Zoodiversity, **58**(3):221–230, 2024
DOI 10.15407/zoo2024.03.221

UDC 595.42:57.017.8

A STUDY ON THE FEEDING BIOLOGY OF SOIL ORIBATID MITE *PAPILLACARUS (PAPILLACARUS) ELONGATUS* (ACARI, LOHMANNIIDAE)

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urn:lsid:zoobank.org:pub:F6D3FA83-87FB-43C3-B11B-C0F8ADEFB4AF

A Study on the Feeding Biology of Soil Oribatid Mite *Papillacarus (Papillacarus) elongatus* (Acari, Lohmanniidae). Praveena, K. K. & Sobha, T. R. — The feeding biology of lohmanniid mite *Papillacarus* (*Papillacarus*) *elongatus* Xavier, 2007 was investigated under laboratory conditions (relative humidity 80 ± 2 % and temperature 27 ± 2 °C) using appropriate feeding preference tests. Different microfungi and semi-degraded leaves were provided as food items. To validate feeding biology, the gut enzymes and structural morphology of mouth parts of *P.* (*P.*) *elongatus* were also examined. The results demonstrated that the *P.* (*P.*) *elongatus* are panphytophages, and could feed on both higher and lower plant elements. These alternative feeding guilds might enable these mites to coexist with other soil organisms; moreover, they can be directly involved in the biodegradation of leaf litter and indirectly influence the microbial activity in the soil ecosystem.

Key words: Lohmanniidae, microfungi, semi-degraded leaves, biodegradation, detritus food chain, panphytophages.

Introduction

The soil is a natural source of minerals and organic materials on the earth's surface and contains about 25 % of global biodiversity in terms of species (Coleman & Wall, 2015; Trap et al., 2016; Voroney, 2007). Biological communities inhabiting soil consist of wide range of life forms and functions, involved in large number of ecological processes and provide major ecosystem services for human population (Barrios, 2007; Wagg et al., 2014; de Vries et al., 2013; Kibblewhite et al., 2008). Soil microarthropods are very important members in soil fauna which can influence litter decomposition, structure and functional activity of the microbial community in the soil (Wolters, 2000).

Oribatid mites are important and dominant arthropod groups in highly organic temperate forest soils (Krantz & Walter, 2009). Strong trophic niche differentiation in decomposer microarthropods was registered with stable isotope ratios (15N/14N) (Schneider et al., 2004). The high species diversity of oribatid mites indicates their different life strategies as well as strong niche differentiation (Erdmann et al., 2007). Oribatid mites explore various food items like bacteria, algae, fungi, leafy and woody material in the leaf litter, and wastes from living organisms and carrions. Based on these feeding habits, they are grouped in to three major feeding guilds namely, microphytophages, macrophytophages, and panphytophages (Schuster, 1956; Hartenstein, 1962, Haq, 1994, 1996, 2019). The macrophytophages mainly feed on higher plant materials like dried leaves and woody materials. Microphytophages can only consume lower plant materials and the panphytophages consume higher and lower plant material. Thus phytophagous feeding behaviour must have certain advantages and it acts as a measure of ecological plasticity that allows diet switching when confronted with competition or shortage of a particular food item (Wallwork, 1958). The feeding habits were revised and more detailed groups were formed; panphytophagous for non- specialist feeders; zoophagous for feeding on living material; necrophagous for feeding on carrion; coprophagous for feeding on faecal material (Luxton, 1972). The oribatid mites is the only group of arachnids known to play multiple roles in the decomposition process (Norton, 1985). They act as a 'catalyst' in the decomposition process, dispersing fungal spores, hyphae and bacteria through their gut and faeces, and on their body surface (Behan & Hill, 1978). In addition, they can alter the chemistry of litter during decomposition and enhance Carbon mineralization rates, stimulating hydrolytic and oxidative enzyme activities (Wickings & Grandy, 2011).

According to Wallwork (1983), the observation of feeding behaviour of oribatid mites helps to understand their role in the soil ecosystem and litter decomposition. Among the Oribatida, the Lohmanniidae is one of the more important taxa in the process of degradation of the residues of higher plants in the litter and the mineralization process (Haq & Prabhoo, 1976; Haq, 1992, 1994, 1996, Haq & Konikkara, 1989). A summary of family Lohmanniidae Berlese 1916 was first published by Grandjean with 15 species in 1950. Now total 22 genera with 236 species have been reported mostly with tropical and subtropical distributions (Hammer & Wallwork, 1979; Balogh & Balogh, 1992; Subías 2022). They are early to intermediate derivatives, exhibit just a little amount of evolutionary radiation and laboratory research has demonstrated that they have parthenogenetic reproduction (Norton, 2010; Shereef 1976). Feeding habits of selected members of family Lohmannidae under laboratory condition disclosed their wood boring and leaf skeletonizing abilities, facilitated by microbial colonies in their gut which release necessary enzymes for mite food degradation (Ramani & Haq 1990). In the present study we are investigating the feeding preferences of the lohmanniid mite *Papillacarus* (*P.*) *elongatus* under laboratory conditions(relative humidity 80 ± 2 % and 27 ± 2 °C) by providing different microfungi (*Pseudopestalotiopsis* sp.*, Ectophoma multirostrata, Curvularia verruculosa*, *Corynespora cassiicola, Lasiodiplodia theobromae,* and *Trichoderma harzianum*) and semi-degraded leaves (*Hevea brasiliensis, Theobroma cacao, Myristica fragrans, Artocarpus hirsutus, Artocarpus heterophyllus, Mangifera indica*).

Material and Methods

Study area

The soil samples were collected from Thusharagiri (11.28° N; 76.3° E), in Calicut district of Kerala, India (fig. 1). The sampling location is characterized by heavy litter accumulation. This area is a part of the Western Ghats and is famous for its waterfalls, and thick evergreen forest comprising wide variety of flora and fauna. Currently, the forest's vegetation dominated mainly by trees of various girths and heights. Notable tree species in the area are *Alexandrian laurel*, *Albizia lebbeck, A. procera, Alstonias cholaris, Ficus callosa, Bauhinia malabarica, Artocarpus hirsutus, Artocarpus heterophyllus, Cassia fistula etc.*

Collection, extraction and identification of mites

The sampling was done from October 2021 to March 2022, in the early morning between 7–8 am. Soil along with decomposed plant parts like leaf and wood, algae, fungi, lichens and mosses were carefully collected from upper 10 cm using a metal corer (5 x 5 x 10 cm). The collected soil samples were kept for extraction in a Berlese Funnel Apparatus. The process of extraction was completed within 3–4 days. From the extracted live

Fig. 1. A view of sample collection site-Thusharagiri, Kozhikode, Kerala. The left insert shows semi-degraded leaf litter from the sampling site in detail.

mites, *P. (P.) elongatus* were separated using a moistened camel hairbrush under a Stereo Zoom Microscope (Labomed) and transferred into individual culture vessels for subsequent rearing.

The images of *P.* (*P.*) *elongatus* were taken using Hitachi SU6600 Variable Pressure Field Emission Scanning electron microscopic (FESEM) (fig. 2). The identification of the mites was done by using taxonomic keys, (Balogh and Balogh, 2002) and relevant studies.

Preparation of culture vessels and rearing of mites

The rearing of selected *P. (P.) elongatus* was carried out under laboratory condition (relative humidity of 80 ± 2 % and temperature — 27 ± 2 °C) within plastic containers (4 cm diameter and 5 cm height). The base of culture vessel was filled with mixture of plaster of paris and activated charcoal in a 4 : 1 ratio. Semi-degraded leaves of *Artocarpus hirsutus* used as food item for rearing the mites.

Analysis of laboratory feeding

The following microfungi and semi-degraded leaves were offered to *P. (P.) elongatus* as food items:

1. Semi-degraded leaves of *H. brasiliensis*, *T. cacao*, *M. fragrans*, *A. hirsutus*, *A. heterophyllus*, and *M. indica*. The semi-degraded leaf litters of *A. hirsutus*, *A. heterophyllus*, and *M. indica* were collected from the sampling site itself. *H. brasiliensis*, *T. cacao*, *M. fragrans* are the important plantation crops in the area near Thusharagiri. All the leaf litter collected were washed with distilled water, oven dried (45 °C for 24 hours), then cut in to small square pieces with 2 cm^2 size.

2. Both beneficial *(T. harzianum)* and pathogenic *(Pseudopestalotiopsis* sp., *E. multirostrata*, *C. verruculosa*,*C. cassiicola*, *L. theobromae)* fungi were used in the experiment. The fungi along with PDA medium cut into small square pieces with 2 cm^2 size.

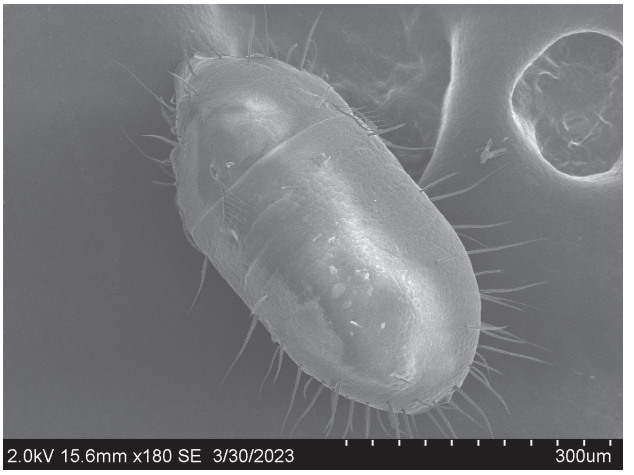

Fig. 2. Scanning electron microscopic image of *P.* (*P.*) *elongatus*.

The presence of *P. (P.) elongatus* on the food (assessment of choice between diets): Twenty adults of *P. (P.) elongatus* were introduced into the culture vessel, and a single food item was placed in the vessel. The number of individuals staying on the food was checked after 24, 48, 72, 96, and 120 hours.

Preference among food items (Cafeteria experiment): Twenty adults of *P. (P.) elongatus* were kept in the culture vessel, and different food items were placed inside the vessel in a circle. The number of individuals staying on each food type was checked after 24, 48, 72, 96, and 120 hours.

Defecation rate of *P.* (*P.*) *elongatus*: *P.* (*P.*) *elongatus* was reared individually in culture vessels with one food item. Then the number of new fecal pellets was counted after 24, 48, 72, 96, and 120 hours.

All the experimental tests were set as ten replicates each.

Statistical analysis

The normality of the data from the experiment regarding the presence of *P. (P.) elongatus* on a single food item was tested with the Kolmogorov-Smirnov test of normality in SPSS 25 software. The non-parametric Kruskal-Wallis H test was used to test the variation in feeding preference among the provided food items.

Gut enzyme analysis

The presence of cellulase and trehalase in the gut of *P. (P.) elongatus* was confirmed by testing the conversion of added cellulose and trehalose to glucose by these enzymes. Initially, 20 individuals of *P. (P.) elongatus* were placed in a glass mortar and added 100 µl of 1 % carboxymethyl cellulose or 100 µl of 1 % trehalose. They were then pulverized with a small glass pestle until no body parts were recognizable. The 400 µl carboxymethyl-cellulose or 400 µl 1 % trehalose was added to the homogenate, mixed well, and transferred into a plastic Eppendorf tip. The mortar and pestle were rinsed twice with 500 µl 1 % carboxymethyl-cellulose or 500 µl 1 % trehalose. This was also transferred to the Eppendorf tip. Then the Eppendorf tip was kept in a water bath for 4 hours at 37 °C. After this, they centrifuged at 12,000 rpm for 10 minutes. 0.5 µl of supernatant was pipetted out and mixed with 0.5 µl colour reagent solution (prepared by adding 1.6 ml of dianisidine solution to a mixture of one capsule of PGO enzyme dissolved in 100 ml of distilled water). Then the mixture was incubated for 45 minutes in a dark room at 18 °C. The mixture was carefully observed for colour changes. For the analysis of chitinase, 20–25 individuals of *P. (P.) elongatus* were put in a small glass mortar. 100 µl of potassium-phosphate buffer was added and pulverized with a glass pestle. Another 400 µl of potassium-phosphate buffer was added to the homogenate. After thorough mixing, 1 ml of potassium-phosphate buffer was added to rinse the mortar and pestle. The homogenate was centrifuged for 10 minutes at 12,000 rpm. A 100 µl supernatant was pipetted out and mixed with 900 µl p-nitrophenol NN-diacetyl chitobiose. The mixture was gently shaken for ten seconds and incubated for three hours in a 37 °C water bath. The mixture was observed for any colour changes (Berg et al., 2004).

Analysis of the morphology of mouthparts

The mouth parts of *P.* (*P.*) *elongatus* were dissected, mounted on the slide, and examined under a compound microscope to study their structural morphology (Haq, 2007 a).

Results

Analysis of laboratory feeding

The experiment regarding the number of *P.* (*P.*) *elongatus* present on a single food item showed that average mite count on *A. hirsutus* and *A. heterophyllus* was 18.6 ± 0.5 and 18.2 ± 0.4, respectively, within 24 hrs. The number of *P.*(*P.*) *elongatus* present on the semi-degraded leaves of *M. indica* was 16.2 ± 0.2 mites while no mites present on the semi-degraded leaves of *H. brasiliensis*, *T. cacao*, and *M. fragrans* (table 1, a).

Among the microfungi, in the case of *C. verruculosa* 17 ± 1.2 mites were present within 24 hours. On *C. cassiicola*, *L. theobromae*, and *T. harzianum* the number of present mites was 15.6 ± 0.5, 16.2 ± 0.1, and 10.2 ± 0.8, respectively. Contrary to the above observations, no *P. (P.) elongatus* mites were present on the microfungi *Pseudopestalotiopsis* sp. and *E. multirostrata*.

According to the Kolmogorov-Smirnov normality test, the data about the number of *P. (P.) elongatus* staying on a single food item was not in a normal distribution (table 1, b). So the non-parametric Kruskal-Wallis H test was used to test the variation. According to the test, there was a significant difference in the number of *P. (P.) elongatus* staying on a single food item ($p < 0.05$) (table 1, c). Thus, it is clearly evident that *P. (P.) elongatus* showed significant variation in preference among the different food items provided.

Table 1, a. Average number of *P. (P.) elongatus* present on a single food item per day (N = 5)

Sl no	Food	Mean	N	Std. Deviation
1	*C. verruculosa*	17	5	1.225
2	*E. multirostrata*	1.9	5	0.742
3	*Pseudopestalotiopsis* sp.	1.5	5	0.458
4	*C. cassiicola*	15.6	5	0.548
5	*L. theobromae*	16.2	5	1.095
6	*T. harzianum*	10.2	5	0.837
7	*H. brasiliensis*	1	5	0.707
8	*A. heterophyllus*	18.2	5	0.447
9	*A. hirsutus*	18.6	5	0.548
10	*M. indica*	16.2	5	0.837
Total		11.64	50	7.11

Table 1, b. Kolmogorov-Smirnov normality test for distribution of the number of *P. (P.) elongatus* staying on a single food item

Tests of Normality			
	Kolmogorov-Smirnov		
	Statistic	Df	Sig.
Number of Mites	0.282	50	0.000

Table 1, c. Kruskal Wallis H test showing the variation in the number of *P. (P.) elongatus* staying on a single food item ($p < 0.05$)

	Number of Mites
Kruskal-Wallis H	44.884
Df	9
P-value	0.000

In the cafeteria experiment, the number of mites present on the semi-degraded leaves of *A. hirsutus*, *A. heterophyllus*, and the microfungus *C. verruculosa* was 4.64 ± 0.2, 3.8 ± 0.2, and 4.01 ± 0.7, respectively. The number of mites present on the semi-degraded leaves of *M. indica* was 2.005 ± 0.2, and on the microfungi *C. cassiicola*, *L. theobromae*, *T. harzianum*, *E. multirostrata*, and *H. brasiliensis* values of this parameter were 1.15 ± 0.3, 1.3 ± 0.2, 0.2 ± 0.4, and 1.3 ± 0.1. There were no mites present on the microfungi *Pseudopestalotiopsis* sp. and semi-degraded leaves of *T. cacao* and *M. fragrans* (table 2).

Table 2. Cafeteria experiment number of *P. (P.) elongatus* present on different food items per day (N = 5)

Sl No	Food	Mean ±SD
1	*C. verruculosa*	4 ± 0.7
2	*E. multirostrata*	0.2 ± 0.4
3	*Pseudopestalotiopsis* sp.	0
4	*C. cassiicola*	1.15 ± 0.3
5	*L. theobromae*	1.3 ± 0.2
6	*T. harzianum*	1.35 ± 0.3
7	*H. brasiliensis*	1.3 ± 0.1
8	*T. cocoa*	0
9	*M. fragrans*	0
10	*A. heterophyllus*	3.8 ± 0.2
11	*A. hirsutus*	4.64 ± 0.2
12	*M. indica*	2.005 ± 0.2

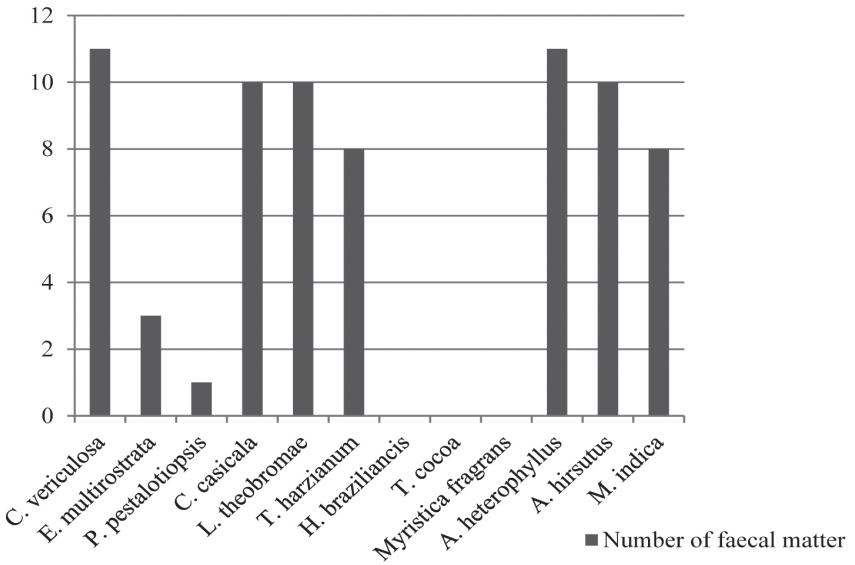

Fig. 3. Average defecation rate of *P.* (*P.*) *elongatus* per day after feeding semi-degraded leaves and microfungi.

Analysis of defecation rate showed that *P.* (*P.*) *elongatus* produced a maximum number of fecal pellets (10–12) after feeding on semi-degraded leaves of *A. hirsutus*, *A. heterophyllus*, and microfungus *C. verruculosa* within 24 hours. At the same time, a moderate number of feces were observed (7–9) after feeding on the semi-degraded leaves of *M. indica* and the microfungi *C. cassiicola*, *L. theobromae*, and *T. harzianum*. A minimum number of fecal matter was observed (1–2) after feeding on the microfungi *Pseudopestalotiops* sp. and *E. multirostrata* and did not produce any fecal matter on the semi-degraded leaves of *H. brasiliensis*, *T. cacao*, and *M. fragrans* (fig. 3).

Analysis of the morphology of mouthparts

The chelicera of *P.* (*P.*) *elongatus* was strong and stout, with well-sclerotized, sharp, and highly developed teeth. The rutellum was sclerotized, strong, with three unsharped incisions (fig. 4). This type of gnathal appendage enabled *P.* (*P.*) *elongatus* to utilize a wide range of food items, including higher plant materials like semi-degraded leaves and lower plant materials like fungi.

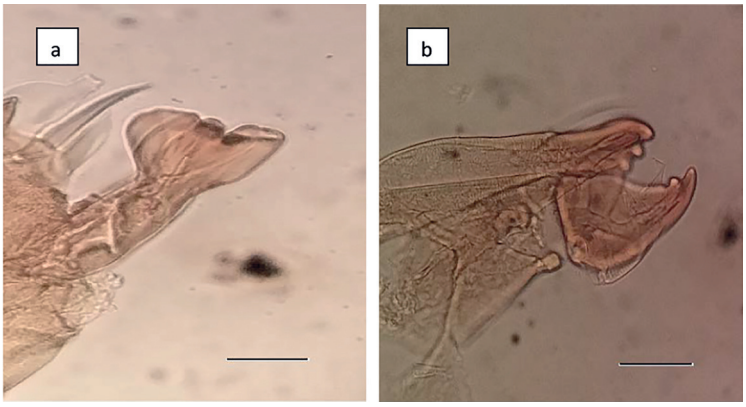

Fig. 4. Mouth parts of *P.* (*P.*) *elongatus*. a — chelicera; b — rutellum. Scale 50μm

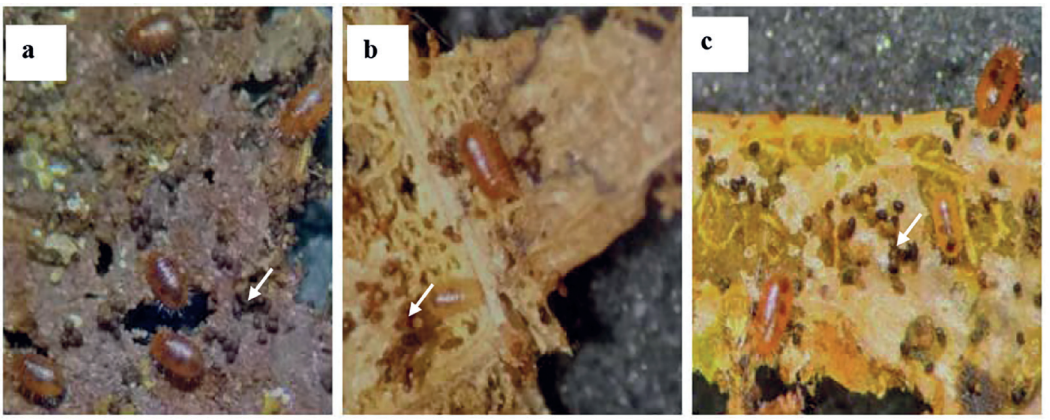

Fig. 5. *P.* (*P.*) *elongatus* feeding on: a — *A. hirsutus*; b — *A. heterophyllus*; c — *M. indica*. The faecal pellets produced are indicated by arrows.

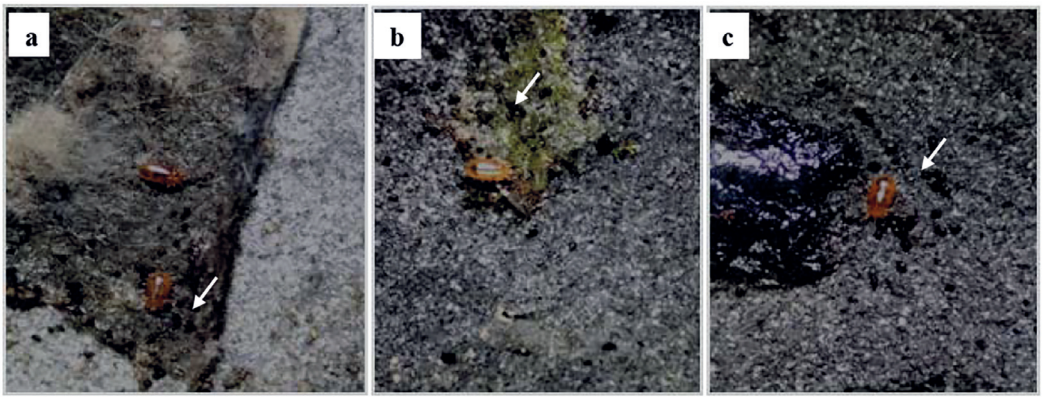

Fig. 6. *P.* (*P.*) *elongatus* feeding on: a — *C. cassiicola*; b — *T. harzianum*; c — *C. verruculosa*. The faecal pellets produced are indicated by arrows.

Gut enzyme analysis

The results of the qualitative analysis of the gut enzymes of *P.* (*P.*) *elongatus* revealed that trehalase and cellulase activity were present, but chitinase activity was absent. The above enzymatic activities confirmed that *P.* (*P.*) *elongatus* mites are opportunistic herbo-fungivores. That is, they can digest the cellulose present in the semi-degraded litter and the trehalose in the fungi.

The results of the present study showed that *P. (P.) elongatus* are panphytophages that can feed on both higher and lower plant materials like semi-degraded leaves (fig. 5) and microfungi (fig. 6) under laboratory condition.

Discussion

The Lohmanniidae is a moderately diverse family of soil oribatid mites. Previous studies on the food choice test and gut analysis of the lohmanniid mites *Hoplophthiracarus rimosus* and *Lohmannia* sp. showed that they are macrophytophages with cellulase and cellobiase present in the gut to digest higher plant materials (Ramani & Haq, 2001). Similarly, *Meristacarus degradatus* mites are macrophytophages with the potential for biodegradation of higher plant residues like wood and leaf materials (Haq & Jaikumar, 1993). Contrary to the above findings, the studies by Haq (2016) found that *Lohmannia* sp. can consume lower plant ma-

terials like algae and fungi in laboratory conditions and concluded that some members of the family Lohmanniidae can be included in the panphytophagous feeding guild. Similarly, the feeding preference test of *P.* (*P.*) *elongatus* showed that they exhibit both macrophytophagous and microphytophagous feeding behavior under laboratory conditions.

Previous studies reported that the total number of faecal matter produced by panphytophagous oribatid mites, *Scheloribates laevigatus, Achiptera coleopteran* and *Galumna elimata* during 5 successive days was different and significantly affected by diet (Hubert et al., 2001). The amount of faecal matter produced per day during the study period did not differ significantly in our investigation; nevertheless the rate of defecation was impacted by the diet given.

The food preference test conducted in laboratory conditions may get biased due to various physical factors like types of food offered, stages of decomposition, quality, and quantity of food. Therefore, gut enzyme analysis is considered the most reliable method for testing food preferences in oribatid mites (Luxton, 1972; Haq, 1987; Haq, 2007 b; Haq, 2016). According to Siepel and Dijkman (1993), the oribatid mites can be grouped into seven feeding guilds based on their carbohydrase activities: herbivorous grazers, herbivorous browsers, fungivorous grazers, fungivorous browsers, herbo-fungivorous grazers, opportunistic herbo-fungivores, and omnivores. Based on the observations of the present study, *P.* (*P.*) *elongatus* can be included in the opportunistic herbo-fungivore group because the mites contain both cellulase and trehalase enzymes in its gut. These enzymes would help *P.* (*P.*) *elongatus* to digest both cellulose in the leaf litter and trehalose in fungi.

According to Kaneko (1988), the shape of chelicerae can affect the feeding habits of oribatid mites. The studies on the feeding behavior of some forest soil acarina showed that macrophytophagous mites have large, well-developed chelicerae with wide bases for muscle attachment, which helps them to consume a large amount of plant litter and make them simpler units in the form of fecal pellets (Wallwork, 1958). Norton (1990) states that oribatid mites are particulate feeders; chelicera and other structures of the mouthparts are used to cut or tear particles into sizes suitable for intake. Microphytophagous oribatid mites possess narrow, compressed, triangular rutella and elongated, narrow chelicera with small, sharp teeth, which help them nibble fungal cushions and small food items (Alphonsa & Haq, 2007). In the case of macrophytophagous and panphytophagous oribatid mites, they have well-developed, strong armored mouth parts to feed on hard plant materials like wood and leaves (Haq, 2016). The species under study, *P.* (*P.*) *elongatus,* has well-developed sclerotized chelicerae with sharp teeth, and the rutellum is also well-sclerotized with incisions. This type of mouthpart may help them to utilize a wide range of food items, including semi-degraded leaves and fungi.

Though oribatid mites are generalists as they can feed on a wide variety of resources, at the same time they can selectively feed on materials offered in laboratory food choice experiments. Therefore, Schneider and Maraun (2005) coined the term "choosy generalists" for oribatid mites. *P. (P.) elongatus* also exhibited different degrees of preference toward provided food items in the laboratory experiments. Sánchez-Chávez et al. (2023) proved that resource partitioning and a degree of preference help oribatid mites to coexist with other soil fauna. Thus, in the case of *P.* (*P.*) *elongatus,* the panphytophagous feeding behaviour and different degree of preference for provided food items may help them to reduce competition and coexist with other soil fauna.

Conclusion

Under laboratory condition (RH 80 ± 2 % and temperature 27 ± 2 °C), *P.* (*P.*) *elongatus* can feed on both lower and higher plant material with different degree of preference.

Therefore *P.* (*P.*) *elongatus* can coexist with hyper diverse soil organisms and allow for diet switching in the face of competition or shortage of one particular dietary item. Through this they would play a significant role in the detritus food chain maintenance as well as secondary decomposition process in the soil ecosystem.

Acknowledgement

The findings of the above paper "Study on the feeding preference of the oribatid mite, *Papillacarus (P.) elongatus* Xavier, 2007 (Acari: Oribatida: Lohmannidae) using microfungal and leaf litter diets" was presented in the XVI International Congress of Acarology December 2022, Auckland, New Zealand. The authors are thankful to Roy A. Norton, for providing relevant studies and to Arun A. Research Scholar from University of Calicut, Kerala, India, for confirming the identity of the species studied. We are thankful to National Institute of Technology (NIT), Calicut, for providing SEM facility. The work has been financially supported by the University Grants Commission (UGC-JRF), New Delhi, India.

Conflict of interest

The authors declare that there is no conflict of interest regarding the publication of this paper.

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Received 29 February 2024
Accepted 6 June 2024