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Research Article

Nutritional Ecology, Developmental Biology and Life Stages of the Model Mite, Archegozetes Longisetosus Aoki, 1965

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Abstract

Archegozetes longisetosus Aoki 1965 by virtue of its extreme adaptational peculiarities has attained convincing evidence of its pantropical distribution. Diversity in food habits, extreme tolerance to hazardous environmental setup, acquisition of 'enemy free life space' marvelous rate of fecundity, cheap and amenable mass culturing inputs and the like have been instrumental to raise the status of the mite as a model organism of Chelicerate group. This has opened further insights into various research studies mainly on nutritional and developmental aspects of the mite, many fold times better than earlier. Obviously, still there exits lacuna of detailed studies on the life stages of this mite. The present study, therefore has been an attempt to gather knowledge on nutritional habits and developmental biology, providing data on the morphological traits of the various life stages of the mite. Detection of profound feeding affinity towards two species of mosses and two species of liverworts, exceedingly well and new inputs on feeding of more higher plant materials could also be evidenced, facilitating wider potential of the species for better biodegradative ability. The most advantageous outcome of the study relates to the provision of life history of individual stages of the mite with duration and diagnostic features of all life stages in the development of the mite.

Keywords: Oribatid Mite; Model Organism; Pantropical Distribution; Biodegradation; Nutrition; Development; Kerala

Introduction

Archegozetes longisetosus represents a common, primitive species of oribatid mite included under the monophyletic family Trhypochthoniidae. The species has been recognized as parthenogenetic [26,28] pan-tropical and widely distributed in all continents [1,2,27]. Its predominance in habitats of frequent human dominance with varied biomass and macronutrient composition reflects its nutritional heterogeneity and efficiency to turn over resources in a much faster way, thereby helping to label it as an opportunistic feeder [1,2,5]. The uniqueness of the species with a combination of supreme evolutionarily advantageous morpho-cum biological characteristics such as the larger body size, simplicity of raising large populations in laboratory cultures, shorter developmental time, high rate of egg production [18,33] and the like raised its status to be considered as the chelicerate model organism [20,36] for over 20 years. The species had been chosen as the subject in tracing chelicerate development [36], parthenogenetic studies of mites [19,24], life cycle studies [6,23], toxicological studies [30], embryological studies [37] and studies on developmental genetics [34,35]. The life history parameters of the species, like the developmental time, number of off spring produced, processing of food and body size with respect to different food [29,33,31] etc. were found considerably influenced by the quality and type of food consumed.

Data procured on the nutritional biology of *A. longisetosus* clearly revealed the ability of the species to thrive on a wide range of feeding substrates and accordingly they were assigned to different feeding categories.

The species had been widely accepted for its pan phytophagous trend, showing preference to fungi, algae and decomposing litter [2,8]. Even toxic fungi like *Alternaria* were found grazed by the species, despite the strong fungal toxins which could be lethal to other animals and plants [25,33]. Dependence of the species in natural conditions on fungal and leafy diet [9,13,12,15] clearly designated its unspecialized feeding trend and its ability to consume wide range of feeding substrates comprising the lower and higher plant materials. The alga, *Protococcus* sp. was found highly palatable to the species by recording its total consumption [33]. A wide range of food resources comprising different species of fungi, yeast, mushroom, leaf litter, cotton thread, boiled rice, filter paper, moss, live animals like earth worm, and even artificial diet like synthetic sponge of polyurethane were found consumed by the mite under laboratory conditions and the species could successfully complete its life history on the latter [32]. The predatory and scavenging habit of the species was also elucidated [22] by supplementing live and dead nematodes. The preference of the species to diets with a high amount of fat also was recorded thereby establishing the role of fatty acids to serve as cue by the species to locate its food [4].

Thus the nutritional habits of the species clearly disclosed the high potential of the species in the biodegradation of organic materials thereby projecting its role in the turnover of nutrients and enrichment of fertility status in terrestrial ecosystem. Further, its wide range of distribution and high adaptability to enjoy extremely

wide panphytophagous feeding mode helps the species to survive and replenish in almost all habitats of the terrestrial ecosystems, over continents. Considering the potential of the species to degrade varied food substrates enhancing organic decomposition, it is intended to concentrate further on the nutritional diversity, developmental biology and life stages of the immature of the mite as objectives in details.

Materials and Methods

Soil, litter and moss samples were collected from various ecosystems distributed in four districts of Kerala viz. Thiruvanthapuram, Thrissur, Malappuram and Wayanad. In the Thiruvananthapuram Dt., four localities with distinct variation in the geographical features and vegetational characteristics such as Kesavadasapuram. Kariavattom, Neyyar Dam and Ponmudi hills were included for collection of lower plants like algae, mosses and litter samples. In Thrissur Dt., Puthukkad and kodungallur were selected for algal and moss collection and in Wayanad Dt., Sulthan Bathery formed the collection locality for a verity of litter samples. In the Malappuram Dt., Calicut University Botanical Garden and Baypore beach had been chosen as nearby sites for collection of available plant materials. An adjacent locality of Calicut University Campus called Olipramkadavu was mainly considered for mass collection of fungus and mosses. The site selection was made duly considering the great diversity in the geographical places, ranging from the hill tops to the planes, and the tremendous variations in the ecological niches available in those sites. Even in the plane situations, collections were made from habitats distributed in the sea shore as well as those available on the compound walls where mosses were grown in plenty.

Live specimens of *A. longisetosus* required for the study were recovered through Open Brass Funnel Extraction [14] of the litter and moss samples collected from the different localities of Kerala mentioned above. Live specimens were extracted into moistened leaf litter powder of *A. integrifolia* taken in collection vials. The live specimens extracted through the Open Brass Funnel [14] method and segregated by spreading the litter powder containing the specimens into the watch glass/petridishes and examination under a stereomicroscope. The specimens thus segregated under the microscope were kept in plastic vials having plaster of Paris- charcoal mixture (4:1 ratio) as their base. Feeding experiments were carried out by offering a variety of food items like two species of fungi (*Alternaria* sp.; *Trichoderma* sp.) two species of mosses (*Hyophila involuta; Philonotis hastata*), two species of liverworts (*Cyathodium cavernarum; Riccia* sp.), leaf litter of three species of higher plants (*Artocarpus integrifolia; A. indicus; Bambusa aurundinacea*) etc. and assessing and recording the mites' response. Gut content analysis of field collected specimens was also carried out following [8,11]. Microbial colonies residing in the gut of field collected individuals by making faecal plating on Mc Beth's medium. Laboratory cultures of the species were raised for making observations on breeding biology, duration of developmental instars, total duration of development etc. by rearing the species on *Alternaria* sp. of fungus in plastic culture rings based with a mixture of plaster of Paris-charcoal in 4:1 ratio. A temperature of 30 ± 1^0C and a relative humidity of 94 have been maintained throughout the study.

Results and Discussions

Nutritional diversity of *Archegozetes longisetosus*

During the study, the presence of *A. longisetosus* was recorded in eight localities of Kerala screened. The food requirements of *A. longisetosus* though considered simple earlier, high degree of diversity could be evidenced in the present study. Based on the diversity in ecological niches of these geographical areas variations could be observed in the population density and distribution of *A. longisetosus*. Habitats available in plane situations disclosed the presence of mite their immatures in high dencities particularly on moss and algae grown on compound walls, during the rainy season. Depending upon the plant species grown in the habitats, the mite population also showed variation and in accordance with the variation in the plants grown, the food requirement of the species also varied [16]. The recovery of the species in abundance was possible from the moss cushions grown on the walls which is an indication that apart from variations in geographical constitution, floral variation also could exert tremendous impact on the population density and distribution pattern of the species. The nutritional requirement of the species also showed considerable variation, depending upon the diversity in the floral components [16]. Possibly, this would have been one of the reasons for getting a high diversity of *A. longisetosus* in most part of Kerala, from ten localities, thereby supporting the earlier designation of the species as an opportunistic feeder [21]. The species exhibited exceedingly tremendous flexibility to enjoy a wider panphytophagous trend, which prompted the conduct of biological studies under experimental conditions by confining the species to individual test food items so as to trace its impact in determining the duration of various instars.

The feeding preference of the different life stages of the species to varied food items such as fungi (*Alternaria* sp., *Trichoderma* sp.), moss (*H. involuta*; *P. hastata*), liverwort (*C. cavernarum; Riccia* sp.), and leaf litter (*A. integrifolia*; *A. indicus; B. aurundinacea)* was also assessed. Rearing of individual instars of the species was possible in the laboratory based on their feeding affinity to two moss species (*H. involuta; P. hastata*) and two liverwort species (*C. cavernarum; Riccia* sp.), which were available in abundance during the rainy season in the nearby locality of Calicut University Campus. The species was found showing more affinity to higher plant materials like the litter of *A. integrifolia*, *A. indicus* and *B. aurundinacea*, which reflected its potential in biodegradation and productivity by hastening the litter decomposition process in soil conditions as evidenced through earlier studies [11,16,17]. The later developmental stages of the species such as the deuto and tritonymphs as well as the adults also exhibited affinity to higher plant materials. Affinity to feed on the moss species was also evident in the laboratory, followed by the liverworts and hence attempts for rearing the species on the above food resources were also made during the study. Concurrently the species also showed greater affinity to *Alternaria* sp. and fungus also. Thus by considering the overall feeding performance, the species could be better included under the feeding mode of 'strong opportunistic' category. During the study

the duration of development of the species was traced on its diet *Alternaria* sp. of fungus.

Detailed studies were also undertaken for the evaluation of the potential of the species in relation to degradation of diverse types of nutritional resources by adopting both qualitative and quantitative measures [11]. The presence and involvement of symbiotic gut microflora in the process of biodegradation was also elucidated by isolating and raising of pure cultures of bacterial colonies and subsequently inoculating the pure strains on Mc Beth's medium containing carboxy methyl cellulose (CMC). Development of clear zones was taken as an index to indicate the ability of bacteria for cellulose degradation. Quantification of the impact of feeding by *A. longisetosus* in relation to mineralization was made by comparing the concentration of micro and macronutrients such as N,P,K and Ca present in the leafy and woody components of litter of *A. integrifolia* before and after feeding by the adult and life stages of the species.

Feeding response shown by the species on the test food items comprising different species of fungi and higher plant materials offered in the laboratory clearly evidenced the adaptability of the species and to a certain extent its life stages also, to thrive on fungal as well as litter diets [10,11] with preference to decomposed leafy and woody elements. Analysis of the gut contents of the field collected specimens of the species also enabled to record the presence of fungal hyphae, spores of *Cladosporium* sp. of fungus, and leafy components such as parenchymatous cells and other particles in varying stages of digestion enabling to identify the species as a confirmed panphytophage member under natural conditions too. Further confirmation of the panphytophagous trend of the species was based on the results of assays made to locate the enzyme compliments which recorded the presence of maltase, raffinase, trehalase and cellulase, essential for the digestion of fungal materials as well as cellulose based plant particles [10,17]. Thus results of the thorough scrutiny of the nutritional ecology of *A. longisetosus* made during the current study based on laboratory cum field studies such as the food choice test, gut content analysis and enzyme assays provided ample support to justify its placement under the panphytophagous feeding category with extremely wide ability for food selection [11]. Such wider panphytophagous feeding trend of the species would be advantageous in focusing further on the nutritional credibility of the above species.

Release of the above enzyme compliments is believed to be a contribution from the microbial flora associated with the gut of the species, thereby pointing out the symbiotic relationship between the mite and its gut microbes. Microbial entities segregated from the gut of the species were found to comprise four numbers of bacterial and fungal colonies each, of which the former comprised of gram +ve and gram -ve nature and of bacillus and coccus shape. These bacterial colonies when tested for cellulose degradation by inoculating on McBeth's medium containing CMC, showed the development of clear zones, thereby confirming their role in the release of cellulose, an essential enzyme required for cellulose digestion [11,17]. In the present study, quantitative studies were also undertaken to evaluate the alterations in the micro and macronutrient composition as a consequence of feeding by the species. A comparative evaluation of the concentration of elements like N,P,K and Ca in the leaf litter of *A. integrifolia* (before feeding) and in the faecal pellets of *A. longisetosus* (after feeding) revealed increase in some elements and decrease in other. The positive impact of the species in the release of nutrients and thereby enriching soil nutrient status was evident in the study by recording increased levels of N and P in the faecal pellets of the mite after feeding on litter of *A. integrifolia* and subsequently converting it to faecal pellets. The concentration of nutrients like K and Ca showed a decrease in the faecal pellets, thereby revealing the mites' dependence on the above minerals for its metabolic activities [16,17]. The decrease in the amounts of the above elements would be a reflection of the intake of these minerals for calcification and incorporation into the exoskeleton of the mite as suggested earlier [7,38].

Breeding biology of *Archegozetes longisetosus*

Oviposition and incubation

The gravid females (Figure 1) could be easily recognized by the presence fully packed eggs, through their transparent body. The females showed a preference to select sites which were not exposed to direct light for oviposition. Several females were found to aggregate in the crevices of the substratum or underneath the food materials and laying their eggs. Eggs were laid in clusters (Figure 2A) along with some amount of fluid. This fluid appeared to be sticky, keeping the eggs adhering together. A maximum of 143 eggs were noticed in one cluster in contrast to the 7 eggs in another cluster. Egg masses consisting of intermediate number between this were also found and they were as 16, 21, 24, 27, 49 and 93. However, the body of gravid females revealed the presence of 21-53 eggs. Freshly laid eggs were smooth, oval in shape and white in colour with an average length of 168.7 µm and a width of 118.6 µm. Average incubation period of the eggs of *A. longisetosus* was found to be 5.7 days.

Figure 1: A gravid female with a batch of eggs and two fecal pellets in the body cavity.

Hatching

At the end of incubation period, the eggs appeared to be transparent when kept in a film of water. In this condition, the moving appendages of the larva could be perceived when observed under

higher magnification. The hatching of the egg was initiated by the splitting of the egg case followed by a break at one third region or more. The break extended deep, but never encircling the egg shell completely and through subsequent movements of the legs, the larva (Figure 2B) emerged out.

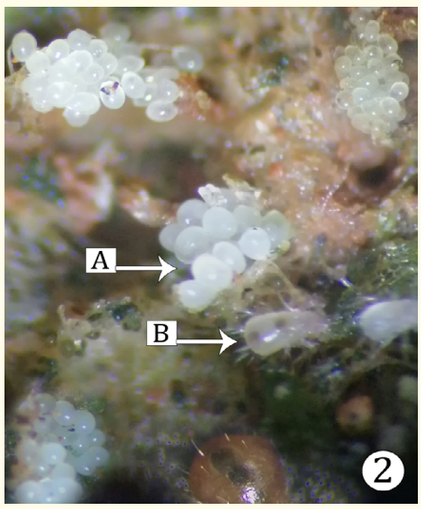

Figure 2: Several batches of eggs laid (A) by the female (B) newly emerged larvae.

Larval period

Soon after hatching, the larva remained inactive for some time and then initiated movements in search of food. The larva bears three pairs of legs and was found feeding on varied items. The larvae, during its course of development underwent four moults and passed through the protonymphal, deutonymphal and tritonymphal stages before reaching the adult stage. The larvae actively feeding the preferred food (Figure 3) and underwent massive aggregation (Figure 4). After an average feeding period of 5.3 days, the larva entered into the first quiescent period of 2.4 days and subsequently moulted into the protonymph (Figure 5 and 6).

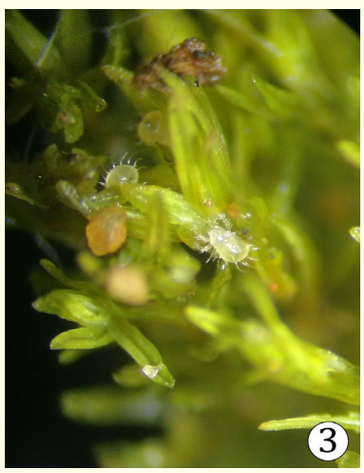

Figure 3: The larvae feeding on moss leaves of *Hyophilainvoluta*.

Figure 4: Massive aggregation of larvae.

Figure 5: Larval quiescence.

Figure 6: Moulted skins of larvae with emerging protonymph.

Protonymphal period

The protonymph (Figure 7) was larger than the larva and was readily distinguishable by presence of four pairs of legs. The feeding period of the protonymph was found to last for an average of 4.1 days. At the end they search and select a spot for aggregation (Figure 8) and invite other members to join the aggregation (Fig-

ure 9) subsequently other members (Figure 10) may also join one by one forming a cluster (Figure 11) or as a mass of aggregation (Figure 12), A in lab culture and B in pot culture). Then it entered into the second quiescent phase (Figure 13) for 2.6 days. Upon subsequent moulting, the deutonymph got emerged (Figure 14).

Figure 7: Protonymphs searching spot for aggregation.

Figure 8: Protonymphs searching spot for aggregation.

Figure 9: Attraction of other protonymph to aggregation spot.

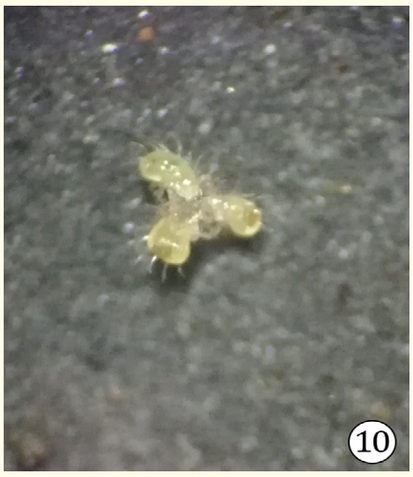

Figure 10: New members joining for protonymph aggregation.

Figure 11: Aggregation process of a cluster of 11 members of protonymph.

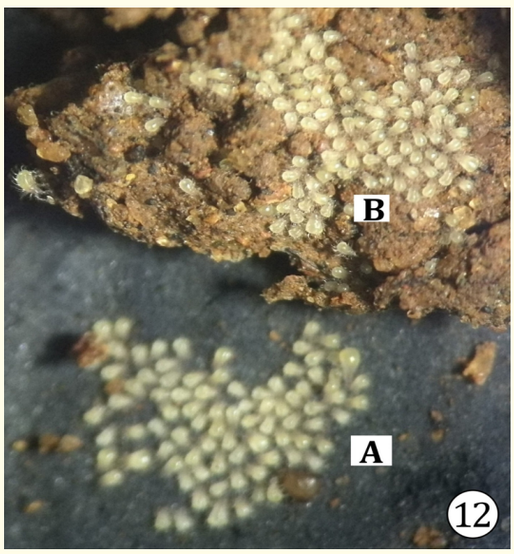

Figure 12: Aggregation of protonymph in the culture vessel in lab (A) and in pot cultures (B) in garden.

Figure 13: Focused view of thirteen members of a batch of protonymph in quiescent stage.

Figure 14: Six moulted skins of protonymphs from a cluster of thirteen aggregated mites of the same.

Deutonymphal period

The deutonymph was larger and fed actively for an average of 4.4 days. When aggregation is formed the initiating member/ members of the cluster may occupy a central position of the cluster (Figure 15). The cluster size may vary depending on the number of individual participate in a life stage/phase (Figure 16). Interestingly enough, availability of surplus food in highly preferred context often provide enthusiastic scene of massive aggregation of hundreds of individuals (Figure 17) mediated through aggregation pheromone [9] as seen in culture cells in the laboratory. The third quiescent phase is completed with an average of 2.5 days and moulted into the tritonymph and the process continued one by one (Figure 18).

Figure 15: Eighteen deutonymphs in aggregation with two initiating ones in the middle.

Figure 16: Cluster formation of fifteen deutonymphs

Figure 17: Portion of massive aggregation view of deutonymph from laboratory culture.

Figure 18: Emergence of one member of deutonymph leaving away of its moulting skin.

Figure 19: Focused view of tritonymph in quiensence.

Tritonymphal period

The tritonymph was the largest among the nymphal stages (Figure 19) and its body was more pigmented and light brown in colour. The duration of the active feeding period of the tritonymph was averaged to 4.1 days and then entered into the fourth quiescent period of 3.2 days. The moulting skins were all well exposed with wide opening and partially detached leg skins (Figure 20). Through the final moult, the adult was found to emerge out from the moulting skin of the quiescent instar, exactly the same manner as in the prior nymphal moulting. Such quiescent, moulting and emerging individuals (Figure 21 and 22) could be seen on two liverworts species of mosses found on compound wall when observed with a hand lens. Apart from these, newly emerged adults and other life stages were found in abundance in the two species of mosses studied presently (Figure 23 and 24). In addition, the nymphs and adults were found feeding and surviving on leaves and woody tissues of higher plants like *Artocarpus* spp. (Figure 25) also. Further, partially mastigated paranchymatous tissue and fungal hyphae (Figure 26) from the gut contents analysis and spores of *Cladosporium* (Figure 27) were also isolated. This has prompted further search for the details of mouth parts, more specifically to the peculiarities of chelicerae. The information gained showed that the chelicerae carried 4-5 teeth for the mastigation of leaves of *Artocarpus* sp. consumed as food (Figure 28).

Figure 20: Newly emerged tritonymph showing wide opening emergence hole of casted skins..

Total duration of life cycle and F_1 generation

The females of *A. longisetosus* initiated laying of eggs on the sixteenth day, after the fourth moult.The total duration of development from egg to the adult stage of *A. longisetosus* was found to require an average of 34.4 days when the rearing of the species was completed on the fungal food, *Alternaria* sp. Thus the duration of F_1 generation of the species could be recorded as 50.4 days at a temperature of 30 ± 1^{0}C and RH of 94.

Figure 21: A field view of liverwort, Cyathodium cavernarum growth from where Archegozetes longisetosus was collected.

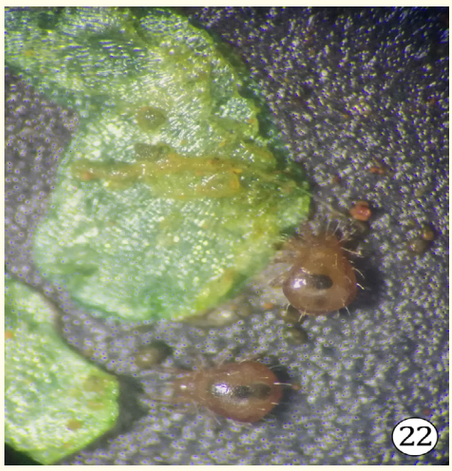

Figure 22: Newly emerged adults feeding on liverwort, *Cyathodium cavernarum.*

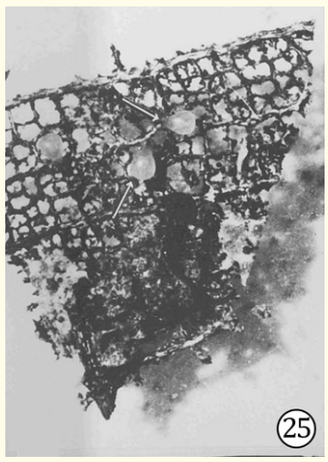

Figure 25: Adults and nymphs feeding on leaf tissue of *Artocarpus* sp.

Figure 23: Adult feeding on moss, *Hyophilainvoluta*.

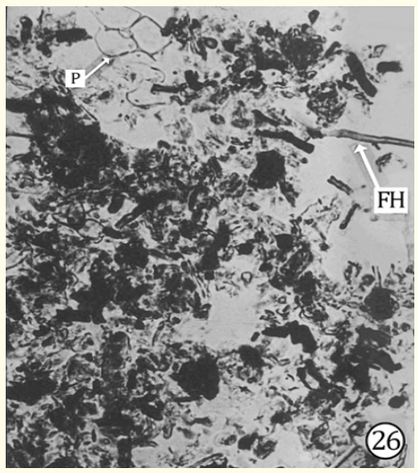

Figure 26: Fungal hyphe and parenchymatous tissue isolated from the gut of *A. longisetosus.*

Figure 24: Active adults and nymph grazing on moss *Philonotishastata.*

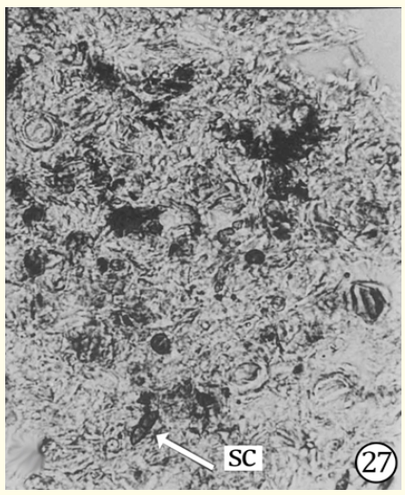

Figure 27: Spores of *cladosprium* sp. isolated from the fecal pellets of *A. longisetosus.*

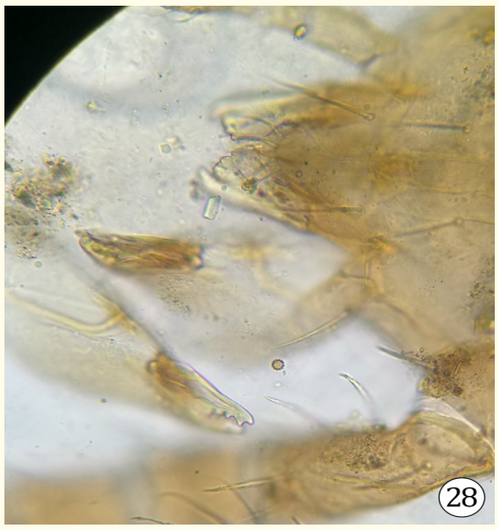

Figure 28: Chelicerae showing 4 - 5 theeths of *A. longisetosus.*

Plate 1: Dorsal view of the larva.

Description of life stages of *Archegozetes longisetosus* Aoki, 1965

Egg

Length: 156.0 - 177.8 µm, Width: 114.0 - 125.8 µm

Freshly laid eggs were smooth, oval in shape and white in colour with an average length of 168.7 µm and a width of 118.6 µm.The mean duration of incubation was found to be 5.7 days.

Larva

Length:184.6 - 200.2 µm, Width: 117.0 - 124.8 µm

The larva was small, (Table 1) active, transparent and shiny. The body was oval in shape. In newly emerged larvae, many wrinkles were seen particularly on the notogaster. The integument was lightly pigmented, the pigment however, was more clearly seen only in the regions of the legs.

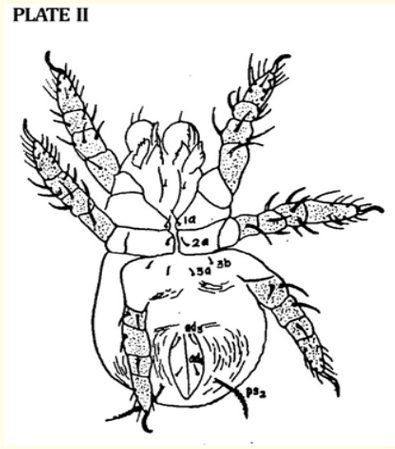

Plate 2: Ventral view of the larva.

Sl. No.	Egg Length - width in µm	Larva Length - width in µm	Protonymph Length - width in µm	Deutonymph Length - width in µm	Tritonymph Length - width in µm	Adult Length - width in µm
1.	166.2/125.2	200.2/124.8	449.2/280.8	468.0/343.02	728.0/520.0	956.8/676.0
2.	177.4/114.0	192.4/118.3	468.0/291.2	572.0/384.8	748.8/520.0	946.4/674.0
3.	156.0/114.0	184.6/117.0	468.0/296.4	540.8/379.6	821.6/551.2	915.2/592.8
4.	177.8/128.8	195.0/119.6	418.4/301.6	520.0/374.4	769.6/530.4	936.0/572.0
5.	166.2/114.2	189.8/117.0	483.6/301.6	488.8/353.6	852.8/572.0	977.6/638.1
Average	168.7/118.6	192.4/119.3	469.4/294.3	517.9/367.1	784.1/538.7	946.4/638.1

Table 1: Average measurements in µm of five individuals at each stages of development of *Archegozetes longisetosus.*

Prodorsum

Anterior end of the rostrum was broad and bore the rostral setae. Rostral, lamellar and interlamellar setae were clearly barbed and about 50 µm, 60 µm and 95 µm long respectively. The pseudostigmatic organ was represented only by the bothridium, as a small irregular circle. Exobothridial seta was small and smooth and inserted lateral to bothridium.

Notogaster

Notogaster was wider anteriorly than posteriorly. There were 10 pairs of barbed notogastral setae which were arranged in transverse rows, in segments c, d, e, h and ps (Table 2) and named. All the notogastral fissures present on the larva were difficult to locate because of the wrinkled nature of the skin. Fissure ia was located posterior to seta c_3, im posterior to cp and ip behind seta e_2.

Ventral region

Infracapitulum was provided with a pair of small and smooth setae. In the larva, there were only three apodemes with seta 1a on apodeme I, 2a on apodeme II and 3a and 3b on apodeme III. All these setae appeared to be smooth. Epimeralsetal formula was 1-1-2-0 (Table 3).

Stage	Segments						Total Number of setae	Setae appeared anew
	c	d	e	f	h	ps		
Larva	c1, c2, c3, cp	d1	e1, e2		h1	ps1, ps2	10	
Protonymph	c1, c2, c3, cp	d1	e1, e2	f2	h1, h2	ps1, ps2, ps3	13	f2, h2, ps3
Deutonymph	c1, c2, c3, cp	d1, d2	e1, e2	f2	h1, h2, h3	ps1, ps2, ps3	15	d2, h3
Tritonymph	c1, c2, c3, cp	d1, d2	e1, e2	f2	h1, h2, h3	ps1, ps2, ps3	15	
Adult	c1, c2, c3, cp	d1, d2	e1, e2	f2	h1, h2, h3	ps1, ps2, ps3	15	

Table 2: Appearance of notogastral setae at different stages of development of *Archegozetes longisetosus.*

Stages	Apodeme				Setae appear anew	Setae disappear	Epimeralsetal formula
	I	II	III	IV			
Larva	1a	2a	3a, 3b				1-1-2-0
Protonymph	1a, 1b, 1c	2a	3a, 3b	4a	1b, 1c, 4a		3-1-2-1
Deutonymph	1a, 1b, 1c	2a	3a, 3b	4b, 4c	4b, 4c	4a	3-1-2-2
Tritonymph	1a, 1b, 1c	2a	3a, 3b, 3c	4a, 4b, 4c	3c, 4a		3-1-3-3
Adult	1a, 1b, 1c	2a	3a, 3b, 3c	4a, 4b, 4c			3-1-3-3

Table 3: Appearance of apodemal setae at different stages of development of *Archegozetes longisetosus.*

Anogenital region

The genital region was bare and anal region was occupied by the adanal segment. Each adanal plate carried two smooth adanal setae ad1 and ad3. The skin surrounding the adanal region was found strongly wrinkled.

Legs

The larva had six legs which were monodactylous. There were 19 setae on leg 1 and they were distributed from trochanter to tarsus as follows: 1-1-3-4-10.

Protonymph

Length: 418.4 - 483.6 µm, Width: 280.8 - 301.6 µm

The length of the protonymph was more than twice that of the larva. It was transparent. Apart from the size, the appearance of the sensillus in the bothridium, the addition of a fourth pair of legs with associated changes on the ventral region of the body and appearance of anal and genital apertures were some of the most important features which enabled one to distinguish the protonymph from the larva.

PLATE III

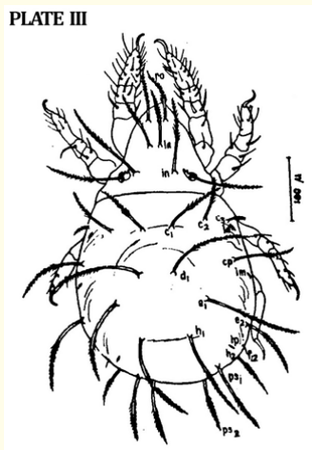

Plate 3: Dorsal view of the protonymph.

PLATE IV

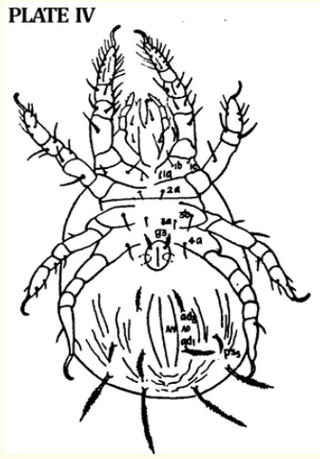

Plate 4: Ventral view of the protonymph.

Prodorsum

The anterior tip of the rostrum was less broader than the condition in the larva. The rostral setae were inserted far behind the anterior end of the rostrum. The rostral, lamellar and interlamellar setae were distinctly barbed and measured about 75 µm, 95 µmand 138 µm long respectively. The bothridium was well developed and cup shaped. The sensillus appeared anew and was densely barbed, about 130 µm long. Exobothridial setae though present in larva were difficult to locate in the protonymph.

Notogaster

Anterior, lateral and posterior margins of the notogaster were clearly visible. The middle portion of the notogaster had the maximum width and a total of 13 pairs of setae were present. Setae f_2, h_2 and ps_3 were added. Setae c_3 and f_2 were smallest among notogastral setae and they remained small in later stages of the development also. Wrinkles of the notogaster were less apparent. Of the notogastral fissures ia, im and ip, only ip was shifted above the seta h_2, whereas ia and im retained their larval position.

Ventral region

On the infracapitulum, a new pair of setae appeared anteriorly. With the introduction of a fourth pair of leg, a new apodeme was formed with seta 4a. Apodeme I, carried two more setae, setae 1b and 1e and therefore, the epimeralsetal formula was 3-1-2-1 (Table 3).

Anogenital region

Anal and genital apertures were widely separated from each other. Anal aperture was long but without setae and seen posterior to adanal segment. The border of the adanal segment was irregular and ad_1 and ad_3 (Table 4) were long and barbed. They were well spaced here than in the larvae. Genital aperture was some what round in appearance and situated in between the fourth pair of legs. The presence of a pair of seta, g_3 (Table 5) at the anterolateral margin of each genital plate and the occurrence of a pair of genital suckers were characteristic features of the protonymph.

Leg

Leg l carried 24 setae which were distributed from trochanter to tarsus as follows: 1-1-3-5-14.

Stages	Adanal Segment			Anal Segment		
	Adanal setae	Total number of setae	Setae appear anew	Anal setae	Total number of setae	Setae appeared anew
Larva	ad:1, ad:3	2	0	0	0	0
Protonymph	ad:1, ad:3	2	0	0	0	0
Deutonymph	ad:1, ad:2, ad:3	3	ad:2	0	0	0
Tritonymph	ad:1, ad:2, ad:3	3	0	a:1, a:2	2	0
Adult	ad:1, ad:2, ad:3	3	0	a:1, a:2	2	0

Table 4: Appearance of adanal and anal setae at different stages of development of *Archegozetes longisetosus.*

Stages	Genital Setae	Total number of setae	Setae appear anew	Setae disappeared
Larva	0	0	0	0
Protonymph	g3	1	g3	0
Deutonymph	g6, g7	2	g6, g7	g3
Tritonymph	g1, g2, g3, g4, g5, g6, g7	7	g1, g2, g3, g4, g5	0
Adult	g1, g2, g3, g4, g5, g6, g7	7	0	0

Table 5: Appearance of genital setae at different stages of development of *Archegozetes longisetosus.*

Deutonymph

Length: 468.0 - 520.0 µm, Width: 343.2 - 384.8 µm

The deutonymphwas slightly larger than the protonymph. The body had a slight yellowish tinge and the cuticle was without much wrinkles. The important changes occurred mainly on the ventral regions of the body.

PLATE V

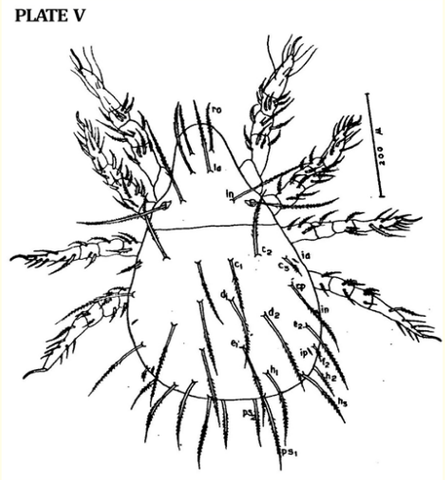

Plate 5: Dorsal view of the deutonymph.

PLATE VI

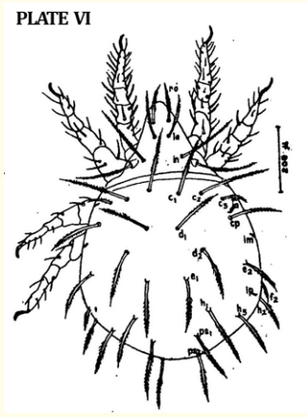

Plate 6: Ventral view of the deutonymph.

Prodorsum

The prodorsal setae were same in appearance as in the protonymph. Rostral, lamellar and interlamellar setae were longer about 105 µm, 155 µm and 200 µm respectively. Sensillus resembled the long body setae and measured about 155 µm in length.

Notogaster

Notogaster was broadly rounded with somewhat straight anterior margins. A total of 15 pairs of setae were present on the notogaster. Two secondary setae, d2 and h3 appeared anew on the notogaster.

Ventral region

A third pair of small setae mostly represented by their alveoli was found in between the long anterior and posterior pair of setae on the infracapitulum. Apodemal seta 4a disappeared instead setae 4b and 4c were added in the apodeme IV. Epimeral setal formula of the deutonymphwas therefore 3-1-2-2.

Anogenital region

Anal segment was bare as in protonymph. Adanal segment showed three pairs of setae by the addition of the smooth seta ad_2. The genital area showed an enlargement in size. Genital aperture was semicircular in shape with each plate having two genital suckers. Genital seta g_3 disappeared and two smooth secondary setae g_6 and g_7 were added near the middle and the posterior region of each genital plate.

Leg

A total of 34 or 35 setae were present on leg l of the deutonymph which were distributed from trochanter to tarsus as followed: 2-5-5-7-15 or 16.

Tritonymph

Length: 728.0 - 852.8 µm, Width: 520.0 - 572.0 µm

The tritonymph was fairly larger in size. It was light yellow in colour and without wrinkles on the body. The important changes noted in the tritonymph were the addition of setae on the apodemes, genital and anal plates.

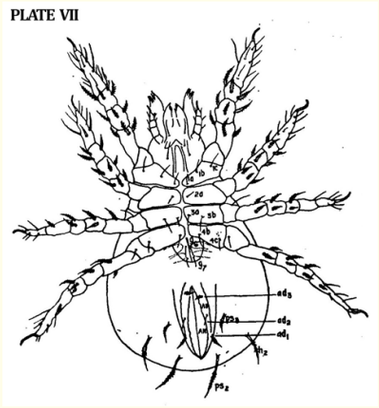

Plate 7: Dorsal view of the tritonymph.

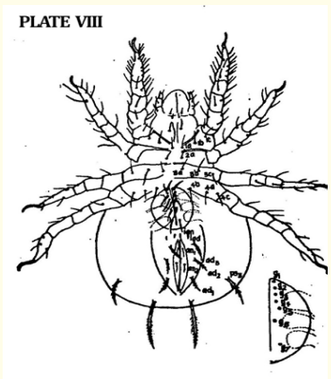

Plate 8: Ventral view of the tritonymph and Genital plate showing the positions of the genital setae.

Prodorsum

Anterior region of the rostrum was more pointed than in the deutonymph. Rostral, lamellar and interlamellar setae measured about 130 µm, 160 µmand 220 µm respectively. Sensillus was much longer, about 180 µm.

Notogaster

Posterior region of the notogaster was much rounded in appearance. The middle portion of the notogaster was well elevated than all preceding stages. There was no change in the number and positions of notogastral setae. Notogastral fissures ia, im and ip were in their usual positions.

Ventral region

Infracapitulum bore three pairs of setae as in deutonymph. Apodemal seta 3c was added to the apodeme III and seta 4a reappeared on apodeme IV. Epimeral setal formula was 3-1-3-3.

Anogenital region

Each anal plate possessed two small setae, a_1 and a_2, of which a1was found at the anterior part of the anal plate and a_2 at the middle of each anal plate. All the adanal setae including ad_2 were barbed and retained by the adult. The widely separated ano-genital aperture became closer in tritonymph. The genital aperture was oval in shape with three pairs of genital suckers. Five tertiary setae, g_1 to g_5 appeared on each genital plate, above the seta g_6.

Legs

A reduction in number of setae on segment 2 and 4 of leg 1 of the tritonymph was noticed. Totally, 31 setae were present on different segments of the leg of the tritonymph which were represented from trochanter to tarsus as:2-4-5-5-15 (Table 6) shows the duration in development of the life stages of *A. longisetosus* from egg to adult. Distinguishing characters of the nymphal and adult stages are given in (Table 7).

An important morphological feature observed in the larva of *A. longisetosus* during the present study was the absence of sensillus on the prodorsum, and made its appearance only in the protonymph. There was a progressive increase in the body size from the larva to the adult but the percentage increase was not uniform in all the stages. There was about one and a half times increase in the protonymph over the larva and in the deutonymph, tritonymph and adult, the increase was respectively 12%, 32% and 20% over that of the previous instar. The increase that was seen in the protonymph was more than in any other succeeding stages and it was very impressive, as the protonymph attained a size of about one and a half times that of the larva. Such remarkable increase in the growth was recorded earlier in species like *Damaeus clavipes* [3] where the increase was observed between proto and deutonymphal stages.

The percentage increase was more in tritonymph than in the adult. The larval complement of notogastral setae was retained in the adult and there was only addition to the basic number in the course of development. Further, since there was no regression of setae in the later stages of development, the adult notogastral

	Egg Incubation Period	Larval Duration	First Quiescent	Protonymphal Duration	Second Quiescent	Deutonymphal Duration	Third Quiescent	Tritonymphal Duration	Fourth Quiescent	Total days
1.	4 days	5 days	2 days	4 days	3 days	5 days	2 days	4 days	3 days	32
2.	5 days	6 days	2 days	4 days	2 days	5 days	2 days	4 days	2 days	32
3.	7 days	5 days	3 days	3 days	2 days	4 days	3 days	4 days	3 days	34
4.	6 days	6 days	3 days	4 days	3 days	4 days	2 days	5 days	3 days	36
5.	5 days	5 days	2 days	4 days	3 days	4 days	3 days	5 days	4 days	35
6.	7 days	5 days	3 days	4 days	2 days	4 days	2 days	4 days	4 days	35
7.	6 days	4 days	2 days	4 days	2 days	4 days	2 days	4 days	3 days	31
8.	4 days	6 days	2 days	5 days	3 days	5 days	2 days	4 days	3 days	34
9.	7 days	5 days	2 days	5 days	2 days	4 days	3 days	4 days	3 days	35
10.	5 days	6 days	3 days	4 days	2 days	4 days	3 days	4 days	3 days	34
11.	7 days	5 days	2 days	5 days	3 days	5 days	2 days	4 days	4 days	37
12.	4 days	6 days	3 days	4 days	3 days	5 days	3 days	4 days	3 days	35
13.	8 days	5 days	2 days	4 days	2 days	4 days	3 days	5 days	3 days	36
14.	5 days	6 days	3 days	4 days	4 days	4 days	3 days	3 days	4 days	36
Average	5.7 days	5.3 days	2.4 days	4.1 days	2.6 days	4.4 days	2.5 days	4.1 days	3.2 days	34.4 days

Table 6: Life stages of *Archegozetes longisetosus* Duration of development in days.

	Larva	Protonymph	Deutonymph		Tritonymph	Adult
Length of rostral setae	50 µm	75 µm	105 µm		130 µm	130 µm
Number of pairs of notogastral setae	10	13	15		15	15
Apodemalsetal formula	1-1-2-0	3-1-2-1	3-1-2-2		3-1-3-3	3-1-3-3
Number of pairs of genital setae	0	1	2		7	7
Number of pairs of genital suckers	0	1	2		3	3
Number of pairs of adanal setae	2 (Smooth)	2 (Barbed)	3		3 All barbed	3 All barbed
			Ad-1 Ad-2	Barbed		
Number of pairs of anal setae	0	0	0		2	2
Length/width in average	190.0/120.0	470.0/295.0	520.0/368.0		785.0/540.0	950.0/640.0

Table 7: Distinguishing characters of various nymphal and adult stages of *Archegozetes longisetosus.*

complement was attained in the deutonymph Generally, the full setal complement appeared first in the anterior segments and subsequently in the posterior segments.

The segment F in the larva was bare and the only segments which were completed in the larva were C and E. Further, the posterior-most segment PS was found completed before D and H. The notogaster was bideficient where the setae d_3 and f_1 were absent. The complement of 15 setae would suggest a unideficient nature-but this was actually due to the appearance of an extra seta Cp on the segment C. The adult complement of setae was attained in the tritonymphal stage. In the case of apodemes also, it was found that the anterior ones had the full complement of setae before the posterior ones.

All the setae on the adanal segment appeared in the deutonymphal stage. The analsegment also appeared in the deutonymph but it was bare, without carrying any setae. In the tritonymphal stage, the full complement of 2 setae on each of the anal plate. The full genital setal complement appeared in the tritonymphal stage. In the deutonymph, the larval seta g_3 appeared only again in the tritonymph. This appeared rather unusual. Thus the body chaetotaxy of the tritonymph was exactly similar to that of the adult.

The average time taken for completion of life cycle was 50 days. It was observed that the newly emerged adults required about 2 weeks for becoming sexually mature to initiate oviposition, as observed in the laboratory condition. The fertility was found rather high, being nearly 100%. The number of eggs present in a single female of *A. longisetosus* was found to range from 19 to 21 at a time. The highest number of eggs recorded in Oribatei so far was 18 in *Ceratozetes cisalpinus* by [39]. Usually the eggs are laid nearby areas in batches comprising 16, 21, 24, 27, 49 and up to 91 by individual females. In the laboratory, usually 3 - 4 batches but rarely 5-7 batches of eggs could also be noted. Based on the life history data procured through laboratory observation, it could be predicted that the species could complete a maximum of seven annual generations in the field, under favourable conditions.

Conclusion

The current study has investigated the nutritional habit of the model mite, *A. longisetosus* in the laboratory and natural habitats through feeding experiments gut content analysis, enzyme assays, microbial analysis and analysis of the nutrients in food before and after consumption. The developmental pattern, morphological ontogeny and duration of the post-embryonic development of the species have been studied and presented. The diverse nutritional habit, parthenogentic reproduction, mass production of eggs, and short duration of development of this species make it a suitable candidate for mass rearing, one of the lacunae in the oribatid mite research. The morphological ontogeny of the species illustrated in the current study is highly relevant in solving the ambiguity in the identification of the life stages of the mite.

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