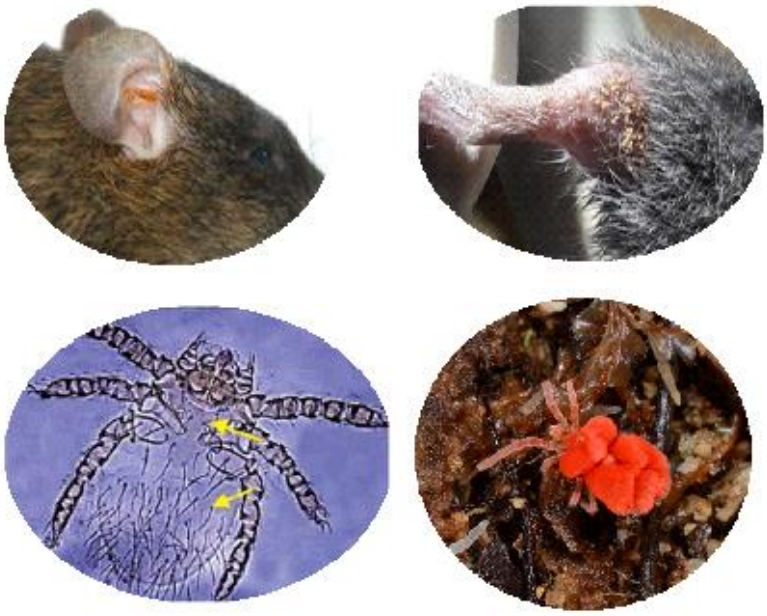
Training Module 02/2022

TRAINING MODULE ON MEDICALLY IMPORTANT MITES: IDENTIFICATION AND SURVEY METHODS



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आई सी एम आर - रोगवाहक नियंत्रण अनुसंधान केन्द्र
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Foreword

Mites are small creatures (usually 0.25-0.75mm long) belonging to class Arachnida, like ticks. Some mites may be larger. They occupy several ecological niches, in soils as decomposers, on plants, as ectoparasites on animals and humans or even act as predators. The oldest mite discovered preserved in amber dates back about 400 million years based on carbon dating. The body composition of mites is very simple. There are four life cycle forms viz., eggs, larvae, nymphs and adults. Mites are known to cause asthma, respiratory allergies, itching, scrub typhus, and scabies besides being very useful for their ecological services. Hence, research on mites is fascinating and assumes significance in the effective prevention, control and management of these diseases.

In this context, “Hands-on Training Module on Medically Important Mites” prepared on the eve of Workshop on Medically Important Mites by Dr. P. Philip Samuel, Scientist C and Mr. R. Govindarajan, Technical Officer at the ICMR-VCRC Field Station, Madurai, is not only timely, but also much needed training resource material for imparting the right knowledge to the Scholars, Entomologists, Medical Officers and Programme Managers so that the mite-borne diseases are effectively studied and controlled. I sincerely laud the efforts by Dr. Samuel and Mr. Govindarajan, and congratulate them for bringing out this important manual. I also thank Prof. S. Sabesan, Senior Consultant, Dr. S. L. Hoti, Emeritus Scientist and Dr. K.H.K. Raju Technical Officer-C for editing and assisting in bringing out publication.

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TROMBICULID MITE IDENTIFICATION AND SURVEY METHODS

1. Introduction:

Recently, emerging and re-emerging diseases are being reported due to numerous anthropogenic factors responsible for their spatial spread and distribution. In this context, it becomes necessary to update and disseminate the knowledge on the disease-transmitting agents. It is highly essential to develop resources through regular capacity-building measures among the Public Health Entomologists of the country. This training module deals mainly with the mite borne zoonotic diseases, currently reported from various parts of India. It covers the pertinent information on the chigger mite vectors, taxonomic identification and survey methods.

This training module will be useful for imparting training, teaching, and research on mites. The personnel who are working in the Public Health Departments, State/National vector-borne disease control programme, Research Students and Faculties from Educational and Medical institutions will find it useful for identification, taxonomic classification and faunistic surveys of mites.

2. Learning objectives

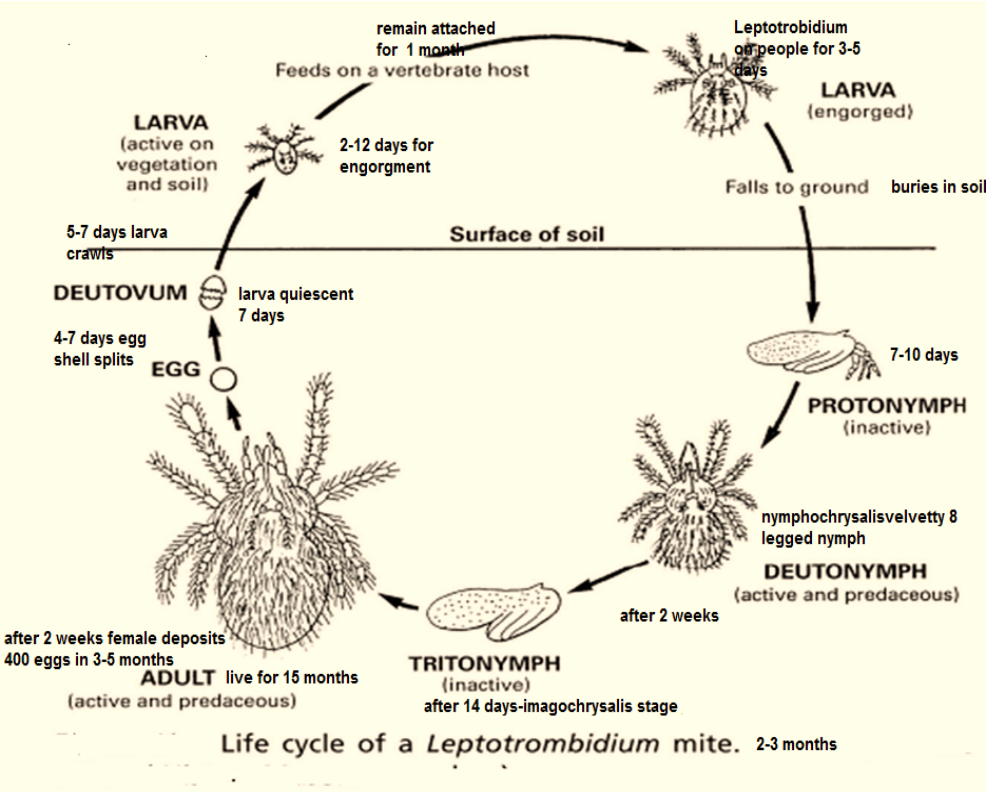
1. To know in detail, the methods to collect mite samples and transport.
2. To determine the prevalence of vector species.
3. To gain knowledge on the biology and systematics of mite species
4. Methods for pinning/mounting and preservation of mite specimens.
5. To gain expertise on mite survey methods and determine indices.

3. Life cycle (Trombiculid mites)

The egg hatches after a period of 4-7 days of incubation into an inactive stage called deutovum, which is a six-legged larva. The larvae are found in small clusters on the blades of grass or branches of shrubs, even drier places such as lawns and parks, waiting for latching onto a host. Clusters of Chiggers found in a particular place is called “mite island". After finding the host, the chiggers crawl over the body and find a suitable place such as the ear or leg region for feeding. Larvae feed on tissue fluids of the host using stylostome or feeding tube. Feeding on the host

may last for five to seven days or months, depending on the species. The unfed larva can remain alive for up to three months without food.

After engorgement, the larvae drop to the soil and enter into a quiescent stage called ‘protonymph’ which has four pairs of legs. The next stage, active deutonymph, feeds on other arthropods such as adult bees. Later, the deutonymph enters into an inactive stage called ‘tritonymph’. The adult emerges from tritonymph and it feeds on small insects. The male mates with females and deposits spermatophore outside of the female body which is pushed in to the gonophore by the male with its legs or through the penis depending upon species. A female lay about 400 eggs during her lifetime. The adults live for about a year.



Life cycle of trombiculid mites [Source: Service, 2008]

4. Survey Method

Rodent capture and collection of mites:

- Animal ethical committee clearance is necessary before starting the rodent/shrew capture.
- Wonder & Sherman traps (specialized rodent traps for live rodent trapping) with bait having oil aroma (e.g., fried coconut or onion vada) are laid in dusk hours in and around households, and also areas of thick vegetation around households.

- Care must be taken when keeping the traps in the thick scrub areas to prevent disturbance by other animals.
- The traps are retrieved the next day before sunrise.
- The trapped rodents are anaesthetized using Chloroform.
- Identify the shrew and rodents at species level using standard taxonomy key.
- In the case of shrews, the common infestation sites i.e. ears, eyes, anal area and leg to be screened for larval trombiculid mites.

- The mites are collected by combing the fur of the shrew and rodents using fine brush.
- Collected mites are preserved in 80% ethanol.
- Preserved mites are mounted in Hoyer's medium.
- Mounted slides can be observed for identifying trombiculid mites using the taxonomy keys.

Mites mounting technique

Clearing agents:

Various chemicals are useful in macerating internal tissues of preserved mites with little or no damage to the exoskeleton. One of the most popular for general use is lactophenol, which is prepared with the following ingredients added in sequence (Philip Samuel et al., 2021b):

1. 50% Lactic acid
2. 25% Phenol crystals
3. 0.5% Iodine resublimed p.a.
4. 25% Distilled water

Hoyer's medium:

The most satisfactory aqueous mountant now in use is Hoyer's medium, which is prepared as follows (Philip Samuel et al., 2021b):

1. Distilled water : 50 ml
2. Gum arabic (crystals) : 30gm
3. Chloral hydrate : 200gm
4. Glycerine : 20 ml
5. Iodine resublimed p.a. : 0.5ml

- These ingredients should be added in the order shown.
- Solid ingredients should be completely dissolved before addition of succeeding reagents.
- The resulting material should be filtered through clean muslin cloth in order to remove bits of wood or other impurities from the gum arable used.

Mounting of Chigger mites: stepwise procedure as given below

Preserve the chigger mites in 70% ethanol

⇩

Take a cavity slide

⇩

Place a drop of clearing solution (lacto phenol) to center of the cavity

⇩

Put the individual chigger mite

⇩

Hold the slide at 60^0-70^0 C for 10-15 minutes (Hot air oven)

⇩

Allow the slide to cool for 5-10 minutes

⇩

Transfer the chigger mites on to a clean glass slide

⇩

Then on a clean slide add a drop of mountant (HOYER'S medium) and place the chigger mite

⇩

Place a cover slip over the specimen carefully

⇩

Hold the mounted slide at 80^0-90^0 for 30 minutes (Hot air over)

⇩

The excess mountant should be removed using a white cotton bud

⇩

Finally seal the edges of cover slip with nail polish

⇩

Subject the slide for microscopic identification

⇩

Label the slide with date & place of collection and name of the species

5. Taxonomical Characteristics

The taxonomic classification of Trombiculid (Chigger) mite, the major vector for scrub typhus is depicted below:

Systematics/Taxonomy:

Phylum: Arthropoda

Subphylum: Chelicerata

Class: Arachnida

Subclass: Acari

Order: Acariformes

Suborder: Actinedida

Family: Trombiculidae

Phylum Arthropods: Invertebrate, bilateral symmetry, chitinous skeleton with articulating segments and /or appendages.

Subphylum Chelicerata: The first appendage is modified as chelicerae, used for grasping, cutting, piercing the skin while feeding. The body consists of mainly two parts, 1) the prosoma 2) the opisthosoma.

Class Arachnida: The second pair of appendages is modified as pedipalps, and four pairs of legs found on the prosoma (gnathosoma + podosoma). Arachnida lack antennae and wings. The anterior body segments bearing the palps and chelicerae, are attached with posterior segments & possess walking legs.

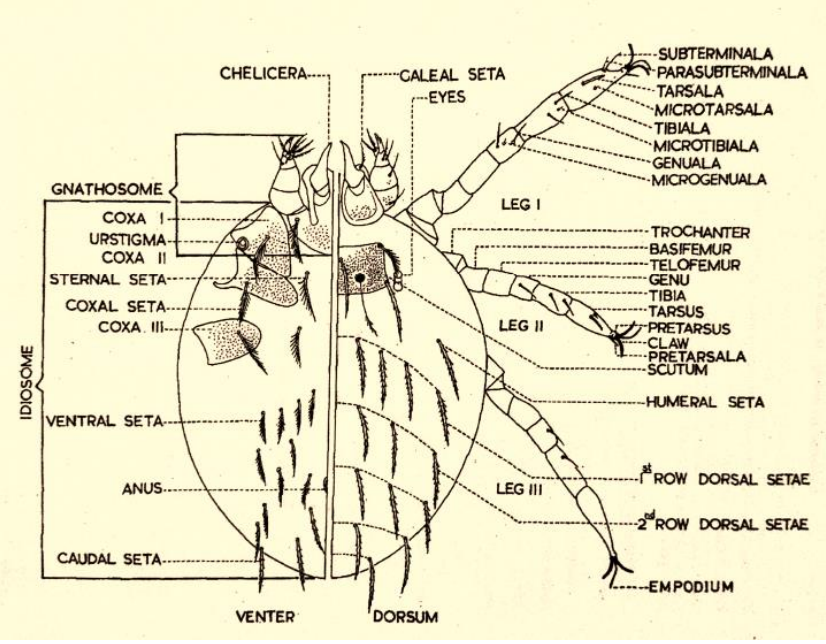
Subclass Acari: This sub-class contains mainly Ticks and mites. In Acari, the body segments are fused. The idiosoma is formed by the fusion of podosoma and the opisthosoma. The mouthparts consist of hypostome and two chelicerae. The two pedipalps form the capitulum which does not penetrate the tissues.

Suborder Prostigmata: Chiggers include a pair of stigma lateral to the gnathosoma.

Family Trombiculidae: It is also called as chigger mite family; a chigger is about 1mm long and figure 8 shaped. Morphologically the cuticle is striated with scutum in the dorsal anterior region which is the main taxonomical character for the genus. The eyes are present on lateral side of the scutum. However, in some chigger mites the eyes are absent. The body and legs are covered with setae which are arranged in order. Larvae have three pair of legs whereas nymph and adult have 4 pairs of legs. The larvae are orange, red or white in colour, whereas nymph and adult are red/deep red in colour. Adult chiggers feed only on arthropod eggs.

6. Morphological identification of trombiculid mites

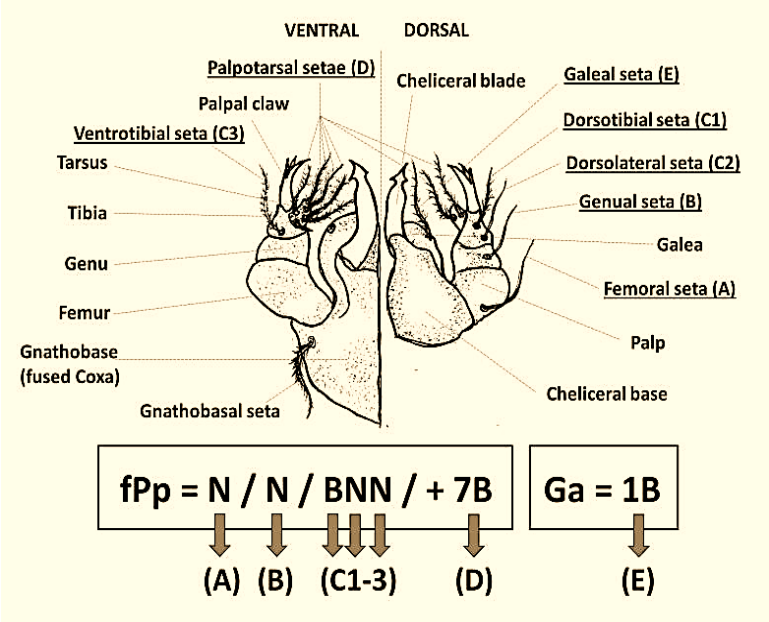
Chigger mites is very small (0.2-0.4 mm), hairy, various in color (white, yellow, orange to red) and possess oval to round shape (Nadchatram and Dohany, 1974). The exoskeleton is soft and thin. Its body can be divided into two parts: 1. Gnathosoma (head/mouthparts) comprising Chelicera, palp and galea for sensory and feeding functions; 2. Idiosoma (abdomen and thorax) which is the major part of the body supporting the eye, scutum (dorsal view), body setae (Hair) and legs (Vercammen Grandjean and Langston 1975). Chiggers have 3 pairs of legs, and others (nymph, and adults) four pairs.



General appearance of ventral and dorsal view of *Leptotrombidium deliense* (Fernandes and Kulkarni 2003)

Gnathosome:

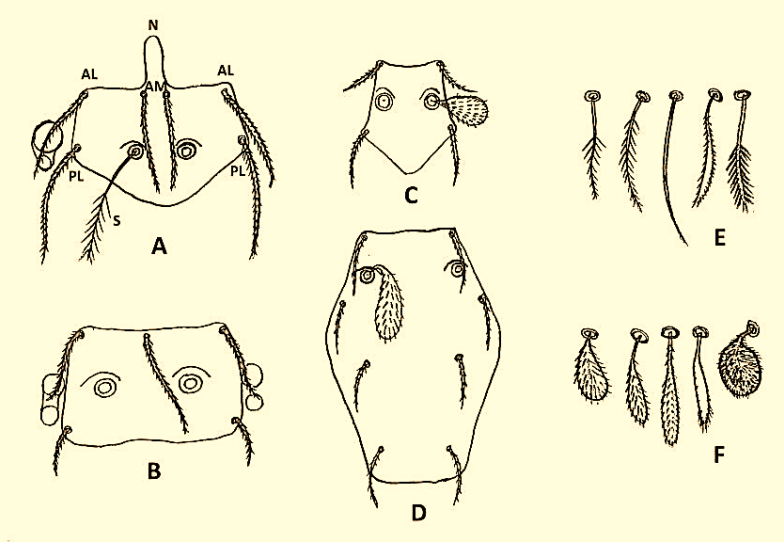
The Gnathosome (mouthparts) consists of palpal coxae at the base of the Gnathosome, a pair of palpi, a pair of moveable cheliceral blades, their immovable bases, and a pair of galeae.



Ventral and dorsal aspects of gnathosome

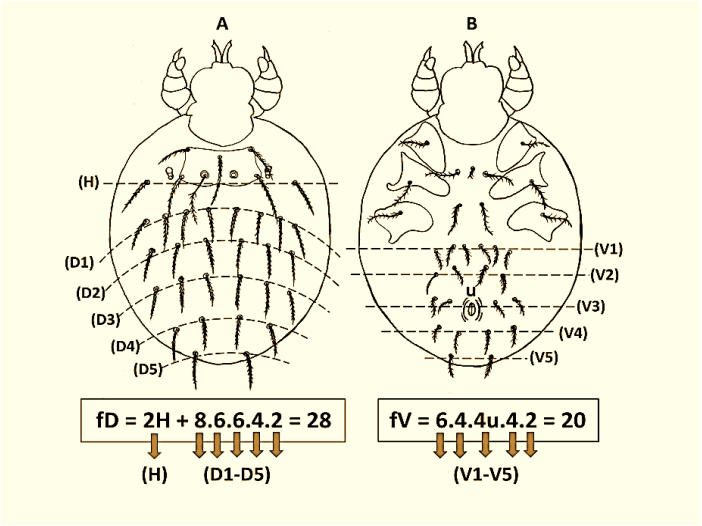
Idiosome The idiosome consists of the globular part of the body behind the mouthparts and to which the legs are attached.

Scutum The scutum or dorsal plate is located in the anterodorsal portion of the idiosome. The general shape, dimentions and composition of scutal setae are characters of great diagnostic importance as they are usually constant at the genus or subgenus level. The shape of the scutum can be rectangular, trapezoid, pentagonal with a slightly convex or undulated anterior/posterior margin. The scutum also bears a pair of sensillary bases or pits from an unexpanded thread-like filament to an expanded globular structure. Some of the common terms used for describing the shape of the sensilla are filiform, lanceolate, fusiform, clavate, globose and so on. The shape of these organs is important for determining various genera.



Scutal variation of trombiculid genera: A. odontacarus, B. Walchiella, C. Walchia and D. Gahrliepia; N=nasus, AL= anterolateral setae, AM=anteromedian setae, PL= posterolateral setae, and S=sensillae. The variation of scutal sensillae shape can be unexpanded or expanded (Nadchatram & Dohany, 1974).

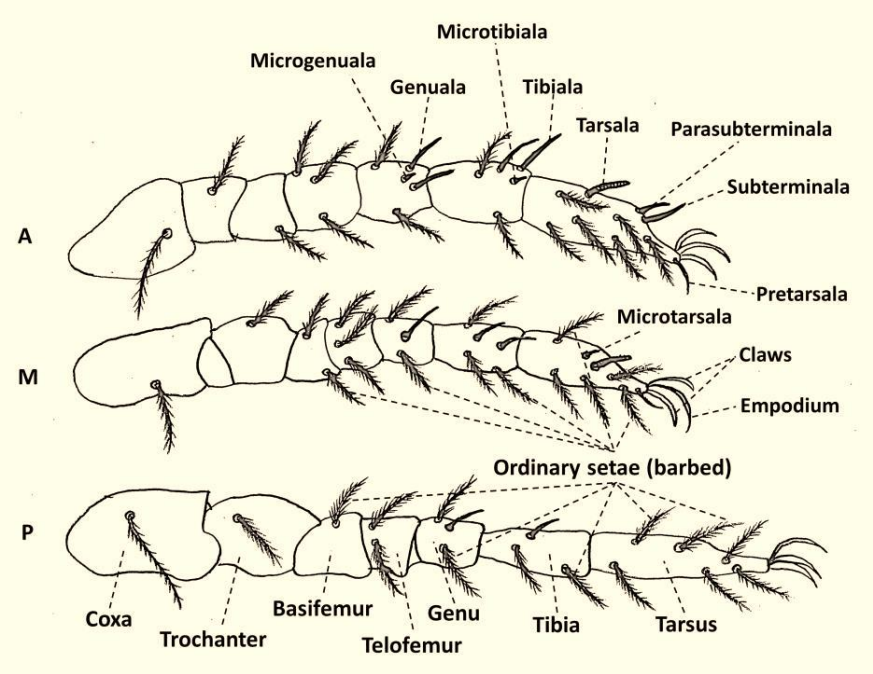
Body chaetotaxy: The number and pattern of the arangement of setae on the arthropod body cuticle is called 'Chaetotaxy'. In the trombiculid species identification, the body chatotaxy is as imoportant as the fPp (palpal pilosity formula) on the gnathosoma described earlier. There are numberous body setae on both the dorsal and ventral sides which are arranged in distinctive pattern of transverse row.



Dorsal (A) and Ventral (B) aspects of *L. deliense* showing body chaetotaxy: dorsal body setation formula (fD), ventral body setation formula (fV), number of dorsal setae (ND=28), number of ventral setae (NV=20) and total number of body setae (NDV=ND+NV=46); u=urogenital pore (Kittipong Chaisiri 2016).

Legs:

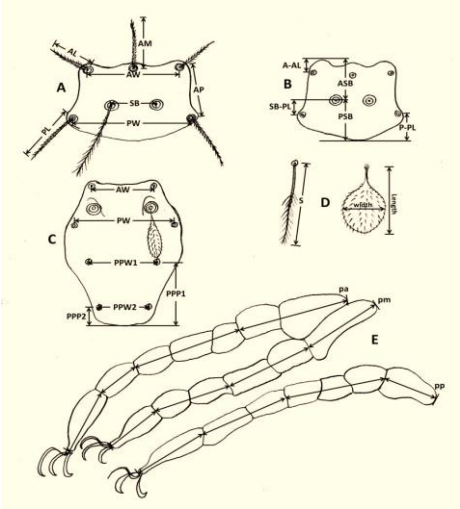
Legs are usually seven segmented and expressed as 7-7-7. In the subfamily Leeuwenhoekiinae, the segments are 6-6-6, while in a few Trombiculinae and in the genus Gahrliepia, the segments are 7-6-6. In a 6-segmented leg, the basi- and telo-femur are fused.



Leg segmentation and setae of *Leptotrombidium deliense*: anterior (A), median (M) and posterior legs (P). Redrawn from Goff et al. (1982).

7. Morphometric identification of trombiculid mites

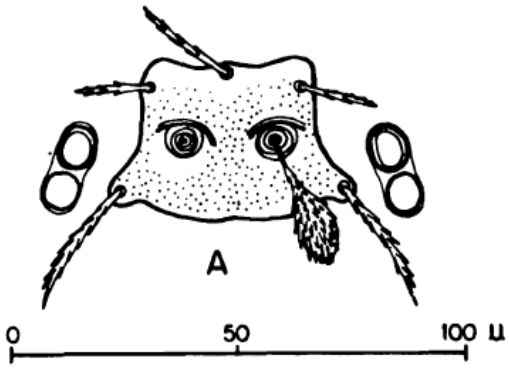
Morphometric or morphometry analysis of the aforementioned important characteristic of trombiculid larval mites-scutum, setae, sensillae and legs are used for definitive taxonomic identification. In this section, the measurement of the key characters used in this module are as per the methods of Nadchatram & Dohany (1974) and Vercammen & Langston (1975). All measurements are in microns, the standardised unit universally used in taxonomic and systematic studies of trombiculid mites.



Measuring approach for the main features of trombiculid larvae: dimention and length of scutum and its setae (A,B, and C); dimension and length of sensillae (D); length of the 3 legs (E) (Nadchatram & Dohany, 1974).

8. **Identification Key for Genera of Trombiculid Mites** (Stan Fernandes and Kulkarni, 2003).

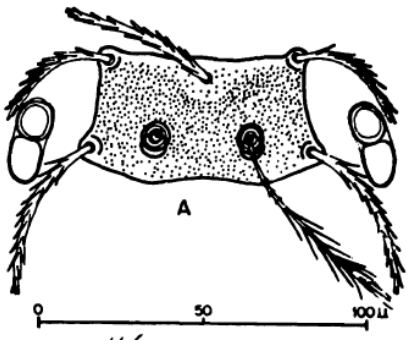
Genus: *Ascoschoengastia*



Diagnosis:

- Eyes 2/2 or 1/1
- Scutum small, subquadrate with anterolateral shoulders and convex or biconvex posterior margin;
- AM base anterior to level of AL bases
- SB anterior to level of PL bases
- PL>AM>AL;
- Sensillary bases little more than their diameter apart;
- Sensillae fusiform to clavate, head with short or long spiked setules.

Genus: *Leptotrombidium*



Diagnosis:

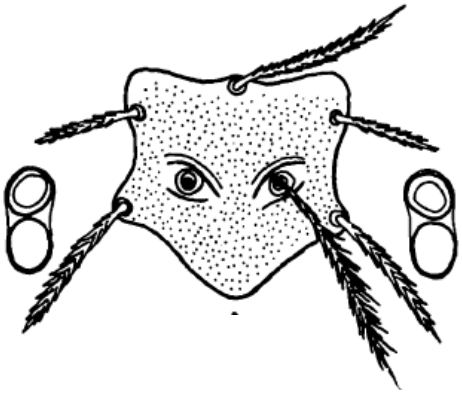
- Anteromedian (AM) setae present

- Scutum subrectangular without anterolateral shoulders
- 2 genulae on Coxa I
- Legs all 7 segmented
- Palpal tarsus 7B
- Galeala with branched setae
- Anteromedian setae is submarginal in scutum
- AL setae marginal
- Sensillae flagelliform, branched distally

Genus *Miicrobtrombicula*

Diagnosis:

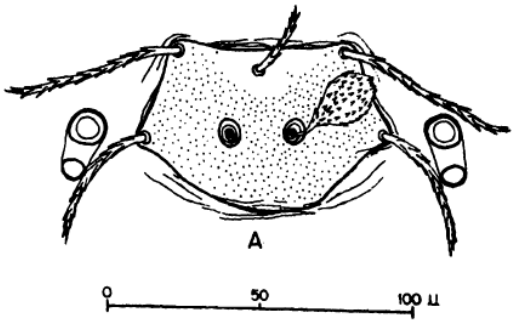
- Anteromedian (AM) setae present
- Scutum with anterolateral shoulders
- Subpentagonal scutum with convex posteromargin
- Palpal tarsus 6B
- Gnathobase and coxae without striations
- Leg III with single genuala



Genus *Schoengastia*:

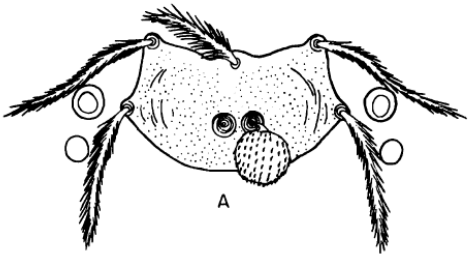
Diagnosis:

- Anteromedian (AM) setae present
- Scutum without shoulder
- Sensilla expanded (lanceolate to globose)
- Scutum with extensive posterior projection
- Cheliceral blade with row of dorsal teeth
- AL>PL;
- Sensilla pyriform
- Sensillary bases = or > diameter of SB apart



Genus *Helenicula*:

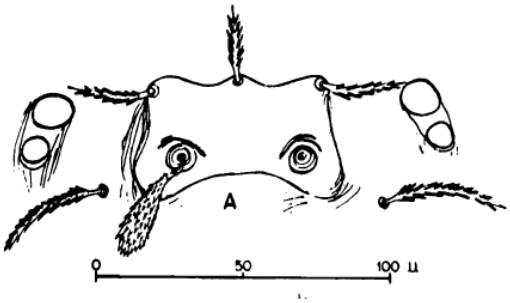
Diagnosis:



- Anteromedian (AM) setae present
- Scutum subtrapezoidal with convex posterior margin
- Sensilla expanded (laceolate or globose)
- Sensillary bases (SB) contiguous, i.e < diameter of SB apart
- AM posterior to level of AL bases; usually AL>PL>PM

Genus: *Schoutedenichia*

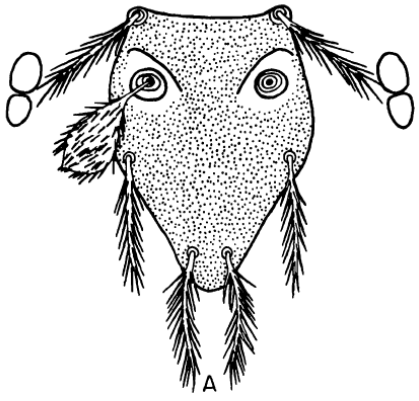
Diagnosis:



- AM Setae present
- Scutum trapezoidal
- Sensilla expanded (laceolate or globose)
- Sensillary bases (SB) contiguous, i.e < diameter of SB apart
- Coxa I and II unisetose
- Tibiala II absent

Genus: *Schoengastiella*

Diagnosis:

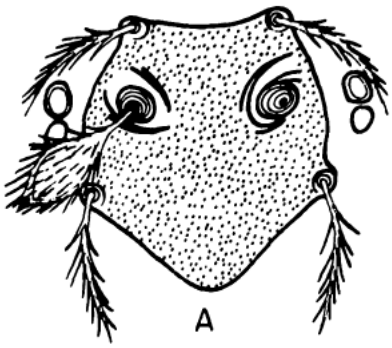


- Anteromedian setae (AM) absent
- Scutal formula 2 AL+ 2PL+ 2PPL.
- Scutum subpentagonal, with anterior margin shallowly convave
- Scutum posteriolateral margins tapering, caudal angle rounded
- Posterior margin of scutum produced beyond PL setae
- Sensillae clavate

Genus: *Walchia*

Diagnosis:

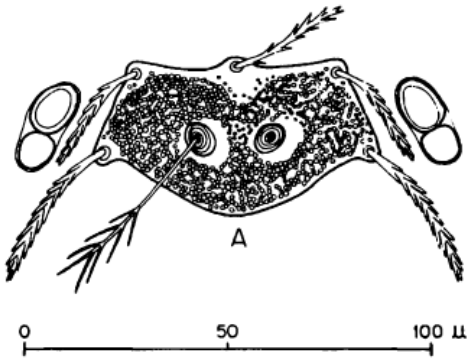
- Anteromedian setae (AM) absent
- Scutum anterior margin shallowly concave
- Scutal setal formula: 2AL + 2PL
- Scutum subpentagonal, with pointed posterior projection
- Sensillae clavate to globose



Genus: *Trombicula*

Diagnosis:

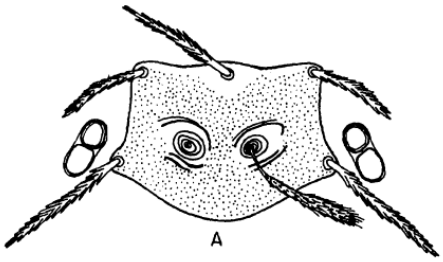
- Eyes2/2.
- Scutum subtrapezoidal with irregular pitting, scrobiculate
- posterior margin convex; sensillae flagelliform with distal branches



Genus *Herpetacarus*

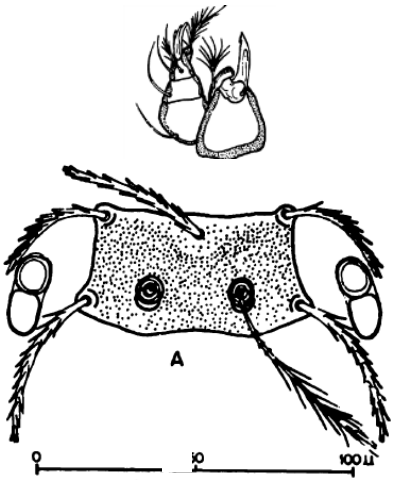
Diagnosis

- Eyes 2/2, well developed.
- Scutum wider than long with convex posterior margin;
- anterolateral shoulders absent;
- sensillae lanceolate to narrowly clavate, head with spiked setules;
- PL setae usually longest of scutal setae



Indian vectors

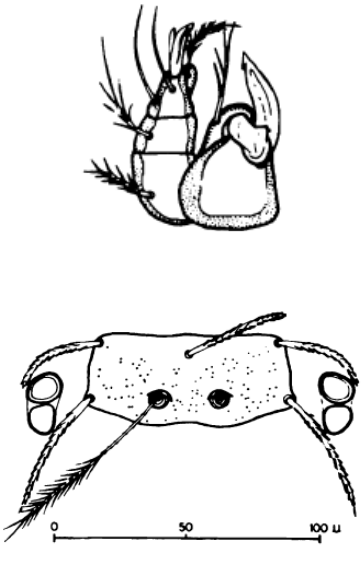
Leptotrombidium deliense



Diagnosis:

- Eyes 2/2 subequal, on ocular plate.
- One pair of humeral setae
- DS: 8-6-6-4-2-;
- Palpal setal formula N/N/BNN/7B;
- Scutum moderately punctate, subrectangular with anterior margin shallowly biconcave; posterior margin shallowly biconvex, posterolateral comers rounded;
- PL = AM> AL; sensillae flagelliform with basal barbs and branches on distal
- PW/SD = 1.62-1.75.
- Measurement: AW 60, 56-66; PW 71, 66-76; SB 28, 26-32; ASB. 27, 24-28; PSB 14, 12-15; AP 26, 26-29; AM 52, 49-56; AL 38, 34-43; PL 54, 50-57; sens. 60, 58-62.

Leptotrombidium jayewickremei

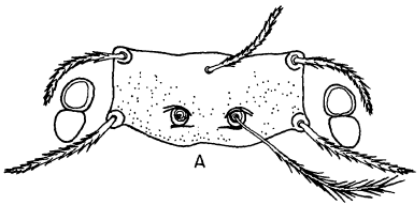


Diagnosis:

- Eyes 2/2, sub equal, on ocular plate.
- One pair of humeral setae
- DS: 8-6-6-4-(4)-2- (2); 2 pairs of sternal setae,
- Palpal setal formula B/B/NNB/7B;
- Scutum sparsely punctate, subrectangular with anterior margin shallowly biconcave; posterior margin shallowly biconvex;
- PL>AM>AL; sensillae flagelliform; PW/SD = 1.78-2.25.
- Scutal measurements : AW 65, (62-67); PW 76, (73-79); SB 23, (21-25); ASB 25,(24-26); PSB 13, (12-15); AP 22, (21-24); AM 41, (37-44); AL 37, (33-40); PL 46,(44-49); sens.64, (64-65).

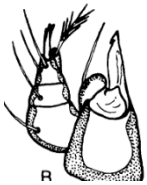
Leptotrombidium rajasthanense

Diagnosis:

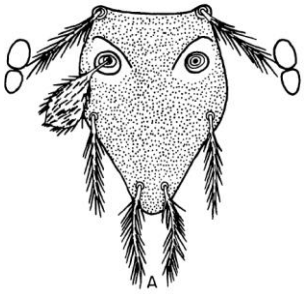


- Eyes 2/2, subequal, on ocular plate.
- One pair of humeral setae
- D.S: 8-6-6-4-2; 2 pairs of sternal setae, One pair of humeral setae
- Palpal setal formula B/B/NNB/7B.S
- Scutum sparsely punctate, subrectangular with anterıor margin shallowly biconcave; posterior margin shallowly biconvex;
- PL>AM>AL; sensillae flagelliform; PW/SD = 2.05.
- Scutal measurements : AW 67 (66, 63-69); PW 78 (77, 72-82); SB 24 (22,) 8-24); ASB 26 (24, 23-26); PSB 13 (13, 12-13); AP 24 (23, 21-24); AM 38 (38, 36-41); AL 39 (37, 35-39); PL 45 (44, 43-47); sens. 65 (58-65).

Schoengastiella ligula



Diagnosis:



- Eyes 2/2
- One pair of humeral setae
- D.S: 4-8-2-8-6, the rest irregular; 2 pairs of sternal setae
- Palpal setal formula: N/N/NNN/4B
- Scutum densely punctate, sub pentagonal, with anterior margin shallowly concave; postero-lateral margins tapering immediately beyond PL bases, caudal angle rounded;
- AL and PL setae finely ciliated; PL>AL; PPL setae similar to scutal setae
- sensillae clavate, head with setules; PW/SD = 0.47-0.80

- Scutal measurements: AW 37,(27-44); PW 48, (38-61); PPW 15, (10-20); SB 32, (29-40); ASB 20, (18-22); PSB 56, (48-68); AP 37,(33-42); APP 65, (56-78); PP 10,(8-12); AL 31, (27-35); PL 33,(28-38); sense 30 x 12, 24- 35 x 9-14.

9. Mite Indices

$$\textit{Chigger Index:} \quad \frac{\textit{Total no. of chigger mites collected}}{\textit{Total no. of hosts examined}}$$

$$\textit{Chigger Infestation Rate:} \quad \frac{\textit{Total no. of chigger mites collected}}{\textit{Total no. of hosts collected with chigger mites}} \textit{ X 100}$$

The chigger index has relevance to the presence of vector *trombiculid* mite i.e. *Leptotrombidium deliense* group. Mite collection should be made both from domestic and wild situations as per the method described earlier.

10. Control Methods for mites

A basic knowledge on the habitats of mites is essential for planning a control programme. The following are the various options available for the control of mites.

i. Environmental control:

- Cracks and crevices in ground, particularly near buildings and paths should be sealed. Maintain close-cut lawns and gardens with well kept paths and weed-free beds.
- If it is possible to identify the patches of vegetation that harbour large numbers of larval mites (mite islands), it may be advantageous to remove them by cutting and then to scrape or plough the top-soil.
- Mowing grass or weeds in these areas also helps. Such measures are recommended in the vicinity of camp sites and buildings.
- Clearing the vegetation (shrubs and dry leaves) around the human dwelling (10-m) in the forest area.

- Collecting dry leaves of forest, storing in the backyard and using for bedding purpose in cattle sheds should be discouraged.

ii. Chemical control:

- Where the removal of vegetation is not possible, mite islands can be sprayed with residual insecticide. The spraying of vegetation up to a height of 20cm around houses, hospitals and camp sites is effective against grass mites.
- The insecticides can be applied as fogs with ultra-low-volume spray equipment. Some suitable insecticides are diazinon, fenthion, malathion, propoxur and permethrin.

iii. Personal protection measures:

- Exposed workers should be encouraged to wear protective clothing impregnated with an insect-repellent.
- Frequently bathing (at least twice a week) cattle by scrubbing and applying repellents and also physically searching for mites on the body and destroying them.
- If persons in chigger infested areas are without the protection of repellents, they should bathe as soon as possible after returning. If welts- raised red bumps have formed, a dab of antiseptic applied to each will kill the chigger.
- Clothing can be treated with any one of the repellents viz., DEET, Benzyl benzoate and Dimethyl phthalate (0.65 – 1g /sq. m.), which will provide long lasting protection even after one or two washes.
- *Metarhizium anisopliae* Repellents like diethyl toluamide, dimethyl phthalate, dimethyl carbamate, benzyl benzoate etc. may be applied to clothing of persons moving in chigger infested areas and to arms or legs that usually do not remain covered by clothing.

iv. Biological control:

- fungal exposure on ticks revealed that the egg laying capacity of ticks was reduced by 50%, weight of larvae and nymphs was reduced significantly, suggesting that the fungus reduced the tick fitness and growth and caused mortality. Also there was a 5–10-fold reduction in the number of tick population.
- A concentration of 50 nematodes/cm caused 100% mortality of ticks in less than 5 days as a spray.

v. Rodent control:

- Bait boxes can be placed along rodent runs at intervals of 60 metres. A suitable bait consists of 100g of rolled oats, dry fish or foods with oil aroma (fried coconut, vada etc) mixed with a rodenticide.

11. Abbreviations of taxonomic terms and morphometric formulae

Abbreviation	Full form
AW	Distance between bases of anterolateral setae
PW	Distance between bases of posterolateral setae
SB	Distance between bases of sensillae
ASB	Distance between a line connecting sensillae bases to anterior-most margin of scutum
PSB	Distance between a line connecting sensillae bases to posterior-most margin of scutum
SD	Scutal depth or ASB+PSB
APO	Distance between bases of anterolateral and posterolateral setae
AM	Length of anteromedian seta
AL	Length of anterolateral seta
PL	Length of posterolateral seta
A-AL	Distance from base of anterolateral seta to anterior lateral margin of scutum
P-PL	Distance from base of poserolateral seta to posterior margin of scutum
SB-PL	Distance between a line connecting sensillae bases and base of posterolateral seta
PPW1	Distance between the bases of 1st pair of usurped setae
PPW2	Distance between the bases of 2nd pair of usurped setae
PPP1	Distance from base of the 1st pair of usurped setae to posterior margin of scutum
PPP2	Distance from base of the 2nd pair of usurped setae to posterior margin of scutum
S	Length of sensillae
Pa	Length of anterior leg (leg I)
pm	Length of median leg (leg II)
Pp	Length of posterior leg (leg III)
Ip	Index pedibus (pa+pm+pp)
fPp	Palpal pilous formula
fSc	Scutal formula

fD	Dorsal body setation formula
fV	Ventral body setation formula
Fsp	Leg segmentation formula
fCx	Coxal setation formula
ND	Number of dorsal setae
NV	Number of ventral setae
NDV	Total number of body setae (ND+NV)
B or N	Barbed (plumose) or nude

12. Acknowledgements

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