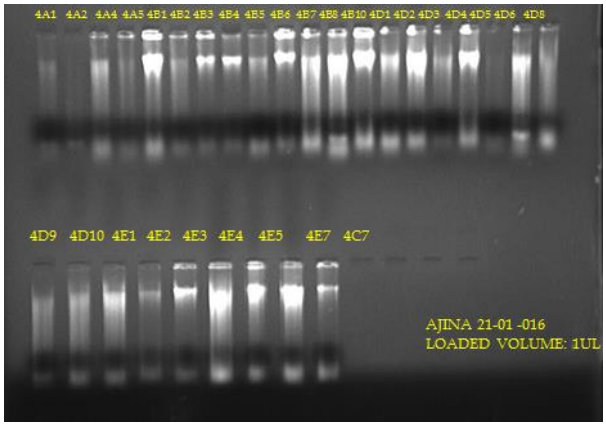


Plate 3.4.5.1: Isolated DNA in agarose gel

3.4.6 Polymerised Chain Reaction

3.4.6.1 Primers used for the amplification (da Silva *et al.,* 2011)

Forward primer -CrustDF1

GGTCWACAAAYCATAAAGAYATTGG

Reverse primer-CrustDR1

TAAACYTCAGGRTGACCRAARAAYCA

3.4.6.2 Master Mix preparation (for 25 µl volume of PCR mix)

- Nuclease free water (18.85 µl)
- Taq buffer (2.5 µl)
- DNTPs (0.5 µl)
- Primer Crustacean DFI (1 µl)
- Primer Crustacean DR1 (1 µl)
- Taq polymerase (0.15 µl)
- DNA template (1 µl)

3.4.6.3 PCR condition

- Initial denaturation temperature 94° C for 5 minutes
- Denaturation temperature 95° C for 1 minutes
- Annealing temperature 43° C for 1 minutes
- Extension 72° C for 1.5 minutes
- Final extension 72° C for 7 minutes

The number of cycles for PCR is 35cycles

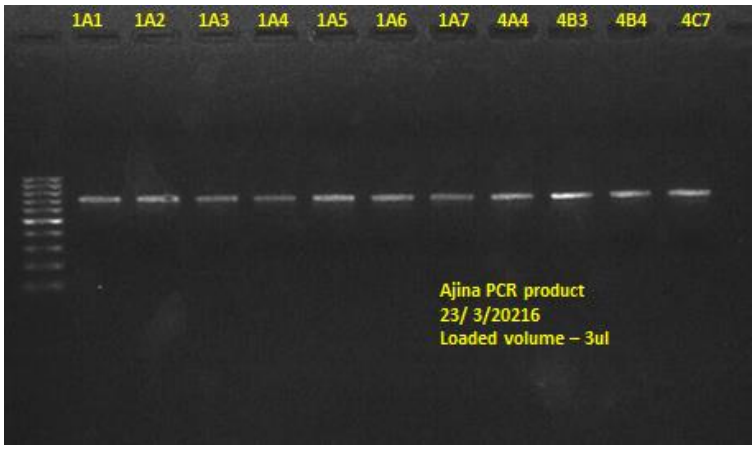


Plate 3.4.6.1: PCR product in agarose gel

3.4.7 DNA sequencing

The Non-purified PCR product of 75 µl sends to Xcelris Labs Ltd, Old Premchandnagar Road, Opp. Satyagrah Chhavani, Satellite, Ahmedabad-380054, Gujarat, India.

3.4.8 Alignment and sequence analysis

Approximate size of 650 bp of cytochrome oxidase region of mitochondrial DNA was sequenced. The alignment of the sequence is done by using Mega 7.0.14 the sequence was trimmed based on the NCBI ORF finder.

3.5 The endemism and the IUCN status of the family Palaemonidae and Atyidae present in the Neyyar river

A list of all the species present in Neyyar river of two families Palaemonidae Rafinesque, 1815 and Atyidae De Hann, 1849 reported from the present study as well as earlier work (collected from the literature) has been made. The distribution information of the organism confirmed with the help of IUCN Red List of Threatened Species (*www.iucnredlist.org)* and the data from the checklist of freshwater shrimp (De Grave and Fransen, 2011). The Threats to the species is observed at the time of sample collection itself following the papers of Abraham *et al.* (2011) Raghavan *et al.* (2015).

3.6 Analysis of data

The data for fulfilling objectives of study was entered in spreadsheets and analysed using statistical tools. Microsoft Excel, Primer, Statistica, Trail Ver. 12 statistical tools used for data analysis.

Results

4. RESULTS

4.1 Species under the Families Palaemonidae and Atyidae Identified From River Neyyar

During the research period (August 2015 to June 2016) about nine species of *Macrobrachium* comes under the family Palaemonidae and three species of *Caridina* comes under the family Atyidae has been collected, identified and described. The classification up to the family level given in fig 4.1.1

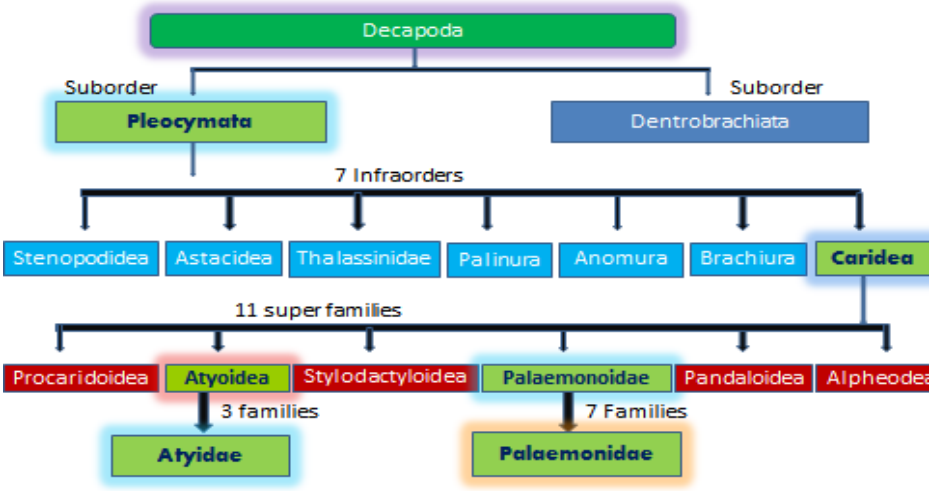


Figure 4.1.1: Taxonomic position of families Palemonidae and Atyidae in Crustacean order Decapoda

4.1.1 Species comes under Family Palaemonidae collected from Neyyar river

Nine species has been collected from three stations of 100m stretch and species were *Macrobrachium abrahami,* Pillai, Unnikrishnan and Kumar 2014, *Macrobrachium aemulum keralauni,* (Pillai and Unnikrishnan, 2013c), *Macrobrachium indicum,* Jayachandran and Joseph, 1986, *Macrobrachium canarae,* (Tiwari, 1958), *Macrobrachium idella idella,* (Hilgendrof, 1898), *Macrobrachium idella georgii,* Jayachandran and Joseph, 1985, *Macrobrachium*

spp., *Macrobrachium prabhakarani,* Pillai and Unnikrishnan, 2012, and *Macrobrachium scabriculum,* (Heller, 1862)

4.1.2 Species comes under Family Atyidae collected from Neyyar river

Three species has been collected from two stations of 100m stretch and species were *Caridina mathiassi, Caridina natarajani* and *Caridina gracilirostris.*

Table 4.1: Details of various species of the genus *Macrobrachium* Bate, 1864 of family Palaemonidae Rafinesque, 1815 and genus *Caridina* H. Milne Edwards, 1837 of family Atyidae De Hann, 1849 in Neyyar river

Station number	**Collection stations and Location**	**Diversity of species under the genus *Macrobrachium* Bate, 1864**	**Diversity of species under the genus *Caridina* H. Milne Edwards, 1837**
1.	Near Amaravila Bridge (8°38'66" N, 77°09'23" E)	*Macrobrachium abrahami, Macrobrachium aemulum keralauni, Macrobrachium indicum, M. scabriculum M idella idella,* M*. idella georgi, M. canarae Macrobrachium prabhakarani*	*Caridina mathiassi C. gracilirostris C. natarajani*
2.	Near Pzhamala Devi temple, Perunkadavila (8°44'87" N, 77°10'15" E)	*Macrobrachium scabriculum, M. lamarrei*	*Caridina mathiassi*
3.	Near Neyyar dam (8°53'26" N, 77°13'91" 77.139192)	*M idella idella Macrobrachium scabriculum*	-

4.2 Description Species Comes Under the Family Palaemonidae

4.2.1 *Macrobrachium abrahami* Pillai, Unnikrishnan and Kumar, 2014

*Macrobrachium abraham*i Pillai, Unnuikrishnan and Kumar, 2014. *Zootaxa* 3768: 546- 556.

Plate 4.2.1.1: *Macrobrachium abrahami*

Materials examined:

Three male specimens ranging in total length 28.86 mm - 40.95 mm were collected and identified as *M. abrahami*

Description:

Rostrum short and extending up to the level of distal part of antennular peduncle. The dorsal margin contains about 12 to 14 teeth in which 4 to 5 are post-orbital. The ventral margin with 2 teeth usually 3. Setae present in between the teeth both dorsal and ventral margin.

Carapace smooth and glabrous the antennal and hepatic spines characteristics of the species present.

The abdomen smooth and glabrous the lateral side of posterior margin of fourth and fifth pleura bear dark dots.

Telson robust and smooth. The dorsal margin of telson contains two pair of dorsal spines. The proximal pair is at the mid length of the telson and the

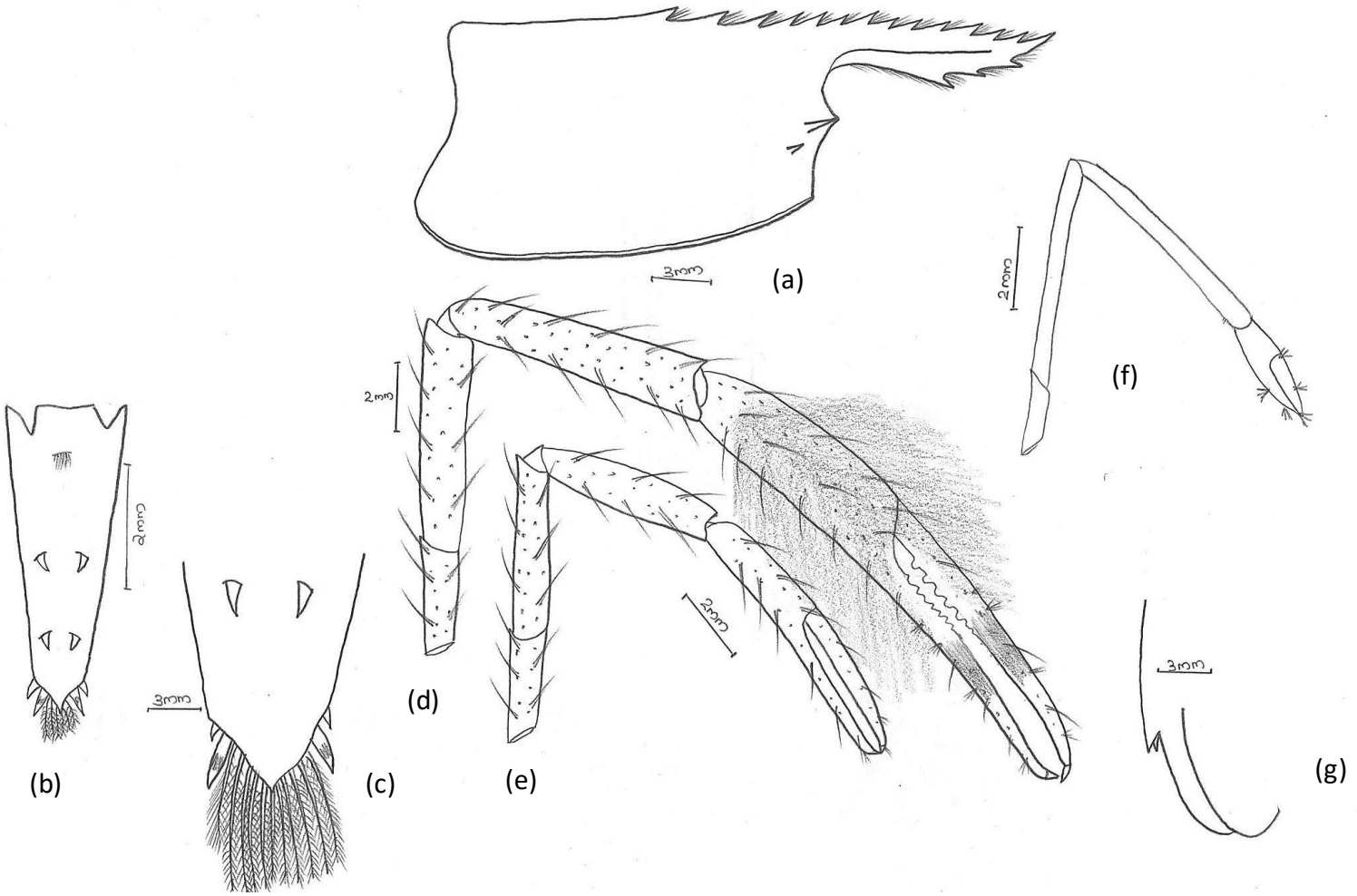


Figure 4.2.1: *Macrobrachium abrahami* (a) carapace, (b) telson, (c) distal portion of telson, (d) first chelate leg, (e) large 2nd chelate leg, (f) small 2nd chelate leg, (g) accessory spine in exopod of uropod

2nd pair is below the mid-level of the first pair and distal end of telson. The distal margin with 2 pairs of spines outer pair smaller and immovable. And the inner pair is large and movable. The plumose setae present in between the teeth and the number is more than 14.

Antennule typical in shape, the outer lateral spine of the antenna is apical in position.

First pereiopod typical, ischium short and flat length is less than that of the merus and carpus. Merus shorter than carpus; Carpus is the longest podomere. The fingers and palm are equal in length. The tuft of setae present in the fingers of first pereiopod.

The second periopod are unequal in shape and structure. The large 2nd pereiopod is slightly longer than the total length of species, the ratio is Total length: length of 2nd pereiopod is 4: 4.2.

In larger second pereiopod ischium smaller than the merus; merus shorter than the carpus. Palm length is equal to the length of merus. Fingers are the longest segment and about double that of ischium the ratio are Ischium: merus: Carpus: Palm: Fingers is 0.68:1: 1.13:1:1.44. The cutting edges of both the fingers has rows of minute denticles up to the ¾th of the length the size of the denticles decreases from anterior to the posterior portion. The minute spinules and stiff setae scattered over all podomeres. The distal portion of palm is covered with soft pubescent setae.

In second smallest pereiopod Ischium flat and equals to the palm, merus equal to carpus. The fingers are longer than all other segments. The ratio of the segments, Ischium: Merus: Carpus: Palm: fingers is 4:5:5:4:6. The entire leg is covered by minute spinules and stiff setae. The cutting edges devoid of denticles.

The Non Chelate legs are typical in shape.

Pleopods typical and appendix musculina just below the level of mid length of the endopod of 2nd pleopod.

Uropod typical and have a pair of accessory spines.

Rostral formula:

12-14 (4-5)/ 2-3

Distribution:

Southern rivers of Kerala (Vamanapuram river and Neyyar river)

Map 4.2.1.1: Distribution of *Macrobrachium abrahami*

(*http://www.mapsofindia.com/*)

Plate 4.2.1.2: Carapace of *M.abrahami*

Plate 4.2.1.3: Large second pereiopod

Plate 4.2.1.4: Fingers of large 2nd pereiopod

Plate 4.2.1.5: Anterior portion of fingers of large 2nd pereiopod *of M. abrahami*

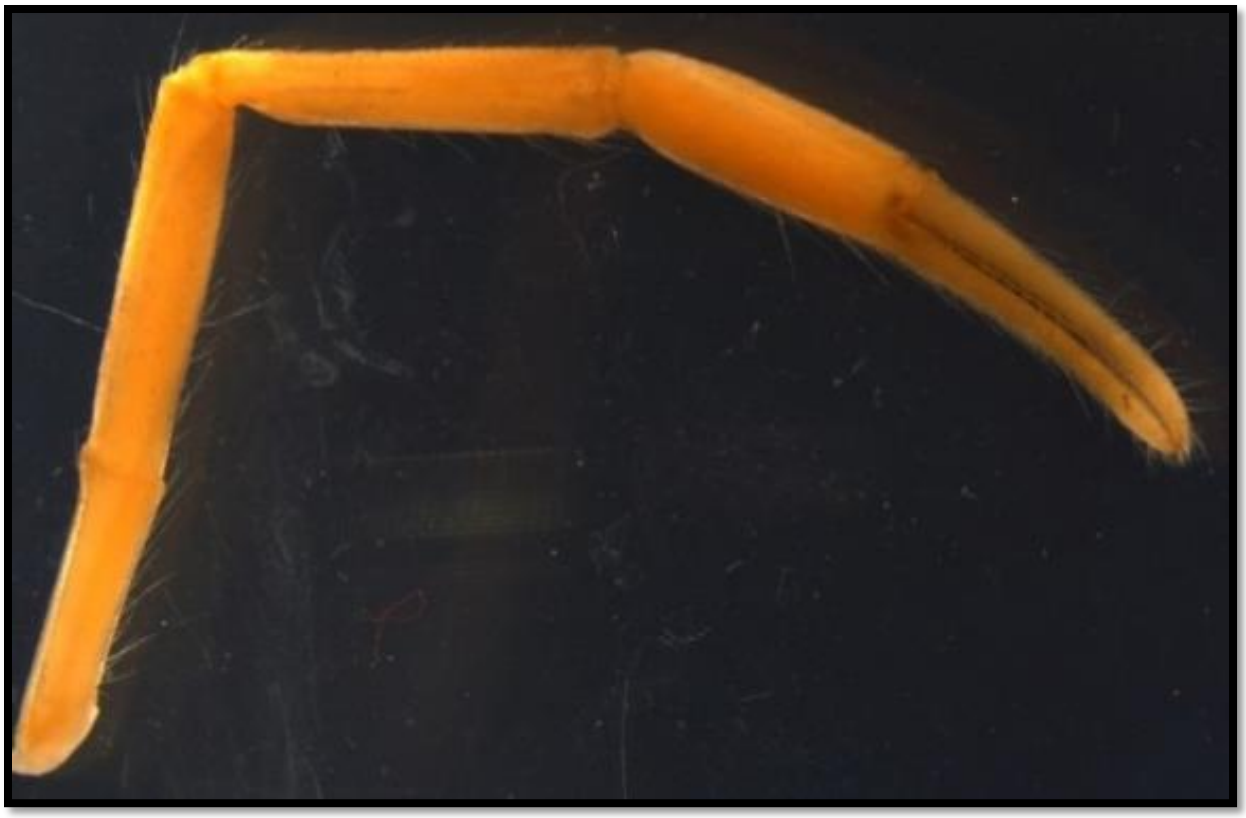


Plate 4.2.1.6: Small second pereiopod of *M.abrahami*

Plate 4.2.1.7: Fingers of small 2nd pereiopod *of M.abrahami*

Plate 4.2.1.7: Accessory spine in uropod of *M.abrahami*

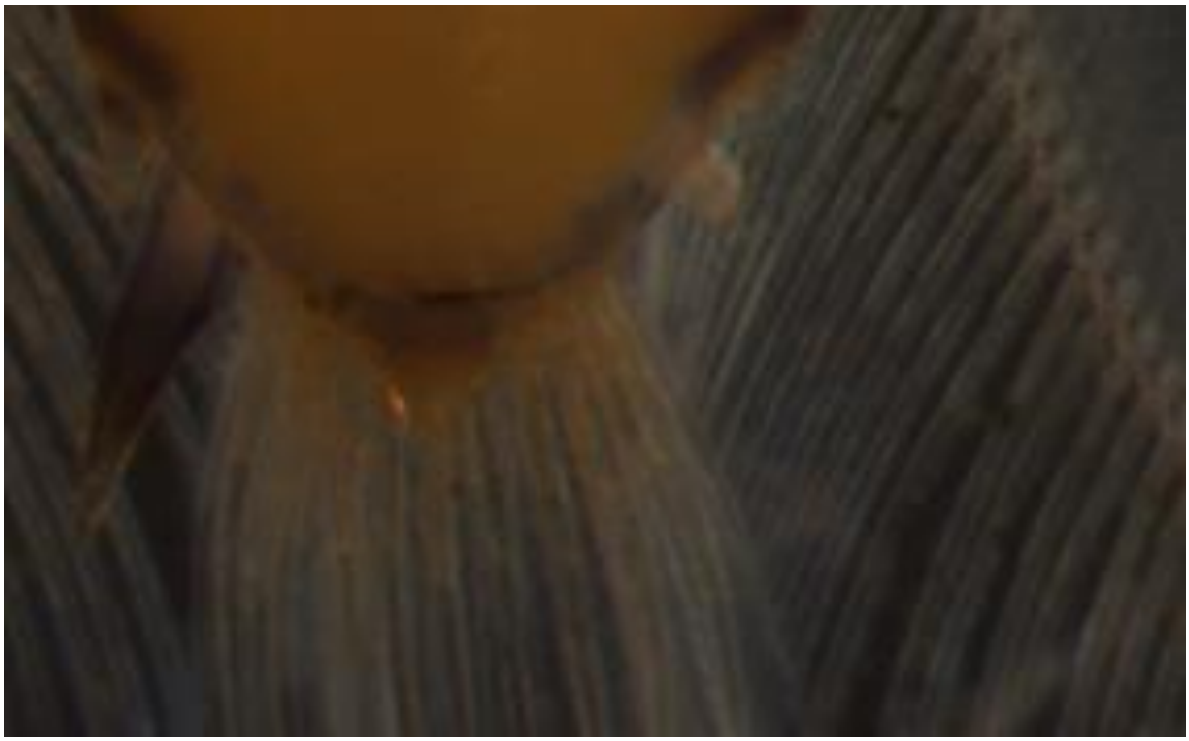


Plate 4.2.1.8: Distal end of telson of *M.abrahami*

4.2.2 *Macrobrachium aemulum keralauni* (Pillai and Unnikrishnan, 2013c)

Macrobrachium aemulum madhusoodani Pillai and Unnikrishnan, 2013c, *Zootaxa* 3741: 400.

Plate 4.2.2.1: *Macrobrachium aemulum keralauni*

Synonymy:

Macrobrachium aemulum madhusoodani Pillai and Unnikrishnan, 2013b

Materials examined:

One male specimen of length 56 +mm collected from station 1 was examined.

Description:

Rostrum short, extending up to the middle of distal segment of Antennular peduncle. Tip of the rostrum is sharp and directed upwards. Upper margin almost straight with 12 teeth of which five are post orbital. The first three dorsal spines are same in size and the gap between them are equal. The fourth and fifth teeth are slightly larger than the first three, sixth tooth above the orbit. The gap found in between teeth from the fourth to the eleventh are same. And from the penultimate to the ultimate it is slightly greater than the remaining.

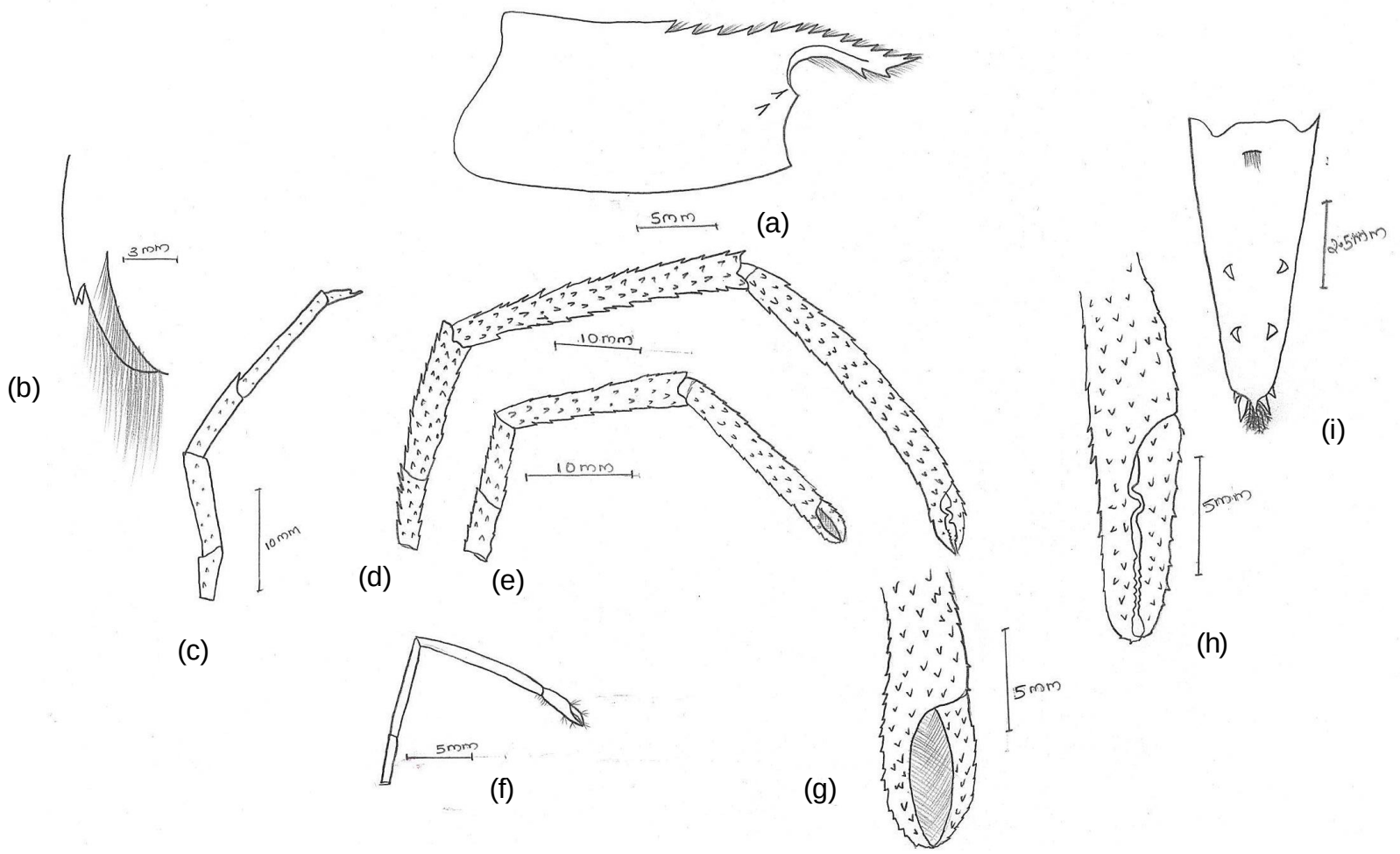


Figure 4.2.2: *Macrobrachium aemulum keralauni* (a) carapace, (b) acessory spine in uropod, (c) 3rd pereiopod, (d) 2nd large pereiopod, (e) 2nd small pereiopod, (f) 1st chelate leg, (g) fingers of 2nd second leg, (h) fingers of large 2nd large leg, (i) telson

Carapace smooth, antennal and hepatic spines present, hepatic spine is smaller and it is behind the antennal spine.

Abdomen smooth, the pleura of first three segments are rounded the other two are directed backward and last is ends in a spine.

Telson is stout and broad reaching up to the level of outer spine uropodal exopod. The dorsal surface bears two pairs of spines; the proximal pair is just below the middle of telson. The distal pair of spines almost the midway between the proximal pair of spines and distal end of telson. Telson ends with two pair of distal spines the outer pair immovable small and inner pair long and movable. About 14 plumose setae present in between the movable distal spines. The plumose setae present in the middle portion are longer than the lateral ones.

Antennular peduncle is normal in structure and the ratio of each segment is from proximal to distal is 4.73: 1.90: 2.69. The lateral spine of scaphocerite is subapical.

The first chelate leg is normal and devoid of spinules and spines, and it extends beyond the distal end of antennal scale. The ischium inflated and about the half of merus. Merus shorter than the carpus while carpus twice the length of chela. Palm is longer than that of fingers.

The 2nd chelate legs are unequal, in the specimen under study, right leg larger, and ischium stout and flattened. Merus shorter than carpus, about half the length chela. Carpus longer than merus and equal to the palm. Palm is about 3 times longer than fingers. The palm of larger chelate leg is narrower than that of smaller one. Fingers stout and equal and have distinct gap between the movable and immovable one. The cutting edge of movable fingers with two strong denticles and immovable with only one denticle. The distal cutting portion of cutting edge with 6-7 weak denticles on both cutting edges. The whole leg is covered by strong spinules.

In the smaller second chelate leg ischium stout and flat, shorter than merus. Merus is about half of chela. Carpus is longer than the merus and slightly shorter than palm. Palm is stout and its length is more than twice of the fingers. The width of palm is greater than the width of distal carpus. Fingers stout and equal, a

wide gap present in between them. Fingers are stout, equal, armed with numerous stiff setae crisscrossing on edges of fingers. Three to fifth pereiopods are non-chelate covered by strong spinules. Pleopods normal in shape. The appendix masculina is extending up to the middle of the exopod of the 2nd Pleopods.

Uropodal exopod with accessory spine.

Distribution:

Neyyar river, Kerala, India

Map 4.2.2.1: Distribution of *Macrobrachium aemulum keraluni*

(*http://www.mapsofindia.com/)*

Plate 4.2.2.2: Carapace of *Macrobrachium aemulum keralauni*

Plate 4.2.2.3 and Plate 4.2.2.4: Fingers of large and small 2nd pereiopods

4.2.3 *Macrobrachium canarae* (Tiwari, 1958)

Palaemon canarae Tiwari, 1958, *Rec. Indian Mus*., 53:298

Plate 4.2.3.1: *Macrobrachium canarae*

Synonym:

Palaemon canarae Tiwari, 1958

Materials examined:

30 specimens of size 12.53 mm to 37 mm were examined, (17 were male and 13 were females)

Description:

Rostrum very long , extending beyond the distal end of antennal scale. Rostrum generally slender and straight with distal end upwards. Upper magin is with about 9 to 10 teeth of which two teeth post orbital. The first tooth is smaller and separated from the 2nd by a little gap. The remaing teeth from the 2nd to 8th are similatr and equidistant. The ultimate teeth separated from the other by a large edentulus portion. Sometimes one or two teeth placed in between this gap. The ventral margin with 3 to 4 teeth. Setae present in beween the teeth, on the dorsal and ventral margin.

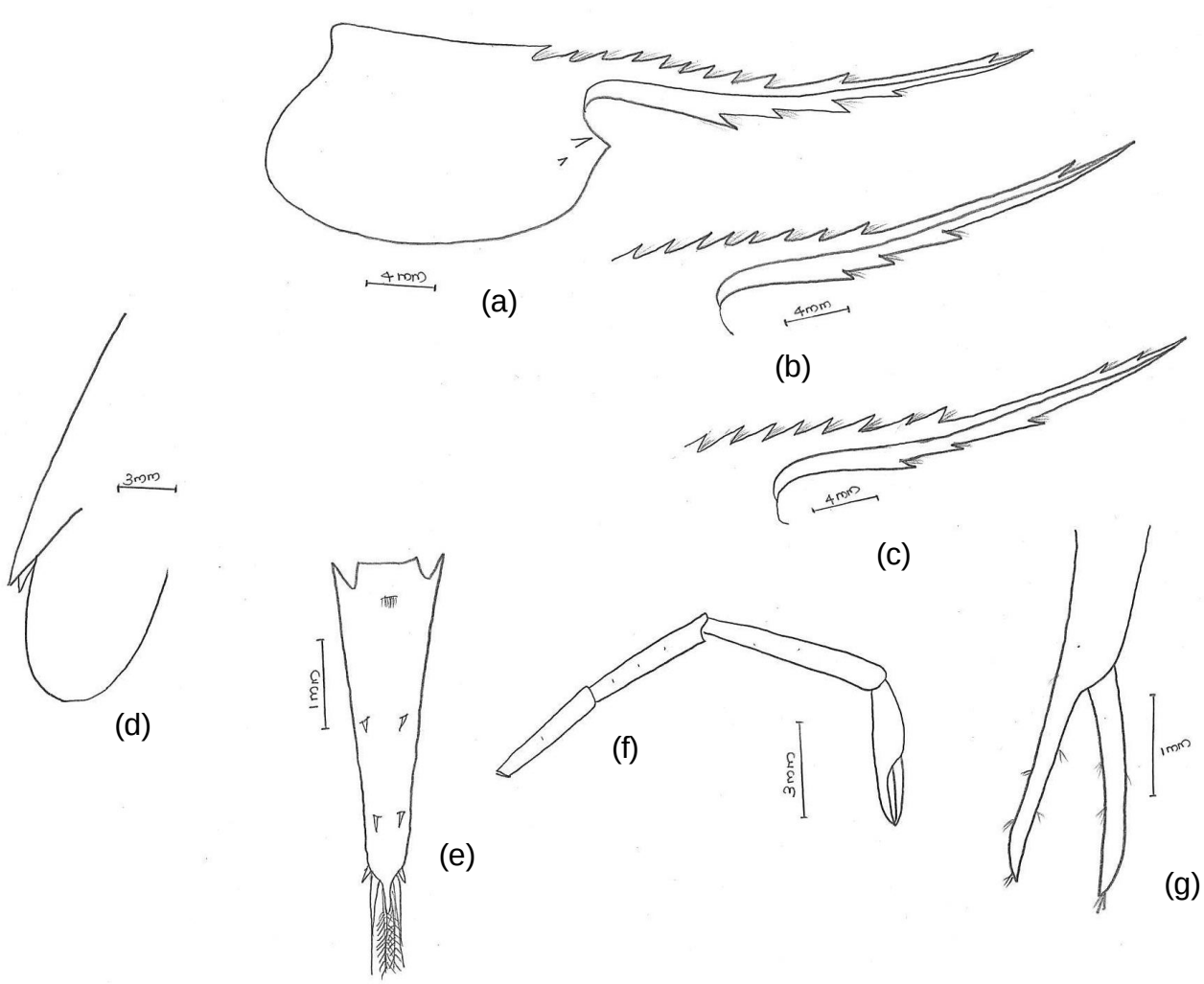


Figure 4.2.3: *Macrobrachium canarae* (a) carapace, (b and c) variation in the rostrum (d) acessory spine in uropod (e) telson, (f) 2nd pereiopod, (g) fingers of 2nd pereiopod

Carapace smooth and glabrous with antennal and hepatic spines, characteristics of the genus present. The sixth segment of abdomen is longer than the other segments and the pleura is ends up in sharp point.

Abdomen smooth. Pleurae of first three segments rounded in posterior margin. The fourth to six is directly backward.

Telson long and narrow and sharply pointed distally, reaching beyond the level of outer lateral spine of uropod. The dorsal surface is smooth with two pairs of spines. The proximal pair midway of the telson and distal pair mid way between proximal pair and distal tip. The distal end also with two pairs spines the first pair is small and immovable and second pair movable and long. Few plumose setae present in between the inner pair of spines.

Antennule normal in shape. Antennal exopod is broad and outer spine is apical in position.

The first pair of cheliped slender, reaching the tip of scaphocerite when extented. Ischium is smaller than the merus. Merus less than carpus. Carpus is the longest segment. Palm inflattened and equal to fingers.

The second chelate periopods equal and size is same size in both sexes. Ischium equal to merus. Carpus longest podomere. Palm is inflated and smaller than ischium and merus. Fingers are smaller in size and smooth. Scattered setae present in the fingers.

Non chelate legs are normal and the 5^{th} leg is longer than the other two.

Pleopods are normal in shape and structure.

Uropodal exopod with acessory spines.

Dstribution:

India (Kerala, Karnataka)

Map 4.2.3.1: Distributional map of *Macrobrachium canarae*

(*https://www.google.co.in*)

Plate 4.2.3.2: Rostrum of *M. canarae*

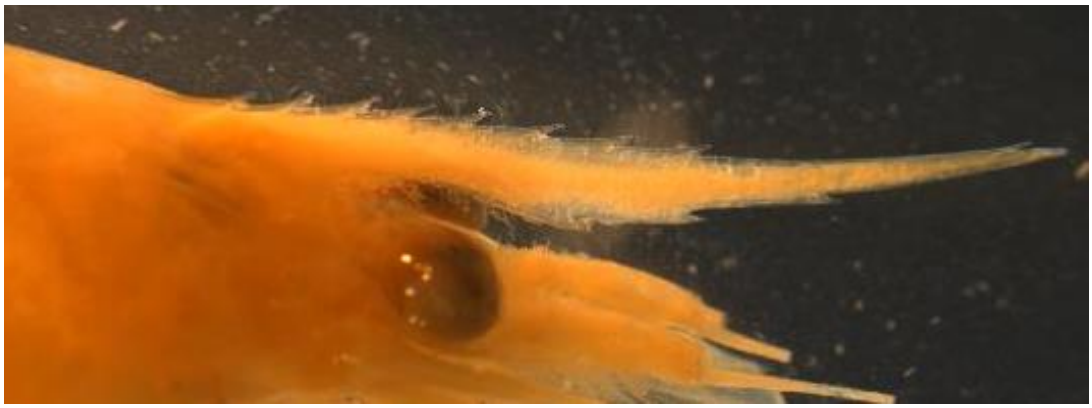


Plate 4.2.3.3: Variation in rostrum of *M. canarae*

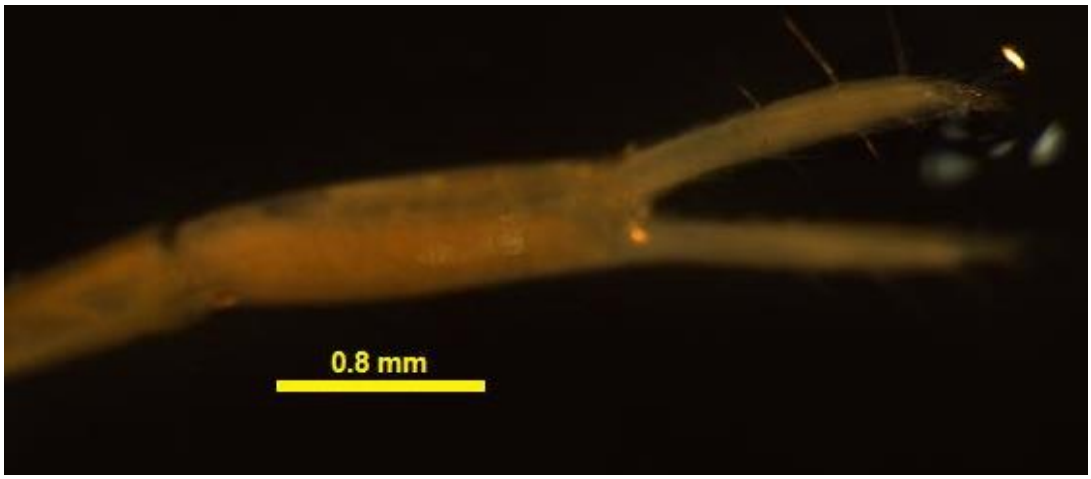


Plate 4.2.3.4: Chelae of 2nd Pereiopod of *M. canarae*

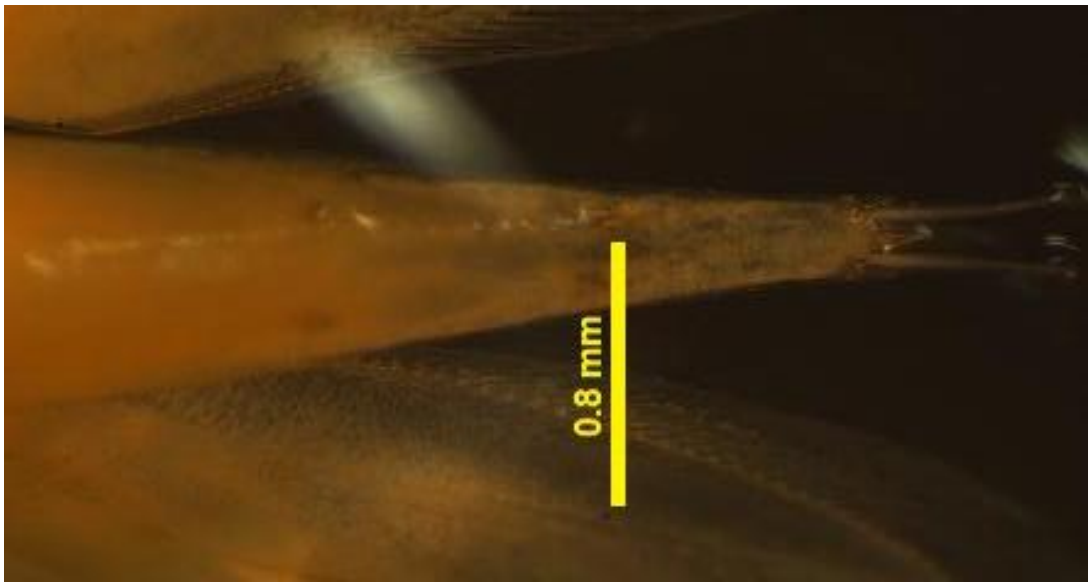


Plate 4.2.3.5: Telson *of M. canarae*

4.2.4 *Macrobrachium idella idella* (Hilgendrof, 1898)

Palaemon (*Eupalaemon*) *idea idella* Hilgendrof, 1898, Deutsch Ost-Afrika, 4(7):29

Plate 4.2.4.1: *Macrobrachium idella idella*

Synonymy:

Palaemon (*Eupalaemon*) *idea idella; Palaemon* (*Eupalaemon*) *multidens*; *Palaemon idea*; *Macrobrachium idella*)

Materials examined:

Seven male specimens of size 54.6 mm to 74.44 mm and non- berried females of size 45.0- 51.3 mm were collected from the station 1, station 2 and station 3 and identified based on key provided by Jayachandran, 2001.

Description:

Rostrum long, extending beyond the antennular peduncle and reaching up to the distal end of antennal exopod (scaphocerite). The dorsal profile slightly convex with about 11 to 13 teeth from which 2 are post orbital. There is a wide gap present in between the first and second dorsal teeth 3rd is closer to the second the closeness repeated up to the ante-penultimate tooth. A wide gap present in between the ante-penultimate and penultimate. The ultimate tooth is subapical in position. The ventral margin armed with 4 to 6 teeth. The posterior most tooth is backward to

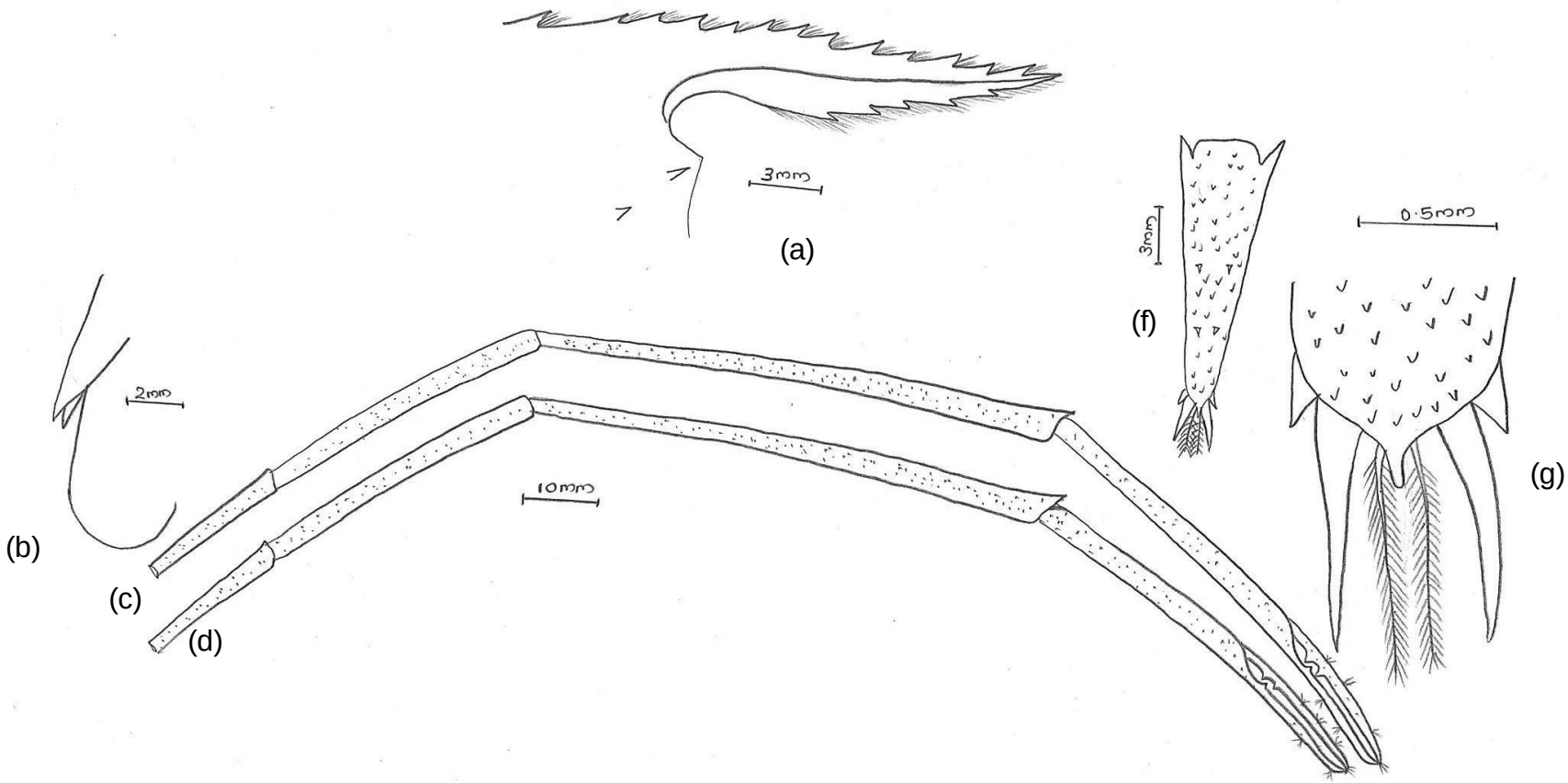


Figure 4.2.4: *Macrobrachium idella idella* (a) rostrum, (b) acessory spine in uropodal exopod, (c and d) 2nd pereiopods, (e) telson. (f) distal end of telson

the level of ante-penultimate teeth. The teeth in the ventral margin are equidistant. The setae are present in between the teeth.

Carapace with minute spinules. Antennal and hepatic spines present.

Abdomen smooth. Posterior margin of first four pleurae are smooth and round. The margins of other two segments (fifth and sixth) are rough with minute tubercles and directing backward and sixth ends with a spine.

Telson slender and long narrowing posteriorly reaching just beyond the level of outer spine of uropodal exopod. The dorsal surface with two pairs of spines. The proximal pair is midway of telson and distal pair is slightly above the midway between the proximal pair and the tip of telson. The tip of telson is pointed with two pair distal spines. The outer small and immovable and the inner is long and movable. One pair of plumose setae present in between the inner pair of spines. The tubercles are scattered over the telson and the dorsal spines are submerged it.

Antennule typical in structure. Antennal scales are broad and the outer lateral spine is subapical in position. Mandible maxilla, maxillula and maxillipid are typical.

The fist pereiopod is long and slender extending far beyond the antennal scale. Ischium flat and smaller than the merus (almost half). Merus shorter than carpus. Carpus three times longer than chela. The palm and fingers are almost equal and the fingers covered with setae.

Both the second chelate leg is same except a small difference in the palm size. Ischium is the smaller segment shorter than merus, merus shorter than carpus, carpus shorter than the Propodus. The fingers are more than half of the palm. The proximal part of the fingers with strong denticles, in fixed finger it is one and in movable it is two. The entire cutting edge is with setae.

Distribution:

The species is known with certainty from Tanzania and Madagascar, where it inhabits rivers and lakes. It has been extensively recorded from India.

Map 4.2.4.1: Distributional range of *Macrobrachium idella idella*

(*https://www.google.co.in*)

Plate 4.2.4.2: Rostrum of *Macrobrachium idella idella*

Plate 4.2.4.3: Palm and fingers of left second pereiopod of *M. idella idella*

Plate 4.2.4.4: Palm and fingers of right and left second pereiopod of *M. idella idella*

4.2.5 *Macrobrachium idella georgii* Jayachandran et Joseph, 1985b

Macrobrachium idella georgii Jayachandran et joseph, 1985b, *Aqua. Biol.,* V: 130

Plate 4.2.5.1: *Macrobrachium idella georgii*

Material examined:

Twelve specimens of males of size 26mm- 51.46 mm and 2 non berried females of size 33 mm - 35 mm collected and identified.

Description:

Rostrum moderately long, extending beyond the third segment of antennular peduncle and extending up to the distal edge of scaphocerite. Dorsal profile elevated and convex and about 9- 12 teeth are arranged, of which first two are post orbital. The first tooth separated from second by a wide gap and remaining are closed arranged up to ante-penultimate tooth. The gap between the antepenultimate and penultimate is slightly greater. Ultimate tooth is small and sub distal. Lower margin of rostrum contains about 4 to 6 teeth. The setae present in both dorsal and ventral margins.

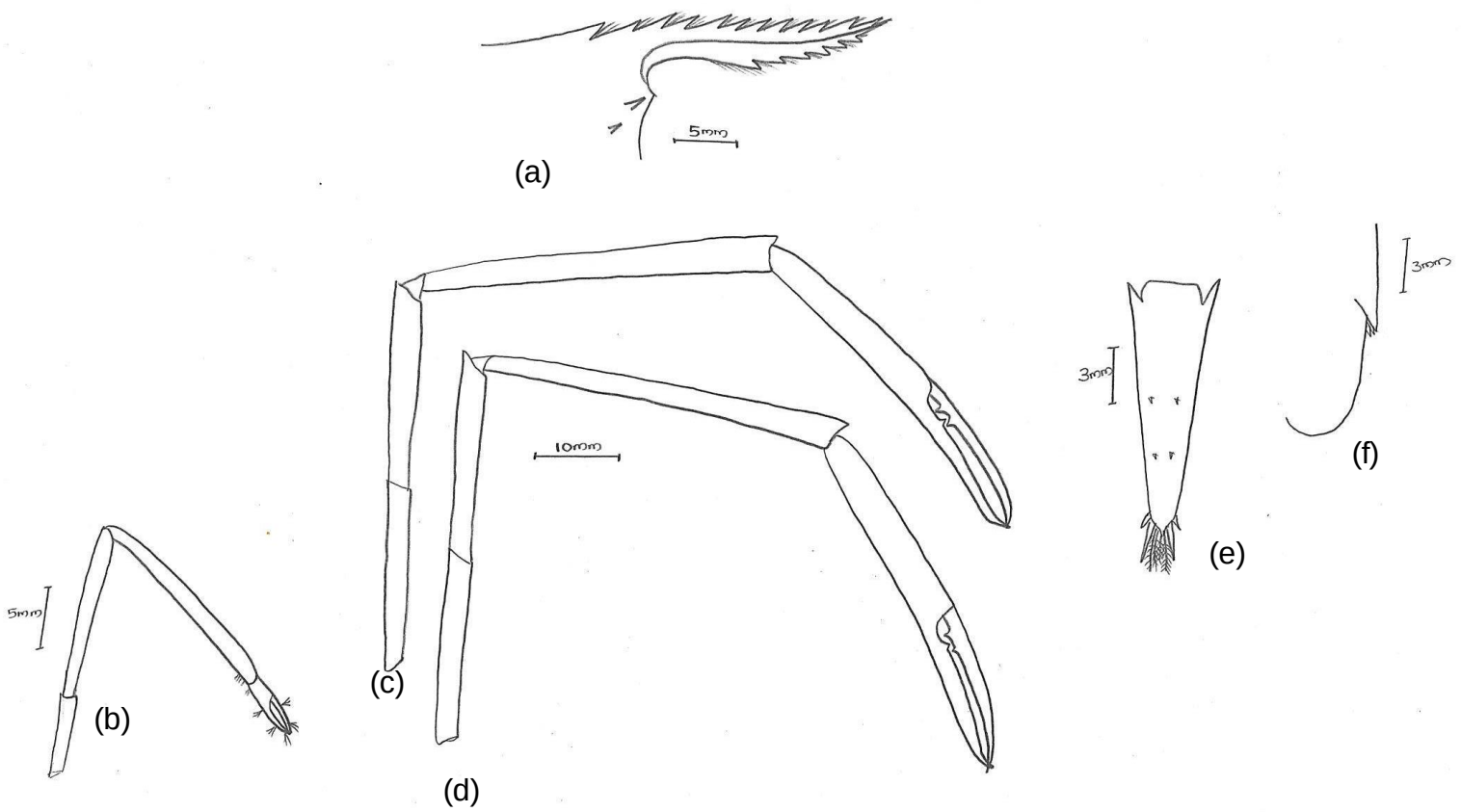


Figure 4.2.5: *Macrobrachium idella georgii* (a) rostrum, (b) 1st pereiopod, (c) 2nd pereiopods, (e) telson, (f) acessory spines in uropodal exopod

Carapace smooth and round both the antennal and hepatic spines present.

Abdomen glabrous, pleurae of first three segments broad and rounded posteriorly. The pleurae of 5th and 6th are directed posteriorly sixth end with a sharp point.

Telson smooth and slender and narrowing posteriorly, reaches as far as the level of outer spine of uropod. Two pair of dorsal spines, proximal pair is just above midway and the distal pair almost middle of the proximal pair and distal end. The distal end is pointed with two pairs of distal spines, outer smaller and immovable and inner pair larger and movable. There are 1 to 2 pairs of plumose setae present in between the movable inner spines.

Ratio of the three segments of antennule is 6.06: 2.49: 3.50. Antennal exopod broad and sub apical.

The first pair of chelate legs slender, ischium slightly inflated, merus longer than the ischium and smaller than the carpus. Carpus long and smooth its length about 2.5 times of the ischium. Palm and fingers are almost same. Fingers covered by tuft of setae.

The second pereiopod are same in size and shape. Ischium flat and long as that of fingers of chela. Merus long and length is greater than that of the ischium. Carpus long and the length is double the length of merus. Carpus equals to chela is a distinguishing character of the subspecies. Palm stout and smooth. Fingers long, slender and length are greater than the half of palm. The cutting edge of movable finger with 2 spines and one interlocking spine present in the opposite side, in the immovable finger. The entire podomeres are smooth and no setae on fingers.

The non-chelate legs are smooth, and normal in structures. All these legs ischium small and flat, merus long and length is almost equal to the Propodus. Carpus small, the inner margin of the Propodus contain small spines, Dactylus slightly curved and end with a spine.

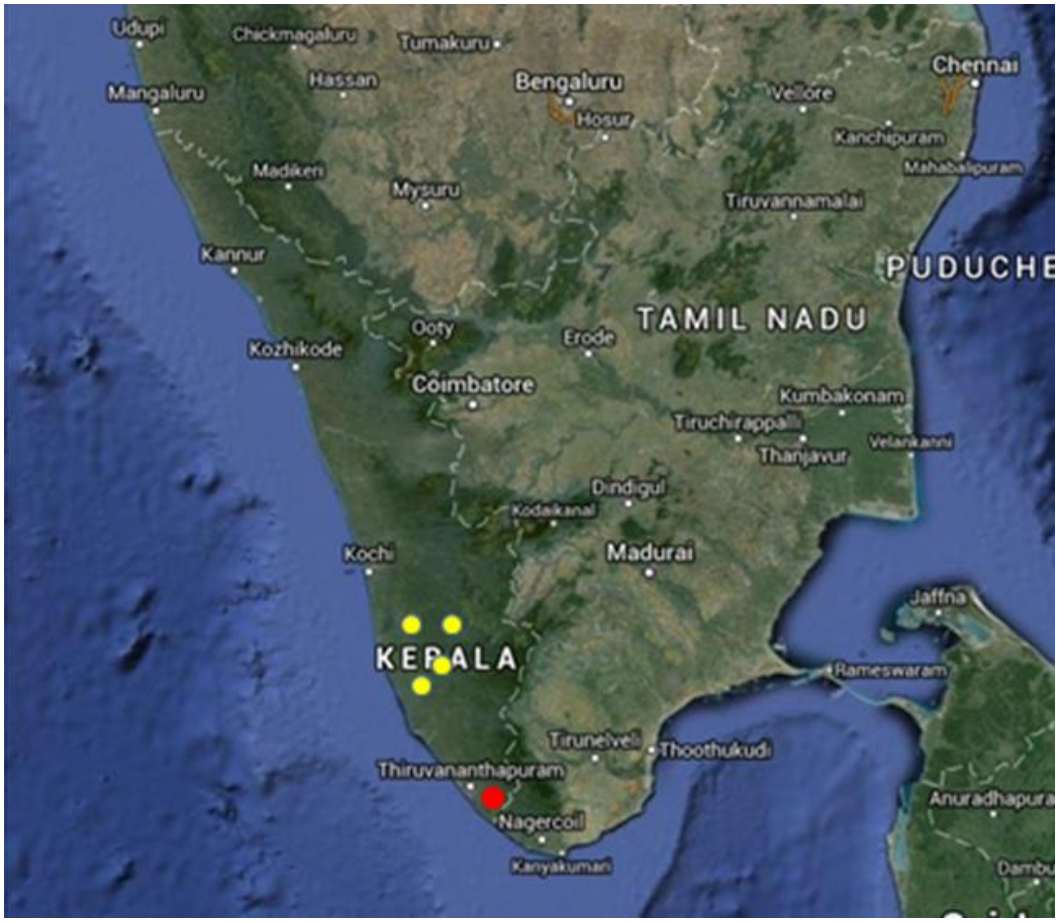
Pleopods are normal in structure.

Outer margin of Uropodal exopod with two pair of spines.

Distribution:

The subspecies is only known from four river systems in central Kerala (Pallickal, Pamba, Manimala, Meenachil rivers)

Map 4.2.5.1: Distributional range of *Macrobrachium idella georgii*



(*https://www.google.co.in*)

Plate 4.2.5.2: Rostrum of *M. idella georgii*

Plate 4.2.5.3: 2nd pereiopods of *M. idella georgii*

Plate 4.2.5.4: Chela of 2nd pereiopod of *M.idella georgii*

4.2.6 *Macrobrachium indicum* Jayachandran and Joseph, 1986

Macrobrachium indicum Jayachandran and Joseph, 1986, *Crustaceana,* 50(2):217

Plate 4.2.6.1: *Macrobrachium indicum*

Materials examined:

Thirteen specimens were taken from Neyyar river at Amaravila (Dt: 22-04-2015- 26-04-2016): 6 males (54.0- 67.2 mm), 6 non-berried females (45.0- 6.2 mm), one berried female (45.3 mm).

Description:

Rostrum moderately long, extending beyond the antennular peduncle and reaching up to the apex of antennal scale or slightly beyond it. It is convex proximally and tip slightly upturned. Upper margin with 9 to 13 teeth (usually 10 to 11), of which 3 to 4 are post orbital. The gap between the dorsal teeth increasing from the proximal to distal part of rostrum. The gap between the ultimate and penultimate and also between penultimate and ante-penultimate are wider. The lower margin with 3 to 5 teeth. Setae are present between the teeth of both dorsal and ventral margins.

Carapace smooth, both antennal and hepatic spines present (Characteristics of the genus) and latter distinctly at the lower level of the first.

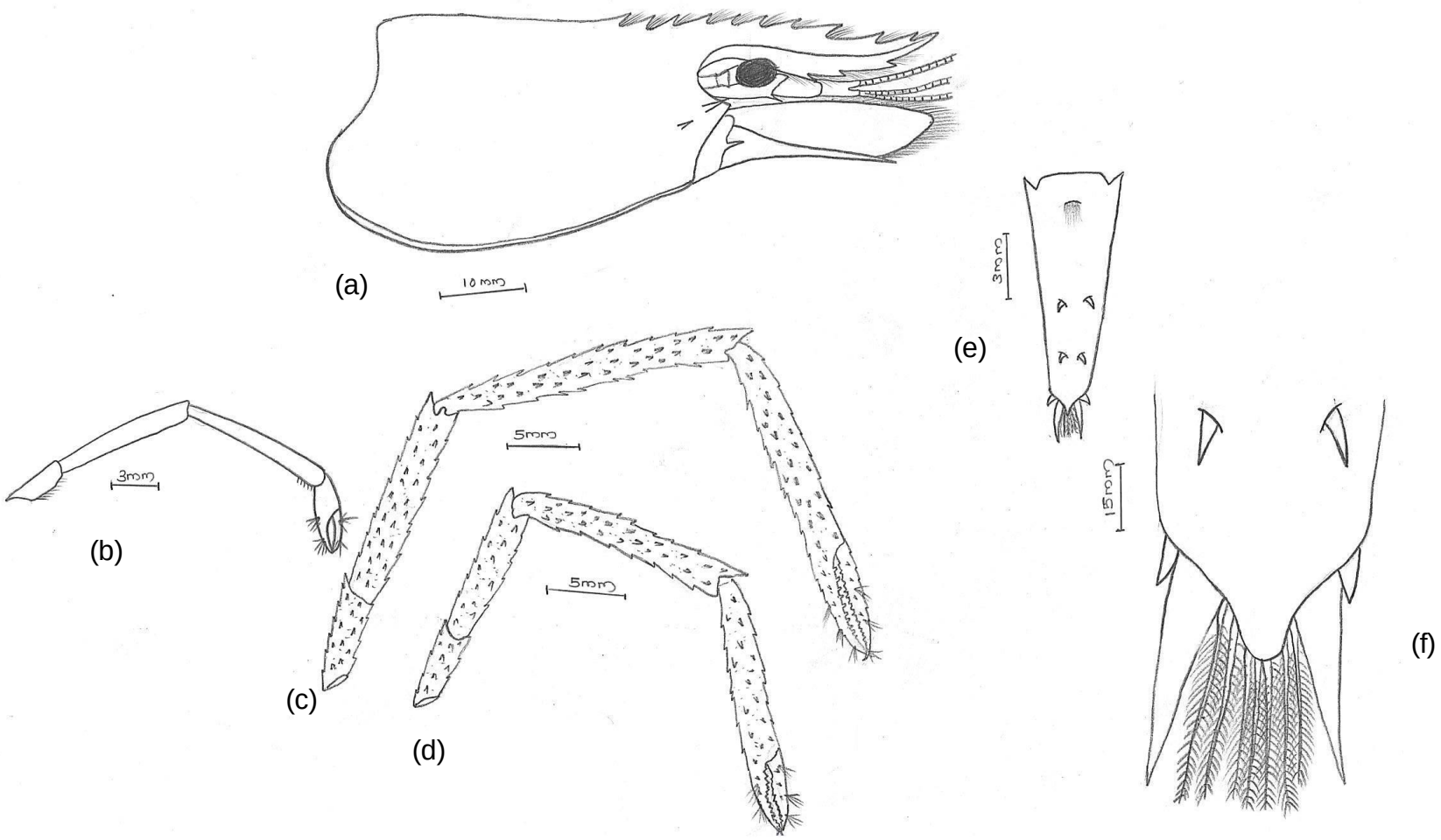


Figure 4.2.6: *Macrobrachium indicum* (a) carapace, (b) 1st pereiopod, (c) large 2nd pereiopod, (d) small second pereiopod, (e) telson, (f) distal tip of telson

Abdominal segments smooth and pleurae of first three segments rounded posteriorly.

Telson basally broad, narrowing distally, about 1.5 times longer than that of the sixth abdominal segment. The dorsal surface with two pairs of spines and proximal pair is posterior to the mid telson and the distal pair is nearly at the tip of the telson. The posterior margin contains two pairs of spines of which outer pairs immovable, inner pair is longer and movable and reached beyond the level of telson tip. Several plumose setae present between the inner pair of spines.

First pair of Chelate legs is slender, reaching beyond the antennal scale. Ischium inflated, with setae on inner side, and half of the carpus and longer than the chela. Merus cylindrical and shorter than the carpus. Carpus is the largest segment and the length is about four times of ischium. The palm and fingers are equal and covered with stiff setae.

Second chelate legs are distinctly unequal in shape and size, either left or right leg larger. In the larger leg ischium flattened and smaller than that of merus and longer than that of fingers, covered with some scattered and minute spinules. Merus cylindrical and sub-equal to palm. Carpus long and double the length of ischium, almost equal to the palm. Palm elongate and cylindrical and covered with strong spinules. Fingers shorter than the palm, with about 7 to 9 denticles throughout the length of cutting edge (in both the cutting edges). Proximal denticles are larger than the distal. The entire podomeres are covered with very close set of spinules. Spinules of the lower surface are larger than that of the upper one. Velvety setae present in ischium, merus and dorsal part of the carpus. Some scattered hairs present in the chela.

In smaller second periopod ischium flat and about the half length of carpus. Merus cylindrical, about half length of chela. Carpus cylindrical, and longer than the merus. The palm is longer than fingers. Fingers with minute denticles at the cutting edge. The palm and fingers covered with long setae.

In the case of females the 2nd pereiopods are equal and very smaller than that of the male.

The non-chelate legs are typical in structure and covered with setae. The length is slightly increasing from third to the fifth.

Pleopods are typical in structure.

Colour:

Transparent colour with light reddish tinge on carapace and antennular peduncle. Dark black spots present in the lateral side of pleura except the third and 6th abdominal segment. The 2nd periopod have dark bands.

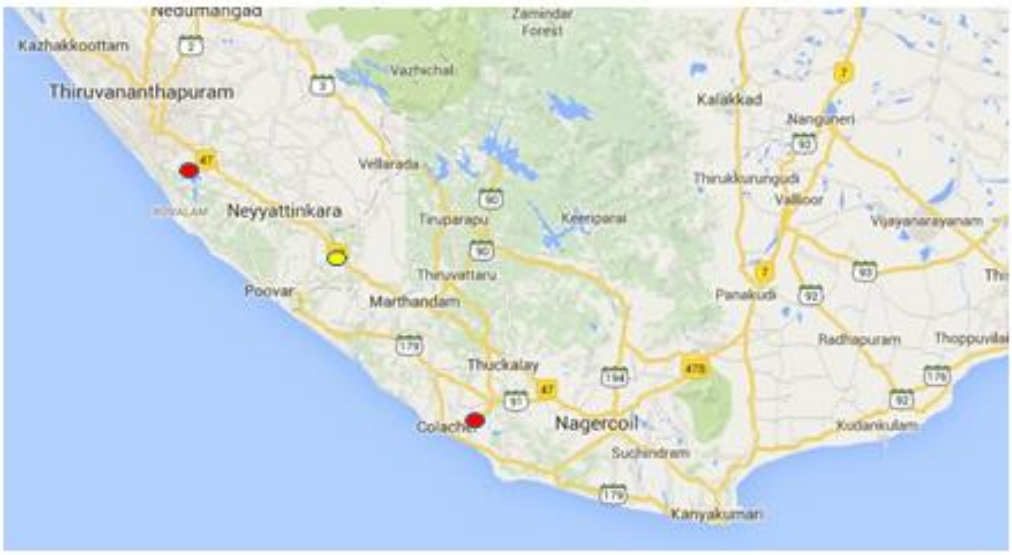
Fecundity:

3000-3500 per 45.5 mm sized female.

Distribution:

South tip of India

Map 4.2.6.1: Distribution range of *Macrobrachium idicum*



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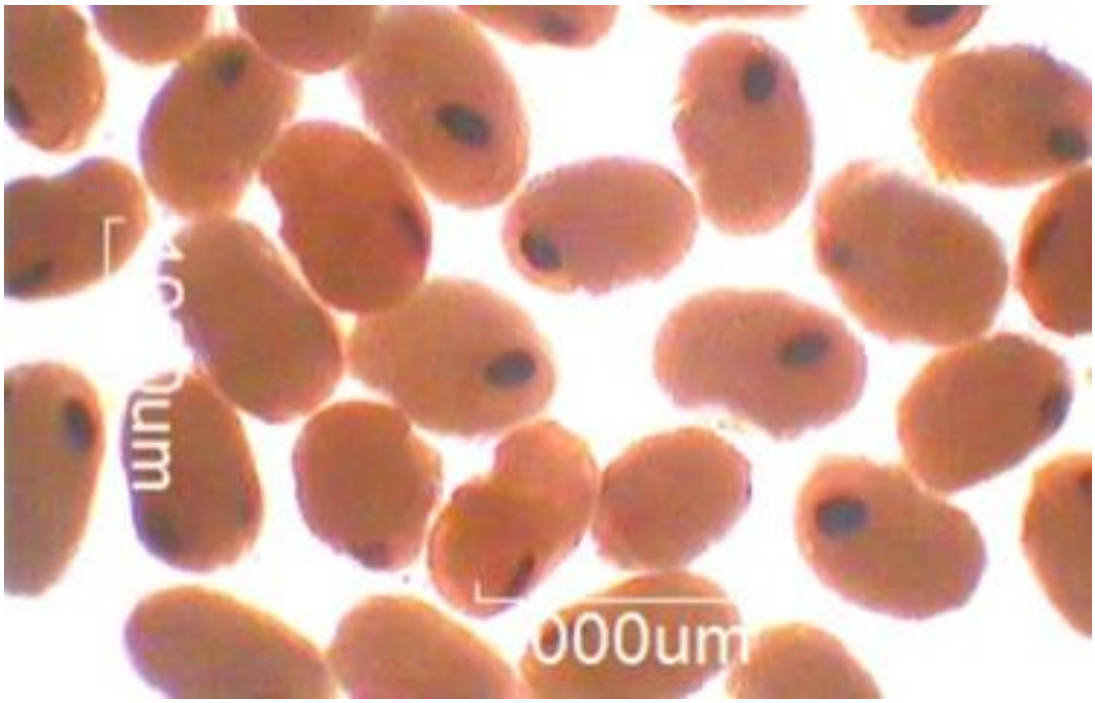


Plate 4.2.6.2: Eggs of *Macrobrachium indicum*

Plate 4.2.6.3: Male specimen of *M. indicum*

Plate 4.2.6.4: Berried female specimen of *M. indicum*

Plate 4.2.6.5: Rostrum of *M. indicum*

Plate 4.2.6.6: Telson of *M.indicum*

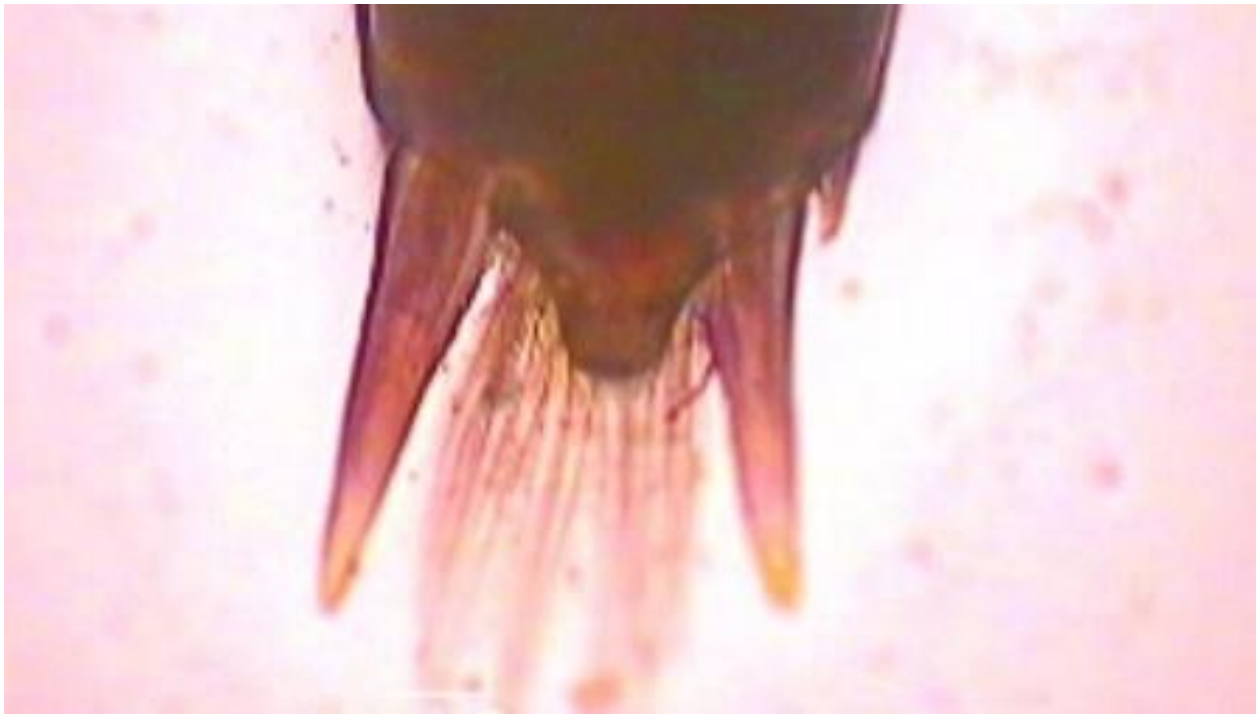


Plate 4.2.6.7: Distal end of telson of *M. indicum*

Plate 4.2.6.8: The chela of larger 2nd pereiopod of *M. indicum*

Plate 4.2.6.9: Largest 2nd pereiopod of *M. indicum*

4.2.7 *Macrobrachium* spp.

Plate 4.2.7.1: *Macrobrachium* spp.

Materials examined:

Seven specimens were collected and examined, 4 non-berried females, a single berried female

Description:

Rostrum extremely long and slender, reaching beyond the antennal scale. Basel crest elevated and distal portion upwardly curved. Upper margin with 8 - 9 teeth of which 2 are post orbital. The first tooth is small have little gap with the second. The proximal series of 6 teeth are equidistant by set. A wide gap found in between the 6^{th} and 7^{th} tooth, sometimes a tooth may be present in this part. The distal two teeth are very smaller. Ventral margin with about 9 to 10 teeth. The proximal most tooth at the level of 5^{th} tooth in dorsal and all the teeth are diminishing in size and shape end before the edentulous region completed in dorsal side. The setae present in both dorsal and ventral margin.

Carapaces smooth with antennal and hepatic spines characteristics of the genus.

Abdomen smooth and pleurae of first three segments are rounded. The sixth segment is comparatively longer than others.

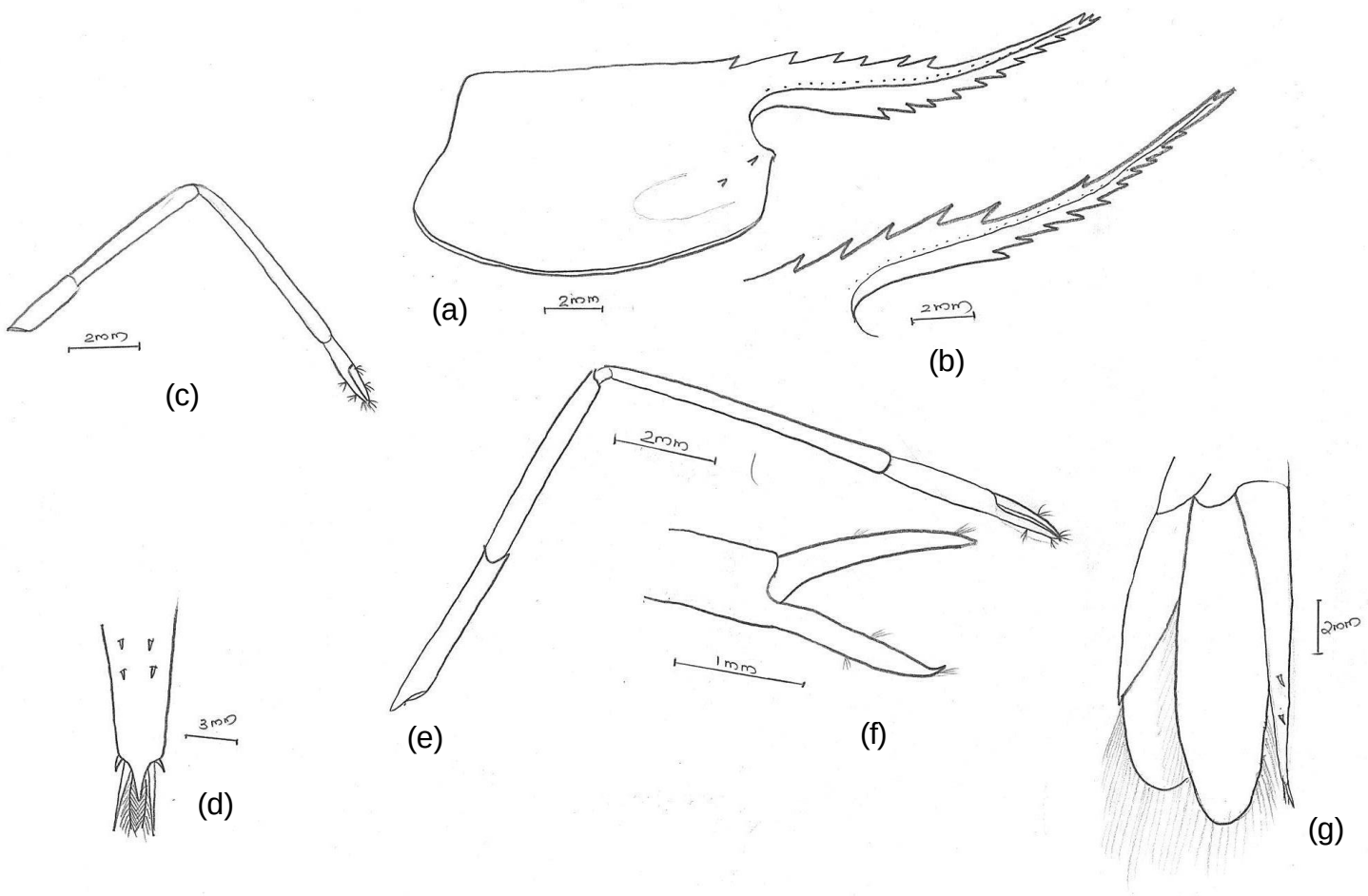


Figure 4.2.7: *Macrobrachium* spp. (a) carapace, (b) variability in rostrum, (c) 1st chelate leg, (d) distal end of telson, (e) 2nd pereiopod, (f) fingers of 2nd pereipod, (g) telson with uropod

Telson narrow and slender. Posterior end sharply pointed and extending almost the end of uropodal exopod. The dorsal surface with two pairs of spines proximal is at the middle of the telson and the distal pair is close to the proximal pair. The distal part contains 2 pairs of spines in which outer is smaller and immovable. The inner is longer and movable. One pair of plumose setae present in between the inner movable spines.

Ratio of three segments of antennule 4.29: 1.50: 2.00.Antennal scale is broad and the outer spine is apical in position. Mandible, maxillula and maxilla are normal.

The first chelate leg slender and smooth extending as far the distal end of antennal exopod. Ischium small and slender, the size is longer than the palm. Merus long and shorter than carpus. Carpus long and twice the size of chela. Palm smooth and slightly longer than the fingers.

The second chelate legs are equal sized. Ischium slender, equal to the length of merus. Carpus long and twice the length of the merus, carpus longer than chela. Palm is broad and shorter than ischium. Fingers are slender, smaller and about half of the ischium. No denticles on cutting edge of both movable and immovable fingers. Small setae present in the posterior part. All the podomere are smooth with scattered setae.

Non-chelate legs are typical. The spinules are present in all the podomeres. The last non- chelate is longer than the other two legs.

Pleopods are normal in structure.

Uropod has no accessory spines in exopod.

Plate 4.2.7.2: Rostrum of *Macrobrachium* spp.

Plate 4.2.7.2: Variation in rostrum of *Macrobrachium* spp.

Plate 4.2.7.4: Exopod of uropod without acessory spines (*Macrobrachium* spp.)

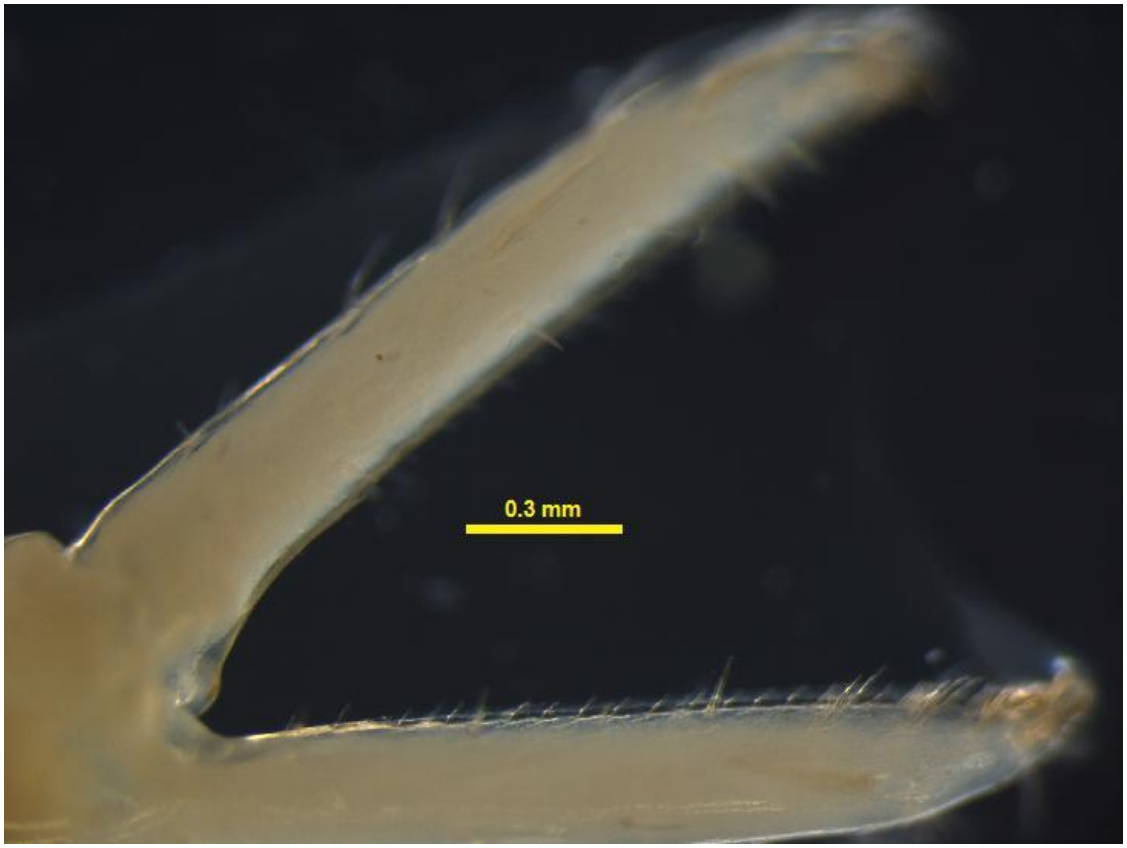


Plate 4.2.7.5: Cutting edge of 2nd pereiopod of *Macrobrachium* spp.

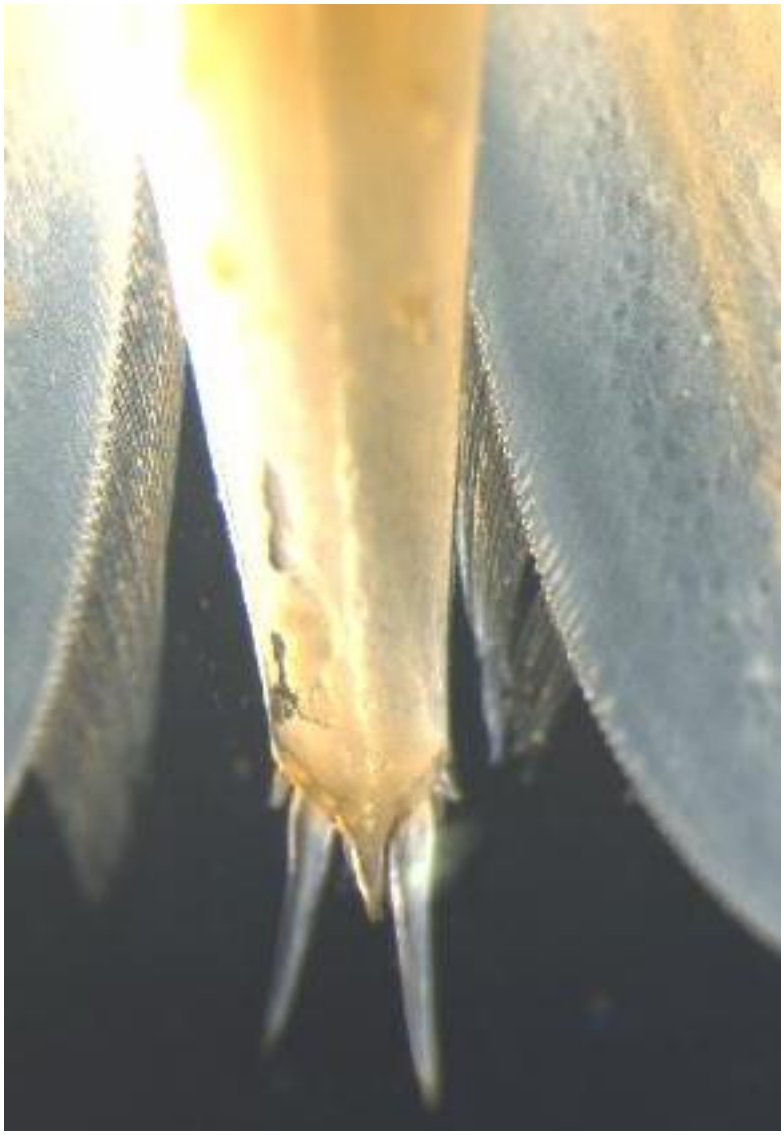


Plate 4.2.7.6: Distal end of telson of *Macrobrachium* spp.

4.2.8 *Macrobrachium prabhakarani* Pillai and Unnikrishnan, 2012

Macrobrachium prabhakaranii Pillai and Unnikrishnan, 2012, *Zootaxa* 3528: 63-68.

Plate: 4.2.8.1: *Macrobrachium prabhakarani*

Materials examined:

Four male specimens of size 29.81 mm to 52.56 mm segregated from the total collection and examined.

Description:

Rostrum short and reaching up to the distal end of third segment of antennular peduncle or slightly beyond it. The dorsal margin of rostrum slightly convex with 13 to 15 teeth, of which 5 are post orbital. The ventral margin is armed with 1 to 3 teeth, usually 2. Setae present in between the teeth.

Carapace smooth and glabrous, antennal and hepatic spines present characteristics of the genus.

The abdomen smooth and glabrous the lateral side of posterior margin of fourth and fifth pleura have dark dots.

Telson robust and smooth. The dorsal surfaces of telson contain two pairs of spines. The proximal pair is at the mid length of the telson and the distal pair

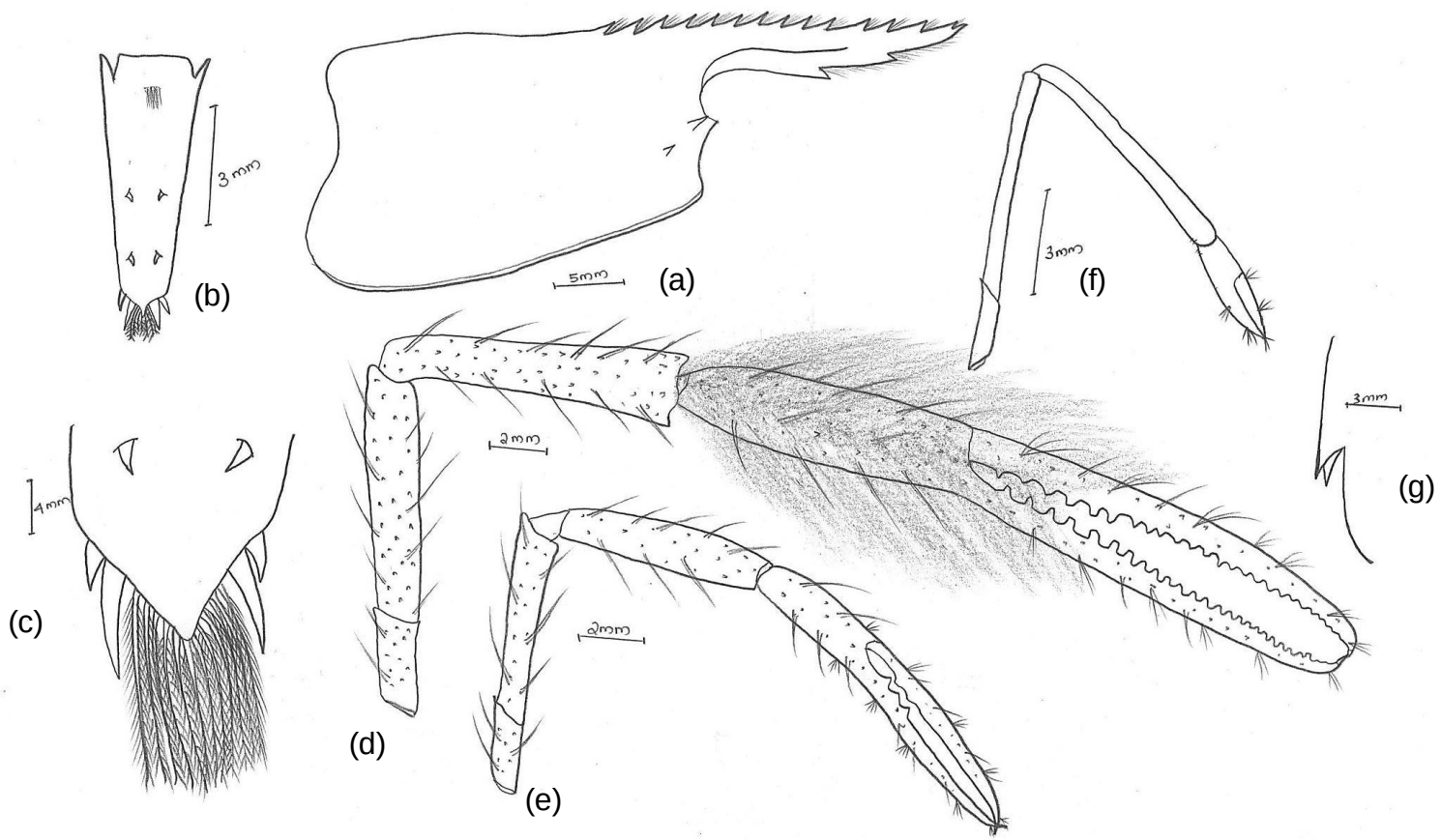


Figure 4.2.8: *Macrobrachium prabhakarani* (a) carapce, (b) telson, (c) distal end of telson, (d) 2nd large pereiopod, (e) 2nd small pereiopod, (f) 1st chelate leg, (g) acessory spine in urpodal exopod

is at below mid-level of the proximal pair and distal end of telson. The distal end with 2 pair of spines outer smaller and immovable. Inner pair is larger and movable. A tuft of more than 15 plumose setae present in between inner pair of spines.

Antennule typical in shape. The outer spine of the antennal exopod is apical.

First pereiopod typical. Ischium shortest. Merus shorter than carpus. Carpus is the longest podomere. The fingers and palm equal in length. The small tuft of setae present in the fingers of first pereiopod.

The second pereiopod are strongly unequal in shape and structure. The large 2nd pereiopod is longer than the total length of species. The ratio of total length and length of large 2nd pereiopod is 1.00: 1.56. Ischium smaller than the merus; merus smaller to the carpus. Carpus smaller to palm, Palm smaller to finger. The ratio of Ischium: merus: Carpus: Palm: Fingers is 1.0: 2.6: 3.0: 3.24: 4.6. The cutting edge of both the fingers has strong denticles the size of the denticles and gapes between the denticles are large in the anterior portion. While coming in to the distal region the size of the denticles and the gap between the denticles are reducing. The each finger bears more than 20 denticles. The entire podomeres are covered by minute spinules and stiff setae. The entire palm and proximal portion of finger covered by thick pubescent setae.

In small 2nd pereiopod Ischium is flat and half of merus; merus smaller than carpus, carpus longer than palm, palm is shorter the carpus and fingers and the merus. The fingers are longer than the all other segments. The ratio of the segments, Ischium: Merus: Carpus: Palm: fingers equals 1:2:2.27:1.75:2.44. The entire leg is covered by minute spinules and stiff setae. The anterior regions of cutting edge bear denticles. In movable finger is with 2 denticles and the immovable with one. All podomeres are covered with spines and stiff setae.

The Non-chelate legs are typical in shape.

Pleopods typical and appendix musculina does not reach of mid length of the endopod of 2nd pleopod.

Uropod typical and have a pair of accessory spine.

Distribution:

Vamanapuram and Neyyar river of Ashambu hills

Map 4.2.8.1: Distribution range of *M. prabhakarani*

(*http://www.mapsofindia.com/maps*)

Plate 4.2.8.2: Carapace of *M. prabhakarani*

Plate 4.2.8.3: 2nd large pereiopod *M. prabhakarani*

Plate 4.2.8.4: Live picture of male and Female of *M. prabhakarani*

4.2.9 *Macrobrachium scabriculum* (Heller, 1862)

Palaemon scabriculus Heller, 1862, *verh. Zool.-bot. Ges. Wien*., 12:527

Plate 4.2.9.1: *Macrobrachium scabriculum* (male)

Synonymy:

Palaemon scabriculus; *Palaemon dolichodactylus; Palaemon (Parapalaemon) dolichodactylus*; *Palaemon (Parapalaemon) scabriculus*; *Palaemon dubius; Macrobrachium scabriculam*

Materials examined:

Thirty male specimens (21.63 mm to 33.49 mm total length), ten female specimen of size 22.3 mm - 31.1 mm segregated from the group and examined.

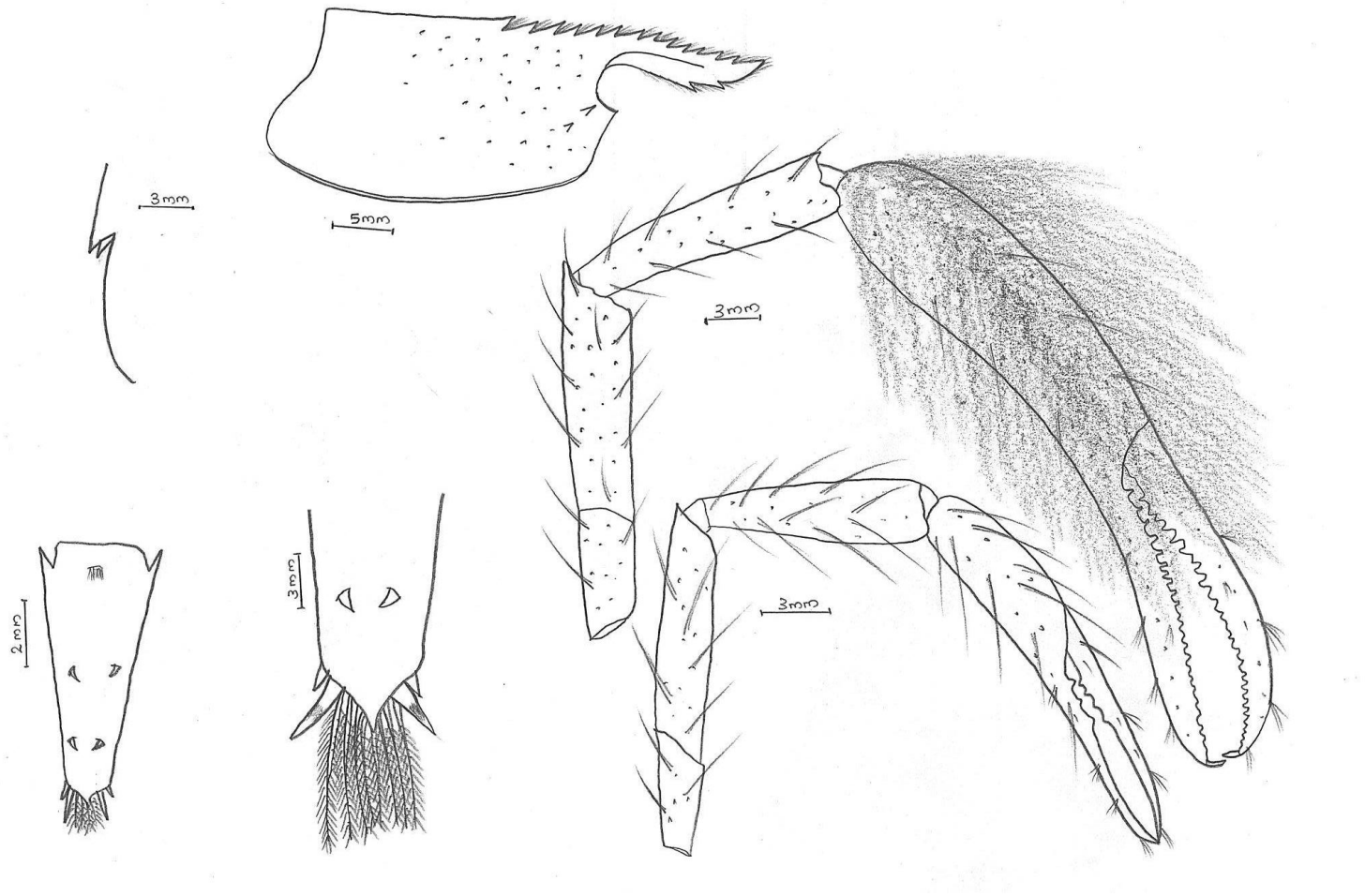


Figure 4.2.9: *Macrobrachium scabriculum* (a) carpace, (b) acessory spine in uropod, (c) telson, (d) distal end of telson, (e) large 2nd pereiopod, (f) small 2nd pereiopod

Description:

Rostrum short and reaching up to the distal end of third segment of antennular peduncle. The dorsal margin of rostrum straight with 13 teeth, of which 5 are post orbital. The ventral margin is armed with 1 to 3 teeth. Setae present in between the teeth.

Carapace rough in anterior portion and covered with minute spinules. The antennal and hepatic spine present characteristics of the genus

The abdomen smooth and glabrous the lateral side of posterior margin of fourth and fifth pleura have dark dots.

Telson robust and smooth. The dorsal surface of telson contains two pairs of spines. The proximal pair is at the mid length of the telson and the distal pair is at the mid-level between the proximal pair and distal end of telson. The distal end with 2 pair of spines outer smaller and immovable, inner pair is large and movable. A tuft of more than 8 plumose setae present in between inner pair of spines.

Antennule typical in shape. The outer spine of the antenna is apical.

First pereiopod typical. Ischium short and flat length is shorter than that of the merus and carpus. Merus shorter than carpus. Carpus is the longest podomere. The fingers and palm are shorter podomeres are equal sized. The small tuft of setae present in the fingers of first pereiopod.

The second pereiopod are strongly unequal in shape and structure. The large 2nd pereiopod is longer than the total length of species the ratio is Total length: length of 2nd periopod is 3.0: 4.29

In larger second pereiopod Ischium shorter than the merus, merus equal to carpus. Palm longer than the merus, carpus and fingers. The ratio of ischium: merus: carpus: palm: fingers is 3.5: 8.2: 8.2: 13.0: 11.0. The cutting edge of both the fingers has strong denticles the size of the denticles and gapes between the denticles are larger in the proximal portion. While coming in to the distal region the size of the denticles and the gap between the denticles are diminishing. Each finger bears more than 20 denticles. The entire podomere is covered by minute spinules

and stiff setae. The entire palm and anterior portion of finger covered by thick pubescent setae.

In second smallest pereiopod ischium flat and small almost equal to the palm, merus equal to carpus and are longer than the palm and ischium. Palm is shorter than fingers. The fingers are longer than the all other segments. The ratio of the segments, Ischium: Merus: Carpus: Palm: fingers equals 0.63: 1.0: 1.0: 0.72: 1.1. The entire leg is covered by minute spinules and stiff setae. The anterior region of cutting edge bears denticles. The movable finger is with 2 denticles and the immovable with one. All podomeres covered with spines and stiff setae.

The Non-chelate legs are typical in shape.

Pleopods typical and appendix musculina does not reach level of mid length of the endopod of 2nd pleopod.

Uropod typical and have a pair of accessory spine.

Distribution:

Map 4.2.9.1: Distribution range of *M. scabriculum*

(https://www.google.co.in)

Plate 4.2.9.2: Carapace and rostrum of *M.scabriculum*

Plate 4.2.9.3: Large 2nd chelate leg of *M. scabriculum*

Plate 4.2.9.4: Small 2nd chelate leg of *M. scabriculum*

4.3 Descriptive Statistics of Morphometric Data of Different Species *Macrobrachium* of Family Palaemonidae

Descriptive statistics (minimum, Maximum, mean, range) were obtained for 24 morphometric characters (one scaled for TL, 6 scaled to CL, two scaled for TEL, 5 scaled to FPL, 5 scaled for SLPL, 5 scaled to SSPL) for all species under genus *Macrobrachium* (Only male are taken because of sexual dimorphism in morphometric characters) collected during the study have been done. Several morphometric variables obtained were found to be important in differentiating between the species. The statistics are presented species wise in tables 4.3.1 to 4.3.9

Table 4.3.1: Descriptive statistics of morphometric measurements of *M. abrahami*

Variable	Valid N	Mean	Minimum	Maximum	Range
TL/CL	3	2.172631	2.138444	2.194305	0.055861
CL/TL	3	0.460330	0.455725	0.46763	0.011905
RL/CL	3	0.36467	0.362676	0.365725	0.003049
FPL/CL	3	0.783810	0.78040	0.78375	0.00335
SLPL/CL	3	1.915949	1.910657	1.92071	0.010053
SSPL/CL	3	1.09674	1.09418	1.09994	0.00576
TEL /CL	3	0.270897	0.26887	0.274706	0.005836
FSTEL/TEL	3	0.505504	0.5	0.508511	0.008511
SSTEL/TEL	3	0.800398	0.792683	0.8	0.007317
FPIL/FPL	3	0.133568	0.133333	0.133793	0.00046
FPML/FPL	3	0.316753	0.313869	0.319149	0.00528
FPCL/FPL	3	0.407096	0.406897	0.407299	0.000403
FPPL/FPL	3	0.069501	0.069343	0.069655	0.000312
FPFL/FPL	3	0.069501	0.069343	0.069655	0.000312
SLPIL/SLPL	3	0.131139	0.131045	0.131268	0.000223
SLPML/SLPL	3	0.190617	0.190448	0.190704	0.000256
SLPCL/SLPL	3	0.214316	0.214085	0.214535	0.00045
SLPPL/SLPL	3	0.188582	0.18806	0.188953	0.000894
SLPFL/SLPL	3	0.273003	0.271642	0.274128	0.002486
SSPIL/SSPL	3	0.166416	0.166257	0.166497	0.000241
SSPML/SSPL	3	0.208145	0.208067	0.208246	0.000179
SSPCL/SSPL	3	0.206579	0.203549	0.208122	0.004573
SSPPL/SSPL	3	0.166416	0.166257	0.166497	0.000241
SSPFL/SSPL	3	0.24797	0.245303	0.249877	0.004574

Table 4.3.2: Descriptive statistics of morphometric measurements of *M. aemulum keralauni*

Variable	Valid N	Ratio
TL/CL	1	3.04448
CL/TL	1	0.328463
RL/CL	1	0.043478
FPL/CL	1	1.355126
SLPL/CL	1	4.952381
SSPL/CL	1	3.239272
TEL /CL	1	0.451709
FSTEL/TEL	1	0.524941
SSTEL/TEL	1	0.747031
FPIL/FPL	1	0.144893
FPML/FPL	1	0.309976
FPCL/FPL	1	0.388757
FPPL/FPL	1	0.091845
FPFL/FPL	1	0.064529
SLPIL/SLPL	1	0.113733
SLPML/SLPL	1	0.183103
SLPCL/SLPL	1	0.296075
SLPPL/SLPL	1	0.304447
SLPFL/SLPL	1	0.103077
SSPIL/SSPL	1	0.081731
SSPML/SSPL	1	0.201757
SSPCL/SSPL	1	0.291777
SSPPL/SSPL	1	0.304211
SSPFL/SSPL	1	0.120524

Table 4.3.3: Descriptive statistics of morphometric measurements of *M. canarae*

Variable	Valid N	Mean	Minimum	Maximum	Range
TL/CL	30	2.00050	1.879205	2.213845	0.33464
CL/TL	30	0.500759	0.451703	0.532139	0.080409
RL/CL	30	0.611553	0.51760	0.646684	0.129084
FPL/CL	30	0.50421	0.44731	0.58375	0.13644
SLPL/CL	30	1.024912	0.939146	1.22286	0.283714
SSPL/CL	30	1.024912	0.939146	1.22286	0.283714
TEL /CL	30	0.2295988	0.20675	0.26649	0.05974
FSTEL/TEL	30	0.48473	0.469256	0.522388	0.053132
SSTEL/TEL	30	0.788395	0.755285	1.007463	0.252178
FPIL/FPL	30	0.207135	0.160964	0.215	0.054036
FPML/FPL	30	0.337264	0.313846	0.406667	0.092821
FPCL/FPL	30	0.379624	0.355805	0.398621	0.042815
FPPL/FPL	30	0.057955	0.046154	0.062687	0.016533
FPFL/FPL	30	0.058549	0.049231	0.062	0.012769
SLPIL/SLPL	30	0.207346	0.204662	0.211852	0.00719
SLPML/SLPL	30	0.208961	0.185383	0.214074	0.028691
SLPCL/SLPL	30	0.31184	0.305344	0.323171	0.017827
SLPPL/SLPL	30	0.14539	0.13923	0.148361	0.009131
SLPFL/SLPL	30	0.122014	0.115702	0.124655	0.008952
SSPIL/SSPL	30	0.207346	0.178253	0.211852	0.033599
SSPML/SSPL	30	0.208961	0.185383	0.214074	0.028691
SSPCL/SSPL	30	0.31184	0.305085	0.316134	0.01105
SSPPL/SSPL	30	0.14539	0.13923	0.148361	0.009131
SSPFL/SSPL	30	0.122014	0.118519	0.124655	0.006136

Table 4.3.4: Descriptive statistics of morphometric measurements of *M. idella idella*

Variable	Valid N	Mean	Minimum	Maximum	Range
TL/CL	7	2.1668847	2.100055	2.198083	0.098028
CL/TL	7	0.461588	0.454942	0.476178	0.02136
RL/CL	7	0.430466	0.41613	0.47306	0.05693
FPL/CL	7	0.90483	0.82078	0.93469	0.11391
SLPL/CL	7	3.85644	3.60857	3.94686	0.33829
SSPL/CL	7	3.64053	3.3741	3.7259	0.3518
TEL /CL	7	0.32610	0.30800	0.3361	0.0281
FSTEL/TEL	7	0.485993	0.4	0.614747	0.214747
SSTEL/TEL	7	0.699819	0.675105	0.739851	0.064745
FPIL/FPL	7	0.159688	0.136986	0.172806	0.03582
FPML/FPL	7	0.299269	0.294521	0.309119	0.014598
FPCL/FPL	7	0.417391	0.401855	0.445205	0.043351
FPPL/FPL	7	0.064221	0.06135	0.065224	0.003874
FPFL/FPL	7	0.059431	0.058219	0.060281	0.002062
SLPIL/SLPL	7	0.123265	0.118601	0.129125	0.010523
SLPML/SLPL	7	0.181287	0.181673	0.186091	0.004418
SLPCL/SLPL	7	0.34718	0.340633	0.347471	0.006839
SLPPL/SLPL	7	0.220647	0.218077	0.227088	0.009011
SLPFL/SLPL	7	0.127621	0.110299	0.132632	0.022333
SSPIL/SSPL	7	0.118274	0.113647	0.129043	0.015396
SSPML/SSPL	7	0.181541	0.173979	0.184708	0.010729
SSPCL/SSPL	7	0.36157	0.360817	0.359689	0.001128
SSPPL/SSPL	7	0.208804	0.205296	0.212383	0.007086
SSPFL/SSPL	7	0.129811	0.113483	0.136157	0.022675

Table 4.3.5: Descriptive statistics of morphometric measurements of *M. idella georgii*

Variable	Valid N	Mean	Minimum	Maximum	Range
TL/CL	12	1.5809874	1.502311	1.61859	0.116279
CL/TL	12	0.6327506	0.617822	0.648052	0.03023
RL/CL	12	0.419340	0.406871	0.431965	0.025094
FPL/CL	12	0.927036	0.903471	0.967450	0.063979
SLPL/CL	12	2.932957	2.868610	3.00090	0.13229
SSPL/CL	12	2.932957	2.868610	3.00090	0.13229
TEL /CL	12	0.4322	0.37037	0.455555	0.085185
FSTEL/TEL	12	0.432217	0.37037	0.455556	0.085185
SSTEL/TEL	12	0.715848	0.651515	0.777778	0.126263
FPIL/FPL	12	0.157843	0.154167	0.166667	0.0125
FPML/FPL	12	0.310795	0.291667	0.326316	0.034649
FPCL/FPL	12	0.660264	0.401122	0.693793	0.292671
FPPL/FPL	12	0.075229	0.065421	0.093103	0.027683
FPFL/FPL	12	0.06406	0.058333	0.067368	0.009035
SLPIL/SLPL	12	0.146751	0.139935	0.159109	0.019174
SLPML/SLPL	12	0.18389	0.17502	0.188229	0.01321
SLPCL/SLPL	12	0.33271	0.32413	0.344456	0.020327
SLPPL/SLPL	12	0.189445	0.182992	0.193123	0.010131
SLPFL/SLPL	12	0.147204	0.138035	0.152461	0.014426
SSPIL/SSPL	12	0.146751	0.139935	0.159109	0.019174
SSPML/SSPL	12	0.18389	0.17502	0.188229	0.01321
SSPCL/SSPL	12	0.33271	0.32413	0.344456	0.020327
SSPPL/SSPL	12	0.189445	0.182992	0.193123	0.010131
SSPFL/SSPL	12	0.147204	0.138035	0.152461	0.014426

Table 4.3.6: Descriptive statistics of morphometric measurements of *M. indicum*

Variable	Valid N	Mean	Minimum	Maximum	Range
TL/CL	6	2.018941	2.0047	2.0333	0.0286
CL/TL	6	0.4953244	0.491803	0.498812	0.007009
RL/CL	6	0.41979	0.4047	0.4411764	0.0364764
FPL/CL	6	0.696333	0.70525	0.728148	0.022898
SLPL/CL	6	1.69861	1.62088	1.803703	0.182823
SSPL/CL	6	1.1756481	1.115294	1.23125	0.115956
TEL /CL	6	0.2798	0.26192	0.29441	0.03249
FSTEL/TEL	6	0.555066	0.545349	0.6	0.054651
SSTEL/TEL	6	0.83149	0.815789	0.9	0.084211
FPIL/FPL	6	0.104616	0.099567	0.107639	0.008072
FPML/FPL	6	0.315117	0.298701	0.321755	0.023054
FPCL/FPL	6	0.422479	0.393939	0.437436	0.043497
FPPL/FPL	6	0.084443	0.071211	0.134199	0.062989
FPFL/FPL	6	0.073344	0.066728	0.078125	0.011397
SLPIL/SLPL	6	0.160511	0.15343	0.164918	0.011488
SLPML/SLPL	6	0.193652	0.184805	0.199717	0.014912
SLPCL/SLPL	6	0.326192	0.311615	0.353183	0.041568
SLPPL/SLPL	6	0.186122	0.179603	0.191218	0.011615
SLPFL/SLPL	6	0.133523	0.12731	0.137931	0.010621
SSPIL/SSPL	6	0.152026	0.1516	0.152954	0.001353
SSPML/SSPL	6	0.224017	0.22288	0.225908	0.003029
SSPCL/SSPL	6	0.268869	0.267857	0.270051	0.002194
SSPPL/SSPL	6	0.200226	0.198312	0.20203	0.003718
SSPFL/SSPL	6	0.154862	0.152284	0.15625	0.003966

Table 4.3.7: Descriptive statistics of morphometric measurements of *Macrobrachium* spp.

Variable	Valid N	Mean	Minimum	Maximum	Range
TL/CL	7	2.200430	2.052809	2.293215	0.240406
CL/TL	7	0.4550812	0.436069	0.487137	0.51068
RL/CL	7	0.569852	0.5429	0.629213	0.086313
FPL/CL	7	0.595703	0.491011	0.60695	0.115939
SLPL/CL	7	0.8743996	0.752808	0.940226	0.187418
SSPL/CL	7	0.8743996	0.752808	0.940226	0.187418
TEL /CL	7	0.28926	0.2471910	0.308296	0.061105
FSTEL/TEL	7	0.586539	0.574163	0.594737	0.020574
SSTEL/TEL	7	0.699883	0.681818	0.731579	0.049761
FPIL/FPL	7	0.158582	0.143372	0.170667	0.027295
FPML/FPL	7	0.321916	0.297483	0.341085	0.043602
FPCL/FPL	7	0.387082	0.346774	0.414634	0.06786
FPPL/FPL	7	0.076265	0.073171	0.08	0.006829
FPFL/FPL	7	0.078721	0.074194	0.082853	0.008659
SLPIL/SLPL	7	0.208502	0.202532	0.21322	0.010688
SLPML/SLPL	7	0.241564	0.231959	0.239872	0.007913
SLPCL/SLPL	7	0.352928	0.349206	0.359808	0.010602
SLPPL/SLPL	7	0.122336	0.103175	0.127932	0.024757
SLPFL/SLPL	7	0.069685	0.065421	0.070959	0.005538
SSPIL/SSPL	7	0.208502	0.202532	0.21322	0.010688
SSPML/SSPL	7	0.241564	0.231959	0.265672	0.033713
SSPCL/SSPL	7	0.352928	0.349206	0.359808	0.010602
SSPPL/SSPL	7	0.122336	0.103175	0.127932	0.024757
SSPFL/SSPL	7	0.069685	0.065421	0.070959	0.005538

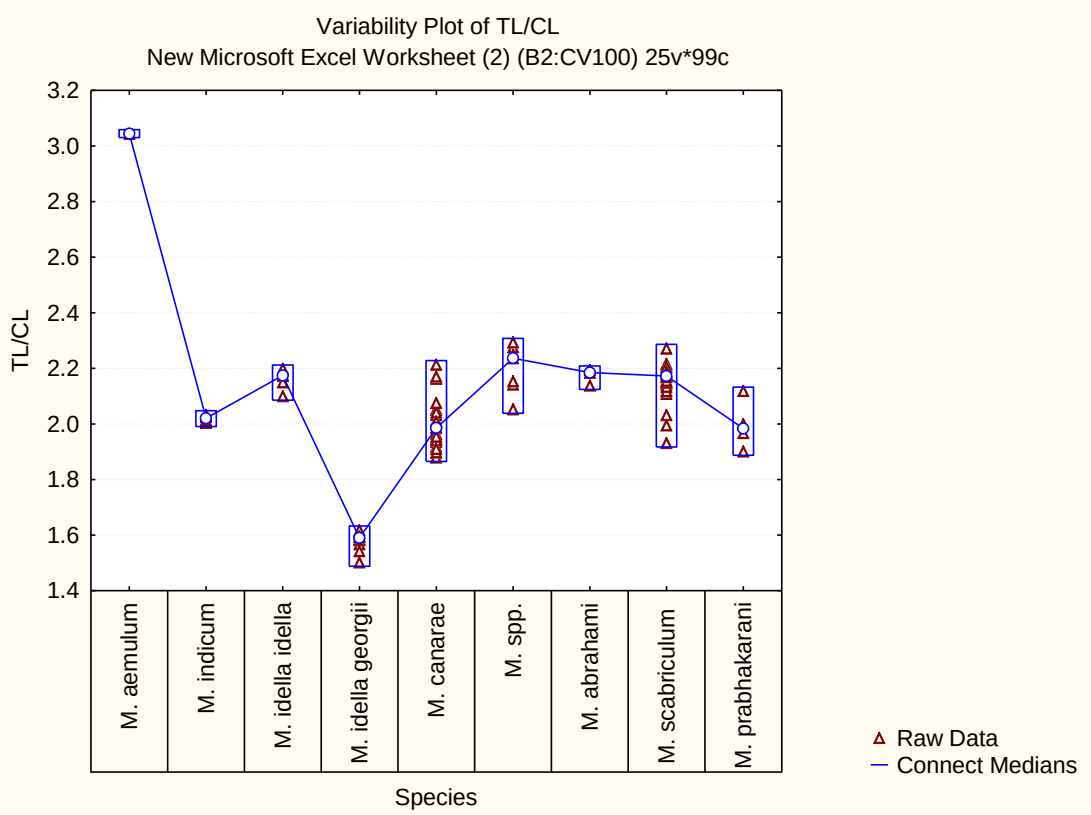
Table 4.3.8: Descriptive statistics of morphometric measurements *of M. prabhakarani*

Variable	Valid N	Mean	Minimum	Maximum	Range
TL/CL	4	1.996930	1.901658	2.118357	0.216699
CL/TL	4	0.501532	0.472064	0.52857	0.056506
RL/CL	4	0.351409	0.3461708	0.35800	0.0118292
FPL/CL	4	0.78274	0.75169	0.78212	0.03043
SLPL/CL	4	2.32034	1.9716	2.5289	0.5573
SSPL/CL	4	1.4028	1.26427	1.60483	0.34056
TEL /CL	4	0.282419	0.27392	0.2923	0.01838
FSTEL/TEL	4	0.497144	0.489499	0.518569	0.02907
SSTEL/TEL	4	0.770515	0.759289	0.789649	0.03036
FPIL/FPL	4	0.153796	0.135831	0.182378	0.046547
FPML/FPL	4	0.293398	0.267406	0.309273	0.041867
FPCL/FPL	4	0.393742	0.375847	0.413095	0.037248
FPPL/FPL	4	0.083414	0.075952	0.10536	0.029408
FPFL/FPL	4	0.075721	0.069008	0.079198	0.01019
SLPIL/SLPL	4	0.089894	0.076772	0.108245	0.031474
SLPML/SLPL	4	0.184828	0.171812	0.192187	0.020375
SLPCL/SLPL	4	0.214009	0.199838	0.222592	0.022755
SLPPL/SLPL	4	0.203387	0.184282	0.227436	0.043154
SLPFL/SLPL	4	0.307882	0.285586	0.329346	0.04376
SSPIL/SSPL	4	0.123345	0.107493	0.140421	0.032928
SSPML/SSPL	4	0.214688	0.202288	0.222668	0.02038
SSPCL/SSPL	4	0.231775	0.218322	0.238035	0.019713
SSPPL/SSPL	4	0.181703	0.170512	0.211835	0.041323
SSPFL/SSPL	4	0.248928	0.231443	0.266707	0.035264

Table 4.3.9: Descriptive statistics of morphometric measurements of *M. scabriculum*

Variable	Valid N	Mean	Minimum	Maximum	Range
TL/CL	29	2.153749	1.931306	2.272	0.340694
CL/TL	29	0.464550	0.440140	0.501228	0.61088
RL/CL	29	0.350924	0.317142	0.378779	0.061637
FPL/CL	29	0.862682	0.816151	0.907452	0.091301
SLPL/CL	29	2.6864097	1.97166	3.00048	1.02882
SSPL/CL	29	1.59311	1.2572842	1.73891865	0.48163445
TEL /CL	29	0.29344	0.271321	0.341728	0.070407
FSTEL/TEL	29	0.510999	0.40871	0.619687	0.210977
SSTEL/TEL	29	0.766966	0.670017	0.930649	0.260632
FPIL/FPL	29	0.158768	0.139836	0.207132	0.067296
FPML/FPL	29	0.344792	0.270204	0.381925	0.111721
FPCL/FPL	29	0.375127	0.340132	0.396999	0.056867
FPPL/FPL	29	0.067543	0.057143	0.093087	0.035944
FPFL/FPL	29	0.070198	0.053737	0.138776	0.085038
SLPIL/SLPL	29	0.088873	0.074949	0.108059	0.033109
SLPML/SLPL	29	0.192701	0.17706	0.206964	0.029904
SLPCL/SLPL	29	0.19319	0.184255	0.211268	0.027013
SLPPL/SLPL	29	0.257227	0.236874	0.285301	0.048426
SLPFL/SLPL	29	0.267393	0.242926	0.285663	0.042737
SSPIL/SSPL	29	0.128918	0.122062	0.150243	0.028181
SSPML/SSPL	29	0.217719	0.204131	0.228864	0.024732
SSPCL/SSPL	29	0.221885	0.211349	0.249036	0.037688
SSPPL/SSPL	29	0.178765	0.162298	0.208556	0.046259
SSPFL/SSPL	29	0.251055	0.21255	0.270175	0.057625

4.4 Variability Plots (The graph showing the various morphometric trait variations in each *Macrobrachium* species collected from the Neyyar river)



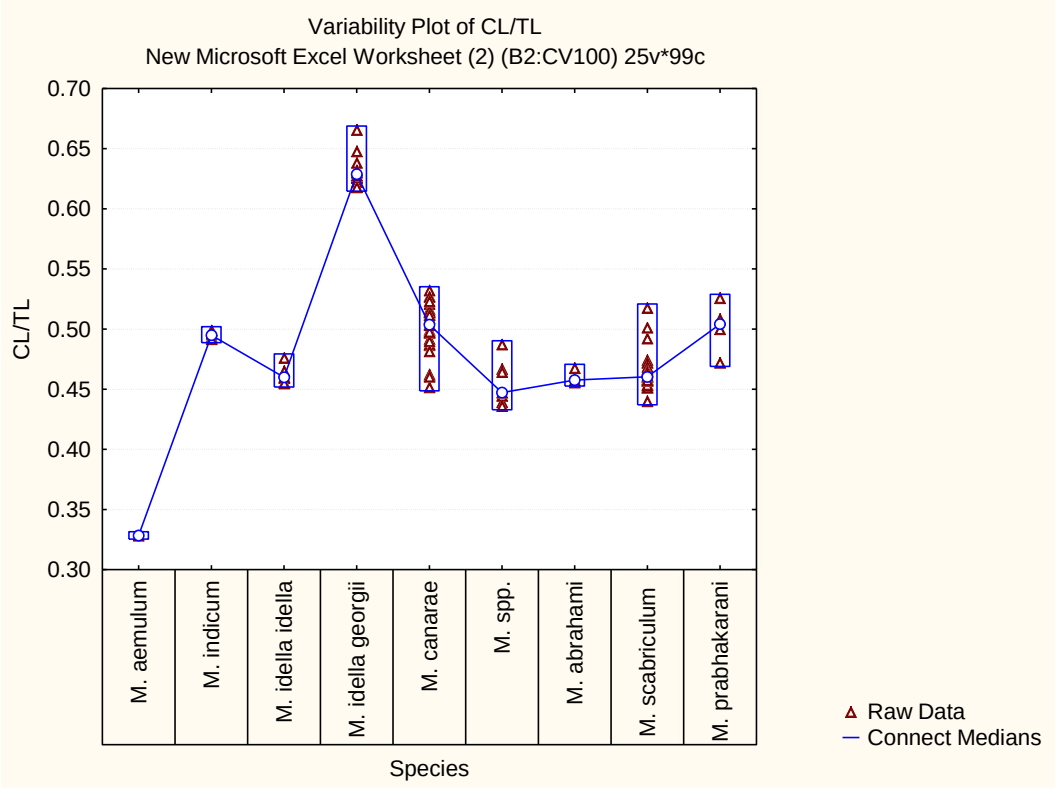
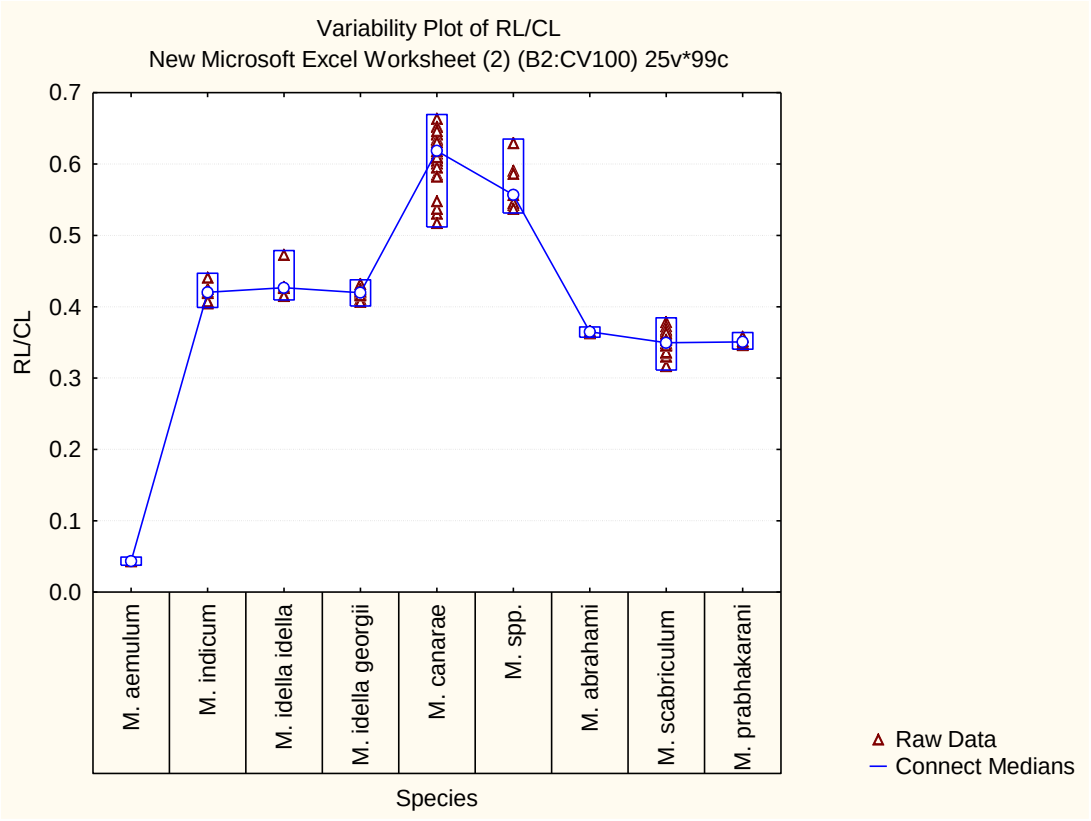


Figure 4.4.1 and Figure 4.4.2: Species wise variability plot of TL/CL and CL/TL



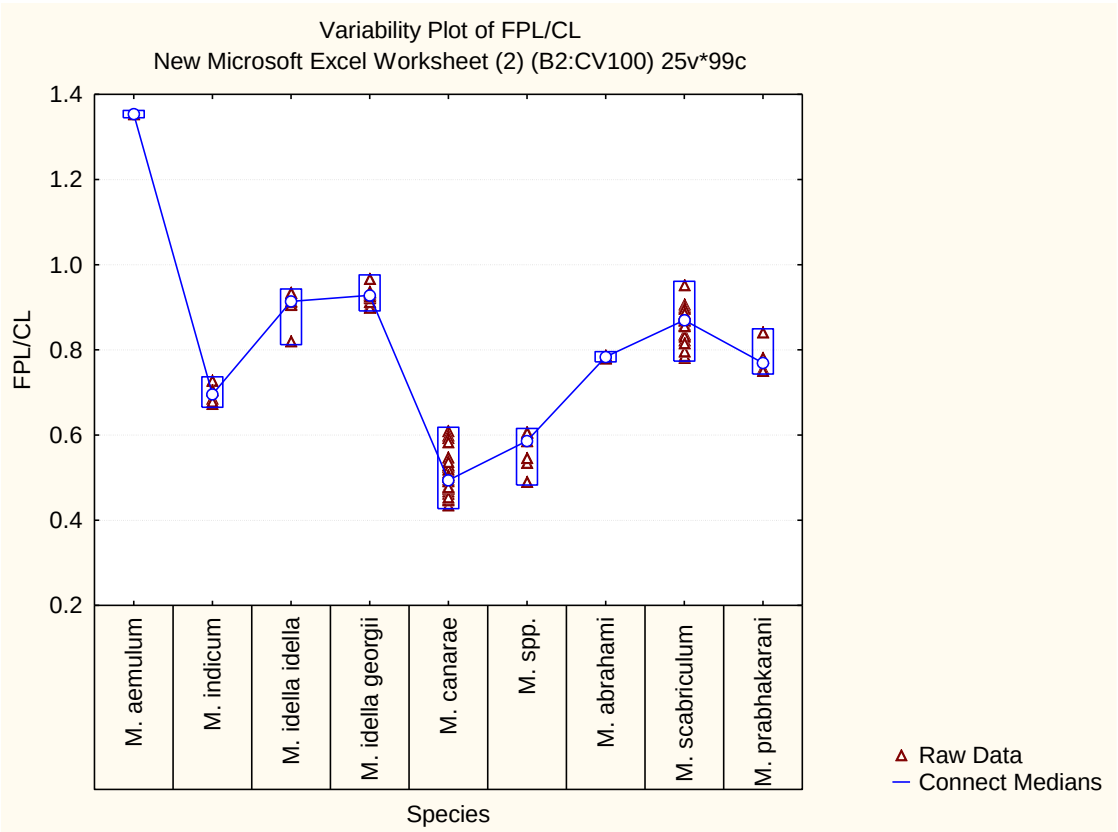
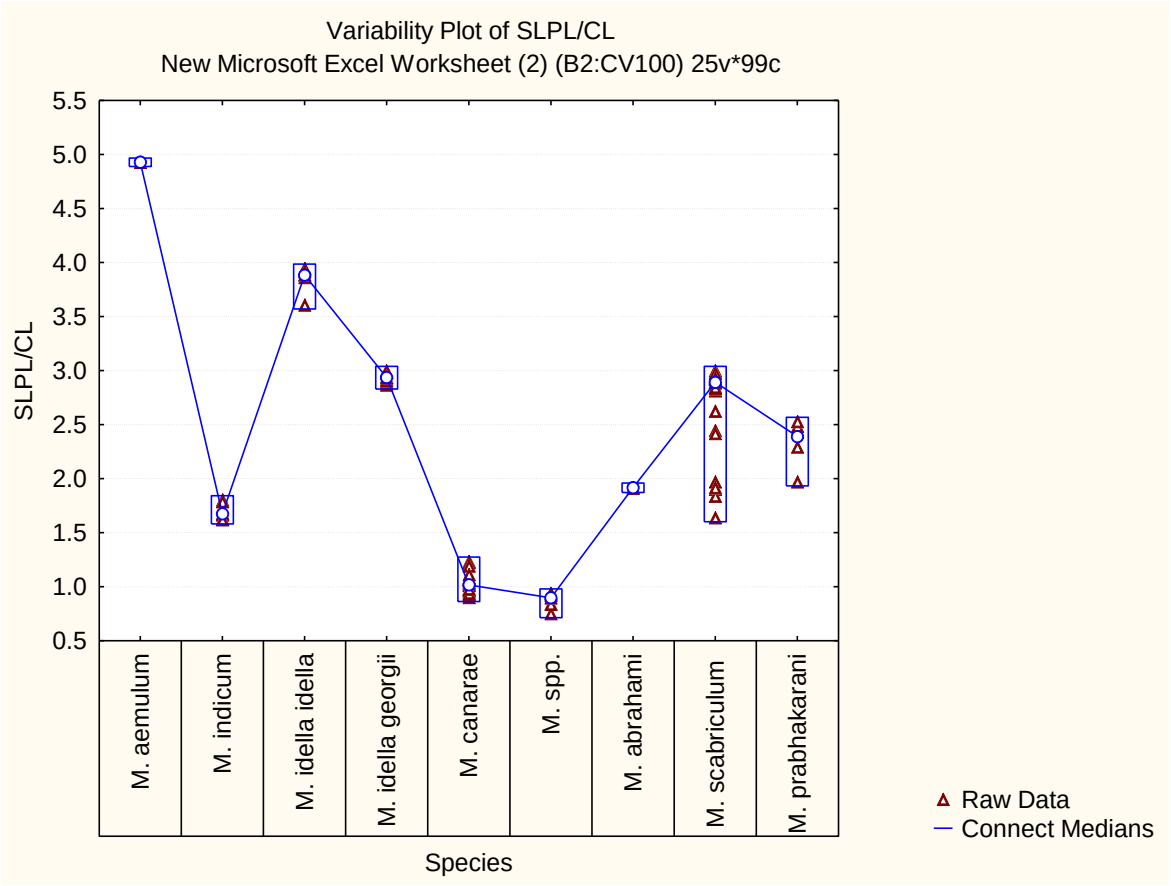


Figure 4.4.3 and Figure 4.4.4: Species wise variability plot of RL/CL and FPL/CL



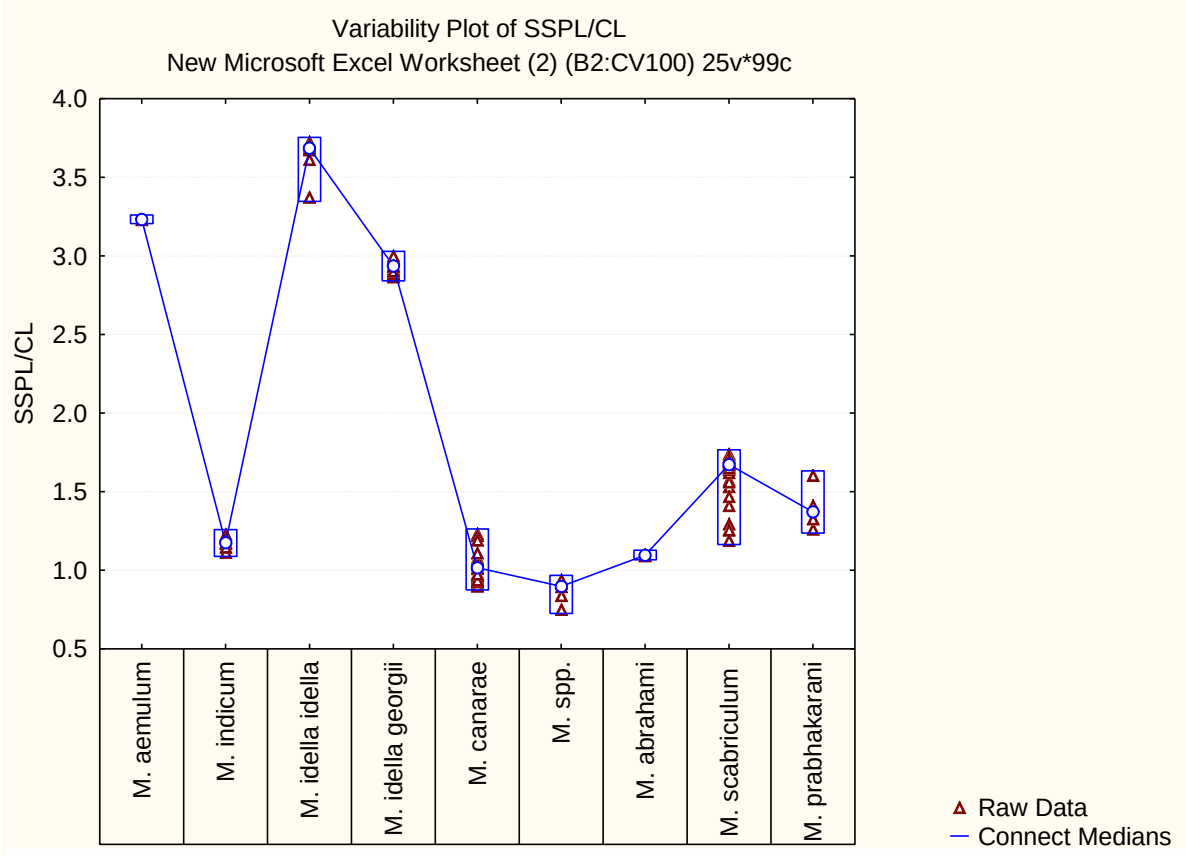
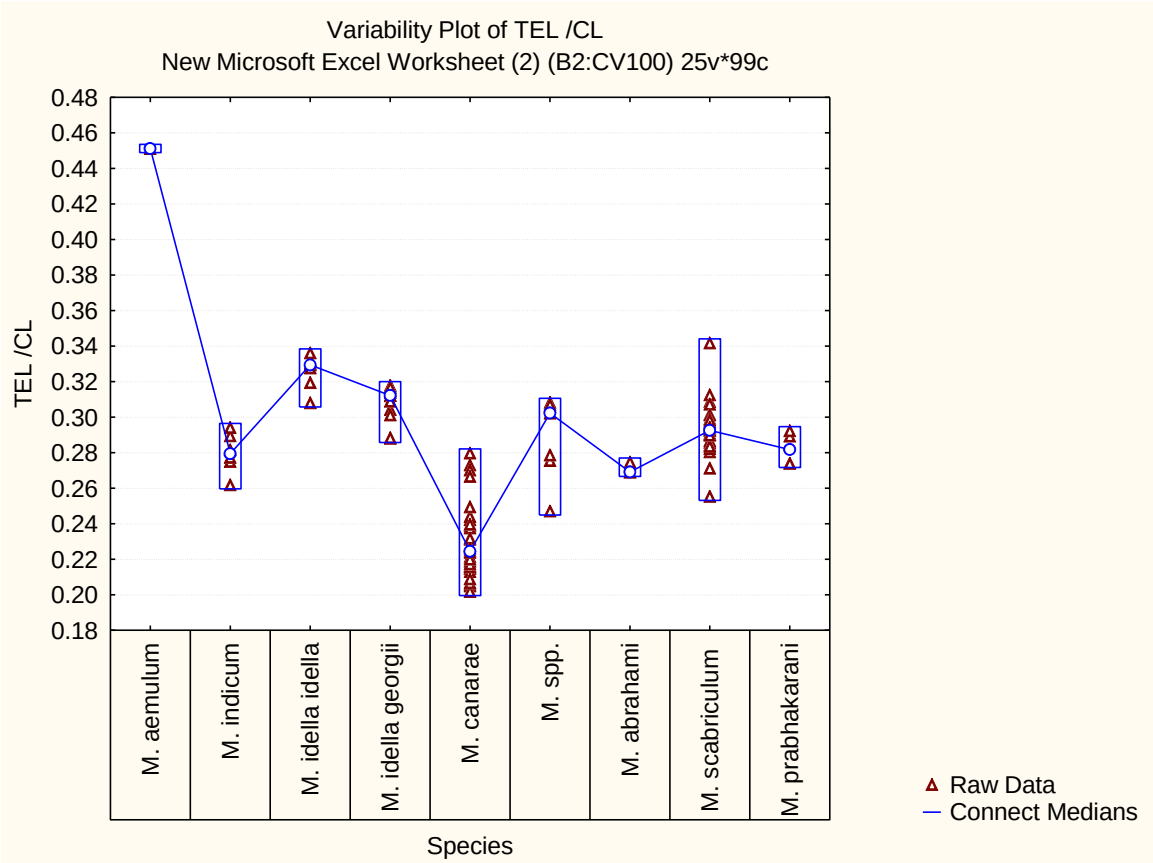


Figure 4.4.5 and Figure 4.4.6: Species wise variability plot of SLPL/CL and SSPL/CL



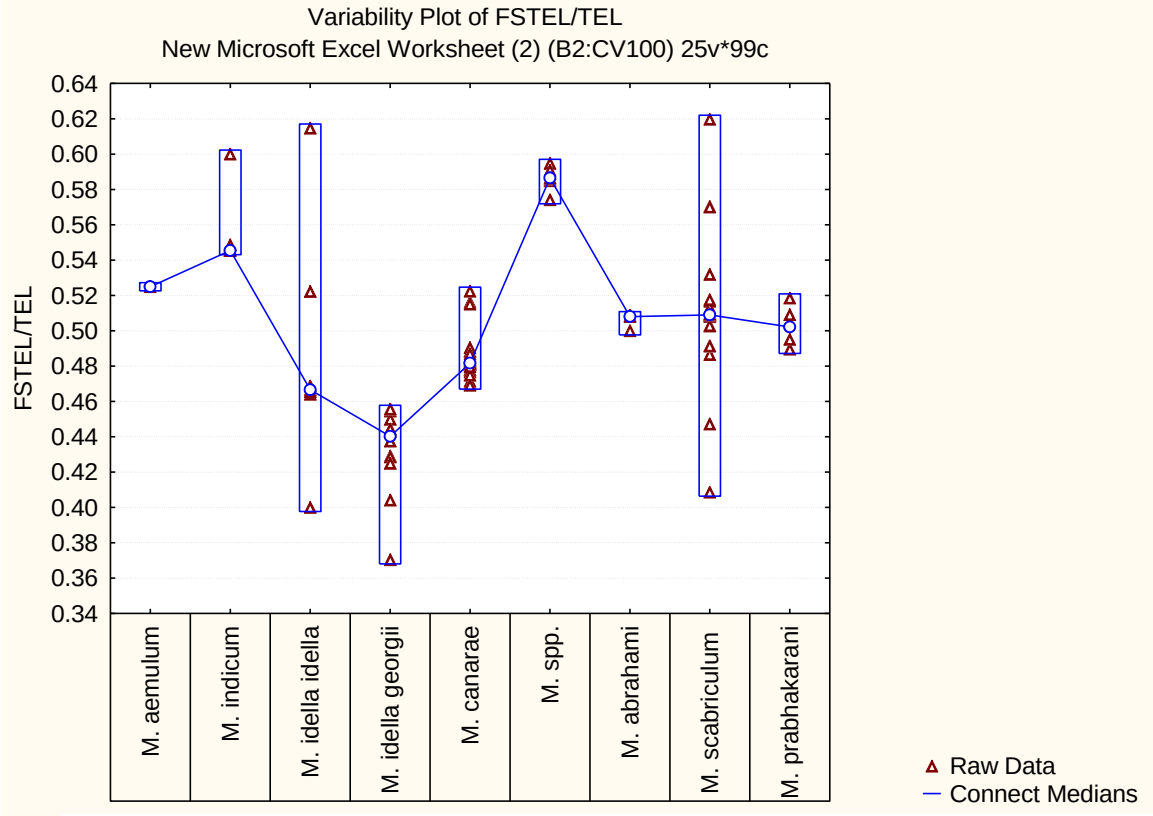
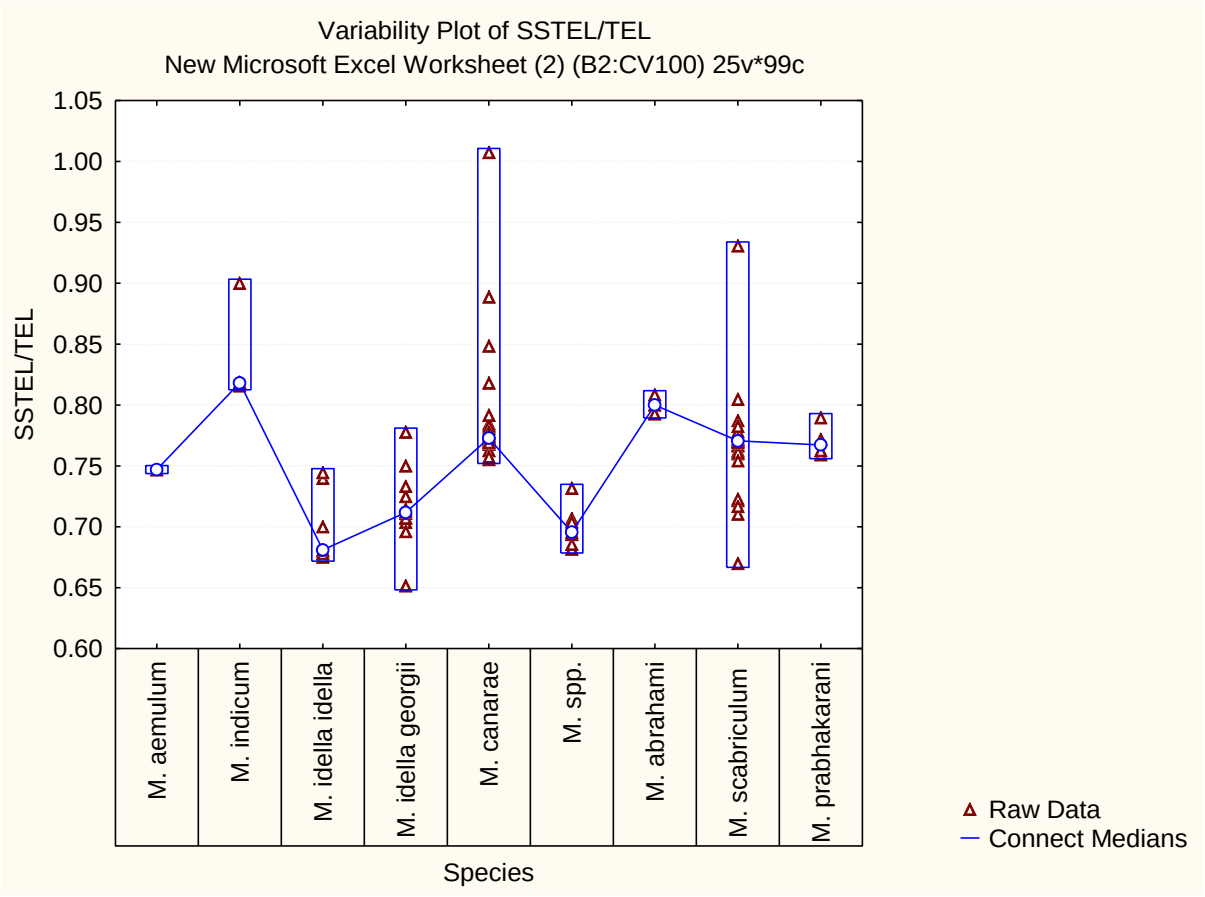


Figure 4.4.7 and Figure 4.4.8: Species wise variability plot of TEL/CL and FSTEL/TEL



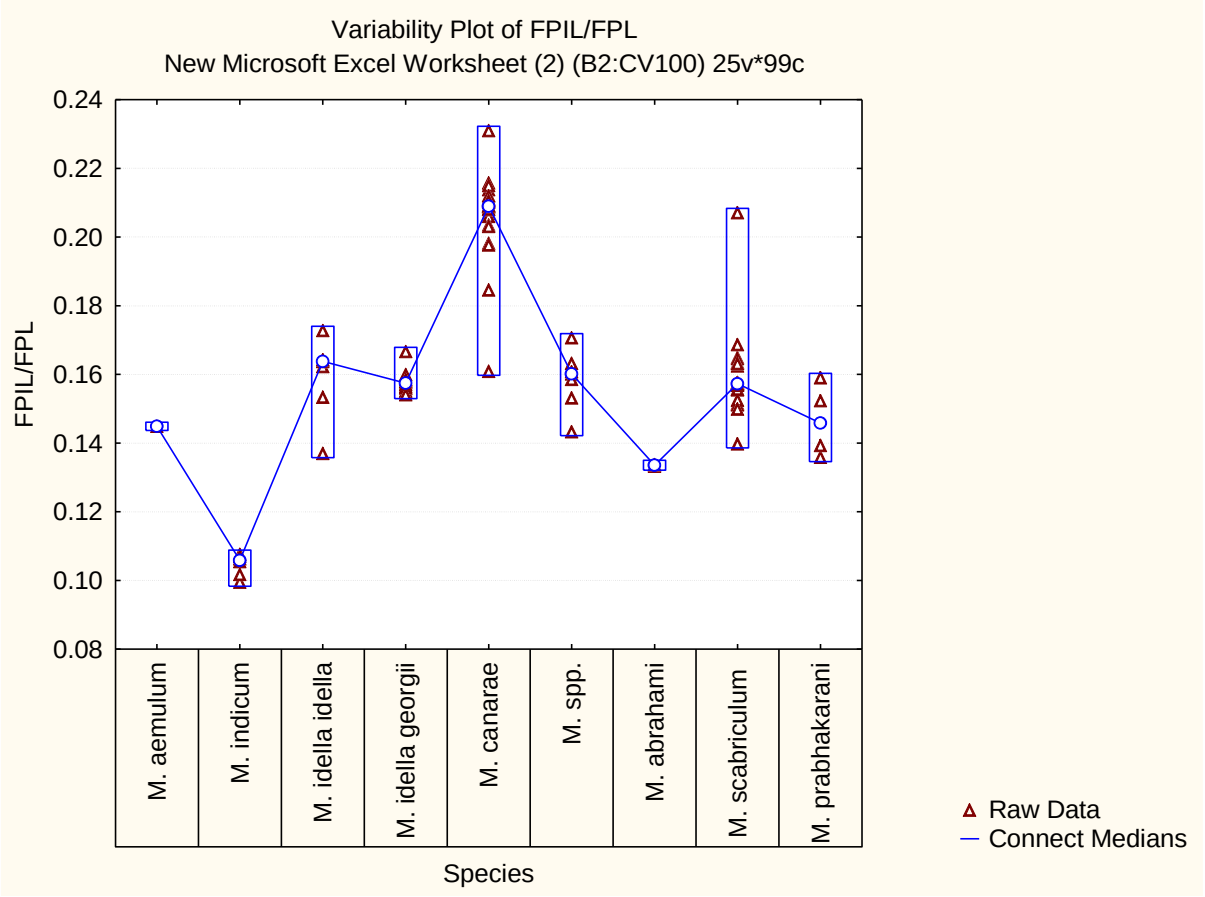
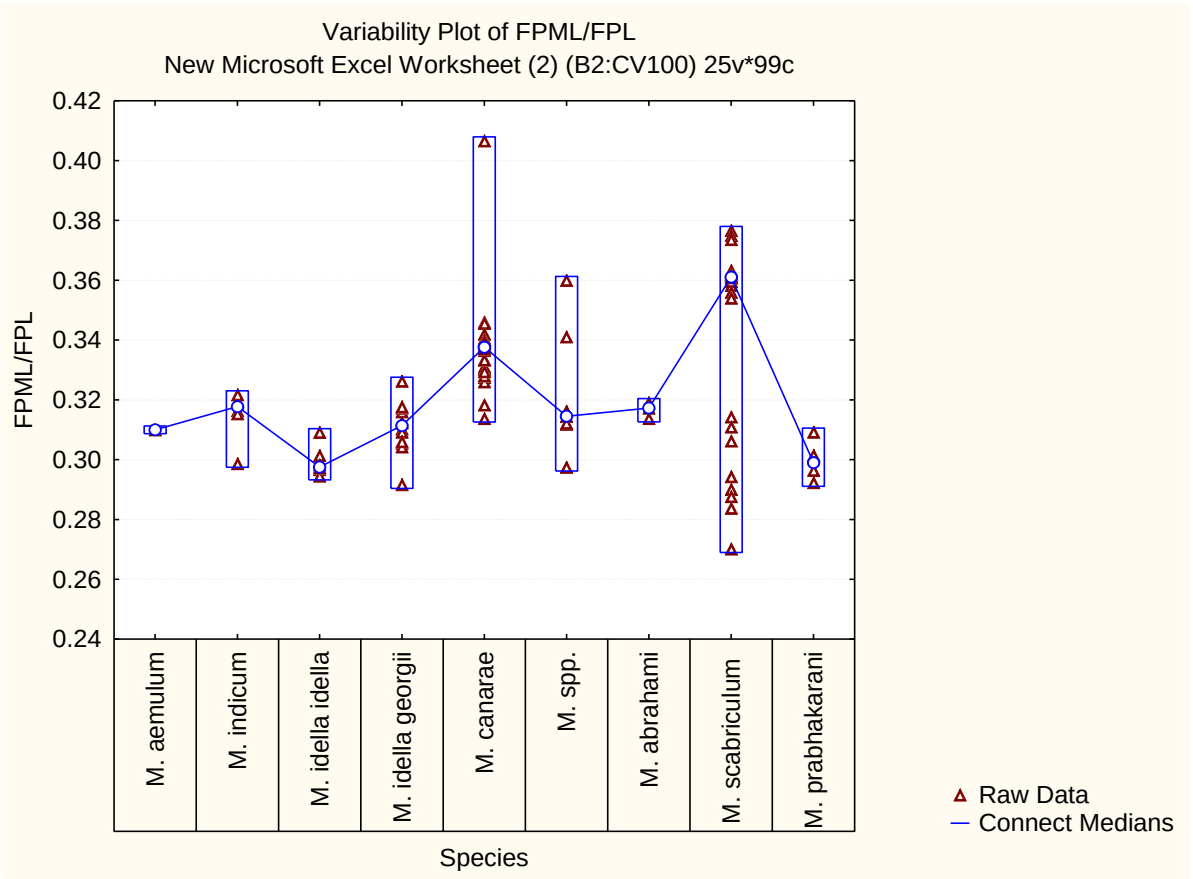


Figure 4.4.9 and Figure 4.4.10: Species wise variability plot of SSTEL/CL and FPIL/FPL



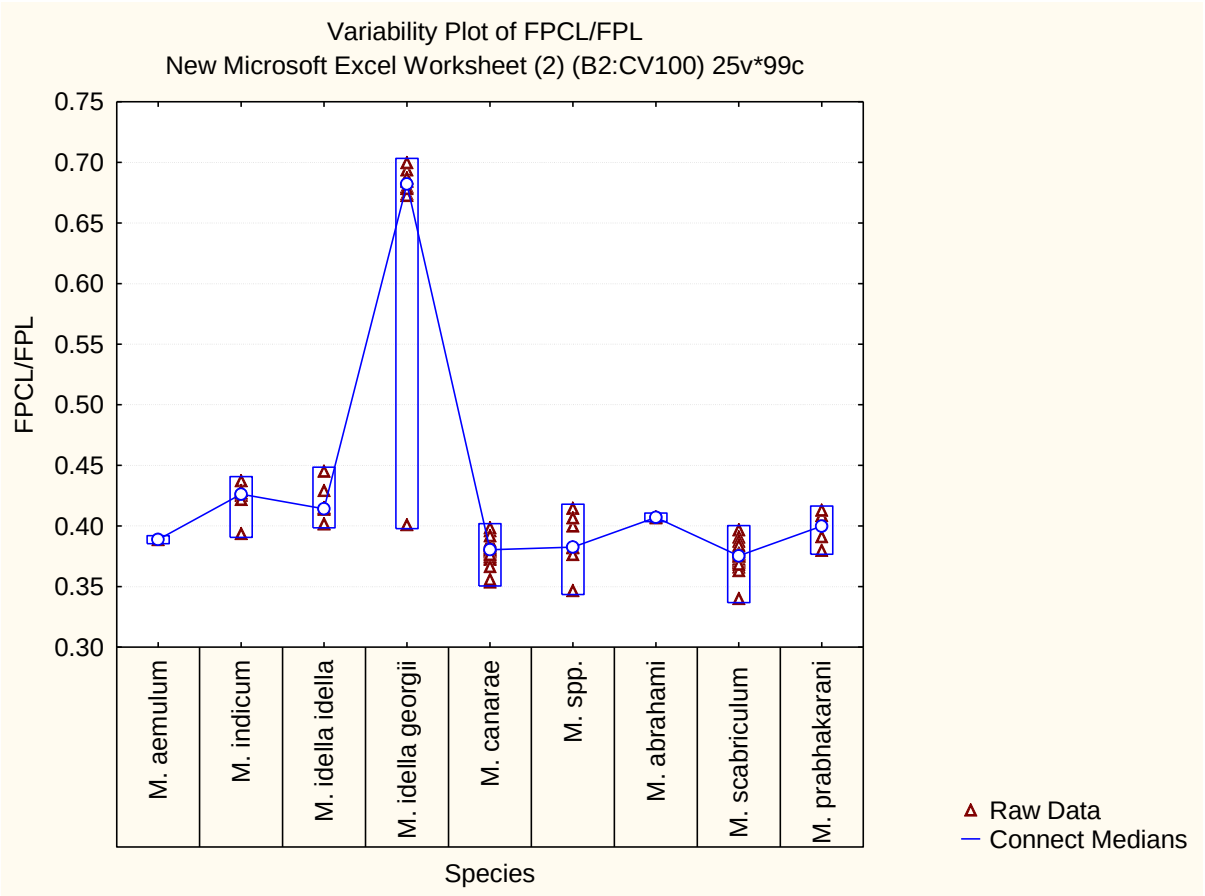


Figure 4.4.11 and Figure 4.4.12: Species wise variability plot of FPML/FPL and FPCL/FPL

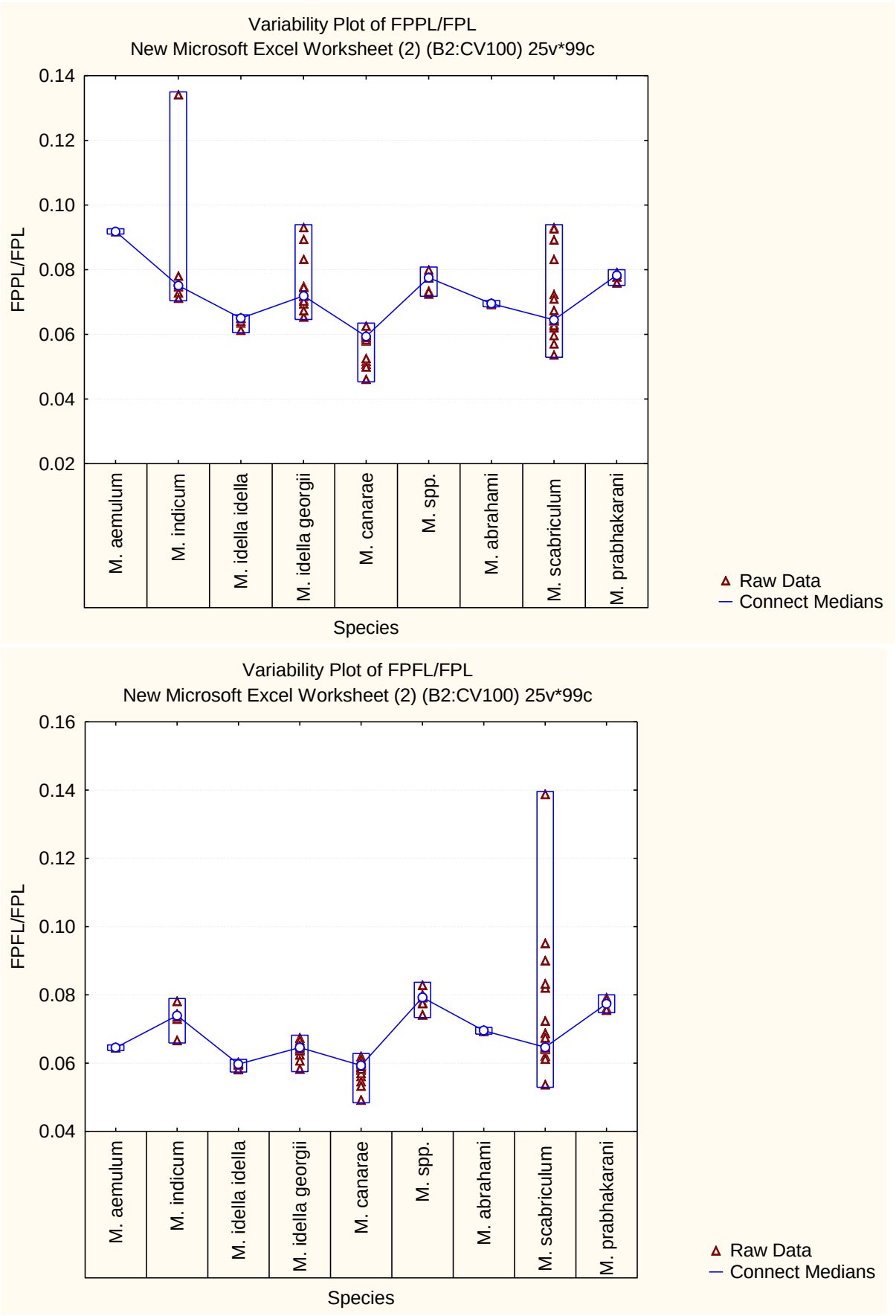
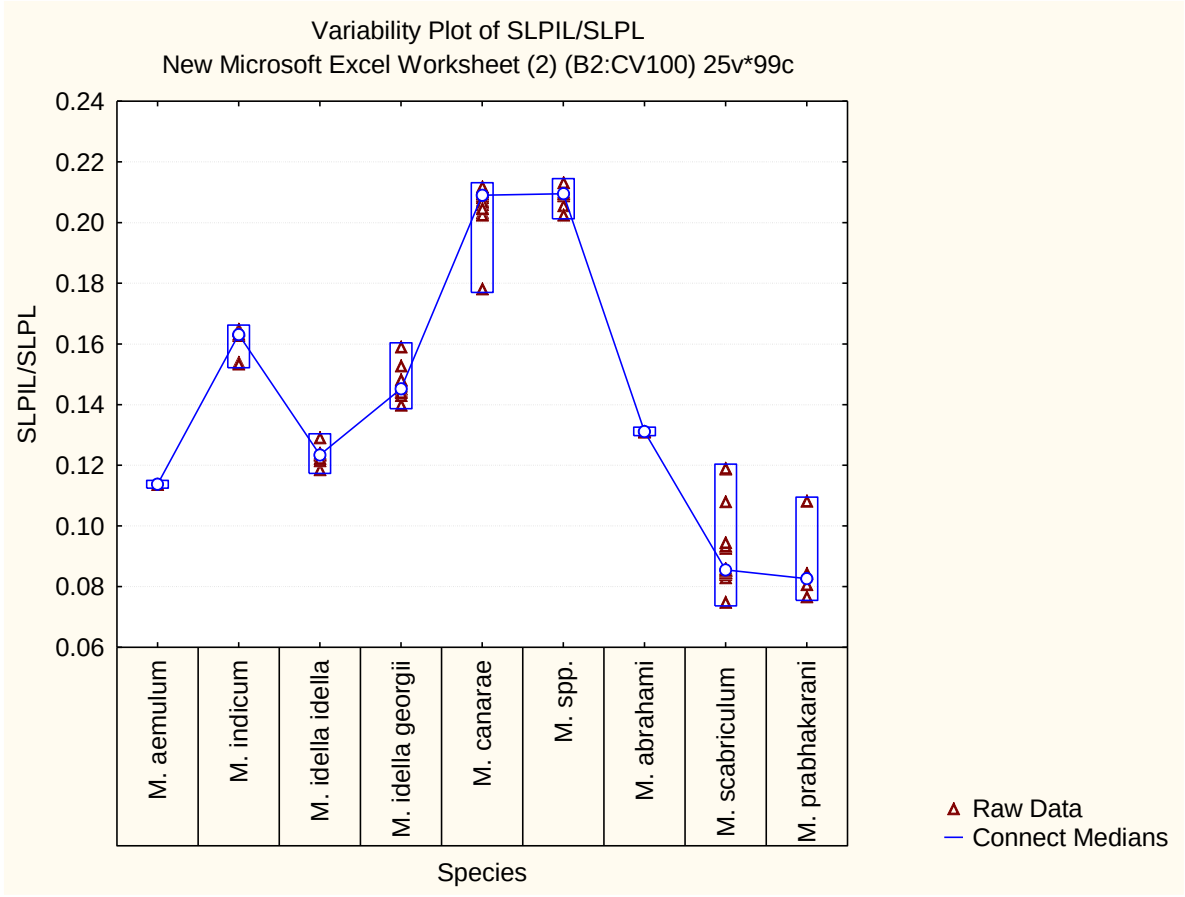


Figure 4.4.13 and Figure 4.4.14: Species wise variability plot of FPPLL/FPL and FPFL/FPL



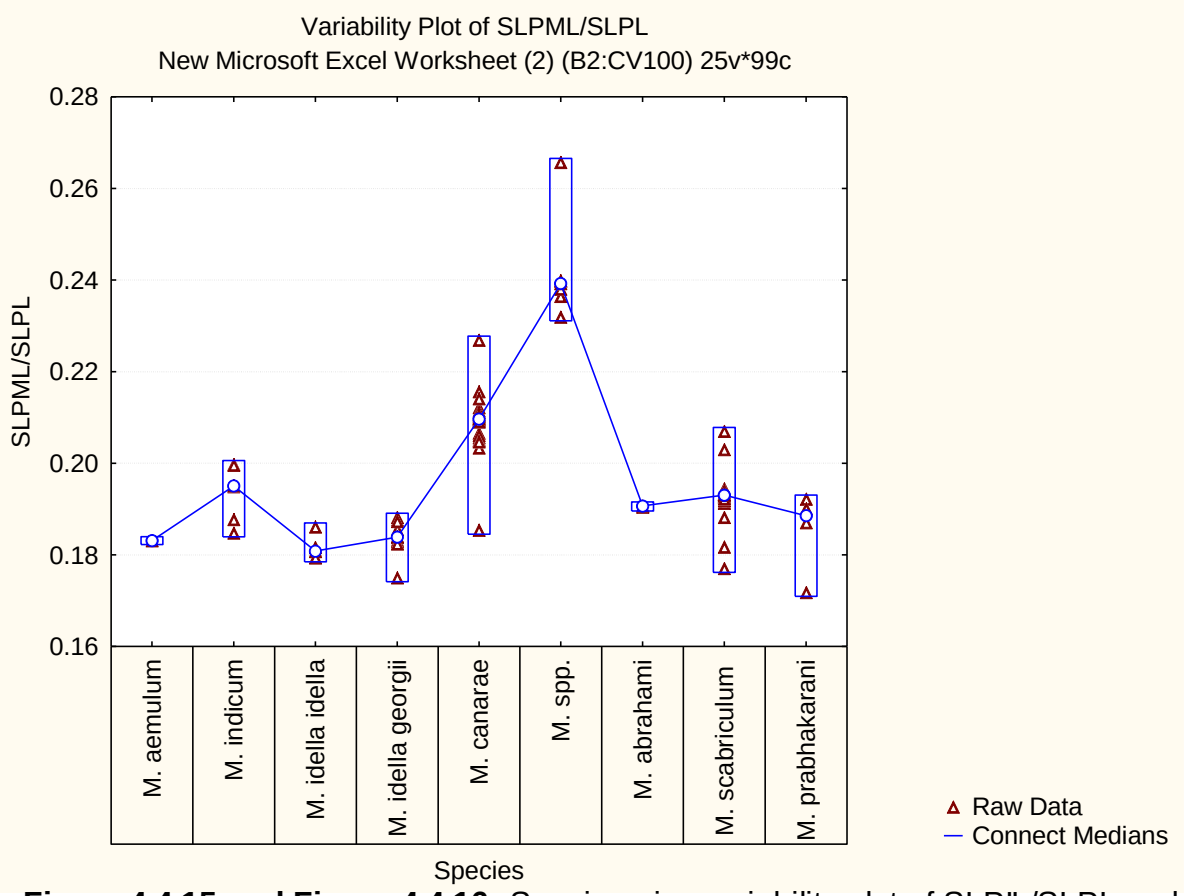
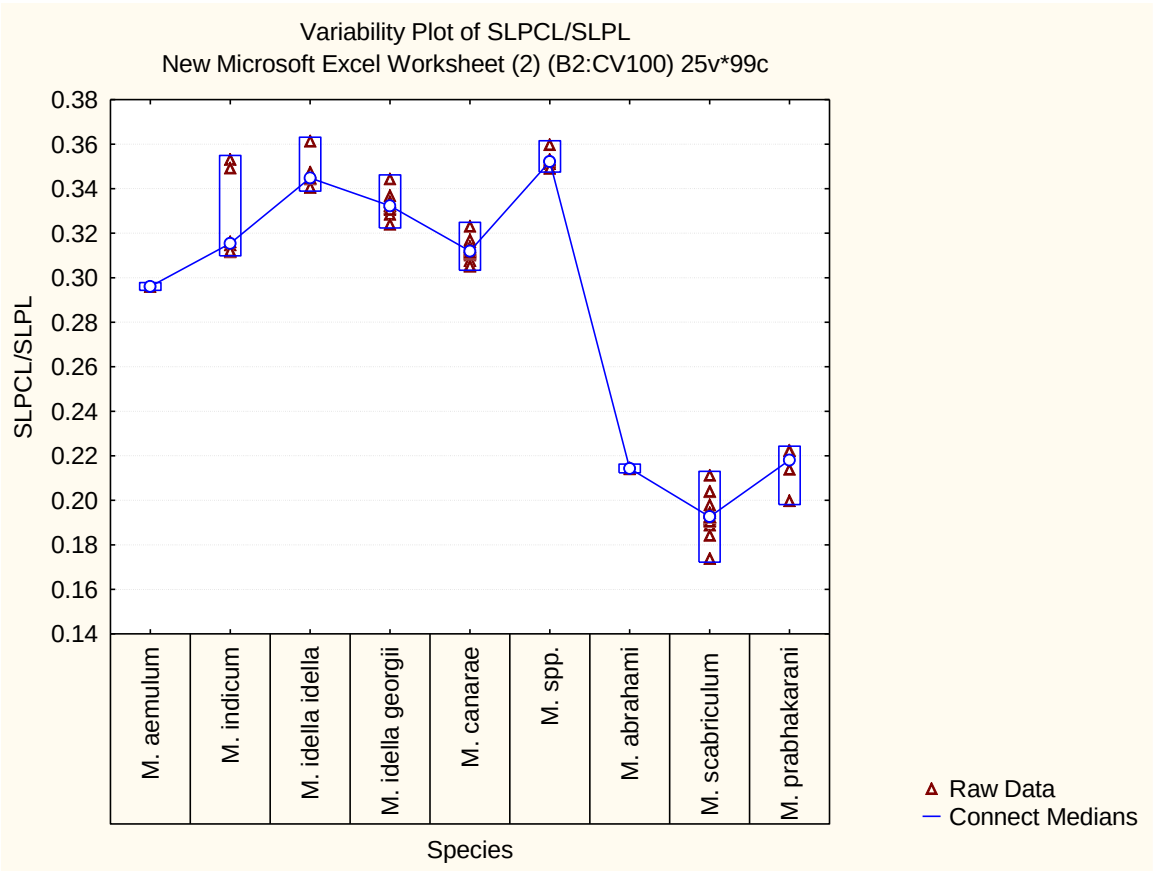


Figure 4.4.15 and Figure 4.4.16: Species wise variability plot of SLPIL/SLPL and SLPML/SLPL



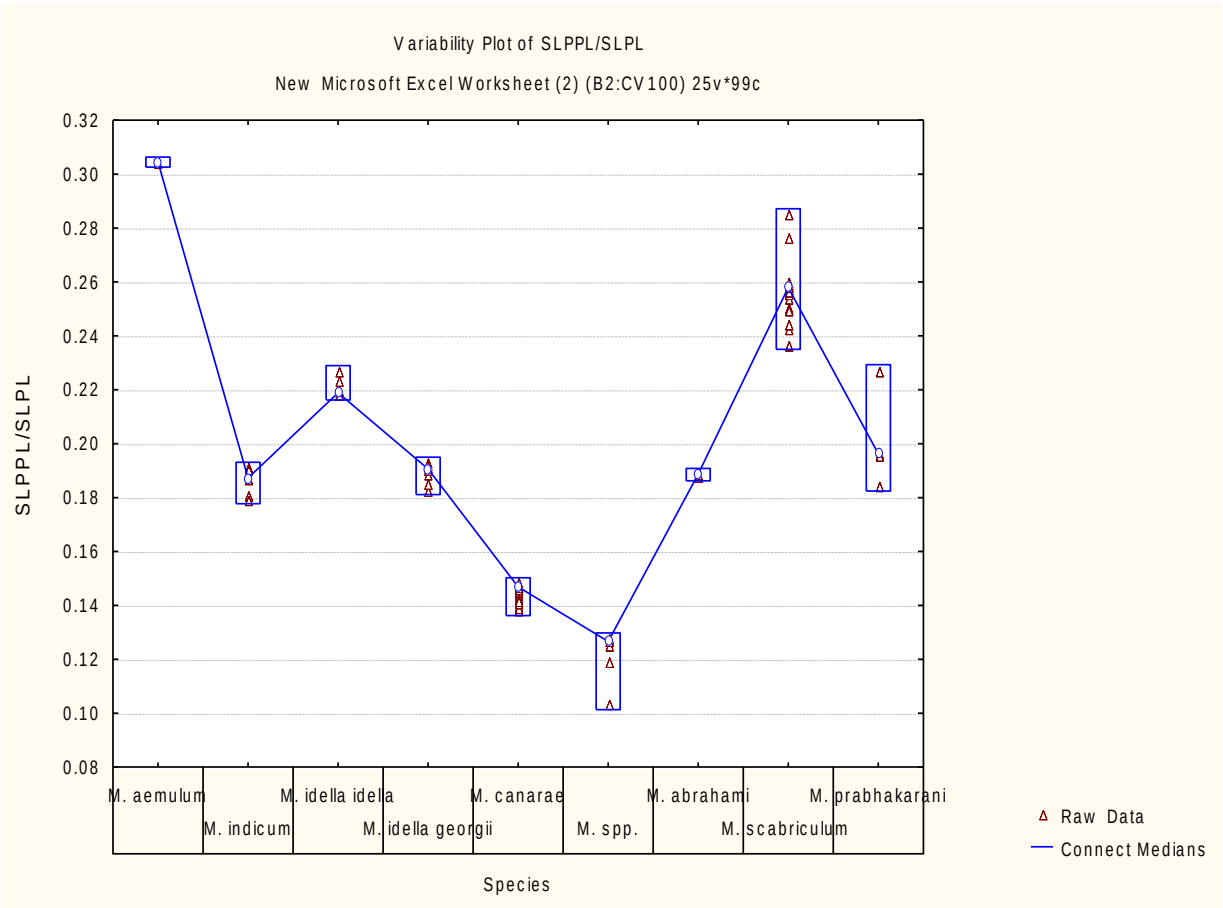
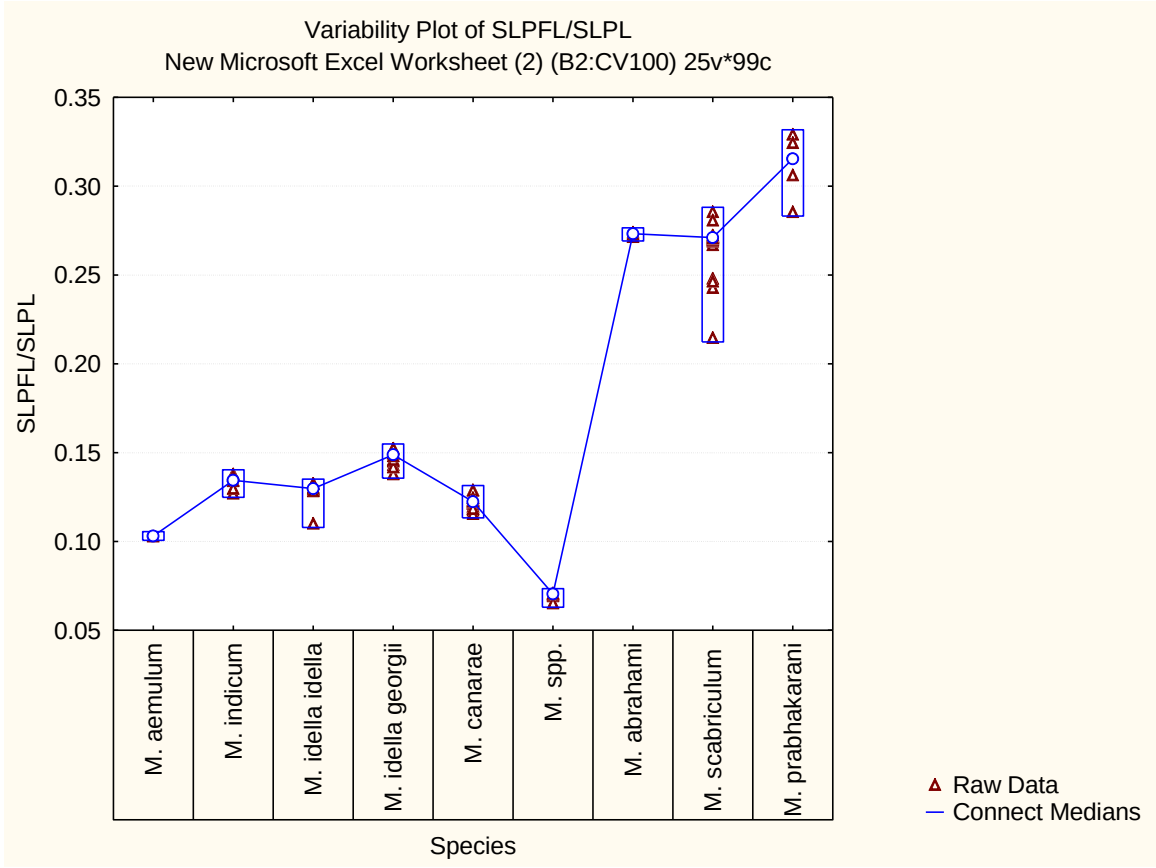


Figure 4.4.17and Figure 4.4.18: Species wise variability plot of SLPCL/SLPL and SLPPL/SLPL



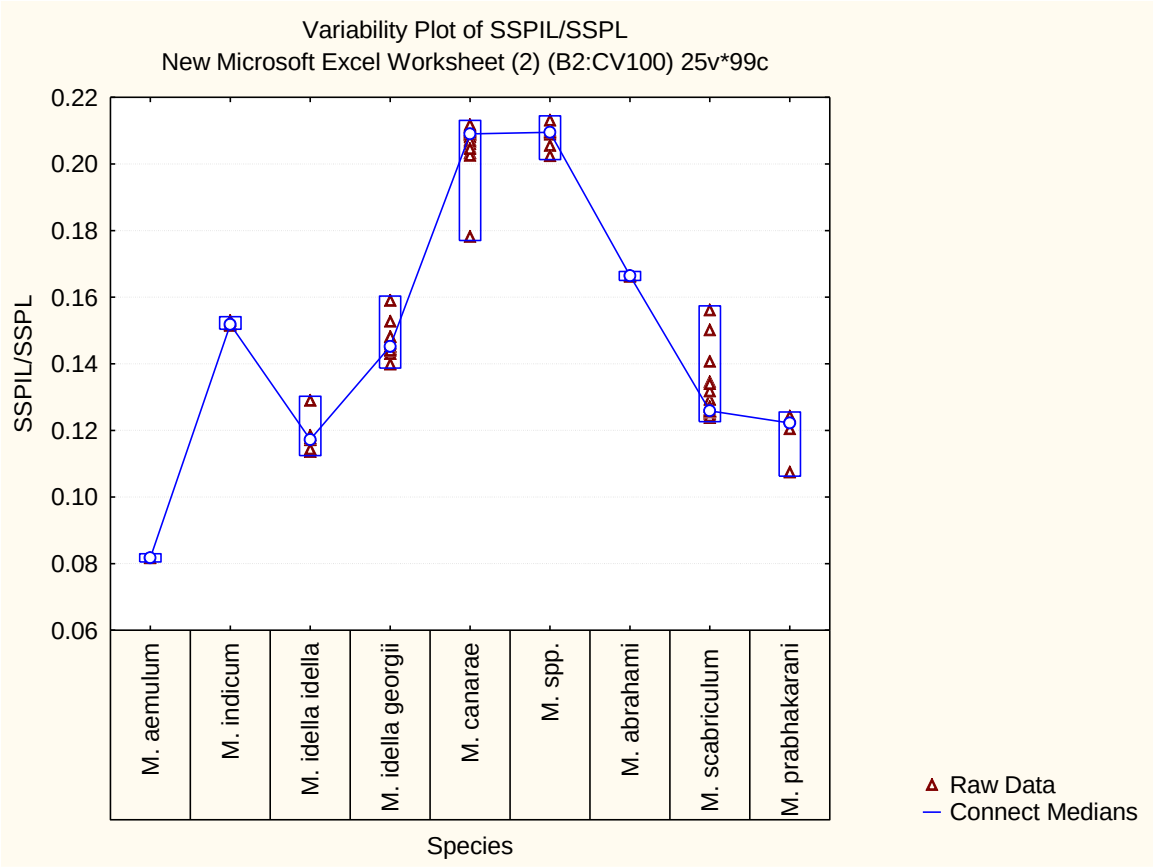


Figure 4.4.19 and Figure 4.4.20: Species wise variability plot of SLPFL/SLPL and SSPIL/SSPL

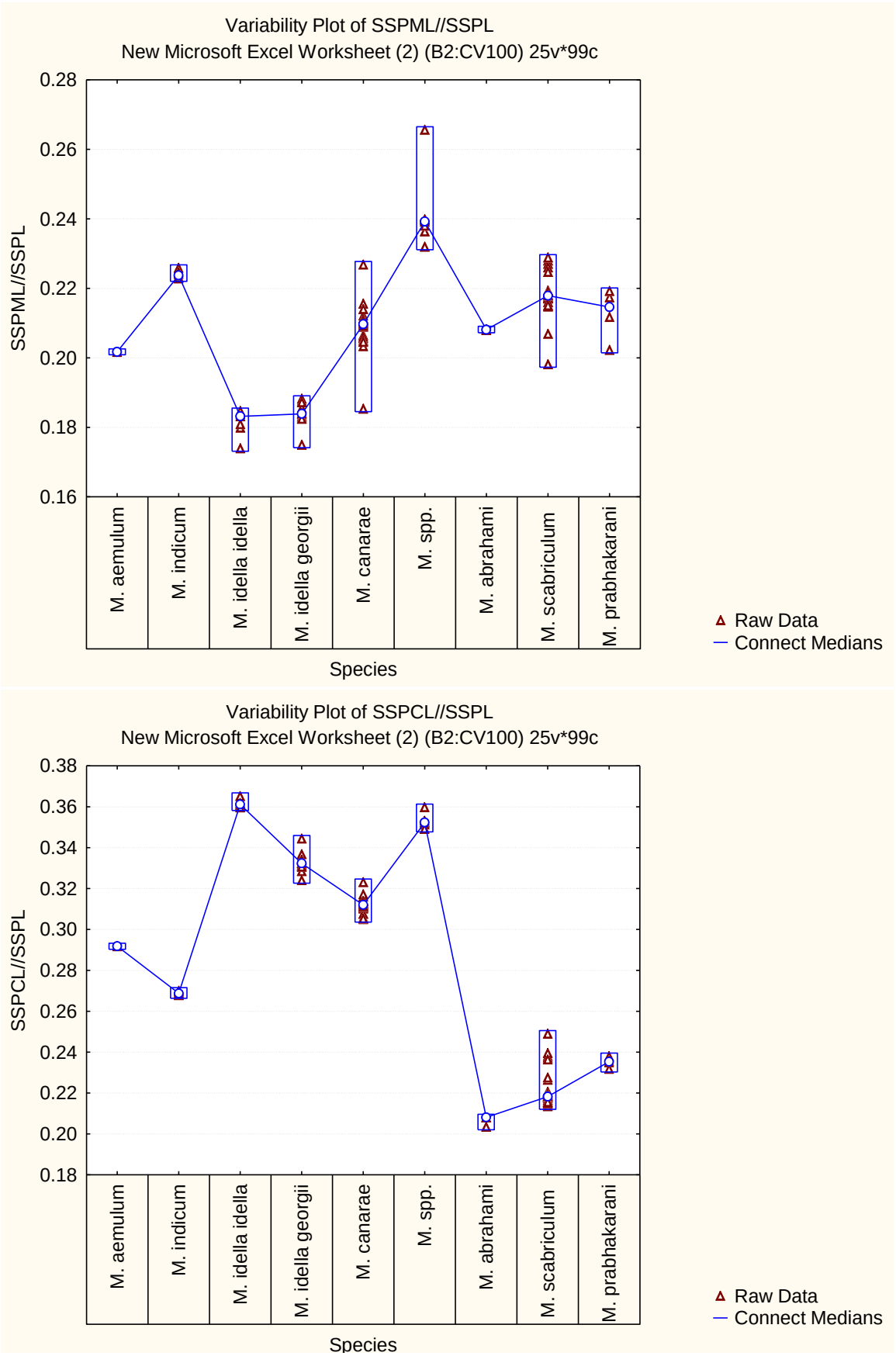
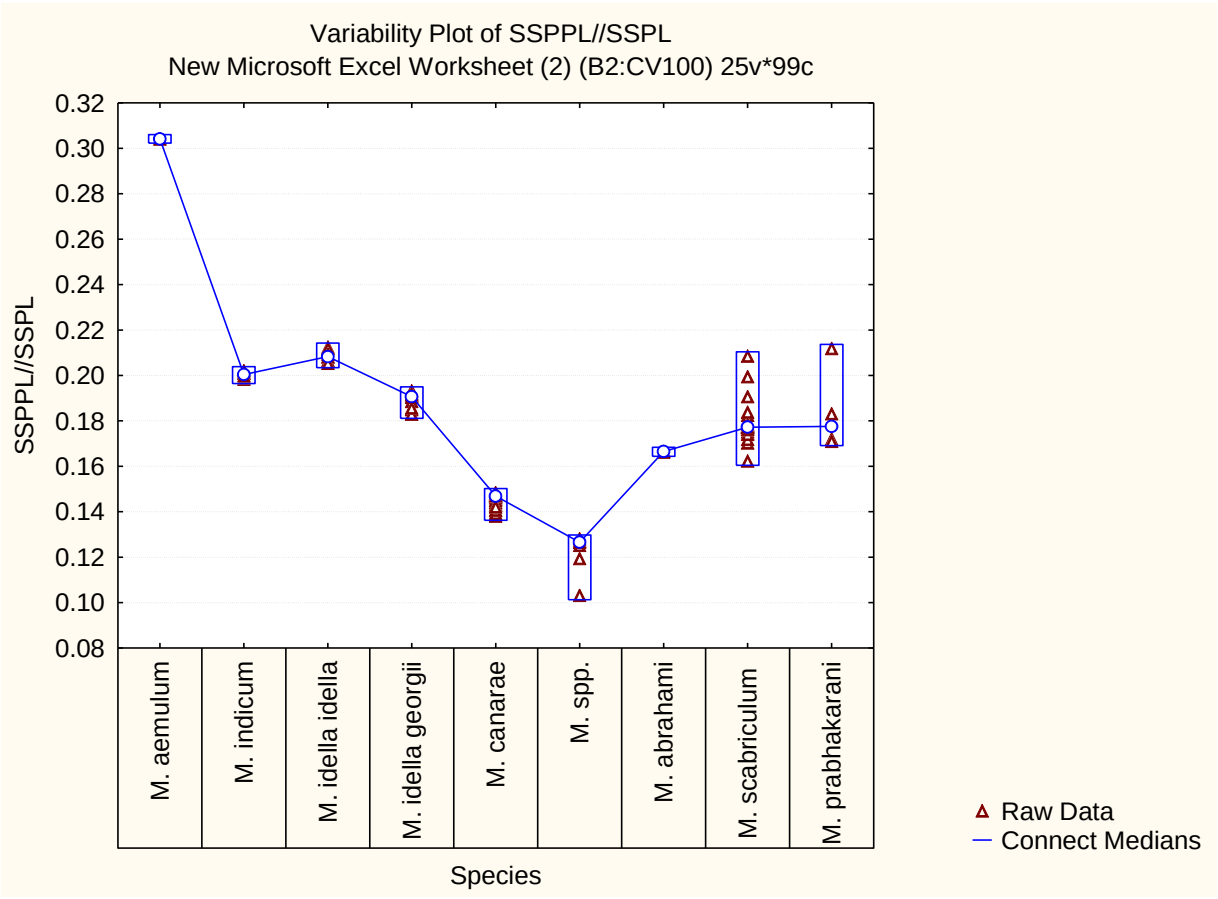


Figure 4.4.21 and Figure 4.4.22: Species wise variability plot of SSPML/SSPL and SSPCL/SSPL



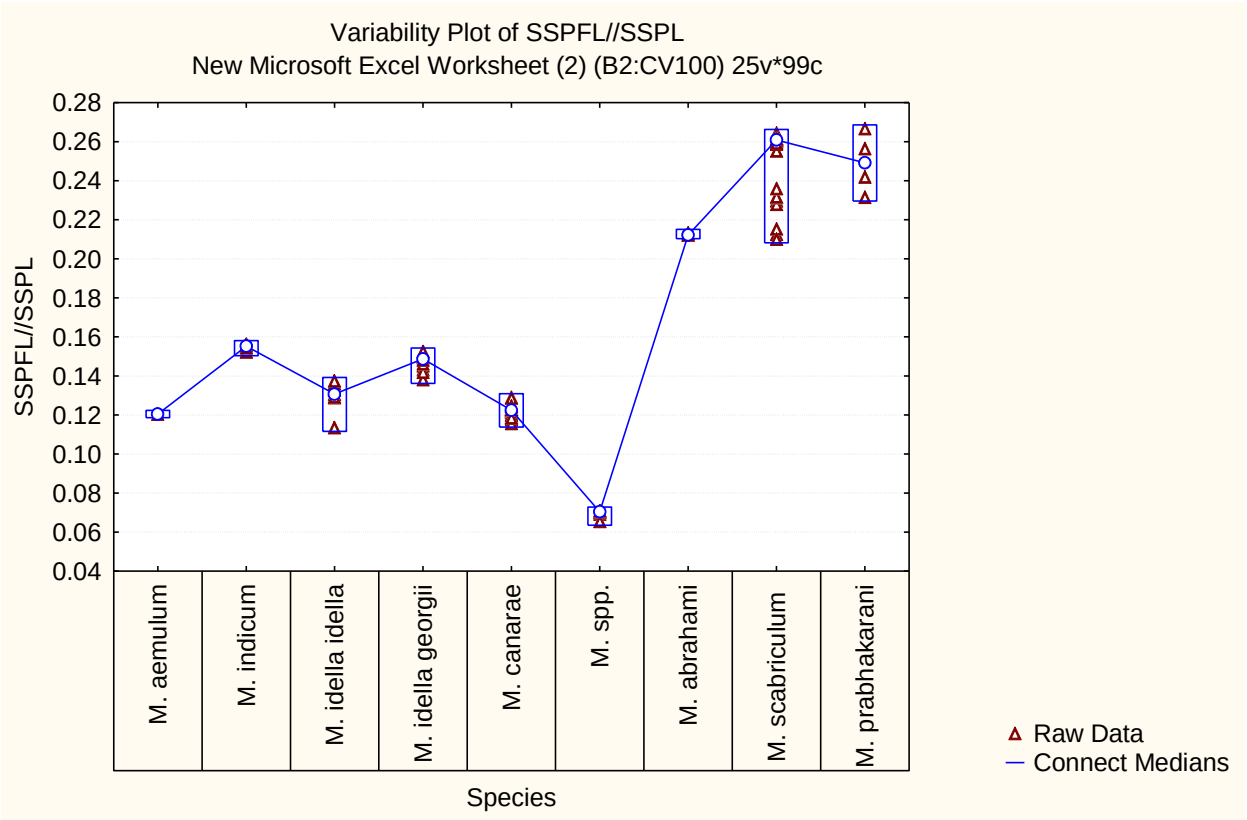


Figure 4.4.23 and Figure 4.4.24: Species wise variability plot of SSPFL/SSPL and SSPFL/SSPL

4.5 Multivariate Analysis of Morphometric Traits

4.5.1 Factor analysis

Twenty four morphometric characters after log transformation were subjected to the factor analysis to generated factors. The factor loading after varimax rotation for the morphometric variables were analysed. The characters having factor loading of above 0.9 on any of the first two factors were selected for subsequent stepwise discriminant analysis. The selected variables (6) are Rostral length (RL), Length of first pereiopod (FPL) Length of large 2nd pereiopod (SLPL), Ischium length of second large pereiopod (SLPIL), palm length of second large pereiopod (SLPPL), ischium length small second pereiopod (SSPIL).

Table 4.5.1.1: Factor loading (Extraction of principle components) - Palaemonidae

	Factor Loadings (Varimax raw) (New Microsoft Excel Workshee Extraction: Principal components (Marked loadings are >.900000)	
Variable	Factor 1	Factor 2
TL	0.12051	-0.80528
CL	-0.12051	0.80528
RL	-0.9778(	0.03907(
FPL	0.9184[	0.29590
SLPL	0.91561	0.32228(
SSPL	0.6533(	0.67071
TEL	0.71773	0.31713
FSTEL	-0.0564(	-0.65075
SSTEL	-0.1050[	-0.47310
FPIL	-0.5620[	-0.08948
FPML	0.04363	-0.55048
FPCL	0.12977	0.87460[
FPPL	0.29551	0.25110
FPFL	0.2297(	-0.15495(
SLPIL	-0.9520	0.21232
SLPML	-0.7547	-0.43966
SLPCL	-0.7090	0.58198
SLPPL	0.9746[	-0.10347[
SLPFL	0.8576(	-0.29432
SSPIL	-0.9627	-0.09991
SSPML	-0.1555(	-0.81056
SSPCL	-0.6843(	0.62818(
SSPPL	0.8045(	0.37100(
SSPFL	0.8706	-0.27419[
Expl.Var	10.58687	5.79955
Prp.Totl	0.44112	0.24164

4.5.2 Stepwise (forward) discriminant analysis

Selected variables (6) after factor analysis were subjected to stepwise discriminant analysis (SDFA). Discriminant analysis generated 6 functions (table 4.5.2.1) with first two explaining 96.47% of total variation. The means of conical variables shows that that Root 1 successfully discriminates between most of the concerned species.

Table 4.5.2.1: Standardized coefficient for Canonical variables- Palaemonidae

	Standardized Coefficients (New Microsoft Excel Worksheet (2) (B2:CN92 for Canonical Variables				
Variable	Root 1	Root 2	Root 3	Root 4	Root 5
SLPPL	-0.8319	0.02575	-0.28367	-0.466117	0.151177
SLPL	0.5487	0.85733	-0.83493	-0.564544	0.634025
SLPIL	0.6449	0.70594	-1.05460	0.419161	0.362571
RL	1.1980	0.80581	1.07741	-0.980695	-0.766430
FPL	0.7242	0.72581	1.99874	-0.366723	-0.287732
SSPIL	0.1441	-0.42934	0.44598	-0.958766	0.817966
Eigenval	163.5283	16.81968	3.45967	2.396327	0.733036
Cum.Prop	0.8748	0.96475	0.98326	0.996079	1.000000

Table 4.5.2.2: Means of Canonical variables- Palaemonidae

	Means of Canonical Variables (New Microsoft Excel Worksheet (2) (B2:CN92				
Group	Root 1	Root 2	Root 3	Root 4	Root 5
M. indicum	-1.4631	-0.18106	-4.16160	4.19784	0.79303
M. idella idella	-1.9607	9.23632	-1.29911	-0.12517	-2.02950
M. idella georgii	2.8907	6.56526	1.74506	-0.14009	1.39300
M. canarae	11.9029	-2.04256	-0.92253	-1.13526	0.02724
M. spp.	15.4027	-3.17058	3.97221	2.84056	-0.95052
M. scabriculum	-16.4514	-2.03035	0.44803	-0.29159	-0.04935

The factor structure matrix (Table 4.5.2.3), the palm length of second large pereiopod and finger (SLPPL), and Ischium length of large 2nd pereiopod (SLPIL), are the main contributors to the Root 1 discriminating the species. Whereas for Root 2 the total length of second large periopod (SLPL), total length of first pereiopod (FPL), contribute more to the species discrimination. While the major contributors to the third and fourth are rostral length (RL) and ischium length of small 2nd periopod (SSPIL).

Table 4.5.2.3: Factor structure matrix - Palaemonidae

Variable	Factor Structure Matrix (New Microsoft Excel Worksheet (2) (B2:CN92) Correlations Variables - Canonical Roots (Pooled-within-groups correlations) Root 1	Root 2	Root 3	Root 4	Root 5
SLPPL	-0.652744	0.391572	-0.199186	-0.444917	0.010294
SLPL	-0.288175	0.708506	0.158090	-0.058511	0.121535
SLPIL	0.458630	-0.117376	-0.376090	0.146986	0.396877
RL	0.378115	-0.257170	-0.317531	-0.533671	-0.611710
FPL	-0.264541	0.564748	0.518436	0.347972	0.470262
SSPIL	0.401955	-0.611831	-0.034178	-0.315213	0.542771

Table 4.5.2.4 Classification matrix- Palaemonidae

Group	Classification Matrix (New Microsoft Excel Worksheet (2) (B2:CN92)) Rows: Observed classifications Columns: Predicted classifications Percent Correct	M. indicum p=.06593	M. idella idella p=.07692	M. idella georgii p=.13187	M. canarae p=.32967	M. spp. p=.07692	M. scabriculum p=.31868
M. indicum	100.0000	6	0	0	0	0	0
M. idella idella	100.0000	0	7	0	0	0	0
M. idella georgii	100.0000	0	0	12	0	0	0
M. canarae	100.0000	0	0	0	30	0	0
M. spp.	100.0000	0	0	0	0	7	0
M. scabriculum	100.0000	0	0	0	0	0	29
Total	100.0000	6	7	12	30	7	29

Table 4.5.2.5: Summary of Stepwise analysis- Palaemonidae

Variable Enter/Remove	Summary of Stepwise Analysis (New Microsoft Excel Worksheet (2) (B2:CN92)) Step	F to entr/rem	df 1	df 2	p-level	No. of vars. in	Lambda	F-value	df 1	df 2	p-level
SLPPL -(E)	1	1238.721	5	85	0.00000(	1.00000(	0.013538	1238.72:	5	85	0.00
SLPL -(E)	2	88.032	5	84	0.00000(	2.00000(	0.00217(	343.881	10	168	0.00
SLPIL -(E)	3	66.955	5	83	0.00000(	3.00000(	0.00043:	238.14(	15	230	0.00
RL -(E)	4	49.991	5	82	0.00000(	4.00000(	0.00010(	201.547	20	273	0.00
FPL -(E)	5	32.845	5	81	0.00000(	5.00000(	0.00003:	179.144	25	302	0.00
SSPIL -(E)	6	27.305	5	80	0.00000(	6.00000(	0.00001:	168.03:	30	322	0.00

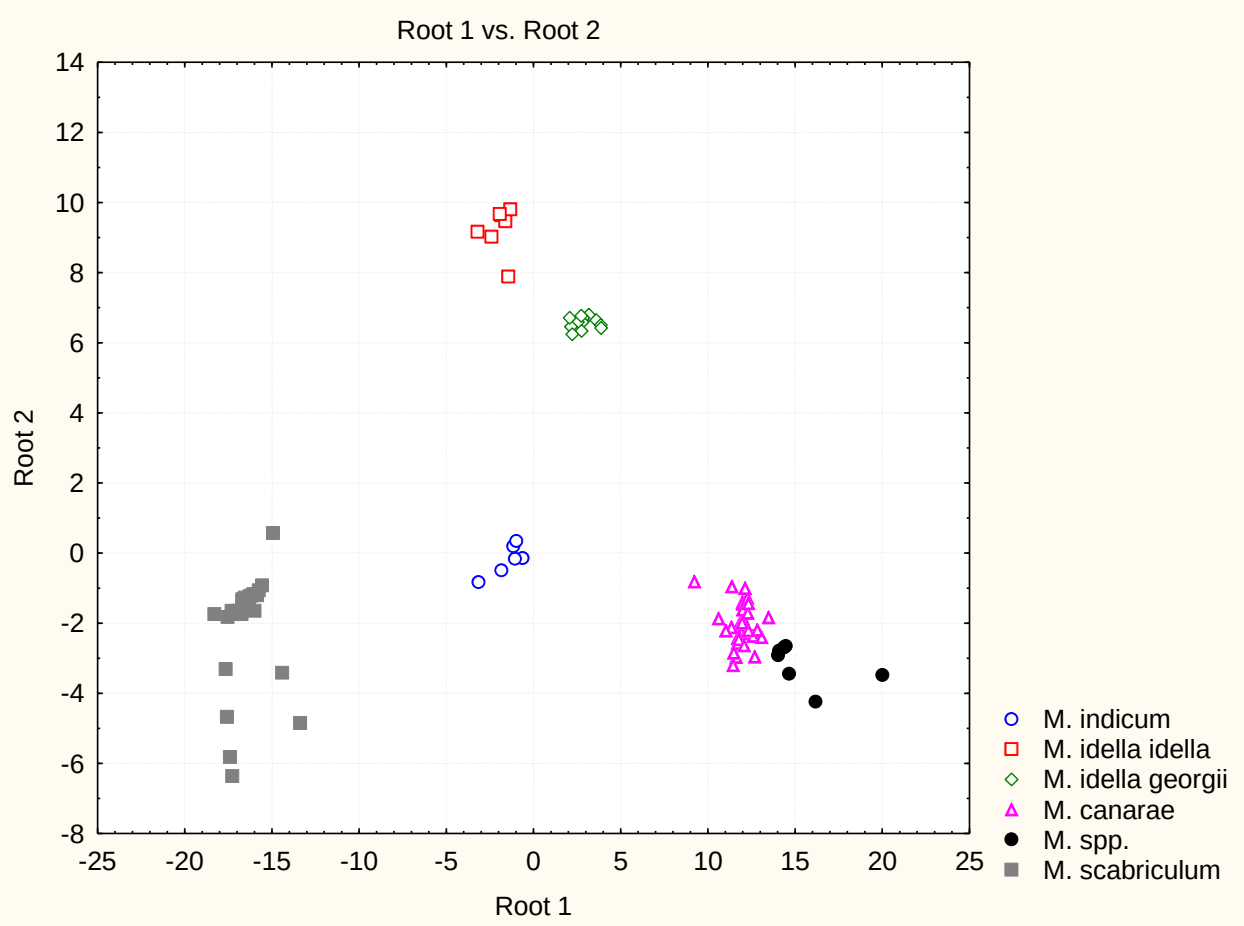


Figure 4.5.1: Scatter plot of six species of *Macrobrachium* (Root1 Vs Root 2)

4.6 Meristic Characters in *Macrobrachium* Species

Nine meristic characters (dorsal teeth, Ventral teeth, Post orbital teeth, accessory spines in uropod, the denticles in movable finger of large periopod. The denticles on the immovable finger of large second periopod, denticles on the movable finger of small second periopod, denticles in the immovable finger of large small second periopod) are taken for the analysis. The numbers of dorsal spines are varying within the species very remarkably. But in the case of ventral teeth it is showing the variation within the species but the range is very less.

In some species it is showing that the post orbital teeth are constant which indicate the species identification based on such characters is quit important in the classical taxonomy. The species collected from the Neyyar river system showing the three different characteristics in their uropodal exopod the totally 9 identified

species didn't have any accessary spine (*Macrobrachium*. Spp.), one has two accessory spines (*M. idella georgii*) but others pose a single accessary spine in uropodal exopod and it is constant in all the specimens of a single species.

The important meristic character is the number of denticles in the fingers of both second periopod. Most of the species like *M. idella idella*, *M. idella georgi*, the number of denticles are same in the species level (there is no variation within the species), similarly those species shows the same characters in both the legs.

The variability plot shows the variation in the meristic trait of all nine Macrobrachium species collected from the river. The fingers (both in the right and left leg) do not poses any denticles in *Macrobrachium* spp.

The number of plumose setae present in between the movable distal spines of telson helps to identify the species which are closely related.

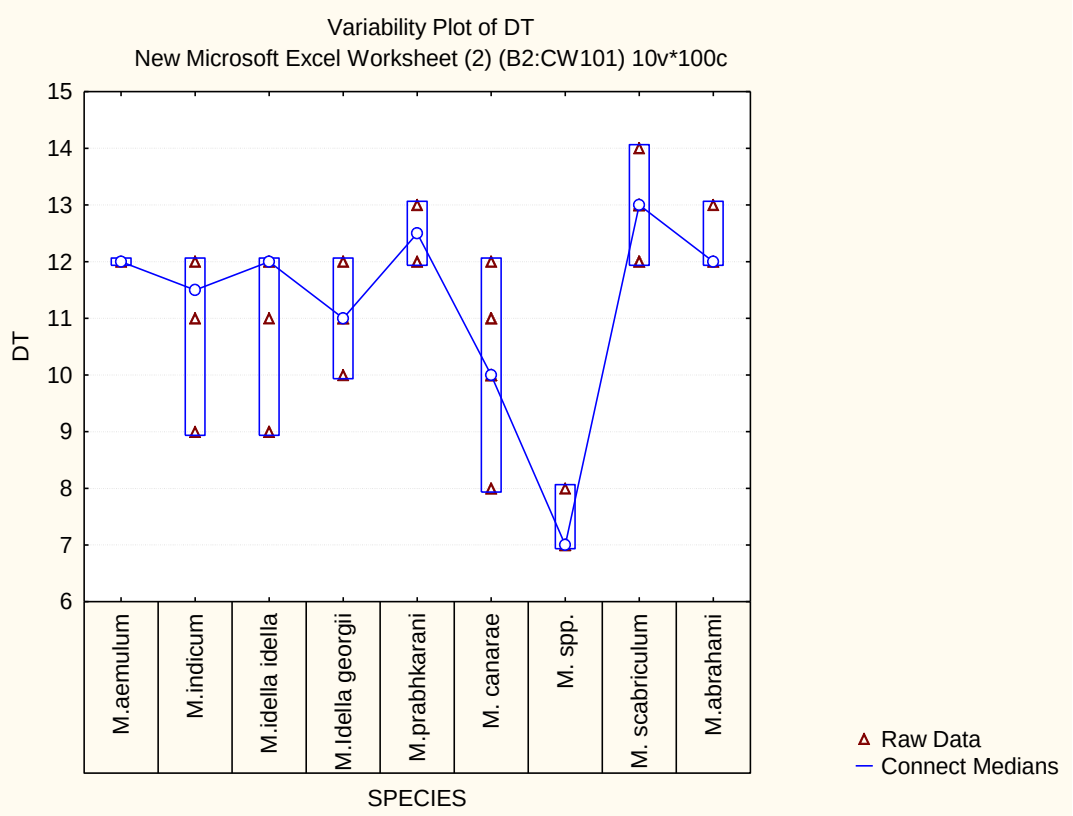
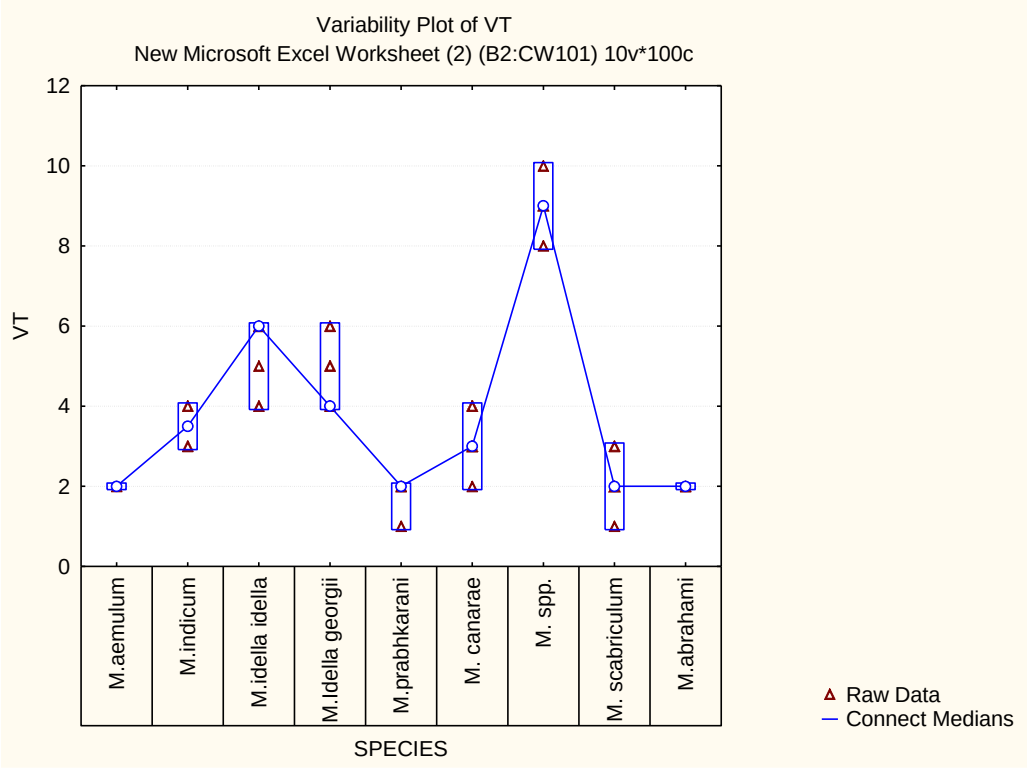


Figure 4.6.1: Species wise variability plot of dorsal teeth of *Macrobrachium* species collected from Neyyar river



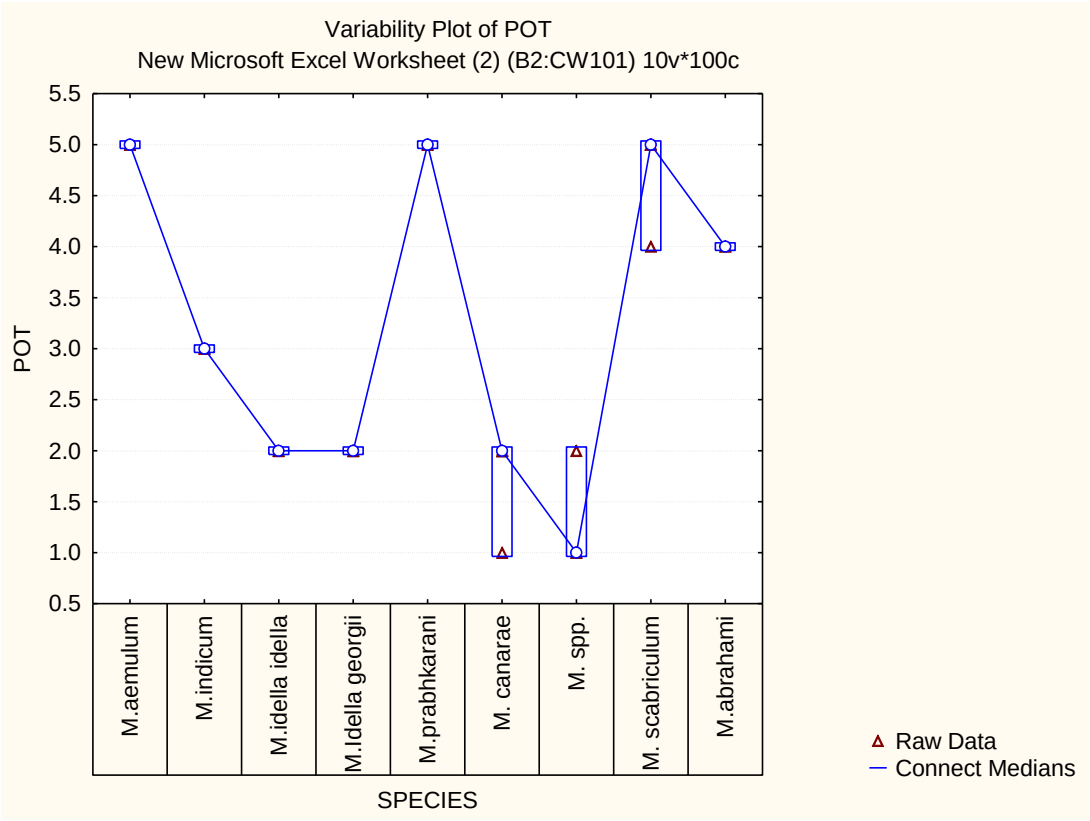
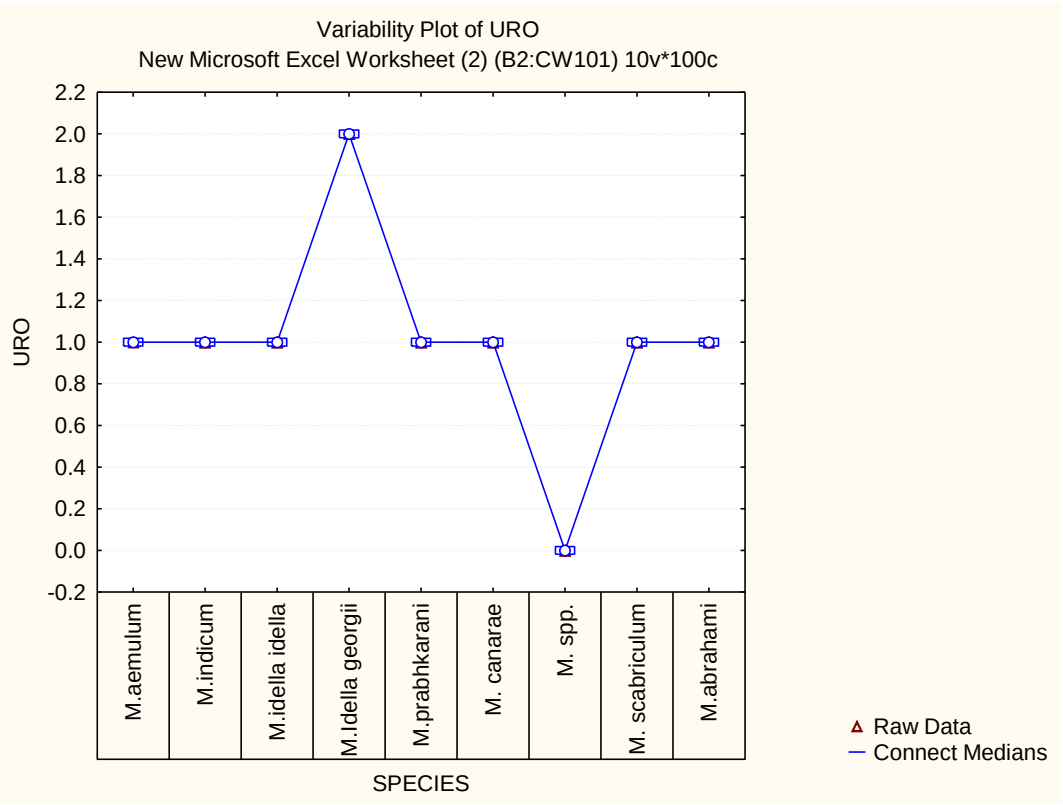


Figure 4.6.2 and figure 4.6.3: Species wise variability plot of ventral and post orbital teeth of *Macrobrachium* species collected from Neyyar river



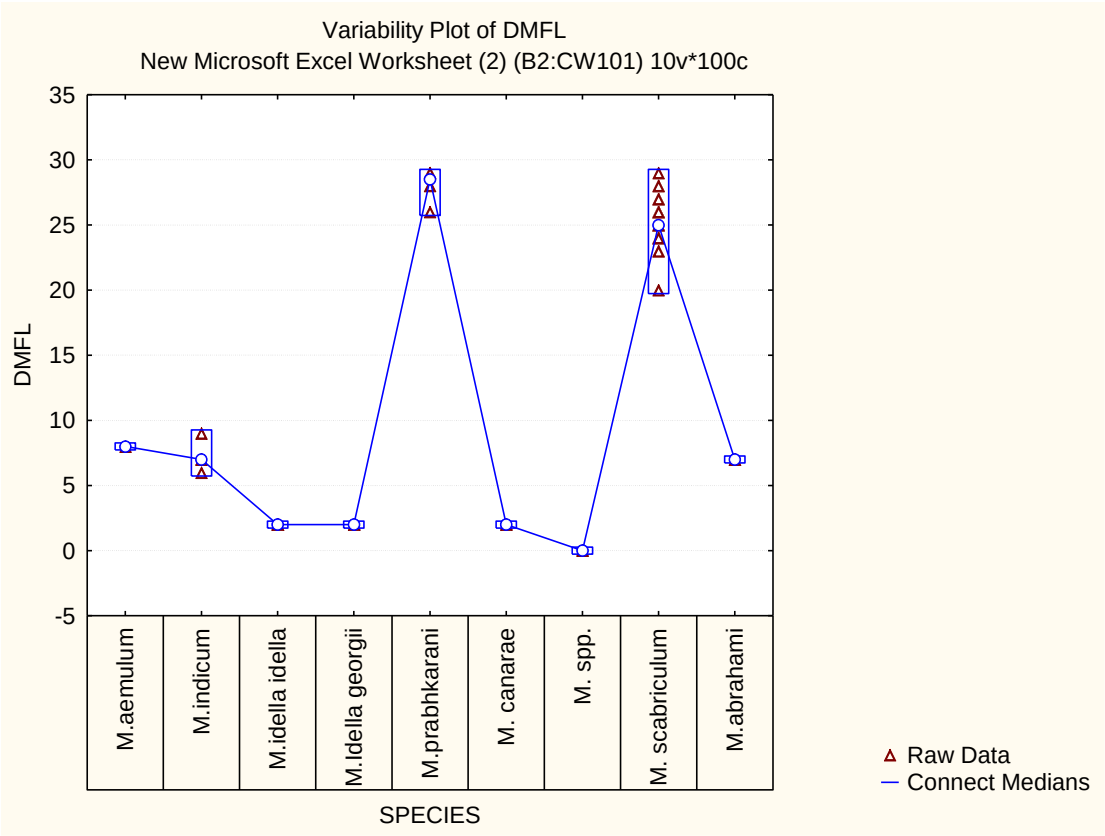
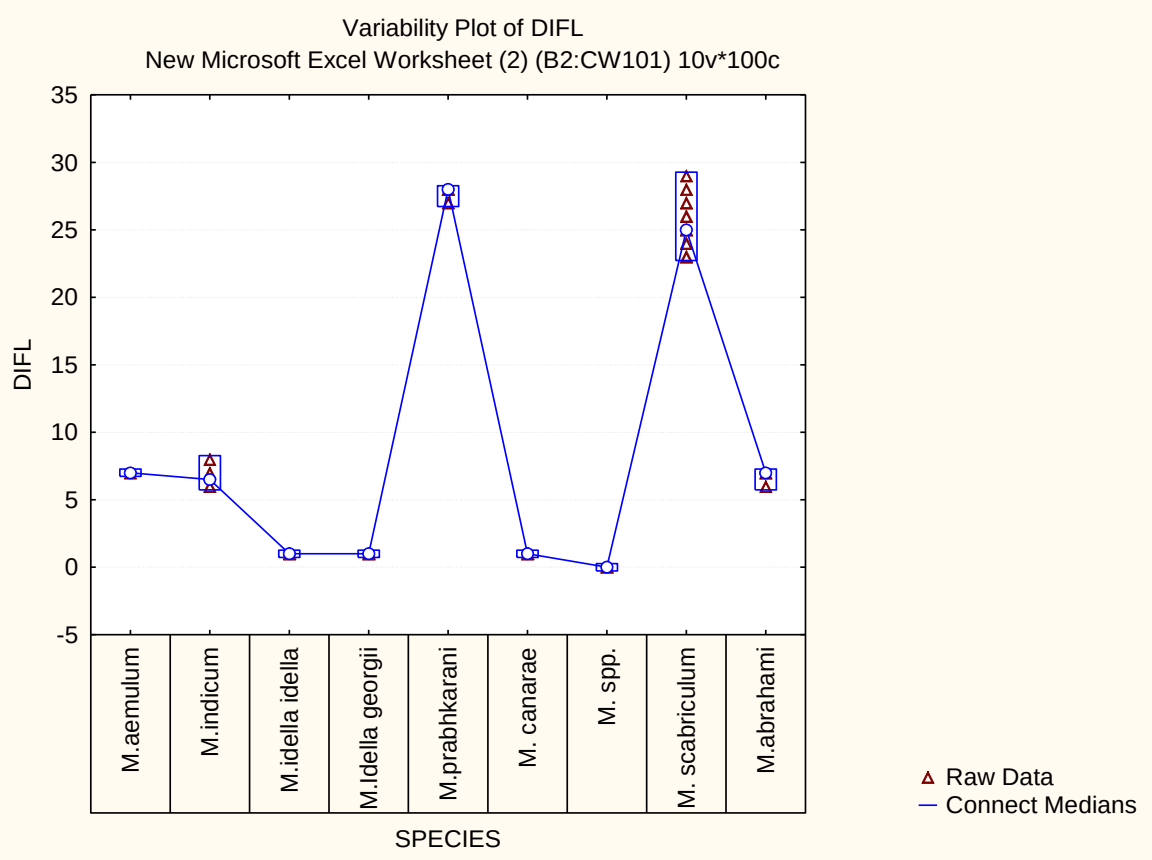


Figure 4.6.4 and Figure 4.6.5: Species wise variability plot of accessory spines in uropod and denticles in movable finger of large chelate leg of *Macrobrachium* species collected from Neyyar river



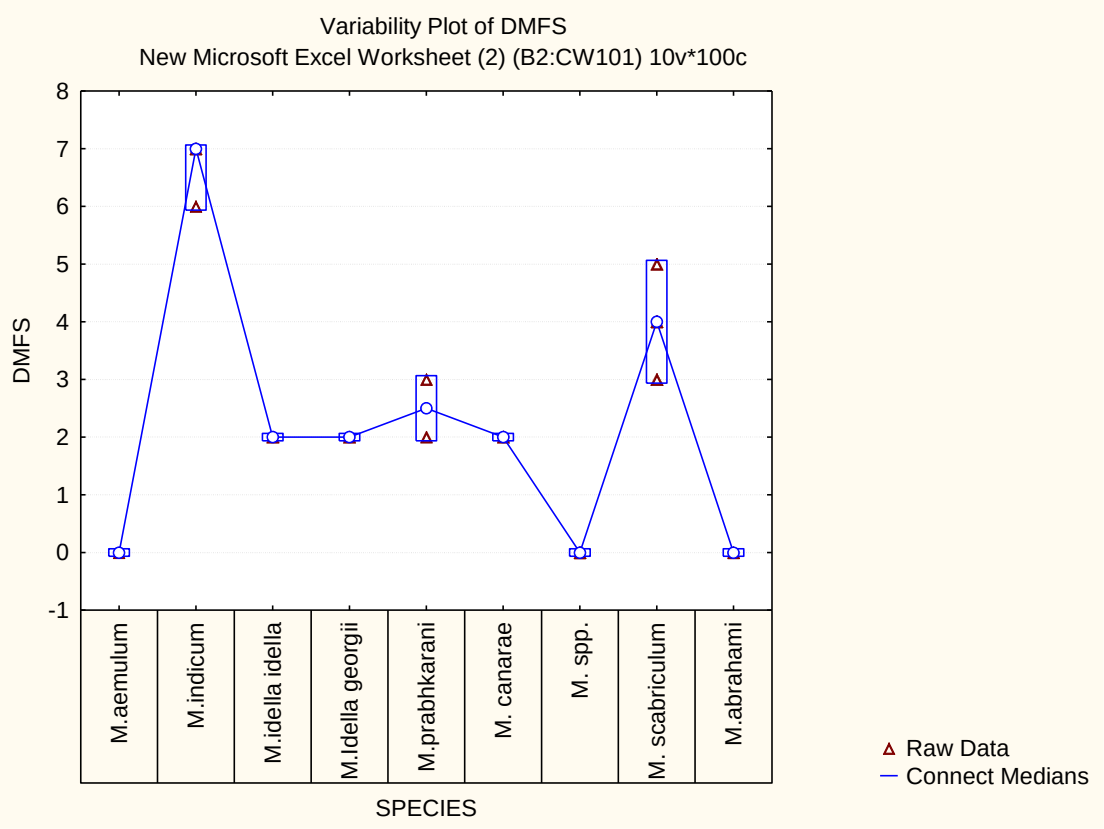
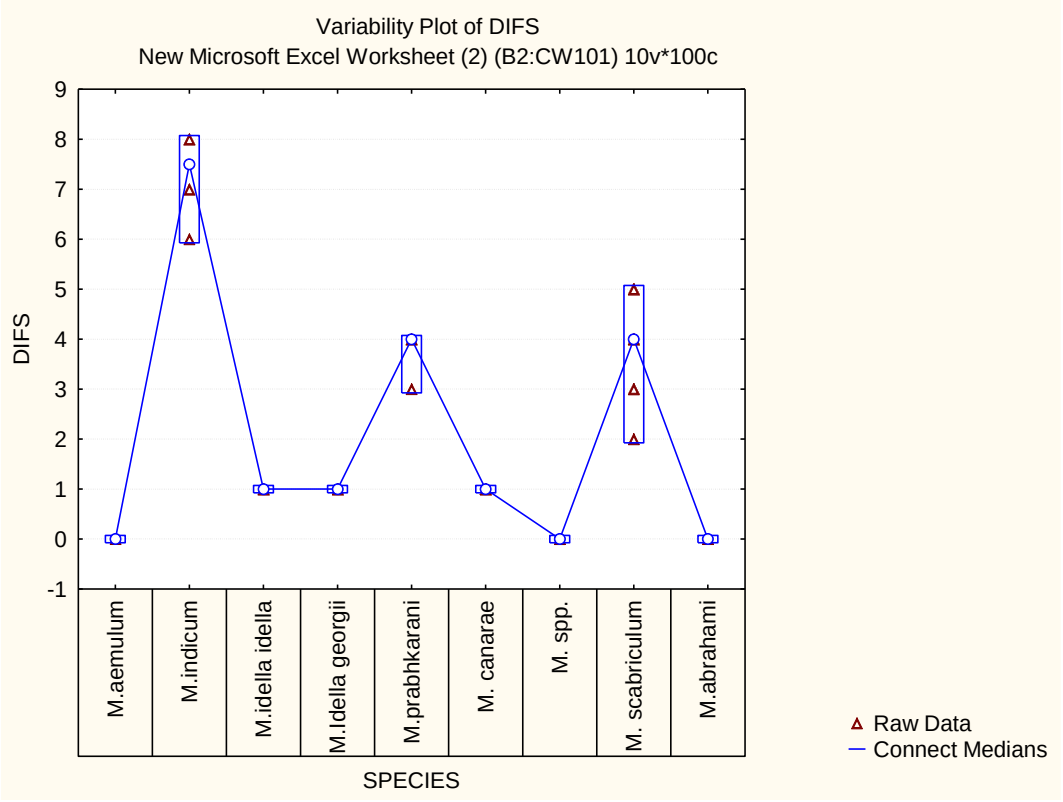


Figure 4.6.6 and Figure 4.6.7: Species wise variability plot of denticles in immovable finger of large 2nd chelate leg and denticles in movable finger of small second chelate leg of *Macrobrachium* species collected from Neyyar river



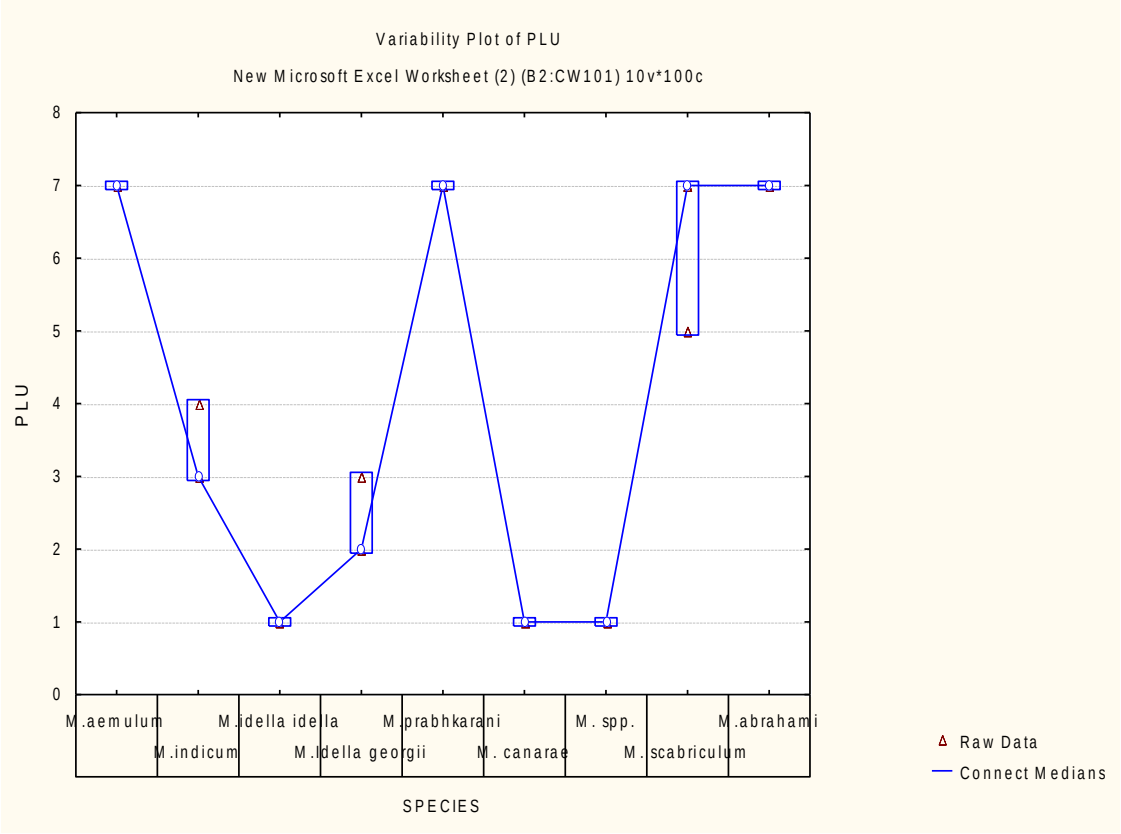


Figure 4.6.8 and Figure 4.6.9: Species wise variability plot of denticles in immovable finger of large 2nd small chelate leg and number of pairs of plumose setae in the distal part of telson of *Macrobrachium* species collected from Neyyar river

4.7 Descriptions *Caridina* spp. comes under the family Atyidae

4.7.1 *Caridina gracilirostris* (de Man, 1892)

Caridina gracilirostris (de Man, 1892) *Zoologische Ergebnisse einer Reise in Nigderlandisch Ost-Indiea* 2:265-527

Plate 4.7.1.1: *Caridina gracilirostris*

Common name:

Red nose shrimp

Materials examined:

Seven specimens of 3 males (12.5, 10.2, 16.2 mm), 3 non- berried females (12.3, 24.3, 16.62 mm) and one berried female (25.4 mm) collected from the station 1 of the Neyyar river and examined.

Description:

Rostrum very long and slender longer than that of the antennal scale. The dorsal margin is concave and distal part is slightly upturned, teeth widely spaced, 1-2 intermediate teeth in the edentulous part, a single post orbital spine present. The ventral margin with close set of teeth except a small gap in the distal region. The size of the teeth is diminishing from the proximal to distal end. Rostral formula: 6+1- 2/26-31 (1 post-orbital).

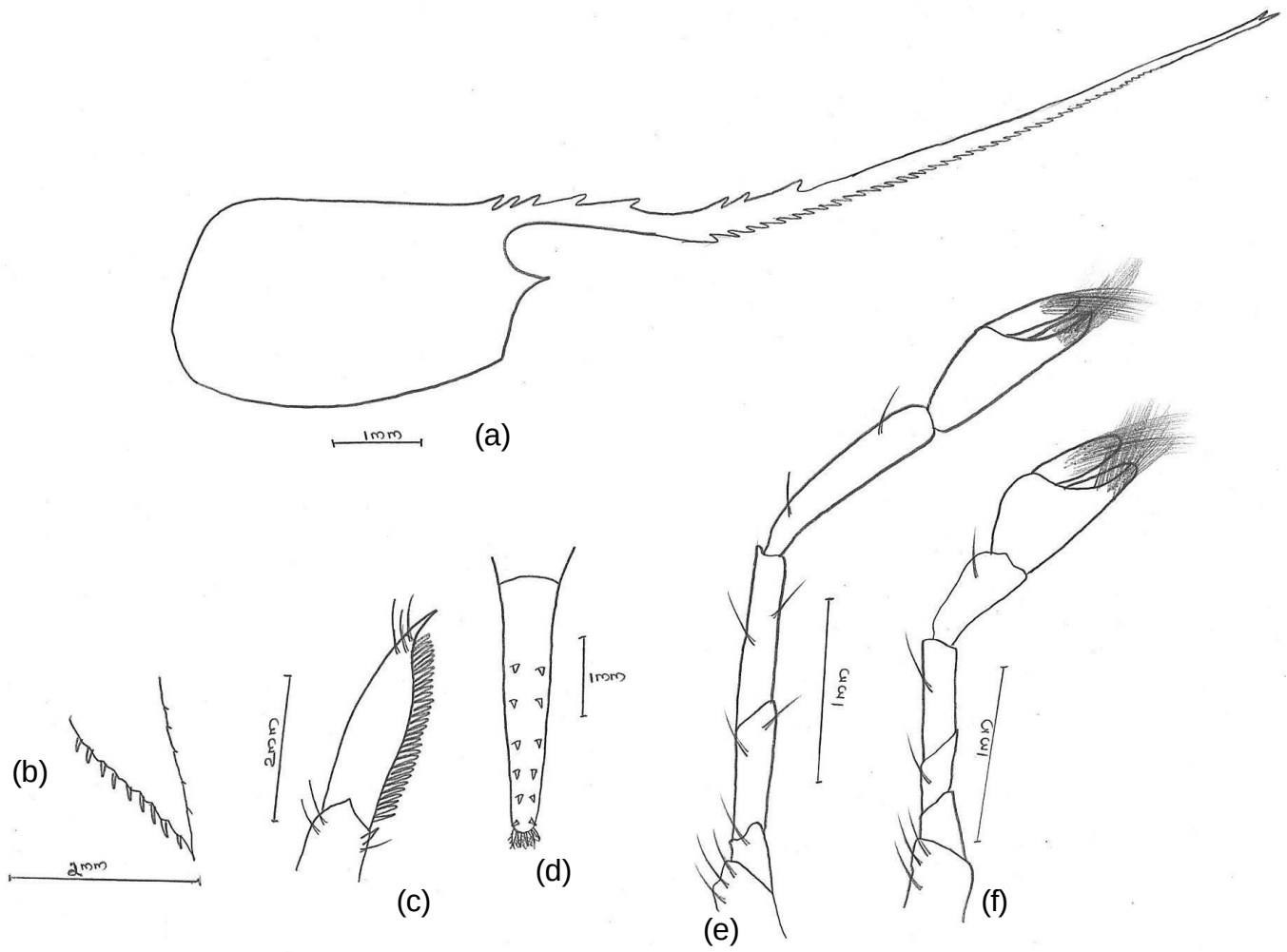


Figure 4.7.1: *Caridina gracilirostris* (a) carapace, (b) uropod diaresis, (c) dactylus of 5th pereiopod, (d) telson, (e) 2nd pereiopod, (f) 1st pereiopod

Telson is smooth with 5 to 6 pair of dorsal spines. Posterior margin of telson rounded and bearing 6 plumose spines. Fingers of 1st pereiopod slightly longer than or equal to palm, carpus with a shallow anterior excavation. Fingers of 2nd pereiopod slightly longer than palm, carpus without anterior excavation.

Dactylus of 3rd pereiopod ending in a sharp spine, with 6-7 spines on its posterior margin. Fourth pereiopod similar to 3rd. Dactylus of 5th pereiopod with 24-33 comb like spinules.

Uropodal diaeresis with 8-10 spinules.

Colouration:

Body translucent, the tip or sometimes the entire upper margin appears red, hence the name red nose shrimp.

Distribution:

India, Fiji, French Polynesia, Philippines, Taiwan, Japan, China

Map 4.7.1.1: Distribution range of *Caridina gracilirostris*

(*http://maps.iucnredlist.org/)*

4.7.2 *Caridina mathiassi* Silas & Jayachandran, 2010

Caridina mathiassi Silas & Jayachandran, 2010, *Indian J. Fish*. 57(4):1-5

Plate: 4.7.2.1: *Caridina mathiassi*

Materials examined:

Twenty eight specimens were collected from the Neyyar river at Amaravila, part of southern Western Ghats.

Description:

Rostrum moderately long and extends up to the middle of the distal segment of antennular peduncle, the dorsal profile is slightly variable in shape and the distal part turned upwards Rostral formula: 11-16/4-6 (3-6 post-orbital). Antennal spine sharp and pterygostomian angle pointed.

Telson with 3-6 pairs of dorsal spines and 4-10 distal spines, posterior margin rounded.

Fingers of 1st cheleped slightly longer than palm, carpus deeply excavated anteriorly. Fingers of 2nd pereiopod 1.43 times as long as palm, carpus slender and long, Propodus slender and shorter than carpus.

Dactylus of 3rd pereiopod carries 4-7 accessory spines, ending in a

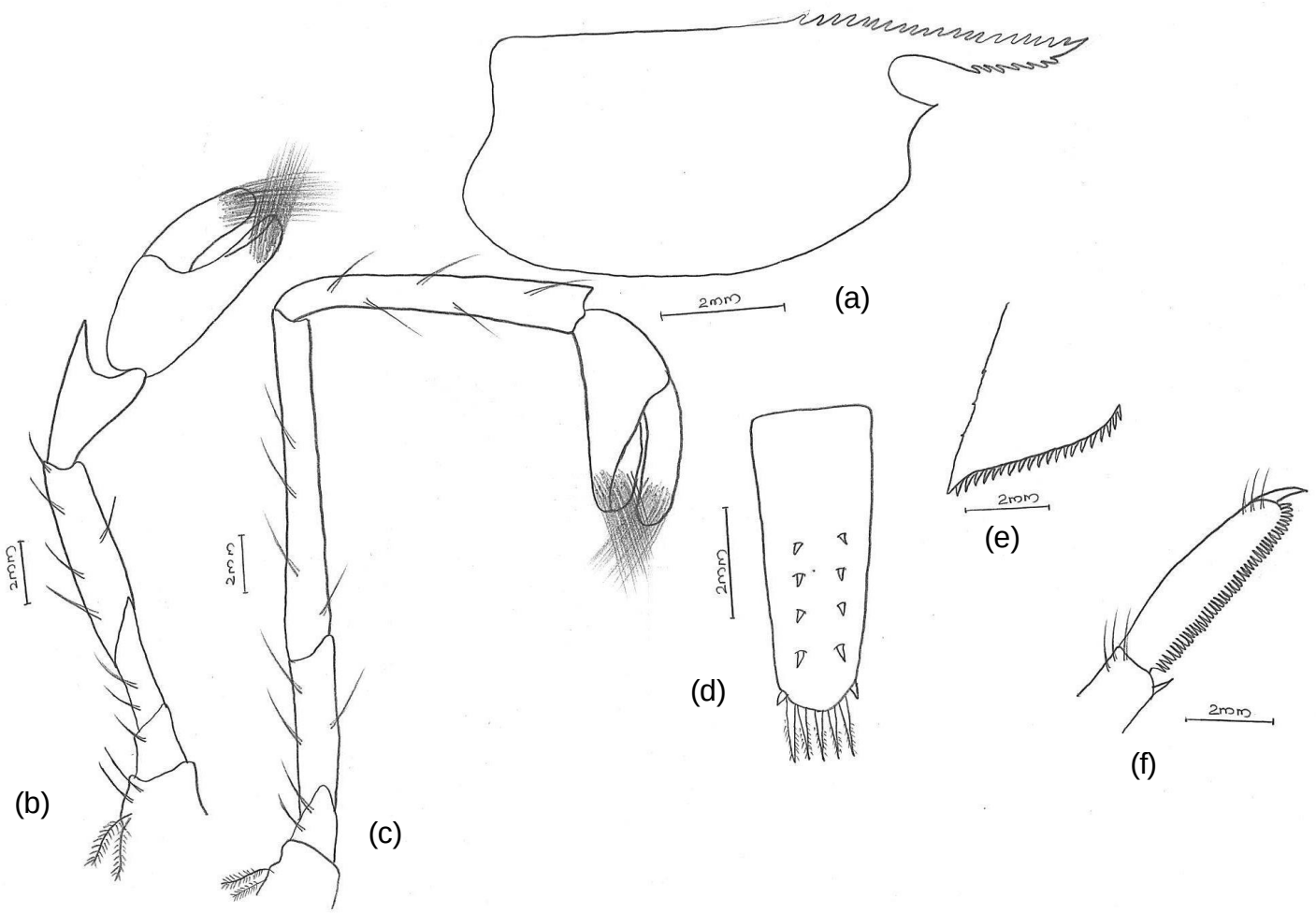


Figure 4.7.3: ***Caridina matiassi*** (a) carapace, (b) 21st chelate leg, (b) 2nd chelate leg, (d) telson, (e) uropod diaresis, (f) dactylus of 5th pereiopod

sharp terminal spine. Fourth pereiopod similar to 3rd. Dactylus of 5th pereiopod with 30-40

Spinules on flexor margin; carpus with a sub-terminal spine and an inner row of 3-4 spinules; merus and ischium with 2-4 and 0-1 spine respectively on the inner margin.

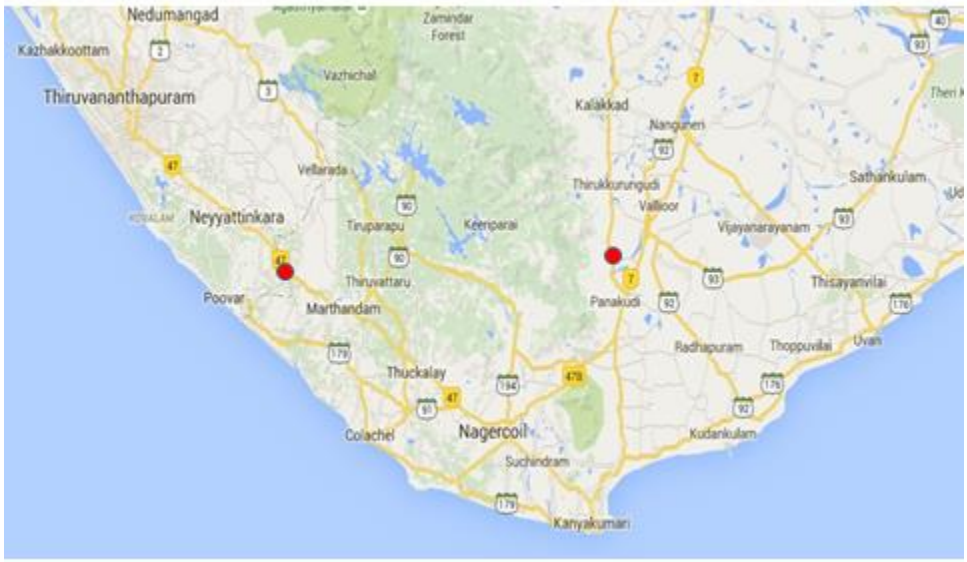
Endopod of male 1st pleopod carries a distinct appendix interna.

Uropodal diaeresis with 16-22 movable spinules

Distribution:

Tamil Nadu, Kerala

Map 4.7.2.1: Distribution range of *Caridina mathiassi*



4.7.3 *Caridina natarajani* Tiwari & Pillai, 1968
Caridina natarajani Tiwari, K.K. & Pillai, R.S., 1968. *Proc*. *Zool*. *Soc*. *Calcutta,* 21: 163

Plate 4.7.3.1: *Caridina natarajani*

Synonym:

Caridina laevis Pillai, 1958

Materials:

Materials collected from the Station 1. The specimens incudes two males (10, 12 mm) three non-berried females of size 15mm, 15.4 mm and 17 mm) 2 berried females (16 and 16.2 mm)

Description:

Rostrum moderately long and slightly convex reaching up to the third segment of antennular peduncle. In male it is shorter than the female proportional rostral length. The tip of the rostrum is slightly up curved and devoid of teeth. Rostral formula: 12-16/3-5 (5 post-orbital).

Pterygostomian angle rounded Antennal spine sharp, and lower than the inferior orbital angle.

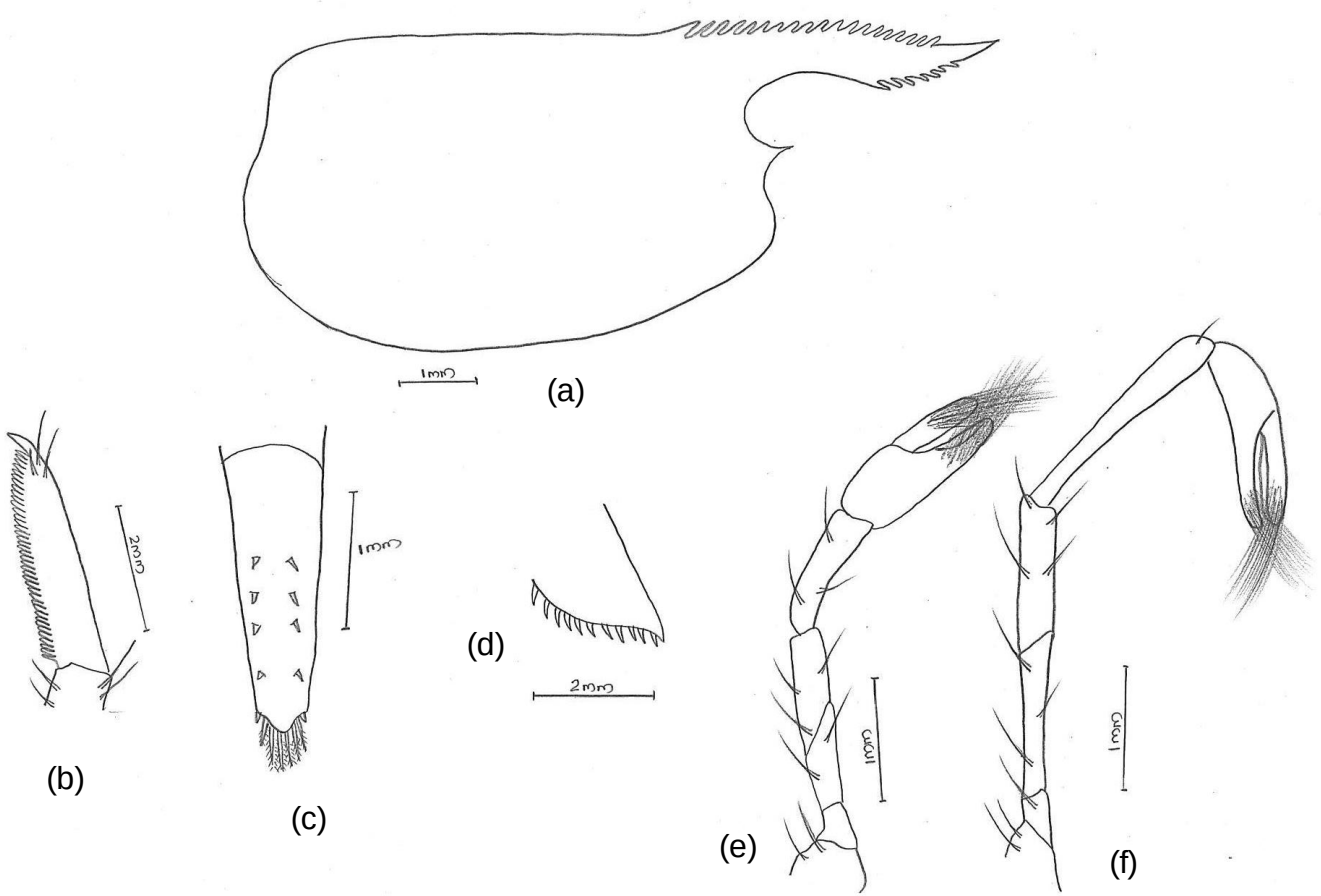


Figure 4.7.3: *Caridina natarajani* (a) carapace, (b) dactylus of 5th pereiopod, (c) telson, (d) uropod diaresis, (e) 1st chelate leg, (f) 2nd chelate leg

Telson with 3-4 pairs of dorsal spines, posterior end rounded and bearing 5-6 distal spines, those at the centre are longer and strongly plumose.

Fingers of 1st pereiopod 1.3 times as long as palm; carpus with a slight anterior excavation and is broad.

Fingers of 2nd pereiopod 1.25-1.45 times as long as palm; carpus slender, and elongated.

Dactylus of 3rd pereiopod ending in a sharp spine, with 8-11 accessories

Spines on its posterior margin. Fourth pereiopod similar to 3rd. Dactylus of 5th pereiopod ending in a sharp claw, its posterior margin armed with 45-70 comb-like spinules.

Uropodal diaeresis with 13-14 spines.

Colouration:

Body translucent with orange, red, blue chromatophores.

Distribution:

Kerala and Tamil Nadu.

Map 4.7.3.1: Distribution range *Caridina natarajani*

4.8 Descriptive Statistics of Morphometric Data of *Caridina* spp. of Family Atyidae

Descriptive statistics (minimum, Maximum, mean, range) were obtained for 5 morphometric characters (one scaled for TL, 4scaled to CL) for all *Caridina* species collected during the study has been done. Several morphometric variables obtained were found to be important in differentiating between the species. The statistics are presented species wise in tables 4.8.1, 4.8.2, and 4.8.3

Table 4.8.1: Descriptive statistics of morphometric measurements of *Caridina gracilirostris*

Variable	Valid N	Mean	Minimum	Maximum	Range
TL/CL	7	1.1478013	1.036364	1.184927	0.148563
AL/CL	7	3.407799	2.777778	3.553114	0.775336
CL/TL	7	0.872766	0.843934	0.964912	0.120978
RL/CL	7	2.5295	2.456929	2.564103	0.107174
TEL/CL	7	0.843519	0.802198	0.883784	0.081586

Table 4.8.2: Descriptive statistics of morphometric measurements of *Caridina mathiassi*

Variable	Valid N	Mean	Minimum	Maximum	Range
TL/CL	32	2.054578	2.012658	2.08462	0.071542
AL/CL	32	2.450515	2.414634	2.488372	0.073738
CL/TL	32	0.486754	0.47929	0.4901969	0.0109069
RL/CL	32	0.448768	0.434783	0.461538	0.026756
TEL/CL	32	0.609926	0.586207	0.652174	0.065967

Table 4.8.3: Descriptive statistics of morphometric measurements of *Caridina natarajani*

Variable	Valid N	Mean	Minimum	Maximum	Range
TL/CL	5	1.845725	1.564103	2.001018	0.436915
AL/CL	5	2.997241	2.884259	3.163717	0.279458
CL/TL	5	0.544928	0.499746	0.639344	0.139598
RL/CL	5	0.598501	0.585714	0.64588	0.060165
TEL/CL	5	0.65041	0.642857	0.655093	0.012235

4.9 Variability Plots (The graph showing the various morphometric trait variations in each *Caridina* spp. collected from the Neyyar river)

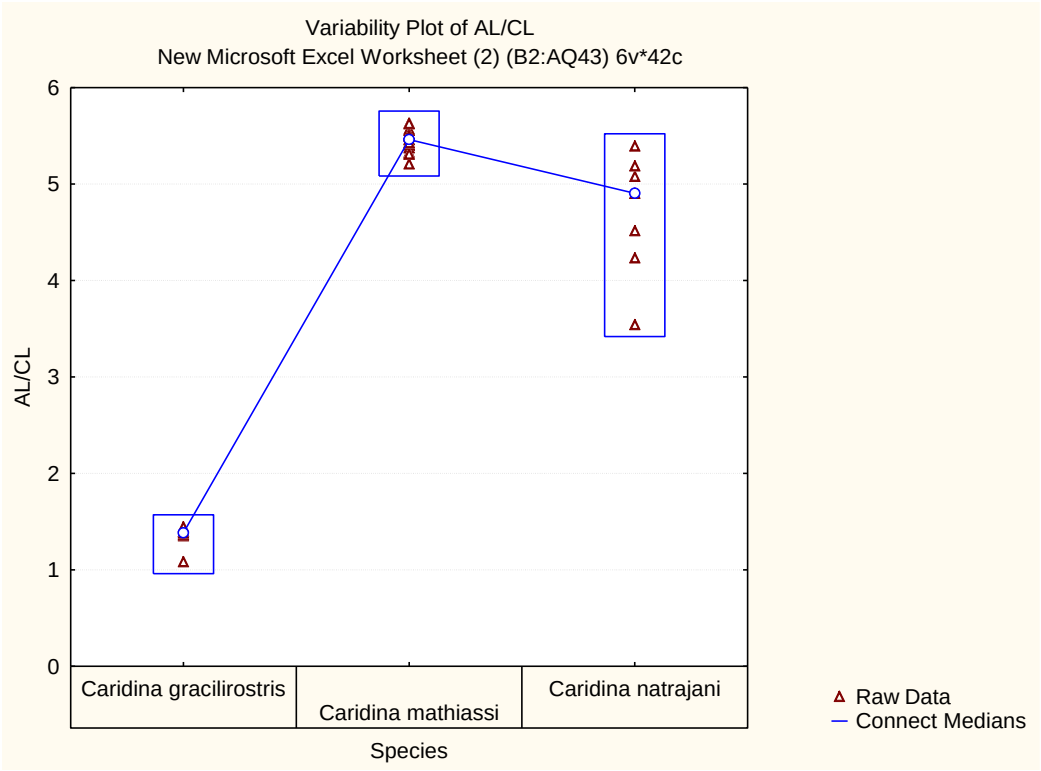
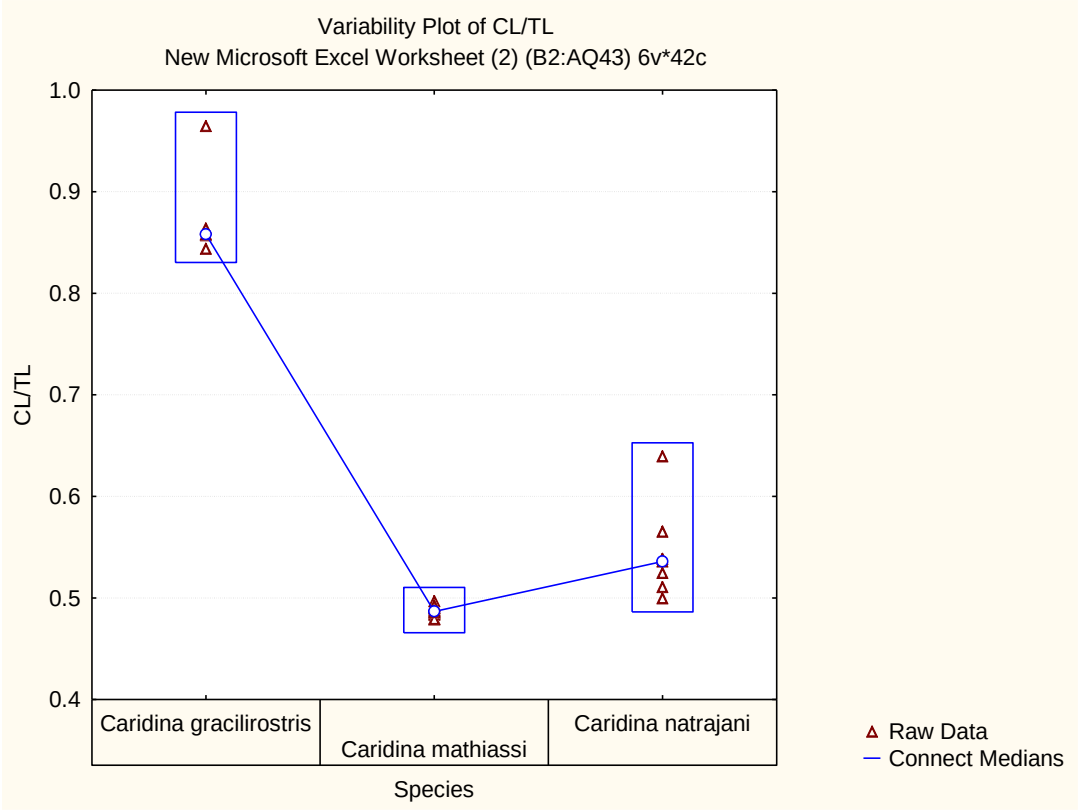


Figure 4.9.1 and Figure 4.9.2: Species wise variability plot of TL/CL and AL/CL



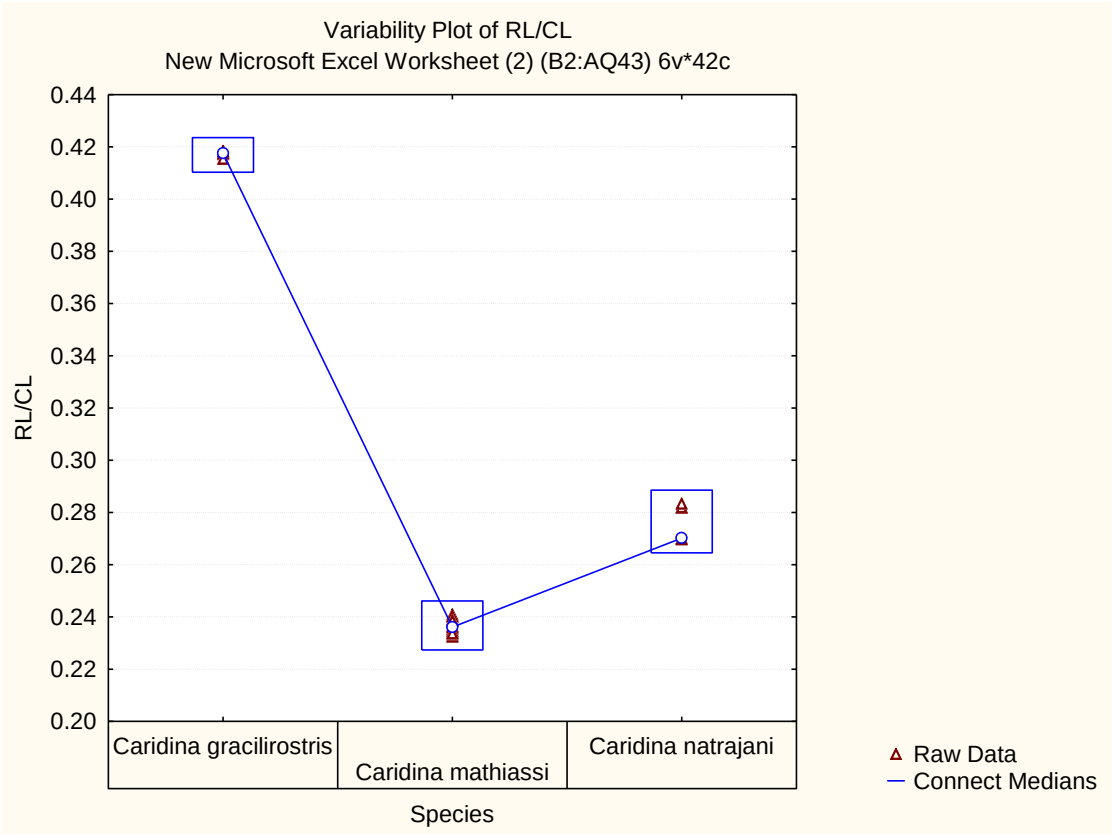


Figure 4.9.3 and Figure 4.9.4: Species wise variability plot of CL/TL and RL/CL

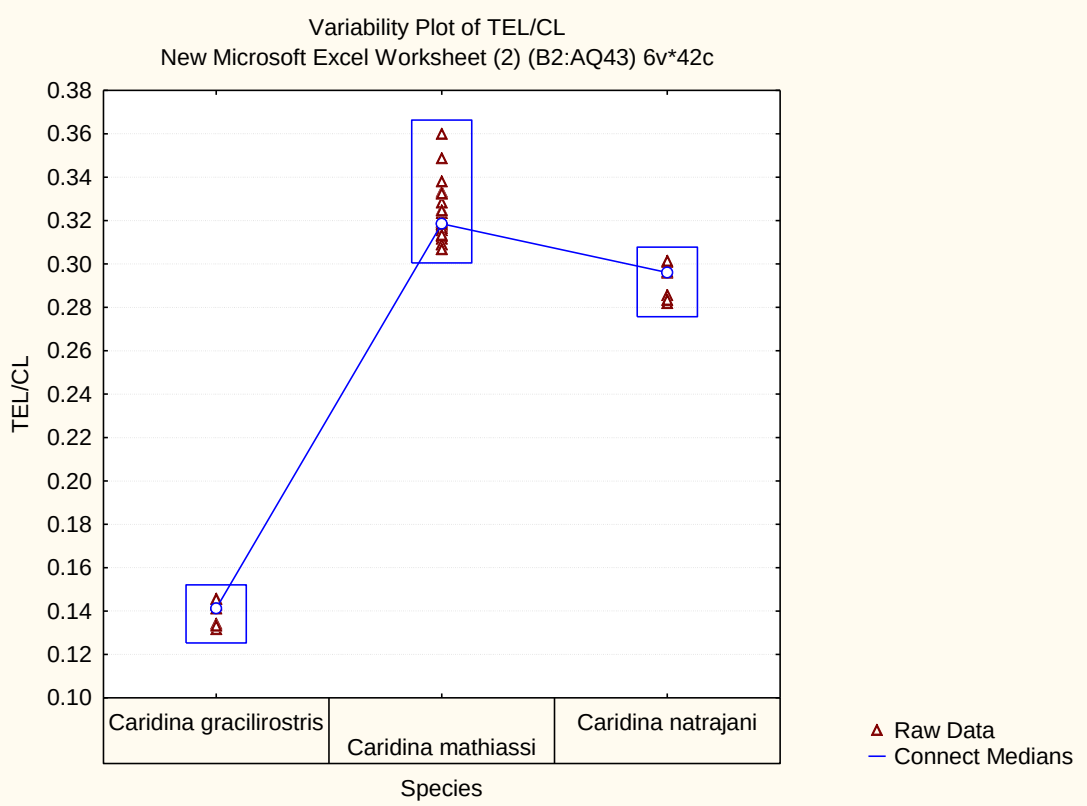


Figure 4.9.5: Species wise variability plot of TEL/CL

4.10 Multivariate Analysis of Morphometric Traits

4.10.1 Factor analysis

Five morphometric characters after log transformation were subjected to the Factor analysis to generated factors. The factor loading after varimax rotation for the morphometric variables were analysed. The characters having factor loading of above 0.9 on any of the first two factors were selected for subsequent stepwise discriminant analysis. All the variables (Total length; TL, Abdominal length; AL, Carapace length; CL, Rostral length; RL, Telson length; TEL) contribute for the species discrimination of *Caridina* collected from Neyyar river Kerala.

Table: 4.10.1: Factor loading extraction- family Atyidae

	Factor Loadings (Unrotated) (Caidina morphometry (B2:AO4 Extraction: Principal components (Marked loadings are >.900000)
Variable	Factor 1
TL	0.998829
AL	0.997616
CL	-0.998829
RL	-0.992869
TEL	0.993778
Expl.Var	4.963941
Prp.Totl	0.992788

4.10.2: Stepwise (forward) discriminant analysis

The variables after factor analysis were subjected to stepwise discriminant analysis (SDFA). Discriminant analysis generated three factions (table 4.10.2.1) with first two explaining 95.38% of total variation. The means of conical variables shows that that Root 1 successfully discriminates between most of the concerned species.

Table 4.10.2.1: Standardized coefficient of canonical variables- family Atyidae

	Standardized Coefficients (Caidina morphometry (B2:AO41)) for Canonical Variables	
Variable	Root 1	Root 2
RL	-1.0383	-0.0301(
AL	-2.0395	6.15272
CL	-1.865(	6.11178
Eigenval	951.582[	65.2935(
Cum.Prop	0.9358	1.0000(

Table 4.10.2.2 Means of canonical variables of *Caridina* species- family Atyidae

	Means of Canonical Variables (Caidina morphometry (B2:AO4	
Group	Root 1	Root 2
Caridina gracilirostris	-62.698(	-3.87175
Caridina mathiassi	16.466[	-2.6978[
Caridina natarajani	-4.4363	20.52864

Table 4.10.2.3 Factor structure matrix of canonical roots- family Atyidae

	Factor Structure Matrix (Caidina morphometry (B2:AO41)) Correlations Variables - Canonical Roots (Pooled-within-groups correlations)	
Variable	Root 1	Root 2
RL	-0.93827(	-0.31039[
AL	-0.10622[	0.123316
CL	0.10231[	0.03794[

Table 4.10.2.4: Classification matrix of morphometric traits of *Caridina* species-family Atyidae

Group	Classification Matrix (Caidina morphometry (B2:AO41)) Rows: Observed classifications Columns: Predicted classifications			
	Percent Correct	Caridina gracilirostris p=.17500	Caridina mathiassi p=.70000	Caridina natarajani p=.12500
Caridina gracilirostris	100.000(	7	0	0
Caridina mathiassi	100.000(	0	28	0
Caridina natarajani	100.000(	0	0	5
Total	100.000(	7	28	5

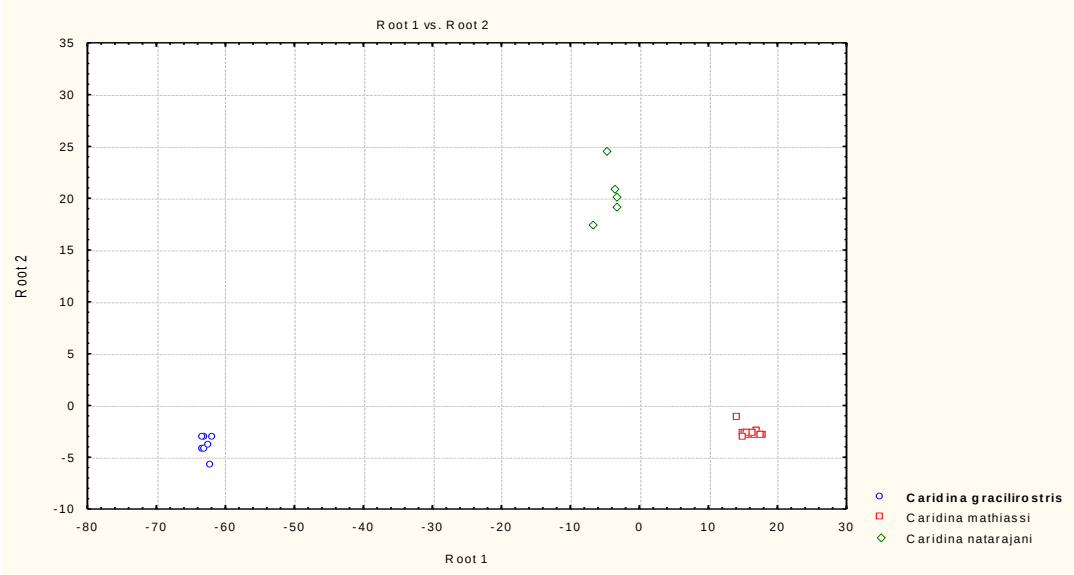


Figure 4.10.1: Scatter plots of *Caridina* species present in the Neyyar river

4.11 Meristic characters

Six meristic characters (number of dorsal teeth, Ventral teeth, Post orbital teeth, spines in uropod diaresis, spines in dorsal margin of telson, spinules in the distal margin) are taken for the analysis. The ventral teeth, spinules in the uropodal distal part are the most discriminating the species meristically.

In some species it is showing that the post orbital teeth are constant which indicate the species identification based on such characters is quit important in the classical taxonomy. From the specimens collected from the Neyyar river Caridina gracilirostris showed the large differentiation among three species.

The important meristic character discriminate the species is spines in uropodal diaresis and the spinules in the distal part of telson.

The variability plot showing the variation in the meristic trait in species and within species variation of each meristic character.

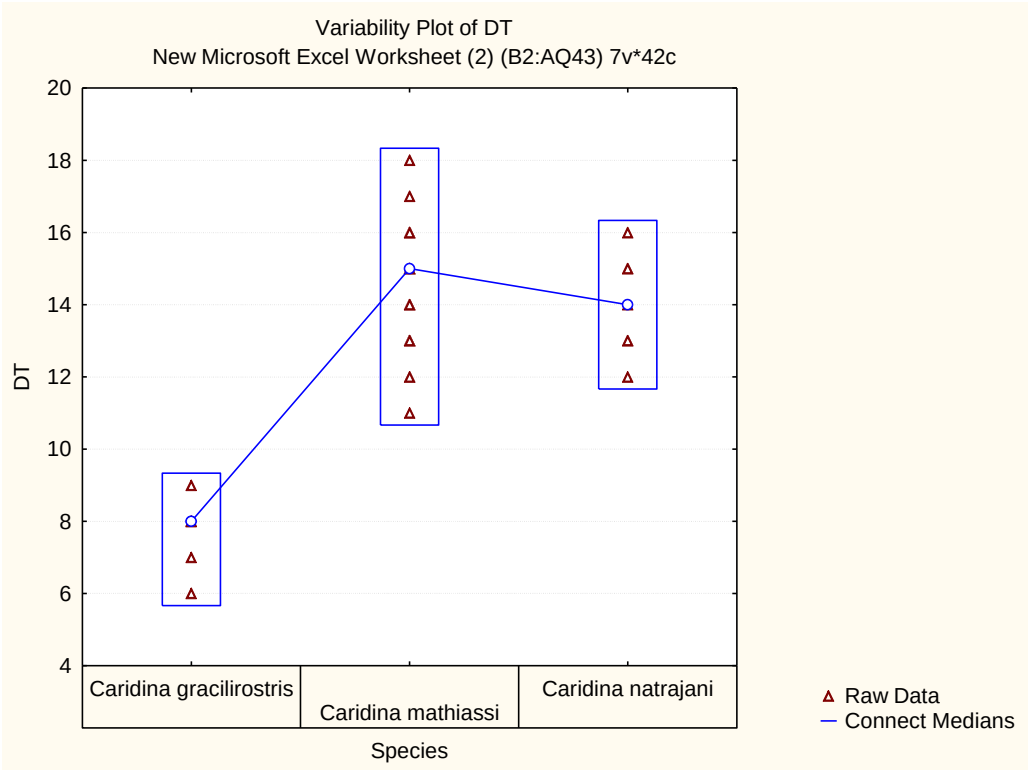
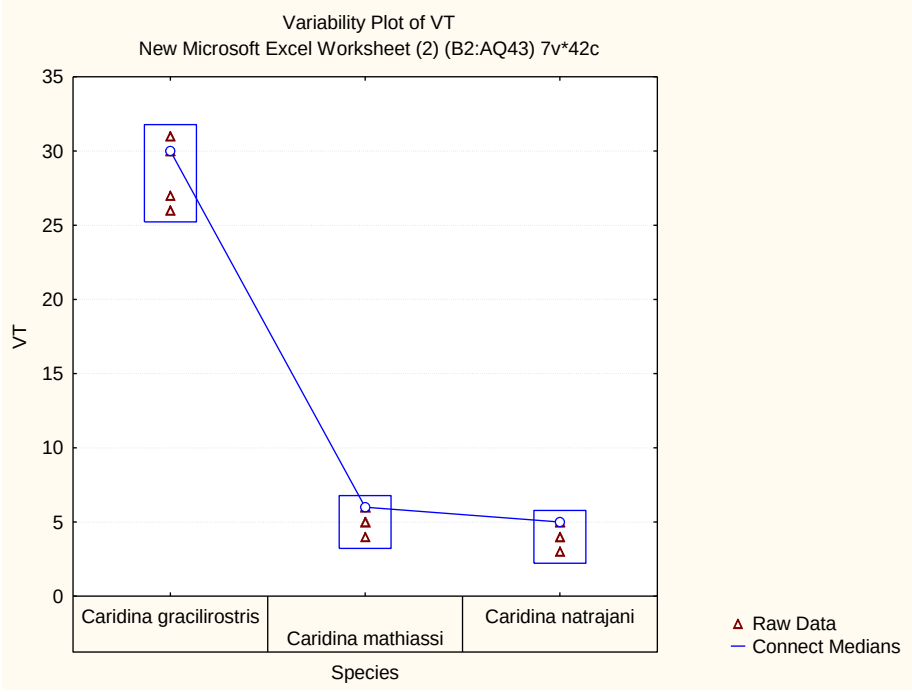


Figure 4.11.1: Species wise variability plot of dorsal teeth of *Caridina* species present in Neyyar river



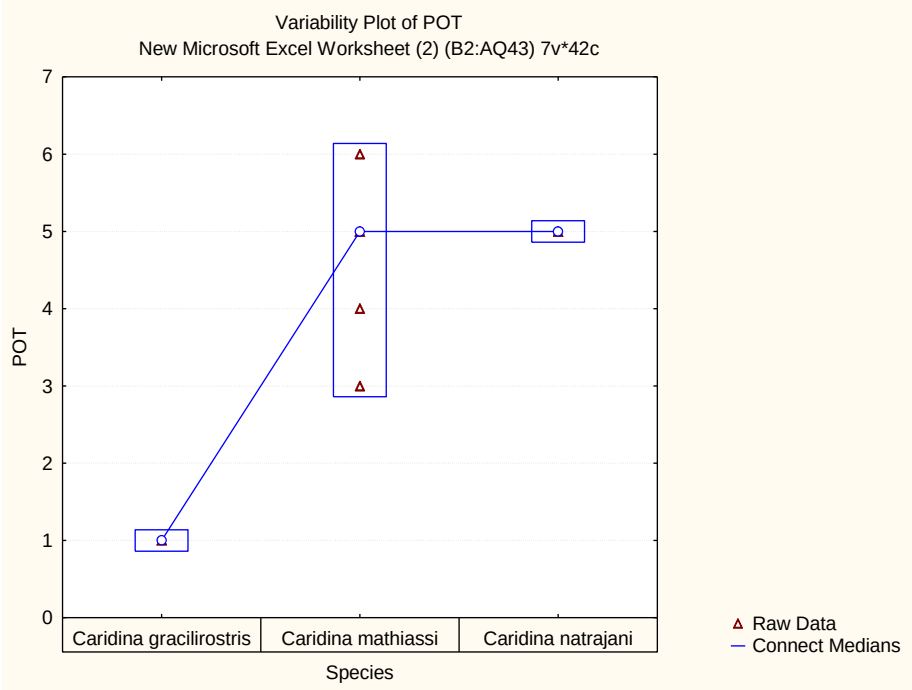
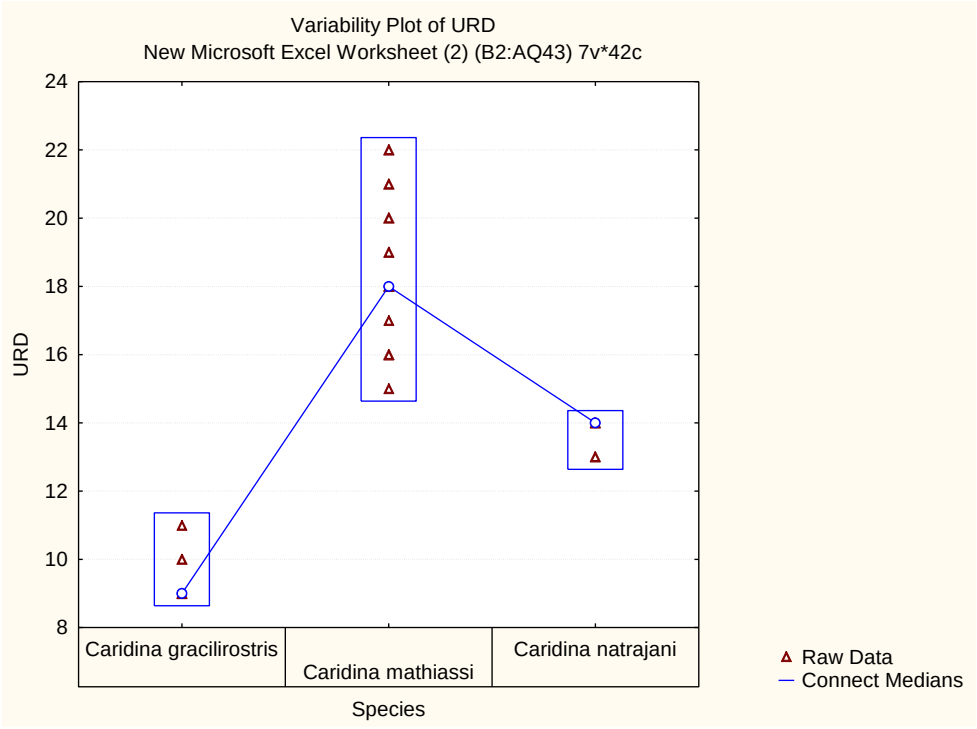


Figure 4.11.2 and Figure 4.11.3: Species wise variability plot of ventral teeth and post orbital teeth of *Caridina* species present in Neyyar river



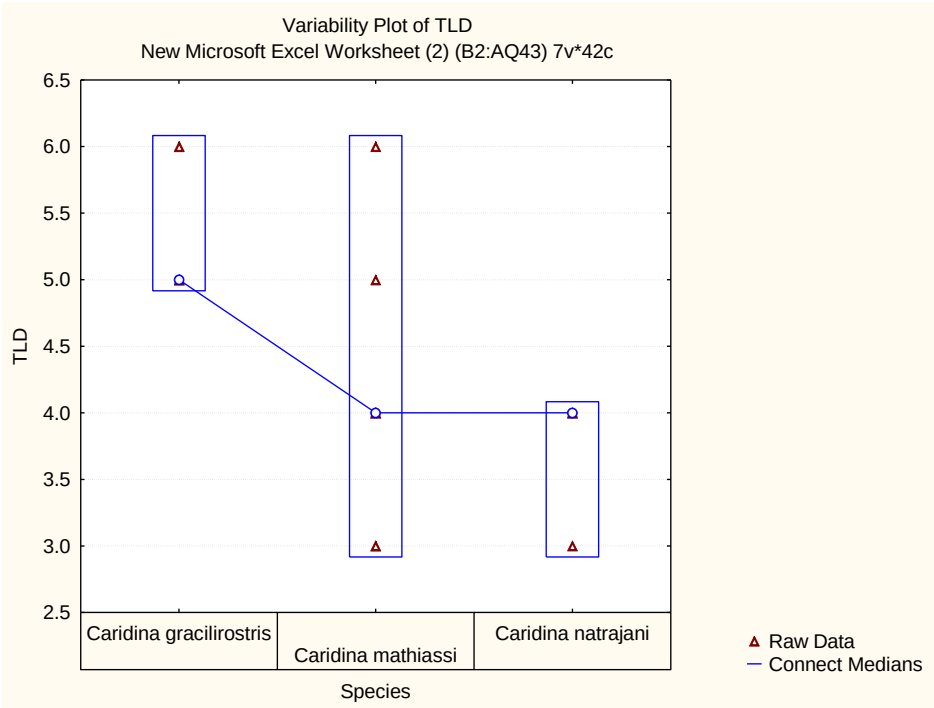


Figure 4.11.4 and Figure 4.11.5: Species wise variability plot spinules in dorsal surface of telson and spinules in uropod diaresis of *Caridina* species present in Neyyar river

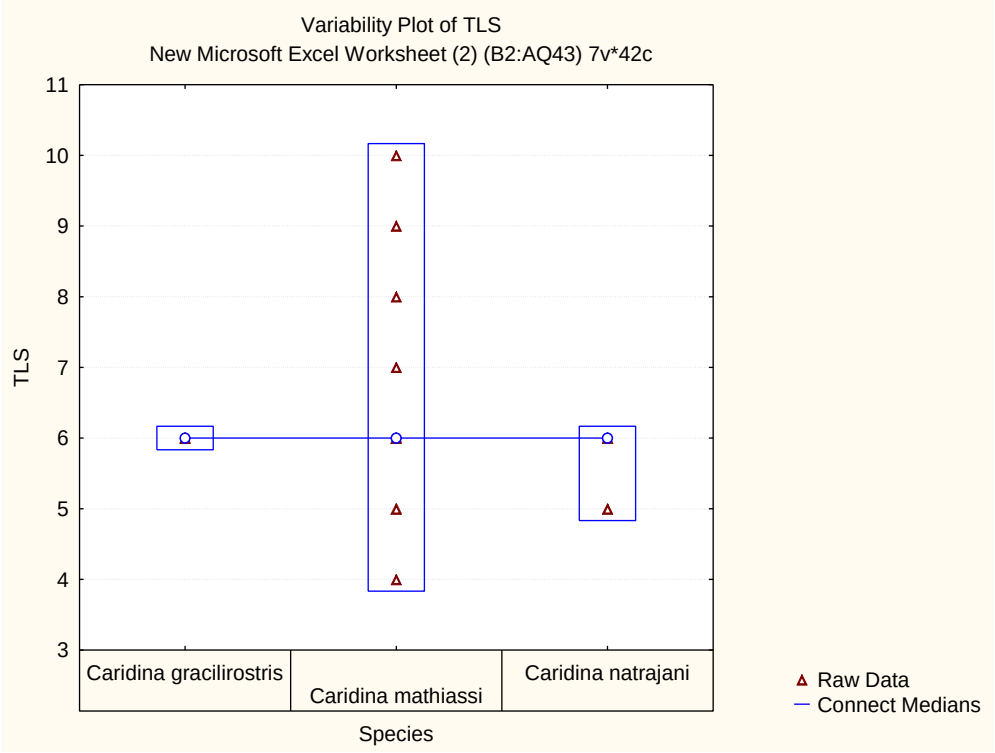


Figure 4.11.6: Species wise variability plot of distal spinules of telson of *Caridina* species present in Neyyar river

4.12 Molecular Taxonomy (Barcoding of species belongs to the family Palaemonidae and Atyidae from Neyyar river)

Total fifty specimens comprising two families, twelve species were collected from upper stretches of Neyyar river (Map 3.1.1). Cytochrome *c* oxidase subunit I (COI) partial gene (650bp) was amplified from all 12 species with primers CrustD F1 and CrustDR1. The COI amplicon was purified and sequenced in both forward and reverse orientation and the average sequence length was 600bp. DNA barcodes were developed for all species using the using the fragment of the COI gene with an average length of ~570-600 base pairs . The COI sequences were checked for the presence of insertions, deletions and stop codons. The lack of stop codons and indels was consistent with all the amplified COI sequences suggesting that NUMTs (Nuclear DNA sequences originating from mitochondrial DNA sequences) were not sequenced (vertebrate NUMTS were typically smaller than 600 bp; Zhang & Hewitt 1996).

4.12.1 General findings

Overall nucleotide content across all samples was estimated (Table 4.12.1.1). Among species, variation in GC content for 3rdcodon position was higher than for position 1, with variation at position 2 being very limited (Figure 4.12.1.1). This showed the absence of synonymous mutations at position 2.

Table 4.12.1.1: GC composition of COI gene across prawns and shrimps of Neyyar river

Nucleotide	Minimum	Mean	Maximum	Standard Error
G%	15.9	18.8	20.1	0.145
C%	20.1	25	27.5	0.194
A%	24.9	26.8	29.9	0.152
T%	28.1	29.4	34.6	0.165
GC%	37	43.8	46.8	0.276
GC% Codon Position 1	49.4	54.4	55.4	0.236
GC% Codon Position 2	42.8	43.5	44.6	0.083
GC% Codon Position 3	18.1	33.5	42.2	0.665

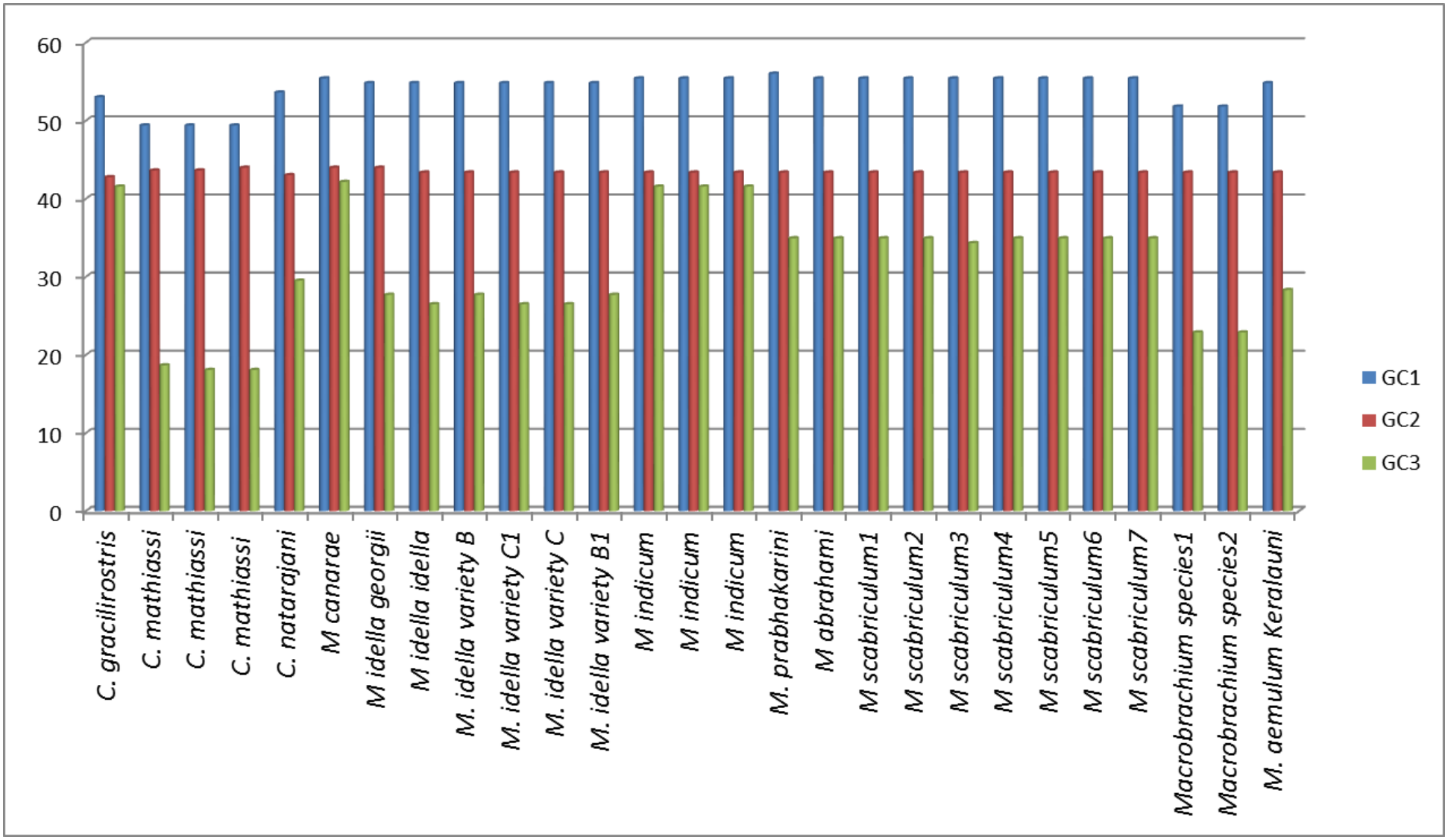


Figure 4.12.1.1: GC composition of COI gene across freshwater prawns and shrimps of Neyyar river

4.12.2 Cytochrome c oxidase subunit I (COI) divergence

The means of K2P (Kimura 2 Parameter) distance for COI increased from lower taxa towards the higher taxonomic rank (i.e. within species, genera, family, Kimura, 1980) (The average congeneric distance (D=21.9%) was ~100 times more than the average conspecific distance (D=0.2%). Whereas, the mean divergence among genera within families was 26.9% (Table 4.12.2.1). The rate of increase in distance was declined in the higher taxonomic groups due to substitution saturation.

Table 4.12.2.1: Distance (K2P model) values of COI gene across Prawns and shrimps

	Min Dist (%)	**Mean Dist (%)**	**Max Dist (%)**	**SE Dist (%)**
Within Species	0	0.2	0.4	0.008
Within Genus	19.6	21.9	26.9	0.048
Within family	24.2	26.9	31.9	0.017

4.12.3 Estimation of the Pattern of Nucleotide Substitution for COI gene in freshwater prawns and shrimps from Neyyar river

The transition / transversion rate ratios are k_1 = 6.78 (purines) and k_2 = 6.098 (pyrimidines). The overall transition/transversion bias is R = 3.183. The pattern of nucleotide substitution was estimated by Tamura-Nei model (Tamura *et al.,* 2013) and the probability values for substitutions were given in Table 4.12.3.1. The average transitional pairs (S_i = 418) were more than transversional pairs (S_v = 49) with an average ratio of 31.

Table 4.12.3.1: Estimate of the Pattern of Nucleotide Substitution for COI gene freshwater prawns and shrimps of Neyyar river

	A	**T**	**C**	**G**
A	-	3.48	2.98	**15.16**
T	3.19	-	**18.18**	2.24
C	3.19	**21.22**	-	2.24
G	**21.66**	3.48	2.98	-

Note: Each entry shows the probability of substitution (r) from one base (row) to another base (column)[1]. For simplicity, the sum of r values is made equal to 100. Rates of different transitional substitutions are shown in bold and those of transversionsal substitutions are shown in italics. The analysis involved 49 nucleotide sequences. Codon positions included were 1st+2nd+3rd+ Noncoding. All ambiguous positions were removed for each sequence pair. There were a total of 497 positions in the final dataset.

4.12.4 Neighbour-joining analysis

The NJ tree was constructed based on K2P values of the COI gene revealed distinct clusters shared by congeneric species with significant bootstrap value. Species belong to the same family clustered together (Fig4.12.4.1)

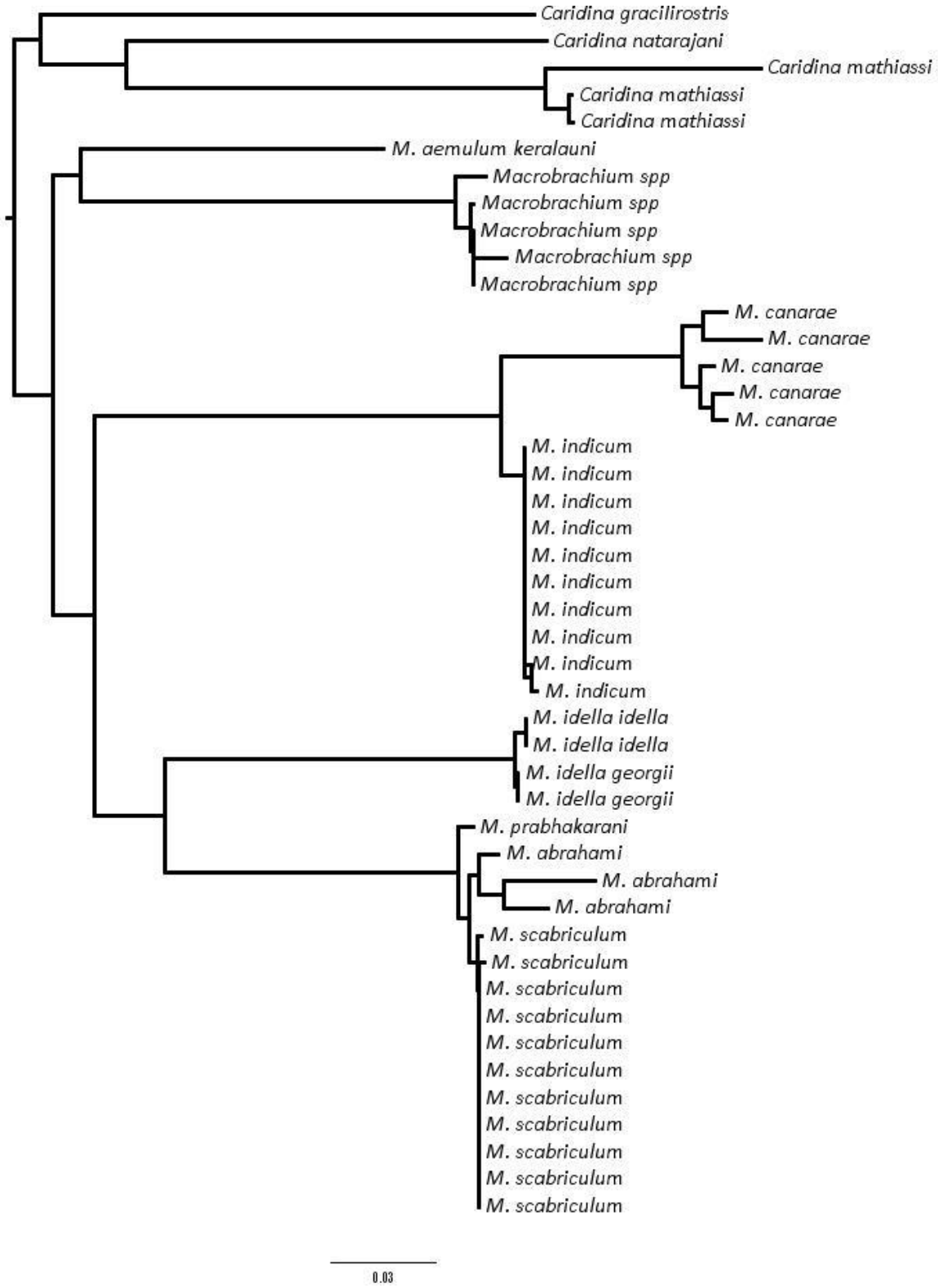


Figure: 4.12.4.1: Neighbour-joining tree

4.13 Diversity, Distribution and Conservation Status of Families Atyidae and Palaemonidae in the Neyyar River Stretch

The organisms collected from the previous studies and present study compiled and made the checklist of the prawns and shrimps occur in the river system and its diversity. The distribution, IUCN status and endemism shown in table 4.13.1 and figure 4.13.1 and figure 4.13.2

Table 4.13.1 Diversity and distribution of *Macrobrachium* species occurs in Neyyar river

Species	Distribution	IUCN status	Endemism
Family: **Palaemonidae**			
Macrobrachium abrahami	Vamanapuram river	NE	Ashambu hill streams
Macrobrachium aemulum	New Caledonia and French Polynesia, Kerala and Tamil Nadu	LC	Not endemic
Macrobrachium aemulum keralauni	Neyyar river, Kerala	NE	Neyyar
Macrobrachium indicum	Vellayani lake, Neyyar river (Trivandrum district of Kerala)	LC	Ashambu hills
Macrobrachium canarae	Karnataka, Tamil nadu, Kerala	DD	South India
Macrobrachium idella idella	Tanzania and Madagascar, India	LC	Not endemic
Macrobrachium idella georgii	Central Kerala and Neyyar river of southern Kerala	LC	Kerala
Macrobrachium lamarrei	All over the India	LC	India

Macrobrachium prabhakarani	Vamanapuram river, Neyyar river	DD	Ashambu hills
Macrobrachium latimanus	India, Fiji, French Polynesia, Philippines Sri Lanka, Japan	LC	Not endemic
Macrobrachium scabriculum	Africa, Madagascar, India, Bangladesh, Sri Lanka and Sumatra	LC	Not endemic
Macrobrachium sankollii	Karnataka and Kerala	LC	South India
	Family: **Atyidae**		
Caridina gracilirostris	India, Fiji, French Polynesia, Philippines, Taiwan, Japan, China	LC	Not endemic
Caridina mathiassi	Kanyakumari district, Neyyar river Kerala	LC	Ashambu hill streams
Caridina natarajani	Rivers of Kerala	LC	Kerala

LC- Least concern

NE- Not evaluated

DD- Data deficient

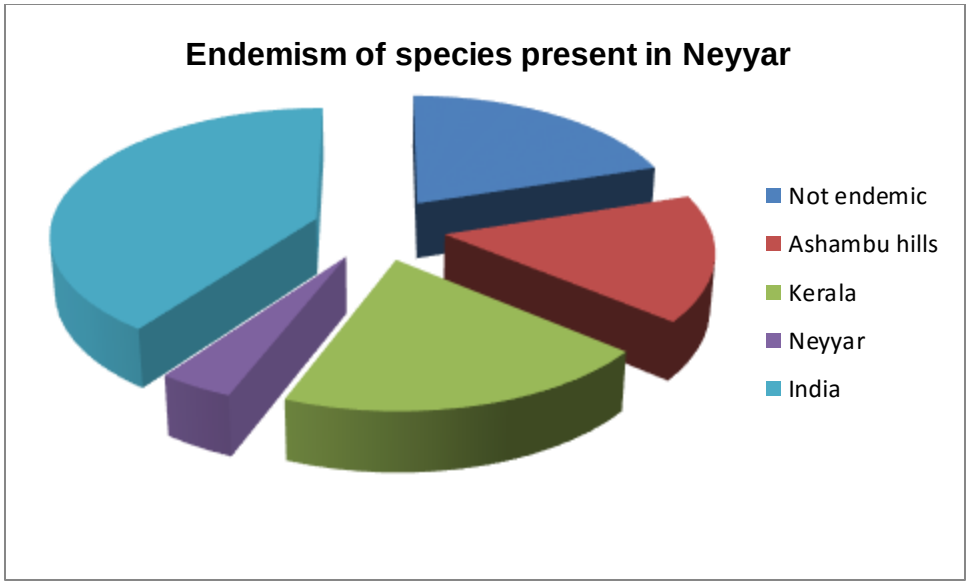


Figure 4.13.1: Pie chart showing the endemism of Palaemonids and Atyids present in the Neyyar river

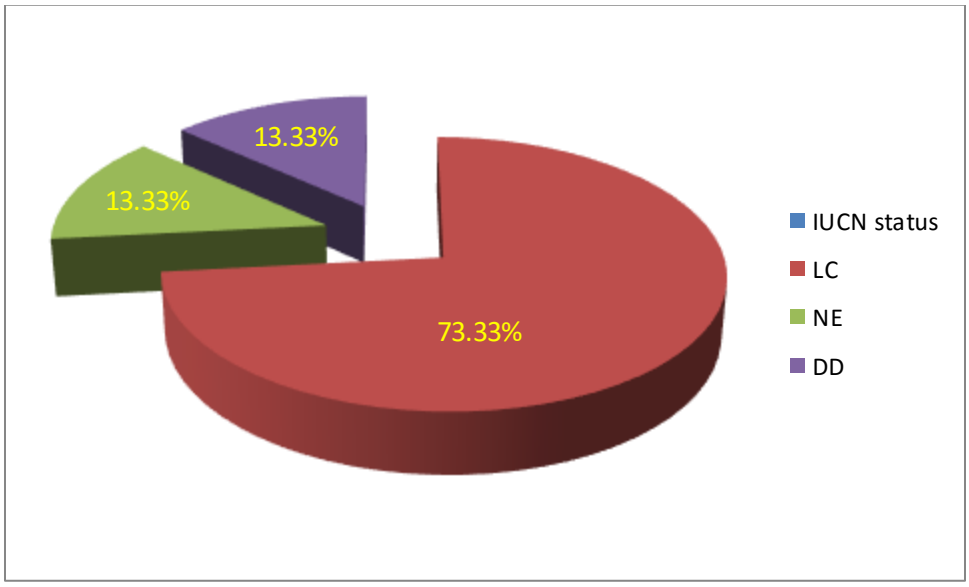


Figure 4.13.2: Pie chart showing the IUCN status of Palaemonids and Atyids present in the Neyyar river

4.14 Habitat Identification of Species Comes under Family Palaemonidae and Atyidae Present in the Neyyar River, Kerala

- Except a single species (*Macrobrachium* spp. Fig 4.2.7.1), all the Palaemonids and Atyids were reported from the site 1, Map 3.1.1)
- In that ecosystem (Site 1) all the species are assembled in different niche
- The large Varieties of *Macrobrachium indicum*, *Macrobrachium idella* idella, *Macrobrachium idella georgii,* and *Macrobrachium aemulum keralauni* hide beneath the large rocks and caves
- The small varieties like *Macrobrachium canarae* and juveniles of *Macrobrachium idella* covered the surface of rocks and caves, where the large organism living and it was like guarders
- *Macrobrachium scabriculum* only distributed beneath the laterite rocks and inside the bamboo spaith
- *Macrobrachium prabhakarani* and *M. abrahami* is assemble near to the alluvial clay bunds
- The *Caridina mathiassi* mainly observed in laterite rocks and *Potamogeton* ground observed in river floors.
- *Caridina gracilirostris* is only found to associated with the roots of surrounding plants
- *Caridina natarajani* found distributed over the roots of bamboo trees and *Potamogeton* ground

4.15 Major Threats to the Diversity Observed During the Study Period.

4.15.1 Illegal sand mining:

Heavy sand mining (banned for three years) observed during the study period in most of the river part. But in the present centres are the small stretches, which are devoid of such threats. But there is a chance of occurrence of such threats in future.

4.15.2 Pollution from surrounding city:

The point source of pollution observed in the two sampling station (Station 1 and Station 2) during sample collection. The pollution indicating organism Chirenemoid larvae, observed in the polluted water and particular zone of river.

4.15.3 Gracing of domestic birds over the prawn ground:

The duck farming in the surrounding house hold causing high damage to the prawn resources of the first station. The main target of the ducks was the shrimps in Potamogneton ground and prawns hide under the laterite rock. The strong beaks helps the birds to pull the rock and catching the pray.

15.4.4 Gold mining in alluvial bunds:

The gold mining (Mining of minute minerals) observed in the alluvial bunds of river basin were the prawns are inhabited. This is a livelihood option of the people living near to the shores.

15.4. 5 Exotic species

During the sampling about 3 numbers of Tilapia and one Loricarid cat fish were captured with the help of mosquito net. It is indicating that the presence of such species in the ecosystem.

15.4.6 Fishing using explosives

The banned fishing practice was observed during the study period from station 1, the practice is observed in most of the holydays as sport fishing.

Discussion

5. DISCUSSION

5.1 Taxonomy of Prawns and Shrimps of Neyyar River

Nine species of *Macrobrachium* under the family Palaemonidae and three species of *Caridina* comes under the family Atyidae has been reported from the river during the study period. Pillai and Unnikrishnan (2013b) reported the six species of *Macrobrachium* earlier; from that list only 3 species were collected during the present study. Among the three *Caridina* species a single species C. natarajani reported earlier from the river (Jayachandran *et al.,* 2008)

5.1.1 *Macrobrachium abrahami* Pillai, Unnikrishnan and Kumar, 2014

Macrobrachium scabriculum is a very common species of India. This species exhibits wide range of variations within the species level. Throughout the world taxonomic studies of this species has been carried out, but its exact taxonomic position is still in its incompletion. In this context the species reported in this also has to be looked into. During the present study specimens belonging to species - *Macrobrachium scabriculum and M. abrahami* and *M. prabhakarani* were collected.

Macrobrachium abrahami belong to "*scabriculum*" group of species and it generally agrees to the descriptions by Pillai *et al.,* 2014. These two species resemble in the nature of rostrum and denticles present on the cutting edge of larger second pereiopods. However the present species differ from Piallai's description in characters, such as, the palm of larger second pereiopods smaller than fingers and also the fingers are with a black band like marking at the middle portion. These differences are coming under the variations reported for "scabriculum" group of species.

The submitted the sequence data in NCBI website is based on Cyt b primer. However the present sequence is based on COI and hence comparison of the sequences is not being attempted. According to phylogenetic tree made from the COI barcode of all the *Macrobrachium* species collected from the river, *M.*

abrahami shows more closeness to *M. prabhakarani* and *M. scabriculum*. Therefore further molecular study using the different gene is required for the species confirmation. In this context the present species is treated as *M. abrahami.*

5.1.2 *Macrobrachium aemulum keralauni* Pillai, Unnikrishnan, 2013

Macrobrachium aemulum keralauni, a newly described subspecies of *Macrobrachiumaemulum*, from the Neyyar river. The same species has been described Jayachandran and Joseph, 1988b from Pamboorivaikal river which is nearer to the present river. The specimens at my disposal share the characters given by Jayachandran and Joseph (1988) and Pillai and Unnikrishnan (2013b, 2013c), Table 5.1.2

Macrobrachium aemulum is the native species of New Caladonia. The COI barcode comparison of New Caladonian and the specimens collected from the Neyyar showing remarkable difference in the nucleotide sequence of the third codon. This is the first sequence analysis of the species from Kerala (outside the type locality). From the Table of comparison it is difficult to segregate the species from the Roux's (1926) description; the species elevation is not attempted to. However there is every merit to elevate it new species. If it is so, then the new species name will be *Macrobrachium keralauni* sp. nov.

5.1.3 *Macrobrachium canarae* (Tiwari, 1958)

The species was originally described by Tiwari (1958) from the Karnataka water, and described as *Palaemon canarae* honouring the type locality. Later Jalihal *et al.* (1988) assigned the correct name for the species as *Macrobrachium canarae*. Jayachandran (1991) described the same species from Meenachil river (Kerala) as the first description out of type locality. The presently collected specimens more similar to the characters of *Macrobrachium canarae* except the variation in the number of teeth in the upper and lower margin of the rostrum. The COI barcode of the present specimens are also not matching with the sequence present in the NCBI and hence detailed study is necessary for confirming the status.

Table 5.2.1.1 Comparison of the description of *Macrobrachium aemulum* collected by different authors from India, present collection and with original description

	Macrobrachium aemulum Jayachandran and Joseph, 1988	***Macrobrachium aemulum keralauni*** Pillai and Unnikrishnan, 2013a,2013b	***Macrobrachium aemulum keralauni*** present collection	***Macrobrachium aemulum*** (Jayachandran, 1988&Pillai and Unnikrishnan, 2013))
Number of dorsal teeth	11	9-13	11	11
Number of post orbital teeth	5	4-5	5	4-5
Nature of carapace	Smooth	Smooth	Smooth	
Telson	Proximal pair slightly posterior to middle of the telson, distal pair at the mid of the proximal pair and distal margin of telson	Proximal pair at the middle of telson and the distal pair is at the midway between the proximal pair and distal margin of telson	Proximal pair at the mid of the telson, the distal pair at about 3/4th length of telson	-
Nature of 2nd chelate leg	Unequal	Unequal	Unequal	Unequal
Large chelate leg (Ratio of each segment with the total length of pereiopod)	I: M: C: P: D 0.13: 0.19: 0.29: 0.38: 0.11	I: M: C: P: D 0.097: 0.16: 0.27: 0.36: 0.11	I: M: C: P: D 0.11: 0.18: 0.30: 0.38: 0.10	I: M: C: P: D 0.13: 0.20: 0.28: 0.40: 0.15

Ratio of legs2nd periopod to total length	1.03for larger 0.87 for smaller	1.36 for larger chelate leg 1.06 for smaller legs	1.7 for longer chelate leg 1.06 for smaller leg	1.01 for larger chelate leg 0.82 for smaller chelate leg
Denticles in fingers of large chelate leg	One large and two smaller denticles on proximal part, and stiff setae on entire cutting edge	Two large denticles on proximal part and 6 weak denticles on distal part of both the fingers	Two large denticles in the proximal and 6 to 7 weak denticles in the distal part of both the fingers.	Basel third of the movable finger with a big tooth preceded at the articulation by two small teeth followed by another big tooth at the middle; another teeth at the ends
Smaller chelate leg (Ratio of each segment with the total length of pereiopod)	I: M: C: P: D 0.15: 0.19: 0.28: 0.37: 0.12	I: M: C: P: D 0.11: 0.17: 0.25: 0.36: 0.11	I: M: C: P: D 0.081: 0.20: 0.29: 0.40: 0.12	I: M: C: P: D 0.14: 0.20: 0.28: 0.38: 0.13
Fingers of smaller leg	Stiff setae crisscrossed in the gap, armed with one or two feeble denticles	The gap is armed with stiff setae	The gap is crisscrossed with stiff setae	-

5.1.4 *Macrobrachium idella idella* (Hilgendrof, 1898)

The species was originally described by Hilgendrof (1898) as *Palaemon idae.* Henderson and Mathai (1910) described the species of *P. idae*. Later Jayachandran and Joseph (1985) described a new subspecies of *Macrobrachium idella* from Central Kerala as *Macrobrachium idella georgii* with the result the names of species are : *Macrobrachium idella idella* (Hilgendorf, 1898) and *Macrobrachium idella* here onwards parent species is known as *M. idella georgii* Jayachandran & Joseph, 1985. Jalihal *et al.* (1988) reported *Macrobrachium idella idella* from waters of Karnataka.

5.1.5 *Macrobrachium idella georgii Jayachandran* et Joseph, 1985

The specimens of the present collection agree fully with the descriptions of Jayachandran and Joseph (1986) described the subspecies from from four river systems in central Kerala (Pallickal, Pamba, Manimala, Meenachil rivers). During the present study this species has been collected and it agrees fully with the descriptions by the above authors. With this report it is the extension of distributional range. Most important characteristic of the species is that the carpus and chela are of equal size. Presence of two accessory spines in the uropodal exopod is the additional character observed in the present collection of specimens and this has not been reported previously.

5.1.6 *Macrobrachium indicum* Jayachandran and Joseph, 1986

The specimens collected during the present study from Neyyar river agrees in all respect to *Macrobrachium indicum* described by Jayachandran and Joseph (1985) from Vellayani lake and Pamboorivaikkal river. These two collection stations are nearer to Neyyar river. This species has been again reported after a period of 30 years. The species resembles Australian species, *Macrobrachium australe*. The most striking resemblance of the two species is hooked nature of the movable finger of the second cheliped. It is quite difficult to identify them also. But for practical purposes we can rely on the less hooked nature of *Macrobrachium australe*. *M. indicum possesses* longer ischium which is longer than merus

The barcode of *M. australe* and *M. indicum* showing a remarkable difference between the two species and hence status of the two species is confirmed. The present barcode generated for the species for the first time and will be submitted to NCBI shortly.

5.1.7 *Macrobrachium spp.*

The morphological characters of the species generally agrees with the previous description of *Macrobrachium lamarrei*, but on blasting the barcode data of the NCBI and the present analysis, it has been found that there is considerable deviation. Further evaluation of morphological characters is necessary to assess the species identity of the collected specimens. Moreover it is worthy to note that *M. lamarrei lamarrei* has not been reported from Southern tip of India.

5.1.8 *Macrobrachium prabhakaranii,* Pillai and Unnikrishnan, 2012

The characters observed in the species agrees with the description of Pillai and Unnikrishnan, 2012 except for the less scabrous anterior portion of the carapace The specimens were collected along with the *M. scabriculum*. It shows close resemblance also. Pillai and Unnikrishnan (2012) submitted the sequence data in NCBI website based on Cyt b primer. However the present sequence is based on COI, and hence a comparison of the sequence could not be made here. The phylogenetic tree made from the COI barcode of all the *Macrobrachium* species collected during the present from the river, the *M. prabhakarani* more close to *M. abrahami* and *M. scabriculum*. Further study is required in this regard.

5.1.9 *Macrobrachium scabriculum* (Heller, 1862)

The characters of the species fully agree with the description of *M. scabriculum* described by Jalihal *et al.* (1988) and Jayachandran (2001). Similarly the COI barcode data generated now of the species is also matching with the sequence in NCBI.

5.1.10 *Caridina gracilirostris* (de Man, 1892)

The characters of the species agree with the all the characters of *C. gracilirostris*. Natarajan (1942) first reported the presence of the species in

Travancore water. Later Thomas *et al.* (1973) described a similar species - *Caridina pseudogracilirostris* from the Cochin backwaters (Brackish water). The Barcode of the C*. pseudogracilirostris* by Jayachandran *et al.,* 2008, shows the similarity with the barcode of the present species. Recently these two species has been synonymized to *Caridina gracilirostris* (de Man, 1892). Therefore this name is retained for the specimens collected from Neyyar river.

5.1.11 *Caridina mathiassi Silas* & Jayachandran, 2010

The specimens of the present collection agree in all respects with the characters of *C. mathiassi* by Silas and Jayachandran, 2010. Present collection is the report of the same other than its type locality Mahendragiri hill stream. The COI barcode generated is the first sequence data of the species.

5.1.12 *Caridina natarajani* Tiwari & Pillai, 1968

The species was originally described by Tiwari and Pillai in 1968 from south Kerala (Museum tank of Trivandrum). Jayachandran *et al.* (2008) reported the presence of species in the Neyyar river. The present species agree the characters of *C. natarajani* in both morphological and molecular ways.

5.2 The Habitat and Threats of the Species Reported From the River

The major threats reported to the Palaemonid and Atyid in the river during the study period were the destruction river bank by sand mining and land cover change. The pollution sources are also observed in the two sampling stations of the river. Destructive fishing and duck gracing are another major threats observed in the prawn resources of the river. Sheeja *et al.* (2010); Harikumar and Kokkal (2010); Abraham *et al.* (2011) reported such threats in the river during their study period. Among them Sheeja *et al*. (2010) reported the impact of land cover change and sand mines to the aquatic fauna by conducting GIS approach study. Harikumar and Kokkal (2010) more specified in the pollution of the river and water quality change from the upstream to the down. Abraham *et al*. (2011) reported all the illegal human activities which harm the precious aquatic fauna of the river. All three literatures specified the more impact region was the stretch below the middle portion of river. In this study the station one is below that range but the species diversity is

higher than the other. But the region is only confined to a 50m stretch succeed and preceded by the large sand mines. Hence conservation method is required for the protection of the region from further degradation and impact.

Summary

6. SUMMARY

An exploratory taxonomic survey for the species of families Palaemonidae and Atyidae conducted in the three sampling station of Neyyar river during august 2015 to April 2016 revealed the presence of 9 species from family Palaemonidae and 3 species of from family Atyidae. A single genus *Macrobrachium* in the family Palaemonidae found in the collection. The species of *Macrobrachium* collected from the river were *Macrobrachium abrahami*, *Macrobrachium aemulum keralauni*, *Macrobrachium idella idella*, *Macrobrachium idella georgii*, *Macrobrachium indicum*, *Macrobrachium* spp., *Macrobrachium prabhakarani*, and *Macrobrachium scabriculum*. Similarly all the species were collected from the river belongs to the family Atyidae comes under a single genera *Caridina* and the species are *Caridina gracilirostris*, *Caridina mathiassi* and *Caridina natarajani.*

Among the *Macrobrachium* species the *Macrobrachium prabhakarani* and *Macrobrachium abrahami*, are the two newly described species from Vamanapuram river near to Neyyar by Pillai and Unnikrishnan in 2012 and 2014. Scientists made phylogenetic tree based on Cyt b. In present study these species shown high affinity towards *Macrobrachium scabriculum* in both morphological and molecular characters. The NJ tree constructed based on the COI barcode the species appears as a single species in phylogenetic tree with a small variation (COI barcode). Further molecular study (using other primers) is required for the confirmation of the existence of species.

Macrobrachium canarae the species collected from the Neyyar River showing some variation with the species character in the original description. The COI barcode of the species have variation with the species barcode in NCBI site. Based on morphometric analysis it was visible that the species is very close to *Macrobrachium* spp. (Specified in results Figure 4.2.7).

Macrobrachium idella idella one of the large sized species collected from the river can identify easily with their large and equal second pereiopods. The

subspecies of *Macrobrachium idella*, *M. idella georgii* is the interesting species got from the river. The present collection reported the range extension of the subspecies from the central Kerala rivers to southern Kerala, which means outside the type locality. Based on the morphometric and molecular study it proven the earlier works these two species are very close and subspecies. In scatter plot of species constructed based on the morphometric characters and the phylogenetic tree constructed based on the molecular characters showing the species are very close.

Macrobrachium indicum, the native species of India and very close to *Macrobrachium australe,* native of Australia. The present study reporting the species after 30 years its original description by Jayachandran and Joseph, 1986. The barcode of the species is new to the science, and going to submit in NCBI.

Macrobrachium spp. a doubtful species of *Macrobrachium* collected from the sampling site 2, which is morphologically similar to the *M. lamarrei* and *M. sankolli* but it is varying in some characters like teeth pattern in rostrum, shape of rostrum. Molecularly the species does not showing any similarity with *M. lamarrei* and *M. sankolli* hence the species may be new to the Palaemonidae family.

In family Atyidae the two species, *C. gracilirostris* and *Caridina mathiassi* are the first report from the river and *Caridina mathiassi* is the first report of the species outside the type locality Mahendragiri estate of Tamil Nadu.

Entire morphometric and meristic characters observed during the study the characters of *M. abrahami*, *M. prabhakarni* and *M. scabriculum* merged with each other. But the numbers of the specimens become the limitation in the case of morphometric comparison of the species. Among the morphometric analysis of six species, *M. indicum*, *M. idella idella*, *M. idella georgii*, *M*. spp, *M. canarae*, *M. scabriculum,* the 6 characters, Rostral length (RL), Length of first pereiopod (FPL) Length of large 2nd pereiopod (SLPL), Ischium length of second large pereiopod (SLPIL), palm length of second large pereiopod (SLPPL), ischium length small second pereiopod (SSPIL) contribute more than 90% share for discriminating the species.

The variability plot constructed by using the meristic characters and ratio of morphometric characters showed the variability of the characters within the species. In the case of *M. scabriculum* variability within species is high than the other species observed. The accessory spines in the uropod not showed any variation within species. But in *Caridina* species all the characters showed a remarkable variation within the species.

The graph plotted using the variation of GC content of each species gave a clear cut identity of the organism and its variation between the species accordance. In phylogenetic tree, it is visible that within species variation most prominent in Crustacean group (may be because of variation in the teeth pattern and number). The *Macrobrachium prabhakarani*, *M. abrahami* and *M. scabriculum* showing such relationship like in the within species. The whole sequence got during the barcoding of the species present in the Neyyar subjected to the further analysis and extracted, the transitional pairs of codon, transversional pairs of codon and Ration of transitional and transversional codon. These indicating the genetic character of the collected Palaemonid and Atyid present in the particular river stretch.

The analysis of status and endemism of entire Palaemonid and Atyid prawns present in the river, a single species *M. aemulum keraluni* only present in the particular stretch of the river. Among the other 14 species 5 are endemic to the Ashambu hill streams. Five distributed other part of the world. The most of the species collected from the river is reported under the LC category due to lacking of proper reported on the threats to the species. Even though its population size less, the further status evaluation the species is required for their conservation and management. The observation and earlier reports on the ecosystem threats to the Palaemonids and Atyids, is high in the station 1; which is just below the middle stretch, were the threats by the human involvement is very high. As the loss of biodiversity is major problem in modern world, the documentation and knowledge on biology and diversity of organism required for the implementation of conservation policy is lacking. In this context the present study can provide a reference on the biodiversity of endemic prawns and shrimps in Neyyar river for further conservation and management measures.

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ABBREVIATION

TL	Total length
CL	Carapace length
RL	Rostral length
FPL	First pereiopod length
SLPL	2nd large pereiopod length
SSPL	2nd small pereiopod length
TEL	Telson length
FSTEL	Telson length up to 1st pair of dorsal spine
SSTEL	Telson length up to 2nd pair of dorsal spine
FPIL	Ischium length of 1st pereiopod
FPML	Merus length of 1st pereiopod
FPCL	Carpus length of 1st pereiopod
FPPL	Palm length of 1st pereiopod
FPFL	Finger length of 1st pereiopod
SLPIL	Ischium length of large 2nd pereiopod
SLPML	Merus length of large 2nd pereiopod
SLPCL	Carpus length of large 2nd pereiopod
SLPPL	Palm length of large 2nd pereiopod
SLPFL	Finger length of large 2nd pereiopod
SSPIL	Ischium length of small 2nd pereiopod

SSPML	Merus length of small 2nd pereiopod
SSPCL	Carpus length of small 2nd pereiopod
SSPPL	Palm length of small 2nd pereiopod
SSPFL	Finger length of small 2nd pereiopod
DT	Number of dorsal teeth
VT	Number of ventral teeth
POT	Number of post orbital teeth
URD	Number of spines Uropod diaeresis
TLD	Number of spines in dorsal surface
TLS	Number of spinules in distal part of telson
URO	Number of accessory spines in uropodal exopod
DMFL	Number of denticles in movable finger of large 2nd pereiopod
DIFL	Number of denticles in immovable finger of large 2nd pereiopod
DMFS	Number of denticles in movable finger of small 2nd pereiopod
DIFS	Number of denticles in immovable finger of small 2nd pereiopod
PLU	Number of plumose setae

APPENDIX I

Equipment used for the collection morphological and morphometric measurement

Equipment	Company
Mosquito net	Mesh size 1mm, 6* 6.5 feet
Camera	Nikon Coolpix L340
Digital Vernier callipers	Camelin , India, Casio, India
Dissection microscope	Warszawa, Poland
Compound microscope	Motic, India
Stereo microscope	Olympus, Corporation, America

APPENDIX II

1. Equipment used for the molecular taxonomic work

Equipment	**Company**
Glassware	Borosil, India
Autoclave	Expo Hi-Tech, India
Microwave oven	Kenstar, India.
Cold water bath	Julabo, Germany.
Gel Documentation System	Syngene, USA
Laminar flow	Yorko Scientific Industries, India.
Orbital Shaker	Saksham Technologies, India
UV Transilluminator	Manisha Enterprises, India.
High speed cooling centrifuge	Stratos, Heraeus, Germany.
Thermal cyclers	ABI (USA), Thakara
UV Biophotometer	Eppendorf AG, Germany.
Electrical balance	APX-100, Denver Instruments, USA.
Millipore water purification system	Milli-Q, ElixTM, Millipore Corporation,Australia
Micropipettes	Eppendorf (Germany), Gene Mate (USA) and Tarson, India.
Plasticware	TarsonPvt. Ltd., India.

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APPENDIX III

Reagent composition

1. Genomic DNA isolation

1.1 **Salting out method chemicals:**

Stock solution	**Chemical in grams.**	**Water (ml)**	**Final volume (ml)**	**For pH adjustment**
500 mMTris – HCl (pH8.0):	60. 55	700	1000	HCl
200 mM EDTA (pH8.0)	58.44	700	1000	NaOH

(Note: Sterilize by autoclaving.)

a) Solution 1: (For 100 ml):

50mM Tris-HCl (pH8.0)

20mM EDTA (pH8.0)

2%SDS

Solution 1 (For 100 ml)	500 mMTris – HCl (pH8.0):	200 mM EDTA (pH8.0)	SDS	Final volume Make up with water
	10 ml	10 ml	2 gms.	100

b) Solution 2: (For 100 ml):

Saturated NaCl solution (6 M)	35.064 NaCl	Make up 100 ml with water

c) Proteinase K (20mg/ml): Store at -20 ^{0}C

d) TE Buffer :

Tris-HCl (pH 8.0)	10 mM
Na_2 EDTA. $2H_2O$ (pH 8.0)	Na_2 EDTA. $2H_2O$ (pH 8.0) 1 mM

e) RNase (10mg/ml): 10 µl and incubated at 37° C for 1 hr