STUDIES ON SYNANTHROPIC SILVERFISH FAUNA (INSECTA: ZYGENTOMA) IN SELECTED AREAS OF KERALA

Thesis submitted to the University of Calicut
for the Award of the Degree of

Doctor of Philosophy in Zoology

By

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Under the Supervision of

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CERTIFICATE

This is to certify that all the corrections/suggestions (minor) recommended by the adjudicators have been incorporated in the PhD thesis of **Ms. Sheeba Raphel**, titled **“Studies on Synanthropic Silverfish Fauna (Insecta: Zygentoma) in Selected Areas of Kerala”,** and the content of the thesis in both hard copy and soft copy are one and same.

Date: Dr. Joyce Jose

Place: Thrissur (Supervisor)

CERTIFICATE

This is to certify that the thesis entitled "**Studies on Synanthropic Silverfish Fauna (Insecta: Zygentoma) in Selected Areas of Kerala**", is an authentic record of research work carried out by **Mrs. Sheeba Raphel** for the award of the Ph.D. degree of the University of Calicut**,** under my guidance. She has carried out work at the Department of Zoology, St. Thomas College (Autonomous), Thrissur, and the results in this thesis have not been included in any other thesis submitted previously for the award of any degree of any other University or institution. Also certified that the contents of the thesis have been checked using an anti-plagiarism database and no unacceptable similarity was found in the check.

Thrissur | Dr. Joyce Jose

Date: | (Research Supervisor)

DECLARATION

I, Sheeba Raphel, hereby declare that the work presented in the thesis entitled "**Studies on Synanthropic Silverfish Fauna (Insecta: Zygentoma) in Selected Areas of Kerala**" is based on original work done by me under the guidance of **Dr. Joyce Jose**, and has not been submitted previously for the award of any degree. The contents of the thesis have undergone plagiarism check using **iThenticate** software C.H.M.K Library, University of Calicut and the similarity index was found within permissible limit. I also declare that the thesis is free from AI generated contents.

Signature

Name of research Scholar: Sheeba Raphel

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Place: Thrissur

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SHEEBA RAPHEL

ABSTRACT

STUDIES ON SYNANTHROPIC SILVERFISH FAUNA (INSECTA: ZYGENTOMA) IN SELECTED AREAS OF KERALA

Silver fish are small, wingless, primitive cosmopolitan insects under the order Zygentoma. Of the, 677 species described under five families world-wide only 32 species from families Lepismatidae and Nicoletiidae have been reported from India.

Many species are synanthropic and are considered pests but silverfish are generally undocumented or poorly documented especially from Asian countries. ZSI based researchers have made substantial contributions to the knowledge of Indian Zygentoma. Based on a recent checklist published in 2024, there is no authenticated reports of silverfish from Kerala. In this context the objectives of this study are to make a checklist of synanthropic silverfish species in selected areas of Kerala; to document the morphology of synanthropic silverfish species in the study area using conventional and modern taxonomic tools; to study the behaviour of selected silverfish species and to document and characterize the gut microbiome of selected silverfish species.

Sample collection was repeatedly done from 69 sites during the period of December 2017 to March 2024, from seven randomly selected districts (Thrissur, Palakkad, Ernakulam, Kannur, Kottayam, Pathanamthitta, and Idukki) of Kerala, India. Silverfish habitus was imaged and measured using Leica S8AP0-MC180 HD, LABOMED Lx 300 compound microscope and SEM-EDAX. Identification was done following published taxonomic literature and consultation with experts. Sequencing of the COI of the species was done and compared with authenicated "DNA barcode" sequences from GenBank.

Five species belonging to family Lepismatidae (*Acrotelsa* cf *collaris*, *Thermobia smithi.* sp. nov., *Ctenolepisma rothschildi, Ctenolepisma longicaudatum* and *Ctenolepisma calvum*-neotype) were documented. This included a species new to science and a neotype. The neotype redesignation led to the reassignment of a species in Europe (*C. phantasma* sp. nov.) as a species new to science.

The behaviour of five synanthropic species of silverfish were observed in both natural and experimental set ups. Twenty unique behaviours were identified and classified into six broad categories Specialized behaviours such as "*thanatosis*" and "*cannibalism*" were documented. All the five species of silverfish showed similar types of behaviour and the major difference lay in the amount of time spent for each behaviour. Gut microbiome of four species of silverfish was documented for the first time. A total of 38 phyla belonging to 2 kingdoms - Bacteria (33) and Archaea (5) were isolated from the gut of the four silverfish species. A total of 684 species belonging to 450 genera, 272 families, 171 orders and 74 classes were documented. *Succinivibrio dextrinosolvens*, a cellulose-digesting bacterium, and many potential pathogenic bacteria were present in the gut microbiome of the silverfish. Recommendations on control of silverfish have been provided.

സംഗ്രഹം

കേരളത്തിലെ തെരഞ്ഞെടുക്കപ്പെട്ട ചില മനുഷ്യവാസസ്ഥലങ്ങളിൽ കാണപ്പെടുന്ന സിൽവർഫിഷുകളെക്കുറിച്ചുള്ള പഠനം. ഷീബ റാഫേ•

സിൽവർഫിഷുകൾ (ഇരട്ടവാലൻ) വളരെ പ്രാചീനവും എന്നാൽ സർവ്വവ്യാപകവുമായി കാണപ്പെടുന്ന ചിറകില്ലാത്ത വളരെ ചെറിയ ഷഡ്പദങ്ങളാണ്. ഇവ 'സൈജെൻന്റോമ' എന്ന ഓർഡറിൽ ഉൾപ്പെടുന്നു. ലോകത്താകമാനം അഞ്ച് ഫാമിലികളിലായി, 677 സ്പീഷിസുകളാണ് ഇതുവരെ രേഖപ്പെടുത്തിയിട്ടുള്ളത്. എന്നാൽ ഇന്ത്യയിൽ നിന്നും 'ലെപ്പിസ്മാറ്റിഡേ' അതുപോലെ 'നിക്കോലെറ്റിഡേ' എന്നീ രണ്ടു ഫാമിലികളിൽ ഉൾപ്പെട്ട 32-ഓളം സ്പീഷിസുകൾ മാത്രമാണ് ഇതുവരെ രേഖപ്പെടുത്തിയിട്ടുള്ളത്.

ചില സിൽവർഫിഷ് സ്പീഷീസുകൾ മനുഷ്യവാസം ഉള്ള ഇടങ്ങളിൽ മാത്രം കാണപ്പെടുന്നവയാണ്. സിൽവർ ഫിഷുകളെ കീടങ്ങളായിട്ടാണ് കണക്കാക്കപ്പെടുന്നത്. എന്നാൽ സിൽവർ ഫിഷിൽ ഉള്ള ഗവേഷണം ഏഷ്യൻ രാജ്യങ്ങളിൽ വളരെ കുറവാണ്. ഇന്ത്യയിൽ 'സൈജെൻന്റോമ' ഗവേഷണത്തിൽ ZSI-യിലെ ശാസ്ത്രജ്ഞരാണ് മികച്ച സംഭാവനകൾ നൽകിയിട്ടുള്ളത്.

2024-ൽ പ്രസിദ്ധീകരിച്ച ചെക്ക്ലിസ്റ്റിന്റെ അടിസ്ഥാനത്തിൽ കേരളത്തിൽ നിന്ന് സിൽവർഫിഷുകളെക്കുറിച്ചുള്ള ആധികാരികമായ പഠനങ്ങൾ ഒന്നുംതന്നെയില്ല. ഇതിന്റെ അടിസ്ഥാനത്തിൽ ഈ പഠനത്തിന്റെ പ്രധാനപ്പെട്ട ഉദ്ദേശ്യങ്ങൾ താഴെപ്പറയുന്നവയാണ്.

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2. പരമ്പരാഗതവും ആധുനികവുമായ വർഗ്ഗീകരണ രീതികൾ ഉപയോഗിച്ചുകൊണ്ട് പഠനമേഖലകളിലെ സിൽവർഫിഷുകളുടെ രൂപശാസ്ത്രപരമായ സവിശേഷതകൾ മനസ്സിലാക്കുക.
3. തെരഞ്ഞെടുക്കപ്പെട്ട ചില സിൽവർഫിഷ് സ്പീഷീസുകളുടെ പെരുമാറ്റശാസ്ത്രം പഠിക്കുക.
4. തെരഞ്ഞെടുക്കപ്പെട്ട സിൽവർഫിഷ് സ്പീഷീസുകളുടെ ദഹനേന്ദ്രിയത്തിലെ സൂക്ഷ്മജീവികളെക്കുറിച്ച് വിശകലനം ചെയ്ത് പഠിക്കുക.

പഠനത്തിനാവശ്യമായ മാതൃകാശേഖരണം നടത്തിയത് 2017 ഡിസംബർ മുതൽ 2024 മാർച്ച് വരെയുള്ള കാലഘട്ടങ്ങളിലാണ്. കേരളത്തിലെ തൃശൂർ, പാലക്കാട്, എറണാകുളം, കണ്ണൂർ, കോട്ടയം, പത്തനംതിട്ട, ഇടുക്കി എന്നീ ജില്ലകളിലെ തെരഞ്ഞെടുക്കപ്പെട്ട 69 സ്ഥലങ്ങളാണ് ഇതിനായി പലയാവർത്തി സന്ദർശിച്ചത്.

സിൽവർഫിഷിന്റെ രൂപശാസ്ത്രം പഠിക്കാനും പ്രതിച്ഛായ നിർമ്മിതി നടത്താനും ഉപയോഗിച്ചത് സ്റ്റീരിയോ സൂക്ഷ്മദർശിനിയും SEM-EDAX ഇലക്ട്രോൺ സൂക്ഷ്മദർശിനിയുമാണ്.

സ്പീഷീസുകളെ തരംതിരിച്ച് മനസ്സിലാക്കിയത് നിലവിൽ പ്രസിദ്ധീകരിച്ച നൂതന വർഗ്ഗീകരണ ശാസ്ത്രത്തിലെ പ്രസിദ്ധീകരണങ്ങളുടെയും വിദഗ്ധരുമായുള്ള ആശയവിനിമയങ്ങളുടെയും അടിസ്ഥാനത്തിലാണ്. കൂടാതെ ഓരോ സ്പീഷീസിന്റെയും COI ജീനുകളുടെ അനുക്രമണം നടത്തുകയും, അങ്ങനെ ലഭിച്ച അനുക്രമങ്ങൾ ജെൻ ബാങ്കിൽ ലഭ്യമായ വിശ്വാസയോഗ്യമായ 'ഡി എൻ എ ബാർകോഡ്' അനുക്രമങ്ങളുമായി താരതമ്യപ്പെടുത്തുകയും ചെയ്തു.

ഈ പഠനത്തിലൂടെ കേരളത്തിൽ നിന്നും 'ലെപ്പിസ്മാറ്റിഡേ' കുടുംബത്തിൽപ്പെട്ട അഞ്ച് സ്പീഷീസുകളെ കണ്ടുപിടിക്കാൻ സാധിച്ചു. ഇതിൽ ശാസ്ത്രലോകത്തിന് പുതുതായി കണ്ടെത്തിയ ഒരു സ്പീഷീസും (തെർമ്മോബയ സ്മിത്തി), ഒരു നിയോടൈപ്പും (ടീനോലെപ്പിസ്മ കാൽവം) ഉൾപ്പെടുന്നു. നിയോടൈപ്പ് പുനർനിർദ്ദേശം യൂറോപ്പിലെ ഒരു സ്പീഷീസിന്റെ പുനഃനാമകരണത്തിന് കാരണമായി.

നാല് സിൽവർഫിഷ് സ്പീഷീസുകളുടെ പെരുമാറ്റരീതികൾ പ്രകൃതിദത്തമായും, പരീക്ഷണാത്മകവുമായ സാഹചര്യങ്ങളിൽ നിരീക്ഷിക്കുകയും, പഠിക്കുകയും ചെയ്തു. ഇവരുടെ ഇരുപതോളം പെരുമാറ്റരീതികൾ മനസ്സിലാക്കുകയും, അവയെ ആറ് വിഭാഗങ്ങളായി തിരിക്കുകയും ചെയ്തു. സവിശേഷമായി 'താനാടോസിസ്', 'കാനിബാലിസം' എന്നീ പ്രത്യേക പെരുമാറ്റരീതികളും രേഖപ്പെടുത്തി. പഠനവിധേയമാക്കിയ നാലു സ്പീഷീസുകളുടേയും പെരുമാറ്റരീതികൾ സമാനതകൾ ഉള്ളതായിരുന്നു. പ്രധാന വ്യത്യാസം ഓരോ പെരുമാറ്റത്തിനും ചിലവഴിച്ച സമയത്തിലുള്ള ദൈർഘ്യം മാത്രമാണ്.

സിൽവർഫിഷുകളുടെ ദഹനേന്ദ്രിയത്തിലെ സൂക്ഷ്മജീവികളെക്കുറിച്ചുള്ള ഈ പഠനം ലോകത്തിലെ ആദ്യത്തെ വിവരണമാണ്. 'ബാക്ടീരിയ', 'ആർക്കിയ' എന്നീ കിങ്ടത്തിൽ ഉൾപ്പെട്ട 38-ഓളം 'ഫൈല' ഈ സ്പീഷീസുകളുടെ ദഹനേന്ദ്രിയത്തിൽ നിന്നും വേർത്തിരിച്ചെടുക്കാൻ സാധിച്ചു. ആകെ 450 ജെനെറ, 275 ഫാമിലികൾ, 171 ഓർഡറുകൾ, 788 ക്ലാസ്സുകൾ എന്നീ വിഭാഗങ്ങളിൽ ഉൾപ്പെട്ട 684 -ഓളം സൂക്ഷ്മജീവികളെ വേർതിരിച്ച് പഠിക്കാൻ സാധിച്ചു.

ഇക്കൂട്ടത്തിൽ 'സെല്ലുലോസ്' ദഹിപ്പിക്കാൻ കഴിവുള്ള 'സക്സിനോവൈബ്രിയോ ഡെക്സിനോസോൾവൻസ്' എന്ന ബാക്ടീരിയയും, കൂടാതെ രോഗകാരികളായ നിരവധി ബാക്ടീരിയകളും ഉൾപ്പെടുന്നു. കീടങ്ങളായി പരിഗണിക്കപ്പെടുന്ന സിൽവർഫിഷുകളെ നിയന്ത്രിക്കാനുള്ള നിർദ്ദേശങ്ങളും ഈ പഠനത്തിന്റെ ഭാഗമായി നൽകിയിട്ടുണ്ട്.

പ്രധാന വാക്കുക•: സജൈ്റോമ, ലപൈിസ്മാറ്റിഡേ, ടാക്സോണമി, പരെുമാറ്റം, ഗട്ട് മകൈ്രോബയോം

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CHAPTER 1

GENERAL INTRODUCTION

Chapter 1

General Introduction

1.1. Introduction

Insects predominate planet earth with their diversity, abundance and myriad ecological roles and precede and outnumber humans. Research on insect groups have contributed immensely to human understanding of genetics, evolution and ecology. While many insects are considered beneficial to humans, many species cause significant economic loss as agricultural pests, disease vectors and invasive species. Detailed studies of insect groups are therefore essential to utilise insects for betterment of human kind and to develop strategies to manage pestilent insects. Many insects of order Zygentoma are well known as pests but other aspects of this group are often overlooked due to their cryptic habit.

Zygentoma Börner, 1904 is a primitive order of insects falling within the apterygote, ectognathan category of the class Insecta and comprises of silverfish and firebrats. Some silverfish species are synanthropic in nature with cosmopolitan nature (Robla *et al.*, 2023). Globally, 677 species have been described under five extant families (Maindroniidae, Protrinemuridae, Tricholepidiidae, Lepismatidae and Nicoletiidae) with 151 genera (Jana *et al.*, 2024).

Zygentoma, in India is represented by 32 species which are placed under 16 genera belonging to two families (Lepismatidae and Nicoletiidae). Of these, twenty-two species under nine genera belong to the family Lepismatidae and 10 species under seven genera belong to the family Nicoletiidae. The status of two species is *incertae sedis* (Jana *et al.*, 2024). According to the checklist published by Jana *et al.*, 2024, there was no authenticated reports of Zygentoma from Kerala. Even though, there is a report of *Lepisma devadasi* (Sukumar & Livingstone, 1993) from the Palghat Gap, South India, it has been considered as *species inquirenda* (Smith, 2018).

1.2. Zygentoma Systematics: Timeline

Zygentoma, a group of insects with ancient lineages, seem to have originated as a distinct branch in the late Silurian before the appearance of insect flight in the Early

Devonian according to the molecular clocks of Misof *et al.*, (2014). Based on these molecular clocks it is further deduced that modern Zygentoma families must have appeared sometime between the Triassic to the Jurassic (~214–160 Ma) (Smith, 2018).

Extant insects are classified into two major sub-classes Apterygota and Pterygota. The sub-class Apterygota was believed to include four insect orders namely Thysanura, Diplura, Protura and Collembola (Alfred *et al.*,1998). The name Thysanura was derived from two Greek words, *thysanos*, meaning “fringed” and *ura*, meaning “tail” because of three terminal tails (Richards and Davies, 1977). Till the middle of the 20th century, the order Thysanura included two sub-orders i.e., Microcoryphia (Verhoeff 1904) or Archaeognatha (Börner,1904) and Zygentoma. The names Archaeognatha Börner, 1904 and Microcoryphia Verhoeff, 1904, were both published in the same year but most taxonomists use the name Microcoryphia (Gaju-Ricart *et al.*, 2015). In the late 20th century, the paraphyletic nature of order Thysanura was recognized and the two sub orders Microcoryphia and Zygentoma were elevated to the level of independent monophyletic orders. (Blanke *et al.*, 2014; Gullan and Cranston, 2014). Microcoryphia (jumping bristletails), was recognized as a distinct and more ancient group of apterygote insects and silverfish and firebrats were placed under the order with the ordinal name Zygentoma Börner, 1904 which replaced the deprecated name “Thysanura” (Gaju-Ricart *et al.*, 2015; Mendes, 2018). This threw up the need to revisit and revise previous knowledge on silverfish.

Currently the order Zygentoma include two suborders (Fig. 1), Archizygentoma, under which the most plesiomorphic extant family Tricholepidiidae is placed, and the Neozygentoma which includes the remaining families (Engel, 2006). Neozygentoma has two infraorders, Parazygentoma and Euzygentoma. Parazygentoma is represented by a single fossil family Lepidotrichidae under which *Lepidotrix pilifera,* discovered from amber in the Baltic region, was placed (Engel, 2006). All other families (Maindroniidae, Protrinemuridae, Lepismatidae and Nicoletiidae) placed under Euzygentoma are extant.

Earlier the family Ateluridae was treated as a separate family and Protrinemurinae as sub-family (Mendes, 1988). Subsequent phylogenetic studies (Mendes, 2002a) led to the following changes in systematic status. Atelurinae became a sub-family of Nicoletiidae and Protrinemurinae was raised to family status as Protrinemuridae. Lepismatidae is the largest family with more than 310 documented species all over the world (Smith, 2018).

1.3. General characters of Zygentoma with emphasis on Lepismatidae

Zygentoma are wingless, elongated or oval shaped insects with small and soft dorsoventrally flattened bodies with or without scales. Adults of the common silverfish normally reach a maximum length of 10-12 mm (Robinson, 2005). Body is mainly divided into head, thorax and abdomen. The head is prognathous except in Lepidotrichidae and Tricholepidiidae (Engel, 2006; Wygodzinsky, 1961), where head is hypognathous. Eyes are usually reduced or absent in most Zygentoma but Lepismatidae have compound eyes with reduced number of large, rounded ommatidia. Ocelli are present in primitive Tricholepidiidae (Blanke *et al.*, 2014). The head bears long, thin and multi-segmented antennae and five segmented maxillary palps. Maxillary palps with six segments present rarely. The head also has smooth or pectinate, often apically bifurcated macrochaetae arranged either in a single line as combs or clumped as a bush.

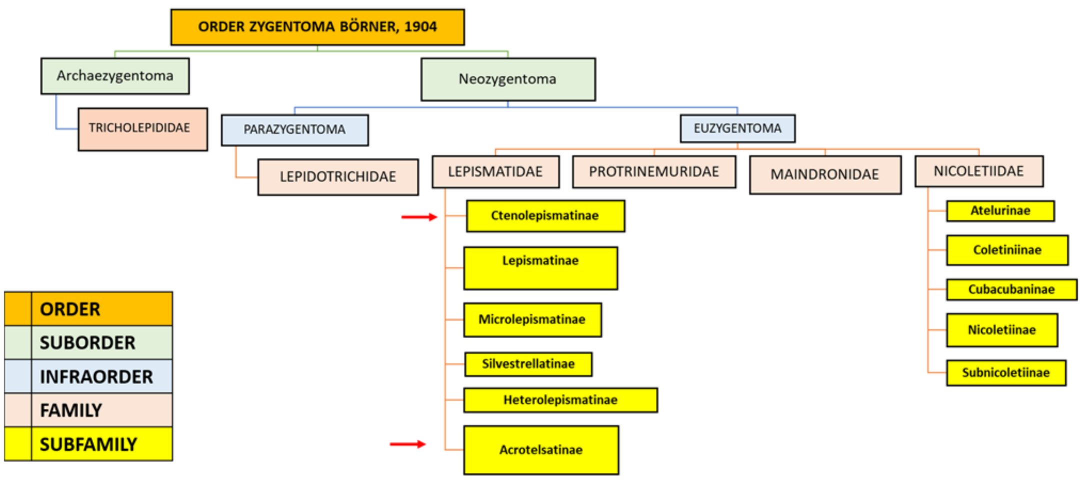


Fig. 1 Prominent taxa in Zygentoma

Thorax is well developed. Lateral margins of thoracic nota in Lepismatidae are characterized by strong combs of one to ten macrochaetae. Long, thin trichobothrial hairs, usually at anterior and posterior areas of lateral margins and 1+1 strong bristle combs in the posterior margins of the nota are characteristics of Lepsimatidae. Thoracic sterna are well developed and cover the inner margins of coxae in most Lepismatidae. The abdomen bears 10 urotergites and nine coxosternites. The posterior macrochaetae are often arranged as combs, known respectively as submedial, sublateral, lateral combs and infralateral combs based on their location. In Lepismatidae, urotergite X is presented in various shapes and can be used as a key character in taxonomy. There are usually nine urosternites. In females the urosternites VIII and IX are divided into separate free coxites, but in males only urosternite IX is divided into separate coxites.

Legs have large, strongly flattened coxae and usually bear four tarsal articles. The pretarsus usually has three claws (two outer claws and a medial empodial claw). The type of scales and arrangement of macrochaetae on coxae and tarsi are taxonomically very relevant characters for species identification.

In Lepismatidae short abdominal appendages called styli are found either on segments 7 to 9 or 8 to 9, and sometimes just on the ninth segment (Smith, 2017) and in some species these styli can be completely absent (Irish, 1986b).

The male genitalia is the penis which is composed of two articles with a circular or longitudinal opening. The penis is flanked by parameres in certain genus of Lepismatidae. The female genitalia is a pseudo-segmented ovipositor usually bearing fine simple setae. Exceptions are seen in some species of Lepismatidae where the apical articles have robust macrochaetae. The ovipositor is formed of four gonapophyses- the anterior pair arise from the urosternite VIII and the posterior pair from urosternite IX. The terminal end of abdomen possesses two lateral (anal cerci) and median caudal filament (epiproct) (Mendes, 2018). The filaments are more or less equal in length. Abdominal vesicles are absent in Lepismatidae (Kahrarian *et al.*, 2014).

Brief description of terms used above are given in Table 1 and have been represented in Fig 2 to 7.

Table 1. Description of terms used in silverfish morphology

Sl. No.	Term	Description	Fig. No.
1.	Notum (Pl. Nota)	The dorsal sclerite of the thorax	Fig. 2a
2.	Sternum (Pl. Sterna)	The ventral sclerite of the thorax or abdomen	Fig. 2b
3.	Pronotal collar	transverse field of macrochaetae arranged connecting the anterolateral corners of the pronotum	Fig. 3a
4.	Trichobothrium (Pl. Trichobothria)	long and straight fine hairs serve as mechanoreceptors.	Fig. 3a
5.	Prognathous	Type of head where the mouthparts are directed anteriorly	Fig. 3b
6.	Coxa (Pl. Coxae)	It is the first or proximal leg segment.	Fig. 4
7.	Femur (Pl. Femora)	It is the largest and stoutest part of the leg	Fig. 4
8.	Tibia (Pl. Tibiae)	Longest leg segment, with spines.	Fig. 4
9.	Tarsus (Pl. Tarsi)	Final segment of insect leg	Fig. 4
10.	Tarsal articles/ tarsomeres	Sub segments of Tarsus (usually 4 in number)	Fig. 5
11.	Pretarsus	Structures beyond the tarsus, usually with a pair of strong claws	Fig. 5
12.	Empodium	Spine between the pretarsal claws.	Fig. 5
13.	Urotergite	Dorsal surface of an abdominal segment.	Fig. 2a
14.	Urosternite	Ventral surface of the abdominal segment	Fig. 2b
15.	Coxite	Pair of plate-like structures seen in the underside of abdominal segments	Fig. 2b
16.	Comb	An arrangement of setae or macrosetae usually found on the thoracic and abdominal segments. Based on the positions, they are classified into various types. (Submedial comb, Sublateral comb, Lateral comb, Infralateral comb)	Fig. 3a
17.	Abdominal stylus (Pl: styli)	appendages inserted at both sides of the posterior margin of some abdominal sternites.	Fig.2b
18.	Parameres	Part of the external reproductive organs of male insects	Fig. 6
19.	Gonapophyses	long appendages of the eighth and ninth segments, which fuse together to form gonapophyses.	Fig. 7

1.4. Habit and Habitats

Longevity (Lindsay, 1940) and cosmopolitan distribution are characteristic features of Zygentoma. Zygentoma species are present on all over the world and have been reported even from islands around Antarctica. They utilize natural or semi-natural diverse ecological niches (Smith, 2017 and Mendes, 2018) and are also known to be synanthropic (Molero-Baltanás *et al.*, 1997a; Mendes, 2002a; Hage *et al.*, 2020; Kulma *et al.*, 2021). Most of the synanthropic species have successfully adapted to a variety of habitats worldwide (Yates,1992) and in some cases, forming commensal or parasitic relationships with ants or termites (Robla *et al.*, 2023).

Synanthropic species such as, *Ctenolepisma longicaudatum*, *C. lineatum*, and *C. villosum* in new geographic regions have shown an expansion of their range (Molero-Baltanas *et al.*, 2024) which indicates passive movement of these species through trade and tourism routes. Zygentoma undergo moulting throughout their lives, even after reaching sexual maturity (Sweetman, 1938). These insects inhabit moist, humid or dry environments, both as free-living or nest-associates (Mendes *et al.*,2009). They are able to absorb moisture directly from the atmosphere, so they can survive in dry conditions. They are fast moving insects, escaping capture with a short-lived but “explosive” burst of speed (Manton, 1972) or “thanotosis” (Smith, 2017). Jumping mechanism is absent except in Thermobia and *Ctenolepisma longicaudatum* (Sturm and Machida, 2001; Mendes, 2018). They exhibit negative phototaxis. Zygentoma engage in indirect sperm transfer during mating (Cranston and Gullan, 2009). Isolated studies have indicated the presence of specialized behaviours such as cannibalism, courtship, etc.

1.5. Challenges of Zygentoma research

The study of Zygentoma pose several challenges:

Silverfish undergo continuous moulting throughout their lives leading to significant variability, in morphology. Morphometric measurements are not absolute but taken relative to each other as the measurements alter with the growth stages of insects.

Insects belonging to the order Zygentoma generally require microscopic analysis for distinguishing species and even genera (Stach, 1946), so photo-identification is usually discouraged. Many key identifying characteristics

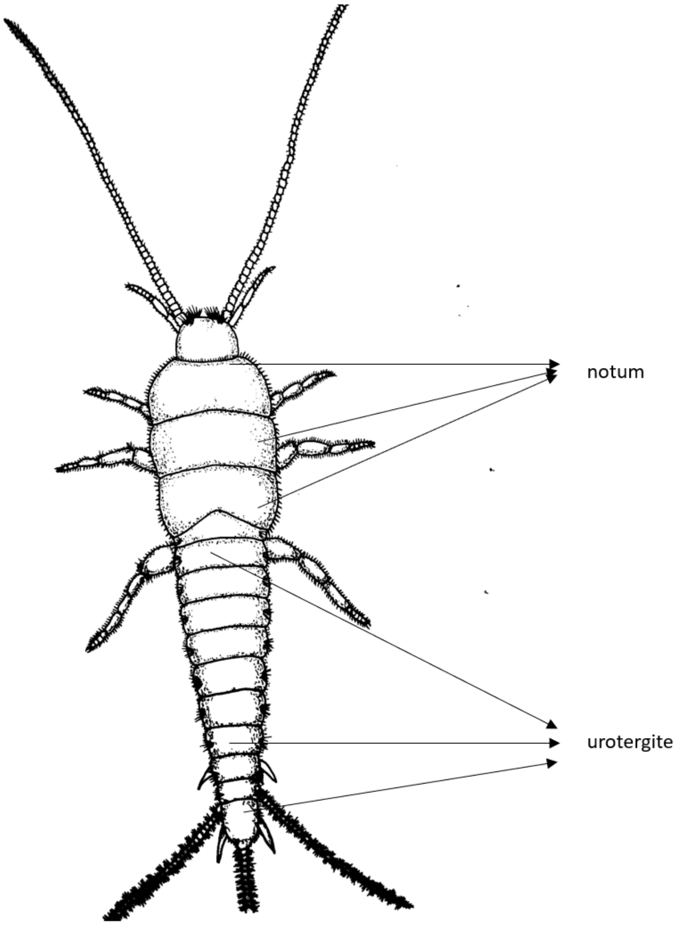


Fig. 2a. Lepismatidae (Dorsal View)

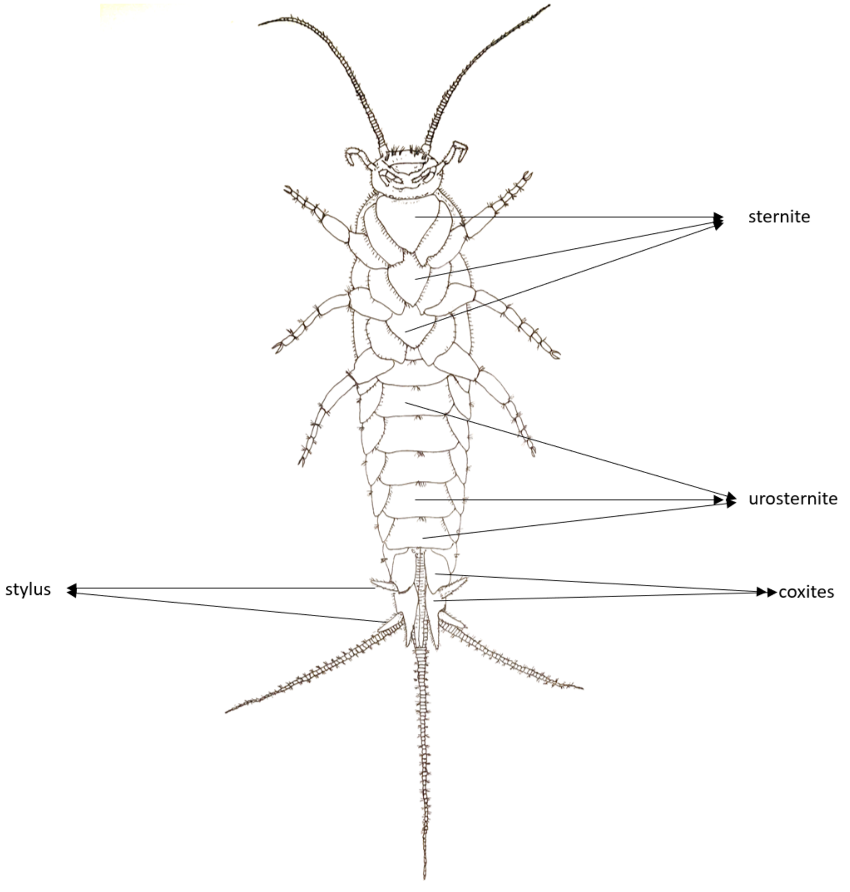


Fig. 2b. Lepismatidae (Ventral View)

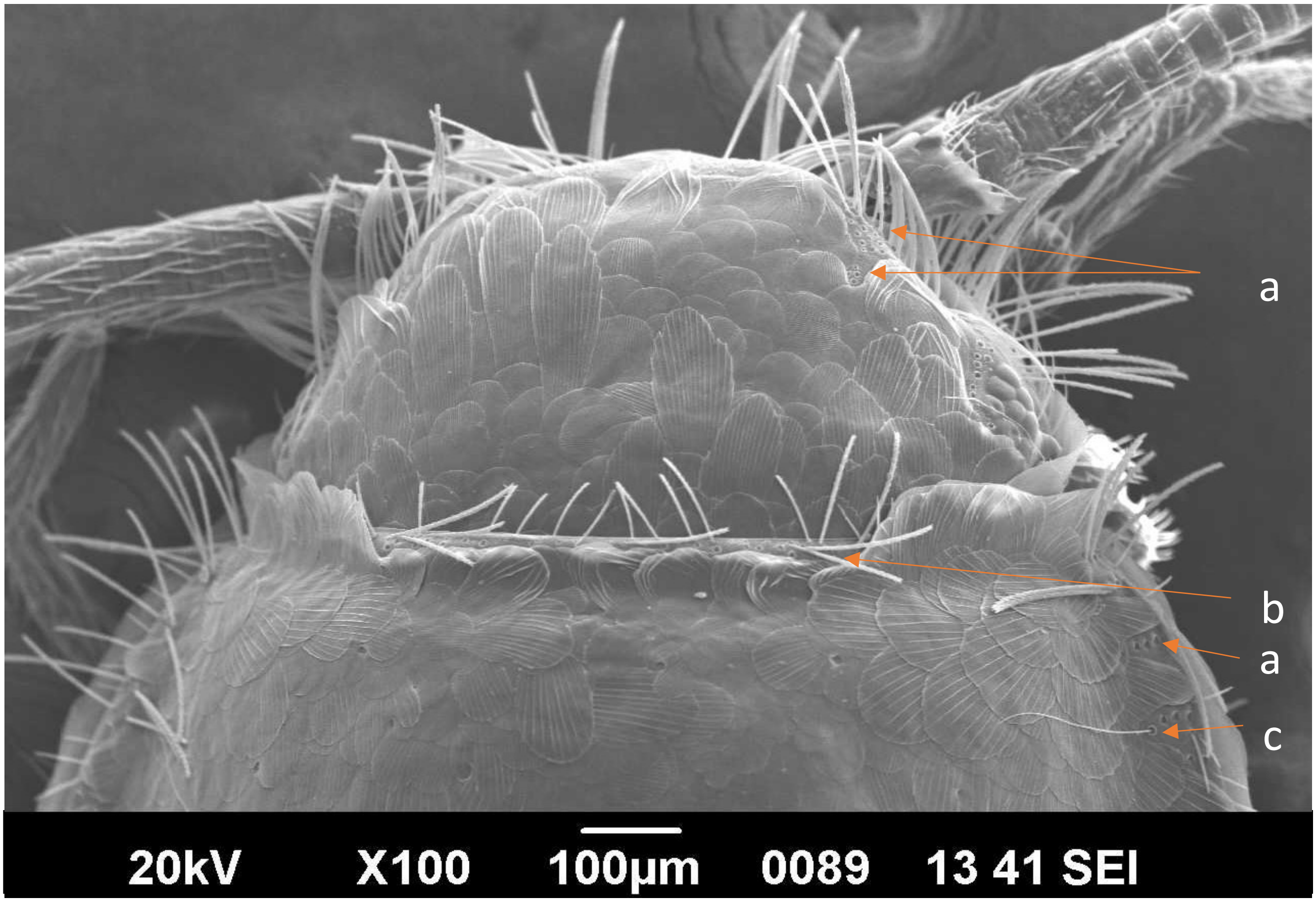


Fig. 3a. Head and part of pronotum of *C calvum* from Kerala, showing a. bristle sockets of frontal bush (right side), b. pronotal collar, c. trichobothrium at N5

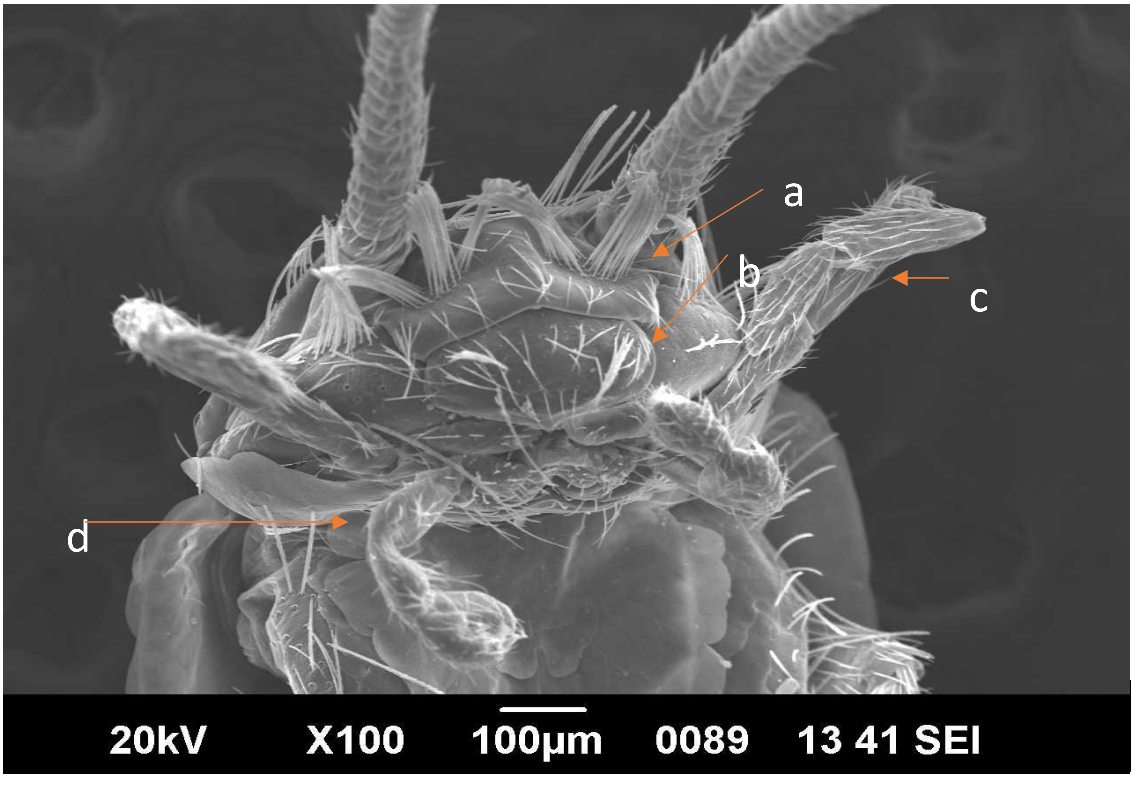


Fig.3b. Ventral view of head of of *C calvum* from Kerala showing a. setae on clypeus, b. setae on labrum, c. five segmented maxillary palp, d. four segmented labial palp

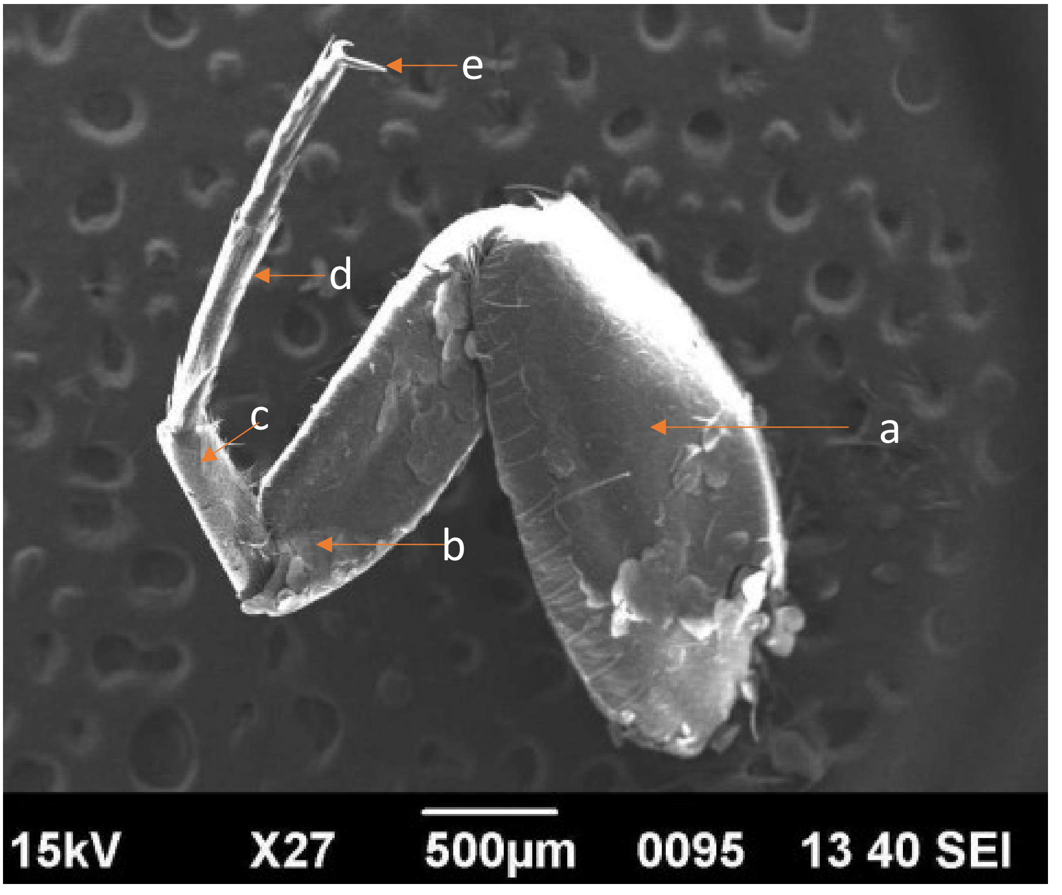


Fig. 4. Segments of the leg (a) coxa (b) femora (c) tibia (d) tarsus (e) pretartsus

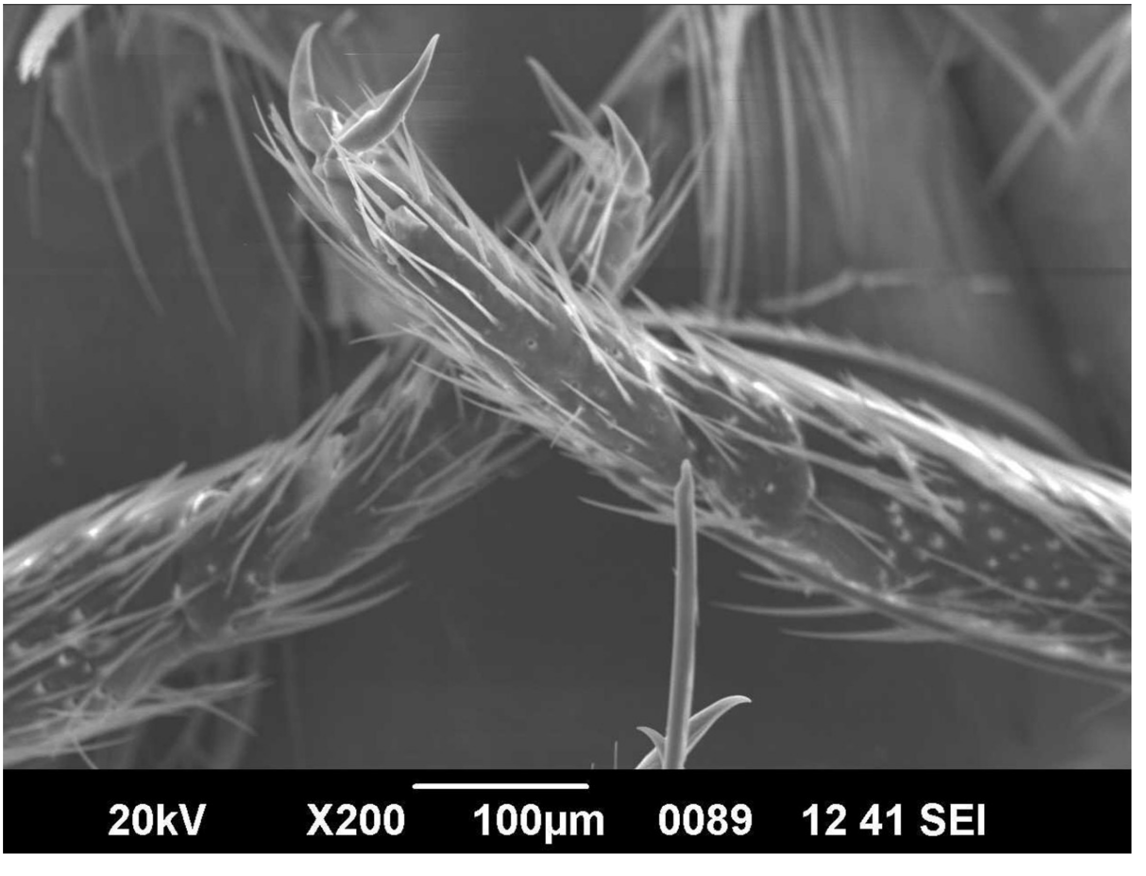


Fig. 5. Enlarged view of the tarsus, pretarsus and empodium

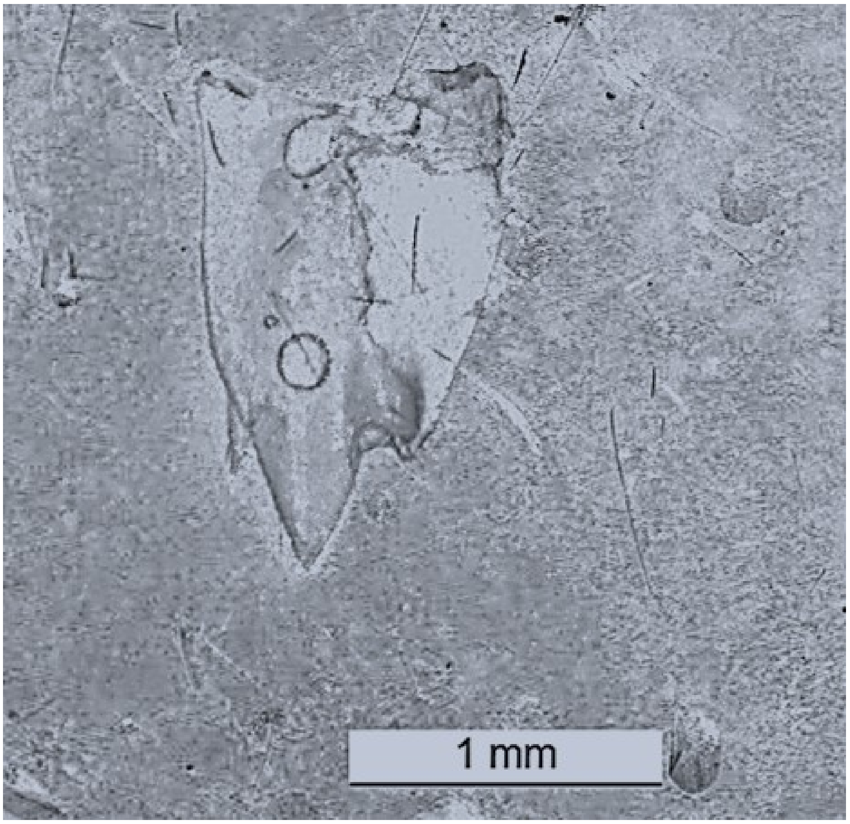


Fig. 6. Paramere

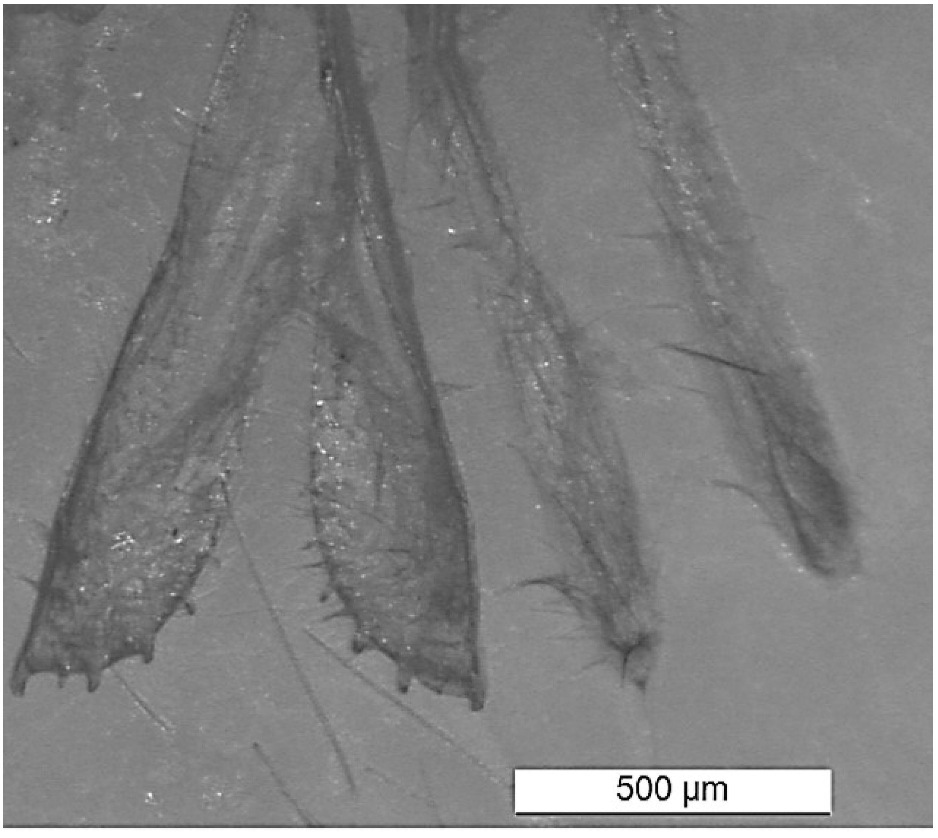


Fig. 7. Gonapophyses

(arrangement of bristle combs, position of trichobothrial areas, sensory papillae on the labial palps, divisions of ovipositor, etc.) are of microscopic nature. Dissection and microscopic observation are tedious and time consuming due to the fragile, small size of and cryptic speciation the insects. (Smith, 2017).

Advanced microscopic techniques, including scanning electron microscopy, are essential for accurately identifying these minute features. In lesser developed countries, the lack of access to such advanced equipment forces researchers to rely on available but inadequate machines, complicating the correct interpretation of species-specific characteristics.

Molero Baltanas *et al.*, (2024) mentioned that many records of silverfish were published without enough taxonomic background to ensure correct identification. They further state that the absence of Zygentoma specialists and updated identification keys leading to inaccurate descriptions have favoured the spread of many identification errors. They point out that non-specialists in this field may inadvertently describe species that are synonyms of previously described species.

There are very little reliable molecular data on silverfish available. It is difficult to extract DNA from specimens stored for more than a couple of years in 70% ethanol. So new, preferably topotypic, material would have to be collected to establish sequences on most currently described species. (Smith, 2017).

This issue is compounded by the possibility of misidentified silverfish species data being deposited in GenBank. Researchers relying on this incorrect molecular data for species identification risk further misinterpretation. The current citizen science platforms which depend on photo-based identification increase the risk of wrong identification.

The lack of regional experts for consultation and trustworthy literature for the region was another challenge.

From the Kerala context, economic and social progress has led to better sanitation, cleanliness and renovation of old buildings which led to difficulty in finding synanthropic silverfish samples from many sites. Getting permission from house/building owners to search for silverfish also posed difficulties.

1.6. Research Gaps and Significance of the study

Despite being present almost all over the world and posing significant threat as a pest, information about the geographical distribution of many synanthropic silverfish, their natural origin, and their morphological diagnosis is still imprecise or needs deep revision (Gaju-Ricart *et al.*, 2015). Silverfish are undocumented or poorly documented in many regions of the world. Another cause of gap in information is uneven research efforts where research is either focused on a single family (eg: subterranean Nicoletiidae in North and Central America) (Smith 2017) or focussed on some regions of a country (eg: India). A detailed review of literature has been presented in **section 2.2** of Chapter 2.

In India the silverfish studies and reports prior to 2010 did not follow the currently accepted rules on systematic classification and taxonomic diagnosis. According to Jana *et al.*, (2024) Zygentoma is presently represented by 32 species in India. Only 20 states and three union territories of India have records of Zygentoma. Based on the checklist published by Jana *et al.*, 2024, there is no authenticated reports of silverfish from Kerala. There are also hardly any studies from the Southern and Western part of India. Therefore, there is a lot of confusion about the distribution and diagnostic characteristics of identification. Some reasons for taxonomic confusions have also been mentioned in **1.5**. Accurate species identification is crucial for determining their actual range and implementing appropriate control measures (Molero-Baltanas *et al*., 2024).

Withing this frame of reference, this is a pioneering study of Zygentoma in Kerala using globally accepted taxonomic rules and methods for identification of species. In addition to the contributions in taxonomy, this study also presents pioneering and baseline information in the behaviour and gut microbiome of silverfish in the study area. This study will serve as a reference for further studies on Zygentoma from Kerala and India, while enriching global knowledge on this insect group.

1.7. Objectives of the study

In this context the objectives of the study are:

- To make a checklist of synanthropic silverfish species in selected areas of Kerala.

- To document the morphology of synanthropic silverfish species in the study area using conventional and modern taxonomic tools.
- To study the behaviour of selected silverfish species.
- To document and characterize the gut microbiome of selected silverfish species

1.8. Outline of the thesis

The thesis consists of six chapters; the first chapter is a general introduction of the study with its relevance. The second chapter provides a brief introduction, a review of selected literature on studies pertaining to Zygentoma taxonomy, methodology, results and discussion for objective 1 and 2. In the third chapter an overview of behaviour of synanthropic silverfish is presented. The fourth chapter presents preliminary information on gut microbiome of four species of synanthropic silverfish. The fifth chapter provides the recommendations. The sixth chapter summarizes the results and conclusions of the study. It is followed by references. Appendices are placed after references. Plates are placed after relevant chapters.

CHAPTER 2

CHECKLIST AND DESCRIPTION OF SYNANTHROPIC ZYGENTOMA FROM KERALA, INDIA.

Chapter 2

Checklist and Description of Synanthropic Zygentoma from Kerala, India

2.1. Introduction

The order Zygentoma (commonly known as silverfish) comprises of one of the earliest insect orders. Most of the species are free living and are distributed in several forms in nature as synanthropic species, as subterranean species and even as parasites or commensals in ant or termite colony (Kulma *et al.*, 2022; Mendes, 1987; Molero-Baltanas *et al.* 2022). Of the 677 species of Zygentoma reported from all over the world 32 species under 16 genera grouped in two families are from India (Jana *et al.*, 2024). Lepismatidae Latreille is a family of primitive wingless insects, generally known as "silverfish," that includes around 43 genera and over 310 documented species distributed among six subfamilies: Acrotelsatinae Mendes, 1991; Ctenolepismatinae Mendes, 1991; Heterolepismatinae Mendes, 1991; Lepismatinae Mendes, 1991; Mirolepismatinae Mendes, 1991, and Silvestrellinae Mendes, 1991 (Smith, 2018).

In India, the family Lepismatidae is represented by 22 species from which 12 belong to genus Ctenolepisma, 3 of Acrotelsella and one each of Acrotelsa, Afrolepisma, Lepisma, Tricholepisma, Xenolepisma, Thermobia and Silvestrella.

As discussed in the previous chapter research on Zygentoma is patchy and has many gaps. The following section provides a brief overview of Zygentoma, research at global and national level with special focus on Lepismatidae.

2.2. A Review of Taxonomic Studies in Zygentoma with Emphasis on Lepismatidae

The pioneers of research in Zygentoma were Karl Escherich (1905, 1906) Silvestri (1911, 1913, 1936, 1938, 1948) and Wygodzinsky (1942, 1944, 1952, 1963, 1972) who each described over 100 new species. Luis Mendes starting from the 1980s described an additional 130 new species and established a coherent suprageneric classification. Of the five extant families, only Lepismatidae and Nicoletiidae each have around 300 described species globally (Smith, 2017). Other early seminal contributors include Oudemans (1890), Folsom (1923), Dufour (1931), Slabaugh (1940), Janetschek (1957), Swan (1961), Bitsch (1968), Paclt (1961, 1967, 1969), and Lefroy (1990).

In 2015, Gaju Ricart *et al.*, reported the dissolution of Thysanura and upgrading of Zygentoma to order level. Following the systematic revision, in recent times many groundbreaking publications have come out from around the globe.

In the African continent majority of the research on Zygentoma and Lepismatidae was led by John Irish who published many taxa new to science, and systematic revisions (Irish, 1986a, 1986b, 1987, 1988a, 1988b, 1989, 1995, 1996a, 1996b). After the systematic revision of silverfish (Gaju Ricart *et al.*, 2015) and Irish (2018) provided a summary of Lepismatidae known from Southern Africa, detailing 78 described and several undescribed species, along with keys to genera and species level, distribution patterns, and host associations of various nidicolous Lepismatidae.

In Europe Molero-Baltanás *et al.*, (1994, 1995a, 1995b, 1997b, 2000, 2002, 2005,2010, 2012, 2013, 2017a, 2017b, 2018, 2022, 2024a, 2024b, 2025), Goddard *et al.*, (2016) Tahami *et al.* (2018), Aak *et al.*, (2019, 2021), Kaplin (2019), Kulma *et al.*, (2018, 2021, 2022), Kaplin and Shakula (2022), Querner *et al.*, (2022), Robla *et al.*, (2023) and Bednar *et al.*, (2023) have contributed significantly to the information about Zygentoma.

In Australia Smith (2006, 2012, 2013, 2014, 2015a, 2015b, 2015c, 2015d, 2016, 2017, 2018), Smith *et al.*, (2011, 2019, 2020, 2021, 2022) Smith and Mitchell (2019, 2021, 2022, 2024) and Smith and McRae (2014, 2016) have been the seminal publications on Zygentoma.

Studies on Zygentoma from Asia are few and mainly concentrated in a few countries like Iran, Japan and India. In addition to the early works of Mendes (1992, 2002a, 2002b, 2018) which included parts of India, Mendes and Wunderlich (2013) reported a new Lepismatidae, *Cretolepisma kachinicum,* preserved in Burmese amber from Myanmar. Kahrarian *et al.*, (2016) provided an overview of the genus Ctenolepisma (Zygentoma: Lepismatidae) in Western Iran and described three new species. Research in Japan has not focussed much on taxonomy.

However, compared to the global fauna, very little is known about these insects in India. Escherich initially identified a thysanuran, *Lepisma indica* from India in 1903 which was later considered as *species inquirenda* by Mendes. A year later, *Lepisma* (Acrotelsa) *collaris* Fabricius, was described from Calcutta. Escherich described three new species from India in 1906; with no note of the collection place. Silvestri (1911, 1913, 1936, 1938 and 1948) contributed a new genus of Machilidae and 10 new species from the families Machilidae, Nicoletiidae, and Lepismatidae from India. Dover, 1922 described three species from the coast of Barkuda Island and Chilka in Orrisa, and contributed to the families Lepismatidae and Machilidae.

Wygodzinsky (1944, 1952, 1954, 1957, 1962 and 1974) recorded a new genus of the family Machilidae, two species of Lepisma from Sikkim and Manipur. He also described five new species of the family Machilidae from the northwest Himalayas. Joseph and Mathad (1963) described a new genus of Ateluridae. Mendes (1990) published a paper on the zoogeographic affinities of Indian Thysanura, and listed out 28 species of Thysanura (eleven species of Microcoryphia and seventeen species of Zygentoma) from India.

Hazra (1980, 1993,1996), Hazra and Mandal (2010), Hazra *et al.*, (1999, 2000, 2003, 2004a, 2004b, 2007, 2012) made substantial contributions to the knowledge of Indian Zygentoma. Subsequently, Hazra and Mandal (2007) published "A Pictorial Handbook of Indian Thysanura". A species, *Lepisma devadasi* was described by Sukumar and Livingstone (1993) which was later considered as *species inquirenda* (Smith, 2018).

After a gap, a checklist of Indian Thysanura was published by Hazra *et al.*, (2020a) based on globally accepted taxonomy of Zygentoma. Additionally, Hazra *et al.*, (2020b) recorded *Ctenolepisma alticola* Silvestri, 1935 and *Ctenolepisma boettgerianum* Paclt 1961 from the states Chhattisgarh, Gujarat, Madhya Pradesh and Jharkhand of India for the first time. This was followed by new reports following modern classification, new taxa and checklists of Zygentoma in India (Hazra *et al.*, 2022a, 2022b, 2023a, 2023b, 2024a, 2024b, Jana and Hazra 2023, Jana *et al.*, 2023, 2024). A new species *Thermobia smithi* from Kerala (Raphel *et al.*, 2024) and new reports of Ctenolepisma species from Kerala (Raphel *et al.*, 2025) will be discussed in detail in the following sections of this chapter.

2.3. Methodology

2.3.1. Study Area

The studies were conducted in randomly selected sites in Kerala (Appendix.1). Kerala nestles between the Arabian Sea and the Western Ghats in the southern-most part of India. It lies between 8°18' and 12°48' North and 74°52' and 72°22' East. Kerala 's coastline runs for some 580 km and has an average width of around 90 km. Its width ranges between 35 to 120 km. Based on geography and climate there, three distinct zones: the eastern highlands, the central midlands and the coastal lowlands have been noted. In addition to forty-four rivers (three east flowing), many ponds and reservoirs, paddy field networks and backwaters make up its inland waterbodies. The Western Ghats a chain of mountains exerts orographic control over both the climatic and vegetational characteristics of the state. Kerala is the third most densely populated state in India with 859 persons per square kilometre.

Due to its position in the humid equatorial tropics an average of 120-140 rainy days per from two monsoons and a premonsoon rainy seasons contribute to a mean annual rainfall of 3,107 mm. The lowest mean annual rainfall is around 1,250 mm and the highest is around 5,000 mm. Daily average temperature range is between 36.7 °C and 19.8 °C (Source of facts: https://www.stateofkerala.in).

Kerala has consistently ranked high based on Human Development Index (HDI) and has scored above 0.75 over the last years. In 2025 the HDI of Kerala was 0.79 which was the sixth highest in India. The HDI is calculated based on sanitation, health, education and poverty reduction.

Based on Census data 92.8% urban growth rate was recorded in Kerala, mostly in Tier III towns which was not accompanied by equal pace of development, in basic amenities (Praveen and Nair, 2018). Urbanization was marked by changes in land use with large proportion of paddy fields and home gardens being converted into small-scale plantations of coconut and rubber which are latter converted to pineapple, spices and finally residential or urban centres (Kumar and Nair, 2004). Thus, residential and service buildings are often found in close vicinity of cultivated land.

Similarly, Kerala’s traditional architectural styles which included *Ettukettu*, *Nalukettu*, *Eksala*, and *Cheri* gradually transformed under the influence of cultural, climatic, and technological changes. In addition to main living areas, outhouses such as *padipura*, *adukala*, *uralupura*, etc which were storage and work areas, were also placed in close vicinity of the houses. These buildings were distinguished by sloping tiled roofs and wooden pillars and were ideally adapted to Kerala's humid environment (Koduveliparambil, 1997).

Mud, laterite, granite stone blocks, lime mortar, wood, bamboo, clay roofing tile, and coconut palm leaves were some of the most prevalent materials used in traditional Kerala architecture (Gupta & Jameel, 2020; Nair *et al*., 2022). Simple palm leaf homes were also typical in rural areas which were slowly replaced by roofing tiled homes with laterite or stone walls as the socio- economic conditions improved. Urbanization and other changes led to the introduction of first the “one-story concrete buildings” followed by multistoried concrete buildings which were more durable and required less maintenance. The drastic change on residential architecture was also reflected in office and institutional buildings where traditional wooden and laterite structures were replaced by reinforced concrete, steel, and glass structures.

Common types of houses and storage areas in Kerala are depicted in Plate I. The discussion of architecture in the study area has been provided because they are the major habitat of synanthropic silverfish.

2.3.2. Collection

The collection of samples was carried out during the period of December 2017 to March 2024, from seven randomly selected districts of Kerala, India (Thrissur, Palakkad, Ernakulam, Kannur, Kottayam, Pathanamthitta, and Idukki) (Fig. 8).

Sixty-nine sites (Appendix I) which included houses, government offices, libraries, storerooms, out houses, storage areas, etc., were visited many times and samples were collected (Plate II). The sites listed in Appendix I do not include the places which were visited but did not have even one silverfish. Such places were not visited again.

Live silverfish were collected following Thomsen *et al.*, (2019) and “paper strip method” (Raphel *et al.*, 2024). The paper strip method was developed as part of this study (thick paper strips were held in front of the silverfishes' path causing them to climb upon these stripes and immediately transferred to wide-mouthed plastic containers). The containers with live insects were carried to the laboratory, sorted and some samples were preserved in plastic vials with 99% ethanol for further studies. Other specimens were transferred to culture boxes.

2.3.3. Procedure of slide preparation and imaging for taxonomic study

Equipment required: Stereo microscope, microscope, needles, brushes, petri dish, glass slides and cover slips.

Chemicals required: Glacial acetic acid, absolute alcohol, purified or distilled water, fuchsin stain powder, Canada balsam, xylene.

Sacrificing Silverfish for dissection: Insects were kept in a 4 °C freezer (causing death after customizing the method suggested by Piou *et al.*, (2022). The insects were then placed in a petri dish; dust particles were removed by gently wiping its body with a soft brush dipped in alcohol. The silverfish to be dissected was placed on a clean slide and kept under the stereomicroscope (Leica S8AP0-MC170 HD Stereomicroscope). Measurements of external body parts (whole body length, length of all terminal appendages, width and length of the head, thorax and abdomen etc) were taken using the facility in the microscope using LAS software.

Dissection method: Dissection and observation was carried out following Smith (2013) and Molero Baltanas *et al.*, (2024). Proper lighting is very essential for dissection and if the lighting is aligned along the length of the body rather than shining on the side of the insect the minute characters would be better discerned. As the study of silverfish is not common detailed steps followed in this study are given in Appendix **III** for further reference.

Imaging: Silverfish habitus was imaged with LEICA MC170 HD stereomicroscope and measurements were taken with LAS software. Imaging was also done with LABOMED Lx 300 compound microscope and SEM-EDAX: (Jeol 639oLA/OXFORD XMX N) at STIC, CUSAT. Appropriate drawings were made using microscope and a drawing tool.

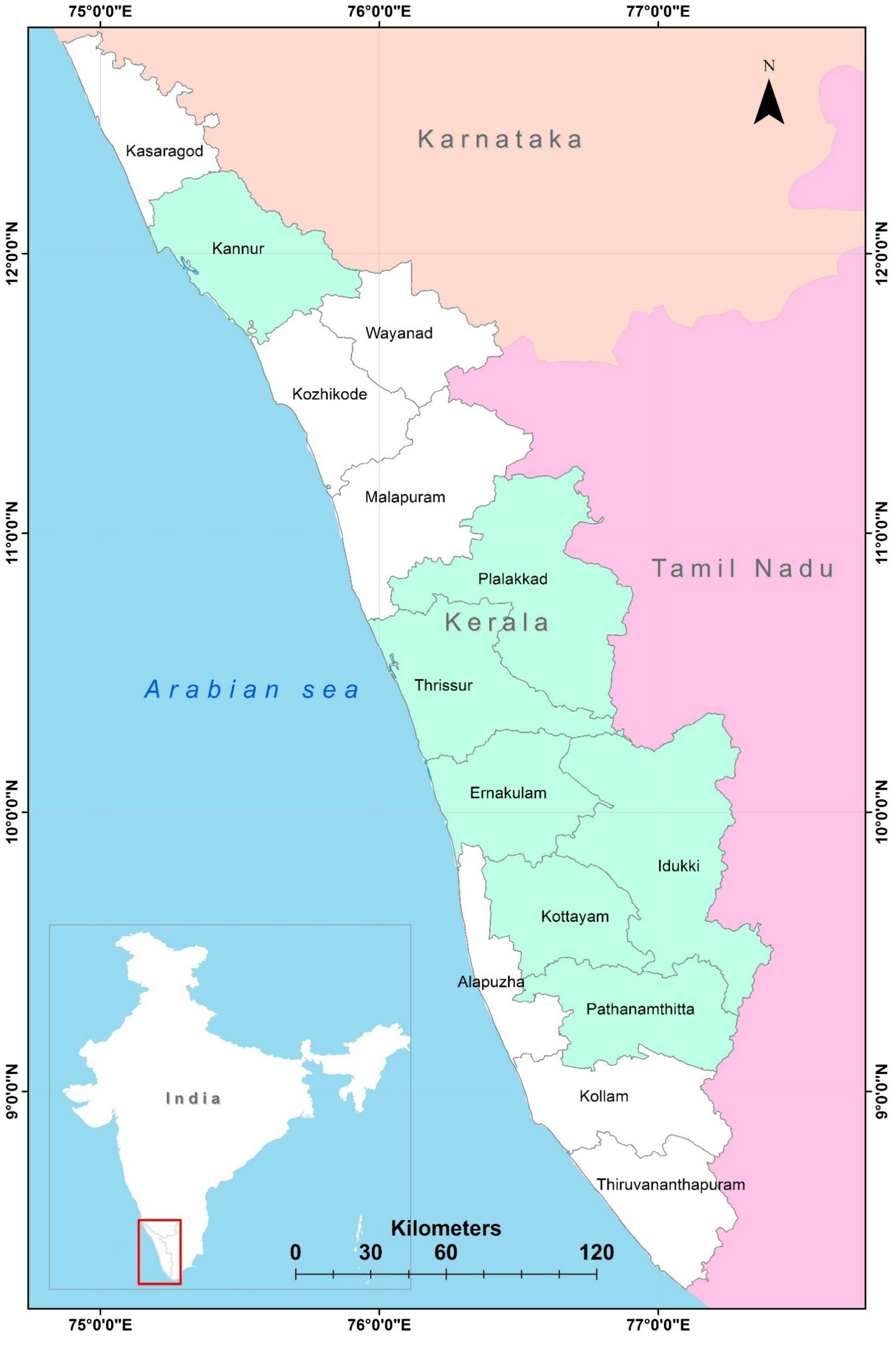


Fig. 8. Map of Study Area- districts selected for sampling are marked in green

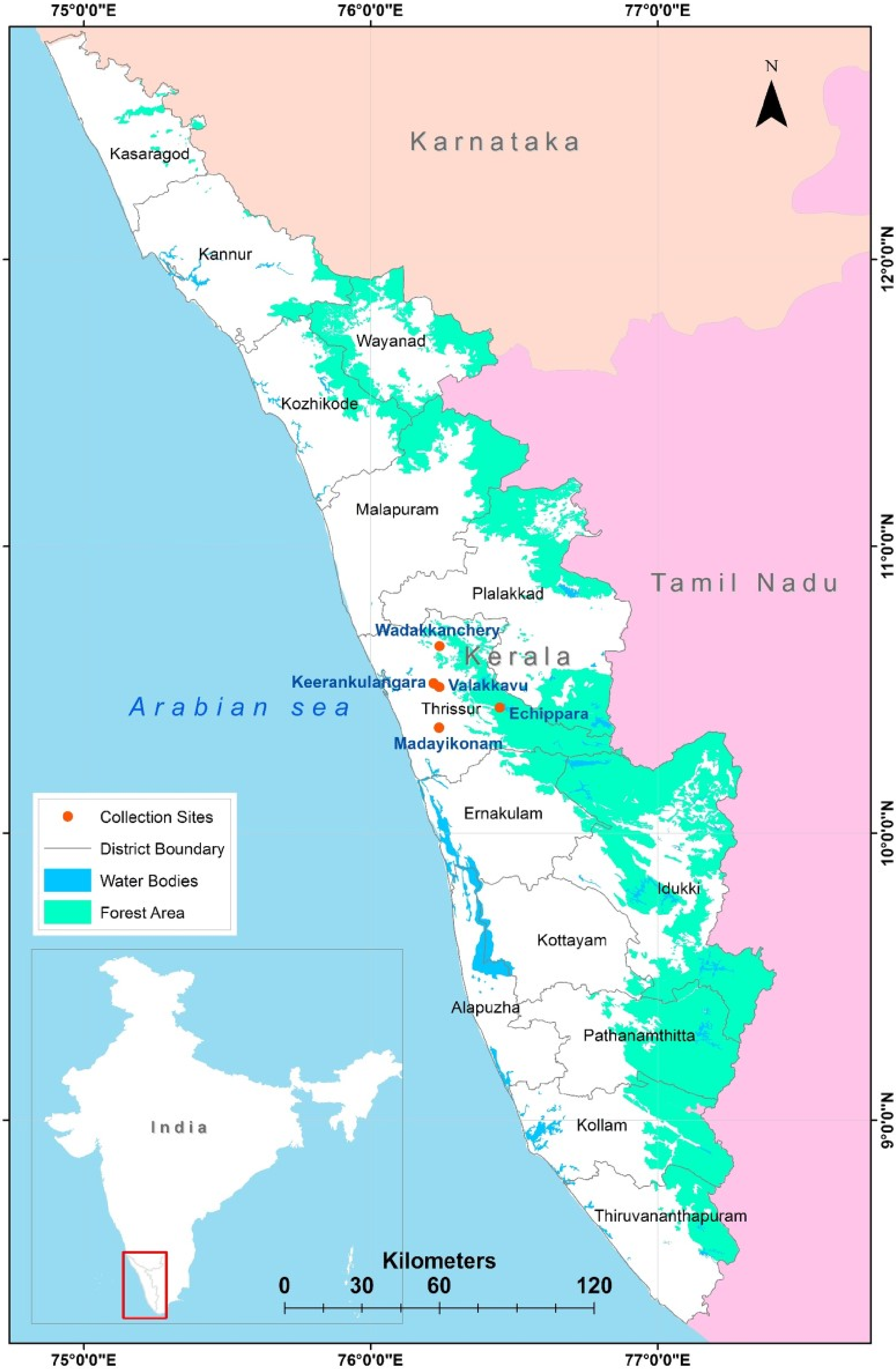


Fig. 9. Collection sites of *C. calvum*

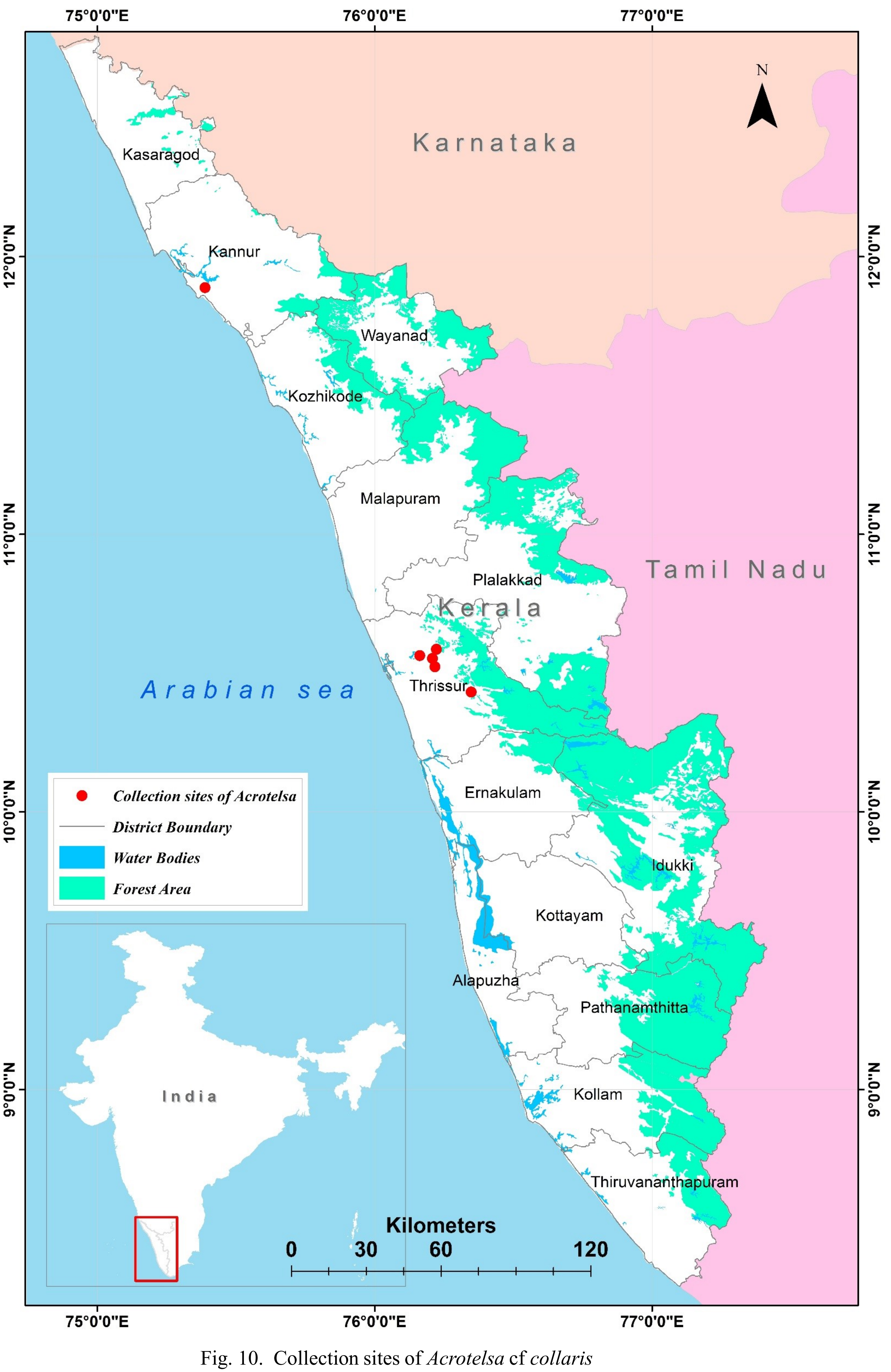


Fig. 10. Collection sites of *Acrotelsa* cf *collaris*

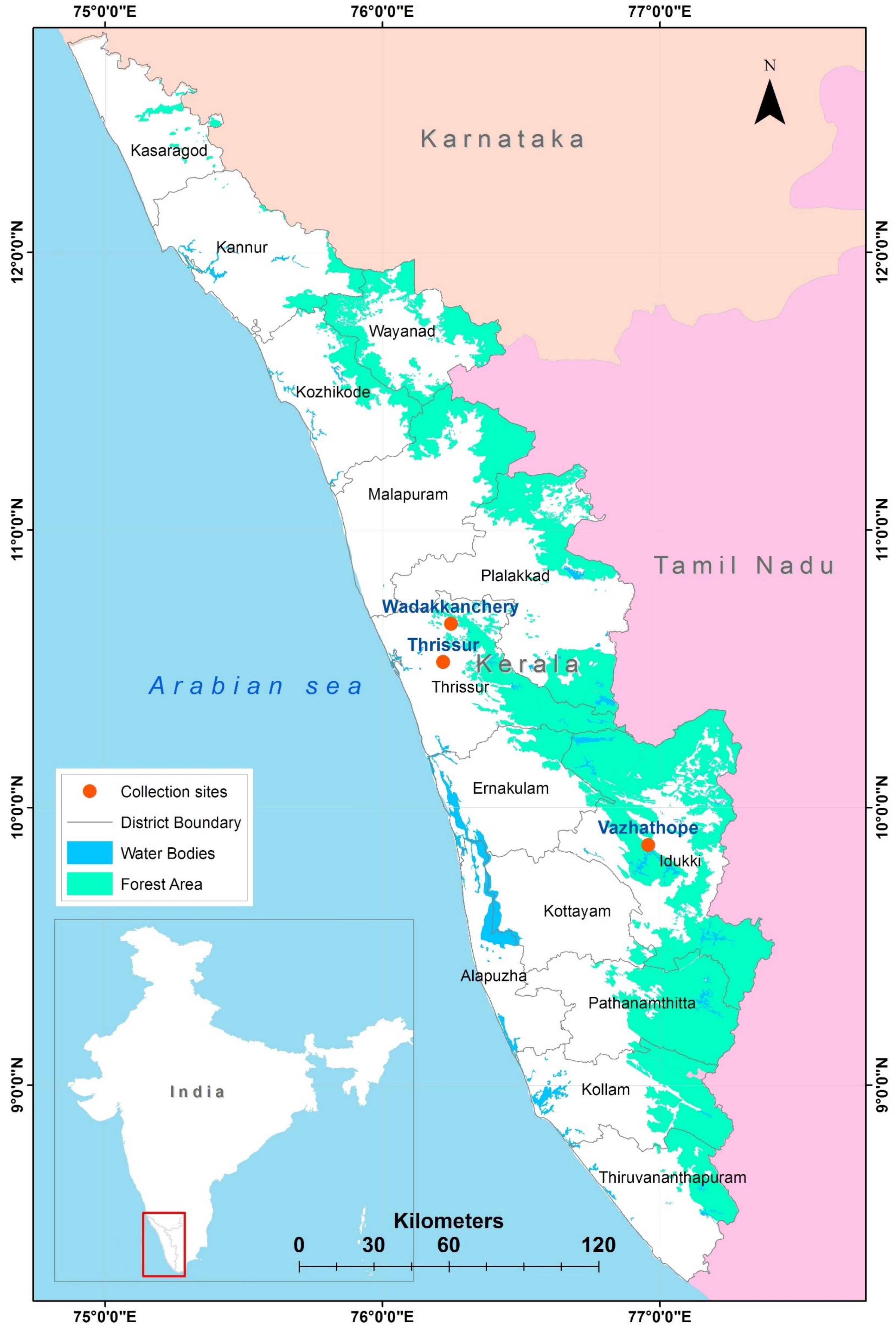


Fig. 11. Collection sites of *Thermobia smithi*

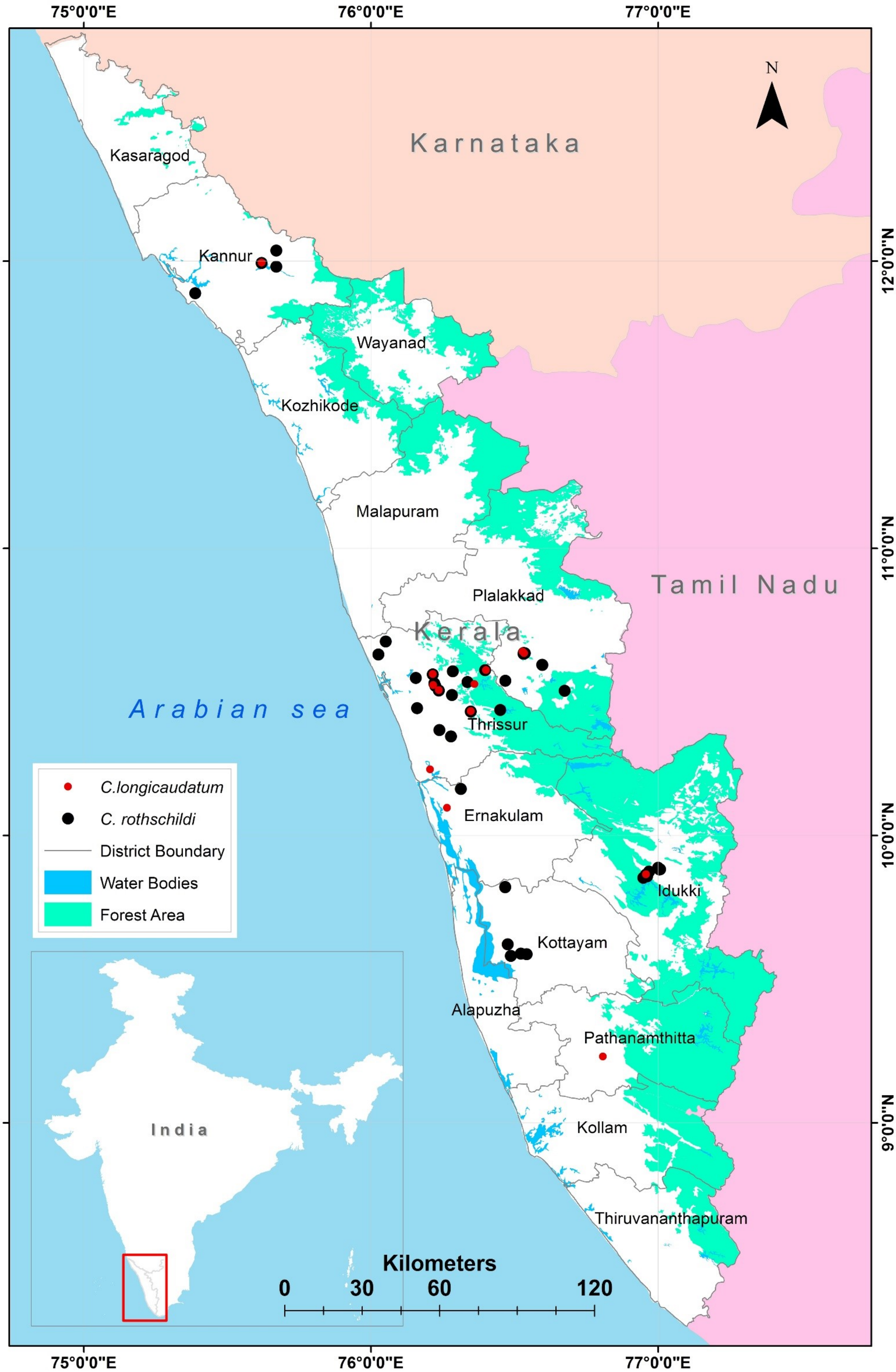


Fig. 12. Collection sites of *C. longicaudatum* and *C. rothschildi*

PLATE 1-Common types of houses and storage areas in Kerala

Sample photos of tile roofed houses

PLATE 1-Common types of houses and storage areas in Kerala

Thatched roofed buildings and storages areas are good habitats for silverfish.

PLATE 1-Common types of houses and storage areas in Kerala

Modern houses often have a lesser degree of silverfish infestations unless there is no periodic cleaning.

PLATE 1-Common types of houses and storage areas in Kerala

Government offices and archives often house large repositories of files and papers and are a good habitat for silverfish.

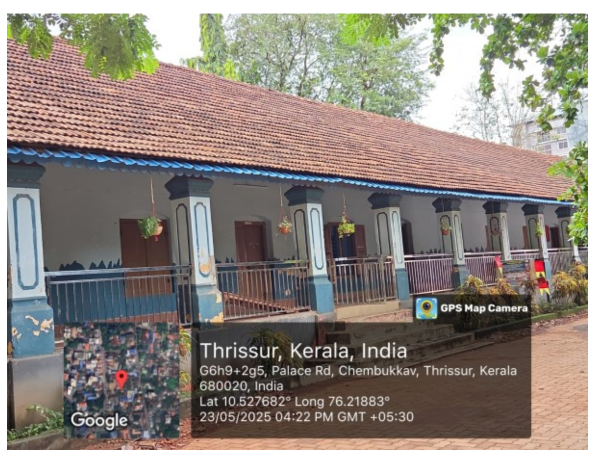
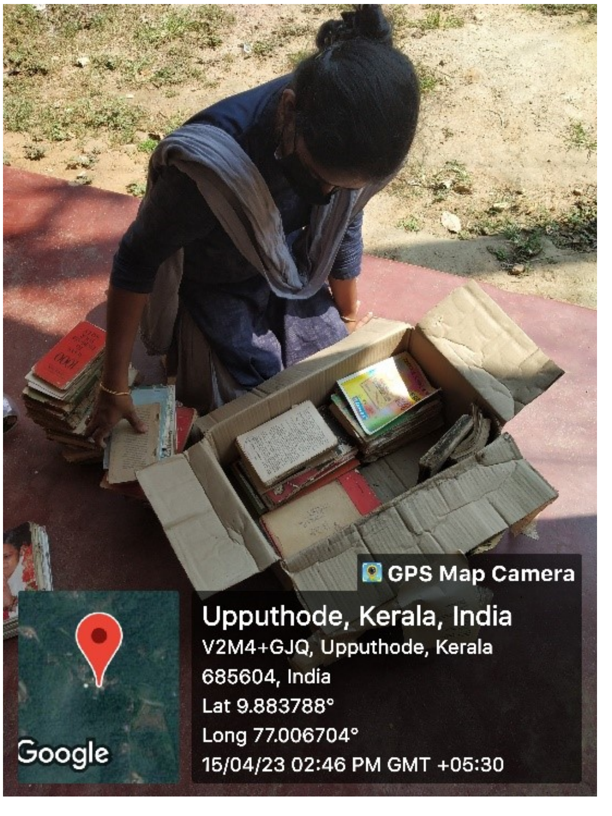
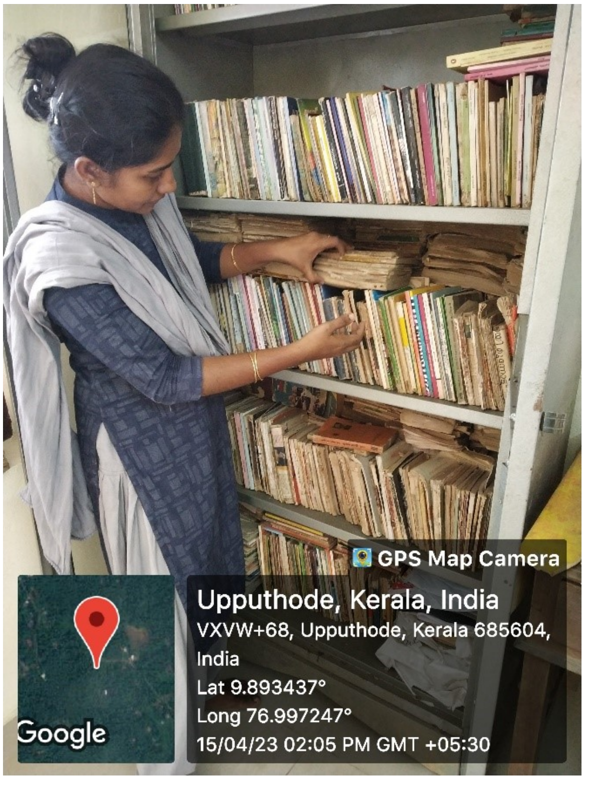


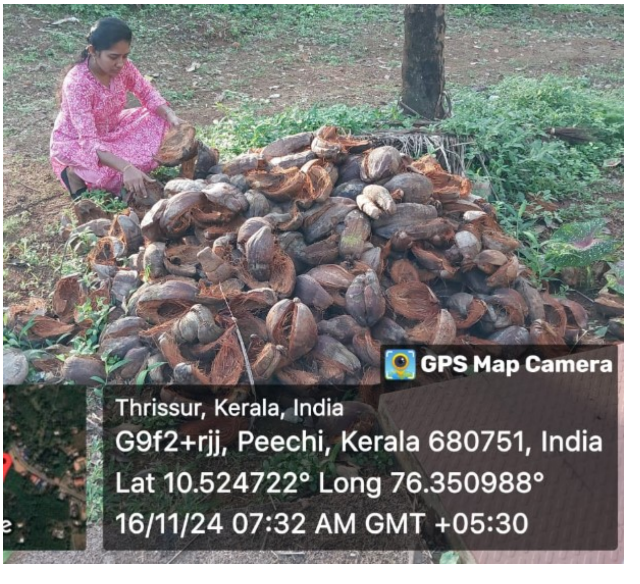
PLATE 1I- Study Sites



Areas adjoining houses and storage areas often have synanthropic silverfish infestations.

PLATE 1I- Study Sites





Areas adjoining houses and storage areas often have synanthropic silverfish infestations.

Procedure followed for SEM imaging

SEM imaging facility in CUSAT (Cochin University of Science and Technology) was utilized. Specimens were prepared for SEM imaging using the following steps. The specimens were fixed using 2% glutaraldehyde at 4 °C for 24 hours. To remove excess fixative and other impurities, the fixed specimens were washed in three changes of Cold Phosphate-Buffered Saline (PBS) at room temperature for 1min each. The specimens were then subjected to a series of ethanol solutions with increasing concentrations (30%, 50%, 70%, 90%, and twice with 100% ethanol) for 3 mins at each concentration. The sample immersed in absolute alcohol was sent to CUSAT for further processing and mounting.

2.3.4. Identification and classification:

The identification of species was done using the diagnostic characters following (Smith, 2013b, 2017; Smith *et al.*, 2019, Molero-Baltanás *et al.*, 2024a and 2024b) are mentioned in Appendix II and Table 1 of the previous chapter. Major published taxonomic literature (Ritter,1910; Smith, 2012, 2013, 2017; Smith, *et al.*, 2019; Molero-Baltanás *et al.*, 2000, 2024a, 2024b, Hazra, *et al.*, 2022a, 2022b) was referred. Wygodzinsky (1972), Irish (1988), Aak *et al.*, (2019), Kulma *et al.*, (2018, 2021 & 2022), Querner *et al.*, (2022), Bednar *et al.*, (2023) were also referred in addition to many descriptions mentioned in review of literature.

2.3.5. COI extraction, isolation and sequencing:

DNA was extracted from the body of the insect. Genomic DNA was isolated from the tissues using NucleoSpin® Tissue Kit (Macherey-Nagel) following manufacturer's instructions. DNA quality was checked using agarose gel electrophoresis. The gels were visualized in a UV transilluminator (Genei) and the image was captured under UV light using Gel documentation system (Bio-Rad). PCR amplification was carried out in a PCR thermal cycler (GeneAmp PCR System 9700, Applied Biosystems). Agarose gel electrophoresis of the PCR products was done following standard protocols and visualized using UV transilluminator (Genei) and the image was captured under UV light using Gel documentation system (Bio-Rad). ExoSAP-IT Treatment (GE Healthcare) was done to remove unwanted primers and dNTPs. Sequencing reaction was done in a PCR thermal cycler (GeneAmp PCR System

9700, Applied Biosystems) using BigDye Terminator v3.1 Cycle sequencing Kit (Applied Biosystems, USA) following kit instructions. The sequence quality was checked using Sequence Scanner Software v1 (Applied Biosystems). Forward and reverse direction sequence trace files for two samples were assembled using Geneious Prime 2022.2.1 (Kearse *et al.*, 2012) and Geneious Prov5.1 (Drummond *et al.*, 2010) and consensus sequences, sequence trace files, and specimen collection data were uploaded to BOLD (www.boldsystems.org) and submitted at GenBank.

Sequencing of the cytochrome oxidase I gene of the species was done at the RGCB, Trivandrum. Samples of the same species were sequenced at Mediomix Diagnosis & Bioresearch Lab, Bengaluru and Enfys Life Sciences, Ernakulam to ensure that there was no error in the molecular data. Folmer's forward and reverse primers, LCO1490 and HCO2198 respectively (Folmer *et al.*, 1994) (Table 2) were used.

Table 2. Primers used for COI sequencing

Target	Primer Name	Direction	Sequence (5' - 3')
COX1	LCO1490	Forward	GGTCAACAAATCA TAAAGATATTGG
	HCO2198	Reverse	TAAACTTCAGGGT GACCAAAA AATCA

2.3.6. Constructing genera level phylogenetic trees:

"DNA barcode" (i.e., mitochondrial COI gene) sequences from GenBank were downloaded (For Genus Thermobia: seven for Thermobia species, and one sequence for *Lepisma saccharinum* to serve as the outgroup. For Genus Ctenolepisma: ten DNA barcodes were downloaded (Table 3). All sequences used were publicly available and determined in a recent review of the world fauna of synanthropic silverfish (Molero-Baltanás *et al*. 2024) to be correctly identified. In addition to the sequences used in this study, there are other publicly available sequences on GenBank and BOLD for the species considered in this study. However, Molero-Baltanás *et al.*, (2024) determined that they had been misidentified at the level of genus or species, therefore those sequences were excluded from this study.

Sequences were aligned using MUSCLE (Edgar, 2004). The DNA sequence alignment was trimmed to 645 (Thermobia) and 646 (Ctenolepisma) base pairs to minimize missing data. MEGA v.11.0.13 (Tamura *et al.*, 2021) was used to calculate uncorrected DNA distances ("p-distances") and to test models of sequence evolution for phylogenetic analysis. The General Time Reversible model with Gamma-distributed rates (GTR+G) had both the lowest Bayesian Information Criterion (BIC) score and the lowest Akaike Information Criterion, corrected (AICc) score, and was selected for the analysis.

Phylogenetic analyses were performed using Maximum Likelihood (ML) as implemented in RAxML 8.2.11 (Stamatakis, 2014) and Bayesian Inference (BI) in MrBayes v.3.2.6 (Ronquist *et al.*, 2012) using the plugins available in Geneious. RAxML analyses used the ML search convergence criterion and performed 1,000 fast bootstrap replicates. MrBayes analyses used four heated chains with chain temperature=0.2, a chain length of 1,000,000 generations, subsampling every 500 generations, and a burnin length of 500 samples (25%).

For *Acrotelsa* cf *collaris* phylogenetic tree was not constructed due to the following reasons. There is some ambiguity about the identity of our specimen as will be discussed in the result section. There are no confirmed COI sequences submitted to public databases for comparison. There is only one species reported from the genus all over the world and consultation with global experts indicate that the genus may need a systematic revision.

Table 3. Details of COI sequences downloaded from public databases and details of COI sequences generated as part of this study

Sl. No.	**Accession Number**	**Species**	**Country**	**Authors**	**Base pair length**
1	@OR800371	*C. longicaudatum*	India	Raphel & Jose, 2023	300
2	@ZGYSTCSR24iv/*TBA/	*C. calvum*	India	Raphel *et al.*, In press	646
3.	@ZGYSTCSR24v/*TBA/	*C. calvum*	India	Raphel *et al.*, In press	646
4.	@OR800372	*C.rothschildi*	India	Raphel & Jose, 2023	480
5.	OR732093	*C. lineatum*	Spain	Molero-Baltanas *et al., 2023*	646
6.	OR732102	*C. nicoletiid*	Spain	Molero-Baltanas *et al.*, 2023	646
7.	OR732091	*C. nicoletiid*	Spain	Molero-Baltanas *et al.*, 2023	646
8.	OR732095	*C. rothschildi*	Australia	Molero-Baltanas *et al.*, 2023	559
9.	OR732096	#*C. phantasma* sp. nov.	Spain	Raphel *et al.* In press	646

Sl. No.	Accession Number	Species	Country	Authors	Base pair length
10.	OR732092	**C. villosum*	Spain	Molero-Baltanas *et al.*, 2023	646
11.	NC-046478	*C. villosum*	China	Chen *et al.*, 2020	15488
12.	OR732105	*C. ciliatum*	Spain	Molero- Baltanas *et al.*, 2023	646
13.	OR732097	*C. longicaudatum*	Australia	Molero- Baltanas *et al.*, 2024	607
14.	MK185702	*C. longicaudatum*	Australia	Smith *et al.*, 2018	646
15.	OP028707	*Lepisma saccharinum*	Poland	Querner *et al.*, 2022	658
16.	OR732103	*Thermobia domestica*	Spain	Molero-Baltanas *et al.*, 2023	646
17.	NC-006080	*T. domestica*	UK	Cook *et al.*, 2004	15152
18.	DQ280136.1	*T. domestica*	USA	Espinasa *et al.*, 2005	653
19.	JN970940*	*T. domestica*	USA	Not published	
20.	OR732099	*Thermobia sp.*	Iran	Molero-Baltanas *et al.*, 2023	528
21.	OR732094	*T. nebulosa*	Namibia	Molero-Baltanas *et al.*, 2023	646
22.	OR732098	*T. nebulosa*	Namibia	Molero-Baltanas *et al.*, 2023	646
23.	[@]PQ046926	*T. smithi*	India	Raphel *et al.* 2024	645
24.	[@]PQ046927	*T. smithi*	India	Raphel *et al.* 2024	645
25.	[@]OR739558	*$T. smithi*	India	Raphel and Jose 2023	656

**C. villosum* is mentioned as *C. targionii* in databases- *Ctenolepisma targionii* (Grassi and Rovelli, 1889) is synonymized with *C. villosum*. (Molero- Baltanas *et al.*, 2024)

Currently under *C. calvum* in databases as the article is still in press

$ not used for phylogenetic tree construction in this study

[@] COI sequences generated during this study

2.4. Results

2.4.1. Checklist of Synanthropic Zygentoma from the study area

Of the six Zygentoma families worldwide (Robla *et al.,* 2023) only family Lepismatidae was recorded in the study area. Of the different subfamilies of Lepismatidae (Acrotelsatinae Mendes, 1991; Ctenolepismatinae Mendes, 1991; Heterolepismatinae Mendes, 1991; Lepismatinae Mendes, 1991; Mirolepismatinae Mendes, 1991, and Silvestrellinae Mendes, 1991) all the collected specimens belonged to two subfamilies: Acrotelsatinae and Ctenolepismatinae and three genera namely:

Acrotelsa, Ctenolepisma and Thermobia. (Diagnostic characters are given in Appendix II.

From subfamily Acrotelsatinae, only a single species, *Acrotelsa* cf *collaris*, was collected. Subfamily Ctenolepismatinae was represented by four species: *Thermobia smithi.* sp. nov., *Ctenolepisma rothschildi, Ctenolepisma longicaudatum* and *Ctenolepisma calvum.* Among these, *Ctenolepisma calvum* was collected exclusively from the Thrissur district (Fig. 9). Similarly, *Acrotelsa* cf *collaris* was recorded only from two districts (Fig. 10) Thrissur and Kannur. *Thermobia smithi* recorded from Thrissur, Palakkad and Idukki, (Fig.11) is a species new to science. All species documented in this study represent first reports for Kerala. *Ctenolepisma rothschildi* was reported from six districts (Kottayam, Ernakulam, Idukki, Thrissur, Palakkad and Kannur) (Fig. 12) and *Ctenolepisma longicaudatum* was reported from -Thrissur, Palakkad, Ernakulam, Pathanamthitta, Kannur and Idukki districts (Fig. 12). Details are given in Table 4.

Ctenolepisma calvum has been designated as the neotype, since this is the first detailed description from India following its original report from Sri Lanka in 1910 by Ritter leading to *Ctenolepisma calvum* earlier recorded from Europe being redesignated as a new species *C. phantasma* n. sp. (Raphel *et al.* 2025).

Thermobia smithi sp. nov. was collected as synanthropic from inside a school library building and college store room in Thrissur district and a store room from a church in Idukki district in Kerala State. The actual distribution of this species needs further survey. *C longicaudatum* was recorded from 15 localities at Thrissur, Palakkad, Ernakulam, Pathanamthitta, Kannur and Idukki districts.

C. longicaudatum preferred (58% of the sightings) old wooden furniture dumped in storerooms, basements and attics and old books and papers stored in houses (25% of the sightings) and between roofing tiles stored on ground (8.5% of sightings each).

C. rothschildi was recorded from 44 localities in six districts (Kottayam, Ernakulam, Idukki, Thrissur, Palakkad and Kannur). *C. rothschildi* was frequently found (40% of all sightings) in old books and papers stored in homes, offices and libraries and with lesser frequency from under shelf liners (20% of all sightings), old cardboard boxes (16%), coconut husk and coconut shell (10% of sightings) stored outside homes, etc. They were also sighted from unused wooden furniture, pulses stored in paper bags (4% of sightings each) and bundles of paddy (2%).

C. calvum, was recorded mostly from old books. It was found from five localities in Thrissur district, namely Keerankulangara, Echippara, Wadakkanchery, Valarkkavu and Madayikkonam.

Acrotelsa was mostly recorded from coconut husk and shells, mixed substrates in store rooms, wood and book and paper bundles.

Table. 4. Checklist of Synanthropic Lepismatidae from Kerala

Subfamily	**Name of Species**	**ZSI registration number**	**Sites of Collection**	**Remarks**
Ctenolepismatinae	*Thermobia smithi*, Raphel (2024)	ZSI/WGRC/I.R.-INV.26920 ZSI/ WGRC/I.R.-INV.26921	Idukki, Palakkad, Thrissur	Species new to science
Acrotelsatinae	*Acrotelsa* cf *collaris,* Fabricius (1793)		Thrissur, Kannur	First report from Kerala
Ctenolepismatinae	*Ctenolepisma rothschildi*, Silvestri, (1907)	ZSI/WGRC/I.R.-INV. 26926 ZSI/WGRC/I.R.-INV. 26927	Thrissur, Palakkad, Ernakulam, Kannur, Idukki, Kottayam,	First report from India
Ctenolepismatinae	*Ctenolepisma longicaudatum*, Escherich, (1905)	ZSI/WGRC/I.R.-INV.26924 ZSI/WGRC/I.R.-INV.26925	Thrissur, Palakkad, Ernakulam, Kannur, Idukki, Pathanamthitta	First report from Kerala
Ctenolepismatinae	*Ctenolepisma calvum*, Ritter (1910)	ZSI/WGRC/I.R.-INV.26922 ZSI/WGRC/I.R.-INV.26923	Thrissur	Rediscovery and Neotype designation

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2.4.2. Taxonomic description of synanthropic Zygentoma from the study area

Subfamily CTENOLEPISMATINAE **Mendes, 1991**

***Thermobia smithi* Raphel *et al.*, 2024**

(PLATE III Figs. a-n)

Type material: Holotype: male, amidst of books and wooden articles present in the library of a secondary school, Wadakkanchery, Thrissur, district, Kerala India (10.523636°N and 76.216588°E), 12.iii.2021, *leg. coll*. Sheeba Raphel. Paratypes: 1 male and 3 females, same locality data as holotype (10.523636°N and 76.216588°E), 12.iii.2021, *leg. coll.* Sheeba Raphel.

Type Material Location:

Type specimens in alcohol deposited at Zoological Survey of India, Western Ghat Regional Centre, Kozhikode and the registration numbers are ZSI/WGRC/I.R.-INV.26920 and ZSI/ WGRC/I.R.-INV.26921.

ZoobankID: https://zoobank.org/References/b0346254-f251-4978-9967-a99d8fb0ae16.

Order Zygentoma Börner, 1904

Family Lepismatidae Latreille 1802

Subfamily Ctenolepismatinae Mendes, 1991

Genus Thermobia Bergroth, 1890

Thermobia smithi sp. nov. Raphel *et al.*, 2024

Etymology: The species is named after Dr. Graeme B Smith (Research Associate at Australian Museum Sydney, Australia) in recognition for his immense contributions to Zygentoma research.

Description:

Dorso-ventrally flattened body, tapers posteriorly with length up to 12 mm; antennae and caudal filaments longer than body (antennae up to 23 mm, cerci up to 15 mm and paracercus up to 16 mm). Body ground colour yellowish-white; sub-triangular to globular, dorsal scales brown, grey, silvery white and golden, mixed, not forming a concrete pattern. Ventral scales and scales on coxae and femora whitish. Overlapping scales all over the body, coxae and femora. Scales absent on antennal flagellum, terminal filaments, palps, tibiae, tarsi, styli and ovipositor. Scales with closely packed scale rays. Bristles golden yellow with pinkish tinge; bifid or single apical end; barbs along shaft on either side present (Plate III a, b).

Head: Head wider (1.57 mm) than long (0.87 mm). Head chaetotaxy as usual for the genus, with strong bushes of setae on the frontal, in front of antennae and close to the base of maxillary palp (Plate III c). Labrum and clypeus with 1+1 tufts of macrochaetae. Prominent well-spaced compound eyes on the lateral side of the head with 12 ommatidia each.

Maxillae with six-segmented maxillary palps. The distal article about 5.5–5.9 times longer than wide and 0.6 – 0.9 times as long as the penultimate article. Labium with four-segmented palps; axe-shaped apical article (L/W 1.6) with 5 oval shaped sensory papillae in a single row ((Plate III d). Mandibles with well-developed molar and incisor regions; a prominent comb of spines on the marginal end of molar region (Plate III c -e).

Thorax wider than abdomen; TW: 3.2 – 5.8mm. TL: 2.5 to 4.5 mm. Pronotal collar with 3–4 rows in the centre, single row towards the lateral margins. Pronotum with (11–14) + (11–14) lateral bristle combs composed of 2–6 macrosetae each. Posterior trichobothrial area of the pronotum associated with the antepenultimate (N-2) comb and the anterior one, on the inner side of N-4 comb.

Mesonotum with (11–13) +(11+14) lateral bristle combs composed of 2–6 macrosetae each; anterior trichobothrial areas associated to the inner side of last (N) combs and the posterior ones in the outer side of penultimate (N-1) combs.

Metanotum with (8–11) +(8–11) lateral bristle combs with 2–6 macrosetae each; trichobothrial areas associated to the inner side of last two combs (N and N-1). Hind borders of thoracic nota with 1+1 bristle combs of 3–4 macrosetae each.

Thoracic sterna heart-shaped with rounded hind margin. Prosternum and mesosternum similar in size and shape. Prosternum with (5–9) +(7–12) bristle combs usually of 3–7 macrosetae each (at most, 12). Mesosternum and metasternum are provided with 1+1 subapical bristle combs of 10–20 macrosetae each (Plate III e - j).

Legs: Coxae and femora with rounded scales. In males, protibiae 3–5.5 times longer than wide; mesotibiae 3.8–4.2 times longer than wide and about 1.12–1.16 times longer than the protibiae; metatibiae 4 times longer than wide and 1.26–1.3 times longer than the protibiae.

In the protarsus, Tarsomere I is 4–5.5 times longer than T2; is 4–4.7 times longer than T3; is 3–3.5 times longer than T4. In the mesotarsus, tarsomere I is 5 times longer than T2; is 5–6.3 times longer than T3; is 3 times longer than T4. In the metatarsus, tarsomere I is 3.3 times longer than T2; is 4 times longer than T3; is 5 times longer than T4.

In females, protibiae 3–3.25 times longer than wide; mesotibiae 3–4.4 times longer than wide and about 1.16–1.3 times longer than the protibiae; metatibiae 4–5.5 times longer than wide and 1.2–1.6 times longer than the protibiae.

In the protarsus, Tarsomere I is 3.5 times longer than T2; is 4–6.8 times longer than T3; is 3.7 times longer than T4. In the mesotarsus, tarsomere 1 is 4.7–5.3 times longer than T2; is 3.8–5 times longer than T3; is 3–5 times longer than T4. In the metatarsus, tarsomere I is 5.6–6 times longer than T2; is 3.5-4 times longer than T3; is 4 times longer than T4. Pretarsus with 3 claws, two lateral and one median.

Abdomen: Urotergite I with 1+1 bristle combs on lateral margins with 4–6 macrosetae. Urotergites II–VII with 2+2 bristle combs. Each lateral bristle comb with 8–10 macrosetae and each submedial bristle comb with 3-4 macrosetae. Urotergite VIII with 1+1 bristle combs with 5–6 macrosetae. Urotergite IX without any bristle combs. The shape of urotergite X is as shown in the (Plate IIIk) and has 1+1 lateral bristle combs having 5–7 macrosetae each; scattered macrochaetae also visible towards the apex. The number of macrosetae per bristle comb on urotergites and urosternites are given in Table 5.

First and second abdominal sternites without bristle combs. Third urosternite with one median bristle comb of 3–6 macrosetae. Fourth, fifth and sixth urosternites with 1+1+1 bristle combs, *i.e.*, two lateral with 15–20 macrosetae and a median one with 5–8 macrosetae. Seventh and eighth urosternites have 1+1 lateral bristle combs of 12–20 macrosetae each.

Three pairs of styli inserted on abdominal segments VII, VIII and IX ((Plate III l). The ratio of length of styli IX/ length of styli VIII in male is 0.8 to 1.5 and in female 0.8 to 1.6; the ratio of length of styli IX/length of styli VII in male is 2 to 2.25 and in female 1 to 2.15.

Inner process of coxite 1X is subtriangular, narrower and longer; in male 1.2–1.5 times longer than wide at its base and 2.6–2.9 times longer than the outer process. In female 2.5– 3 times longer than wide at its base and 3.5–4 times longer than the outer process.

Ovipositor long with 51 –53 segments (3.5 - 4.5 mm from the base of urosternite VIII); surpassing the apex of the long internal processes of coxites IX by 2.7-3.18 times the length of the coxite IX. Male genitalia as shown in (Plate III m -n).

Details of characteristic features of the species and a comparison with other species of the genus is given in Table 6.

Table 5. Number of macrosetae per bristle comb of abdominal plates in *Thermobia smithi* sp. nov.

Segment	**Urotergites**		**Urosternites**	
	Lateral	**Submedial**	**Lateral**	**Median**
I	4−6	-----	----	----
II	8−10	3−4	----	-----
III	8−11	3−4	----	3−6
IV	8−10	3−4	15−20	5−8
V	8−10	3−4	15−20	5−8
VI	8−10	3−4	15−20	5−8
VII	8−10	3− 4	12−20	----
VIII	5−6	---	11−20	----
IX	----	----	---	---
X	5−7	----	-----	---

PLATE III- *Thermobia smithi Raphel et al., 2024*

Fig. a Dorsal view of *T. smithi*

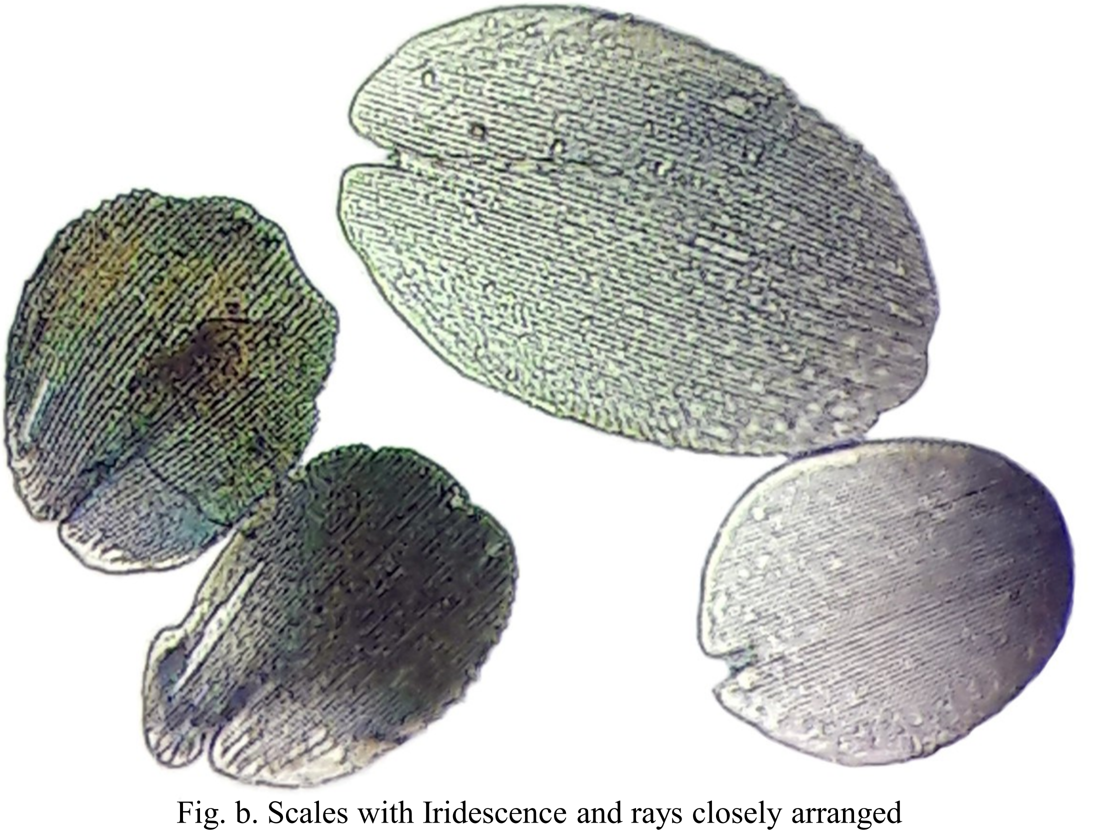


Fig. b. Scales with Iridescence and rays closely arranged

PLATE III- *Thermobia smithi Raphel et al., 2024*

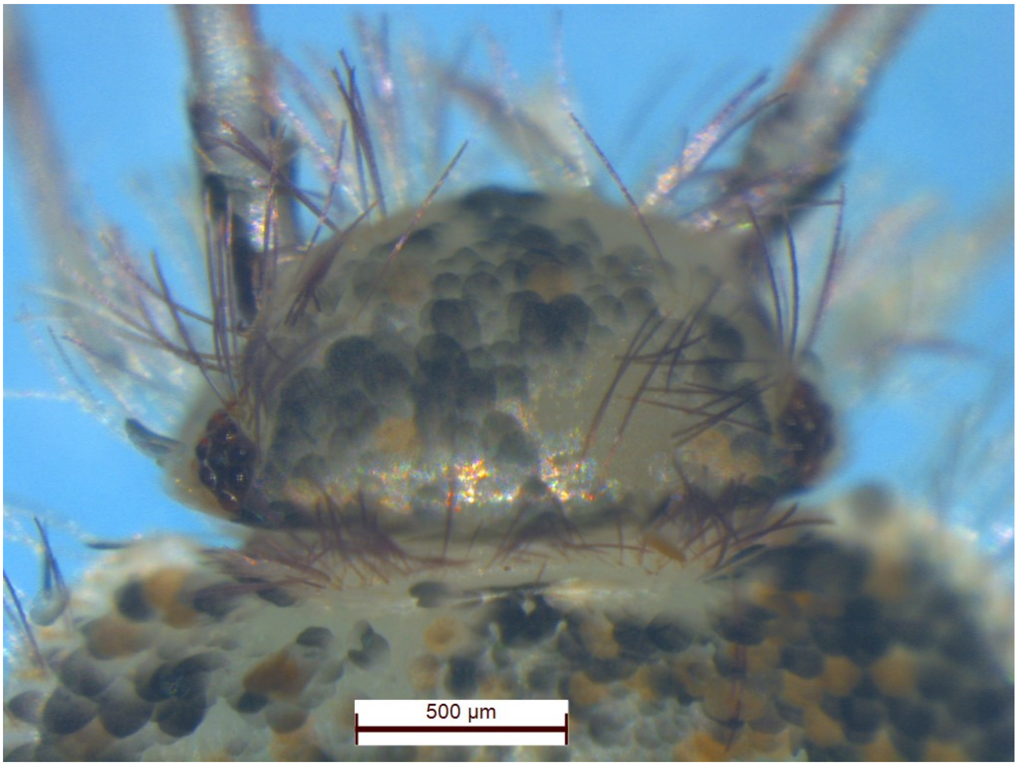


Fig c. Dorsal view of head of *Thermobia smithi* sp. nov. showing ommatidia and clusters of setae

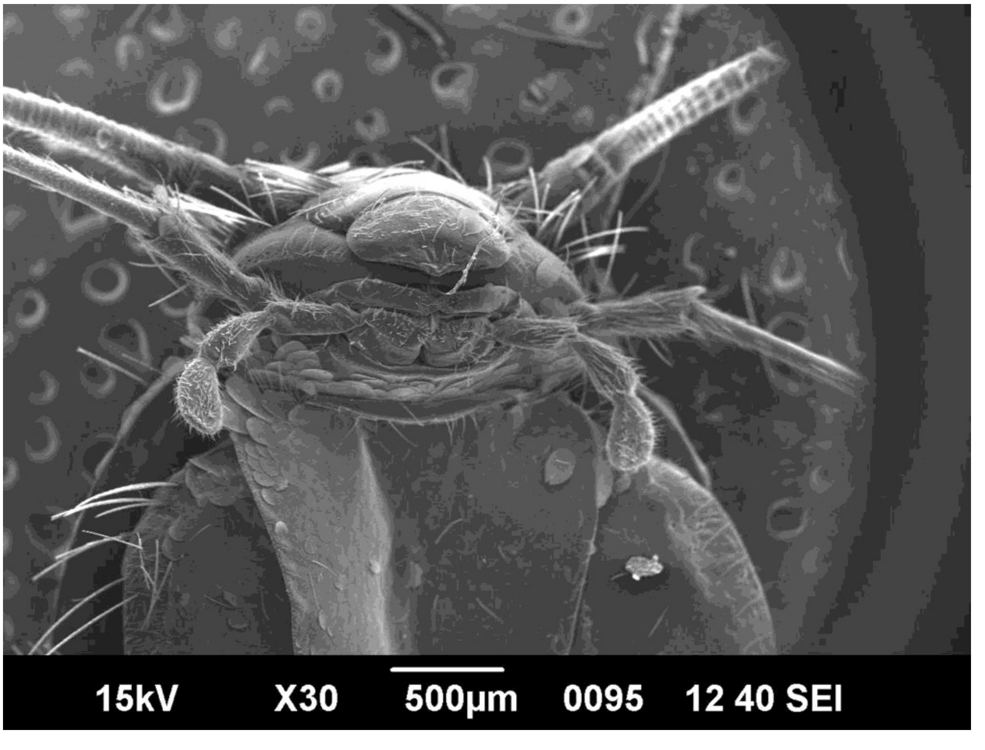


Fig d. Ventral view of head of *Thermobia smithi* sp. nov. showing mouthparts , labial palp with sensillae and imsertion of of maxillary palp and antennae

PLATE III- *Thermobia smithi Raphel et al., 2024*

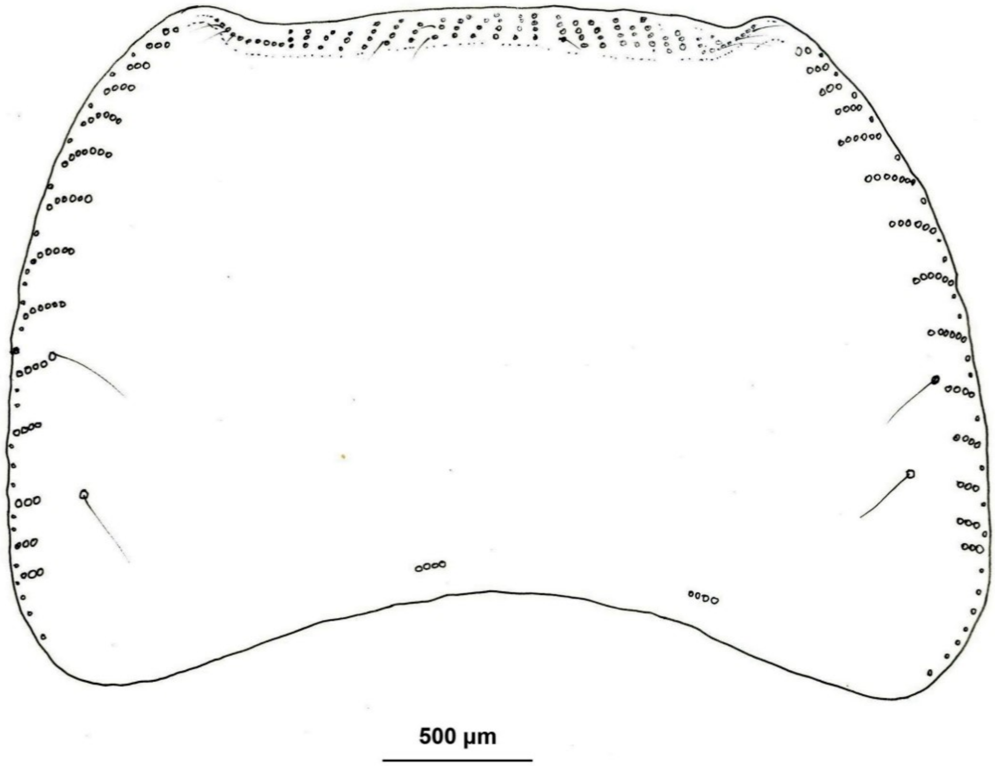


Fig. e. Arrangement of bristles combs and trichobothria on pronotum

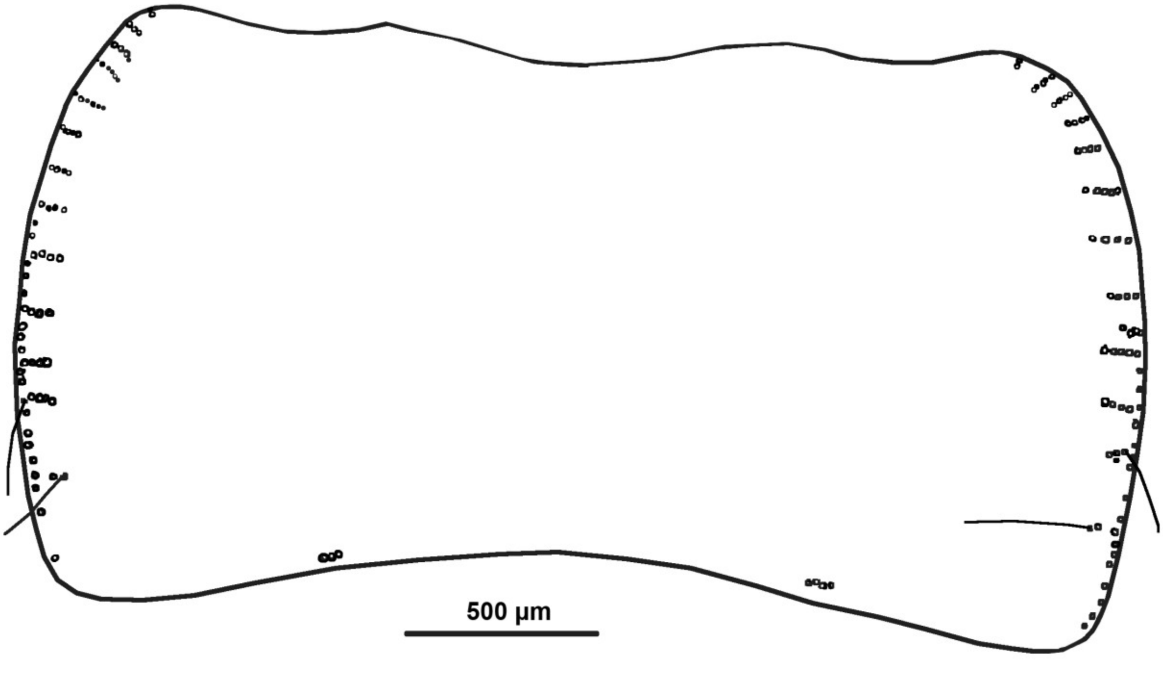


Fig. f. Arrangement of bristles combs and trichobothria on mesonotum

PLATE III- *Thermobia smithi Raphel et al., 2024*

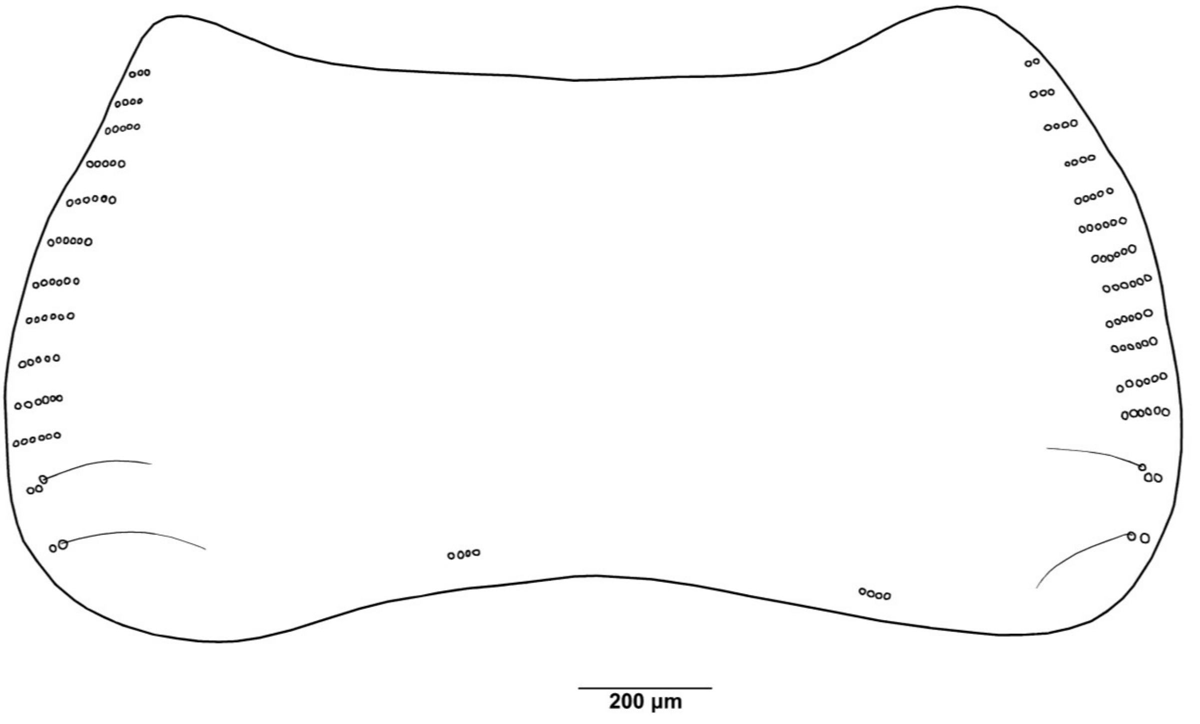


Fig. g. Arrangement of bristles combs and trichobothria on metanotum

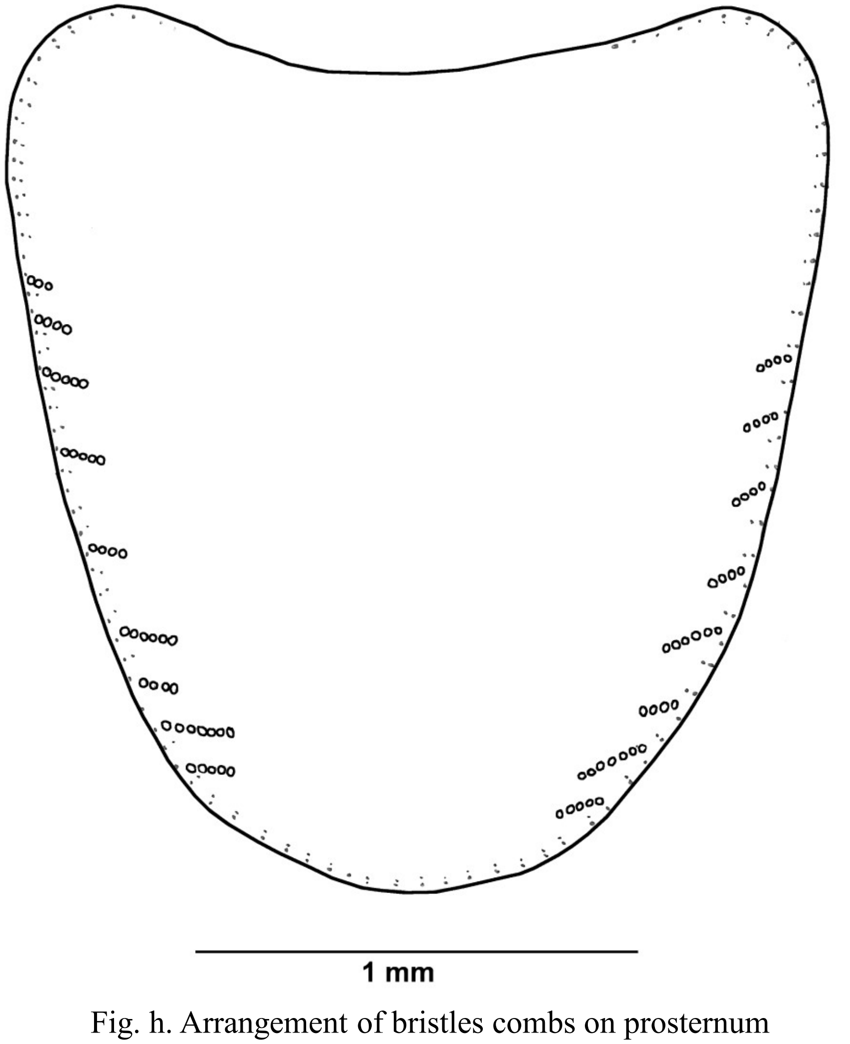


Fig. h. Arrangement of bristles combs on prosternum

PLATE III- *Thermobia smithi Raphel et al., 2024*

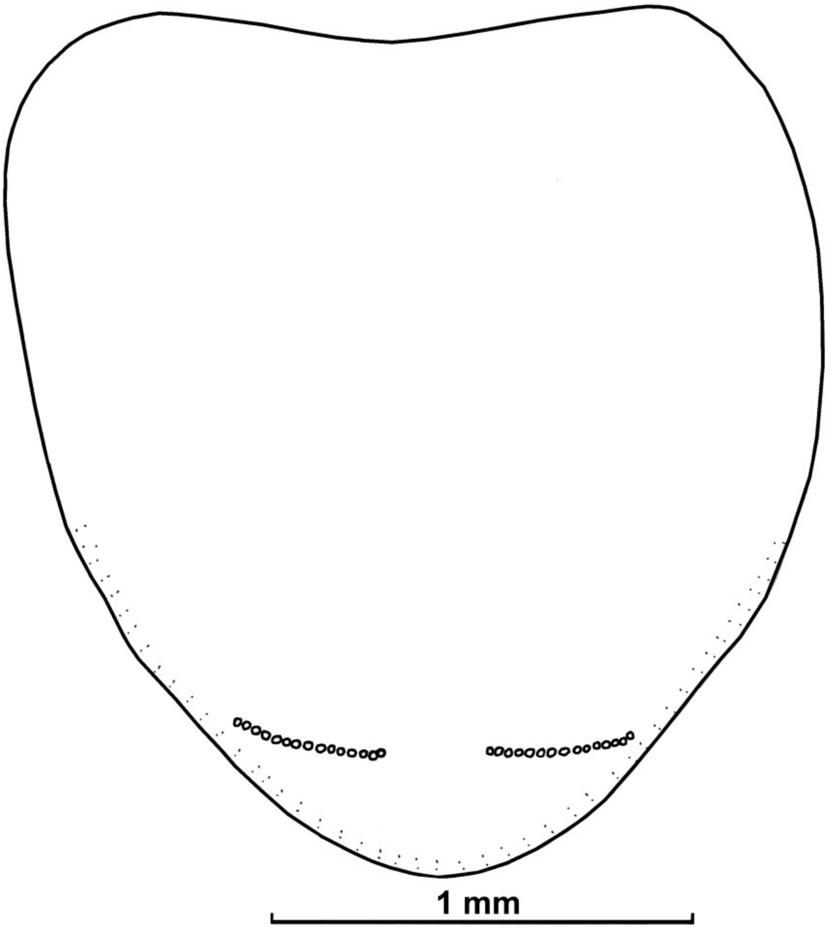


Fig. i. Arrangement of bristles combs on mesosternum

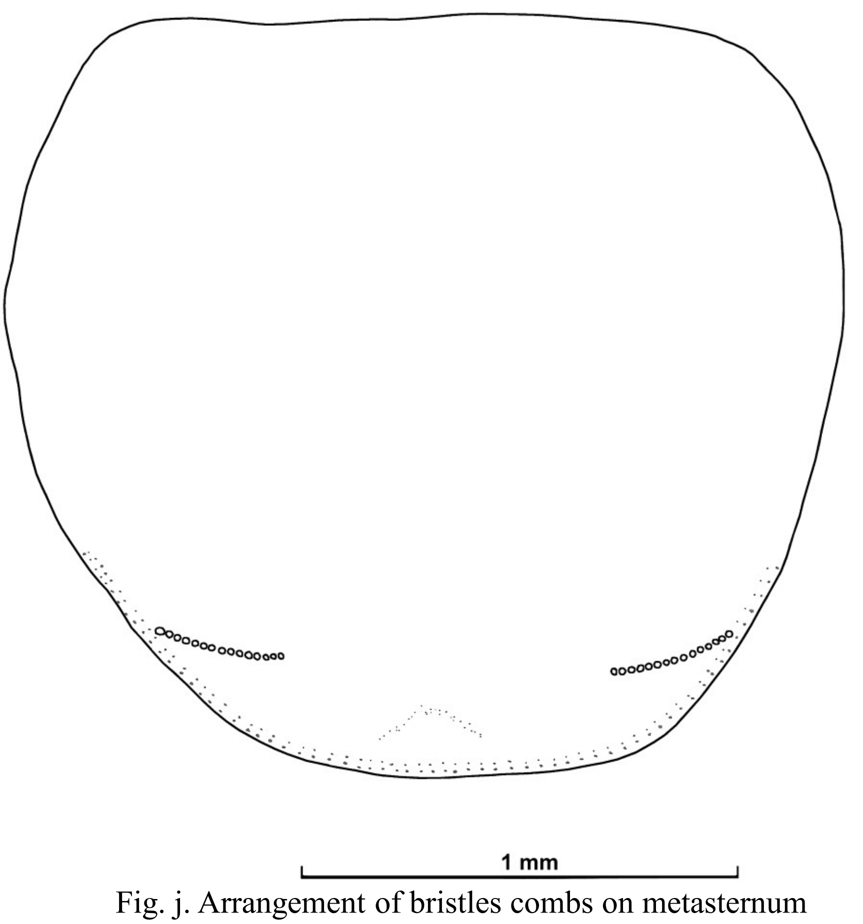


Fig. j. Arrangement of bristles combs on metasternum

PLATE III- *Thermobia smithi Raphel et al., 2024*

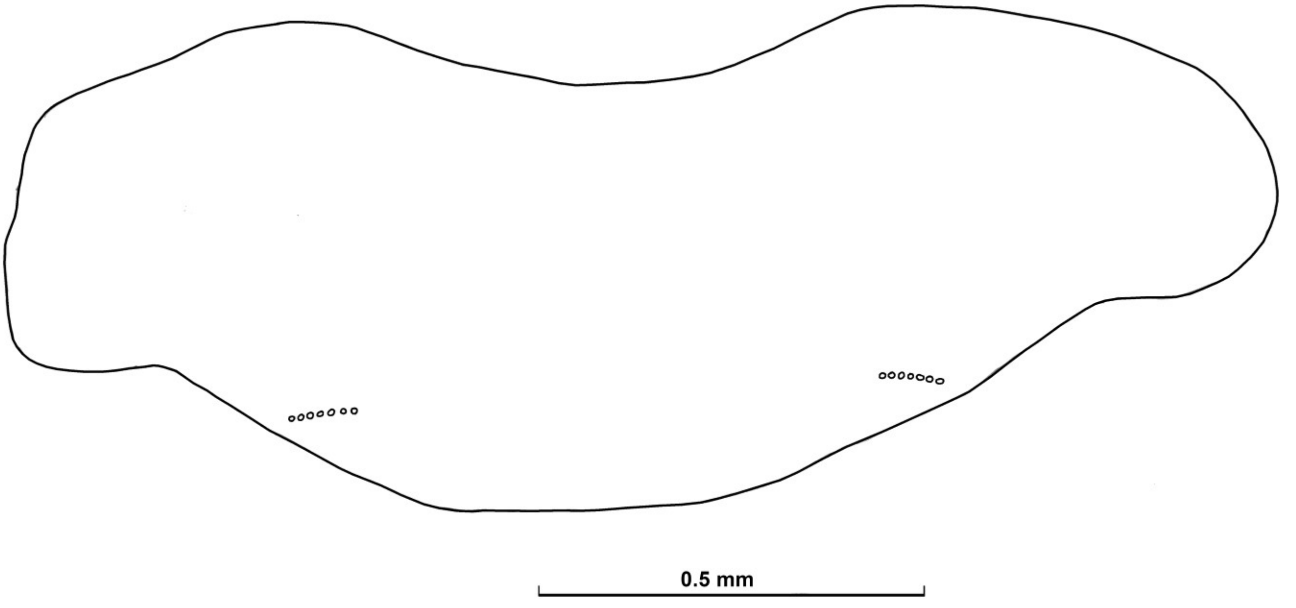


Fig. k. Trapezoidal Urotergite X

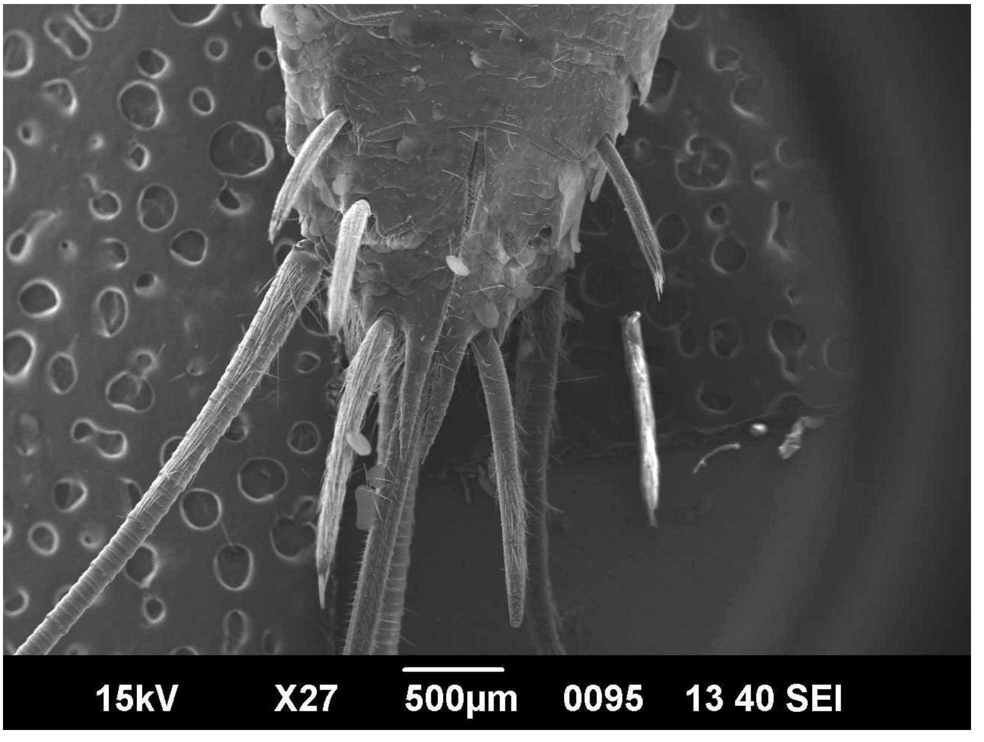


Fig. l. VII, VIII and IX coxite with styli

PLATE III- *Thermobia smithi Raphel et al., 2024*

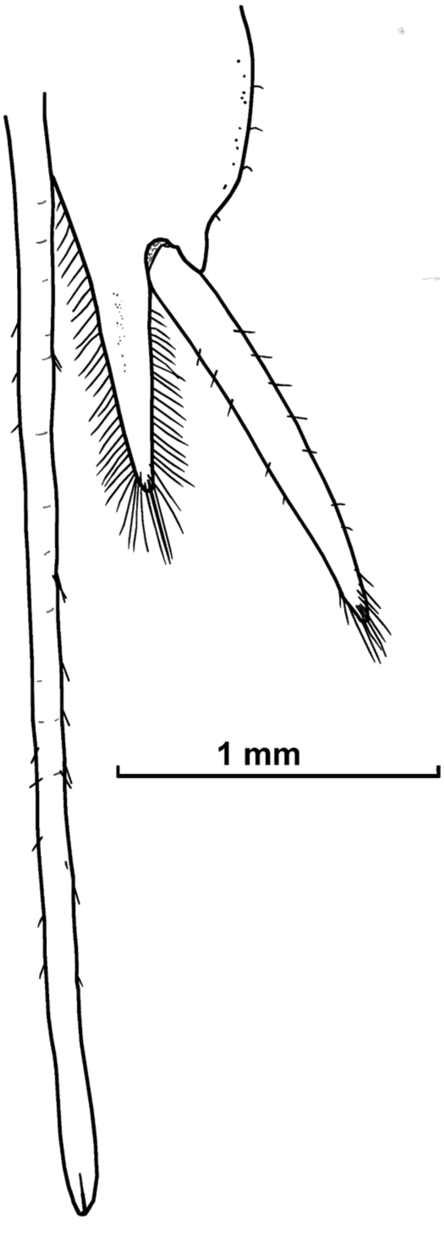


Fig. m Ovipositor with IX left coxite and stylus

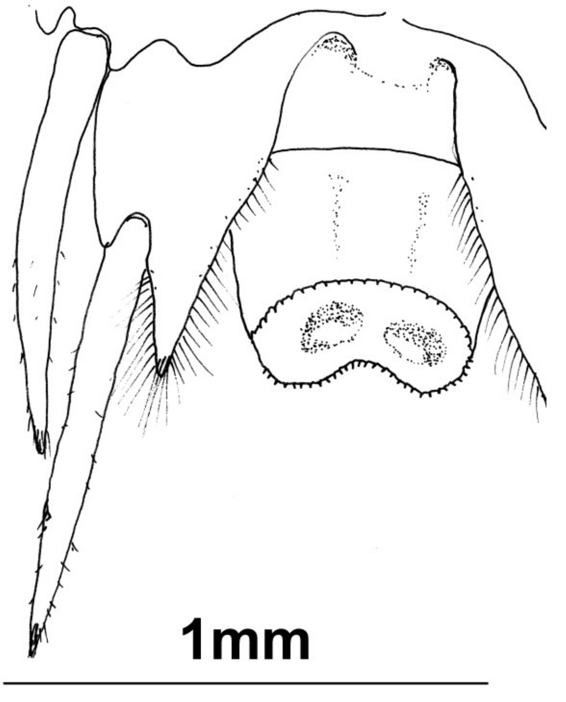


Fig. n. Male genitalia with IX left coxite, VIII and IX styli

Table 6. Comparison of morphological characters in all described species of the genus *Thermobia*

Character	***T. smithi* sp. nov.**	***T. domestica***	***T. aegyptiaca* Palaearctic /S. African**	***T. vallaris***	***T. nebulosa***
Segments of maxillary palp	6	6	5	5	5
Lateral combs (number)	8−14	7−12	8−12/8−12	11−13	7−11
Number of macrochaetae of lateral combs of thoracic nota	2−6	4−12	2−6	2−6	2−6
Number of macrochaetae of posterolateral combs of thoracic nota	3−4	10−12	6−8/7-8	11−13	10−16, multiseriate in one subspecies
Number of combs of prosternum	5−11 +7−12	5−8 + 5−8	5−8 + 5−8	6−8 + 6−8	4−6 (10) + 4−6
Number of combs of mesosternum	1+1	2+2	1−2+1−2 / 1−2+1−2	2+2	2−3+2−3
Number of combs of metasternum	1+1	2+2	1+1/1+1	2+2	2+2
Number of macrochaetae of mesosternal combs	10−20	6−10	Not described	9−14	6−9
Number of macrochaetae of metasternal combs	10−20	6−10	22*	9−14	6−9
Scales on femora	Rounded	Rounded	Rounded / Not described	Truncated, subtriangular (**)	Not described
Lateral chaetotaxy of urosternite III	Absent	Absent	Absent / A small cluster of setae	Absent	A small group of 1−3 setae
Macrochaetae of lateral combs of urotergites II-VII	8−10	10−12	9−13	10−13	5−10
Macrochaetae of submedial combs of urotergites II-VII	3−4	4−10	6−8	8−11	8−12
Number of combs of urotergite VIII	1+1	2+2	2+2 / 1+1 and an additional small comb	2+2	2+2
Number of macrochaetae of combs on urotergite X	5−7	8−11	10−11*/9−11	8−9	7
Number of macrochaetae on median urosternal combs	3−8	7−10	9−15	12−19	8−11
Number of macrochaetae on lateral urosternal combs	15−20	15−20	14−22	15−22	18−32
Sclerotized apex of ovipositor	No	No	No	No	Yes

Description of this species published in Raphel, S., Baltanás, R.M., Mitchell, A. Jose J, *Thermobia smithi* sp. nov. a new species of synanthropic silverfish (Zygentoma: Lepismatidae) from Kerala, India. *Int J Trop Insect Sci* **44**, 2371–2380 (2024).

Ctenolepisma rothschildi **(Silvestri, 1907)**

(Plate IV. Figs. a- m)

Materials Examined: India, Kerala, (specimens from 54 sites see Appendix I for details) mostly from books and papers: *leg. coll*. Sheeba Raphel. Specimen in alcohol currently housed in STC Thrissur, Ref: ZGYSTCSR26i-v. and will be deposited after the publication of this paper in ZSI (Zoological Survey of India), Calicut, Kerala.

Zoobank id: http://zoobank.org/urn:lsid:zoobank.org:pub:2769486A-D505-4EAA-89DD-5E0C5894795D

Materials deposited in ZSI: Specimens in alcohol deposited at Zoological Survey of India, Western Ghat Regional Centre, Kozhikode and the registration numbers are ZSI/WGRC/I.R.-INV.26926 and ZSI/WGRC/I.R.-INV.26927

Order Zygentoma Börner, 1904
Family Lepismatidae Latreille, 1802)
Subfamily Ctenolepismatinae Mendes, 1991
Genus Ctenolepisma Escherich, 1905
Ctenolepisma rothschildi **(Silvestri, 1907)**

Description:

Elongated body, more or less parallel sided and gradually tapering towards the posterior end. Body length up to 9.8 mm (♀) and 7.3 mm (*♂*). Epidermic colour light straw; covered with dark brownish to blackish scales dorsally ((Plate IV a) and whitish yellow scales ventrally. Length of antennae up to 7.05 mm and that of cerci and paracercus 4.75 mm and 4.5 mm respectively. Body length up to 8.5 mm. Macrosetae plumose.

Dorsal Scales: Different types of scales; based on shaped- oval, globular and sub triangular; based on size large and small; Based on rays- one with 20–30 fine rays and the other with 10-16 widely spaced thicker rays. The three different shaped scales were in both small and large sizes with either wide spaced or narrow spaced rays. (Plate IV b).

Head wider (0.9–1.1 mm) than long (0.5–0.7 mm); anterior margin of head with tufts of bifid and pectinate macrochaetae laterally extending up to the eye and 1+1 frontal tufts of macrosetae on the frontal margin; clypeus and labrum also with 1+1 tufts of setae. Five segmented maxillary palps; apical article 5–8.7 times longer than wide and about the same length as the penultimate article (0.9–1.2). (Plate IV c). Four segmented labial palps with its apical article wider than long (L/W 0.75–0.86mm) and with 5 papillae in a single row (Plate IV d).

Thorax wider than abdomen. Maximum thorax width 2.18 mm; maximum thorax length 3.3 mm. Pronotal collar with 2–4 rows of bristle combs in the centre and single towards anterolateral margins. Pronotum (Plate IV e) with (8–9) + (8–9) bristle combs on the lateral margins composed of 2–4 macrosetae. Mesonotum (Plate IV f) with (10–11) + (11–12) bristle combs composed of 2 to 4 macrosetae and metanotum (Plate IV g) with (8–10) +(9–11) lateral combs of 1–3 macrosetae. All nota have 1+1 posterior combs of 2–3 macrosetae. Trichobothrial areas in pronotum, posterior on inner side of the last (N) comb and the anterior one, on the innerside of N–3 bristle comb. In mesonotum posterior associated to the innerside of last (N) combs and posterior on the outer side of antepenultimate (N–2) comb and to the two last combs (N and N–1) in the metanotum.

Prosternum (L/W 1.24) (Plate IV h) and mesosternum (L/W 1.26) (Plate IV i) of approximately equal size and shape (rounded subtriangular). Both have 1+1 posterior submarginal combs of 3–4 and 5–6 macrosetae respectively and also have some marginal setae in between the comb and margin. Metasternum broader (width 1.073mm) with 1+1 posterior submarginal combs of 6 macrosetae; with some marginal setae (Plate IV j).

Legs: Protibiae 2.65–2.74 times longer than wide; mesotibiae 3–3.6 times longer than wide and about 1–1.2 times longer than the protibiae; metatibiae 3.25–4.3 times longer than wide and about 1.3–1.6 times longer than the protibiae. Tarsi L/W: PI 7.5, PII 7.5 and PIII 9.3–11. The basal article of tarsi longer than the remaining three. Pretarsi have two strong, curved lateral claws and a smaller medial claw. coxae with broad and rounded scales, along outer margin. Femur with small, narrow, scales along proximal two-thirds of outer margin; the remaining articles are devoid of legs.

Abdomen: Urotergite I with infralateral (1+1) combs of 3–4 macrosetae, urotergites II–V with 3+3 bristle combs, urotergites VI–VIII with 2+2 combs, urotergite IX without combs and urotergite X trapezoidal in shape (Plate IV k) with 1+1 combs of 3 macrosetae and several marginal setae also present. Urosternites I and II glabrous, urosternites III–VIII with 1+1 lateral combs of 4–10 macrosetae. Details of the number of macrosetae per comb on urotergites and urosternites are given in Table 7

Styli two pairs in both sexes inserted on abdominal segments VIII and IX, armed apically with several strong spines. stylets of IX more developed than that of VIII. The ratio length of styli IX/ length of styli VIII in female is about 1–1.67. The ratio length of styli IX/ length of styli VIII in male is about 1.3–1.6. Coxite IX in male with fringe of setae along internal margin; internal process about 2.6–4 times longer than the external process and 1–1.3 (L/W) times as long as broad at its base. Penis with numerous apical setae. Parameres absent (Plate IV l). Genital region of female as in (Plate IV m), coxite IX also with fringe of setae along internal margin, internal process about 0.5–1.5 times longer than wide at its base and 2.2–2.8 times longer than the external process. Ovipositor long with 34–41 divisions and its length measures 2.24–2.66 mm from the base of urosternite VIII; surpassing the apex of the internal processes of coxites IX by 4.4–4.8 times coxite IX length.

Table: 7. Number of macrosetae per bristle comb on Urotergites and Urosternites in *C. longicaudatum* and *C. rothschildi*

	C. longicaudatum				*C. rothschildi*			
Segment	Urotergite			Urosternite	Urotergite			Urosternite
	Infra lateral	lateral	Submedial	Lateral	Lateral	Sub lateral	Submedial	Lateral
I	6 –8	—	—	—	3 –4	—	—	—
II	6 –8	5	6 –7	—	3 –4	2	2	—
III	8	5	6	17	3 –4	3	2	6
IV	8	5	7	20	4 –6	3	2	8
V	9 –10	5 –7	6	19-20	6	3	2 –3	9 –10
VI	9 –10	5 –6	6	18-20	5 –6	—	2 –3	9
VII	—	11	7-8	20-21	4 –5	—	3	9
VIII	—	11	7	12-13	4 –5	—	2	4 –6
IX	—	—	—	—	–	—	—	—

This description has been published in Raphel, S. & Jose, J. (2025) Description of two synanthropic silverfish (Insecta: Zygentoma), *Ctenolepisma longicaudatum* (Escherich, 1905) and *Ctenolepisma rothschildi* (Silvestri, 1907), from Kerala, India. *Zootaxa*, 5570 (2), 309–324.

PLATE IV -*Ctenolepisma rothschildi* (Silvestri, 1907)

Fig. a Dorsal view of *C. rothschildi*

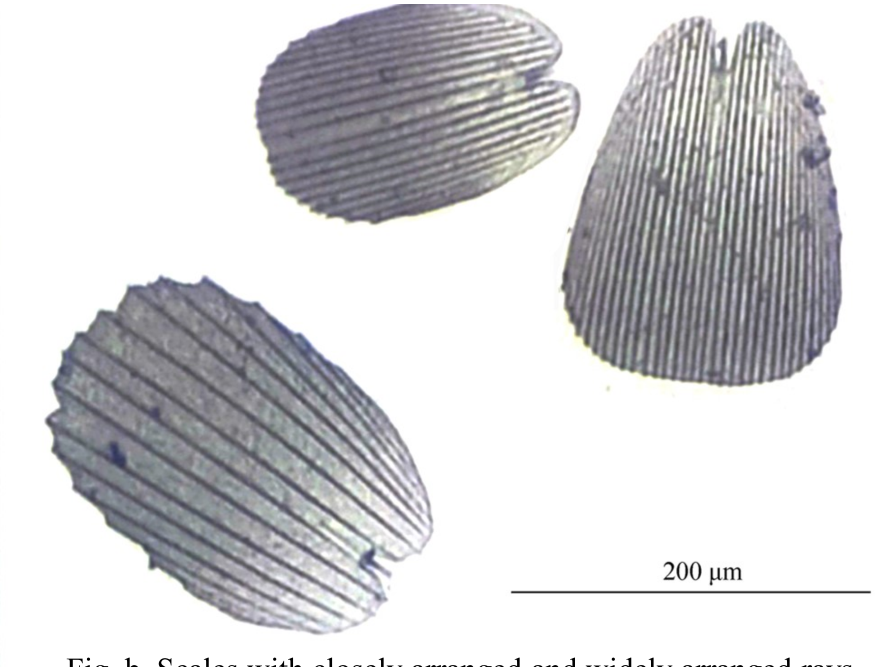


Fig. b. Scales with closely arranged and widely arranged rays

PLATE IV -*Ctenolepisma rothschildi* (Silvestri, 1907)

Fig. c. Ventral view of head with mouthparts and cephalic bristle comb arrangements as bushes

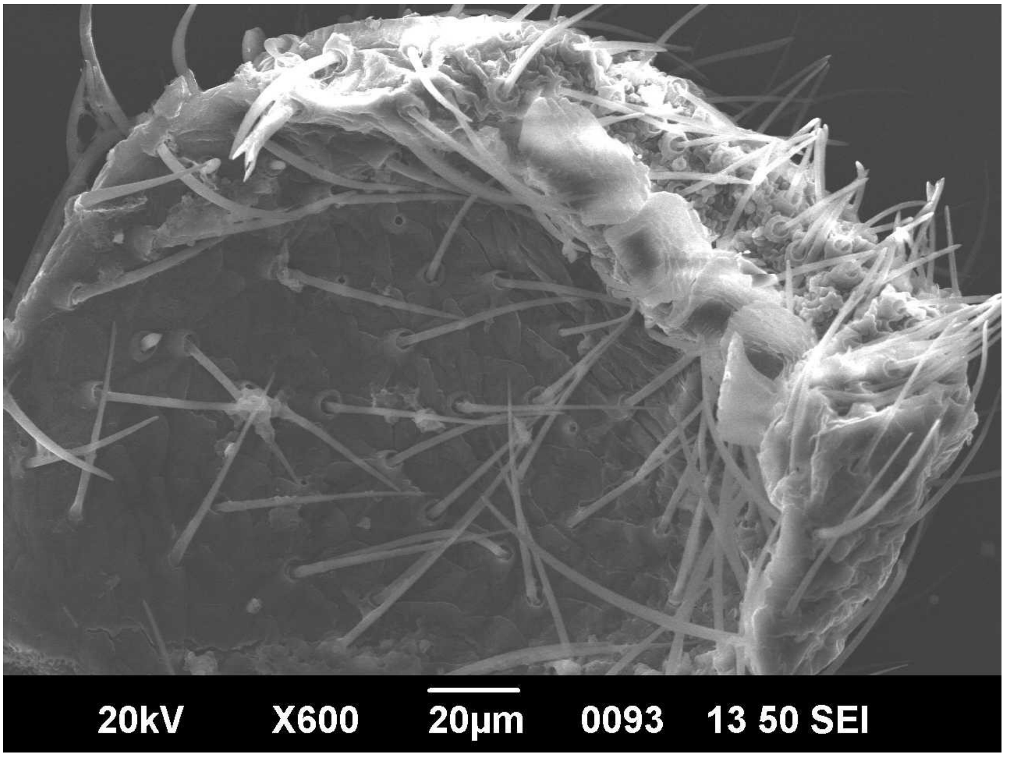


Fig. d. Labial papillae

PLATE IV -*Ctenolepisma rothschildi* (Silvestri, 1907)

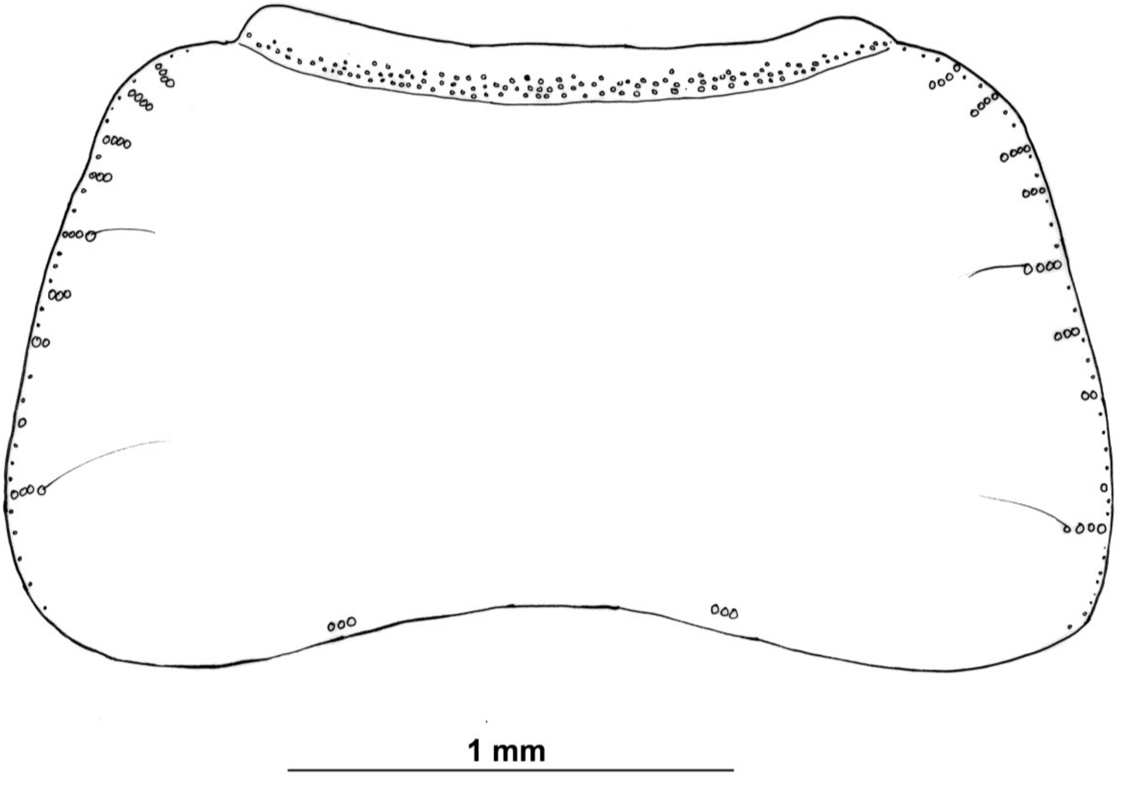


Fig. e. Pronotum with pronotal collar, trichobothrial regions, lateral and posterior bristle combs

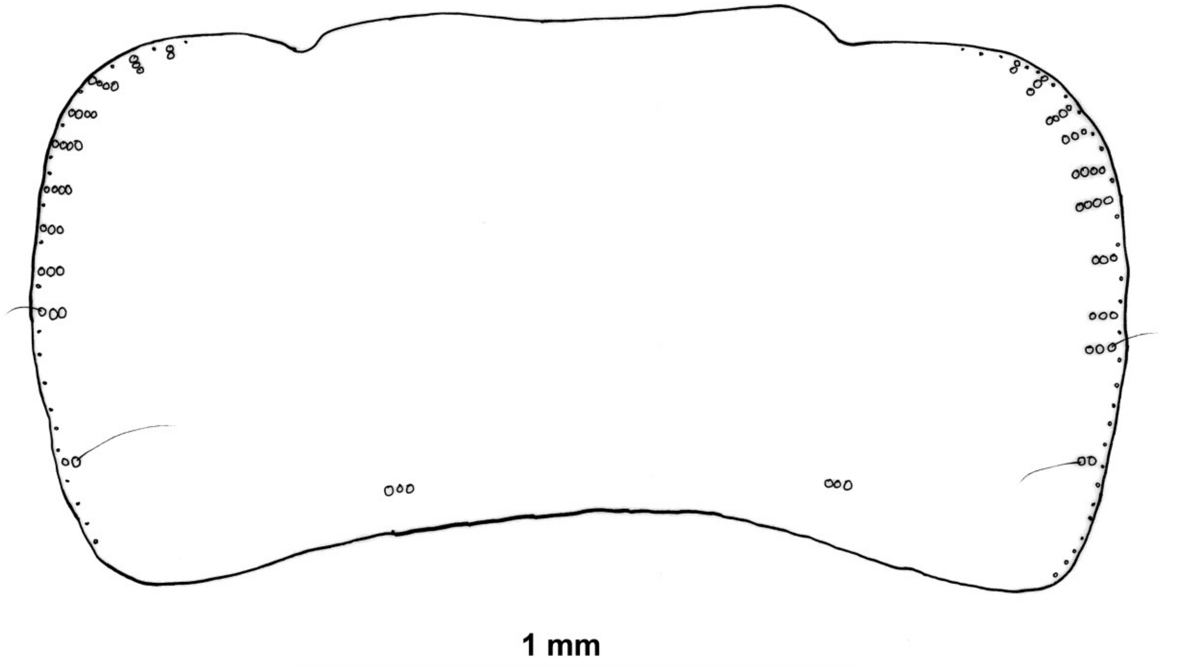


Fig. f. Mesonotum with trichobothrial regions, lateral and posterior bristle combs

PLATE IV -*Ctenolepisma rothschildi* (Silvestri, 1907)

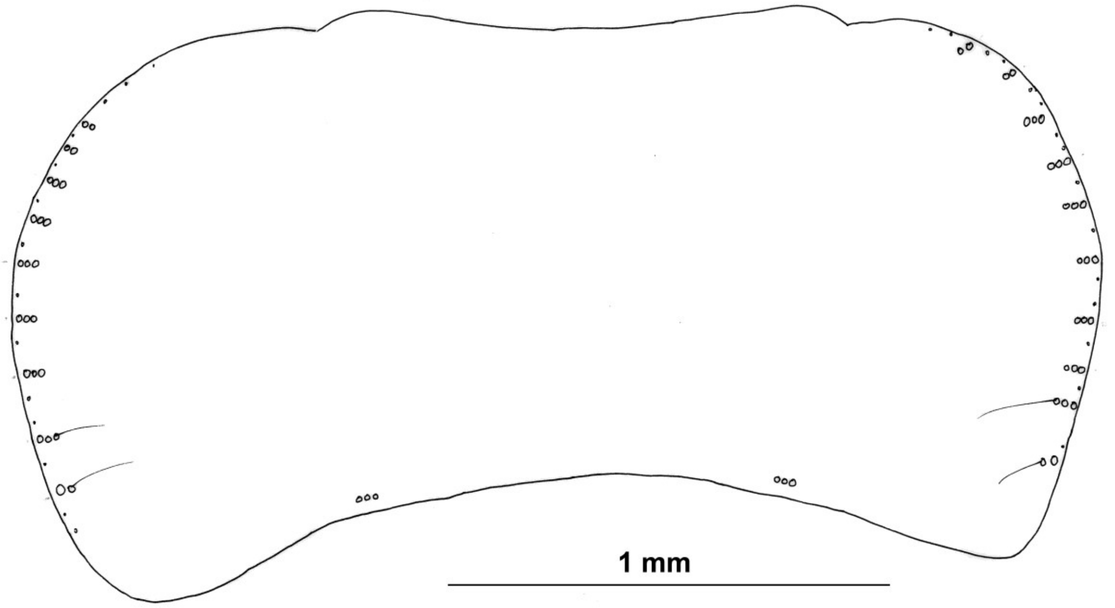


Fig. g. Metanotum with trichobothrial regions, lateral and posterior bristle combs

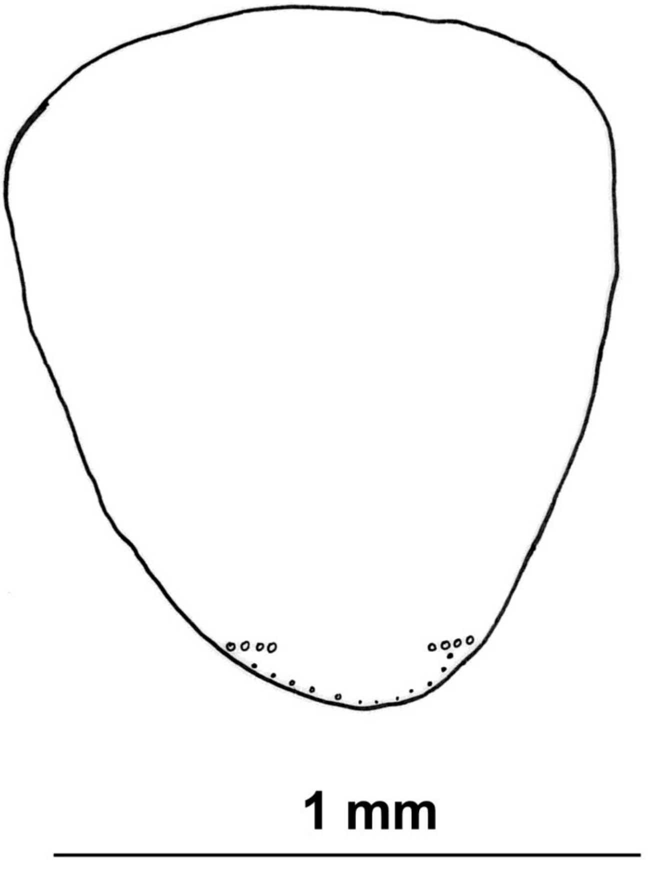


Fig. h. Prosternum with posterior bristle combs

PLATE IV -*Ctenolepisma rothschildi* (Silvestri, 1907)

Fig. i. Mesosternum with posterior bristle combs

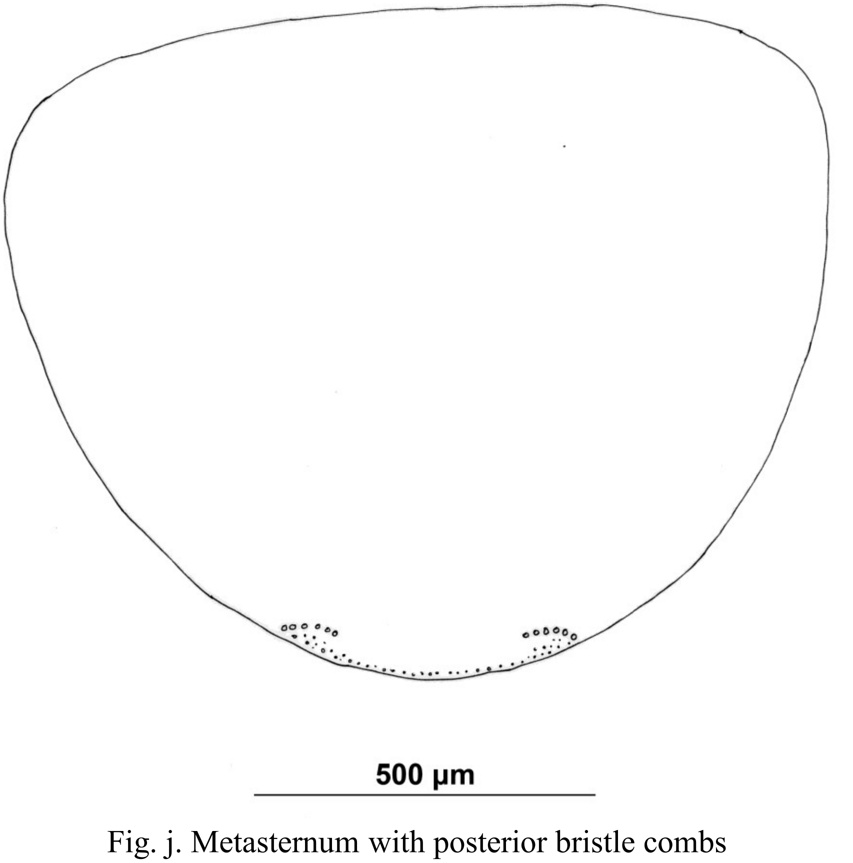


Fig. j. Metasternum with posterior bristle combs

PLATE IV -*Ctenolepisma rothschildi* (Silvestri, 1907)

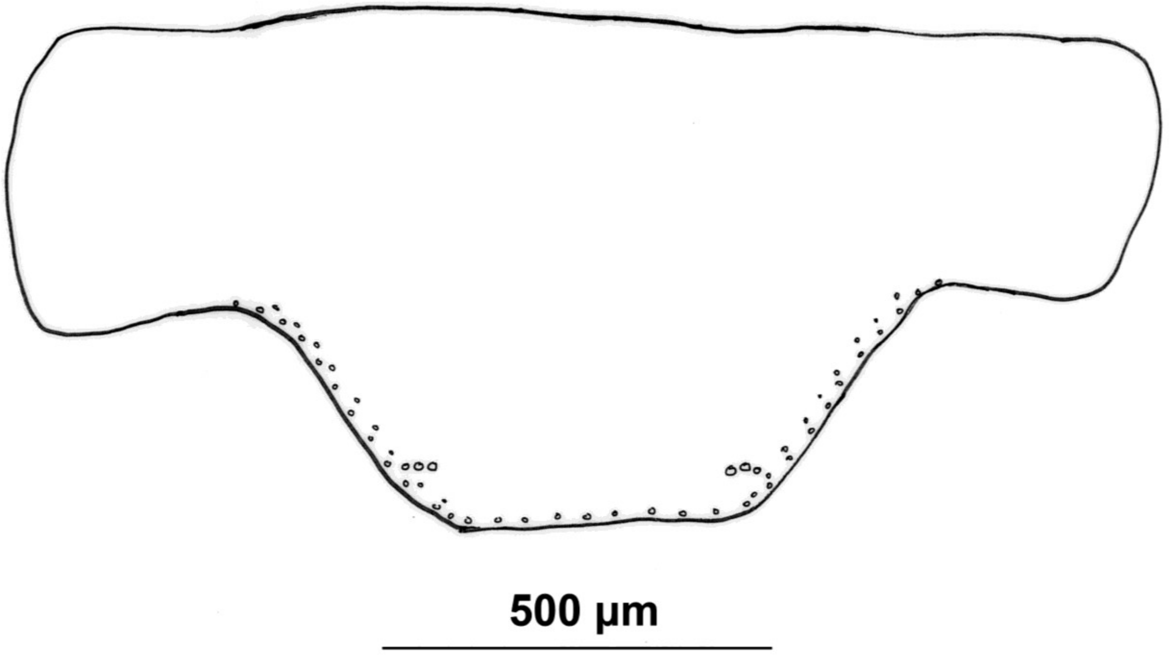


Fig. k. Trapezoidal Urotergite X

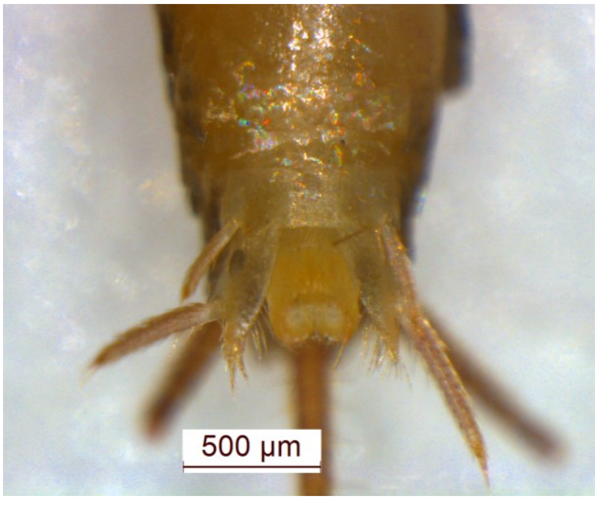


Fig. m. Penis

Fig. l. Ovipositor

Ctenolepisma longicaudatum **Escherich,1905**

(PLATE V Figs. a to l)

Materials Examined: India, Kerala, (specimens from 13 sites see Appendix I for details) amidst of old wooden articles, *leg. coll*. Sheeba Raphel.

Specimens deposited at Zoological Survey of India, Western Ghat Regional Centre, Kozhikode, with registration numbers ZSI/WGRC/I.R.-INV.26924 and ZSI/WGRC/I.R.-INV.26925

Zoo Bank id: http://zoobank.org/urn:lsid:zoobank.org:pub:2769486A-D505-4EAA-89DD-5E0C5894795D

Order Zygentoma Börner, 1904

Family Lepismatidae Latreille, 1802

Subfamily Ctenolepismatinae Mendes, 1991

Genus Ctenolepisma Escherich, 1905

Ctenolepisma longicaudatum **Escherich, 1905**

Description:

Body sub cylindrical, elongated and tapering posteriorly; length up to 13 mm (Plate V a). Epidermic pigmentation is light golden yellow dorsally and ventrally lighter. Dorsally covered with dark grayish or brownish scales and ventrally with light yellowish scales. Round shaped scales with dense parallel ribs; relatively homogeneous structures, only difference in its size (Plate V b).

Head wider (1.35–1.7 mm) than long (0.9–1.1 mm), with tufts of bifid and pectinate macrosetae on frontal region, 1+1 tufts of macrosetae on clypeus and 1+1 small tufts of macrosetae on labrum. Eyes dark black with 12 ommatidia, located well behind the antennae. Antennae length ranges from 10 –13 mm. Five segmented maxillary palp, with the apical article about 5.87– 6.5 times longer than wide and almost same length as that of the penultimate article (Plate V c). Four segmented labial palps with distal article wider at the apex than at the base (L/W 1–1.3) and usually with five sensory papillae (some samples also show more than 10 papillae (Plate V c) arranged in a single row.

Thorax wider than abdomen; maximum thorax width up to 3.25 mm; maximum thorax length up to 4.1 mm. Pronotal collar with 4–5 rows of bristles in the centre and single row towards the anterolateral side; lateral margin of pronotum with (9–10) + (10 –11) bristle combs of 3–6 macrosetae. Trichobothrial areas of the pronotum situated on innerside of last (N) and the anterior on the innerside of antepenultimate (N-4) bristle-combs (Plate V d). Mesonotum (Plate V e) lateral margins with (11–12) + (12–15) bristle combs of 3–6 macrosetae. Trichobothrial areas of the mesonotum situated on last (N) and the anterior on outer side of antepenultimate (N–2) bristle-combs. Metanotum (Plate V f) lateral margins with (10–11) + (10–11) bristle combs of 2–6 macrosetae. Trichobothrial areas of the metanotum located on last (N) and the anterior on outer side of (N–1) bristle-combs. All nota provided with 1+1 bristle combs of 6–7 setae on the posterolateral region.

Prosternum (L/W: 1.3–1.4) and mesosternum (L/W: 1.1–1.3), slightly longer than wide; metanotum wider than long (L/W: 0.85–0.1) Prosternum with 3–5+ 3–5 bristle combs of 3–10 macrosetae on its subapical area. Mesosternum with 2–5 + 2–5 bristle combs of 4–12 macrosetae on its subapical area. Metasternum with 1 + 1 subapical combs of more than 20 macrochaetae, arranged in a single row; few scattered setae also visible between the comb and the margin (Plate V g-i).

Legs stout; protibiae 2.4–2.8 times longer than wide; mesotibiae 3.1–3.82 times longer than wide and about 1.2–1.3 times longer than the protibiae; metatibiae 4–5 times longer than wide and about 1.87–1.9 times longer than the protibiae. Tarsi L/W: PI 6.7–7.5, PII 7.7–12.6, PIII 10.5–13.66. Pretarsi with two curved lateral claws and a medial claw. Coxae and femora are covered ventrally by rounded scales similar to those of the body; remaining articles of leg are devoid of scales.

Abdomen: Urotergite I with 1+1 infra lateral bristle-combs composed of 6–8 macrosetae. Urotergites II–VI with 3+3 bristle-combs; infra lateral bristle combs with 5–10 macrosetae, lateral combs with 5–7 macrosetae and sub-median bristle combs with 6–7 macrosetae. Urotergite VII and VIII with 2+2 bristle-combs, each lateral bristle comb composed of 11 macrosetae and sub-median bristle combs with 7–8

PLATE V-*Ctenolepisma longicaudatum* Escherich,1905

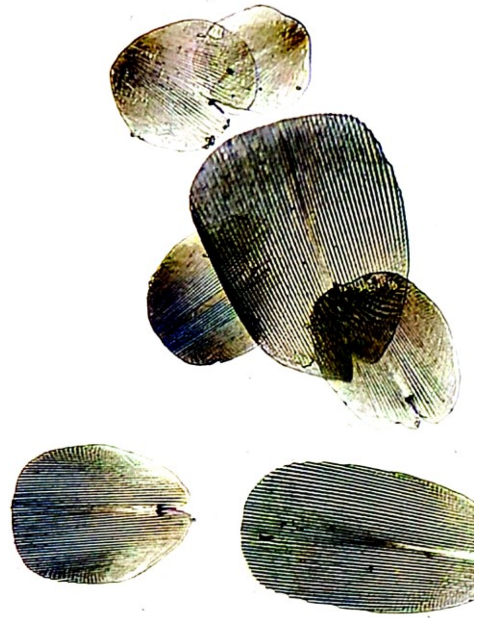


Fig. b. Scales

Fig. a. Dorsal view of *C. longicaudatum*

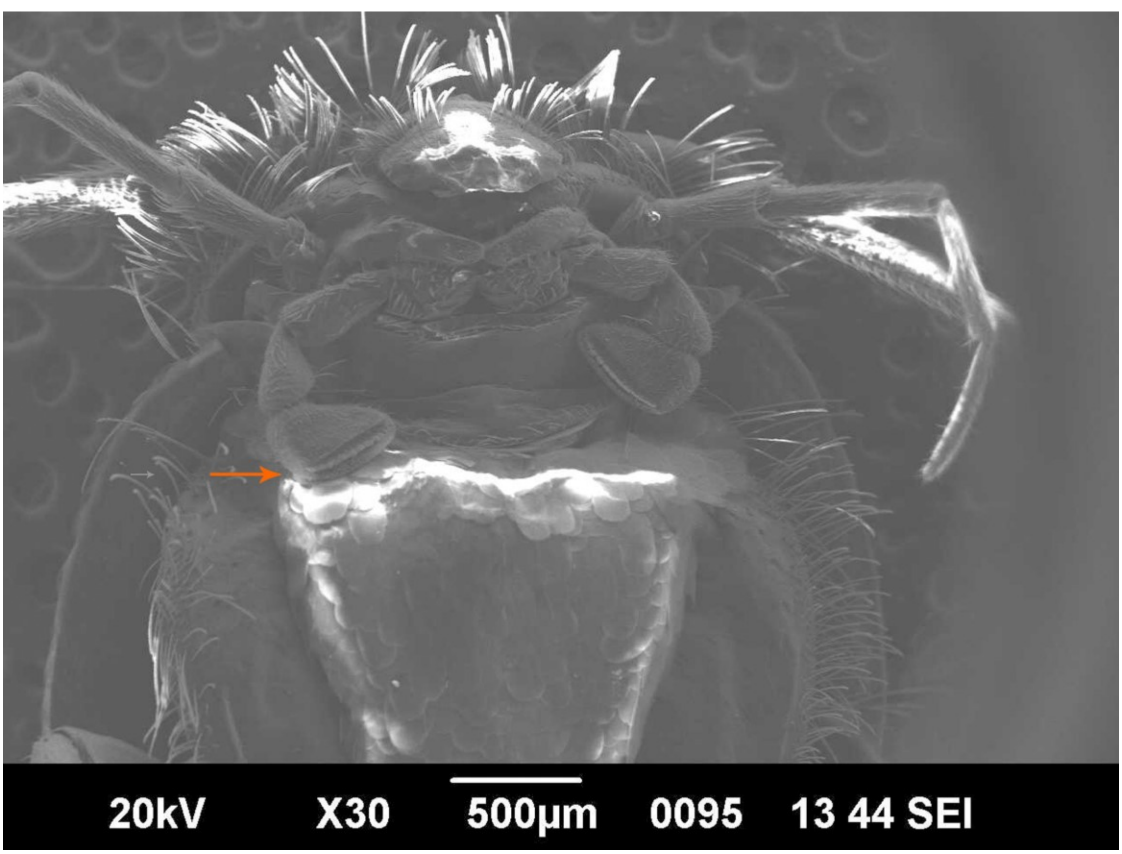


Fig. c. Ventral view of *C. longicaudatum* with labial papillae

PLATE V-*Ctenolepisma longicaudatum* Escherich,1905

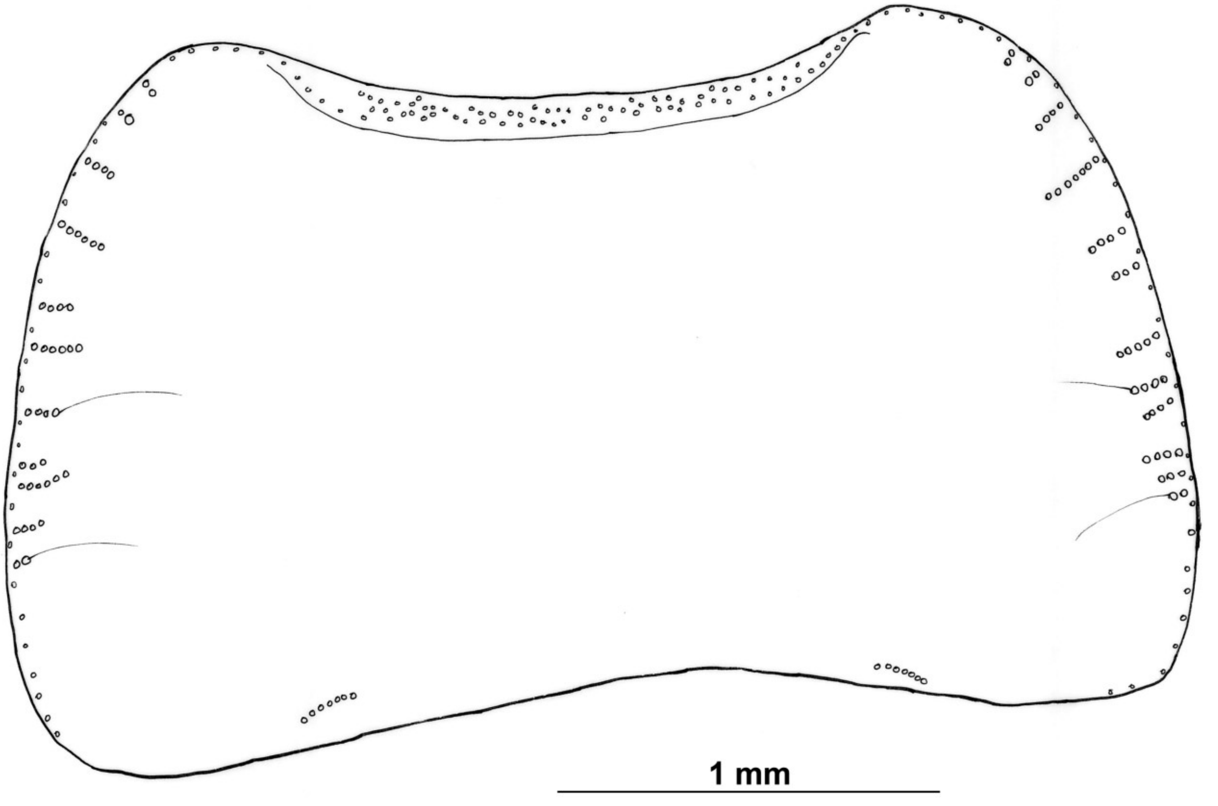


Fig. d. Pronotum with notal collar, trichobothrial and bristle comb regions

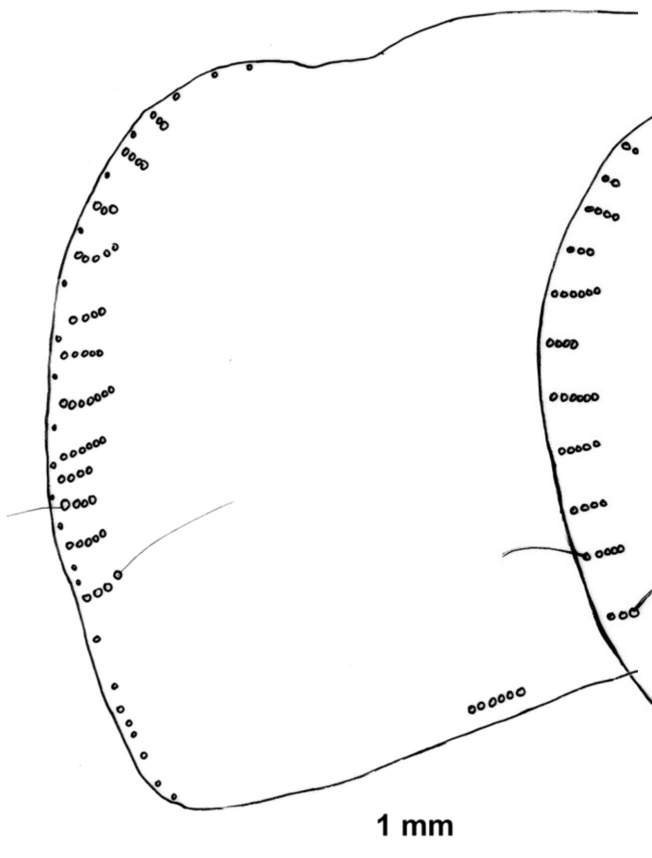


Fig. e. Mesonotum with trichobothrial and bristle comb regions

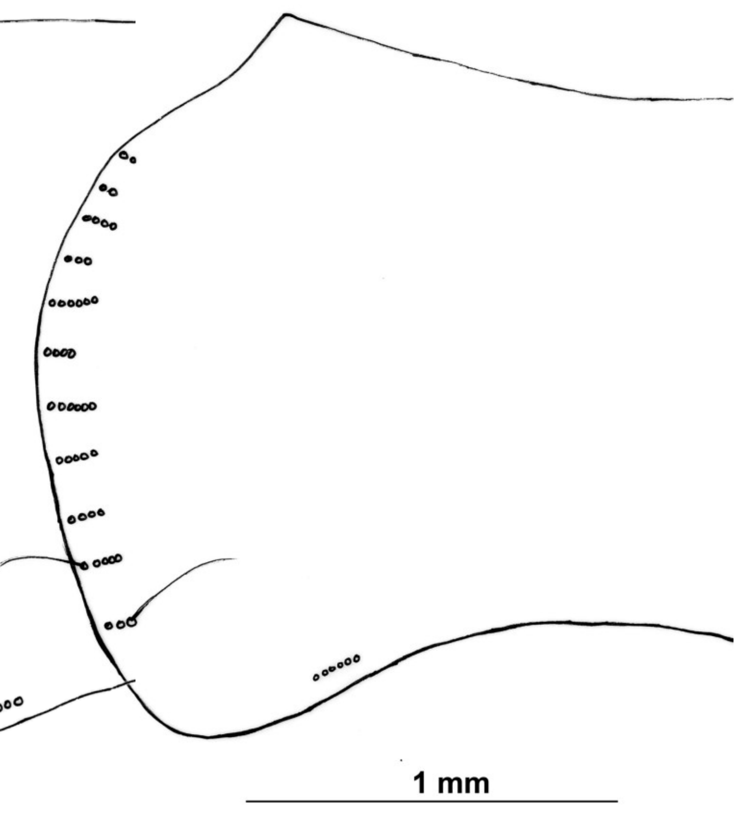


Fig. f. Metanotum with trichobothrial and bristle comb regions

PLATE V-*Ctenolepisma longicaudatum* Escherich,1905

Fig. g. Prosternum with and bristle comb regions

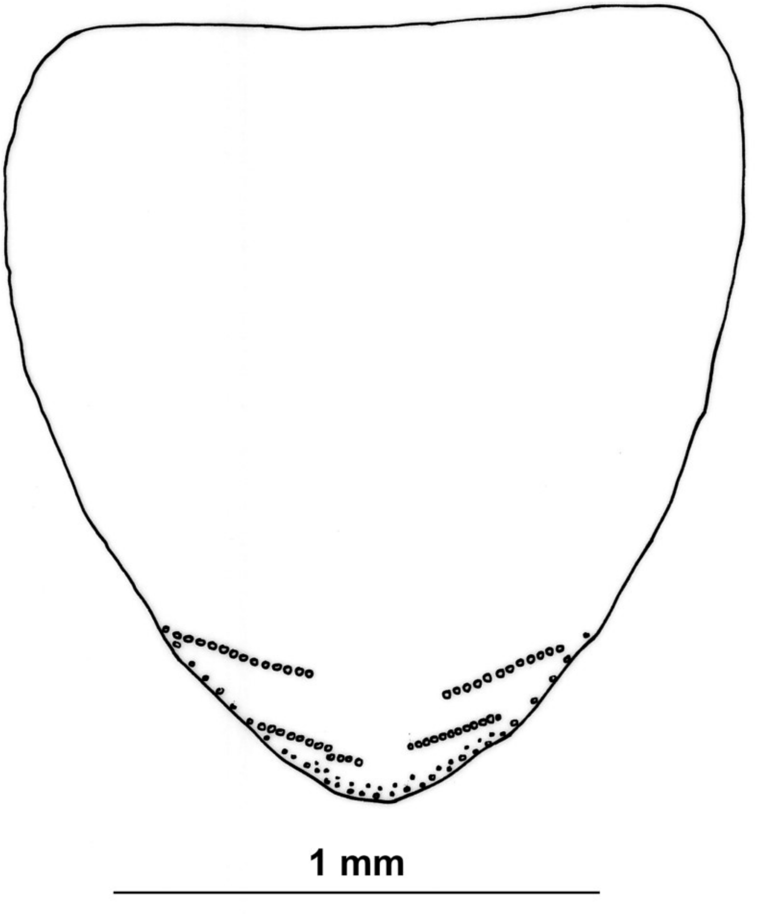


Fig. h. Mesosternum with bristle comb regions

PLATE V-*Ctenolepisma longicaudatum* Escherich,1905

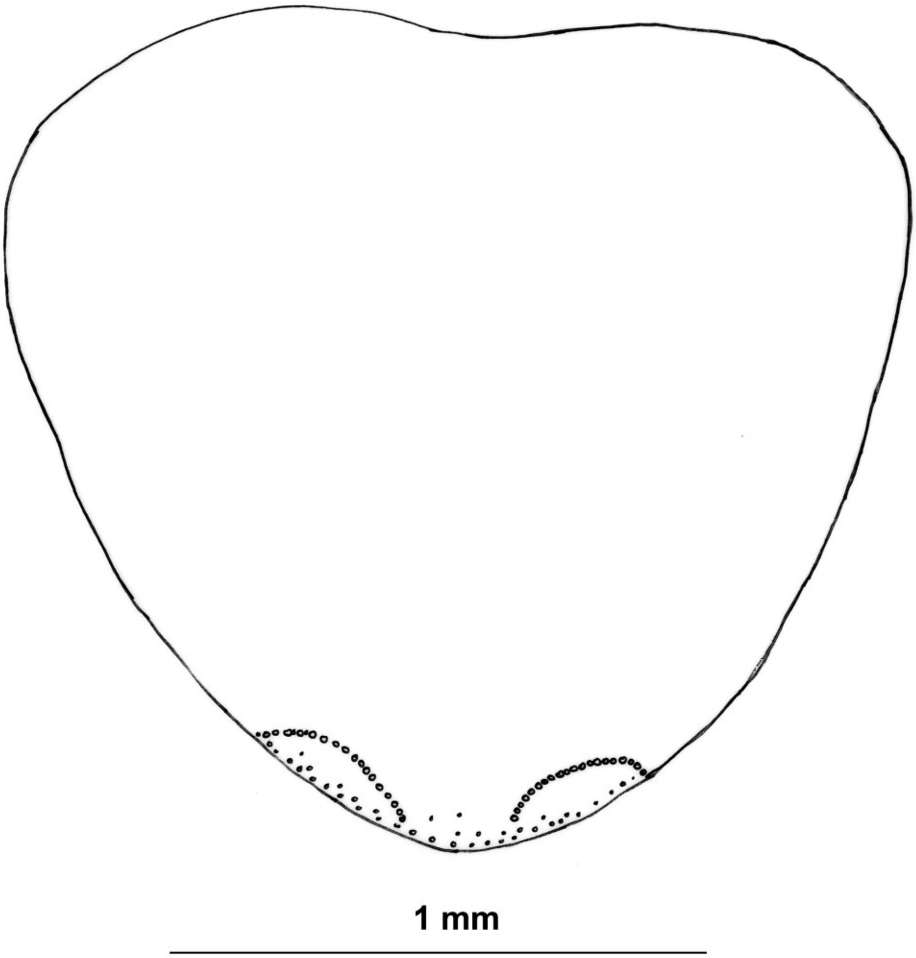


Fig. i. Metasternum with bristle comb regions

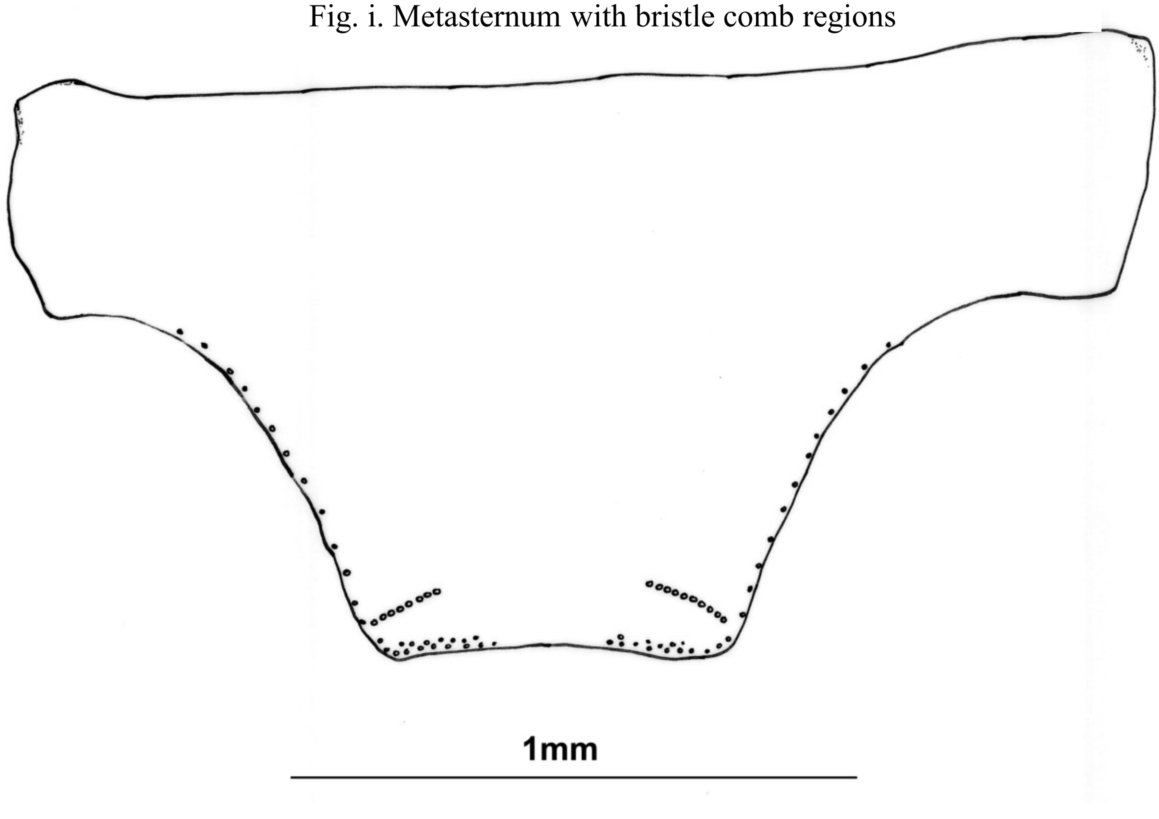


Fig. j. Trapezoidal shaped Urotergite X

PLATE V-*Ctenolepisma longicaudatum* Escherich,1905

Fig. l. Male genital region

Fig. k. Female genital region

macrosetae. Urotergite IX without bristle combs. Urotergite X (Plate V j) trapezoidal in shape with 1+1 bristle-combs of 8–10 macrosetae; few scattered setae towards the tip.

Urosternites I and II without setae. Urosternites III–VII with 1+1 lateral combs of 17–20 macrosetae. The lateral combs of urosternite VIII with 12–13 macrosetae. The number of macrosetae per bristle comb on urotergites and urosternites as in Table 8. Both sexes bear two pairs of stylets inserted on abdominal segments VIII and IX (Plate V k, l), armed apically with several strong spines. The ratio of length of styli IX/ length of styli VIII is 1.25–2.

The genital region of female is as shown in (Plate V k). Inner process of the coxite IX long, triangular and pointed at tip about 2–2.7 times longer than wide at its base and 2.7–5 times longer than the external process. Very long ovipositor with 40–48 divisions and its length measures 2.5–3.8 mm from the base of urosternite VIII; surpassing the apex of the internal processes of coxite IX by 2–3.75 times coxite IX length. Coxite IX in male with fringe of setae along internal margin; internal process about 1.8–4.5 times longer than the external process and 1.08–1.18 (L/W) times as long as broad at its base. Penis with numerous apical setae. Parameres absent (Plate V l).

This description has been published in Raphel, S. & Jose, J. (2025) Description of two synanthropic silverfish (Insecta: Zygentoma), *Ctenolepisma longicaudatum* (Escherich, 1905) and *Ctenolepisma rothschildi* (Silvestri, 1907), from Kerala, India. *Zootaxa*, 5570 (2), 309–324.

Ctenolepisma calvum Ritter, 1910

(Plate VI Figs. a- n)

Type material Examined

Neotype. India • 1 female; Kerala, Thrissur District, Valarkkavu, bookcase in a house; 22.IX.2022; *leg. coll*. Sheeba Raphel, specimen in alcohol; ZSI/WGRC, registration number I.R.-INV.26922.

Paraneotype. India • 1 female; same locality as neotype; 12.IV.2023; specimen in alcohol; ZSI/WGRC, registration number I.R.-INV.26923.

Order Zygentoma Börner, 1904

Family Lepismatidae Latreille, 1802

Subfamily Ctenolepismatinae Mendes, 1991

Genus Ctenolepisma Escherich, 1905

Redescription: Male unknown. Body of female elongated, more or less parallel sided, slightly tapering towards the posterior end. Maximum body length: 9.75 mm, excluding terminal filaments. Macrosetae pectinate. Epidermic pigment whitish dorsally, with slightly grey pigmentation; under artificial light the colour appears to be light straw, covered with dull golden scales. Maximum length of cerci up to 4.5 mm and that of paracercus up to 5.25 mm (Plate VI a, b, c, k).

Scales: Body covered with different types of scales; based on shape, they can be oval, globular and sub triangular; based on size, they can be large (more than 100 µm) and small (less than or equal to 3 µm). Based on rays, two types can be distinguished: one with 30–40 fine rays and the other with 10–16 widely spaced thicker rays. The three different shaped scales are both small and large sized, with either wide spaced or narrow spaced rays (Plate VI c). Femoral scales different in shape, elongated, with bifid apex (Plate VI k).

Head wider (maximum 1.1 mm) than long (maximum 0.70 mm). Setation similar to other species of the genus, with both clypeus and labrum having setal bushes of bifid and pectinate macrosetae (Plate VI c). Antennae up to 6 mm long. Maxillary palp 5 segmented; apical article 4.2–6.5 times longer than wide and 0.74–1.00 times as long as the penultimate article. Four segmented labial palps, its apical article is slightly longer than wide (L/W: 1.1–1.2) with five sensory papillae arranged in single row (Plate VI d).

Thorax wider than abdomen. Maximum thorax width: 2.12 mm; maximum thorax length: 3.1mm. Pronotal collar with 2 rows of bristle combs in the centre and single row towards the anterolateral corner. Pronotum (Plate VI e) with (8–9) + (8–10) bristle combs on lateral margins, each one with 2–5 macrosetae; posterior trichobothria of pronotum on inner side of the last lateral comb (N) and anterior trichobothria on inner side of N–4 or N–5 combs. Mesonotum (Plate VI f) with (8–9) + (8–9) lateral combs of 2–3 macrosetae; posterior trichobothria on the inner side of the last lateral comb (N) and anterior on the outer side of the penultimate lateral comb (N–1). Metanotum (Plate VI g) with (5–6) + (5–7) bristle combs of 1–3 macrosetae each on its lateral margins;

arrangement of trichobothria similar to that of mesonotum. All nota possess 1+1 posterior submarginal combs with 2–3 macrosetae.

Thoracic sterna as in (Plate VI h-j). Prosternum and mesosternum longer than wide; ratio L/W of the prosternum: 1.16–1.20, and ratio L/W of the mesosternum: 1.20–1.26. Metasternum as long as wide or slightly shorter (ratio L/W:0.92–1.00). All thoracic sterna with one pair of oblique antedistal combs; those of prosternum and metasternum with 7–10 macrosetae, and those of mesosternum consisting of 5–8 macrosetae. In all sterna, the macrosetae of the combs are arranged in two irregular rows, and few submarginal setae are also present between the comb and the margin.

Legs stout; femora shorter than tibiae and tarsi. Tarsi of hind legs moderately longer than that of other two legs. Protibiae 3.0–3.5 times longer than wide; mesotibiae 3.0–3.8 times longer than wide and about 1.14–1.19 times longer than the protibiae; metatibiae 3.6–5.2 times longer than wide and 1.5–1.6 times longer than the protibiae.

In the tarsi of the first legs, tarsomere I (T1) 3.5–5.5 times longer than the second one (T2), 3.7–4.8 times longer than third one (T3) and 3.2–5.6 times longer than fourth one (T4). In the mesotarsus, the first tarsomere is 5.3–6.0 times longer than T2, 4.0–6.4 times longer than T3 and 4.6–4.8 times longer than T4. In the metatarsus, T1 is 5.30–6.65 times longer than T2, 6.5–9.0 times longer than T3 and 4.0–5.6 times longer than T4. Femur with subtriangular elongated bifid scales (Plate VI k) on their inner side. Pretarsus with 3 claws, two lateral and one median.

Abdomen: Urotergite I with 1+1 infralateral combs of 2–3 macrosetae, urotergites II–V with 3+3 bristle combs, urotergites VI and VIII with 2+2 combs and urotergite IX bare. Urotergite X trapezoidal in shape (Plate VI l) wider than long (in the neotype, length: 0.56 mm and base width: 1.4 mm), with 1+1 subapical bristle combs consisting of 2–3 macrosetae; scattered setae arranged as in (Plate VI l). Urosternites I and II glabrous, urosternites III–VII with 1+1 lateral combs of 10–13 macrosetae. The number of macrosetae on abdominal bristle combs is indicated in Table 8.

Two pairs of styli inserted on abdominal segments VIII and IX (Plate VI b, m), apically with several spines. Styli IX longer than styli VIII; the ratio of length of styli IX/ length of styli VIII is 1.06–2.08. Inner process of the coxite IX long, triangular and pointed at tip, about 1.00–1.66 times longer than wide at its base and 2.0–2.4 times longer than the outer process. Ovipositor with 30–33 divisions; its length measures 1.36–1.76 mm from the base of urosternite VIII, surpassing the apex of the inner processes of coxites IX by 2–4 times its length. Comparison between *C. calvum* (Kerala Specimens) and *C. calvum* now redesignated as *C. phantasma* (Raphel *et al.* in press) (European specimens) with Ritter's original description is given in table 9. Table 10 presents a detailed comparison between *C. calvum* and *C. phantasma*.

Table 8. Number of macrosetae per bristle comb on urotergites and urosternites of *C. calvum* from Kerala

Segment	Urotergite			Urosternites
	Infra lateral	Sublateral	Submedial	Lateral
I	2–3	–	–	–
II	3–4	2	2	–
III	3–4	2	2	8–12
IV	3–4	2	2	10–13
V	3–4	2	2–3	10–13
VI	4–5	2	–	10–12
VII	4–5	–	2–3	10–11
VIII	4–5	–	2	4–6
IX	–	–	–	–

Table 9. Comparison of significant similarities and differences between *C. calvum* in Kerala, *C. calvum/ C. phantasma* sp. nov. in Europe and Ritter's description

Character	**In Kerala species**	**In European Species**	**In Ritter's description**
Relative length of Antennae to body*	Body longer than antennae	Body longer than antennae	Antennae almost as long as body
Relative length of caudal filaments to antennae*	antennae slightly longer than caudal filaments	antennae slightly longer than caudal filaments	Caudal appendages slightly longer than antennae
Number of segments in ovipositor	30-33	19-21	More than 35 as in diagram, not mentioned in text

Bristles of tergites	Well developed, but less than other Ctenolepisma	Well developed, but less than other Ctenolepisma	Rudimentary (probably overlooked by Ritter)
Shape of Urotergite X	Trapezoidal, wider than long	Trapezoidal, wider than long	Trapezoidal, wider than long
Number of Styli	Two pairs	One pair	Two pairs

* relative length can vary if appendages are broken in their apical part

Table 10: Comparison of *C. calvum* (Kerala, India) and *C. phantasma* sp. nov. (European specimens) described in Molero-Baltanás *et al.*, (2024) as *C. calvum*

Character	*C. calvum* from Kerala	*C. phantasma* sp. nov. from Europe
Body length	Up to 10 mm	Up to 12 mm (usually 8-9 mm)
Number of sensory papillae in labial palp	5 in a single row	3 in single row
Number of lateral combs on pronotum	(8-9)+ (8-10)	8
Number of macrosetae of lateral combs of pronotum	2–5	1–4
Number of lateral combs on mesonotum	(8–9) +(8–9)	(7–8) +(7–8)
Number of macrosetae of lateral combs of mesonotum	2–3	1–3
Number of lateral combs on metanotum	(5–6) +(5–7)	(5–8) +(5–8)
Number of macrosetae of lateral combs of metanotum	1–3	1–3
Number of macrosetae of posterolateral combs of thoracic nota	2–3	1–3
Anterior trichobothrial position of pronotum	N-4 (or N-5)	N-3
Anterior trichobothrial position of mesonotum	N-1	N-2
Number of macrosetae of prosternal combs	7–10	3–4
Number of macrosetae of mesosternal combs	5–8	4–5
Number of macrosetae of metasternal combs	7–10	4–6
Metasternum ratio length / width	0.9–1.0	0.87
Number of macrosetae of infralateral combs of urotergites II-VIII	3–5	3–4
Number of macrosetae of sublateral combs of urotergites II-VIII	2	2–3
Number of macrosetae of submedial combs of urotergites II-VIII	2–3	1–2

Number of macrosetae of lateral combs of urosternites	4–13	4–8
Number of pairs of styli	2 pairs (on VIII & IX)	1 pair (on IX)
Scales on femora	Subtriangular, elongate, bifid or truncate apically	Subtriangular, elongate, bifid or truncate apically

This description has been published in Zoosystema (Raphel *et al*. 2025).

PLATE VI- *Ctenolepisma calvum* Ritter, 1910

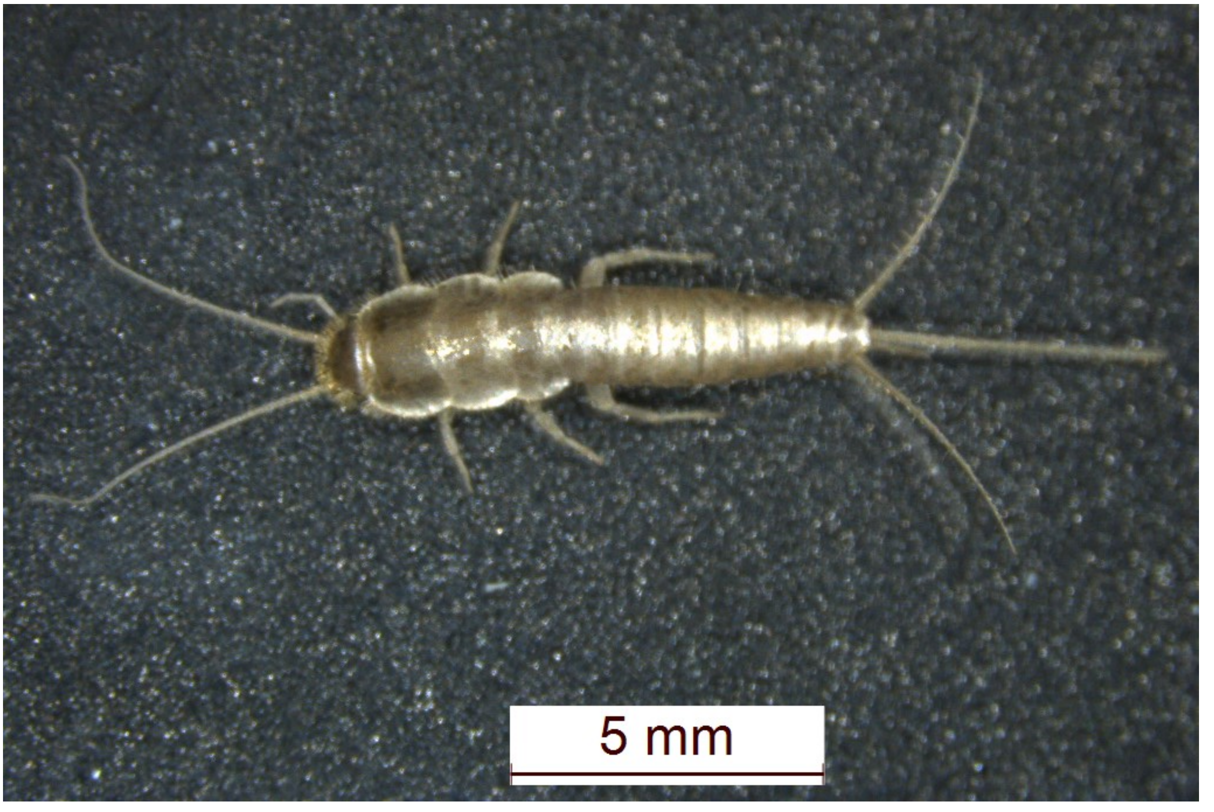


Fig. a Dorsal view of *C.calvum*

Fig. b Ventral posterior end showing two styli on left side, ovipositor and caudal filaments. On the right only the stylus on Coxite IX is present but it is not in erect position

PLATE VI- *Ctenolepisma calvum* Ritter, 1910

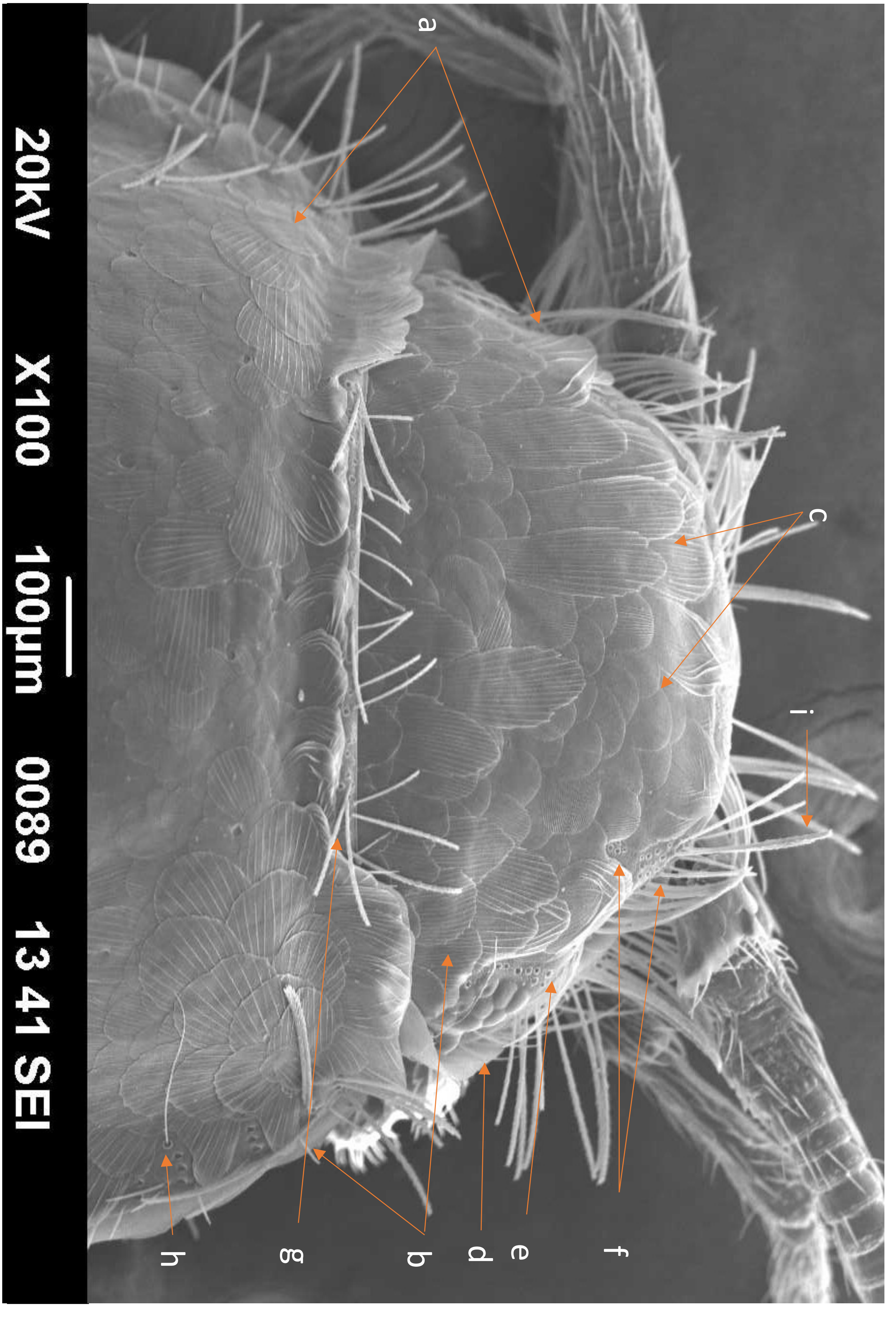


Fig.. c Head and part of pronotum of *C calvum* from Kerala, showing , a. arrangement of scales, b. two types of scales narrow and broad ribbed, c. two types of scales- elongated and rounded, d. ommatidia (right side) , e. periocular bristle comb sockets (right side), f. bristle sockets of frontal bush (right side), g. pronotal collar, h. trichobothrium at N5, i. feathered macrosetae

PLATE VI- *Ctenolepisma calvum* Ritter, 1910

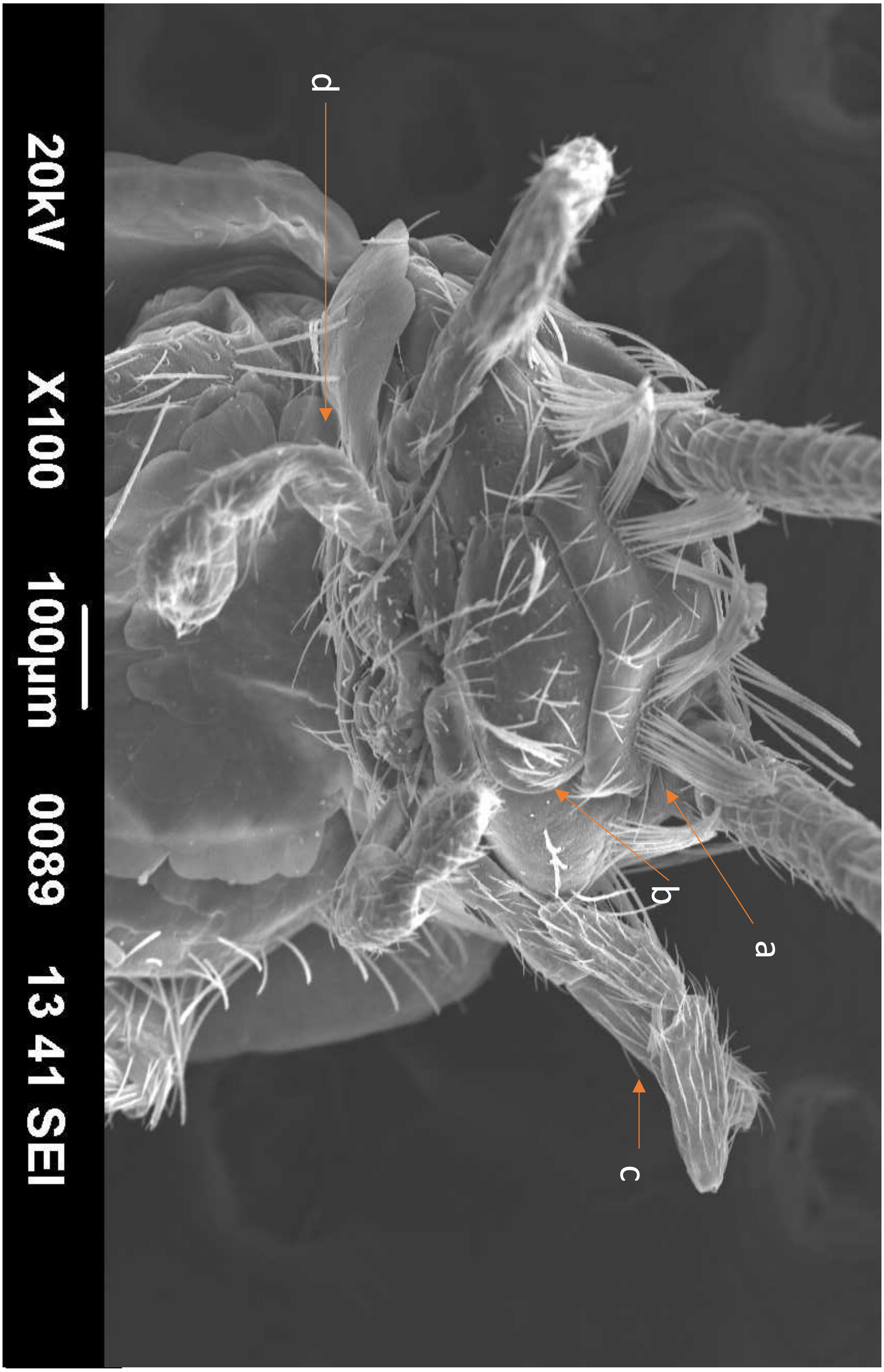


Fig. d Ventral view of head of of *C calvum* from Kerala showing a. setae on clypeus, b. setae on labrum, c. five segmented maxillary palp, d. four segmented labial palp

PLATE VI- *Ctenolepisma calvum* Ritter, 1910

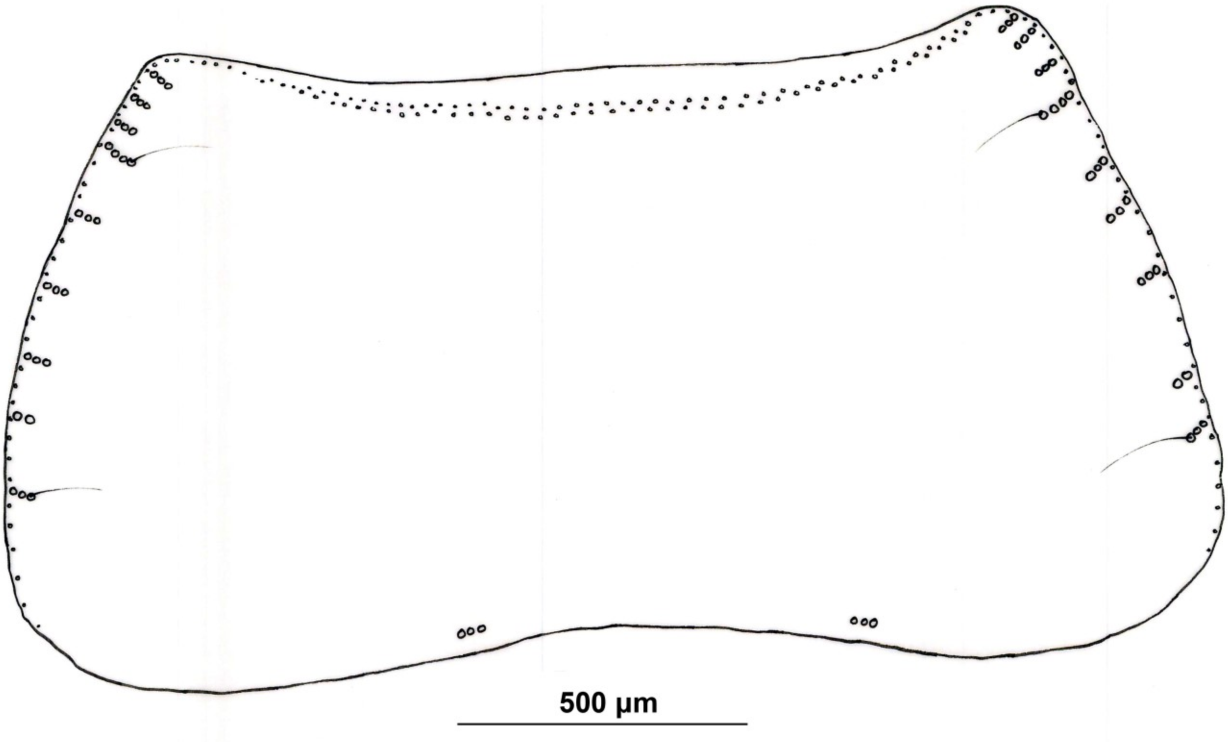


Fig. e Pronotum of *C calvum* from Kerala

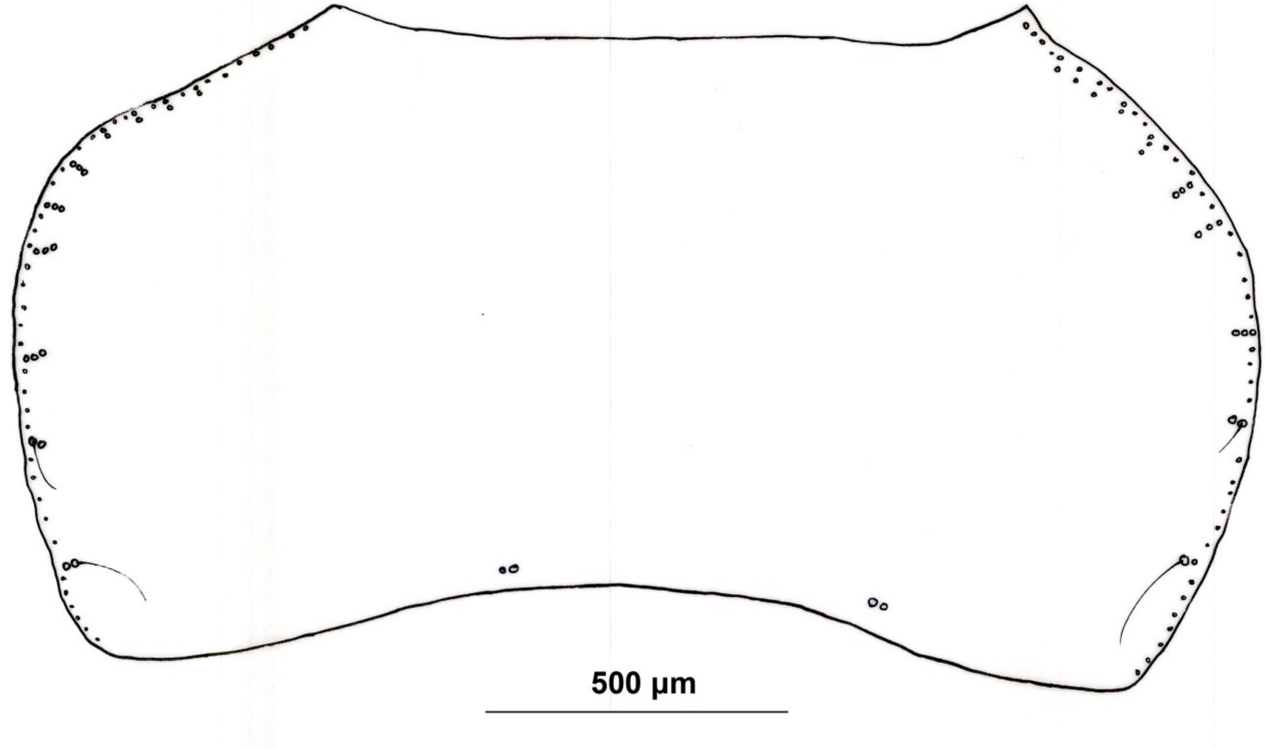


Fig. f. Mesonotum of *C calvum* from Kerala

PLATE VI- *Ctenolepisma calvum* Ritter, 1910

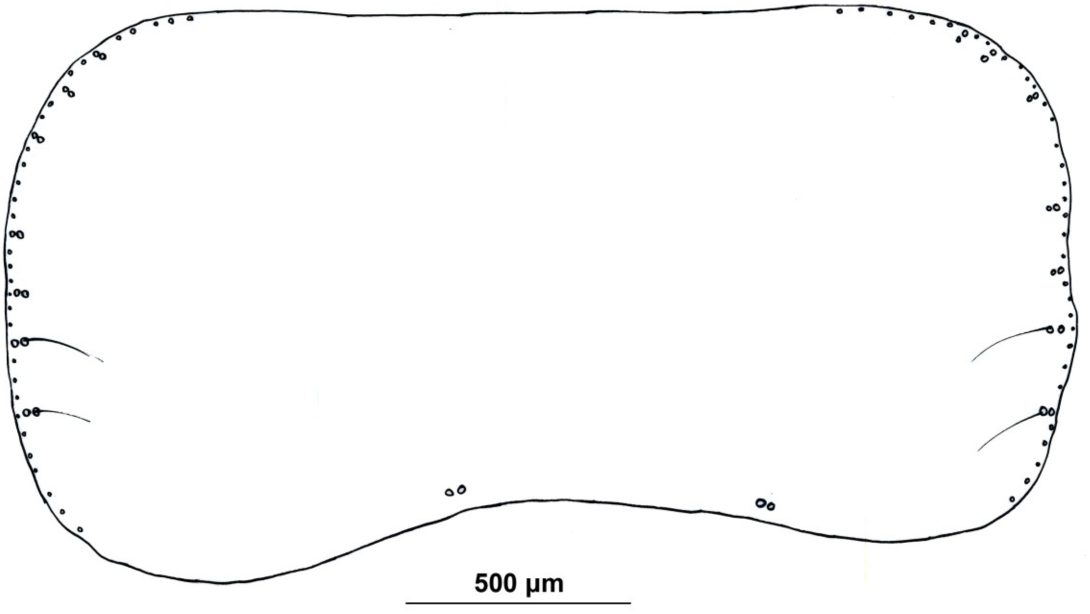


Fig. g Metanotum of *C calvum* from Kerala

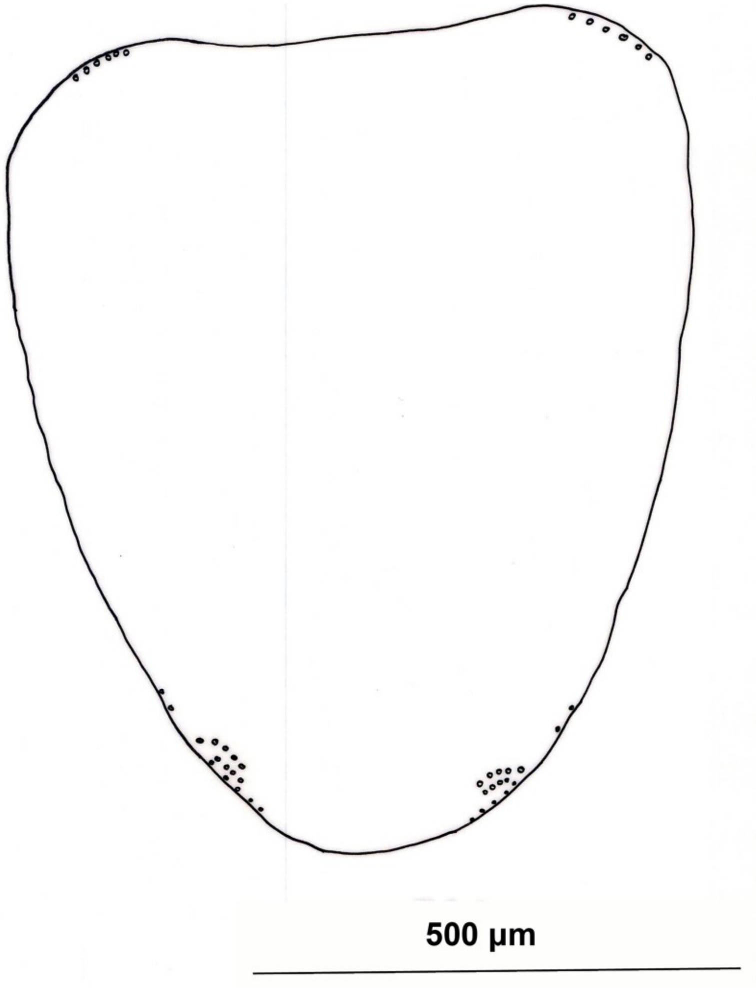


Fig. h Prosternum of *C calvum* from Kerala

PLATE VI- *Ctenolepisma calvum* Ritter, 1910

Fig.i. Mesosternum of *C calvum* from Kerala

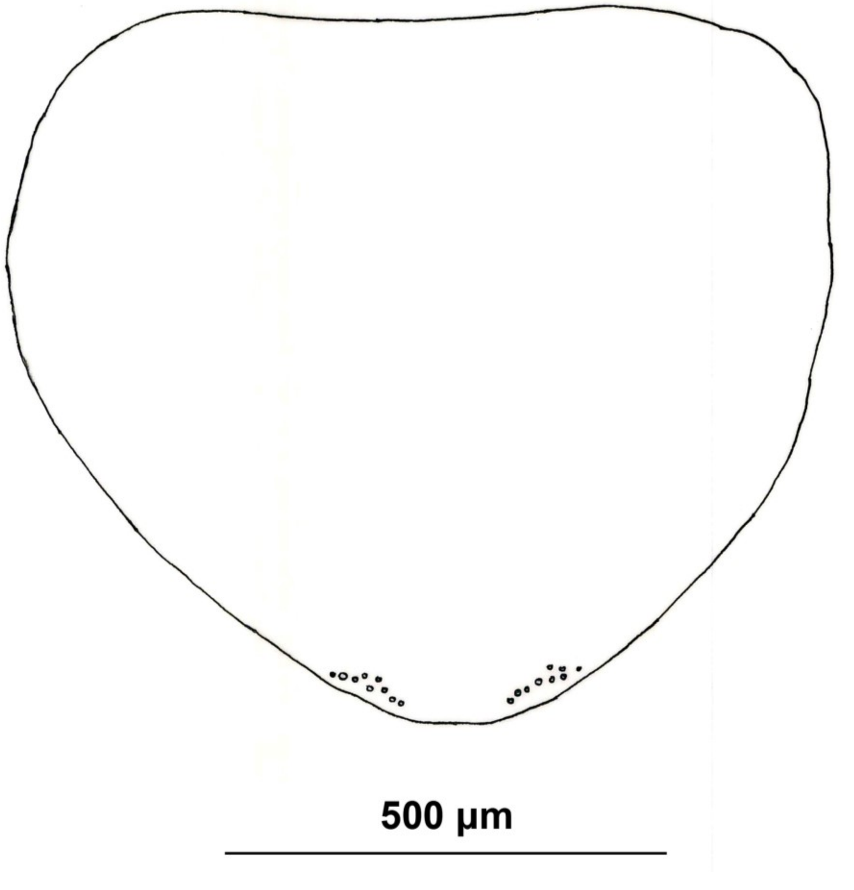


Fig. j Metasternum of *C calvum* from Kerala

PLATE VI- *Ctenolepisma calvum* Ritter, 1910

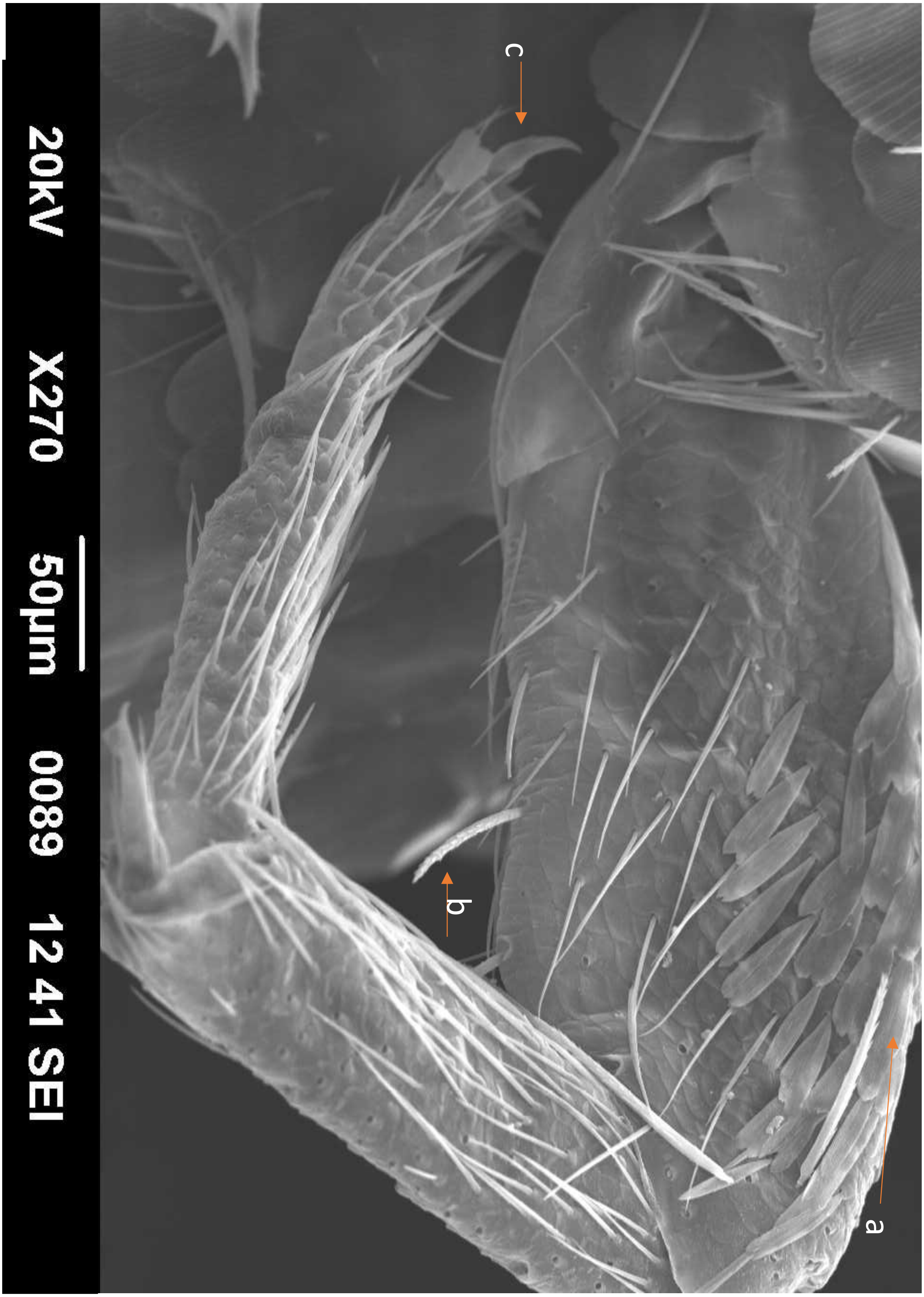


Fig. k Part of a leg of of *C calvum* from Kerala showing the femur with . elongated bifid scales (a) and pectinate macroseta. The pretarsus is also shown (c)

PLATE VI- *Ctenolepisma calvum* Ritter, 1910

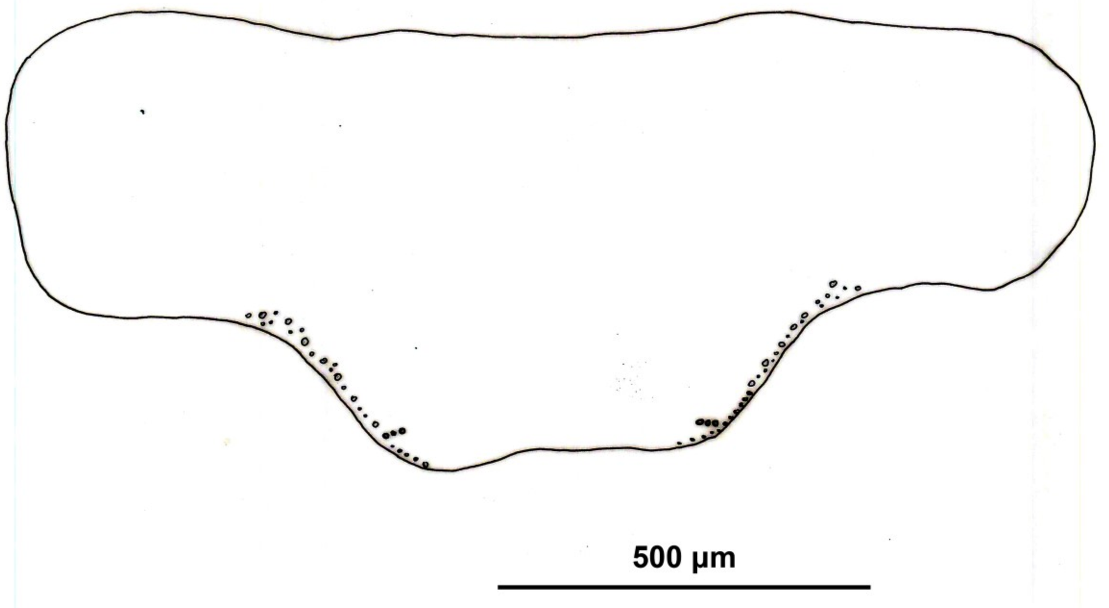


Fig. l Urotergite X of *C calvum* from Kerala

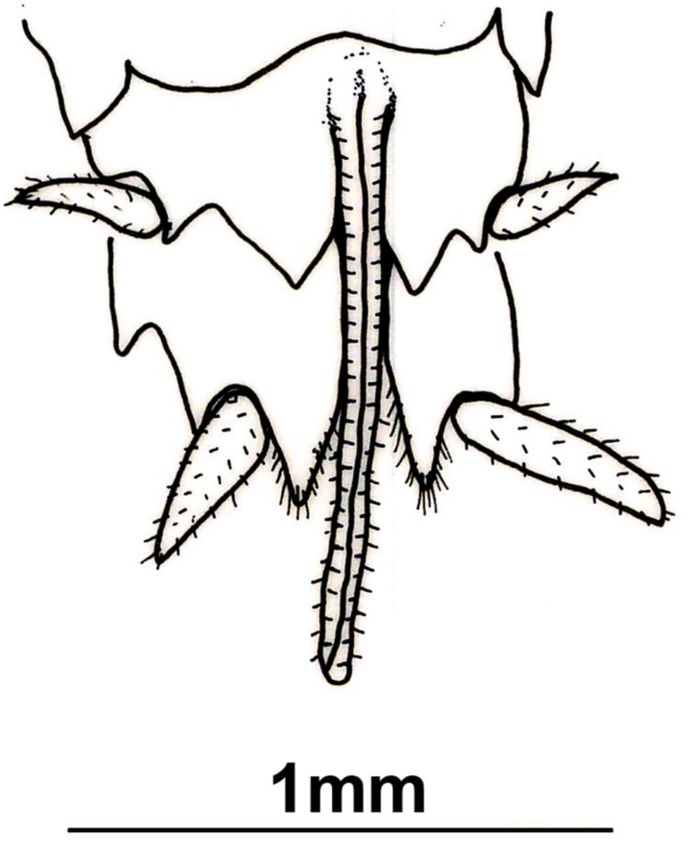


Fig. m Ventral view of abdominal segments VIII and IX of *Ctenolepisma calvum* from Kerala, showing the two pairs of styli and the ovipositor

Subfamily ACROTELSATINAE **Mendes, 1991**

***Acrotelsa* cf *collaris* (Fabricius, 1793)**

(Plate VII Figs. a-n)

Type material: Holotype: male, amidst old books and wooden articles dumped in terrace of a house, Velupadam, Thrissur district, Kerala India (10.432764°N and 76.347399°E), 07.iii.2023, *leg. coll*. Sheeba Raphel. Paratypes: 1 male and 1 female, same locality data as holotype, 07.iii.2023, collected Sheeba Raphel.

Order Zygentoma Börner, 1904

Family Lepismatidae Latreille, 1802

Subfamily Acrotelsatinae Mendes, 1991

Genus Acrotelsa Escherich, 1905

Acrotelsa collaris **(Fabricius, 1793)**

Specimens are currently housed in the museum at the department of Zoology, St. Thomas College (Autonomous), Thrissur (Ref: STCZGTS001 to 5). It will be transferred to Zoological Survey of India (ZSI), Calicut sub centre after and resolving taxonomic ambiguities regarding the genus and publishing it as a research article

Description:

Elongated and almost parallel sided body, dorso-ventrally compressed anteriorly and almost cylindrical posteriorly (Plate VIIa) Body length in males up to 14 mm and in females up to 14.5 mm. Epidermic pigmentation light golden yellow dorsally and lighter ventrally; a white band of scales extending dorsally between prothorax and mesothorax, posterior margins of urotergites VIII and IX and lateral margins of urotergite X; legs lighter coloured. Caudal filaments with dark brown coloured pigments interrupted by many light-coloured bands.

Scales brownish black; either globular or oval shaped with closely spaced rays; scales on posterior margins of the pronotum, urotergites VIII and IX and lateral margins of urotergite X white; ventral scales whitish yellow.

Head: Semi-circular anteriorly, wider (1.35-1.9 mm) than long (0.5-0.85 mm); bunches of light golden coloured bifid and pectinate macrochaetae, four each on either side of the vertex (**Plate VII b)**; numerous small macrochaetae on clypeus and labrum; eyes small, located well behind the antennae; maximum antennae length 9.7 mm with uniform brown pigmentation; maxillary palp is 5 segmented with apical article 6.3-7.5 times longer than wide and 0.7– 0.8 times as long as the penultimate article. Four segmented labial palps (L/W: 2); its apical article axe-shaped bearing five oval sensory papillae arranged in two rows, outer row with three and inner row with two sensory papillae each. Labial and maxillary palps uniformly covered with fine setae. Both palps covered by scales. Macrochaetae are positioned in whirl like patterns on the legs and cerci.

Thorax wider than abdomen. Maximum thorax width 4.5 mm. Maximum thorax length: 4.8 mm. Setal collar on anterior margin of pronotum absent. Pronotum (**Plate VII c)** with (20-27) +(20-27) bristle combs on the lateral margins with 2-3 macrosetae. A pair of setal tufts present behind the anterior margin of pronotum. Mesonotum (**Plate VII d)** lateral margins with (16-22) +(16-22) bristle combs with 2–3 macrosetae. Metanotum (**Plate VII e)** lateral margins with (14-16) + (14-18) bristle combs with 2–3 macrosetae. No bristle combs on the hind margin of pro, meso and meta notum.

Three trichobothrial areas noticed; anterior trichobothrial areas of the pronotum associated to last(N) lateral comb; posterior on outer side of N3 and N7; those of the mesonotum associated with inner side of last (N) lateral comb, outerside of penultimate (N-1) and outer side of N-3 combs.: those on the metanotum associated with innerside of last(N), outer side of antepenultimate(N-2) and on the outer side of N-4 lateral comb.

Prosternum anteriorly rectangular and posteriorly semi-elliptical in shape (**Plate VII b, f)** covered by fore coxae from below and has tuft of bifid setae at the baso-medial part; its length/width ratio is 1.35-1.5; No bristle combs on apical part.

Mesosternum (**Plate VII g)** heart shaped and its lateral margin with 33 to 34 macrosetae; its length/width ratio is 0.5-1.1, with no bristle combs apically.

Metasternum is cordate shaped with its anterior margin extended strongly on both sides and sharply pointed posterior end; its length/width ratio is 0.6-0.7 with no bristle combs apically. lateral margins with 30 to 40 macrosetae on both sides (**Plate VII h)**.

Leg: Stout; femora shorter than tibiae and tarsi. one strong seta distally near the junction of femora and tibiae; Tibiae and tarsi moderately longer than other segments (**Plate VII**

i) all articles provided with scales. Pretarsi with two curved lateral claws and a medial claw.

In males, protibiae 2.5-3.5 times longer than wide; mesotibiae 2.5-–3.5 times longer than wide and about 1.1–1.3 times longer than the protibiae; metatibiae 4-4.3 times longer than wide and 1–1.8 times longer than the protibiae. In the protarsus, Tarsomere I is 2 times longer than T2; 2.5-2.8 times longer than T3; 2.7-3.2 times longer than T4. In the mesotarsus, tarsomere 1 is 1.6-2 times longer than T2; 2.8-3 times longer than T3; 3.5-3.7 times longer than T4. In the metatarsus, tarsomere 1 is 2.3-3 times longer than T2; 3-3.3 times longer than T3; 4.8-5.2 times longer than T4.

In females, protibiae 2.2 -3.2 times longer than wide; mesotibiae 2.7-3.6 times longer than wide and about 1.2–1.4 times longer than the protibiae; metatibiae 3.7-4.9 times longer than wide and 1.5–2 times longer than the protibiae. In the protarsus, Tarsomere I is 1.7-1.8 times longer than T2; 2.2-2.5 times longer than T3; 3.1-3.4 times longer than T4. In the mesotarsus, tarsomere 1 is 2.2 times longer than T2; 2.3-times longer than T3; 3.7-4.2 times longer than T4. In the metatarsus, tarsomere 1 is 2.8-3.4 times longer than T2; 3.6-5.6 times longer than T3; 2.5 -3 times longer than T4.

Abdomen: The urotergite I with 2+2 bristle combs; both lateral and submedial combs composed of 4–6 macrosetae each. Urotergites II−VII with 3+3 bristle combs, each lateral bristle comb composed of 6-8 macro setae, sublateral comb with 5-6 macrosetae and submedial bristle comb with 4–6 macrosetae. Urotergite VIII with 2+2 bristle-combs, each lateral bristle comb composed of 7 macrosetae and sub-median bristle comb with 4–5 macrosetae. Beneath each comb, few simple setal hairs are also visible. Urotergite IX without bristle combs. Urotergite X (**Plate VII j)** triangular in shape and apically pointed (length 1.5-2.25 mm, base width 1.8- 2.3 mm, lateral margin length 2-2.6 mm, ratio length/width 0.75-0.95). It bears (6- 8) + (6-8) bristle-combs of 1–6 macrosetae.

The urosternites I and II without setae, III−VII with 2+2 bristle-combs, both lateral and median. Each lateral bristle comb composed of 5–9 macrosetae and median comb with 11–14 macrosetae. Urosternite VIII with 1+1 bristle combs of 15-20 macrosetae. The number of macrosetae per bristle comb on urotergites and urosternites are given in Table 11.

PLATE VII-*Acrotelsa* cf *collaris* (Fabricius, 1793)

Fig. a Dorsal *Acrotelsa* cf *collaris*

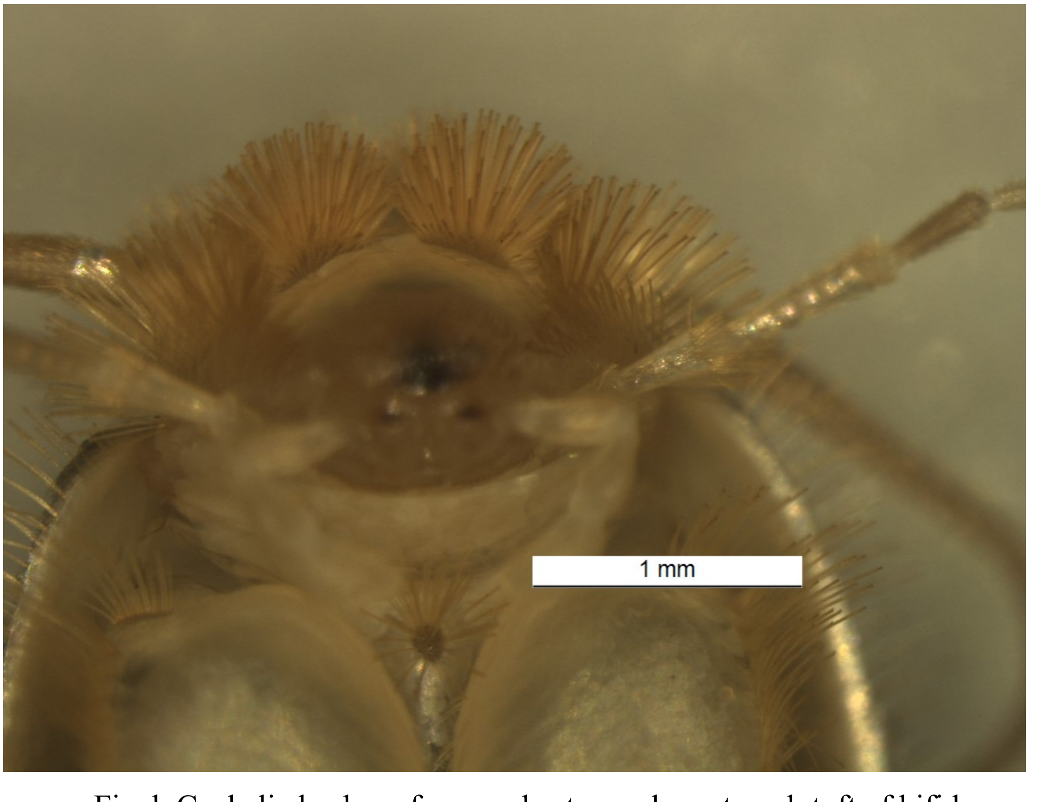


Fig. b Cephalic bushes of macrochaetae and prosternal tuft of bifid setae characteristic of *Acrotelsa* cf *collaris*

PLATE VII-*Acrotelsa* cf *collaris* (Fabricius, 1793)

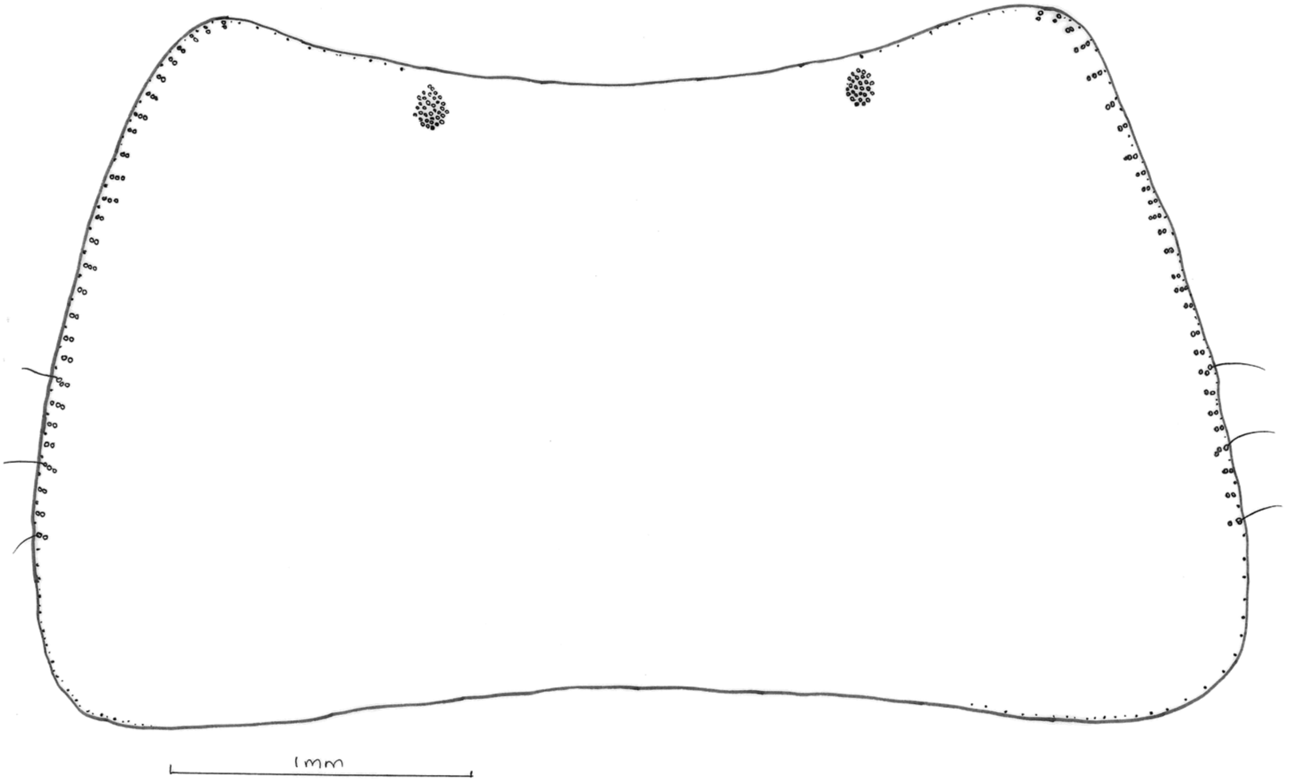
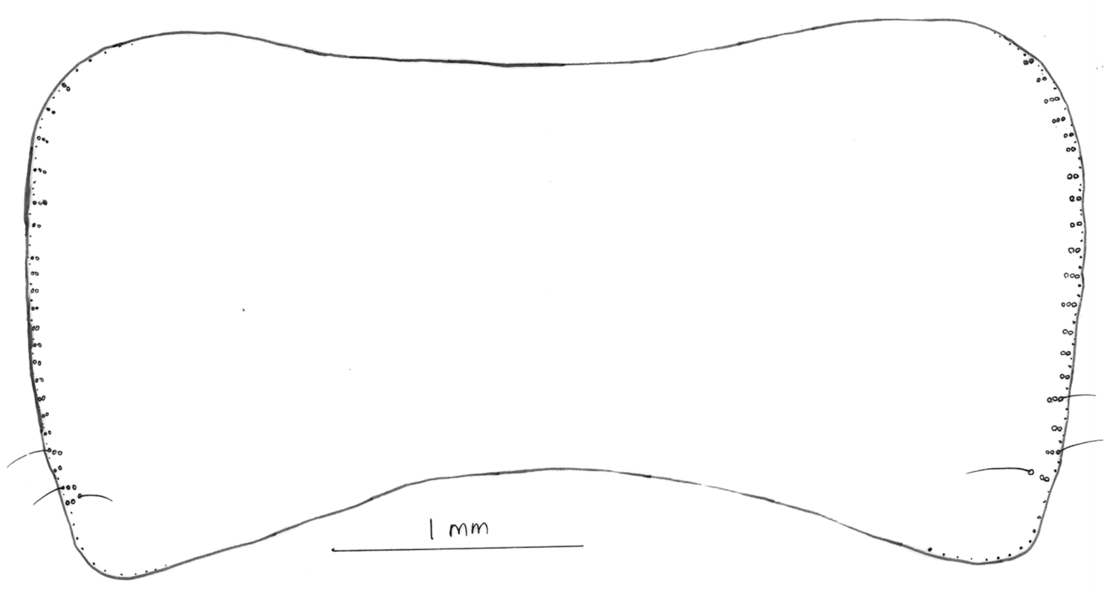


Fig. c Pronotum of with two setal tufts in the anterior margin in *Acrotelsa* cf *collaris*



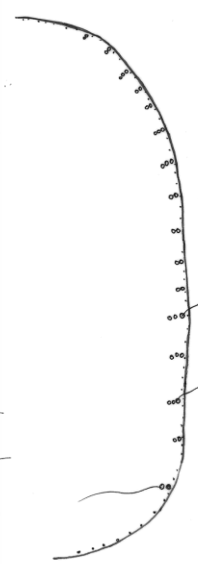


Fig. d Mesonotum of *Acrotelsa* cf *collaris*

Fig. e Metanotum of *Acrotelsa* cf *collaris*

PLATE VII-*Acrotelsa* cf *collaris* (Fabricius, 1793)

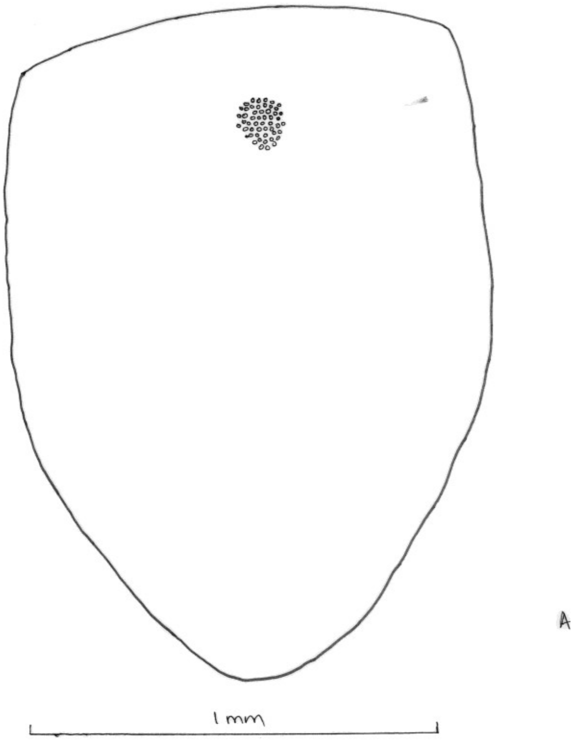


Fig. f Prosternum of *Acrotelsa* cf *collaris*

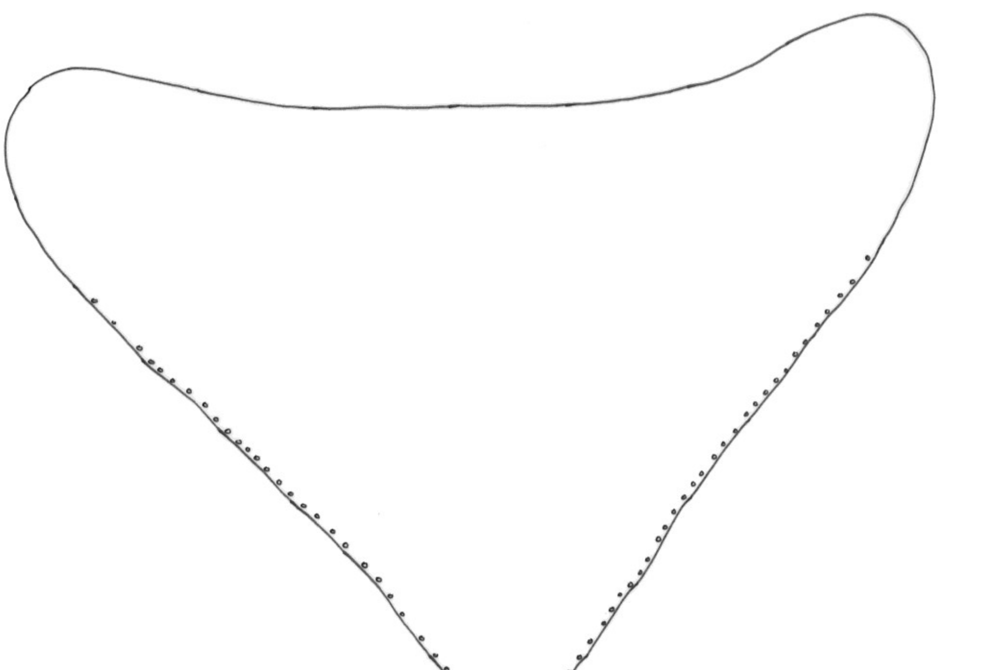


Fig. g Mesosternum of *Acrotelsa* cf *collaris*

PLATE VII-*Acrotelsa* cf *collaris* (Fabricius, 1793)

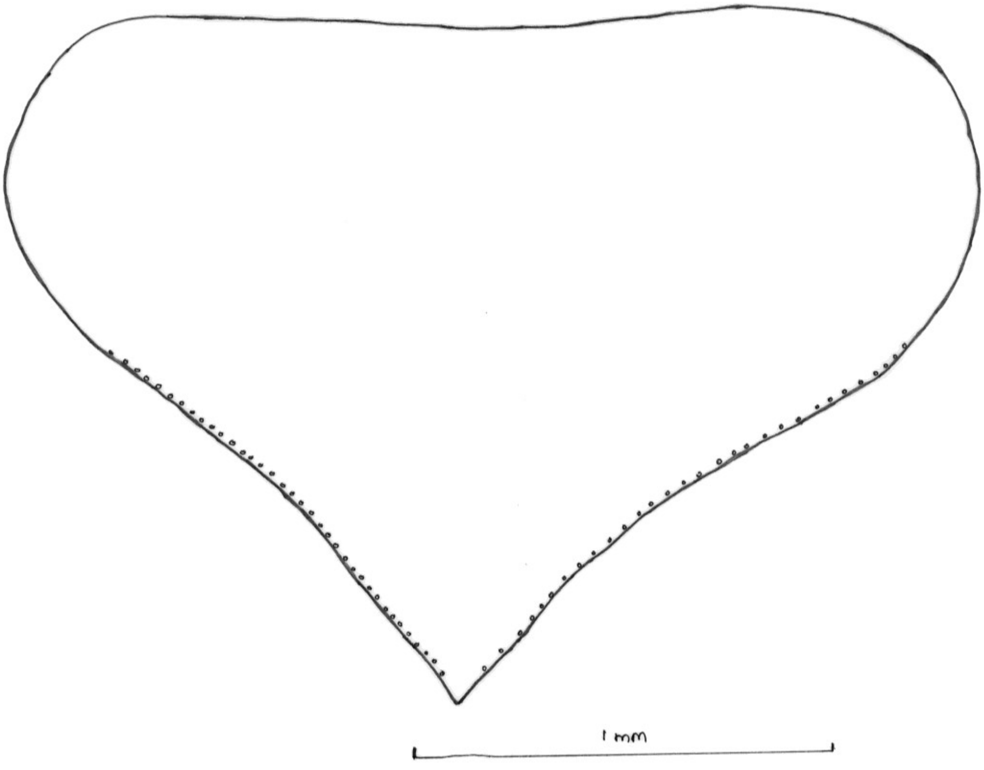


Fig. h Metasternum of *Acrotelsa* cf *collaris*

Fig. i Leg of *Acrotelsa* cf *collaris*

PLATE VII-*Acrotelsa* cf *collaris* (Fabricius, 1793)

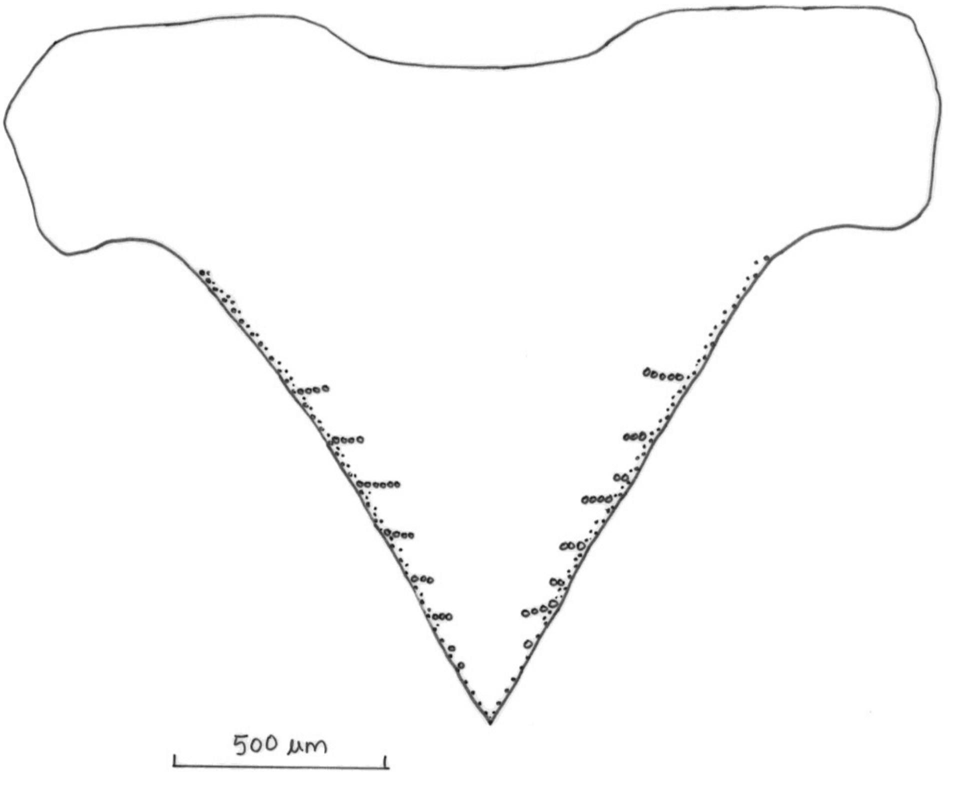


Fig. j Triangular urotergite X of *Acrotelsa* cf *collaris* with distinct tapering end and bristle comb arrangements

Fig. k Ovipositor and styli

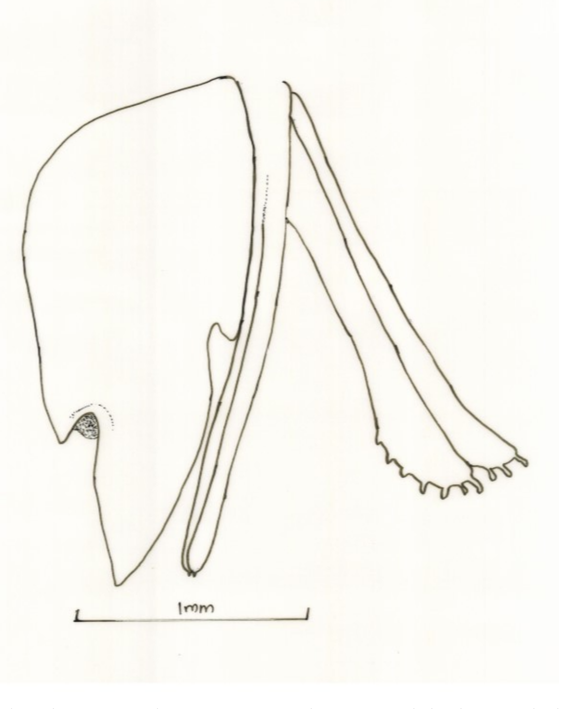


Fig. l Posterior gonapophyses with fossorial spines

PLATE VII-*Acrotelsa* cf *collaris* (Fabricius, 1793)

Fig. m Penis and styli

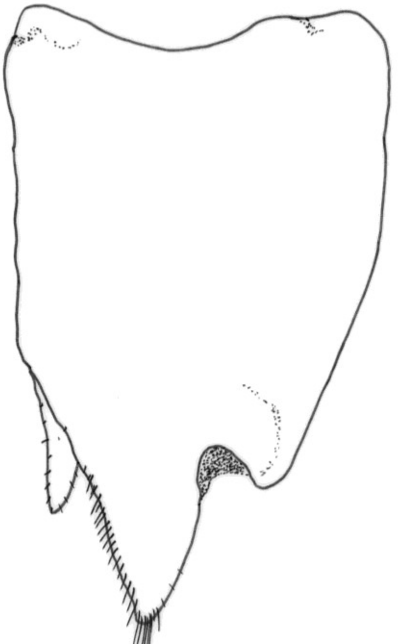


Fig. n. Paramere on IX th coxite characteristic of males in this species

Two pairs of styli on abdominal segments VIII and IX. The ratio length of styli IX/ styli VIII is 1-1.36; Inner process of the coxite IX long, triangular and pointed at tip, in the male about 1.1 times longer than wide at its base and 3.8 times longer than the outer process; in the female about 1.4 times longer than wide at its base and 3.1 times longer than the outer process.

Ovipositor measures 2-2.8 mm, from the base of urosternite VIII (**Plate VII k)** reaching almost up to the apex of the inner process of the coxite IX. The apical part of posterior gonapophyses with seven fossorial spines (**Plate VII l).** Male genital organ along with coxite IX (**Plate VII m)**. Presence of small paramera, not surpassing the inner process of ninth coxite (**Plate VII n)** The length/ width ratio of paramera with respect to inner process of coxite IX is 3.75. Paracercus 11.5 mm in length, cerci 8.5 mm in length.

Table 11. Number of macrosetae per bristle comb on Urotergites and Urosternites in *Acrotelsa* cf *collaris.*

Segment	Urotergite			Urosternite	
	Lateral	Sublateral	Submedial	Lateral	Median
I	4-6	_	4-6	_	_
II	7-8	5-6	4-6	_	_
III	7	5-6	4-6	6-8	12-14
IV	7-8	5-6	4-6	5-9	12-13
V	6-7	5-6	4-6	7-9	12-14
VI	7-8	5-6	5-6	7-8	11-12
VII	6-7	5-6	5	7-8	12-13
VIII	6-7	--	4-6	15-20	_
IX	--	--	--	--	--

Table 12: A comparative study of major characters of Acrotelsa specimens from Kerala. and *Acrotelsa collaris* (Hazra *et al.* 2022) and *Acrotelsa collaris* in (Molero-Baltanas *et al*. 2024)

Character	*Acrotelsa* cf *collaris (Kerala sps)*	*A.collaris (Hazra et al. 2022)*	*A.collaris (Molero-Baltanas et al. 2024)*
Maximum Body length observed	14.5 mm	8.1mm	18 mm
Number of macrochaetae of lateral combs of thoracic nota	2-3	1-3	Data not available
Number of macrochaetae of posterolateral combs of thoracic nota	Nil	Nil	Nil
Number of apical bristle-combs on pro, meso and meta sternum	Nil	Nil	Nil
Scales on femora	Present	?	present
chaetotaxy of urotergite I	(2+2) Lateral and submedial	(2+2) Sublateral and submedial	1+1 (sublateral)
chaetotaxy of urotergite VIII	(2+2) Lateral and submedial	(2+2) Sublateral and submedial	Data not available
Chaetotaxy of urosternite VIII	1+1	2+2	Data not available
Macrochaetae of lateral combs of urosternite VIII	15-20	6-7	Data not available
Shape of last abdominal tergite	Triangular	Triangular	Triangular
Chaetotaxy of last abdominal tergite	1+1	1+1	1+1
Number of lateral combs of last abdominal tergite	(6- 8) + (6-8)	5+6	(7–9) + (7–9)
Number of bristle combs on lateral margin of pronotum	(20-27) +(20-27)	16+16	Data not available
Number of bristle combs on lateral margin of mesonotum	(16-22) + (16+22)	12+12	Data not available
Number of bristle combs on lateral margin of metanotum	(14-16) + (14-18)	11+12	Data not available
Trichobothrial position of pronotum	N, N-3 & N-7	N & N-2	Data not available
Trichobothrial position of mesonotum	N, N-1 & N-3	N	Data not available
Trichobothrial position of metanotum	N, N-2 & N-4	N & N-1	Data not available
Relative length of ovipositor with respect to inner process of coxite IX	not exceeding the apex of the inner process of the ninth coxite	just surpassing the apex of the inner process of the ninth coxite.	not exceeding the apex of the inner process of the ninth coxite

2.4.3. Phylogenetic study of synanthropic Zygentoma from the study area

DNA sequence analysis and phylogeny of *T. smithi*

Both the Maximum Likelihood trees and Bayesian Inference trees had similar topology. *Thermobia smithi* sp. nov. was placed as a sister-group to *T. nebulosa* from Namibia. *T. domestica* was more distant (Fig. 13). Interspecific DNA distances between *T. domestica* and *T. smithi* sp. nov was 16 %.

COI sequence of Ctenolepisma species from Kerala and comparison with other previously sequenced specimens with confirmed identification as per Molero Baltanas *et al.*, 2024.

The COI sequences of the *C. calvum* from Kerala were compared with the GENBANK COI sequences of Japanese and European specimens previously identified as *C. calvum,* (LC719153, LC719154, LC719155, LC719156-Japan, OP028702, OP028703-Poland) following Molero-Baltanás *et al.,* (2024). Sequences affiliated to Japan were 100% similar to each other whereas sequences from Poland were similar to each other too. Sequence (sample ID gbs006285) of freshly dissected and identified specimen from Spain (courtesy Dr. Rafael Molero-Baltanás) showed only a very slight difference when compared to the sequences from Japanese and European specimens which indicated that the specimens of Japanese, Polish and Spanish affiliation were the same species. But the he Kerala specimens showed only 82.83% and 82.87% similarity with the Japanese and European specimens respectively but was placed distantly when compared to other *Ctenolepisma* species.

In addition to the morphological and chaetotaxic uniqueness, COI sequencing indicated that Kerala specimens were a different species from both the *C. calvum* identified from Europe and Japan and other synanthropic species of *Ctenolepisma* with available sequences (Fig. 14)

The COI sequences for *C. longicaudatum* and C. rothschildi showed very high similarity to that of COI specimens confirmed as for *C. longicaudatum* and *C. rothschildi* (OR732097.1. and of OR732095.1) following Molero Baltanás *et al.*, (2024) confirming their identification.

The sequences of Acrotelsa were not used in the phylogeny study because no confirmed sequence of *Acrotelsa collaris* was available in public databases.

Phylogenetic trees constructed was not for the purpose of studying phylogenetic lineages but to confirm the distinct nature of a species and to support the taxonomic work presented in the previous section.

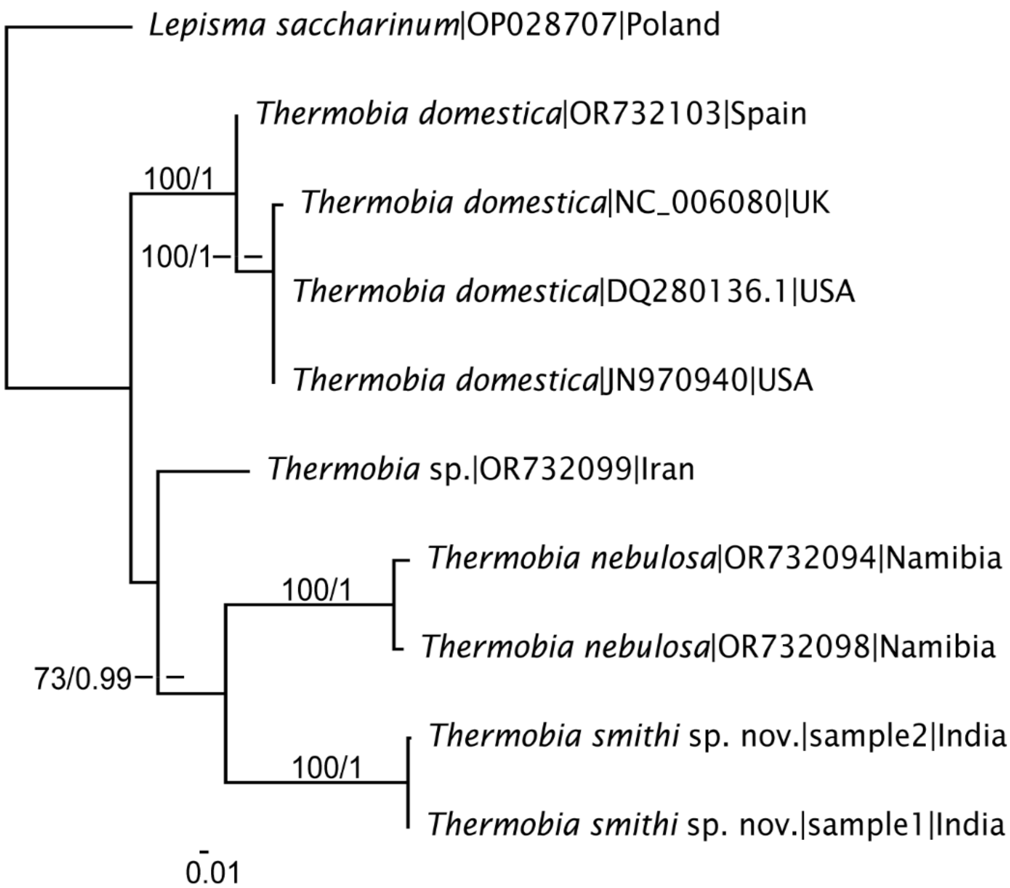


Fig. 13 Maximum Likelihood (ML) tree for Thermobia Genus (constructed following Mitchell (pers. comm.)

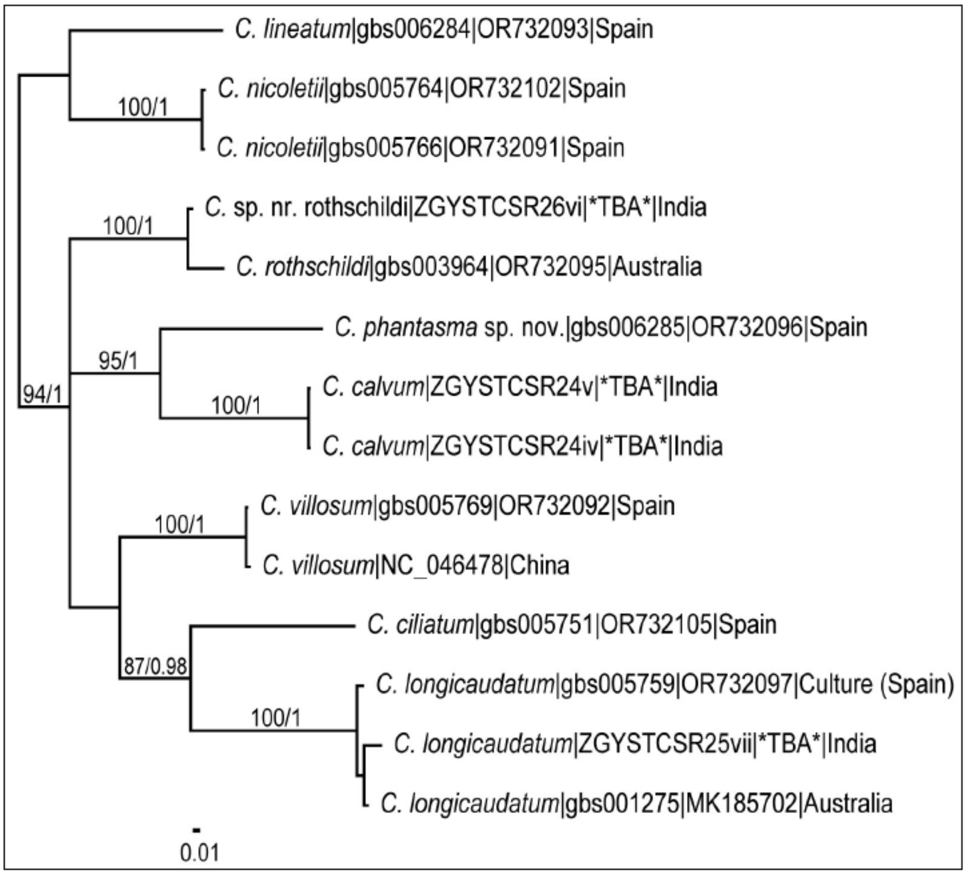


Fig. 14. Bayesian tree of COI sequences of Ctenolepisma species (constructed following Mitchell (per.comm.))

2.5. Discussion

Despite being cosmopolitan in distribution and widely studied, there is a lacuna in the silverfish research especially because of microscopic diagnostic characters and wrong molecular data in public databases and reasons already mentioned in **Section 1.5** of Chapter 1.

Molero Baltanás *et al.*, (2024) mention that the true distribution and morphological diagnosis of synanthropic silverfish are still imprecise. In this light all studies and observations on Zygentoma contribute in unravelling the intricacies of this group.

2.5.1. *Thermobia smithi*

At least some specimens of Thermobia in India were misidentified as *T. domestica* for a long time because many diagnostic characteristics were overlooked.

In many previous studies relevant diagnostic characteristics could not be examined due to lack of resources. As a result, a detailed comparison of all such studies with *T. smithi* sps nov. is not possible at this time but a summary of the main differences between all previously known Thermobia species and *Thermobia smithi* sps nov. is presented in Table 6.

DNA sequence analysis of COI sequences also distinguished *T. smithi* sp. nov. from the three other species of Thermobia. DNA variation found among Thermobia species than within Thermobia species, as sampled to date was higher indicating that DNA barcoding and multiple sequence analysis through phylogenetic trees is an efficient method for distinguishing species of Thermobia, as in the case of other Zygentoma, (Smith *et al.*, 2019) and Smith and Mitchell (2024). Although care must be taken when assessing sequences in public databases to ensure that the specimens they are derived from have been reliably identified (MoleroBaltanás *et al.*, 2024).

2.5.2. *Ctenolepisma rothschildi* and *Ctenolepisma longicaudatum*

C. rothschildi and *C.longicaudatum* specimens from Kerala had almost all the major characteristics described in Molero Baltanas *et al.*, (2024) which includes chaetotaxy of different nota, sterna and tergites; trichobothrial positions on nota; shape and length-width aspect of sterna, scales on coxae and femora, and two pairs of styli. Minor differences were due to intraspecific variations.

The *C. longicaudatum* specimens collected from Kerala could be classified into two populations based on the number of sensory papillae on the last article of labial palp. This follows Molero Baltanás *et al.*, (2024) who mention that *Ctenolepisma longicaudatum* usually has five labial papillae but some specimens having more labial papillae have also been observed. This study also concurred with their conclusion further examination is needed to understand if the two morphs could belong to two different species.

Another surprising observation was that *C. rothschildi* was very commonly observed in the study area and caused substantial damage. Yet there has been no formal report from India and evaluation of the damage caused by it has not been done.

COI sequence analysis in this study also concurred with Molero Baltanás *et al.*, 2024 which indicate intraspecific molecular variation for reliably identified species. This could be caused by many reasons including difference in protocols, inadequacy of existing primers (Gibson *et al.,* 2011), geographical variations, etc.

2.5.3. *Ctenolepisma calvum*

Specimens from Kerala differed significantly from modern descriptions of *C. calvum* recorded from Europe and Japan but showed more similarity to Ritter's original description (1910) of *Peliolepisma calva.*

Many relevant characters for the taxonomy of Ctenolepismatinae were not mentioned in the antique description by Ritter, such as the number of papillae of labial palps, the shape and chaetotaxy of thoracic sternites, etc., possibly due to lack of appropriate microscopes but many characters such as the trapezoidal shape of the urotergite X etc matched with those shown by the specimens from Kerala (Tables 9, 10). The European specimens and their descriptions showed similarity in body size, body colour, femoral scales, shape of urotergite X and chaetotaxy of the nota (Table 10). But while the European descriptions showed many similarities with specimens from Kerala and with the description of Ritter, there were two significant differences in the ovipositor and number of styli. Ritter's description and drawing of genital region showed two pairs of styli inserted on coxites VIII and IX, with long ovipositor surpassing the apex of ninth styli. The number of divisions, on the drawing of the ovipositor was higher than 35. These characters were closer to the specimens of this study than to those described for European specimens previously attributed to *C. calvum*, which had only one pair of styli and a shorter ovipositor only slightly surpassing the apex of ninth styli and having 19–21 divisions. COI sequence analyses also proved specimens from Kerala and Europe had distinct species identity.

In this context, Zygentoma experts who had recently published articles on *C. calvum* were consulted who re-examined the specimens in Europe. Based on their inputs the following was concluded.

"Molero-Baltanás *et al.*, (2024), when examining European specimens, interpreted that Ritter could have made a mistake when illustrating the genital region of their *Peliolepisma calva*, because he also collected *C. longicaudatum* in the same locality, and this genital region is more similar to this frequent synanthropic species than to the one that is recently spreading over Europe." But the discovery of the specimens of

Kerala with more similarities to the Sri Lankan taxon forced European experts to reconsider the identity of the taxon studied in Europe."

In collaboration with the European expert, specimens from this study and European specimens were compared. Table 10 presents a comparison of remarkable morphological traits of both species.

Thus, it was concurred that the specimens in this study and the specimens from Europe were two distinct species. The specimens from Kerala were reassigned as a neotype of *C. calvum* and the European specimens were designated as a species new to science.

2.5.4. *Acrotelsa cf collaris*

Acrotelsa is a monotypical genus with ***Acrotelsa collaris*** as the only known species from all over the world. On comparison with a recent publication from India (Hazra *et al.*, 2022) specimens shows some differences. But in addition to characteristics of Acrotelsatinae, such as prosternum largely covered by fore coxae from below, and with a tuft of macrochaetae centrally; ovipositor with fossorial spines apically on posterior gonapophyses; male with parameres, the specimens also show some diagnostic characteristics of the species (Appendix II, Table 12). Reliable COI sequences were not yet available in public databases. On consultation with global experts, it was advised that currently the name ***Acrotelsa collaris*** may be retained for the specimens until further details of specimens from other parts of the world can be obtained through collaborative work.

For the purpose of this thesis the specimens have been designated as ***Acrotelsa* cf *collaris***

2.6. CONCLUSION:

Jana and Hazra (2023) reported that there were no published reports of silverfish Lepismatidae from Kerala State. In this context all discoveries and progress in Zygentoma research from the state is of great consequence. Examination of the synanthropic Zygentoma in Kerala led to the discovery of a hitherto undescribed species *Thermobia smithi* sps. nov. Neotype for *C. calvum* was described and specimens from Europe wrongly attributed as *C. calvum* was designated as a new species *C. phantasma* sps. nov. The description of *C. calvum* is the first report of the species from

India and is the 10th from the genus *Ctenolepisma*. Detailed description of *C. rothschildi* and *C. longicaudatum* both of which are first reports from the state also significantly adds to our understanding of Zygentoma from India. For specimens designated as *Acrotelsa* cf *collaris*, it would be better to treat it as *species inquerida* -a species being investigated further due to discrepancies in its characteristics and relationship to other known species. While existing protocols, tools and techniques to find solutions to Zygentoma taxonomy must be explored, global collaborations are also necessary to accurately document the presence of these species and evaluate their pest status. All findings from this chapter except for *Acrotelsa* cf *collaris* have been published as research articles (Raphel *et al.*, 2024; Raphel and Jose, 2025; Raphel *et al.*, 2025).

CHAPTER 3

BEHAVIOUR IN FIVE SPECIES OF SYNANTHROPIC SILVER FISH FROM KERALA.

Chapter 3

Behaviour of Five Species of Synanthropic Silverfish from Kerala

3.1. Introduction

Silverfish have been subjected to many experiments to document their behaviour especially focussing on feeding (Modder, 1962, 1969, 1975; Parmentier *et al*.,2024), courtship (Ambrose, 2004; Walker *et al.*, 2013), predation, effect of abiotic factors on the circadian rhythm (Heeg, 1967a, 1967b; Kaufman,1996) microhabitat selection (Tremblay and Gries, 2006), maze learning behaviour and mating behaviour (Inada *et al.*, 2023) and pheromone-based arrestment behavior among *Lepisma saccharina*, *C. longicaudatum* and *Thermobia* (Woodbury and Gries, 2007, 2008 2013a, 2013b, 2013c: Woodbury *et al.*, 2013). But majority of these studies have focused on a few species listed above.

Due to the secretive nature of these insects, it is very difficult to study the natural behaviour of silverfish *insitu*. The behaviour of five synanthropic silverfish species (*Ctenolepisma longicaudatum* Escherich 1905, C. *rothschildi* (Silvestri 1907), *C. calvum* Ritter 1910, *Thermobia smithi* Raphel *et al.,* 2024 and *Acrotelsa* cf *collaris* (Fabricius, 1793)) documented from the study area was examined by creating a pseudo-natural culture set up. It is anticipated that learning more about the behaviour of this pestilent insect will assist in better control and prevention of silverfish infestations. Moreover, there have been no behavioural studies on silverfish from India and detailed studies at global level are also few and far between.

3.2. Methodology

3.2.1. Method of collection

Collection of silverfish has been detailed in the previous chapter. After each field trip, live insects were sorted based on size and morphological characters visible to the naked eye, in the laboratory. Insects collected from same substrate of a collection site on the same date and having same external appearance were initially kept in one rearing box (PLATE VIII). Silverfish were randomly selected from each box, sacrificed, dissected and microscopically examined to confirm species identification following Molero

Baltanas *et al.*, (2024). Some specimens were preserved in plastic vials in 100% alcohol for further taxonomic studies and the rest were reared (Fig. 15).

3.2.2. Method of rearing and culture

Grouped and identified silverfish (25-50) were housed in food grade plastic boxes ((38 x 25 x8.5 cm) at room temperature ranges from 28°–34°C (as explained in the earlier paragraph) to facilitate the observation of their specific behaviour and life cycle. Food (tissue paper, old newspapers, freshly printed newspapers, pulses, rolled oats and broken wheat) was introduced once a month. Folded and rolled paper was placed to provide hiding spaces. Water was not kept in the rearing boxes but moistened cotton balls were kept at the box corners (Smith, 2020) during summers. The cotton balls were replenished with water droplets every week. Cypermethrin (1%) chalk was smeared outside the rearing boxes to prevent insect predators.

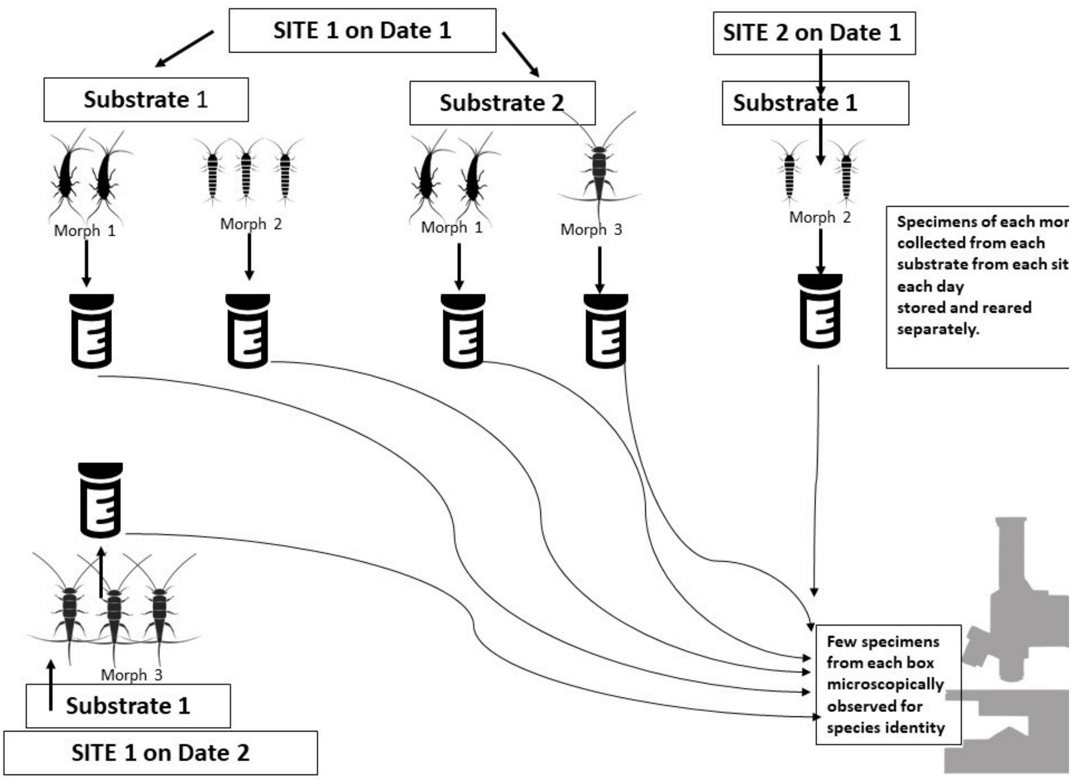


Fig. 15. Diagrammatic representation of how different species have been identified for the purpose of this study

PLATE VIII- REARING SET UP

3.2.3. Method of observing and quantifying behaviour

A single silverfish (at a time) was randomly selected for observation in a rearing box and observed for 600 seconds (10 mins). Time of the observation was randomly chosen between 6 am and 9 pm. Video recordings of individual selected for observation were made to facilitate classification and timing of behaviour. The type of behaviour and timings were then consolidated in excel sheets and used for statistical analysis. The different types of behaviour observed are listed in Table 1. Silverfish in aggregation formation were not included in the timed observation due to arrestment behaviour persisting over extended periods. Behaviour which was observed outside of timed observation periods has also been described here.

Aggregation and arrestment observations could not be completed in 10 min observation periods. Instead, observations were noted during opportunistic sightings of these behaviours. Similarly separate experimental set ups were designed to study cannibalism and damage rate on different papers, etc.

3.2.4. Method to study dispersion pattern

A grid of squares, each square measuring $4cm^2$ was drawn on transparent sheet and placed above the lid of the rearing boxes. The arrangement was left for some time to ensure that the silverfish were not disturbed. Photograph of the arrangement was taken from the top so that the number of silverfish in each grid could be counted. Dispersion pattern was determined using Dispersion index (Variance/mean ratio) following Krebs (1999).

3.2.5. Method to study effect of starvation and different diets on cannibalism

To understand if cannibalism was induced by different diets or lack of food and to observe species specific differences, a simple experiment was devised. Silverfish of each morph was kept in different rearing boxes and observed for 30 days. Four types of diet plans were followed for each species, *viz*; complete starvation where no food source was provided; tissue paper; printed old paper and printed new paper. These three types of paper were added because during collection from sites and in rearing boxes silverfish preferred tissue paper and old printed paper over new printed paper in that order. Rate of cannibalism per box was calculated by the following method.

No of deaths= Number of silverfish at the beginning of the experiment - number of silverfish after 30 days#.

Percentage of cannibalism= (number of deaths/initial number of silverfish) x 100

This number also included any intact dead bodies to ensure that natural or starvation induced deaths were not attributed to cannibalism.

3.2.6. Method to quantify damage on different types of papers

Nine silverfishes each belonging to the five species under study, were kept in different rearing boxes. Different types of papers- glossy paper, paper with matt finish, tissue paper, old printed paper (newspapers older than 3 years), new printed papers were cut into rectangles with an area of 440 cm^2 and provided as food. Three replicas of each experiment were kept for observation. Measurement of paper damage were taken after one month. Perforations caused by feeding were marked on a graph paper by overlaying the damaged paper on it. Perforations were observed only in tissue paper, and old and new newspapers. Therefore, the percentage of paper damaged was calculated only for these three types of papers.

3.2.7. Statistical Analysis:

Data was consolidated in MS Excel. Average time spent for each behaviour by each species was calculated. It was observed that four behaviours took most of the time for all species, therefore, these have been represented separately in Fig.16 and the rest of the behaviours were represented in Table 13. PerMANOVA with Euclidean distance was also performed after transforming (Centered Log Ratio) the compositional data to determine if there was significant difference in the behaviours of the species. It was visualized using PCoA (Principal Coordinates Analysis). One Way ANOVA (5% significance) was performed to interpret the observations of cannibalism experimental set. Two-way ANOVA was done to see if there was any significant difference between the damage caused by different species and in different types of papers. Diversity indices (Shannon_H, Evenness_e^H/S and Simpson's dominance) were calculated to understand the distribution of species and individuals among different substrates (books and papers, coconut husk, coconut shell, straw, pulses, wood, kitchen shelves, moist soil, cloth, roofing tile, etc). It was visualized in a PCA map. All statistical analysis was done using PAST ver 4.17.

3.3. Results

Twenty distinct behaviours were identified based on observations, which were classified into six general categories – feeding, locomotion, immobile, aggression, communication and other behaviours (Table 13). Behaviours placed in "other behaviour" category were rarely observed during timed observations.

Table 13: Behaviour types observed in laboratory reared synanthropic silverfish species

No.	Category of Behaviour	Behaviour	Brief Definition
1.	FEEDING	Feeding	any isolated feeding behaviour excluding cannibalism
2.		Cannibalism	feeding on members of the same species (PLATE XI)
3.		Co feeding	in contact with the same food particle with other silverfish, often leading to feeding (PLATE IXa)
4.	LOCOMOTION	Vertical movement	a silverfish attempts to move on a vertical substrate
5.		Running	characterized by fast movement on a horizontal surface
6.		Walking	characterized by slow movement on a horizontal surface
7.	IMMOBILE	Immobile	no movement at all for few seconds to hours but cannot be defined as arrestment because only one individual is involved
8.	AGGRESSION	Chasing	a silverfish chases another silverfish as without touching it
9.		Antennae fighting	aggressive interactions with other silverfish - waving of antennae, hitting with antennae and entangling and disentangling of antennae with that of the adversary (PLATE X)
10.		Antenna hitting	few taps with antennae to chase away other silverfish which disturb their resting or feeding behaviour (PLATE X)
11.		Hitting with caudal filament	few taps with caudal filament to chase other silverfish which touch on their caudal filament during their immobile or feeding time (PLATE X)

No.	Category of Behaviour	Behaviour	Brief Definition
12.	COMMUNICATION	Antennae-to-Antennae touching	antennae of two silverfish entangle together and remain still for a considerable period of time (more than 30 secs). (PLATE X)
13.		Antennae-to- Caudal filament touching	antennae of one insect touches/entangles with caudal filament of the fellow insects for a duration of few seconds or extends to considerable time (more than 30 secs) (PLATE X)
14.		Touching with body sides	sides of bodies of two insects stay in contact (PLATE X)
15.		Head-to-Head touching	while walking a silverfish makes head-to-head contact for a few seconds with another silverfish approaching from opposite directions and then move apart
16.		Touching on cerci	touching between cerci of two silverfish or between antennae of one silverfish and cerci of the second one (PLATE X)
17.		Antennae wagging	motionless insect wags its antennae for a few seconds when another silverfish is near (PLATE X)
18.	SPECIAL BEHAVIOURS	Thanatosis	evasive behaviour quick gliding movement, followed by immobility (Smith, 2017)
19.		Aggregation	many silverfish of a species group together with or without body touching (IXb)
20.		Arrestment	sudden cessation of all movement in aggregation or in response to stimuli

3.3.1. Comparison of behaviour in different species

Remaining immobile, walking and feeding was the most common behaviours. All the five silverfish species remained immobile for long periods of time. *C. rothschildi* and *C. longicaudatum* was immobile for 54% and 37% of the observation time respectively. *Ctenolepisma calvum* showed most mobility (it was walking during 38% of the observation time) followed by *C. rothschildi* (21%). Other species spent less than 20% of the observation time walking. *Acrotelsa collaris* spent the 33% of the observation time feeding while *Thermobia smithi* spent 25% of the time feeding. For all other species feeding was observed during less than 20% of the time (Fig. 16).

PLATE IX a- CO FEEDING

PLATE IX b- AGGREGATION

PLATE X- COMMUNICATION & AGRESSION USING ANTENNAL AND CAUDAL FILAMENT

PLATE XI-CANNIBALISM

Acrotelsa cf *collaris*

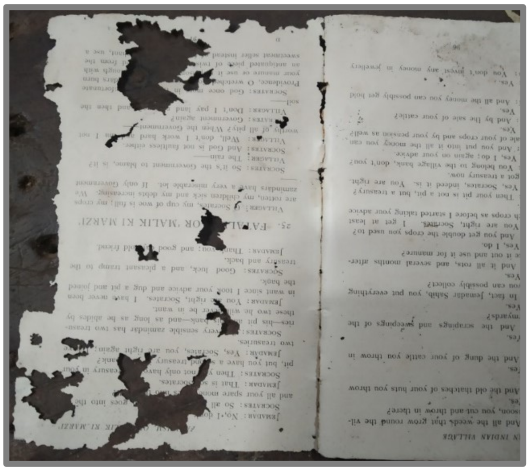
Thermobia smithi

C.rothschildi

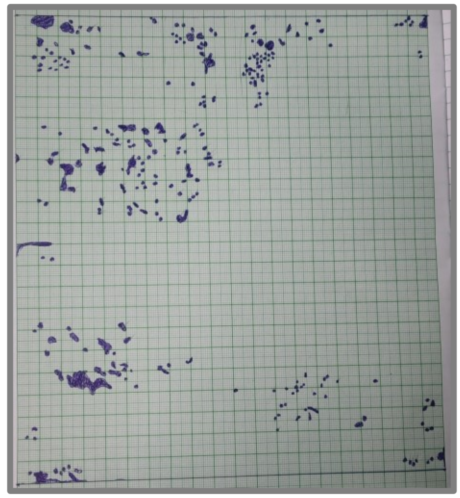
C. calvum

C. longicaudatum

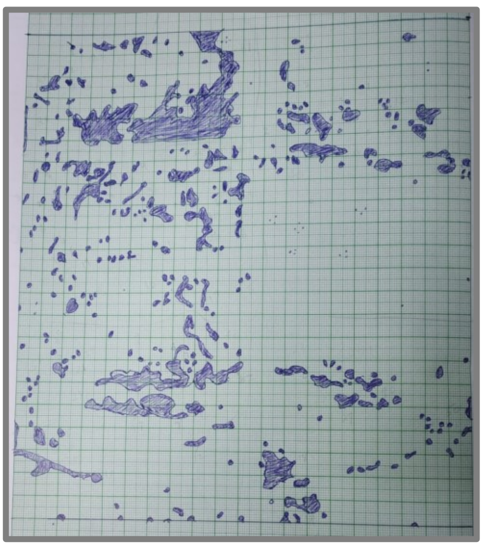
PLATE XII- PATTERNS OF PAPER DAMAGE AND MEASUREMENT OF PERFORATIONS DAMAGE BY DIFFERENT SPECIES



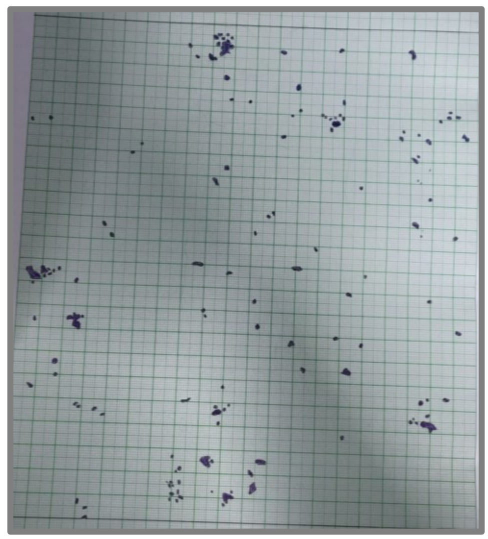
Sample of damage by silverfish



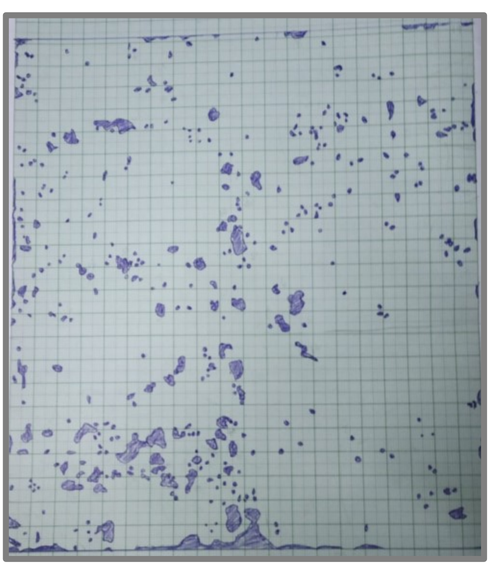
Damage by *Thermobia smithi*



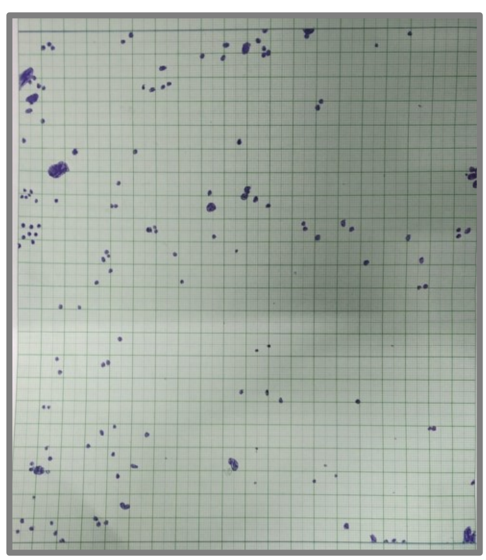
Damage by *C. longicaudatum*



Damage by *C. calvum*



Damage by *Acrotelsa (cf) collaris*



Damage by *C. rothschildi*

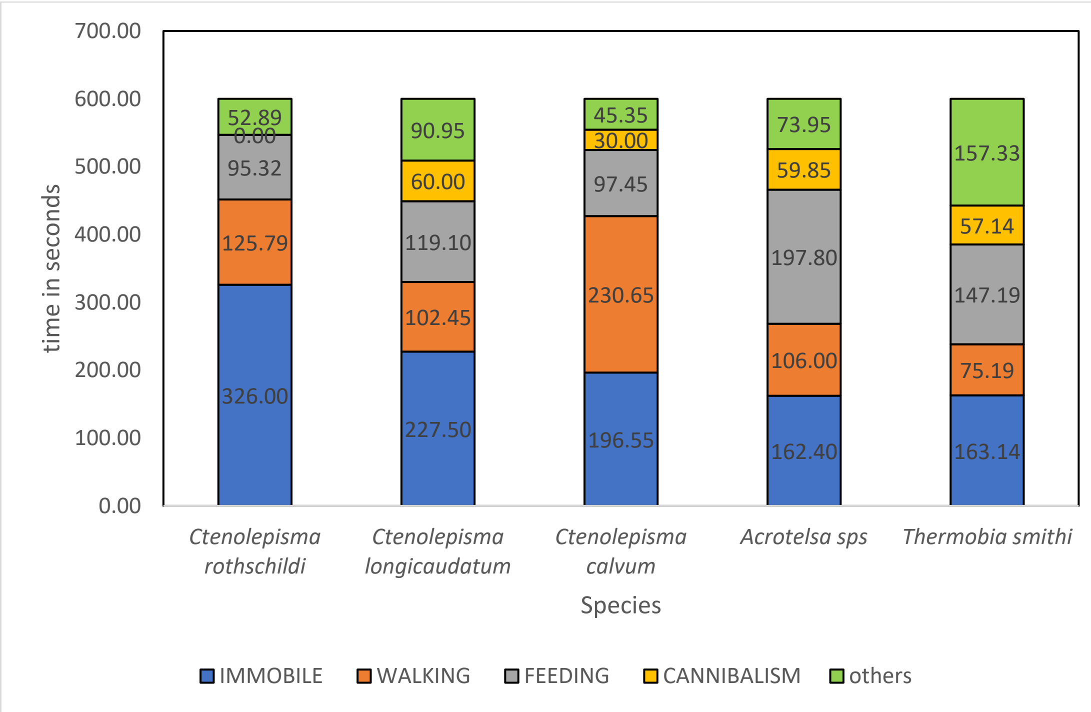


Fig. 16. Mean time spent for each behaviour by the five different species of silverfish.

In all species except *Thermobia smithi*, more than 75% of the time was spent in these three behaviours (immobile, feeding and walking). *C. rothchildi* spent 91% of the time exhibiting these three behaviours. For *C. longicaudatum* (87.44%), *C. calvum* (77.7%). In *Themobia* these three behaviours constituted only 64.25 % of the observation time.

Cannibalism was observed for at least 10% of the observation time in all species except *C. rothschildi*. Cannibalism was observed in *C. rothschildi*, but outside of the observation time. The rest of observation time was spent for other behaviours detailed in Table 1. In *Thermobia* 26.22% of the observation time was spent in behaviours other than being immobile, walking feeding and cannibalism. For all the other species total time spent for other behaviours was less than 16%.

One-way Permanova was performed and it indicated that overall, there was a significant difference in the behaviour of the five species (F=2.67 and p=0.0001) When pairwise comparisons were done it was found that there was no significant difference between the behaviour of *C. longicaudatum* and all other species but there was a significant difference between *C. rothschildi* and *C. calvum* (p=0.0253), *C. rothschildi* and *Acrotelsa* (p=0.0007) and *C. rothschildi* and *Thermobia* (p=0.0016). The behaviour of

C. calvum was also significantly different from the behaviour of *Acrotelsa* (p=0.008) and *Thermobia* (p=0.0001). *Acrotelsa* and *Thermobia* were not significantly different from each other (p=0.3894). Species placement based on behaviour has been visualized in the PCoA map (Fig. 17).

3.3.2. Aggregation and arrestment behaviour: In genera *Acrotelsa* and *Thermobia*, the intriguing phenomenon of aggregation followed by arrestment behaviour within groups was observed. Details of aggregation followed by arrestment shown by *Acrotelsa* (6 instances) and *Thermobia* (4 instances), in this study is given in Table 14.

Table 14: Details of aggregation shown by Acrotelsa and Thermobia species

Substrate	% of individuals aggregating on different substrates		Substrate	Number of aggregations on different substrates		Substrate	Mean number of individuals and range of individuals per aggregation	
	Acrotelsa	Thermobia		Acrotelsa	Thermobia		Acrotelsa	Thermobia
dry leaf	34.18	25.93	dry leaf	6	3	dry leaf	9 (6-11)	4.6 (4-5)
husk	55.06	42.59	husk	5	3	husk	17.4 (7-24)	7.6(7-9)
tissue paper	5.06	31.48	tissue paper	1	3	tissue paper	8	5.7 (4-7)
plastic floor	5.69	0	plastic floor	1	0	plastic floor	9	0

It was observed that in Acrotelsa maximum aggregations were found in dry leaf followed by coconut husk, whereas in Thermobia dry leaf, husk and tissue paper had equal number of aggregations. In both species coconut husk had more individuals in the aggregation. Aggregations in Acrotelsa lasted more than one hour in all observations whereas in Thermobia it lasted more than 45 minutes.

Only two species in the study showed the behaviour of aggregation. The aggregation behaviour of both species was slightly different in the number of individuals per substrate. While these differences could be purely coincidental, understanding factors that drive aggregation could be a key in controlling the population of this insect.

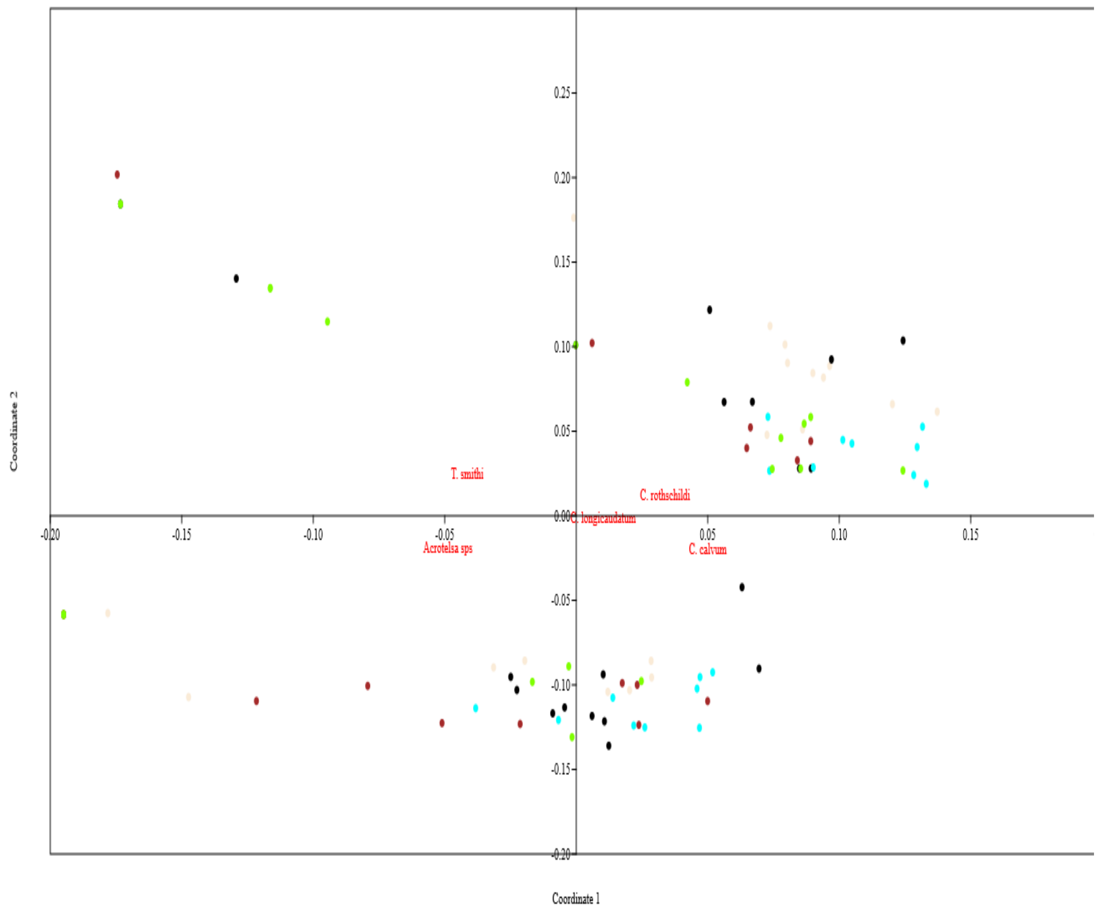


Figure 17. PCoA Map showing species placement based on behaviour *Ctenolepisma rothschildi* (pink), *Ctenoleisma logicaudataum* (black), *Ctenolepisma calvum* (blue), *Acrotelsa* (red) and *Thermobia* (green).

3.3.3. Dispersion pattern:

In the breeding boxes majority of the silverfish irrespective of species were seen congregating towards one end or side of the experimental space, often leaving a large part of the box area unoccupied. *C.longicaudatum* showed uniform dispersion for 75% of the observations and the variance mean ratio was between 0.89 and 0.99. In 25% of the observations the variance mean ratio was just over 1(1.01 to 1.03). Consolidating all the observations from all boxes and times, the variance mean ratio was 0.94 which indicated uniform distribution according to Krebs (1999).

In *C. calvum* 75% of the observations showed uniform distribution with the variance mean ratio between 0.88 and 0.99. In the rest of the boxes the distribution was clumped as the mean variance ratio was well over 1 (1.78-1.88). The consolidated variance-mean ratio for *C. calvum* was also just over one (1.22) indicating a slightly clumped distribution.

Thermobia smithi showed the most diverse dispersion patterns. In around 33% of the observations the variance mean ratio was over just over 1(1.02-1.07) showing a very slight clumped distribution. But the rest of the observations had variance mean ratio of less than 1 (0.74- 0.99). The overall ratio was also less than 1 (0.94) indicating a uniform distribution.

Acrotelsa cf *collaris* in all observations showed clumped distribution (variance mean ratio range was 1.15-2.96). When all observations were consolidated the variance mean ratio was 2.31.

C.rothschildi showed uniform distribution in only about 20% of the observations (variance mean ratio was 0.95). Other observations showed clumped distribution (variance mean ratio 1.31 to 2.95). When all observations were consolidated the variance mean ratio was 1.84. Except for *C. longicaudatum* and *Thermobia smithi* all the other species studied showed clumped distribution with *A. collaris* showing most propensity to clumped distribution.

3.3.4. Cannibalism and different diets

No cannibalism was observed in boxes where food was provided but varying rates of cannibalism was seen all silverfish on starvation diet. There was a significant difference between the cannibalism rate of boxes with different species following starvation diet plan (ANOVA F=4.234; p= 0.0206). But outside of the experimental setup cannibalism was observed in all species even when food was available. Also, three boxes where no food was provided showed zero cannibalism (Fig. 18). It is therefore surmised that while starvation could drastically increase the rate of cannibalism, it may not be the only factor causing it. Is it a solution to remove sick or aged insects from a substrate or a population control measure? Only further study and observation can solve the silverfish cannibalism puzzle.

	Acrotelsa	*Thermobia*	*C. rothschildi*	*C. longicaudatum*	*C. calvum*
STARVED	16.67 33.33 0	42.86 66.67 55.56	38.46 42.86 0 40	12.51 14.29 33.33 11.11	18.18 26.67 0
TISSUE PAPER	0 0 0	0 0 0	0 0 0	0 0 0	0 0 0
OLD PRINTED PAPER	0 0 0	0 0 0	0 0 0	0 0 0	0 0 0
NEW PRINTED PAPER	0 0 0	0 0 0	0 0 0	0 0 0	0 0 0

Fig. 18. Rate of cannibalism for each silverfish species under different feeding experimental set ups

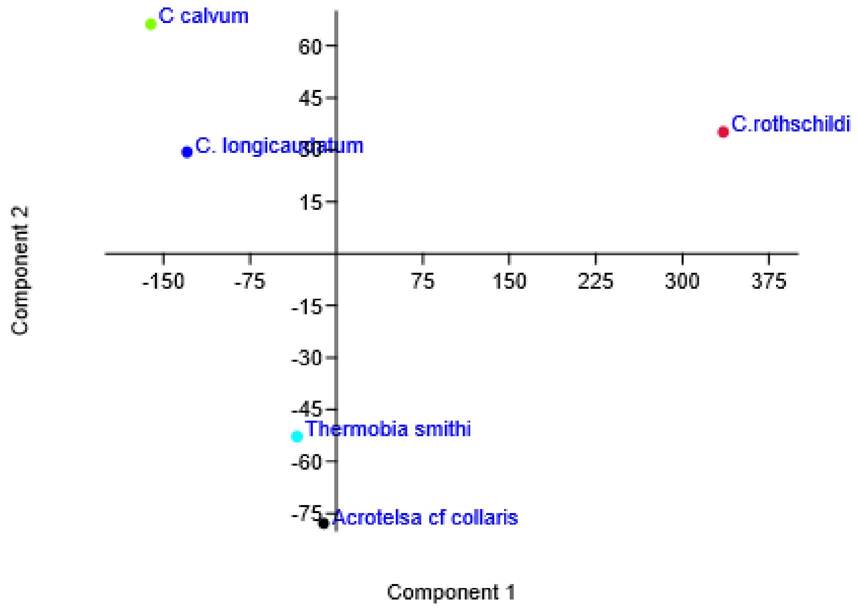


Fig. 19 PCA plot visualizing the substrate preference of five synanthropic silverfish species

3.3.5. Substrate preference in collection sites

Based on the observations, the following conclusion were drawn regarding the substrate choices of different species of silverfish. Despite the varied substrates available in a building, all the sightings of silverfish were from books and papers, coconut husk, coconut shell, pulses stored in packets, unused wood, kitchen shelves, moist soil near the buildings, cloth, roofing tiles and stored junk (jumble of many unused things in storeroom). All the five synanthropic species were found from old books and papers in large numbers (39.44 % of all sightings) and hence inferred that books and papers are the preferred substrate by all species. Storerooms of houses which were dumped with various materials were also occupied by all species (17.71 % of all sightings). All species except *C. calvum* were recorded from unused wood (broken furniture, pores in wooden shelves and almirahs, wooden planks, firewood heaps, etc). Collection from wood made up 10.37 % of all sightings. Certain substrates, such as coconut shells, coconut husks, paper shelf liners on kitchen shelves and moist soil were exclusively occupied by *C. rothschildi*. Roofing tiles were solely occupied by *C. longicaudatum* (0.33%). Only *C. rothschildi* and *C. calvum* were collected from cloth. Even though more individuals of *C. rothschildi* were recorded from cloth it makes up only 2.92% of all *C. rothschildi* sightings whereas while the number of individuals recorded for *C. calvum* from cloth was less it was 10 % of the total sightings of the species from all media. Other species were not sighted from cloth.

Figure 19 shows the PCA plot generated based on the presence and absence and abundance of the five species from different substrates. The two axes of the PCA covered 96.14% variance indicating a robust test. In the PCA map, each species showed a distinct identity with *T. smithi* and *A. collaris* showing similarity in substrate preference. *C. rothschildi* was found from all the substrates and was the most common and abundant species and thus stood out from other species as a true generalist. The preference for books and papers, wood and stored junk is also reflected in the alpha diversity indices. Substrates like coconut shell, pulses, kitchen shelves, moist soil and roofing tile show complete dominance by a single species which is reflected in high dominance and "0" Shannon diversity index values. Books and papers, wood and mixed substrates showed relatively lower dominance and higher diversity indices because of the presence of at least four species. Cloth and Coconut husk had only two species each.

C. rothschildi and *A*. cf *collaris* was present in coconut husk while *C. rothschildi* and *C. calvum* was present in cloth (Table 15).

Table 15. Diversity indices showing the diversity of silverfish in different substrates

	Taxa_S	Dominance_D	Shannon_H	Evenness_e^H/S	Margalef
Books	5	0.3018	1.335	0.7599	0.5948
Coconut husk	2	0.9079	0.1956	0.608	0.1912
Coconut shell	1	1	0	1	0
Storeroom/Mixed Media	5	0.3546	1.216	0.6747	0.6752
Pulses	1	1	0	1	0
Wood	4	0.2725	1.339	0.9542	0.5567
Kitchen shelves	1	1	0	1	0
Moist soil	1	1	0	1	0
Cloth	2	0.8195	0.3319	0.6968	0.2693
Roofing tile	1	1	0	1	0

Paper damage:

It was found that all the five silverfish significantly favoured tissue paper above all other papers and old newspapers over new printed ones (F value=83.1; p value 5.84E-13). Maximum damage was caused by *C. longicautdatum*, Acrotelsa and Thermobia. The damage caused by each species was significantly different (F value15.28; p=6.40E-07). Combined effect of both species difference and paper types were also significantly different (F value 12.37; p value 1.24E-07). In thicker papers damage could not be measured as there were no visible perforations. Perforations were randomly placed and had irregular shapes (PLATE XII and Fig. 20).

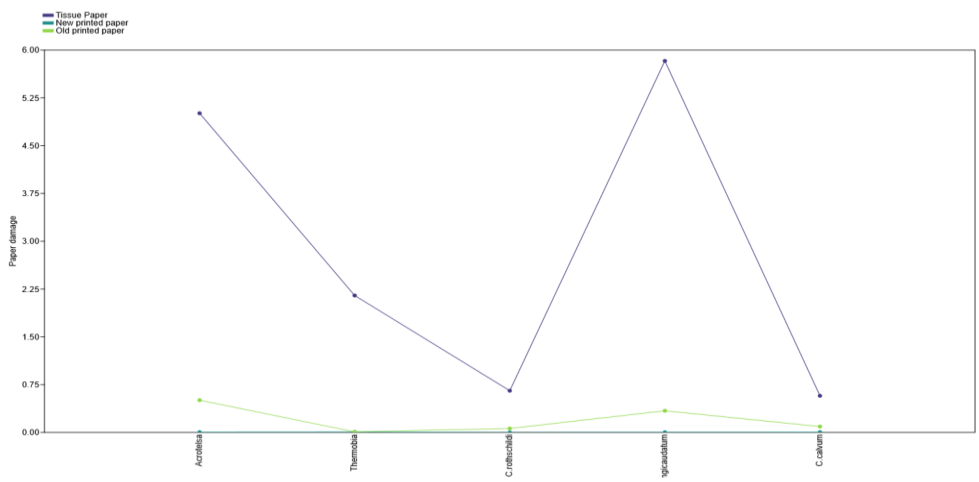


Fig. 20. A comparison of damage in different types of papers by different silverfish species

3.4. Discussion:

Studies on the behaviour of silverfish is fraught with many challenges. The major challenge is that one cannot sacrifice an animal to confirm its identity and study its behaviour at the same time. There are hardly any studies providing a comprehensive account of silverfish behaviour. Previous studies (Modder, 1962, 1969, 1975; Heeg 1967a, 1967b; Kaufman 1996) which describe the behaviour of silverfish were mostly conducted before Mendes (2002a) revised silverfish taxonomy. Observations of synanthropic silverfish behaviour in natural environments or in environments simulating its natural substrate has also not been done. Many studies concentrated on the effect of pheromones (Woodbury and Gries, 2007, 2008 2013a, 2013b, 2013c; Woodbury *et al.*, 2013) or specific behaviour like courtship (Ambrose, 2004; Walker *et al.*, 2013) and cohabitation (Parmentier *et al.*, 2024). Therefore, any new data on silverfish behaviour would serve as a baseline for further intensive and specialized behaviour on silverfish. While behaviour of silverfish has not been classified or observed and quantified, special behaviour like cannibalism, aggregation and arrestment and thanatosis has been mentioned previously (Smith, 2017, Woodbury and Gries 2007, 2008 2013a, 2013b, 2013c, Woodbury *et al.,* 2013). Previously, it was hypothesized that a contact pheromone played a pivotal role in eliciting the observed aggregation and arrestment behaviour in Zygentoma (Woodbury *et al.*, 2007). However, subsequent investigations revealed that the initiation of aggregation behaviour is not attributable to pheromones; rather, it is instigated by specific chemicals released by gut microbes. Notably, endosymbiotic entities such as the fungus *Mycotypha microspora* (Mycotyphaceae) and the bacterium *Enterobacter cloacae* (Enterobacteriaceae) were identified in the faecal matter of the firebrat, *Thermobia domestica*, shedding light on the microbial influence on this behaviour (Woodbury and Gries, 2013a,2013b & 2013c).

All the five species of silverfish showed similar types of behaviour and the major difference lay in the amount of time spent for each behaviour. *Ctenolepisma rothschildi* spent a lot of time immobile but *C calvum* spent more time moving around. Cannibalism was most prominent in *Ctenolepisma rothschildi* and *Acrotelsa*. But structured experiments with different diets including starvation for the five species of silverfish indicated that lack of nutrients while increasing the incidence of cannibalism need not be the only reason. This is especially true for Acrotelsa which showed many instances of cannibalism outside of structured experiments but in the structured experiments starved Acrotelsa showed relatively less cannibalism during one month observation. Aggregation and arrestment were characteristics of Acrotelsa and Thermobia and was not observed in Ctenolepisma species during this study. Previous studies are not available for comparison.

Dispersion patterns in this study showed that all the species except for *C. longicaudatum* and *Thermobia smithi* were prone to clumped behaviour. In nature clumped behaviour is a characteristic of social animals. In other animal groups clumped behaviour is exhibited when resources are concentrated or as a strategy for avoidance of predation or selection of mate. But in the pseudo natural setup negative pressures like predators, lack of resources was absent. It can only be hypothesized that such dispersion patterns could be genetically predetermined to facilitate communication (Woodbury and Gries, 2007). Previous studies are not available for comparison.

Very few studies on substrate selection studies are available for reference. Substrate selection experiments were not conducted in the laboratories due to the difficulty of rearing; allocating a room exclusively for the purpose of silverfish rearing; the risk of silverfish escaping and causing infestation. De Vries and Appel (2014) demonstrated that *Lepisma saccharina* preferred carbohydrates over protein and fats in that order and that increased temperature decreased the consumption of carbohydrates even though the reason for such a change could not be found. They hypothesized that it could be due to different dietary requirements at different temperatures. Silverfish have a small home range, inhibiting small microhabitats, staying close to their food source (Hippisley-Cox, 2017; Woodbury and Gries, 2007).

In this study when a building was randomly selected, all possible parts of it was thoroughly searched for silverfish and the substrate from which the silverfish was collected was recorded as the preferred substrate because it could also have moved on to any other substrate in the house. Substrates without silverfish was not recorded in this study. All the species preferred books and papers followed by other carbohydrate sources which reiterates common knowledge that carbohydrates are the preferred food source for silverfish for all the five species in the study area. *C.rothschildi* demonstrated maximum variation in substrate selection which could be an indication that it has a more varied diet than other species. But such hypothesis can be confirmed only by further studies.

Behavioural studies on silverfish are very significant considering their pestilence at global level. (Austin and Richardson, 1941; Molero-Baltanas *et al.*, 1997a; Barnhart, 1951: Mallis, 1941; Nierop and Hakbijl, 2002; Bennett *et al.*, 2010; Mallis *et al.*, 2011; Trematerra and Pinniger, 2018; Aak *et al.*, 2020) and their contribution to the bio-deterioration of organic monuments (Abdallah, 2023) such as degradation of Ajanta wall paintings (Dhawan *et al.*, .1988) and agricultural, stored and transport products (Seín, 1930, Baeta-Neves *et al.*, 1956; de Carvalho, 1979; Hill, 1983). An allergenic component Lep s 1(Tropomyosin) extracted from silverfish has been found to cause allergic reactions in susceptible individuals (Barletta *et al.*, 2002, 2005 and 2007; Boquete *et al.*, 2008; Morfín Maciel and Mendoza, 2003). Aak *et al.*, (2021) documented the increase in infestations by *Ctenolepisma longicaudatum* in Norway through substantial increase in insurance claims and legal disputes. Such detailed studies are not available from other countries but studies from some European countries (Meineke & Menge, 2014; Schoelitsz & Brooks, 2014; Goddard *et al.*, 2016; Querner 2015; Kulma *et al.*, 2018; Gutsmann, 2019 and Thomsen *et al.*, 2019) have documented an increase in infestations by *Ctenolepisma longicaudatum*. In this context recognizing their feeding habits, communication methods and other natural behaviours is crucial for developing ecologically sound pest management strategies. Effective pest management in tropical countries presents unique challenges compared to Western countries, where fumigation or widespread application of pesticides might be feasible. In tropical regions, high human population density, architectural styles of buildings and unique health concerns necessitate more localized pest control measures. Knowledge of silverfish behaviour and preferred habitats enables targeted application of pesticides, minimizing human exposure. This study aimed to address this gap. Recommendations to avoid the silverfish infestations have been given in Chapter 5.

3.5. Conclusion

The behaviour of five synanthropic species of silverfish were observed in both natural and experimental setups Twenty unique behaviours were identified and classified into six broad categories: feeding, locomotion, immobile, aggression, communication and ‘other behaviour’. While none of the species had a unique behaviour, inter species differences lay in the time spent for different behaviours. All species except *C. longicaudatum* and *Thermobia smithi* showed clumped dispersion patterns. Soft and unprinted papers or very old papers were more prone to damage by silverfish. *C. rothschildi* was found in varied substrates and had the most diverse feeding habits.

CHAPTER 4

GUT MICROBIOTA OF FOUR SYNANTHROPIC SILVERFISH SPECIES

Chapter 4

Gut microbiota of Four Synanthropic Silverfish Species

4.1. Introduction

Silverfish (Zygentoma: Insecta) are cellulose feeding pests causing damage in libraries, museums, kitchens, bakeries, storage areas and households; contamination of stored products by body parts, saliva and feces (Sidebottom,1996); allergic reactions in susceptible persons (Witteman *et al.*, 1995; Barletta *et al.*, 2002, 2005 and 2007; Boquete *et al.*, 2008; Maciel and Mendoza, 2003). Mason *et al.*, (2019) states that most insects secrete digestive enzymes with the help of symbiotic microbes to perform metabolic activities because no insect can completely digest lignocellulose using their own enzymes (Sun & Chen, 2010). While there are many studies on the microbiome of various insects such as termites, moths, beetles, etc, (Cazemier *et al.*, 1997; Broderick *et al.*, 2004; Brune, 2006; Broderick and Lemaitre, 2012; Engel and Moran, 2013) there are hardly any studies about the microbiome of silverfish.

Even though silverfish feed on cellulose rich materials its gut is relatively simple consisting of a thin-walled oesophagus and crop, a proventriculus with well-developed teeth, a glandular midgut which connects to a smooth-walled hindgut and rectum. 'Fermentative chambers' or proctodaeal pouches where extra-cellular micro-organisms could possibly inhabit is absent (Lasker and Giese, 1956). Cellulase activity in Zygentoma has been reported by (Zinkler and Götze, 1987; Lasker and Giese, 1956; Treves and Martin, 1994; Oppert *et al.*, 2010; Sabbadin *et al.*, 2018; Pothula *et al.*, 2019). Similarly, cellulase producing microbe '*Cellulomonas cellulans'* from silverfish has been isolated and identified (Premkumar *et al.*, 2015).

Other than the above there are no studies cataloguing the gut microbiome of silverfish. In this context this study is the first attempt to document and compare the gut microbial diversity of four synanthropic species- *Acrotelsa* cf *collaris*, *Thermobia smithi*, *Ctenolepisma rothschildi* and *Ctenolepisma longicaudatum* of Zygentoma from Kerala, India using 16S rRNA gene high-throughput amplicons.

4.2. Materials and Methods

4.2.1. Collection, Identification and rearing of morphs for the study

Collection, identification and rearing methods have been described in the methodology section of Chapter 2 and Chapter 3 of this thesis. Gut microbiome of *C. calvum* has not been studied due to the extremely fragile and small nature of the insect.

4.2.2. Preparation of the sample

Four different morphs of silverfish; *A. cf. collaris*, *T. smithi*, *C. rothschildi* and *C. longicaudatum* were used for the gut microbiome studies. Twenty-five fully grown insects from each group were randomly selected regardless of sex. They were anesthetized by refrigerating for 5 min at 4°C or killed by refrigerating for a longer duration. Dissection set up was prepared by sterilizing needles, scalpel and slides. Stage region of microscope was disinfected with 70% ethanol. The digestive tract (Fig. 21a, b, c, d) was separated from the rest of the body in this sterile setup. Tissues dissected from individual insects were placed in labelled RNAse free microcentrifuge tubes containing 100 µl of sterile water and kept in a special ice set up (Fig.22). After dissection, the microcentrifuge tubes containing gut samples were immediately stored in a freezer at −20°C for further procedure.

Fig. 21a. Dissected gut of *A.* cf *collaris*

Fig. 21b. Dissected gut of *T. smithi*

Fig. 21c. Dissected gut of *C. rothschildi*

Fig. 21d. Dissected gut of *C. longicaudatum*

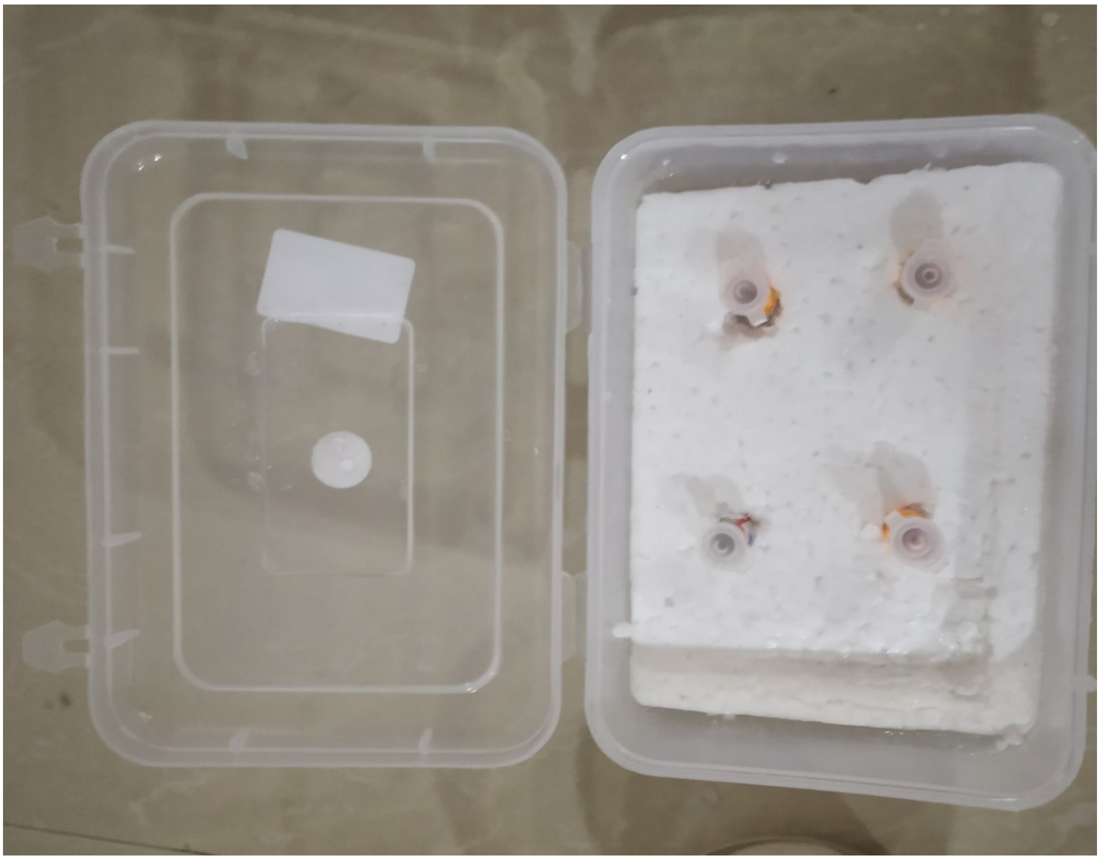


Fig. 22. Storage setup of gut in sterile microcentrifuge to be placed in freezer

4.2.3. Metagenomic DNA Extraction, Qualitative and Quantitative analysis

Metagenomic DNA was extracted from the gut samples using commercially available Quick DNA MiniPrep Kit (Zymo Research). The quality of the extracted metagenomic DNA samples were analyzed using Nanodrop by determining A260/280 ratio.

Generation of First Amplicon

First amplicon PCR was set up with the received metagenomic DNA along with bacterial 16S V3-V4 region specific primer set. 3µl of PCR product was resolved on 1.2% Agarose gel at 120V for approximately 60 min or till the sample reached 3/4th of the gel (Fig. 23).

Table 16: Primers used in the PCR set-up

16S rRNA F	GCCTACGGGNGGCWGCAG
16S rRNA R	ACTACHVGGGTATCTAATCC

Table 17. Quantification of extracted metagenomic DNA samples on NanoDrop

Sr. No.	**Sample ID**	**NanoDrop Readings** (ng/µl)	**NanoDrop** OD A260/280	**NanoDrop** OD A260/230
1	Species 1	27.7	1.73	1.02
2	Species 2	16.7	1.79	1.30
3	Species 3	13.7	1.74	0.91
4	Species 4	20.0	1.73	1.17

Analysis of 16s rRNA gene sequences (Table 16, 17) from Silverfish samples were carried out in QIIME 2 (Estaki *et al.*, 2020). Paired-end reads of the sequences after PCR were joined using FLASH (Magoc and Salzberg, 2011). Quality filtering of the raw sequences was done using FASTQC and Trimmomatic. Taxonomy was assigned with sequences from the SILVA database (Quast *et al.*, 2013).

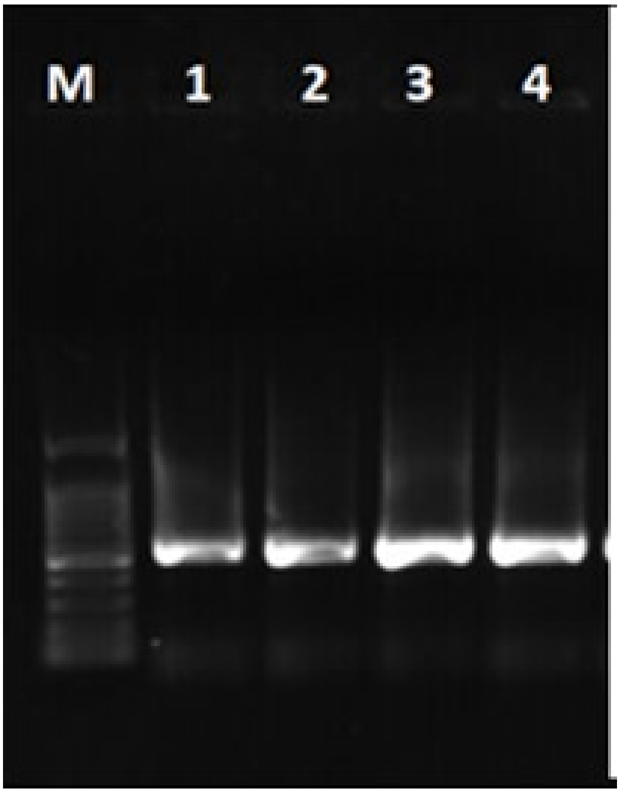


Fig. 23. QC of first amplicon generated on 1.2% agarose gel

4.2.4. Data Analysis

The results were consolidated in MS Excel and summarized in tables. PAST ver 4.17 was used for statistical analysis. Selected alpha diversity indices were calculated. The results were visualized using PCA and dendrogram (Neighbour joining method- Euclidian distance).

4.3. Results

4.3.1. Diversity of microbiome at phylum level

A total of 38 phyla belonging to 2 kingdoms: Bacteria (33) and Archaea (5) were isolated from the gut of the four silverfish species. A total of 684 species belonging to 450 genera, 272 families, 171 orders and 74 classes were documented. A detailed breakup of number of microbial taxa in the four species is given in Table 18.

For *A. cf. collaris* sps, the gut microbiota belongs to 24 phyla, 50 classes, 97 orders, 147 families, 252 genera, and 367 species. The gut microbiota of *T. smithi*, belong to 24 phyla, 44 classes, 100 orders, 143 families, 222 genera, and 294 species. In the case of *C. rothschildi*, the gut microbiota is distributed across 19 phyla, 29 classes, 60 orders, 87 families, 157 genera, and 231 species. The gut microbiota of *C. longicaudatum* is similarly classified under 32 phyla, 58 classes, 124 orders, 189 families, 295 genera and 411 species.

Table 18: Overview of gut microbe of four synanthropic silverfish species of Kerala

Silverfish species	Kingdom	Phyla	Class	Orders	Families	Genera	Species
A. cf. *collaris*	2	24	50	97	147	252	367
T. smithi	2	24	44	100	143	222	294
C. rothschildi	2	19	29	60	87	157	231
C. longicaudatum	2	32	58	124	189	295	411

The microbiome communities of the four silverfish species showed some similar characteristics but the microbiome of *T. smithi* was quite distinct from others. More than 89 % of the gut microbes in all the four silverfish species belonged to eight phyla under kingdom Bacteria (Firmicutes, Bacteroidota, Proteobacteria, Actinobacteriota, Chloroflexi, Planctomycetota, Patescibacteria and Verrucomicrobiota. Phylum

Firmicutes followed by Bacteroidota were most dominant. Phylum Firmicutes made up 36.74%, 51.24%, 45.58 % and 36.09% of species in *A. cf. collaris*, *T. smithi*, *C. rothschildi* and *C. longicaudatum*.

A single phyla Euryarchaeota of kingdom Archaea made up just over 7% of the microbiome in *A. cf. collaris*, *C. rothschildi*, and *C. longicaudatum* and just over 5 % in species *T. smithi* (Table 19). These nine phyla constituted above 97% of the microbiota in all the four species. It is notable that the most dominant phyla Firmicutes and Bacteroidota constituted 68% of species in *A. cf. collaris* and *C. longicaudatum* and just over 75% in *T. smithi* and *C. rothschildi*.

Table 19. Dominant gut microbiome phyla in four synanthropic silverfish species of Kerala

Phylum	*A.* cf. *collaris*	*T. smithi*	*C. rothschildi*	*C. longicaudatum*
Firmicutes	36.74	51.24	45.58	36.09
Bacteroidota	31.22	24.27	30.34	31.84
Proteobacteria	10.40	9.40	5.46	6.75
Actinobacteriota	2.46	1.64	2.23	3.64
Chloroflexi	2.82	1.65	2.51	3.01
Planctomycetota	2.55	1.61	2.23	2.83
Patescibacteria	2.75	1.66	2.46	3.29
Verrucomicrobiota	2.52	1.21	1.43	2.45
Euryarchaeota*	7.23	5.75	7.02	7.78
Total	98.69	98.44	99.26	97.67

* Kingdom Archaea

4.3.2. Diversity of microbiome at class level

The most dominant classes in *A. cf. collaris* and *Ctenolepisma* species were Bacteroidia and Clostridia contributing to around 60% of species in these silverfish. In *A. cf. collaris,* Bacteroidia was most dominant with 31.22 % of species followed by Clostridia with 28.30% of species. In *C. longicaudatum*, Bacteroidia made up 31.84 % of species and 27.82 % of species belonged to Clostridia. In *C. rothschildi,* Clostridia made up 34.89% of species and Bacteroidia made up 30.34 % of species. In *T. smithi*, Bacilli made up 29.33 % of the microbiome community followed by Bacteroidia (24.25%). Clostridia and Bacilli belongs to Firmicutes and Bacteroidia is under Bacteroidota. In Table 20 all the classes which made up more than 1% of species have been listed.

C.longicaudatum was unique in that Class Actinobacteria made up 1.21% of the gut microbiome but in other silverfish this class was less than 0.42.

4.3.3. Diversity of microbiome at order level

In *A. cf. collaris* a single order Bacteroidales of Bacteroidia (31.22%) made up 31.19% of species. Bacteroidales was dominant in both *Ctenolepisma* species with 30.33 and 31.58 % of species each. Order Oscillospirales, Lachnospirales and Christensenellales of Class Clostridia was the next most dominant orders in these species. The microbiome of *T. smithi* was different from the other silverfish species in that Order Lactobacillales (27.05%) and Bacteroidales (24.15%) were most dominant. Order Methanobacteriales of Kingdom Archaea made up over 7% of microbiome species in *A. cf. collaris* and both *Ctenolepisma* species and over 5% in *T. smithi*. Table 21 shows the relative abundance of Orders making up more than 3% of the population in at least one silver fish species.

4.3.4. Diversity of microbiome at family level

The family Prevotellaceae was most dominant in *A. cf. collaris* and *Ctenolepisma* species and made up above 18% of the microbiome. Families of Firmicutes, Clostridia such as Lachnospiraceae, Ruminococcaceae and Oscillospiraceae took the second and third positions in relative abundance. In *T. smithi* Prevotellaceae was the second dominant family (15.62%). The most dominant family was Lactobacillaceae a Firmicute belonging to Bacilli with over 23% relative abudance. A single family of Archaea Methanobacteriaceae made up over 7% of all bacteria in the three species except *T. smithi* which had over 5% of this family. Bacteria of family Rickettsiaceae was present only in *A. cf. collaris*. The relative abundance of Christensenellaceae was slightly higher in the *Ctenolepisma* species (Table 22).

4.3.5. Diversity of microbiome at genus level

The Genus Prevotella was the most dominant among all the four species of silverfish except *T. smithi* and made up just above 10% of the microbiome. In *T.smithi, Prevotella* was at second position while *Pediococcus* of Bacteriodota was first with 23% of relative abundance. In the other three species Pediococcus was negligible. Genus *Methanobrevibacter* (Archaea) took the second positions in all the two species except *A.*cf. *collaris* and *T. smithi* and contributed 6.70% and 7.43% of microbiome respectively in *C. rothschildi* and *C. longicaudatum*. In *A.*cf. *collaris* and *T. smithi* it contributed 6.9%, and 5.5%, of the microbiome respectively. In *A. cf. collaris*, Rickettsia also made upto 6.9% of the gut microbiome but it was absent in the other three species of silverfish (Table 23).

Table 20. Dominant gut microbiome classes in four synanthropic silverfish species of Kerala

	Kingdom	Phylum	Class	*A. cf. collaris*	*T. smithi*	*C. rothschildi*	*C. longicaudatum*
1	Bacteria	Firmicutes	Clostridia	28.3	19.26	34.89	27.82
2	Bacteria	Bacteroidota	Bacteroidia	31.22	24.25	30.34	31.84
3	Bacteria	Proteobacteria	Gammaproteobacteria	1.52	7.18	4.26	3.75
4	Bacteria	Proteobacteria	Alphaproteobacteria	8.87	2.22	1.2	2.99
5	Bacteria	Firmicutes	Bacilli	3.03	In t33	5.57	3.07
6	Bacteria	Firmicutes	Negativicutes	5.24	2.54	4.88	4.99
7	Archaea	Euryarchaeota	Methanobacteria	7.23	5.75	7.02	7.78
8	Bacteria	Chloroflexi	Anaerolineae	2.81	1.57	2.51	2.94
9	Bacteria	Actinobacteri-ota	Coriobacteriia	1.97	1.2	1.8	2.04
10	Bacteria	Patescibacteria	Saccharimonadia	2.62	1.57	2.37	3.08
11	Bacteria	Planctomycet-ota	Planctomycetes	2.49	1.54	2.23	2.69
12	Bacteria	Verrucomicrob-iota	Kiritimatiellae	2.31	1.04	1.25	2.26

Table 21 – Dominant gut microbiome orders in four synanthropic silverfish species of Kerala

No.	Kingdom	Phylum	Class	Order	*A. cf. collaris*	*T. smithi*	*C. rothschildi*	*C. longicaudatum*
.	Bacteria	Bacteroidota	Bacteroidia	Bacteroidales	31.19447	24.15686	30.33276	31.58302
.	Bacteria	Firmicutes	Clostridia	Oscillospirales	11.66998	8.283284	14.46303	11.37633
.	Bacteria	Firmicutes	Clostridia	Lachnospirales	8.352702	5.269451	9.941486	7.413674
.	Archaea	Euryarchaeota	Methanobacteria	Methanobacteriales	7.226182	5.753186	7.024647	7.778548
.	Bacteria	Firmicutes	Negativicutes	Veillonellales	4.143545	1.869718	3.770908	3.928004
.	Bacteria	Firmicutes	Clostridia	Christensenellales	4.732938	3.80194	5.083043	5.240735
.	Bacteria	Patescibacteria	Saccharimonadia	Saccharimonadales	2.624468	1.568063	2.367161	3.084105
.	Bacteria	Firmicutes	Bacilli	Lactobacillales	0.220188	27.05384	3.04687	0.252762

Table 22 - Dominant gut microbiome families in four synanthropic silverfish species of Kerala

Kingdom	Phylum	Class	Order	Family	*A. cf. collaris*	*T. smithi*	*C. rothschildi*	*C. longicaudatum*
Bacteria	Bacteroidota	Bacteroidia	Bacteroidales	Prevotellaceae	18.7994	15.6154	19.1471	18.4924
Bacteria	Firmicutes	Clostridia	Lachnospirales	Lachnospiraceae	8.27597	5.19064	9.8085	7.31583
Bacteria	Firmicutes	Clostridia	Oscillospirales	Ruminococcaceae	6.26758	5.3238	8.2629	6.41282
Bacteria	Firmicutes	Clostridia	Oscillospirales	Oscillospiraceae	4.66288	2.59804	5.39335	4.33161
Archaea	Euryarchaeota	Methanobacteria	Methanobacteriales	Methanobacteriaceae	7.22618	5.75319	7.02465	7.77855
Bacteria	Proteobacteria	Alphaproteobacteria	Rickettsiales	Rickettsiaceae	6.94	0	0	0
Bacteria	Firmicutes	Bacilli	Lactobacillales	Lactobacillacea	0.003	23.23	0.026	0.006
Bacteria	Firmicutes	Clostridia	Christensenellales	Christensenellaceae	4.732	3.801	5.08	5.24

Table 23. Dominant gut microbiome genera in four synanthropic silverfish species of Kerala

Kingdom	Phylum	Class	Order	Family	Genus	Acrotelsa	Thermobia	C. roth	C. longi
d__Bacteria	Bacteroidota	Bacteroidia	Bacteroidales	Prevotellaceae	Prevotella	11.28743	12.18034	11.84172	10.57116
d__Archaea	Euryarchaeota	Methanobacteria	Methanobacteriales	Methanobacteriaceae	Methanobrevibacter	6.913693	5.549365	6.714345	7.432019
d__Bacteria	Firmicutes	Clostridia	Oscillospirales	Oscillospiraceae	NK4A214_group	4.295898	2.511074	5.032803	3.985079
d__Bacteria	Firmicutes	Clostridia	Oscillospirales	Ruminococcaceae	Other	3.045939	2.769247	4.554052	3.597782
d__Bacteria	Bacteroidota	Bacteroidia	Bacteroidales	Muribaculaceae	Muribaculaceae	4.867498	4.022067	4.784562	4.973705
d__Bacteria	Firmicutes	Clostridia	Christensenellales	Christensenellaceae	Christensenellaceae_R-7_group	4.709585	3.80194	5.059401	5.240735
d__Bacteria	Firmicutes	Clostridia	Oscillospirales	Ruminococcaceae	Ruminococcus	3.004793	2.437699	3.608369	2.629541
d__Bacteria	Bacteroidota	Bacteroidia	Bacteroidales	Prevotellaceae	Prevotellaceae_NK3B31_group	4.249191	0.100552	3.759087	4.975743
d__Bacteria	Bacteroidota	Bacteroidia	Bacteroidales	Rikenellaceae	Rikenellaceae_RC9_gut_group	3.345084	1.986575	3.244873	3.554976
d__Bacteria	Proteobacteria	Alphaproteobacteria	Rickettsiales	Rickettsiaceae	Rickettsia	6.908133	0	0	0
d__Bacteria	Bacteroidota	Bacteroidia	Bacteroidales	F082	F082	3.212749	1.858847	2.231219	3.542745
d__Bacteria	Firmicutes	Bacilli	Lactobacillales	Lactobacillaceae	Pediococcus	0	23.02362	0.026597	0
d__Bacteria	Firmicutes	Bacilli	Lactobacillales	Leuconostocaceae	Fructobacillus	0	3.568226	2.024351	0

In *A.* cf. *collaris* and *Ctenolepisma* species unidentified species belonging to Prevotella, Methanobrevibacter and Muribaculaceae was most dominant. Rickettsia species was unique to the gut of *A.* cf. *collaris*. In *T. smithi* unidentified species belonging to Pediococcus, Prevotella, Methanobrevibacter and Muribaculaceae were most dominant (41%). The Shannon (log2) diversity index was 8.812, 7.617, 7.678 and 9.262 respectively for *A.* cf. *collaris, T. smithi, C. rothschildi* and *C. longicaudatum*. Margalef index was 91.33, 72.16, 57.39 and 103.3 respectively for *A.* cf. *collaris, T. smithi, C. rothschildi* and *C. longicaudatu*m. *C. longicaudatum* had the highest diversity followed by *A.* cf. *collaris.*

Based on the composition of the gut biome the position of each silverfish species was visualized in a PCoA plot. The results showed a clear divergence between the two *Ctenolepisma species* and the other groups. The first principal component explained 84.357% of the variation, and the second principal components explained 12.76% of the variance. *A.* cf. *collaris* and *T. smithi* were placed far apart with *T. smithi* being the most distinct (Figure 24). Both *A.* cf. *collaris* and *T. smithi* are distinct from the other two species due to the presence of Rickettesia and Pediococcus respectively in their gut microbiome. These two bacteria are negligible in the other species.

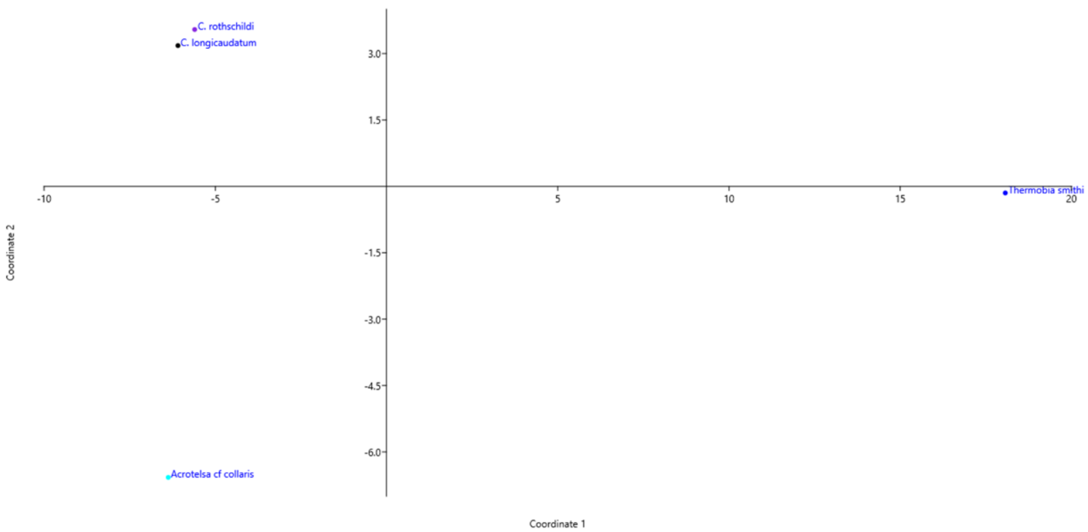


Fig. 24. PCoA map visualizing the similarities between the gut microbiome of silverfish species.

This difference could also be due to the presence of unique microbes in the gut biome of each silverfish species. *C. longicaudatum* had 146 unique microbe species; *C.*

rothschildi had 33 unique species; *T. smithi* had 86 unique species and *A.* cf. *collaris* had 105 species of microbe unique to its gut. Among the bacteria which could not be identified till species level 107 belonged to uncultured or unidentified rumen bacteria; 24 belonged to unidentified or uncultured marine/deep sea bacteria and two belonged to bacteria similar to those found in hydrothermal vents.

Of the 684 species of microbes identified from the guts of the four silverfish species, only a total of 55 microbes could be identified till species level. Based on previous studies (see supplementary document/appendix) these bacteria were classified into different groups based on their economic significance (Table 24).

Table 24. Classification of known species based on anthropogenic relevance

No	SPECIES	REMARKS	Found in Gut of	Literature Source
1	*Pseudomonas geniculata*	EN	*A.* cf. *collaris*	Wu *et al.*, 2020
2	*Acinetobacter_baumannii*	EN; PA	*C. rothschildi*	Moubareck and Halat 2020
3	*Acinetobacter_marinus*	EN; ME	*A.* cf. *collaris*	Van Bergeijk *et al.*, 2020
4	*Aestuariibacter aggregatus*	EN	*C. longicaudatum*	Wang *et al.* 2010
5	*Alcanivoracaceae_bacterium*	EN; ME	*C. longicaudatum*	Silveira and Thompson 2014
6	*Alloprevotella tannerae*	EN; PA	*T. smithi*	Parker and Garrity 2008
7	*Alpha_proteobacterium*	EN; PA	*C. longicaudatum*	Imhoff 2006, Rizzatti *et al.*, 2017; Rosenberg 2006; Spieck and Bock, 2014
8	*Alteromonas macleodii*	EN; ME	*T. smithi*	Wietz *et al.*, 2022
9	*Anaerovibrio_lipolyticus*	EN; RU	*C. longicaudatum*	Merchen, 2002; d Privé *et al.,* 2013
10	*Aureimonas_frigidaquae*	EN; FW	*A.* cf. *collaris*, *T. smithi* and *C. rothschildi*	Kim *et al.,* 2008
11	*Bacteroides_coprocola*	EN; PA	*C. longicaudatum*	Chen *et al.,* 2017
12	*Bacteroides_fragilis*	EN; PA	*A.* cf. *collaris*, *C.rothschildi*	Baughn and Malamy 2004, Wexler 2007
13	*Bacteroides_plebeius*	EN; G	*A.* cf. *collaris*, *T. smithi*	Hehemann *et al.,* 2010, 2012
14	*Campylobacter_showae*	PA	*T. smithi*	Etoh *et al.,* 1993, Silva *et al.,* 2018, Macuch and Tanner 2000, Hsu *et al.,* 2019
15	*Candidatus_fonsibacter*	EN; ME	*T. smithi*	Henson *et al.,* 2018
16	*Capnocytophaga_gingivalis*	EN; PA	*C. longicaudatum*	Piau *et al.,* 2013

No	SPECIES	REMARKS	Found in Gut of	Literature Source
17	*Cardiobacterium_valvarum*	PA	*C. longicaudatum*	Lang *et al.,* 2002, Nisbet and Lang 2002
18	*Clostridiales_bacterium*	EN; CD; RU; PA	*A.* cf. *collaris*, *C. longicaudatum*	Wiegel *et al.,* 2006 and Fujimori 2021
19	*Clostridium pasteurianum*	EN	*A.* cf. *collaris*	Dabrock 1992, Abbad-Andaloussi *et al.,* 1997, Martin & David, 2012
20	*Commensalibacter intestini*	EN; G	*C. longicaudatum*	Roh *et al.,* 2008, Ryu *et al.* 2008
21	*Denitrobacterium detoxificans*	EN; RU	*A.* cf. *collaris*	Anderson *et al.,* 2000, Li *et al.,* 2021
22	*Erysipelotrichaceae_bacterium*	EN; G; RU; PA	*A.* cf. *collaris*, *C. rothschildi*, *C. longicaudatum*	Zhao *et al.,* 2013, Palm *et al.,* 2014, Dinh *et al.,* 2015
23	*Eubacterium_sp*	EN; G; PA	*T. smithi*, *C. longicaudatum*	Mukherjee *et al.,* 2020
24	*Flavobacteriaceae_bacterium*	EN; ME; PA	*T. smithi*	McBride, 2014
25	*Flavobacterium_sasangense*	EN; ME; PA	*A.* cf. *collaris*	Mcbride 2014
26	*Kandleria_vitulina*	EN; RU	*A.* cf. *collaris*	Salvetti *et al.* 2010
27	*Lactobacillus_faecis*	EN; RU	*C.rothschildi*	Yang *et al.* 2021
28	*Lactobacillus_ruminis*	EN; G; PA	*A.* cf. *collaris*	Michelle M O' Donnell *et al.*(2015)
29	*Leptotrichia_wadei*	EN; PA	*C. longicaudatum*	Ranganath *et al.* 2023, Shah 2023
30	*Marinimicrobia_bacterium*	ME	*C. longicaudatum*	Tarn *et al.* 2016
31	*Methanobrevibacter_ boviskoreani-*	EN; RU	*A.* cf. *collaris*, *C. rothschildi*, *C. longicaudatum*	Li *et al.* 2023
32	*Natronorubrum sediminis*	EN	*A.* cf. *collaris*	Gutiérrez *et al.,* 2010
33	*Oleibacter_marinus*	EN; ME	*T. smithi*, *C. longicaudatum*	Teramoto *et al.,* 2011
34	*Ottowia_sp*	EN	*T. smithi*	Cao *et al.,* 2014, Zhang *et al.,* 2021
35	*Paenibacillus_daejeonensis*	EN; CD	*C. longicaudatum*	Lee *et al.,* 2002
36	*Paenibacillus_lautus*	EN; CD; PA	*T. smithi*	Loong *et al.,* 2018, Yadav and Dubey 2018
37	*Parabacteroides_merdae*	EN; G; PA	*A.* cf. *collaris*, *C. longicaudatum*	Cui *et al.,* 2022
38	*Planctomycetes_bacterium*	EN; ME	*C. longicaudatum*	Boersma *et al.,* 2020, Kaboré *et al.,* 2020, Graça *et al.,* 2016
39	*Porphyrobacter_mercurialis*	EN	*C. longicaudatum*	David *et al.,* 2015
40	*Prevotella_baroniae*	EN; PA	*T. smithi*	Rôças *et al.,* 2009
41	*Prevotella_intermedia*	EN; PA	*T. smithi*	Karched *et al.,* 2022

No	SPECIES	REMARKS	Found in Gut of	Literature Source
42	*Prevotella_melaninogenica*	EN; PA	*T. smithi*	Holdeman and Johnson, 1982
43	*Prevotella_nigrescens*	EN; PA	*T. smithi*	Yakob *et al.,* 2011, Fukui *et al.*, 1999
44	*Prevotella_oulorum*	EN; G; PA	*T. smithi*	Tett *et al.,* 2021
45	*Prevotella_pallens*	EN; PA	*T. smithi*	Prasoodanan *et al.,* 2021
46	*Prevotella_pleuritidis*	PA	*T. smithi*	Cobo *et al.,* 2022
47	*Prevotella_salivae*	EN; PA	*T. smithi*	Könönen *et al.,* 2022
48	*Prevotellaceae_bacterium*	EN; G; PA	*T. smithi*	Hahnke *et al.,* 2016, Meier-Kolthoff, 2022
49	*Roseomonas_cervicalis*	EN; PA	*C.rothschildi*	Rihs *et al.,* 1993, Romano-Bertrand *et al.,* 2016
50	*Roseomonas_genomospecies*	PA	*C. longicaudatum*	Bello-López *et al.,* 2017
51	*Ruminococcaceae_bacterium*	EN; CD; RU; PA	*T. smithi*	Biddle *et al.,* 2013
52	*Ruminococcus_flavefaciens*	EN; CD; RU	*T. smithi*, *C. longicaudatum*	Dai *et al.,* 2015
53	*Ruminococcus_sp*	EN; CD; PA	*A.* cf. *collaris*	Rajilić-Stojanović and De vos, 2014
54	*Schwartzia_succinivorans*	EN; CD	*A.* cf. *collaris*	Gylswyk *et al.,*1997
55	*Selenomonas_noxia*	EN; CD; PA	*C. rothschildi*	Williams *et al.,* 2024
56	*Succinivibrio_dextrinosolvens*	EN; CD; PA; RU	*A.* cf. *collaris*, *T. smithi*, *C. rothschildi*, *C. longicaudatum*	Bryant and Small, 1955, Patterson and Hespell, 1985
57	*Treponema_medium*	EN; PA	*T. smithi*	Umemoto *et al.,* 1997
58	*Treponema_ruminis*	EN; CD	*A.* cf. *collaris*	Newbrook *et al.,* 2017
59	*Veillonella_sp.*	EN; G; PA	*T. smithi*	Megrian *et al.,* 2020

EN: Enzymatic activity; ME: from Marine environment; G: from Gut; CD: Cellulose digesting; RU: from Rumen; PA: Pathogenic activity; FW: from fresh water environment

Almost all the bacteria identified till species level have been previously documented for enzymatic activity of various kinds. Five species have been previously reported from rumen of herbivores. Nine bacteria have been identified from the gut of various animals including insects. Thirty-four bacterial species have been previously documented for pathogenic activity. Ten species of bacteria have been previously reported for cellulose digesting activity. Nine species were previously reported from marine environment and one species from freshwater environment.

Succinivibrio dextrinosolvens was a cellulose digesting bacteria found in all the four species of silverfish. More species of bacteria having this ability was found mostly in *A.* cf. *collaris* and *T. smithi*. Majority of the bacteria with previously known pathogenic activity was reported from *T. smithi*.

4.4. Discussion

Gut microbiome studies on silverfish were conducted for the first time in the world. Due to the lack of previous references and financial constraints the study has many shortcomings. But this preliminary work opens the window to a large unexplored realm of possibilities. In addition to understanding how pests such as silverfish digest cellulose, knowledge of insect microbiome has many applications in pest control. Experiments on gut microbiome benefit models, potential pathogenicity of gut microbiome of synanthropic silverfish which co-habits with humans can be conducted.

Knowledge of synanthropic insect gut microbiome could also help in understand if emergence of virulence in commensal bacteria is related to insects which cohabit with humans. Even though answers to such questions is out of the scope of this study, it is hoped that the results of this preliminary study can be enhanced with more samples and better molecular techniques. Hence, this study can serve as a starting point for such investigations.

A total of 38 phyla belonging to 2 kingdoms -Bacteria (33) and Archaea (5) were isolated from the gut of the four silverfish species. A total of 684 species belonging to 450 genera, 272 families, 171 orders and 74 classes were documented. While identification of all taxa till families were complete, identification at genus and species level was mostly incomplete as there were no documented matches in the SILVA database. Most of the gut microbes in all the four silverfish species belonged to eight phyla under kingdom Bacteria (Firmicutes, Bacteroidota, Proteobacteria, Actinobacteriota, Chloroflexi, Planctomycetota, Patescibacteria and Verrucomicrobiota) and a single phyla Euryarchaeota of kingdom Archaea. There were some marked differences between the gut microbiome of the four species with at least Rickettesia being unique to *A. cf. collaris* and Pediococcus being unique to *T. smithi* were placed far apart with *T. smithi* being the most distinct. Some species of bacteria were also found to be dominant in certain silverfish species. The presence of

Succinivibrio dextrinosolvens, a cellulose digesting bacterium, in all the four species of silverfish is a significant discovery. Most of the bacteria with previously known pathogenic activity was reported from *T. smithi*. Cellulase producing microbe '*Cellulomonas cellulans'* which was earlier identified (Premkumar *et al.* 2015) was not documented in this study.

Unidentified bacteria with similarity to gut and rumen of many other organisms have also been documented. While the findings are preliminary in nature the study underscores the potential of further exploration.

4.5. Conclusion

Gut microbiome of four species of silverfish was documented for the first time. Majority of the microorganisms remain unidentified but they could be matched to unidentified species of known provenance (sources) in the database. Many bacteria have earlier been identified from gut and rumen of other animals. The presence of *Succinivibrio_dextrinosolvens*, a cellulose digesting bacterium in all the four silver fish species and the presence of many previously known pathogenic bacteria is a major outcome of the study. Further studies with more samples and better molecular techniques and assistance of expert microbiologists is recommended.

CHAPTER 5

RECOMMENDATIONS

Chapter 5

Recommendations

Every academic study throws up insights which can help academicians, policy makers and society in general. Studies fraught with challenges also tend to leave many unanswered questions as in the case of this study. Based on this study recommendations for different stakeholders have been enlisted below.

5.1. Recommendations for academic purposes

1. The present study has not covered forest lands, plantations, agricultural lands- study of Zygentoma in such habitats is recommended.
2. Extensive field surveys and detailed taxonomical studies with molecular characterization are recommended to expand the checklist of Zygentoma species in Kerala and India
3. Identification of Silverfish includes morphology, morphometry, chaetotaxy, anatomy and molecular studies. It is suggested that identification shortcuts using apps and morphology be avoided
4. Proper control measures should be developed for established pest species in the area.
5. Study of gut microbiome has a lot of potential as described in Chapter 4. Different aspects of gut microbiome should be studied.

5.2. Recommendations to home-owners/librarians/curators

A. Insights gained from aggregation, substrate selection, paper damage and dispersion are:

1. Regularly declutter areas
2. Immediate recycling/selling/disposal of unused articles
3. Use airtight containers for food storage
4. Shelf liners/wall papers made of long- lasting synthetic material may be used.
5. Old papers commonly used as wrappers and liners should be avoided.
6. Holes and cervices in shelves and walls should be sealed

7. Paper documents of importance may be stored in plastic wrappers (not an environment friendly option)

8. Frequent checking of book shelves; providing light source and dehumidifiers.

9. Undertake immediate repair of damaged walls and water leaks especially in kitchens, bathrooms and storage regions.

10. In professional settings 24- hour lighting and dehumidifiers driven by solar power may be introduced

11. It is common to store unused coconut shells, husks, old clothes, newspapers etc either inside or near the house before they are disposed/sold to recyclers, leading to habitats most preferred by silverfish. This practice is indirectly an effect of inefficient centralized waste disposal services.

B. Based on observations on locomotion it is suggested that

12. The inner walls of kitchen shelves/document storage shelves etc be made smooth and devoid of crevices and cracks.

C. Based on previous reports on food habits we suggest that

13. Old book covers having starch-based glue be replaced with synthetic glue

5.3. Recommendations to Policy makers

A. Eradication of silverfish pestilence

Ground surveys undertaken for during this study found that maximum infestation was found in the ill maintained homes of people from lower economic and educational strata, old offices, archives and homes where regular maintenance and cleaning was difficult. An efficient waste disposal service sensitive to the social needs of the communities with community participation and helmed by local governments as suggested by (Knickmeyer, 2020) could help people to discontinue the practice of storing coconut husks, shell, old newspapers etc inside or near homes. Public awareness and education, changing the culture of hoarding to giving and a general civic sense could mitigate some degree of infestation. But ultimately it is the economic upliftment of the most underprivileged which can reduce breeding sources of synanthropic pests. Sutherland *et al.*, (2020) provides unique empirical evidence of the disproportionate allocation of public health burdens upon neighbourhoods facing multiple dimensions of disadvantage.

B. Support for academicians

It is necessary to increase the number of specialists studying taxonomy, even though the knowledge on other aspects of these insects is progressing, biodiversity studies are very poor. The ultrastructure of Zygentoma presents challenges for study, necessitating the introduction of new technologies. Molecular biological techniques, in particular, are essential for accurate specimen identification. However, the scarcity of advanced machinery in localities can impede research progress. So, funding becomes mandatory for introducing advanced technologies (high magnification microscopes, camera, drawing devices and advanced molecular study equipment. Efforts to attract more researchers in this field is also necessary.

CHAPTER 6

CONCLUSION

Chapter 6

Conclusion

Major information gaps concerning family Lepismatidae of Order Zygentoma, from Kerala State, India is filled by this research and summarized in this thesis. The taxonomy of Zygentoma saw a significant shift in research methodology, which resulted in the necessity to reassess many earlier findings and also rendered many earlier studies obsolete. While the Zoological Survey of India has done substantial work on Zygentoma of Northern and Eastern regions of India, there were no authenticated and published research on Zygentoma in Kerala. Given this, this study is crucial in fostering understanding on Lepismatidae of Kerala and India.

The objectives of this study were: to make a checklist of synanthropic silverfish species found in the study area; to record the morphological characteristics of synanthropic silverfish species using both traditional and contemporary taxonomic methods: to study the behaviour of selected silverfish species, and to examine the gut microbiota of selected silverfish species. While there were many references regarding the currently accepted methods for taxonomic examination of silverfish and assistance from global experts could also be procured there was no previous benchmark to decide the methodology for behavioural and gut microbiome studies. Hence methodology for these objectives were formulated after many pilot studies, consultation with experts and the perusal of literature on similar taxa of insects.

The thesis is organized into six chapters with chapter one introducing the subject, objectives and gap areas. Chapter two reviews the existing literature on Zygentoma taxonomy, presents the methods and findings related objective one and two. Chapter three and four does the same for objective three and four respectively. Specific recommendations for further research, policy making and various stakeholders are provided in chapter five. Chapter six consolidates the findings of the study.

Five species of synanthropic silverfish were documented from the study area. The discovery and description of a species new to science, *Thermobia smithi* sp. nov., is a significant outcome of this work. A neotype for *Ctenolepisma calvum* was designated, amending historical misattribution of European specimens to this species. Rediscovery of *Ctenolepisma calvum* from the study area also led to the recognition of the European specimens as a new species, *C. phantasma* sp. nov. *C. calvum* is being reported from India for the first time, and is now the 10th recognized species of the genus from the country. *C. rothschildi* and *C. longicaudatum* were described from Kerala for the first time, contributing new and authenticated distributional data.

The study also identified taxonomic ambiguities in specimens identified as *Acrotelsa* cf. *collaris*, and concluded that the specimens in the study area be designated as *species inquirenda* pending further investigation. All findings, with the exception of *Acrotelsa* cf. *collaris*, have been published in peer-reviewed journals.

This comprehensive study strengthens regional biodiversity documentation by providing correct identification and thereby providing an essential foundation for effective conservation in non- synanthropic settings. From the conservation viewpoint there are many questions which can be resolved only with further studies. Do these synanthropic species exist in the wild? What are their contributions to ecosystem services? Are conservation measures necessary in light of its pest status?

Behavioural studies revealed twenty distinct behaviours, among five synanthropic silverfish species, which were categorized under six major behaviour classes- feeding, locomotion, immobility, aggression, communication, and miscellaneous behaviours. Though no species displayed behaviour unique to itself, interspecific differences in behavioural time budgets were evident. *C. rothschildi* exhibited diverse feeding habits and was found on a wide range of substrates. Specialized behaviours such as "*thanatosis*" and "*cannibalism*" were documented. It was hypothesized that substrate selection and food preferences of species could be closely related. Experiment to determine distribution patterns and paper damage was also conducted. Insights from these experiments provided baseline data about behaviour of this little-known insects and helped to formulate pest management strategies.

The gut microbiome analysis of four synanthropic silverfish species has been undertaken for the first time in the world and disclosed a largely unknown microbial community. Less than sixty species making up the gut microbiome communities could be identified but many taxa matched previously documented but unnamed entries in global databases. The presence of *Succinivibrio dextrinosolvens*, a cellulose-digesting bacterium, in all four silverfish species and several bacteria previously identified from the gut or rumen of other animals indicate the possibility of understanding cellulose digestion mechanisms in silverfish. Many potential pathogenic bacteria were present in the gut microbiome of the silverfish, which raises questions about health implications of silverfish in human-inhabited spaces.

This study provides the first comprehensive account of synanthropic silverfish in Kerala, encompassing taxonomy, behaviour, and gut microbiology. It lays a base for further research and highlights the need for sustained studies on different aspects of Zygentoma.

This research would also help in comprehending the intricate interactions between humans and the arthropods in built spaces and presents many recommendations to limit infestation in tropical and highly populated regions. The study also highlights the persistent difficulties in silverfish taxonomy and the need of combining global cooperation with cutting-edge taxonomic techniques to address them.

CHAPTER 5
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APPENDIX I

LIST OF SITES FROM WHERE SPECIMENS WERE COLLECTED

No.	District	Location	Species * present	Latitude (N)	Longitude E
1	Thrissur	Puthur	R	10.4904	76.2819
2	Thrissur	Thiroor	A	10.5869	76.2218
3	Thrissur	Wadakkanchery	S, A, C, L	10.523636	76.216588
4	Thrissur	Thozhiyoor	R	10.6317	76.0258
5	Thrissur	Peringottukara	R	10.4448	76.1607
6	Thrissur	Kattilapoovam	R	10.573	76.2851
7	Thrissur	Kannara	R	10.5356	76.3362
8	Thrissur	Kombazha	L, R	10.5769	76.3974
9	Thrissur	Eastfort, Thrissur	R	10.5223	76.2238
10	Thrissur	Chembukkavu	R	10.5305	76.2204
11	Thrissur	Keerankulangara	S, C	10.524289°	76.217867
12	Thrissur	Kallettumkara	R	10.22578	76.1210
13	Thrissur	Punkunnam	A, T	10.55359	76.207919
14	Thrissur	Echippara	R	10.4381	76.4504
15	Thrissur	Velupadam	A, L, R	10.4328	76.3474
16	Thrissur	Madayikonam	C, R	10.3681	76.2382
17	Thrissur	Kallettumkara	R	10.22578	76. 1210
18	Thrissur	West Mangad	R	10.6766	76.0513
19	Thrissur	Puranattukara	R	10.5499	76.1564
20	Thrissur	Valarkkavu	C, R, L	10.506	76.2365
21	Thrissur	Peramangalam	A	10.56412	76.16188
22	Thrissur	Padukad	R, L	10.5621	76.2152
23	Thrissur	Peechi	L	10.527	76.3608
24	Thrissur	Vaniyampara	L	10.575684	76.400557
25	Thrissur	Chazhikad	L	10.2300.5	76.2059.5
26	Thrissur	Arimbur	L	10.49356	76.14723
27	Thrissur	Puthukkad	R	10.42119	76.27034
28	Thrissur	Nadathara	R	10.50813	76.26924
29	Palakkad	Alathur	R	10.6359	76.5358
30	Palakkad	Alathur	L	10.63505	76.53314
31	Palakkad	Alathur	R	10.6371	76.5319
32	Palakkad	Shop Near stadium bus stand	S	10.4625	76. 3945
33	Palakkad	Alathur	R	10.634	76.5311
34	Palakkad	Alathur	R	10.63587	76.535801
35	Palakkad	Alathur	R	10.63707	76.531869
36	Palakkad	Alathur	L	10.6403	76.5280
37	Palakkad	Nooradi	R	10.505	76.6744
38	Palakkad	Pattambi College	T	10.8095	76.1993
39	Palakkad	Nenmara	R	10.595	76.5969
40	Palakkad	Nenmara	R	10.5954	76.5962
41	Palakkad	Kizhakkenchery	R	10.5399	76.4679

No.	District	Location	Species * present	Latitude (N)	Longitude E
42	Palakkad	Palakkad fort	R	10.46336	76. 394104
43	Kannur	Padiyur	R	11.9944	75.619
44	Kannur	Ullikkal	R	12.0375	75.6703
45	Kannur	Kakkad	A, R	11.8894	75.3879
46	Kannur	Iritty	R	11.9819	75.6703
47	Kannur	Puzhathi	R	11.9000	75.3850
48	Idukki	Painavu	R	9.85392	76.9481
49	Idukki	Painavu	R, S	9.86493	76.9577
50	Idukki	Cheruthoni	R	9.85885	76.9617
51	Idukki	Upputhode	R	9.88916	77.0001
52	Idukki	Upputhode	R	9.88379	77.0067
53	Idukki	Upputhode	R	9.88332	77.0048
54	Idukki	Thadiyampad	R	9.87592	76.9684
55	Kottayam	Velloor	R	9.82199	76.4672
56	Kottayam	Velloor	R	9.8212	76.4672
57	Kottayam	Velloor	R	9.82198	76.4672
58	Kottayam	Kottayam	R	9.62248	76.4762
59	Kottayam	Kottayam	R	9.59014	76.5218
60	Kottayam	Kottayam	R	9.59016	76.5218
61	Kottayam	Kottayam	R	9.588164°	76.542347°
62	Kottayam	Thiruvarpu	R	9.5821	76.4873
63	Kottayam	Kottayam	R	9.58211	76.4873
64	Kottayam	Kottayam	R	9.58816	76.5423
65	Ernamkulam	Kunnukara	R	10.1634	76.3131
66	Ernamkulam	Koonammavu	L	10.096332	76.264766
67	Ernamkulam	Kochi	R	10.133731	76.241795
68	Ernamkulam	Kochi	R	10.133751	76.24179
69	Pathanamthitta	Konni	L	9.2304085	76.8077604

*Abbrevtions used in this table:

A=Acrotelsa, S= *T. smithi*, L= *C. longicaudatum*, R= *C. rothschildi*, C=*C. calvum*

APPENDIX II

MAJOR DIAGNOSTIC FEATURES USED FOR THE IDENTIFICATION OF SILVERFISH AND ABBREVIATIONS USED

List of Abbreviations used

HW: head width (mm)

TW: thorax width (mm)

TL: thorax length (mm)

H+B: head and body length (mm)

L/W: length to width (ratio)

PI, PII, PIII: legs of prothorax, mesothorax and metathorax respectively

N, N-1, N-2, N-3, N-4 etc referring to positions of bristle combs N starting from posterior most end (N: last, N-1: penultimate, N-2: antepenultimate, etc.)

RGCB Rajiv Gandhi Centre for Biotechnology, Trivandrum

STIC- Sophisticated Test and Instrumentation Centre.

CUSAT- Cochin University of Science and Technology

UCO: Department of Zoology, University of Córdoba (Spain)

ML: Maximum Likelihood Bayesian Inference

BI : Bayesian Inference

MNCN_Ent: Entomology collection of the Museo Nacional de Ciencias Naturales (MNCN), Madrid (Spain).

ZSI: Zoological Survey of India collection.

WGRC: Western Ghat Research Center, Calicut, Kerala (India)

TABLE:1 Currently relevant terminology for identification followed after Smith et al. 2019

No	Characters for identification
1.	Chaetotaxy of the head
2.	Relative length of the ultimate and penultimate article of maxillary palp
3.	Shape of the last article of the labial palp
4.	Arrangement and Number of the sensory papillae on labial palp
5.	Scale type and shape of scales on different body parts
6.	Arrangement of macrochaetae on the anterior collar of the pronotum
7.	Lateral and posterior chaetotaxy of pro, meso and metanota
8.	Shape, size and chaetotaxy of all thoracic sterna
9.	The chaetotaxy (position of combs as well as the number of macrochaetae of the urotergites and urosternites

10.	Shape and chaetotaxy of urotergite X.
11.	The number and length of the styli.
12.	The length of the ovipositor.
13.	The presence or the absence of the paramera

Diagnostic characteristics of Family Lepismatidae (Latreille, 1802) at different taxonomic ranks

The subphylum Hexapoda (Insecta *sensu lato*) is taxonomically classified into two major classes: Entognatha and Ectognatha (Insecta *sensu stricto*) (Hennig *et al*., 1953). Order Zygentoma *(Börner, 1904)* includes the silverfish or fishmoths, and the firebrats, with 2 sub orders, 2 infra orders, 6 families, 650 described species, distributed among 148 genera, organised into 5 families (6 families worldwide--Robla *et al* 2023) and 16 subfamilies. Each of the two larger, widespread families (Lepismatidae and Nicoletiidae) contain about 320 described species worldwide (Smith *et al.* 2022, Smith and Mitchell 2024). The subfamily Nicoletiidae is further subdivided into five tribes (Smith *et al.* 2022).

Family Lepismatidae (Latreille, 1802): Lepismatidae is the largest family of Zygentoma (Kahrarian et al .2014). Globally Lepismatidae comprises around 43 genera and over 310 documented species distributed among six subfamilies: The key features of this family following Mendes (1991) and Smith (2017) are:

- presence of smooth or pectinate setae that are arranged into combs or bushes
- an elongated and flattened body covered in multiradiate scales
- the pronotum may have a setal collar
- thoracic nota usually with lateral combs and anterior and posterior trichobotria on either side of the notum, the posterior margin of the nota may have a 1+1 comb of macrochaetae.
- the thoracic sterna is plate-like and covers the majority of the coxae (apart from Acrotelsatinae).
- the urotergites may have rows of macrochaetae or posterior combs.
- the urosternites may have posterior combs but lack abdominal vesicles, and there are multiple pairs of abdominal styli. either with or without parameres.
- ovipositor without subgenital plate

The different subfamilies of Lepismatidae are Acrotelsatinae Mendes, 1991; Ctenolepismatinae Mendes, 1991; Heterolepismatinae Mendes, 1991; Lepismatinae Mendes, 1991; Mirolepismatinae Mendes, 1991, and Silvestrellinae Mendes, 1991 (Australian Digital thesis of Zygentoma by Smith 2018).

The key features of each sub family given as follows:

1) Acrotelsatinae Mendes, 1991 (Wygodzinsky 1972 and Kahrarian 2014)
    - ✓ body elongated (16-18 mm)

- ✓ macrochaetae plumose
- ✓ pronotum with 1+1 setal tufts on the anterolateral angles.
- ✓ prosternum largely covered by fore coxae from below, and with a tuft of macrochaetae centrally.
- ✓ urosternites III–VII with 2+2 bristle-combs
- ✓ tenth urotergite long and triangular, with acute apex and with several pairs
- ✓ of bristle combs on its sides.
- ✓ ovipositor with fossorial spines apically on posterior gonapophyses
- ✓ male with parameres

2) Ctenolepismatinae Mendes, 1991 (Kahrarian 2014, Smith 2017)
   - ✓ pectinate macrochaetae.
   - ✓ heterogeneous dorsal scales with a big pauci-radiate scale and a smaller multiratiate one.
   - ✓ hind border of nota with 1+1 combs of setae
   - ✓ with 3–5 papillae on the distal article of the labial palp arranged in one row
   - ✓ the thoracic sterna as large plates attached only at their anterior margins
   - ✓ some urotergites (at least II–V) with 3+3 combs of macrosetae
   - ✓ urotergite X trapezoidal
   - ✓ adults with 1 or 2 pairs of abdominal styli
   - ✓ males without paramera

3) Heterolepismatinae Mendes, 1991
   - ✓ macrochaetae smooth
   - ✓ ultimate article of the
   - ✓ labial palp usually with 3+2 papillae
   - ✓ thoracic sterna free.
   - ✓ urotergites II-VII with 3+3 combs
   - ✓ urotergite VIII with 2+2 combs
   - ✓ urotergite X rounded posteriorly, without combs.
   - ✓ urosternite I with or without medial comb
   - ✓ parameres present
   - ✓ scales absent from terminal filaments.

4) Lepismatinae Mendes, 1991
   - ✓ antennae often with specialised asteriform or campaniform sensillae.
   - ✓ macrochaetae smooth, apically bifid or trifid.
   - ✓ apex of last article of
   - ✓ maxillary palp usually with a single cylindrical sensillum.
   - ✓ apical article of labial palp with 3+2 papillae.
   - ✓ anterior margin of pronotum without setal collar.
   - ✓ thoracic sterna large and free.
   - ✓ tarsi of three articles.

- ✓ parameres primitively with single glandular area, secondarily pseudoarticulated
- ✓ ovipositor with simple setae apically.

5) Microlepismatinae Mendes, 1991 (not reported from Indian Subcontinent)
6) Silvestrellatinae Mendes, 1991
   - ✓ parameres absent
   - ✓ ovipositor is a cluster of minute spinelets on the apex of posterior gonapophyses and their apophyses are short and conical
   - ✓ anterior margin of pronotum devoid of setal collar, at most with a row of a few isolated setae.

Papillae of apical article of labial palp arranged in two rows

APPENDIX III

STEPS FOLLOWED IN MICROSCOPIC EXAMINATION AND DISSECTION

1. Observe and photograph the head chaetotaxy, also count the number of the ommatidia in each compound eye.
2. Types of scales covering the body and appendages can be variable. So, before dissection, few scales from dorsal and ventral surface should be removed and studied.
3. Cephalic appendages such as maxillary palps and labial palps are removed one by one using two fine micro needles and their details such as number of segments, shape and L/W ratio of the ultimate and penultimate segments and number of sensory papillae in the case of labial palps are recorded.
4. To study chaetotaxy, one can begin either from the dorsal region or ventral region and carefully separate all the thoracic and abdominal tergites without applying much pressure.
5. Immediately after separating each thoracic and abdominal plates from the body using two micro-needles or fine tip forceps, transfer to clean and labelled slides. Remove any debris by gentle wiping by a fine brush dipped in alcohol.
6. The bristle insertion sockets (chaetotaxy) and the trichobothrial areas should be observed and recorded after mounting them on slides.
7. Chaetotaxy of the lateral and posterior margins of each thoracic nota was counted. Anterior margin of pronotum must be observed for pronotal collar and the arrangement of macrochaetae in it.
8. In the case of urotergites, the shape of the last abdominal tergites must be noted down, in addition to chaetotaxy.
9. Drawings are made using drawing tool.
10. In the case of thoracic sternites, in addition to chaetotaxy the shape and the ratio length/width (L/W) of each sternite is an interesting metric.
11. Pro, meso and metathoracic leg was carefully detached using a fine forceps; macrosetae arrangement, type of femoral scales, L/W ratio of tibiae, relative length of the tarsomeres of the tarsus, were the taxonomically relevant characters observed.
12. The eighth and ninth coxite is holds the genital part, the styli and are of taxonomic relevance. In rare cases (eg: Thermobia sps) 7^{th} sternite also carries the styli.

13. The number of styli and relative length of each stylus with respect to the 9th styli was measured. The styli many be separated from the body to take measurement.
14. The relative measurement (length and width) of the inner and outer process of the 8th coxite was also measured.
15. The length of the ovipositor is measured from the base of the 8th coxite to the tip of the ovipositor. Type of ovipositor (sclerotised or un sclerotised) and number of divisions of ovipositor was noted.
16. The length of the ovipositor that surpasses the apex of the ninth styli or the apex of the ninth coxites relative to the length of these appendages was noted.
17. In male genitalia, the presence or absence of paramera was noted as it has suprageneric relevance.

18. For mounting, the dissected samples were washed in *distilled* water and stained with acid – Fuschin stain. (Acid – Fuchsin stain powder 5 mg is dissolved in 100 ml of acetic acid).*
19. The stained sample was transferred to the xylene solution for clearing.
20. After clearing, a drop of Canada –balsam is applied over the sample and covered by coverslips.
21. The prepared slides were then properly labelled and photographed by using a Leica stereo microscope S8APO and the structure was studied.

Challenges of working with parts very small

1. Silverfish are small and soft-bodied which makes the dissection procedure tedious and tough. Many specimens have to be sacrificed to observe even a single character.
2. Body scales are lost if specimens are not fresh; Scales are small, usually hyaline, and visible clearly with light microscopy (above 40X) or SEM.
3. Macrochaetae and Trichobothria are shed or broken during handling, dissection, preservation, etc and only the insertions of macrochaetae are visible as bristle combs which are again visible only under high magnifications.
4. Sometimes it is difficult to describe the position of the anterior and posterior trichobothria of each side referring to lateral combs.
5. Macrochaetae of urotergites can be hyaline or detached and difficult to see in photographs or stereomicroscope. High power microscopic examination

is necessary to confirm their presence. Intraspecific variability in several taxa adds to the confusion and therefore the practice is not to consider the number of combs or isolated macrochaetae on one specific segment as the only diagnostic character for defining a species (Molero et al. 2010).

6. The number of divisions of ovipositor and the number and arrangement of sensory papillae are very difficult to observe even using high power microscope.
7. Globally Tendeiro solution is used as mounting medium for slide preparation, but it is banned in many countries including India so the quality and life of permanent slides prepared cannot be compared with international studies. Here the dissected sample with treated with fuchsin stain and mounted in Canada balsam which led to short life span of the slides.
8. In some dissected specimens, the abdominal chaetotaxy is difficult to observe because of the presence in the slides of numerous drops of fat coming from the gut contents.
9. Many published descriptions by non-specialists have errors due to mistakes in detecting the macrosetae or its insertion combs Eg; Querner et al. (2022) mentioned that *Lepisma saccharinum* lacks abdominal combs, which is an evident error. Also, in *Ctenolepisma lineatum*, the third pair of styli is not developed until the insect reaches about 8 mm of length. So, this character is not appropriate to identify young specimens. Moreover, the number can be different in both sexes of the same species. In *Ctenolepisma villosum*, males bear one pair of styli and females bear two pairs.

APPENDIX IV

DETAILS OF SPECIMENS DEPOSITED IN ZOOLOGICAL SURVEY OF INDIA (ZSI-WGRC)

Annexure II

Acknowledgement for receiving the deposits by the Designated Repository

Sl. No.	Data Field	Data specification
1.	NBA Reference	—
2.	Repository Reference Number / Registration Number	ZSI/WGRC/I.R.-INV. 26920
3.	Name and Address of the Designated Repository	ZSI-WGRC, Kozhikode
4.	Taxon / Species Name	~~Silverfish (smithi)~~ T. smithi
5.	Collection Details	
	Phylum	Arthropoda
	Class	Insecta
	Order	Zygentoma
	Family	Lepismatidae
	Genus	Thermobia
	Subgenus	—
	Species	T. smithi
	Subspecies	—
	Author and Date	Raphel S, Molero-Baltanas R, Mitchell A, Jose J (2024)
	No. of Specimens	1
	Locality	Wadakkanchery
	Taluk	~~Wadakkanchery~~ Thalapilly
	District	Thrissur
	State	Kerala
	Date of Collection	12/03/2021
	Collected by	Sheeba Raphel
	Determined by	Molero-Baltanas R & Mitchell A
	Type Category	Holotype
6.	Name and address of the depositor	Sheeba Raphel Assistant Professor Dept. of Zoology, St Thomas College Thrissur-1
7.	Date of Receipt	17/07/2024
8.	Date of Acknowledgement	17/07/2024

Name and Signature of the authorized signatory of the Repository with seal.

For o/C, ZSI-WGRC, Kozhikode

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Western Ghat Regional Centre
कोझिकोड, केरल / Kozhikode, Kerala - 673006

Annexure II

Acknowledgement for receiving the deposits by the Designated Repository

Sl. No.	Data Field	Data specification
1	NBA Reference	
2.	Repository Reference Number / Registration Number	ZSI/WGRC/I.R.-INV. 26921
3.	Name and Address of the Designated Repository	ZSI. WGRC, Kozhikode
4	Taxon / Species Name	~~Silverfish~~ ~~(smithi)~~ - T. smithi
5	Collection Details	
	Phylum	Arthropoda
	Class	Insecta
	Order	Zygentoma
	Family	Lepismatidae
	Genus	Thermobia
	Subgenus	
	Species	T. smithi
	Subspecies	
	Author and Date	Raphel S, Molero Baltanas R, Mitchell A, Jose J
	No. of Specimens	1
	Locality	Wadakkanchery
	Taluk	~~Wadakkanchery~~ Thalappilly
	District	Thrissur
	State	Kerala
	Date of Collection	12/03/2021
	Collected by	Sheeba Raphel
	Determined by	Molero Baltanas R
	Type Category	Paratype
6.	Name and address of the depositor	Sheeba Raphel, Assistant Professor Dept of Zoology, St Thomas College, Thrissur-1
7.	Date of Receipt	17/07/2024
8.	Date of Acknowledgement	17/07/2024

Name and Signature of the authorized signatory of the Repository with seal

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Acknowledgement for receiving the deposits by the Designated Repository

Sl. No.	Data Field	Data specification
1.	NBA Reference	—
2.	Repository Reference Number / Registration Number	ZSI/WGRC/I.R.-INV. 26922
3.	Name and Address of the Designated Repository	ZSI-WGRC Kozhikode
4.	Taxon / Species Name	~~Silverfish (calvum)~~ C. calvum
5.	Collection Details	
	Phylum	Arthropoda
	Class	Insecta
	Order	Zygentoma
	Family	Lepismatidae
	Genus	Ctenolepisma
	Subgenus	—
	Species	C. calvum
	Subspecies	—
	Author and Date	Ritter, 1910
	No. of Specimens	1
	Locality	Valarkkavu
	Taluk	Thrissur
	District	Thrissur
	State	Kerala
	Date of Collection	17/03/2022
	Collected by	Sheeba Raphel.
	Determined by	Molero-Baltanas R.
	Type Category	Neotype.
6.	Name and address of the depositor	Sheeba Raphel, Assistant Professor Dept. of Zoology, St. Thomas College, Thrissur-680 001
7.	Date of Receipt	17/07/2024.
8.	Date of Acknowledgement	17/07/2024.

Name and Signature of the authorized signatory of the Repository with seal.

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Acknowledgement for receiving the deposits by the Designated Repository

Sl. No.	Data Field		Data specification
1.	NBA Reference		—
2.	Repository Reference Number / Registration Number		ZSI/WGRC/I.R.-INV. 26923
3.	Name and Address of the Designated Repository		ZSI/WGRC, Kozhikode
4.	Taxon / Species Name		~~Silverfish (calvum)~~ C. calvum
5.	Collection Details		
		Phylum	Arthropoda
		Class	Insecta
		Order	Zygentoma
		Family	Lepismatidae
		Genus	Ctenolepisma
		Subgenus	–
		Species	C. calvum
		Subspecies	–
		Author and Date	Ritter (1910)
		No. of Specimens	1
		Locality	Valarkkavu
		Taluk	Thrissur
		District	Thrissur
		State	Kerala
		Date of Collection	17/03/2022
		Collected by	Sheeba Raphel
		Determined by	Molero-Baltanas R
		Type Category	Paraneotype
6.	Name and address of the depositor		Sheeba Raphel, Assistant Professor Dept. of Zoology, St. Thomas College Thrissur-680 001
7.	Date of Receipt		17/07/2024.
8.	Date of Acknowledgement		17/07/2024.

Name and Signature of the authorized signatory of the Repository with seal.

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Sl. No.	Data Field	Data specification
1.	NBA Reference	—
2.	Repository Reference Number / Registration Number	ZSI/WGRC/I.R.-INV. 26924
3.	Name and Address of the Designated Repository	ZSI/WGRC/ ~~I.R- INV 269~~ Kozhikode
4.	Taxon / Species Name	Silverfish (longicaudatum)
5.	Collection Details	
	Phylum	Arthropoda
	Class	Insecta
	Order	Zygentoma
	Family	Lepismatidae.
	Genus	Ctenolepisma
	Subgenus	—
	Species	longicaudatum
	Subspecies	—
	Author and Date	Escherich, 1905
	No. of Specimens	1
	Locality	Painavu
	Taluk	Idukki
	District	Idukki.
	State	Kerala
	Date of Collection	22/05/2021.
	Collected by	Sheeba Raphel
	Determined by	Molero-Baltanas R.
	Type Category	Voucher specimen
6.	Name and address of the depositor	Sheeba Raphel, Assistant Professor Dept. of Zoology, St. Thomas College, Thrissur-1
7.	Date of Receipt	17/07/2024
8.	Date of Acknowledgement	17/07/2024.

Name and Signature of the authorized signatory of the Repository with seal.

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Sl. No.	Data Field	Data specification
1.	NBA Reference	—
2.	Repository Reference Number / Registration Number	ZSI/WGRC/I.R.-INV.26925
3.	Name and Address of the Designated Repository	ZSI-WGRC, Kozhikode
4.	Taxon / Species Name	Silverfish (longicaudatum).
5.	Collection Details	
	Phylum	Arthropoda
	Class	Insecta
	Order	Zygentoma
	Family	Lepismatidae
	Genus	Ctenolepisma
	Subgenus	-
	Species	longicaudatum
	Subspecies	-
	Author and Date	Escherich, 1905
	No. of Specimens	1
	Locality	Pairavu
	Taluk	Thrissur
	District	Thrissur
	State	Kerala
	Date of Collection	22/05/2021
	Collected by	Sheeba Raphel
	Determined by	Molero-Baltanas R.
	Type Category	Voucher Specimen
6.	Name and address of the depositor	Sheeba Raphel Assistant Professor Dept. of Zoology, St. Thomas College, Thrissur-680001
7.	Date of Receipt	17/7/2024
8.	Date of Acknowledgement	17/7/2024.

Name and Signature of the authorized signatory of the Repository with seal.

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Sl. No.	Data Field	Data specification
1.	NBA Reference	—
2.	Repository Reference Number / Registration Number	ZSI/WGRC/I.R.-INV. 26926
3.	Name and Address of the Designated Repository	ZSI- WGRC, Kozhikode
4.	Taxon / Species Name	~~Silverfish~~ (rothschildi)
5.	Collection Details	
	Phylum	Arthropoda
	Class	Insecta
	Order	Zygentoma
	Family	Lepismatidae
	Genus	Ctenolepisma
	Subgenus	-
	Species	C. rothschildi.
	Subspecies	-
	Author and Date	Silvestri, 1907
	No. of Specimens	1
	Locality	Puthur
	Taluk	Thrissur
	District	Thrissur
	State	Kerala
	Date of Collection	19/8/2020.
	Collected by	Sheeba Raphel
	Determined by	Molero- Baltanas R
	Type Category	Voucher specimen.
6.	Name and address of the depositor	Sheeba Raphel, Assistant Professor Dept. of Zoology, St. Thomas College, Thrissur-680 001
7.	Date of Receipt	17/07/2024.
8.	Date of Acknowledgement	17/07/2024.

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Sl. No.	Data Field	Data specification
1.	NBA Reference	–
2.	Repository Reference Number / Registration Number	ZSI/WGRC/I.R.-INV.26927
3.	Name and Address of the Designated Repository	ZSI-WGRC, Kozhikode
4.	Taxon / Species Name	Silverfish (C. rothschildi)
5.	Collection Details	
	Phylum	Arthropoda
	Class	Insecta
	Order	Zygentoma
	Family	Lepismatidae
	Genus	Ctenolepisma
	Subgenus	–
	Species	C. rothschildi
	Subspecies	–
	Author and Date	Silvestri, 1907
	No. of Specimens	1
	Locality	Puthur
	Taluk	Thrissur
	District	Thrissur
	State	Kerala
	Date of Collection	19-8-2020
	Collected by	Sheeba Raphel
	Determined by	Molero-Baltanas R
	Type Category	Voucher specimen
6.	Name and address of the depositor	Sheeba Raphel, Assistant Professor Dept. of Zoology, St. Thomas College, Thrissur-680001
7.	Date of Receipt	17-07-2024
8.	Date of Acknowledgement	17-07-2024

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LIST OF PAPER PUBLICATIONS AND PAPER PRESENATIONS

	RESEARCH ARTICLES
1.	Raphel, S., Baltanás, R.M., Mitchell, A. & Jose, J. *Thermobia smithi* sp. nov. a new species of synanthropic silverfish (Zygentoma: Lepismatidae) from Kerala, India. Int J Trop Insect Sci 44, 2371–2380 (2024). https://doi.org/10.1007/s42690-024-01343-0
2.	Raphel, S. & Jose, J. (2025) Description of two synanthropic silverfish (Insecta: Zygentoma), *Ctenolepisma longicaudatum* (Escherich, 1905) and *Ctenolepisma rothschildi* (Silvestri, 1907), from Kerala, India. *Zootaxa*, 5570 (2), 309–324. https://doi.org/10.11646/zootaxa.5570.2.4
3.	Raphel, S., Baltanás, R.M., Mitchell, A. & Jose, J. (2025) Rediscovery of synanthropic silverfish species *Ctenolepisma calvum* (Insecta: Zygentoma: Lepismatidae: Ctenolepismatinae) from Thrissur, Kerala, India and designation of European *C. calvum* as *C. phantasma* n.sp. *Zoosystema*. 47(21), 425-443.

PAPER PRESENATIONS

Sr. Dr. Rani George Endowment Lecture
&
Intercollegiate Paper/Poster Presentation Competition

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St. Mary's College, Thrissur, Kerala

CERTIFICATE

This is to certify that ~~Mr.~~/Ms./~~Dr.~~ Sheeba Raphel presented a paper/~~poster~~ entitled "Cellulose Feeding Insects as an innovative remedy For organic waste management with emphasis on SilverFish" on 5thDecember 2017.

Sr.Dr.Magie Jose
Principal

Dr.Julie P Lazar
IQAC Co-ordinator

Dr.Meera C R
Program Convenor

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UNIVERSITY OF CALICUT
DEPARTMENT OF ZOOLOGY

Golden Jubilee National Seminar on
Frontiers in Biological Research

Certificate

This is to certify that ~~Prof.~~/~~Dr.~~/~~Mr.~~/ Ms. SHEEBA RAPHEL has participated/presented a paper entitled Preliminary characterisation of carbohydrate, cholesterol and protein content in Silverfish (Zygentoma) in the Three-Day Golden Jubilee National Seminar on **Frontiers in Biological Research** organized by the **Department of Zoology, University of Calicut, Kerala**, held on March 12-14, 2018.

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14-03-2018

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(Re-accredited by NAAC with A grade)

42nd Annual Conference of ESI & National Symposium on ANIMAL BEHAVIOUR, BIODIVERSITY AND HUMAN FUTURE

Certificate

This is to certify that Prof./Dr./Mr./Ms. Sheeba Raphel *has participated/ presented a paper titled* Feeding behaviour and distribution patterns of a peridomestic Zygentoma species with a note on cannibalism.

*in the 42nd **Annual Conference and National Symposium on Animal Behaviour, Biodiversity and Human future** jointly organized by the **Department of Zoology, University of Calicut and Ethological Society of India (ESI)** on 04 to 06, December 2018.*

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Calicut University
06-12-2018
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Dr. Y. Shibu Vardhanan
General Convener

Dr. S. Faizi
President, Ethological Society of India (ESI)