I. Mangrove Ecosystems Sampled:

Being the most numerically dominant mesofaunal elements of the edaphic ecosystem, oribatid mites exhibit diverse types of interaction with their natural environment and thus occupy almost all available ecosystems in the world. Despite their well-known habitats like the forestlands and other litter-accumulated areas, mangrove soils containing organically derived substrata comprising the litter, pneumatophores and remnants of true mangrove plants or mangrove associates were selected as the sampling sites during the present study for the recovery of oribatid mites. Being highly challenging habitat with frequent exposure to tidal currents, mangrove ecosystems generally support highly specialized floral and faunal elements and this is applicable to the oribatid mite fauna also, necessitating the adoption of special collection procedures with utmost care. In the present study, a total of 15 sampling sites from various mangrove ecosystems distributed over 4 districts of North Kerala viz. Malappuram (MPM), Kozhikode (KKD), Kannur (KNR) and Kasaragod (KSD) (Table-1; Plate-2, Figs.1-5) were screened for the collection of oribatid mites. Regular/intermittent sampling was carried out from the study sites mentioned below to
recover most important and locally available oribatid mite fauna. All the sites sampled were found to support diverse vegetation composed of characteristic true as well as associate mangrove species (Plates,3-4,Figs.1-8; Plate-5, Figs.1-9).

II. Sampling Localities Screened:

A. MALAPPURAM DISTRICT (Sites1&2; Plate-6, Figs. 1&2)

Malappuram district lies on the” top of hills” and bounded North by Wayanad and Kozhikode districts and Southeast and Southwest by Palakkad and Thrissur districts respectively, and enriched with well-known rivers viz. the Chaliyar, Kadalundi and Bharathappuzha. Two sampling sites, viz. Brahmasampadam (BRSPM) and Olipramkadavu (OPKDV) were selected from the Malappuram district for collection of mangrove soil/litter/bark/pneumatophores/leaves/twigs etc. for the extraction of oribatid mites. The mangrove vegetation of the area mainly comprises A. marina, A. officinalis and Acanthus ilicifolius. (Table.3).

1. Brahmasampadam (BRSPM) (Plate- 6, Fig.1)

This site supports small patches of true and associate mangrove vegetation, located 4 kilometers away from the Calicut University Campus and 5 kilometers away from the seashore with coordinates, 11° 7’35.36”N and 5°52’8.46”E respectively. From this site, narrow water channels run up to
2. Olippuzha (Kadalundi River). The major vegetation includes *A. marina*, *A. officinalis* and *Acrostichum areum* (Table 3)

2. Olipramkadavu (OPKDV) (Plate -6, Fig.2)

This site is a small sandy area located at 11° 7′ 41.40″N 75° 51′ 50.72″E in the banks of the river Olippuzha. The vegetation is dominated by true mangrove species, *A. ilicifolius*, *A. marina*, *Pongamia pinnata* and *A. areum*. (Table.3)

B. KOZHIKODE DISTRICT: (Sites 3-7; Plate -6, Figs. 3-7a)

There are 5 study sites from this district were Kadalundi- Vallikkunnu community reserve mangrove (KVCR-I&II) Kottakkadavu (KTKDV), Mankavu (MNKV) and Kallai (KLAI).

Kadalundi-Vallikkunnu Community Reserve Mangrove (KVCR-I&II) (Plate-6, Figs.3&4)

This was the major study site located at 11°07′33.76″ N. and 75°49′49.40″ E. This is the State’s first community reserve in India, declared in 2007. It is a home and breeding site for various shoreline migratory birds, fishes and crustaceans. It is distributed across an area of 1.5 km² at the estuary of the Kadalundi River, Kozhikode and Malappuram districts of North Kerala.
3. Kadalundi-Vallikkunnu Community Reserve Mangrove –I (KVCR-I)
(Plate-6, Fig.3)

KVCR-I is a mangrove area situated at (11° 7’37.51”N and 75°49’54.64”E), at the mouth of the Kadalundi River, below the Railway Bridge. This site is composed of sandy mudflats with relatively good patches of true and associate mangroves and constantly exposed to regular tidal flooding. The flora includes the mangrove species viz. *A.marina*, *A.officinalis*, *R. mucronata*, *Derrris trifoliata*, *Clerodendrum inerme*, *P. pinnata*, *Excoecaria agallocha*, *Mariscus javanicus* and *Wedelia chinensis* etc. (Table.3)

4. Kadalundi-Vallikkunnu Community Reserve Mangrove –II (KVCR-II) (Plate-6, Fig.4)

KVCR-II is a site adjacent to the terrestrial area and just away from the coastline habitat and is located at (11° 7’54.89”N and 75°49’44.55”E). This site is mainly formed by both sandy and loamy soils. Either sides of this site are bordered by true mangrove species like, *A. ilicifolius* and *A. officinalis*. This area is partially flooded by the seawater during high tidal waves. The mangrove vegetations are *A.marina*, *A.officinalis*, *M.javanicus* (Table.3)
5. Kottakkadavu (KTKDV) (Plate -6, Fig.5)

This site is bordered by the Kozhikode and Malappuram districts and is located at 11°8'15.99"N and 75°50'28.26"E. The site is characterized by mangrove vegetations viz. *A. officinalis*, *E. agallocha* are growing along the banks of the Kadalundi River. (Table.3)

6. Kallai (KLAI) (Plate- 6, Fig.6)

Kallai is a well-known timber-trading place in south India. The study site selected in this area is located on the bank of the Kallai River at 11°14'16.72"N and 75°47'14.12"E. The vegetation of the site mainly comprises mangrove trees like *A. marina* and *A. officinalis*. (Table.3)

7. Mankavu (MNKV) (Plate -6, Figs.7 & 7a)

This site was located at 11°14'7.69"N and 75°48'13.52"E on the banks of the Mampuzha River, some 5 Km away from the Kozhikode city. This site was dominated by true mangrove species like *A. ilicifolius* and *A. officinalis* and a mangrove associate *A. marina, A. ilicifolius* and *Ipomea- pes- caprae*, (Table.3)

C. KANNUR DISTRICT: (Sites 8-15; Plate- 7, Figs. 1-8)

Kannur is popularly known as “the land of looms and lore’s”. The district lies between latitudes 11°40'12.48"N and longitudes 74°52'76.07"E. Kannur is geographically divided into highland, midland and lowland regions
where the lowland is comprised of a narrow stretch containing rivers, deltas and coastal regions. Six collection sites as listed below were considered for the sampling purpose during the present study.

**8. Thalassery (TLSRY) (Plate -7, Fig.1)**

The collection site in Thalassery is very near to the Thalassery railway station and it lies at 11°45'18.12"N and 75°29'30.58"E in the Kannur district. This area embraces a number of small patches of mangroves scattered over the water logged area and is with mixed mangrove vegetation. The most dominant species are *A. marina, R. mucronata and D. trifoliata*. (Table.3)

**9. Koduvalli (KDVLY) (Plate -7, Fig.2)**

This is a small site in Kannur Dt. and is located on the banks of the Kuyyali River, at 11°45'59.30"N and 75°28'42.06"E, supporting mangrove plants viz. *A.marina, A.ilicifolius and R. mucronata* (Table.3)

**10. Valapattanam River Bank (VLMRVR) (Plate- 7, Fig.3)**

This site is situated in the Kannur Dt. at the banks of the Valapattanam River at 11°56'3.92"N and 75°21'4.73"E. Densely growing mangroves are found along either sides of the river, giving scenic beauty to the site. The materials mainly collected from this site include the mangrove litter, pneumatophores and decayed twigs. This site characterized by the most
dominant mangrove species viz. *A. marina*, *A. ilicifolius*, *A. officinalis* and *Aegiceras corniculatum* (Table.3)

11. Dharmadam (DRMDM) (Plate -7, Fig.4)

The selected sampling site at Dharmadam is a mud flat situated on the Anjarkandki River at 11°46'51.88"N and 75°27'42.69"E, in the Kannur Dt. It is a mangrove growing area comprising of *A. marina*, *A. ilicifolius* and *R. apiculata*. (Table.3)

12 & 13. Ezhome (EZHM) -I & II (Plate- 7, Figs. 5&6)

It is a small village in Kannur district and is known for the saline-tolerant Kaipad rice cultivation in Kerala. Two sampling sites selected in this locality (Ezhome I & II) are characterized by the presence of almost all types of mangrove vegetation including *A. marina*, *A. ilicifolius*, *A. officinalis*, *M. javanicus*, *A. corniculatum*, *E. agallocha* etc. (Table.3)

14. Pazhayangadi (PNGDY) (Plate -7, Fig.7)

It is a small township located approximately 22 kilometers away from the Kannur town and lies at 12° 1'36.90"N and 75°16'24.05"E. One side of the sampling site in this locality is bordered by the Pazhayangadi River in which small patches of mangroves are found distributed in small islands. This site is dominated by mangrove vegetation comprising of *A. ilicifolius* and *E. agallocha*. (Table.3)
D. **KASARAGOD DISTRICT** (Site15; Plate- 7, Fig. 8)

Kasaragod, the northernmost part of Kerala covers an area of 1,992km² and lies north of Kannur district. It is famous as “land of gods”, awesome hills, rivers and beautiful beaches and well known for the preservation of the largest fort in the state. Only one site as listed below has been screened in this district for collection of oribatid mites.

15. **Nileswaram (NLSRM)** (Plate- 7, Fig.8)

It forms a small area in the Kasaragod District and it lies at 12°15'58.03"N and 75° 7'13.61"E. The sampling site in this area is located 2kms away from the Nileswaram railway station and the vegetation includes *A.ilicifolius* *A.officinalis* and *A.marina* etc. (Table.3)

### III. Collection of Oribatid Mites

1. **Sampling (Plate- 8, Figs. 1-8)**

Collection of samples of soil/ litter/ pneumatophores and other mangrove debris from the above-mentioned sites was carried out for a period of 5 years (2010 – 2015). Sample collection was mainly performed during the early hours of morning and late evenings, depending up on the prevalence of tidal currents. In the case of undisturbed areas of the selected mangrove habitats, soil samples were collected from the soil surface under the different vegetation types, using a shovel. Samples were also retrieved from the
mangrove litter, decayed twigs, decomposing bark of flooded trunks, pneumatophores etc. of true and associate mangrove plants. The collected samples were transferred into polythene bags and labeled with detailed field data like the topographic features of the site, vegetational characteristics, soil texture etc. Individual samples bearing field labels were brought to the laboratory for extraction of mites.

2. Extraction of samples

The extraction method used in the present study is based on Berlese’s (1905) funnel extraction principle modified by Tullgren (1918). Based on their negatively phototactic habit, samples were subjected to extraction under the Open Brass Funnel Apparatus (Haq and Ramani, 2002) (Plate-9, Figs. 1-3) until the samples were completely dried. (Extraction time was found varied depending on the moisture content of the sample).

(i). Open Brass Funnel Apparatus (Plate-9, Figs.1-3)

The frame of this rectangular unit (183cm x 46cm x 170cm) is made up of steel and resting on four legs. The bottom and top of the unit are covered by steel sheets. Two rows of wooden planks with 12-13 holes respectively, are provided with funnels and sample container on each plank. At the tail end of each funnel, a glass/plastic collection vial (6cm X 2cm) is placed which in turn is kept on a spring mounted over a brass block to ensure
firm attachment between the funnel end and the collection vial. The Open Brass funnel apparatus contains 25 sampling units.

Each sampling unit is composed of 4 parts:

(A) **Heat source** (B) **Sample container and Resting Shield**

(C) **Collection vial** & (D) **A metal spring with base**

**A. Heat source:**

In the present study, electric bulbs of 40/60/100 Watts were used as the heat source, depending up on the nature and moisture contents of samples collected. The distance between the bulb above and the sample below was normally kept 12 cm, but it was increased or reduced by lifting or lowering the wooden planks with the help of screws attached at the corners, as per requirement.

**B. Sample container and Resting Shield**

Each sample container is made of brass and was circular with a height of 10 cm and diameter of 15 cm. A fine wire mesh of 0.8 mm size and 15 cm in diameter is attached to the container, which served as the bottom of the sample container. Larger animals could escape through the gap between the base and lower rim of the sample container. Below the sample container, a round resting brass made shield of 17 cm diameter and larger mesh size of 0.5 cm is kept which served as the resting shield. A conical brass funnel
measuring 22 cm in length and 15 cm in diameter at the mouth end with steep and smooth inner sides supports the resting shield and the sample container. The sample container is held well in position on the resting shield and the latter is placed above the holes in the wooden plank because of the raised edges of the funnel. The tail region of the funnel has a diameter of 2 cm.

(C) Collection vial

A glass or plastic specimen tube of 6 cm x 2.5 cm was used as the collection vial. The mites extracted out of the collected samples were collected either in the preserved condition or as alive in to 70% alcohol/distilled water taken in the collection vial. It was placed beneath the tail end of the funnel with the help of a spring mounted on a brass square block.

(D) Metal spring with base

This helps to hold the vial appropriately below the tail end of the funnel in close proximity. The soil mites escaping from the collected soil/litter would descend through the filter in to the 70% alcohol/water kept in the collection vial, for subsequent taxonomic/biological studies.

(ii). Direct Sampling Method (Plate -9; Fig. 4)

Live mites were collected from epiphytic algal cushions, dead plant twigs, decaying bark and pneumatophores of the true mangrove plants and
mangrove associates through direct examination under a stereozoom research microscope.

(iii) Washing Method (Plate -9; Figs. 5-8)

The washing method adopted in the present study was based on the technique of Karasawa and Hijii (2005) with slight modification. Oribatid mites colonizing the arboreal habitats like leaves and small twigs of true and associate mangroves were collected and immersing these substrates in water mixed with approximately 2 teaspoons of detergent (Surf Excel, Hindustan Uniliver Ltd.; Plate-9, Fig. 5) in a bucket (ca. 5 L). The bucket was then covered with a cloth/white paper to prevent invasion by other animals and kept overnight (10 to 12 hrs) in indoor condition (Plate-9, Fig.6). Leaves and branches were then washed manually in the bucket spiny plant species like A. ilicifolius and C. crista were washed with extra care and the solution was filtered through a nylon mesh of around 100-μm mesh size (Plate-9, Figs.7&8). Added 3-5 ml of ethanol to the solution to remove the excess soap bubbles. The oribatid mites thus recovered from the sediments through the nylon mesh, were segregated under a stereozoom microscope.

3. Separation of Mites

The mites extracted under the Open brass funnel apparatus in to the collection vials were transferred in to a watch glass and thoroughly examined under a stereomicroscope. The oribatid mite specimens were separated with
the help of a moistened camel hair brush and kept in 70% alcohol contained in a cavity block.

III. Preparation of Oribatid Mites for Taxonomic Studies

(i) Dehydration and Clearing

The oribatid specimens recovered through Open Brass funnel extraction/ direct examination/ washing method/ and preserved in 70% alcohol were transferred into cavity blocks/ watch glasses and sorted out using a fine needle and the camel hairbrush under 10x and 40x magnification of a Stereozoom microscope (Leica ES2 and Macro Vis, USA) and the mite specimens were then dehydrated by upgrading in alcohol series (80%, 90%, and 100%) and finally transferred to cap eppendorf vials containing clearing medium prepared by mixing absolute alcohol and lactic acid in the ratio 1:1. The time taken for clearing depended on the sclerotization of the mite species.

(ii) Slide Mounting

The cleared specimens were then slide mounted in various mounting media. For easy examination, temporary mounts were prepared in glycerin or lactic acid. Permanent mounts were made in polyvinyl alcohol/Hoyer’s medium (Baker and Wharton, 1952)
A. Preparation of Mounting Media

a. Polyvinyl Medium

1. Elvanol 74-24 (Du port polyvinyl alcohol) was dissolved in 4 volumes of water at 90°C.

2. Then the solution was filtered.

3. Concentrated the clear filtrate on a water bath until the solution became syrupy.

4. 22 parts of lactic acid were added to 56 parts of the PVA solution and used for slide mounting.

b. Hoyer’s Medium

1) Chloral hydrate - 200 gms

2) Gum arabic - 30 gms

3) Distilled water - 50 ml

4) Glycerine - 20 ml

I- Transferred items (1&2) into 1000ml beaker

II- Added 50ml of distilled water

II- Added 20ml of glycerin and mixed well at room temperature
IV- Mixture filtered, and used for mounting.

a) **Temporary Mounting:** Temporary slide mounts were prepared either on normal glass slides or in cavity slides for examination of morphological features and for easy identification of the species, by making ready comparison of key characters.

1- **Normal slide mounting:** For this, a drop of glycerin was placed at the center of a clean microscopic slide. The mite specimen was then transferred carefully into the glycerin drop to avoid air bubbles and to keep the specimen well immersed in the glycerin drop. The specimen was then properly oriented and a glass bristle of slightly larger size than that of the mite specimen was placed near the specimen. The glass bristle would serve to avoid the crushing of the specimen on mounting and ensure better orientation of the specimen so as to reveal the desired characters. A round cover glass of 18 mm diameter was then carefully placed over the specimen, without trapping any air bubbles.

2- **Cavity slide mounting:** This method necessitated the use of a clean cavity slide, in place of the normal glass slide discussed above and a round coverslip. The mite specimen was kept inside the cavity at the center, and then added a drop of glycerin. After properly orienting the specimen, slightly larger glass bristle was placed adjacent to the
specimen and coverslip was applied without trapping any air bubbles. Excess glycerin was wiped out with a tissue paper.

b) Permanent Mounting:

Permanent slides were prepared either by using polyvinyl alcohol or Hoyer’s medium (Baker and Wharton, 1952). The specimen was placed in the centre of a clean slide with the help of a fine Camel hairbrush. It was then aligned in the desired position using glass bristles, and covered by a coverslip. The mounted slides were kept at 50°C in an oven/incubator until the desired clarity was attained. The cover glass was sealed with commercially available nail polish, labeled properly and stored in slide boxes.

(iii). Scanning Electron Microscopy (SEM)

SEM images of selected species of mangrove dwelling oribatid mites were taken at the National Institute of Technology (NIT), Calicut by using Hitachi SU6600 Variable Pressure Field Emission Scanning Electron Microscope (FESEM).

IV. Identification of Oribatid Species

Identification of slide mounted specimens of oribatid mites was made under a Carl Zeiss research microscope by following the identification keys of Balogh and Balogh (1992 and 2002). Further, relevant literature/data on various taxa of oribatid mites were also referred for identification and
confirmation of taxonomic position of the species involved. Help from renowned oribatid taxonomists/experts outside India also was sought for confirmation of species with doubtful taxonomic status. Drawings of the various species included in the study were made with the help of a prism type Camera Lucida attached to a Unitron research microscope. Measurements of the specimens were made using an ocular micrometer and recorded in a calibrated micrometer.

Taxonomic rank and present systematic position of the various species assigned by following the most recent World Catalogue of Oribatid Mites published by Subias (2004) updated in 2015.