

## APPENDIX G

**Appendix G.1.** List of characters and character states from unchanged data set of Hong & Zhang (1996a). Some terminology was changed.

**Appendix G.2.** List of modified characters and character states of Hong & Zhang (1996a) to coincide with the data set defined for the present study.

**Appendix G.1.** List of characters and character states from unchanged data set of Hong & Zhang (1996a). Some terminology was changed.

0. Setae *vi*: (0) present; (1) absent.
1. Setae *ve*: (0) present; (1) absent.
2. Scapular setal tubercles: (0) absent; (1) present.
3. Setae *sc*: (0) present; (1) absent.
4. Frontal lobe (naso): (0) absent; (1) present.
5. Spine(s) on frontal lobe: (0) absent; (1) present.
6. Location of *sc*: (0) ahead of shield rear margin; (1) at shield rear margin.
7. Direction of *sc*: (0) anterior; (1) posterior; (2) upward or inward.
8. Body shape: (0) vermiform (worm-like); (1) fusiform (spindle-shaped).
9. Cheliceral curvature: (0) evenly curved; (1) abruptly curved downwards.
10. Location of genital area: (0) not appressed to coxae II; (1) appressed to coxae II.
11. Opisthosomal setae *c1*: (0) present; (1) absent.
12. Opisthosomal setae *d*: (0) present; (1) absent.
13. Opisthosomal setae *e*: (0) present; (1) absent.
14. Coxal setae *1b* on coxae I: (0) present; (1) absent.
15. Seta *bv* on femur I: (0) present; (1) absent.
16. Seta *l''* on genu I: (0) present; (1) absent.
17. Seta *l'* on tibia I: (0) present; (1) absent.
18. Seta *bv* on femur II: (0) present; (1) absent.
19. Seta *l''* on genu II: (0) present; (1) absent.
20. Tibia: (1) separate segment (“normal”); (1) reduced or fused.
21. Solenidion on tibia I: (0) present; (1) absent.
22. Ridge(s) or through(s) on opisthosoma: (0) absent; (1) present.
23. Empodium: (0) simple (“normal”); (1) divided, palm-shaped etc. (not “normal”).
24. Opisthosomal setae *c2*: (0) present; (1) absent.
25. Ridges on the female genital coverflap: (0) absent; (1) one longitudinal row; (2) two longitudinal rows or transverse lines.
26. Spatulate or shovel-shaped projections on legs: (0) absent; (1) present.
27. Lateral opisthosomal differentiation: (0) absent; (1) differentiated into broader dorsal and narrower ventral annuli.
28. Extensions on dorsal annuli: (0) not extended laterally; (1) extended laterally or with indentations.
29. Prosternal apodeme (sternal line): (0) absent; (1) present.
30. Opisthosomal setae *h1*: (0) present; (1) absent.
31. Spermathecal tubes in female: (0) long; (1) short.
32. Length of setae *sc*: (0) very long; (1) long; (2) short; (3) absent.
33. Microtubercles on dorsal annuli: (0) absent; (1) present.
34. Comparison between locations of coxal setae *1a* and *2a*: (0) *1a* ahead of *2a*; (1) *1a* in line with *2a*; (3) *1a* behind *2a*.

**Appendix G.2.** List of modified characters and character states of Hong & Zhang (1996a) to coincide with the data set defined for the present study.

0. Setae *vi*: (0) pair present; (1) one seta mid-anteriorly; (2) absent.
1. Setae *ve*: (0) present; (1) absent.
2. Scapular setal tubercles: (0) primary absent; (1) present; (2) secondary absent.
3. Setae *sc1*: (0) present; (1) absent.
4. Frontal lobe (naso): (0) absent; (1) very short or indistinct; (2) present.
5. Spine(s) on frontal lobe: (0) absent; (1) one spine; (2) three spines.
6. Location of *sc*: (0) ahead of shield rear margin; (1) well ahead of shield rear margin; (2) on or near shield rear margin.
7. Direction of *sc*: (0) anteriad diverging; (1) anteriad parallel or converging; (2) medially; (3) to outside; (4) posteriad diverging; (5) any direction.
8. Body shape: (0) rounded to oval; (1) vermiform (worm-like); (2) cylindrical; (3) vermiform-elongated; (4) fusiform-fat; (5) fusiform-elongated; (6) fusiform-flattened; (7) fusiform-very-long; (8) flattened-narrow-tail.
9. Cheliceral curvature: (0) recurved in stylophore; (1) evenly curved or straight; (1) abruptly curved downwards.
10. Location of genital area: (0) caudally; (1) 9-15 annuli removed from coxae II; (2) near coxae II; (3) appressed to coxae II.
11. Opisthosomal setae *c1*: (0) present; (1) absent.
12. Opisthosomal setae *d*: (0) present; (1) absent.
13. Opisthosomal setae *e*: (0) present; (1) absent.
14. Coxal setae *1b* on coxae I: (0) present; (1) absent.
15. Seta *bv* on femur I: (0) present; (1) absent.
16. Seta *l''* on genu I: (0) present; (1) absent.
17. Seta *l'* on tibia I: (0) present; (1) absent.
18. Seta *bv* on femur II: (0) present; (1) absent.
19. Seta *l''* on genu II: (0) present; (1) absent.
20. Tibia: (1) separate segment (“normal”); (1) reduced or fused with tarsus.
21. Solenidion on tibia I: (0) present in ventrodiscal position; (1) absent.
22. Ridge(s) or through(s) on opisthosoma: (0) absent; (1) present.
23. Empodium on tarsus I: (0) pad-like with numerous unbranched rays; (1) simple (“normal”); (2) divided; (3) palmate.
24. Opisthosomal setae *c2*: (0) present; (1) absent.
25. Ridges on the female genital coverflap: (0) genital overlap absent; (1) smooth; (2) ornamented basally; (3) entirely ornamented with basal and distal area; (4) entirely ornamented.
26. Spatulate or shovel-shaped projections on leg tarsi: (0) absent; (1) present.
27. Lateral opisthosomal differentiation: (0) annuli absent; (1) subequal and similar; (2) subequal and differentiated; (3) subequal and narrow; (4) subequal and broad; (5) differentiated into slightly broader dorsal than ventral annuli; (6) differentiated into broader dorsal than ventral annuli; (7) differentiated into extremely broader dorsal annuli than ventral annuli.
28. Extensions on dorsal annuli: (0) not extended laterally; (1) with slight lateral extensions.
29. Prosternal apodeme (sternal line): (0) present, very wide; (1) absent; (2) absent, coxae widely separated; (3) separated; (4) apodeme present (coxal inner margins touching); (5) coxae fused.
30. Opisthosomal setae *h1*: (0) present; (1) minute; (2) absent.
31. Spermathecal tubes in female: (0) eriophyoid-type spermathecal tubes absent; (1) short; (2) long.
32. Length of setae *sc*: (0) exceptionally long; (1) very long; (2) long; (3) average; (4) very short.
33. Microtubercles on dorsal annuli: (0) absent; (1) present; (2) obscure; (3) elongated, in rows.
34. Comparison between locations of coxal setae *1a* and *2a*: (0) *1a* ahead of *2a*; (1) *1a* slightly ahead of *2a*; (2) *1a* in line with *2a*; (3) *1a* slightly behind *2a*; (4) *1a* behind *2a*.

## APPENDIX H (H.1, H.2, H.3)

**APPENDIX H.1.** Original data matrix (Hong & Zhang, 1996a) of morphological characters for 17 eriophyoid species and one outgroup Tydeidae for the 18-original analyses. ? = uncertain or unknown character states or inapplicable states.

**APPENDIX H.2.** Data matrix of morphological characters for 17 eriophyoid species and one outgroup species (*Orphareptydeus*) for the 18-correct analyses. ? = uncertain or unknown character states, - = inapplicable states.

**APPENDIX H.3.** Data matrix of morphological characters for 17 eriophyoid species and one outgroup species (*Orphareptydeus*) for the 18-modify analyses. ? = uncertain or unknown character states, - = inapplicable states.

**APPENDIX H.1.** Original data matrix (Hong & Zhang, 1996a) of morphological characters for 17 eriophyoid species and one outgroup Tydeidae for the 18-original analyses. ? = uncertain or unknown character states or inapplicable states.

	<b>0</b>	<b>1</b>	<b>2</b>	<b>3</b>
	<b>0123456789</b>	<b>0123456789</b>	<b>0123456789</b>	<b>01234</b>
<i>Tydeidae</i>	0000000000	0000000000	0000000000	00000
<i>Pentasetacus</i>	0010100000	0000000000	0001000001	00011
<i>Trisetacus</i>	0110000000	0000000000	000000000?	00010
<i>Nalepella</i>	0110100010	0100000000	000000010?	000?0
<i>Novophytoptus</i>	1010001100	0100010010	0100000001	00012
<i>Phytoptus</i>	1010000000	0000000000	0000000001	00010
<i>Sierraphytoptus</i>	1010110210	0000000000	0100000111	00200
<i>Mackiella</i>	1010100000	0100000000	0000000101	00001
<i>Aberoptus</i>	1110001110	1100000100	1101011100	11111
<i>Nothopoda</i>	1110000100	0100100100	1100000000	11210
<i>Ashieldophyes</i>	110000?200	1111100000	0100000000	01200
<i>Cecidophyes</i>	110110??10	1100000000	0100020100	11310
<i>Eriophyes</i>	1110000?00	0100000000	01000?000?	?1110
<i>Phyllocoptes</i>	1110100210	0100000000	01000?0101	?11?0
<i>Diptacus</i>	1110100011	0100010010	01110?0101	?12?0
<i>Diptilomiopus</i>	1111100?11	0100011111	011110010?	?13?0
<i>Rhinophytoptus</i>	1110100011	0100000000	0100000101	?12?0
<i>Rhyncaphytoptus</i>	1110100011	0100000000	01?00?0101	?11?0

**APPENDIX H.2.** Data matrix of morphological characters for 17 eriophyoid species and one outgroup species (*Orphareptydeus*) for the 18-correct analyses. ? = uncertain or unknown character states, - = inapplicable states.

	<b>0</b>	<b>1</b>	<b>2</b>	<b>3</b>
	<b>0123456789</b>	<b>0123456789</b>	<b>0123456789</b>	<b>01234</b>
<i>Orfareptydeus_stepheni</i>	0000000--0	0000000001	0101000000	0-010
<i>Pentasetacus_araucaria</i>	0010100000	0000000000	0001000001	00011
<i>Trisetacus_ehmanni</i>	0110000000	0000000000	0000000000	00011
<i>Nalepella_tsugifoliae</i>	0110100010	0100000000	0000000001	00010
<i>Novophytoptus_rostratae</i>	1010001100	0100010010	0100000001	01012
<i>Phytoptus_avellanae</i>	1010000000	0000000000	0100000001	01110
<i>Sierraphytoptus_alnivagrans</i>	1010110010	0000000000	0100000111	01100
<i>Mackiella_phoenicis</i>	1010100000	0100000000	0000000101	01212
<i>Aberoptus_samoae</i>	1110001210	1100000100	0101011001	11211
<i>Nothopoda_rapanae</i>	1110001100	0100100100	1100020000	11210
<i>Ashieldophyes_pennademensis</i>	1100000210	1111000000	0100000000	01200
<i>Cecidophyes_rouhollahi</i>	110110--10	1100000000	0100020101	11-10
<i>Eriophyes_pyri</i>	1110001000	0100000000	0100010000	01?10
<i>Phyllocoptes_calisorbi</i>	1110100210	0100000000	0100020001	01110
<i>Diptacus_sacramentae</i>	1110101011	0100010010	0101000101	11110
<i>Diptilomiopus_assamica</i>	1111100-11	0100111111	0111100101	11-00
<i>Rhinophytoptus_concinnus</i>	1110001011	0100000000	0100000001	1??1?
<i>Rhyncaphytoptus_ficifoliae</i>	1110001011	0100000000	0100000111	01110

**APPENDIX H.3.** Data matrix of morphological characters for 17 eriophyoid species and one outgroup species (*Orphareptydeus*) for the 18-modify analyses. ? = uncertain or unknown character states, - = inapplicable states.

	<b>0</b>	<b>1</b>	<b>2</b>	<b>3</b>
	<b>0123456789</b>	<b>0123456789</b>	<b>0123456789</b>	<b>01234</b>
<i>Orfareptydeus_stepheni</i>	0000000500	0000000001	0100000000	00310
<i>Pentasetacus_araucaria</i>	1010200030	2000000000	0002010103	02312
<i>Trisetacus_ehmanni</i>	1110001010	2000000000	0001010101	02212
<i>Nalepella_tsugifoliae</i>	1110100140	2100000000	0001010303	02011
<i>Novophytoptus_rostratae</i>	2010002420	1100010010	0101010303	01114
<i>Phytoptus_avellanae</i>	2010000110	2000000000	0101020103	01311
<i>Sierraphytoptus_alnivagrans</i>	2010210160	2000000000	0101010613	01301
<i>Mackiella_phoenicis</i>	2010200010	2100000000	0001010603	01333
<i>Aberoptus_samoae</i>	2110002380	3100000100	0103041103	21312
<i>Nothopoda_rapaneae</i>	2110002410	2100100100	1101030102	21310
<i>Ashieldophyes_pennademensis</i>	2120001370	3111000000	0101010404	01400
<i>Cecidophyes_rouhollahi</i>	212110--40	3100000000	0101030203	21-10
<i>Eriophyes_pyri</i>	2110002030	2100000000	0101040104	01210
<i>Phyllocoptes_calisorbi</i>	2110200250	2100000000	0101030103	11311
<i>Diptacus_sacramentae</i>	2110202051	2100010010	0102010603	21321
<i>Diptilomiopus_assamica</i>	2111100-41	2100111111	0112110503	21-01
<i>Rhinophytoptus_concinnus</i>	2110002051	2100000000	0101010103	2?31?
<i>Rhyncaphytoptus_ficifoliae</i>	2110002051	2100000000	0101010713	01311

## APPENDIX I

**Glossary.**

## Appendix I. Glossary. Definition of selected terms used in the dissertation.

<b>Term</b>	<b>Definition</b>	<b>Source</b>
<b>apomorphy</b>	Derived character or character state.	Kitching <i>et al.</i> (1998)
<b>arrhenotokous</b>	Males hatching from haploid (unfertilized), and females from diploid (fertilized) eggs	
<b>artificial group/taxon</b>	Polyphyletic or paraphyletic group or taxon (not monophyletic).	
<b>character</b>	An observable feature or attribute of an organism, which may or may not have alternative manifestations (character states) which can be used to distinguish between different organisms.	Modified from Kitching <i>et al.</i> (1998)
<b>character state</b>	One of two or more alternative manifestations of a character.	Kitching <i>et al.</i> (1998)
<b>characteristic, feature</b>	Used as an alternative term for character state. See definition of character state.	
<b>ci (consistency index)</b>	Measure of the fit of a character to a tree. It is calculated by the minimum number steps a character will have on any cladogram (e.g., for a binary character this will be 1), divided by the minimum number of steps a character has on a particular cladogram.	Kitching <i>et al.</i> (1998); Lipscomb (1998)
<b>CI</b>	Average fit of all characters to a tree or in other words it measures the relative amount of homoplasy in a tree.	Kitching <i>et al.</i> (1998)
<b>clade</b>	Alternative term for monophyletic group (see monophyletic group for definition).	Kitching <i>et al.</i> (1998)
<b>EM</b>	Electron microscopy or electron microscope, depending on the context.	
<b>erineum</b>	abnormal plant hair growth	
<b>gall-inhabiting eriophyoid mites</b>	Non-vagrant eriophyoid mites living in plant microhabitats created by symptomatic growth caused by their feeding, such as galls, erineum and blisters.	Sabelis & Bruin (1996)
<b>homologize</b>	<ol style="list-style-type: none"> <li>1. To make homologous.</li> <li>2. To show to be homologous.</li> </ol>	
<b>metric tree</b>	Cladogram in which the length of each branch is proportional to the amount of character changes that occurs along it.	Kitching <i>et al.</i> (1998)
<b>monophyletic group</b>	Group which contains the most recent common ancestor plus all and only all its descendants. The group is diagnosed as monophyletic by the discovery of shared homologies (synapomorphies). This group is also known as a clade.	Definition by Hennig in Kitching <i>et al.</i> (1998), wording modified
<b>monophyletic, monophyly</b>	See monophyletic group	
<b>node</b>	Point in a cladogram where three or more branches meet to form a group.	Modified from Kitching <i>et al.</i> (1998)
<b>non-vagrant eriophyoid mites</b>	Eriophyoid mites living a more sheltered life: in natural plant microhabitats e.g., in buds, underneath needle and leaf sheaths, and between bulb scales (refuge-inhabiting mites), or in microhabitats created by symptomatic growth caused by their feeding, such as galls (gall-inhabiting mites).	Sabelis & Bruin (1996)
<b>organule</b>	Association of small numbers of integumental cells which perform some specific function different from that of the general population of epidermal cells, eg.,	Lawrence (1966)

	setae, scales, dermal glands and lyrifissures.	
<b>over-resolved cladogram</b>	“A cladogram with spurious resolution due to the presence of one or more zero-length branches.”	Kitching <i>et al.</i> (1998)
<b>paraphyletic group</b>	A group of which one or more parts were removed. If these parts are added to the paraphyletic group, it will be monophyletic. (Can also be defined as group recognized by symplesiomorphies.)	Definition by Hennig in Kitching <i>et al.</i> (1998), wording modified
<b>polyphyletic group</b>	A group that does not include the most recent common ancestor of all its members. It is also defined as a group based on homoplastic (homoplasious) assumed to have been absent in the most recent common ancestor of all its members. Groups based on convergent characters are also regarded as being polyphyletic.	Definition by Hennig in Kitching <i>et al.</i> (1998), wording modified and added on by Kitching <i>et al.</i> (1998) and author
<b>refuge-inhabiting eriophyoid mites</b>	Non-vagrant eriophyoid mites living in natural plant microhabitats e.g., in buds, underneath needle and leaf sheaths, and between bulb scales, and not in symptomatic growth caused by their feeding.	Sabelis & Bruin (1996)
<b>retention index (ri)</b>	A measure of the fit of a character to a tree. It measures the relative amount of homoplasy required by a character to fit a cladogram.	Kitching <i>et al.</i> (1998); Lipscomb (1998)
<b>SEM</b>	Scanning electron microscope or scanning electron microscopy, depending on the context.	
<b>sister group / taxon</b>	Two taxa that are more closely related to each other than either is to a third taxon	Kitching <i>et al.</i> (1998)
<b>stepwise addition</b>	The sequence by which taxa are added to a developing cladogram during the initial building phase of an analysis.	Kitching <i>et al.</i> (1998)
<b>synapomorphy</b>	Shared apomorphy, which is not homoplasious, that unites two or more taxa into a monophyletic group; also known as homology. It can also be defined as a secondary homology, depending on the definition of homology.	Modified from Kitching <i>et al.</i> (1998)
<b>tree</b>	Alternative term for cladogram, generally used in cladistics, and in the present dissertation.	
<b>unweighted tree</b>	A tree (cladogram) found under equal weighting of characters.	

## APPENDIX J

### Published abstracts.

**J.1.** Craemer, C. & Hall, A.N. 2003. The use of low-temperature scanning electron microscopy for studying eriophyoid mites (Acari: Eriophyoidea). p. 76 In: *Proceedings of the Microscopy Society of Southern Africa* 33.

**J.2.** Craemer, C. 2006. Morphology of eriophyoid mites (Eriophyoidea) as elucidated by scanning electron microscopy: trivial pursuit or valuable systematic contribution? p. 45 In: Bruin, J. (Ed.). *Abstract Book*. 12<sup>th</sup> International Congress of Acarology, 21-26 August 2006, Amsterdam, The Netherlands.

## THE USE OF LOW-TEMPERATURE SCANNING ELECTRON MICROSCOPY FOR STUDYING ERIOPHYOID MITES (ACARI: ERIOPHYOIDEA)

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Mites of the superfamily Eriophyoidea are entirely phytophagous, generally host-specific, and may occur on the majority of perennial higher plant species, with some causing conspicuous deformities or diverse symptoms. Some species are economically important plant pests in agriculture, while others are beneficially used in the control of weeds.

The diversity of eriophyoid mites is virtually unknown. Approximately 3 700 species are known world-wide<sup>3</sup>, which accounts for a scant estimated 1% or less of extant species. Therefore the most important requirement in the systematics of this group remains the description of new taxa. An exact study of morphology is a prerequisite for the adequate description of a taxon, and for hypotheses of homology for phylogenetic studies.

Eriophyoids are a morphologically unique group of mites with elongated, annulated bodies, and four legs in all life stages. They are soft bodied and microscopic, on average about 100-200 µm long and 30 µm wide. During recent years conventional scanning electron microscope (SEM) studies have been sporadically used to supplement light microscope studies of these mites, and this has contributed towards the understanding of their morphology, but it has not been routinely and widely used. Unfortunately conventional SEM preparation methods are associated with artifacts, and particularly for the preparation of eriophyoid mites. Low-temperature SEM, as one of the alternative methods<sup>1,4,5</sup> seems to be the most successful in obtaining highly magnified, largely artifact-free images of these mites<sup>2</sup>. Based on this information, the latter method was chosen to undertake the current study on eriophyoid mites of South Africa. In this study a JEOL 840 SEM with a cryo stage was used. After plunge-freezing in nitrogen slush, specimens were etched for ca. 30 minutes to remove surface condensed water vapor and then sputter coated with gold palladium.

The frozen specimens remained turgid and the form of the mites remained mainly unaltered. The mechanical damage caused by preparatory manipulation to delicate structures, including wax secretions (Fig. 1), was largely prevented. In addition mites could be studied *in situ* in galls and on plant material, facilitating observations on their biology (Fig. 2). Useful data on the morphology was obtained which will be incorporated in the taxonomy of these mites. However, results were not always satisfactory: surface ice could not always be entirely removed; material often degraded after prolonged examination; and some mites intact on plant pieces were washed off<sup>4</sup> during sample preparation. The use of a field emission SEM with cryo-attachment<sup>5</sup> will enhance results, especially for the resolution of fine

structure on e.g. specialized setae, but this option was not available.

We strongly recommend that SEM studies should be incorporated in the systematics of eriophyoid mites.

### References

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Fig. 1. Dorsal view of a *Calacarus* sp. showing intact wax secretions. Bar = 10 µm.

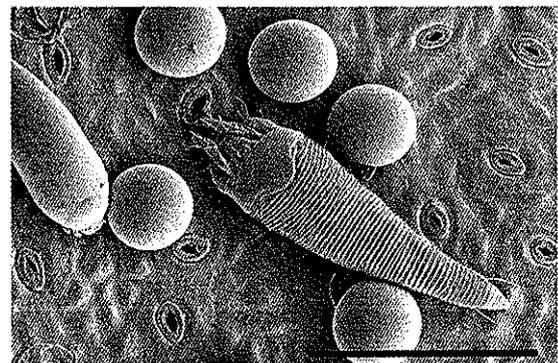


Fig. 2. *Cisaberoptus* sp. on a leaf, e = egg. Bar = 100 µm.

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Wed 23, F001

## **Morphology of eriophyoid mites (Eriophyoidea) as elucidated by scanning electron microscopy: trivial pursuit or valuable systematic contribution?**

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Eriophyoids are the smallest of all mites. Their morphology is unique, and their relatively soft bodies are simplified. Despite many characters available for taxonomy, some are not clearly delimited or readily discernible, and too few are available for phylogenetic studies.

Light microscopic studies of cleared, slide mounted specimens are almost exclusively used for studying the taxonomy of these mites using morphology. This method is still the simplest and the most practical. However, artifacts caused by slide mounting and inefficient resolution of detailed morphology may be detrimental to eriophyoid taxonomy. Scanning electron microscope (SEM) studies have been used only sporadically to supplement conventional descriptions of eriophyoid mites. This has contributed towards the understanding of their morphology, but it has not been routinely used. The conventional SEM preparation methods commonly used may also cause artifacts in specimens. Low-temperature SEM seems to be the most successful in obtaining highly magnified, largely artifact free images of these mites. In this study, the latter method was utilized to study ca. 30 eriophyoid species from South Africa. The frozen specimens remained turgid and the shape of the mites remained largely unaltered, and mechanical damage to delicate structures, including wax secretions, was prevented.

Although results were not always satisfactory, partly due to the quality of equipment available, useful morphological data was obtained. Discrepancies between species descriptions from slide mounted specimens and the SEM images were found. This included body form, interpretation of structures and the presence of secretions. Additional morphological characters were found in the detailed morphology not generally used in the conventional taxonomy such as on the gnathosoma and legs.

The general incorporation and reliance on information from SEM studies in the classification and identification of these mites remain a problem: it is time consuming, expensive, requires additional suitable material; and inaccessibility of electron microscope facilities. SEM studies are also not able to replace the datasets obtained from slide mounted specimens and the investigator's interpretation thereof in morphological illustrations. Furthermore, with printing and subsequent photocopying of publications, the quality of SEM images, in many cases, lose their degree of clarity.

Despite these, the usefulness of SEM studies to mite morphology cannot be disputed, and the contribution of taxonomically useful morphology is clearly demonstrated for the Eriophyoidea. The possible incorporation of artifacts in the description and classification of the group by studying slide mounted specimens should not be ignored. It is also clear that SEM studies can contribute additional characters needed for systematic studies. More importantly, the incorporation of SEM images provides additional information to type material, especially where the type specimens have either deteriorated or were destroyed over time. Eriophyoid taxonomists should thus ideally plan for and enhance their studies with high quality SEM studies.

## APPENDIX K

Published article. Craemer, C.; Amrine, J.W. Jr.; De Lillo, E. & Stasny, T.A. 2005.  
Nomenclatural changes and new synonymy in the genus *Diptilomiopus* Nalepa, 1916 (Acari:  
Eriophyoidea: Diptilomiopidae). *International Journal of Acarology* 31: 133–136.

## NOMENCLATURAL CHANGES AND NEW SYNONYMY IN THE GENUS *DIPTILOMIOPUS* NALEPA, 1916 (ACARI: ERIOPHYOIDEA: DIPTILOMIOPIDAE)

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**ABSTRACT** - The concept of the genus *Diptilomiopus* Nalepa, 1916 is re-examined. Several species in *Vilaia* Chandrapatya and Boczek, 1991a, a junior synonym, are reassigned to *Diptilomiopus*, namely *aglaiae* Chandrapatya and Boczek, 2002a, *anthocephaliae* Chandrapatya and Boczek, 2002a, *benjaminiae* Boczek and Chandrapatya, 2002, *boueae* Chandrapatya and Boczek, 2002a, *cerberae* Chandrapatya, 1998, *combretae* Chandrapatya and Boczek, 2002a, *coreiae* Chandrapatya and Boczek, 2002b, *jasminiae* Chandrapatya and Boczek, 2001, *melastomae* Boczek and Chandrapatya, 2002, *meliae* Boczek, 1998, *morindae* Boczek, 1998, *musae* Chandrapatya, 1998, *racemosae* Chandrapatya and Boczek, 2001, *riciniae* Boczek and Chandrapatya, 2002, *swieteniae* Chandrapatya, 1998, and *thunbergiae* Boczek and Chandrapatya, 2002. *Diptilomiopus septimus* Huang, 2001 is a junior synonym of *Rhynacus championi* Huang, 1992, which is transferred to *Diptilomiopus*.

**Key words** - New combinations, new synonymy, Acari, Eriophyoidea, Diptilomiopidae, Diptilomiopinae, *Diptilomiopus*, *Vilaia*.

### INTRODUCTION

This is the first in a series of articles on the Diptilomiopidae Keifer, 1944. Nalepa (1916) described the then monotypic genus, *Diptilomiopus* with *Diptilomiopus javanicus* type species by original designation; the genus was placed at that time in the Phyllocoptinae. His original description reads: "Dorsalseite des Abdomens von zwei seichten, nach hinten verstreichenden Längsfurchen durchzogen. Keine Patella, Beine daher fünfgliedrig. Prätarsus (Fiederklaue) gegabelt. Beinglieder mit Ausnahme des Tarsus borstenlos". ["Dorsal surface of the abdomen with two shallow (weak) longitudinal furrows, extending to the rear. No patella (genu), legs therefore five segmented. Pretarsus (featherclaw = empodium) forked (divided). Leg segments, with the exception of the tarsus, without setae."] An additional description of this genus, probably meant by Nalepa to be the original description, was received by the publisher in November 1916, but not published until 20 January 1918 (Nalepa, 1918) (Newkirk, 1984). The latter description reads: "Abdomen ungleichartig geringelt, Rückenhal-

bringe breiter als die Bauchhalbringe. Beinglied 3 fehlend, Beine daher fünfgliedrig. Schaft der Fiederklaue gegabelt". ["The abdomen unequally ringed (annulated), dorsal half rings (dorsal annuli) broader than the ventral half rings (ventral annuli). Leg segment three (genu) absent, legs therefore five segmented. Shaft of the featherclaw (empodium) forked (divided)."] Lamb (1953) also translated the 1918 description to English.

Chandrapatya and Boczek (1991a) described *Vilaia*, with type species *Rhynacus pamithus* Boczek and Chandrapatya, 1989, differentiating this new genus from the diptilomiopine genera *Asetadiptacus* Carmona, 1970 and *Neodiptilomiopus* Mohanasundaram, 1982, but not from *Diptilomiopus*.

In their study on the systematics of the Diptilomiopinae, Hong and Zhang (1997) concluded that *Vilaia* is a junior synonym of *Diptilomiopus* based on a comparison of the character states of *Diptilomiopus* and *Vilaia* spp. The confusion may have been created by the fact that the definition of *Diptilomiopus* by Keifer (1975) and later, the generic keys of Boczek *et al.* (1989) and Amrine (1996) implied that *Diptilomiopus* species do not have

scapular setal tubercles; however, many *Diptilomiopus* species do possess scapular setal tubercles (Hong and Zhang, 1997). Although the scapular setae are described as being absent in the type species of *Diptilomiopus* (*D. javanicus*) (Nalepa, 1916, 1918), the presence or absence of the scapular tubercles is not mentioned. No figures of *Diptilomiopus javanicus* are available, and it should be noted that the drawings published by Boczek *et al.* (1989) (page 182, plate 190) are of *Diptilomiopus jevremovici* Keifer, 1960, and not of *D. javanicus* as wrongly indicated.

Hong and Zhang (1997) acted on the synonymy of *Vilaia* with *Diptilomiopus* by transferring the 13 *Vilaia* species (Chandrapatya and Boczek, 1991a, b; Boczek and Chandrapatya, 1992) known at that time to *Diptilomiopus*:

Since the synonymy of *Vilaia* by Hong and Zhang (1997), 16 additional *Vilaia* species have been described (Boczek and Chandrapatya, 1998; Chandrapatya and Boczek, 1998; Chandrapatya and Boczek, 2001, 2002a, b; Boczek and Chandrapatya, 2002), without any indication whether the authors accepted or rejected the synonymy. An attempt should be made to study specimens of *D. javanicus* for the presence or absence of the scapular setal tubercles in the type species of *Diptilomiopus*, which may influence the synonymy of *Vilaia* with *Diptilomiopus*; *Diptilomiopus javanicus* was described as an inquiline in 2-mm pouch-like galls caused by *Aceria hemigraphidis* (Nalepa, 1916) on the upper surfaces of leaves of *Hemigraphis confinis* (Nees) T. Anders (Acanthaceae), collected by W. Docters van Leeuwen-Reijvaan in 1914 in Semarang, Java (Nalepa, 1916, 1918). At this time we concur with the synonymy proposed by Hong and Zhang (1997), and hereby transfer *Vilaia* species in question to *Diptilomiopus*.

We also assign a new species synonymy within *Diptilomiopus*.

#### *Diptilomiopus* Nalepa, 1916

*Diptilomiopus* Nalepa, 1916: 283; Nalepa, 1918: 228-230; Keifer, 1938: 305; Lamb, 1953: 369; Keifer, 1975: 521; Newkirk and Keifer, 1975: 585; Chakrabarti and Mondal, 1983: 299; Amrine and Stasny, 1994: 173; Boczek *et al.*, 1989: 25, 182 (plate 190 not of *D. javanicus*, but of *D. jevremovici*); Kuang, 1995: 168; Amrine, 1996: 111, 140; Hong and Zhang, 1996: 71; Hong and Zhang, 1997: 321; Amrine *et al.*, 2003: 137, 195-196.

*Sectipes* Keifer, 1962a: 18-19; Keifer, 1962b: 19, **synonym**; Newkirk and Keifer, 1975: 585.

*Vilaia* Chandrapatya and Boczek, 1991a: 427-428; Boczek and Chandrapatya, 1989: 139-140; Amrine and Stasny, 1994: 311-312; Amrine, 1996: 114,

141; Hong and Zhang, 1997: 321, **synonym**; Amrine *et al.*, 2003: 137, 215.

Type species - *Diptilomiopus javanicus* Nalepa, 1916, by original designation.

#### NEW COMBINATIONS

##### *Diptilomiopus aglaiae* (Chandrapatya and Boczek, 2002), n. comb.

*Vilaia aglaiae* Chandrapatya and Boczek, 2002a: 129-131, fig. 3.

##### *Diptilomiopus anthocephaliae* (Chandrapatya and Boczek, 2002), n. comb.

*Vilaia anthocephaliae* Chandrapatya and Boczek, 2002a: 126-129, fig. 2.

##### *Diptilomiopus benjaminiae* (Boczek and Chandrapatya, 2002), n. comb.

*Vilaia benjaminiae* Boczek and Chandrapatya, 2002: 25-27, fig. 1.

##### *Diptilomiopus boueae* (Chandrapatya and Boczek, 2002), n. comb.

*Vilaia boueae* Chandrapatya and Boczek, 2002a: 123-126, fig. 1.

##### *Diptilomiopus cerberae* (Chandrapatya, 1998), n. comb.

*Vilaia cerberae* Chandrapatya, 1998, *in* Boczek and Chandrapatya, 1998: 37-38, fig. 4.

##### *Diptilomiopus combretae* (Chandrapatya and Boczek, 2002), n. comb.

*Vilaia combretae* Chandrapatya and Boczek, 2002a: 132-134, fig. 4.

##### *Diptilomiopus coreiae* (Chandrapatya and Boczek, 2002), n. comb.

*Vilaia coreiae* Chandrapatya and Boczek, 2002b: 135-138, fig. 1.

##### *Diptilomiopus jasmintiae* (Chandrapatya and Boczek, 2001), n. comb.

*Vilaia jasmintiae* Chandrapatya and Boczek, 2001: 96-99, fig. 3.

***Diptilomiopus melastomae* (Boczek and Chandrapatya, 2002), n. comb.**

*Vilala melastomae* Boczek and Chandrapatya, 2002: 31-33, fig. 3.

***Diptilomiopus meliae* (Boczek, 1998), n. comb.**

*Vilala meliae* Boczek, 1998, in Boczek and Chandrapatya, 1998: 35-36, 38, fig. 3.

***Diptilomiopus morindae* (Boczek, 1998), n. comb.**

*Vilala morindae* Boczek, 1998, in Boczek and Chandrapatya, 1998: 32, 34-35, fig. 2.

***Diptilomiopus musae* (Chandrapatya, 1998), n. comb.**

*Vilala musae* Chandrapatya, 1998, in Chandrapatya and Boczek, 1998: 45-46, fig. 4.

***Diptilomiopus racemosae* (Chandrapatya and Boczek, 2001), n. comb.**

*Vilala racemosae* Chandrapatya and Boczek, 2001: 99-101, fig. 4.

***Diptilomiopus riciniae* (Boczek and Chandrapatya, 2002), n. comb.**

*Vilala riciniae* Boczek and Chandrapatya, 2002: 31, 34-36, fig. 4.

***Diptilomiopus swieteniae* (Chandrapatya, 1998), n. comb.**

*Vilala swieteniae* Chandrapatya, 1998, in Chandrapatya and Boczek, 1998: 43-44, fig. 3.

***Diptilomiopus thunbergiae* (Boczek and Chandrapatya, 2002), n. comb.**

*Vilala thunbergiae* Boczek and Chandrapatya, 2002: 28-31, fig. 2.

**NEW COMBINATION AND NEW SYNONYM**

***Diptilomiopus championi* (Huang, 1992), n. comb.**

*Rhynacus championi* Huang, 1992: 226, 232, figs. 5-8 (SEM images); Amrine and Stasny, 1994: 274; Hong and Zhang, 1996: 72; Hong and Zhang, 1997: 326.

*Diptilomiopus septimus* Huang, 2001: 67-68, fig. 67, **new synonym.**

**REMARKS** - Huang (2001) redescribed *Rhynacus championi* Huang, 1992 (Huang, 1992) as a new species, *Diptilomiopus septimus* Huang, 2001, without mentioning that the two mites are the same. The fourth author (T. Stasny) uncovered the problem and Huang (pers. comm., 2002) commented that *R. championi* will now be *Diptilomiopus septimus*. As stated by article 23.1 of the International Code for Zoological Nomenclature (ICZN, 1999) the valid name of a taxon is the oldest available name applied to it, unless that name has been invalidated or another name is given precedence by any provision of the Code or by any ruling of the Commission. *Rhynacus championi* is thus the valid name, and *championi* is hereby transferred to *Diptilomiopus* based on the redescription as given for *D. septimus*.

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## APPENDIX L

Published article. De Lillo, E.; Craemer, C.; Amrine, J.W. Jr. & Nuzzaci, G. 2010.  
Recommended procedures and techniques for morphological studies of Eriophyoidea (Acari:  
Prostigmata). *Experimental and Applied Acarology* 51: 283–307. DOI 10.1007/s10493-009-  
9311-x

## Recommended procedures and techniques for morphological studies of Eriophyoidea (Acari: Prostigmata)

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Received: 13 April 2009 / Accepted: 1 September 2009 / Published online: 22 September 2009  
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**Abstract** Methods used for sample storage, specimen clearing, slide mounting, species illustration and morphometric description in alpha-taxonomic studies are essential for the Eriophyoidea. Eriophyoid mites are very tiny and delicate, for which truly permanent specimen slides currently cannot be prepared, resulting in eventual loss of material, including type specimens. Often, published descriptions and drawings have not achieved the required level of quality, and thus many relevant taxonomic details have been permanently lost or neglected. These shortcomings can make certain identifications impossible and cause significant confusion. Consequently, there is a considerable need for accurate and uniform descriptive and illustrative data for the Eriophyoidea. Based on their expertise on this topic, the authors provide guidelines and advices, assisted also by illustrations, of the main critical aspects in managing eriophyoid mites in order to supplement and improve techniques for handling and preparation of specimens, and for improving their taxonomic study. The effects of the short- and long-term preservation methods (i.e., fresh, dried and liquid preservative choices) on digesting the internal tissues of the mites are discussed. Clearing and mounting procedures are analyzed, and special tips are suggested for handling mites and designing tools needed during these steps. Methods for recovering specimens from unsuitable slides (i.e., undercleared and overcleared specimens) are proposed and described. Techniques and tricks to produce descriptive line drawings of good

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quality are highlighted, and the content to include in plates is stressed. Finally, detailed instructions for standardization of measurements are given.

**Keywords** Eriophyoidea · Storage · Clearing · Mounting · Illustrations · Descriptions

## Introduction

As for other mites, eriophyoid systematics depends on the quality of studied specimens and morphological description. Conversely, the microscopic size and ultra fine structural details of these tiny and fragile mites make their morphological study more difficult. Furthermore, the accuracy and correctness of descriptions and associated drawings depend on the methods used in processing, mounting and studying the mites.

Several comprehensive accounts (e.g., Nalepa 1906; Hassan 1928; Keifer 1952, 1975; Amrine and Manson 1996) are available on methods for sample preservation and storage, specimen clearing and mounting, drawing, descriptive arrangements and other activities related to taxonomic/systematic investigations/publications. Hassan (1928) extensively described preservation and mounting methods, eventually mounting mites in Canada balsam (miscible with 100% xylene) or euparal (miscible with 95% ethanol). Hassan's material has not been found by the authors, so it is unknown how long such specimens can remain in useable conditions. Amrine mounted mites, including eriophyoids, in both Euparal<sup>®</sup> and balsam (Permount<sup>®</sup>) media. In all cases, the mites were slowly dehydrated through a graded series of ethanol solutions (passing at 5% ethanol intervals usually from 60 to 100%) for Euparal<sup>®</sup>, or they were transferred from 100% ethanol to xylene for Permount<sup>®</sup>. Despite careful processing, mounted specimens were always badly crumpled and deformed and no useful descriptions could be made.

Keifer experimented with several methods and chemicals for clearing and mounting, and set an excellent standard for making illustrations and taxonomic descriptions of eriophyoids during his career ranging from 1938 to 1991 (see Baker et al. 1996, for a compendium of Keifer's descriptions of species from the USA, including drawings not published before). However, even in 1975, he commented about the general lack of standards for describing eriophyoids. His particular style and high standards for accuracy is conveyed in his reply (Fig. 1) to Nuzzaci who, novice eriophyoidologist, had sent him microscope slides, a draft description and line drawings of a new eriophyoid mite for his assessment and advice. Since Keifer's publications, the major contributions to the interpretation of the external morphology of eriophyoid mites were by Lindquist (1996). Besides, Amrine and Manson (1996) gave a further contribution on the preparation, mounting and descriptive methods of study of these mites; their main intent was to strongly urge "authors to achieve greater uniformity in presenting descriptive, illustrative and biological data" and this seems to have been partially accomplished in the past decade.

However, today many descriptions and drawings still often do not achieve the required standard and quality, even as set by Keifer, and many relevant taxonomic details may be permanently lost or obscured as a result. These shortcomings can lead to incorrect classification, sometimes making certain identifications impossible, or misinterpretation (for example, the prodorsal shield, scapular setae *sc* and coxal setae *1b* and *1a* of *Ashieldophyes* were not clearly described in Mohanasundaram 1984) which can cause considerable confusion. These inadequacies cannot be justified considering the quality of the

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However, I take most direct exception with the correctness and adequacy of your drawings. Admittedly all drawings of these mites are but diagrams, but one must ask - how clearly do the delineations show the precise features of the species in question? Are the diagrams bare, or do they convey specificity? Drawings of these mites are in no sense art. But they must be done with precise recognition of line direction, of relative size of the parts, and they must also include angles and points, when they are present. To do a good job on such depictions takes practice and study.

You have done the usual thing in regard to drawings - produced rather bare diagrams. We have too many of such being continually published. If I can get you to rise above bare delineations or diagrams, and show specific features of these mites I will have accomplished something worthwhile.

Dr. Nuzzaci, what I'm trying to do is get you started correctly. All of this takes practice and study. Actually the study of eriophyids is a full time job, and if you dilute your time with publications on other mites your efforts on eriophyids will more or less suffer.

Sincerely



H. H. Keifer

**Fig. 1** Letter by Keifer commenting on slides, description and descriptive drawings of a new species by Nuzzaci

microscopes and cameras available today. Moreover, a method universally accepted and used for preparing and mounting mites is not available and those methods commonly applied fail to give permanent slides.

Appropriately, Lindquist (2001) emphasized the importance of optimizing the quality of description of mites, including Eriophyoidea. Therefore, standardized descriptions are always imperative and must be continuously promoted, especially in view of the current high rate of description of new eriophyoid genera and species (de Lillo and Skoracka 2009), and also because the best slide mounted specimens rarely last very long and frequently become opaque or precipitated, or too transparent for study (Amrine and Manson 1996). Keifer's slide collection at the US National Museum of Natural History, in Beltsville, Maryland, USA, is a sad example of these shortcomings (Fig. 2). In addition, it should be emphasized that proper interpretation of morphological details certainly support systematic studies, but they are also required for many non-systematic investigations including plant-mite relationships and pest control, identification for quarantine purposes, vectored pathogens, and biological control of weeds.

The present paper provides guidelines and recommendations for techniques that researchers should employ when preparing, studying and describing eriophyoids that supplement techniques previously presented in other articles (e.g., Keifer 1975; Amrine and Manson 1996).

## Preservation

### Temporary preservation

When working with fresh, mite-infested plant samples, the researcher needs to prevent damage caused by desiccation or fungal degradation.



**Fig. 2** Slides in the original eriophyoid collection of Keifer at the US National Museum of Natural History, Beltsville, Maryland, USA. Many slides cannot be used any more because they appear to be completely dark or the mounting medium is dried out

Fresh samples should be brought to the laboratory as soon as possible. They should not be exposed to heat and, therefore, they should be contained in plastic bags and stored in a cooler. Similarly, fresh plant material shipped by courier should be kept cold in a thermally insulated package with a frozen fluid pack or dry ice pack during extended shipment. Mites on plant samples not properly packed and shipped in luggage usually do not survive high altitude aircraft flights or ground transport because of excessive low or high temperatures during transport. Live mites can be extracted from plant samples using a washing solution (Monfreda et al. 2007) and can be stored in water containing a few drops of a commercial surfactant (household detergent or polysorbate as Tween®). Eriophyoids preserved in this manner were able to survive the shipment and stayed alive for 3–4 weeks in a refrigerator at about 4°C. Finally, live mites were successfully collected directly in the field by washing bunches of grapes without cutting them from the plant (de Lillo et al. 2005).

When fresh plant samples with live mites are returned to the laboratory, the mites must be processed within a short time after field collecting. In order to keep the material fresh, the sample can be wrapped in damp (not wet) paper towel, or other paper-like material, then sealed in a plastic bag, preventing it from drying out, and stored in a cold place (e.g., a refrigerator, climate controlled room or cabinet) without freezing the material. Fine holes can be punched in the plastic bag to reduce humidity if necessary (S. Ozman-Sullivan, pers. comm.). Depending on the type of sample and its quality, live mites can still be collected from the plant samples even after a month's, or as in the case of filbert big bud mites (*Phytoptus avellanae* Nalepa), 2 months' storage (S. Ozman-Sullivan, pers. comm.).

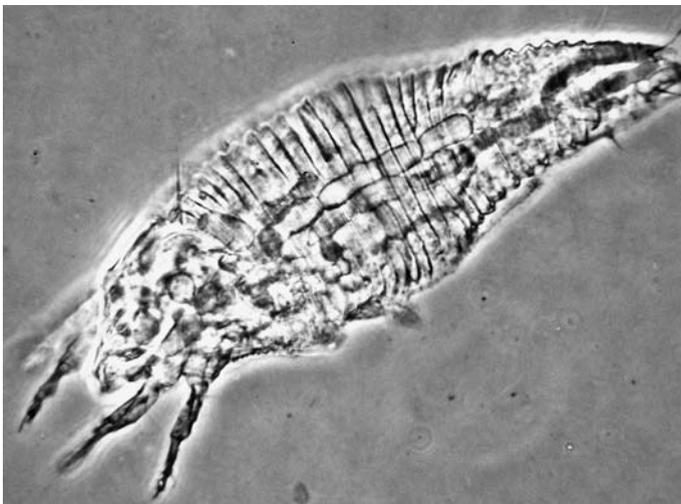
#### *Permanent preservation as dried samples*

In addition to slide mounted specimens, there is a requirement for additional long term preservation of mite-infested plant material or of mites themselves. Permanent preservation

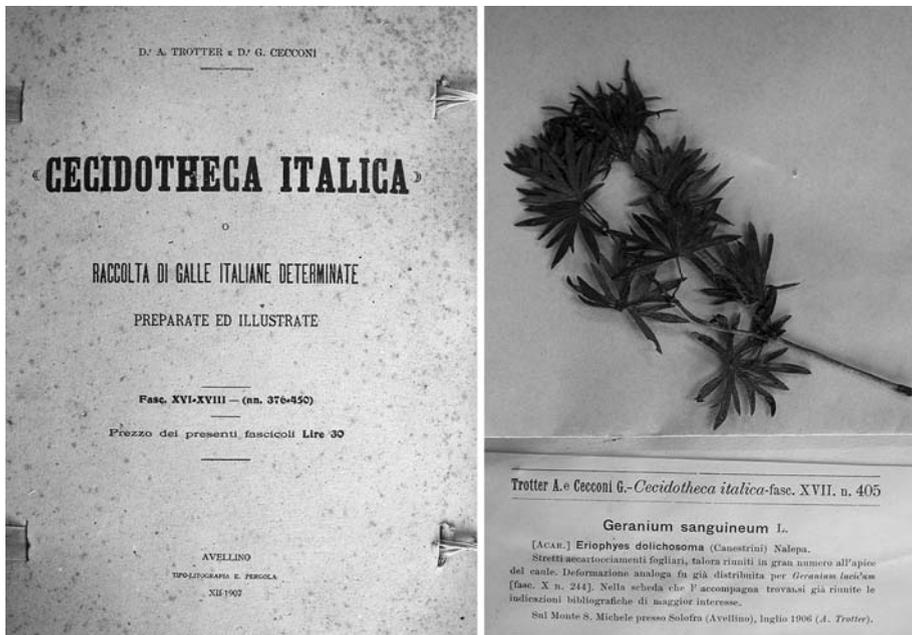
may be necessary because mite specimens cannot always be processed and slide mounted before being stored or accessed in a collection (also see Keifer 1975), and additional material can be used when slide mounted specimens deteriorate or become totally destroyed, or are lost.

For practical convenience, mainly related to sample transportation, handling and storage, mite samples are often permanently preserved and managed as dried (mummified) specimens on leaves and other plant organs. Particular care must be taken on how the plant sample is dried out. In case of improper desiccation, the mite body may be destroyed or nearly completely invaded by fungi (Fig. 3). As a consequence, morphological details can be obscured, making mite identification frequently difficult or impossible. Therefore, plant samples should be dried out as soon as possible after field collecting and prepared as herbarium specimens for sending to specialists for mite identification or for deposition in a dry specimen collection (i.e., a zoo-ecidotheca). Dehydration of plant material should be carried out applying all possible techniques to prevent fungal infection of the mites (e.g., use of desiccating papers, frequent paper change, sample pressing between absorbent pads, slight warming in sunlight or in an oven). The properly dried samples should afterwards be enclosed in an envelope (letter envelope, transparent paper envelope, transparent plastic specimen bag, etc.) and labeled with all relevant data. A repellent or a deterrent compound (PDB, thymol, etc.) or other protective methods should be applied for preventing museum beetle attacks and deterioration over time. Trotter and Cecconi, authors of the *Cecidotheca Italica* (Fig. 4), were familiar with this method (Trotter 1904) and their dried specimens are still well suited for the identification of species after about one century, as demonstrated by Boczek and Nuzzaci (1988), and Petanović et al. (1993).

Well dried and properly preserved specimens, similarly to freshly collected mites, need usually only a few minutes in a mounting or clearing medium on a hot plate in order to become perfectly cleared and to return to their original shape and size.



**Fig. 3** A *Metaculus* specimen completely invaded by fungi obscuring its morphological details



**Fig. 4** *Cecidotheca Italica*: front page of a publication by Trotter and Cecconi (1902) (on the left); original sample of *Geranium sanguineum* L. from which specimens of *Aceria dolichosoma* (Canestrini) were slide mounted and re-described by Petanović et al. (1993) (on the right)

#### *Permanent preservation in liquid preservatives*

In contrast with the general good and relatively quick results of clearing fresh and dried specimens, the digestion of non-cuticular structures of specimens preserved and fixed for years in alcoholic solutions (60–70% ethanol in water) requires much more time. This difficulty increases proportionally with length of preservation, and the results are often poor or insufficient for an exhaustive and reliable identification and description (Keifer 1975). Moreover, the alcoholic solutions tend to evaporate, and specimens usually become completely dried out (Fig. 5). Amrine needed to prepare a few specimens of the genus *Phytoptochetus* from a sample preserved in a vial, originally containing ethanol, for about 70 years and belonging to Nalepa's collections (Amrine and Manson 1996; Amrine et al. 2003). A few specimens were found after careful examination of a yellow powder; clearing took a period of 2 months and needed particularly careful and extensive processing. Specimens were heated at about 90°C in a few drops of Keifer's booster (Amrine and Manson 1996) for a few days. Then they were washed in water and transferred to a few drops of lactic acid, heated for a few days, washed in water once more, and hereafter transferred back to Keifer's booster again. This entire procedure was repeated many times. Eventually a collection of suitable mite fragments were found to correctly illustrate the shield, the coxi-genital region, the legs and the opisthosoma to make adequate drawings to define the essential characteristics of *Phytoptochetus*. On the contrary, notwithstanding similar attempts, the re-description of *Aceria sonchi* (Nalepa) from original powder remnants of dried ethanol preserved material was not possible (D. Knihinicki, pers. comm.).



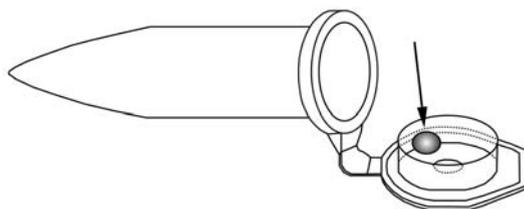
**Fig. 5** Original vials from Nalepa's collection containing ethanol preserved *Galium cruciata* (L.) Scop. and associated mites

Usually, the addition of glycerol to the preservatives (as in AGA and Oudemans's solution) makes the tissues softer and less rigidly fixed, allowing them to be more susceptible to the clearing agents. Glycerol also prevents the specimens from completely drying out, as usually happens when other solvents evaporate over time.

In addition, Keifer (1975) found that a mixture of thin sorbitol syrup in a 25% solution of isopropyl alcohol kept the eriophyoids well preserved and suitable for slide preparation. Craemer commonly uses this fluid, composed by 25% solution of propan-2-ol in water to D-sorbitol powder (e.g., add about 4 ml propan-2-ol diluted with 12 ml water to 30 g D-sorbitol powder) until forming a thin syrup with the consistency of heated honey, at most. When the liquid is added to the powder, the mixture is milky white and after a few hours it dissolves properly, becoming clear and slightly thick. At warm and humid environmental conditions, a very small amount of potassium iodide and an iodine crystal should be added to the mixture to prevent mould growth. The mixture should be kept in a sealed and well closed container, because it quickly becomes too thick and crystallizes when exposed to air. Mites are very easily transferred to and from a small amount of this "sorbitol fluid". S. Nesar (pers. comm.) uses a novel way to collect and transport mites in this fluid also facilitating easy recovery. A small droplet of this solution is placed inside the lid of a polypropylene micro centrifuge tube (Fig. 6). About 100 specimens can easily be collected in this droplet, and when the vessel is closed, it can be safely transported and mailed. The droplet becomes very sticky, dries out over time and it can be re-hydrated by breathing over it. Otherwise, the entire droplet, even when crystallized, can be added to the clearing medium and processed as normal. The suitability of the mites to be slide mounted over extended periods of preservation in sorbitol has not been tested yet, but the mites are well suited if mounted within a few months.

Alternatively and for populations of a few dozen to a few hundred specimens, eriophyoids can also be kept on work slides (Amrine and Manson 1996). Unfortunately, high

**Fig. 6** A small droplet of “sorbitol fluid” into which eriophyoid specimens can be collected (*arrow*), positioned in the side corner of the lid of a 1.5 ml micro centrifuge tube



environmental humidity and temperature can reduce the quality of the specimen preservation on these work slides.

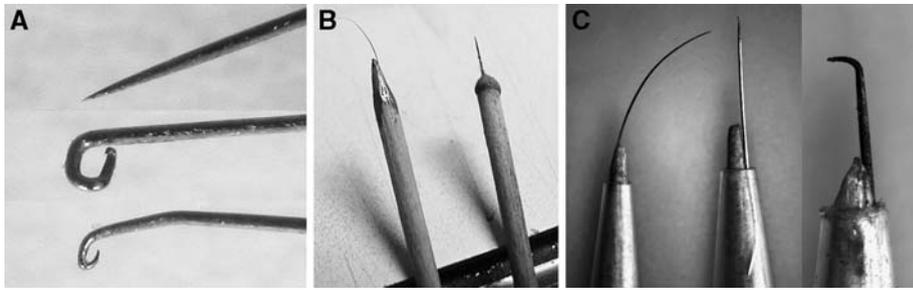
Finally, specimens can be preserved in ATL buffer (a buffer containing edetic acid and sodium dodecyl sulphate) stored in a refrigerator some time before DNA isolation according to Dabert et al. (2008). After the DNA extractions, the mite exoskeleton can be mounted and its morphology can be efficiently studied (Skoracka and Dabert 2009).

### Handling eriophyoids and tools

Mites are usually found on plant samples with the aid of a stereo dissecting microscope, and can be picked up using pin-like or other tools, even if the plant material is deformed. The moistening of the tip of the tool with water or other media can enhance the ease with which the mites are picked up. When mites are rare on the plant sample or the plant organs are severely modified and architecturally intricate, especially when dried, finding and collecting eriophyoids can become time-consuming and inefficient. Then, collecting can be greatly improved by concentrating the mites (Monfreda et al. 2009). In the case of dried material, mites can be easily recovered as described by Amrine and Manson (1996), or by soaking part of the sample overnight in a water solution with a few drops of a surfactant and bleach at room temperature (Monfreda et al. 2009). Hereafter the suspension is stirred and sieved: the specimens can be more easily detected, because of their restored shape, and picked up from a filter paper or from a filtered sediment (through a 20–25  $\mu\text{m}$  sieve) poured into a Petri dish using water plus a small amount of a surfactant (Monfreda et al. 2007).

Commercially available laboratory needles are usually too thick and robust to be used in picking up, transferring and generally handling eriophyoid mites. Several types of apparatus can be specifically made for this purpose, and each laboratory usually has its own design. Some of these tools are mentioned in the materials and methods of many articles concerning Eriophyoidea. They include an eyelash, or several kinds of fine needles or pins, attached to or stuck into some sort of pen-like rod or wooden dowel in different ways (Fig. 7; Keifer 1975; Amrine and Manson 1996).

Insect mounting pins are suitable for constructing an eriophyoid handling tool. These come in different sizes and materials and stainless steel is recommended. Keifer (1975) proposed a size 00 insect pin for “needling” mites from solution to solution and slide to slide. A pair of size 3 insect pins in wooden dowels is useful for dissecting galls and unrolling leaf margins. These needles can be sharpened as needed on Arkansas soap-stones or other fine grindstones. They are commonly used for mounting delicate insect specimens such as microlepidoptera and small flies. Stainless steel micro-pins, known as Austerlitz<sup>®</sup> minutens or minuten pins, headless, 0.1 mm in diameter and about 12 mm long, with one sharpened end, can be particularly recommended. They do not chemically react with the preserving, clearing, and mounting media, and they can be manipulated to suit a



**Fig. 7** Handling tools for eriophyoid mites: **A** details of variously shaped micropins; **B** details of an eyelash held in place with nail polish (on the *left*) and short minuten pin held in place with epoxy (on the *right*); **C** eyebrow hair (on the *left*), micropin (on the *center*) and bent pin (on the *right*) inserted into the narrow end of a micropipette and held in place by inserting a toothpick from the other end

researcher's needs, and their physical properties allow them to be dipped into reagents without being altered. These needle probes should be personally prepared by each researcher for making specimen handling comfortable and convenient. They can be mounted on wood or plastic handles. In particular, exhausted fine- or medium-tip markers can be re-cycled, and the blunt end of the micro-pin can be inserted into their felt-tip and fixed to it by a drop of a cyanoacrylic glue which hardens the felt. If preferred or needed, the sharp end of the pin can be curved or bent into a loop for producing a sort of spoon (Fig. 7A), using tweezers or micropliers under a dissecting microscope. Pointed and looped pins are suitable for transferring individuals without injuring or damaging them.

Disposable plastic micropipette tips (1 ml or c. 60 mm long  $\times$  8 mm diameter) can also be used for making a variety of handling tools (S. Nesor, pers. comm.). A firm, pointed short hair (e.g., from an eyebrow), or micro-pins as above can be inserted into the narrow end and held in place by inserting a toothpick, or other probes of appropriate length from the other end (Fig. 7C). Alternatively root canal files (size 30, c. 0.3 mm in diameter, or thinner) as discarded by dentists, or available from dentist tool suppliers, may be inserted into holders as above.

Comprehensive information on equipment (hot plates, coverslips, plain and cavity slides, tweezers, etc.) and other useful facilities and supplies can be found in Keifer (1975), and Amrine and Manson (1996).

## Clearing

An historical review of this aspect is in Keifer (1975), and in Amrine and Manson (1996) in which they underlined the difficulties in preparing adequately cleared specimens on slides.

Currently, many researchers have developed and improved a preferred medium on the basis of the personal experience and convenience, sometimes changing method over time. Eriophyoidologists have been applying the following media with satisfactory results: Heinze's medium (A. Skoracka, S.-G. Wei, pers. comm.), F-medium with Booster medium plus phenol according to Keifer (1975) (C. Craemer, P. Natchev, S. Ozman-Sullivan, pers. comm.), lactic acid (M. Lewandowski, P. Natcheff, R. Petanovic, C.-Q. Wang, pers. comm.), modified Berlese's medium (J.W. Amrine Jr., A. Chandrapatya, pers. comm.),

modified Keifer's Booster solution including water saturated phenol (E. de Lillo, E. Denizhan, R. Monfreda, G. Nuzzaci, pers. comm.), Nesbitt's medium (C.-Q. Wang, pers. comm.), and a stained mixture of Nesbitt's medium with lactophenol (Faraji and Bakker 2008). The applied clearing procedure should always be reported in publications.

A modified Berlese's medium was described by Amrine and Manson (1996). To about 15 ml of freshly made medium, 10–20 drops of glacial acetic acid, ca 100 mg of metallic iodine crystals and 100 mg of potassium iodide powder are added. This medium is placed on a hot plate at 90°C for about 30 min to dissolve the metallic iodine (or left from overnight to 48 h at room temperature). Iodine, included also in Keifer's medium, stains the fine sculptured details of the cuticle, the microtubercles and other cuticular structures, especially the internal apodemes and genitalia (Keifer 1975). Consequently, the brown color often enhances the contrast of fine or delicate features.

A further improvement of the image quality can be offered by digital cameras on microscopes. They can be adjusted to correct the 'white balance' or intensity and contrast adjustments to obtain excellent micrographs, even though the specimen may appear too dark or too pale at first glance.

When the medium used for digesting the mite internal tissue serves also for mounting (e.g., Heinze's medium), most eriophyoids can be placed directly into a small, shallow drop of medium on the slide. Live mites will right themselves and orient in proper position as they attempt to crawl in the thin film to leave the medium; this will not happen if they are immersed or are previously killed. Their orientation can be adjusted by stroking with the sharp tip of a micropin; the opisthosoma can be stroked several times to "right" a lateral mite into dorso-ventral orientation. In some cases, the uncovered slide with adjusted mites can be put on the hot plate margin for a few moments to thicken the medium and hold specimens in proper position. A small drop of the final medium can be added to a clean coverslip. A drop of glacial acetic acid can be added to this drop (e.g., modified Berlese's medium) which is stirred on the coverslip. Then, the coverslip is placed over the uncovered mounted specimen, using the tips of the forceps to guide specimen orientation and position as the coverslip settles. This step allows more rapid spread of the medium (eliminating air bubbles), keeps the orientation of the mites, and aids more rapid and complete clearing of the mites. The fresh slide is then placed on the edge of a hot plate at about 80–90°C to clear within about 30 min. Most live mites can be prepared in this way to make excellent slides in about 1 h. The boiling of the slide must be avoided because it moves the mites from the center to the margin of the coverslip where they cannot be studied.

In case the medium used for clearing is different from that used for mounting, (e.g., mounting in F-medium, after clearing with Keifer's booster medium with added phenol) cavity slides can be used for the clearing process. Mites can be placed directly into a drop of clearing medium and the slide can be heated until the mites are cleared. The mixture must not boil or become too viscous or hard. When the mite body is sufficiently cleared, drops of water or fresh medium can be added to the mixture to make it fluid enough for further passages. Then, mites are transferred to the mounting medium by means of micropins and a coverslip is added.

A few modifications to clearing procedures are used by eriophyoidologists: live mites are cleared, or alternatively are killed in a preserving solution before clearing; mites are cleared at room temperature taking a long time (days or weeks); mites in the clearing medium are heated carefully over an open alcohol flame, or they are kept in an oven or on a hot plate set at 40–60°C.

Overheating and overclearing can easily occur and are the major sources of error. If the covered slide is placed on a spot of the hot plate that is too hot for too long (about 1 h or more), numerous small air bubbles can develop and cannot be removed. These can often obliterate fine details needed for descriptions or photographs. Using new infrared thermometers, the researcher can carefully map the temperatures on a hot plate and know exactly where to place the slide(s) for best results.

For species difficult to clear using this technique, slides can be left in a cooler portion of the hot plate at about 70°C overnight or for 24–48 h. If your hot plate is too hot at the lowest setting, a thick thermal glass can be placed on the plate and will drop the surface temperatures for several degrees. Alternatively, the temperature of the hot plate can also be regulated by means of a rheostat between the receptacle and the hot plate (be sure to match wattage of the rheostat to that of the hot plate).

These methods are tedious, but they allow a researcher to routinely make excellent slides from which type specimens can be selected. This part of specimen preparation represents 90% of quality control for professional preparation of eriophyoid specimens, and a successful method needs to be learnt well. Amrine and de Lillo have prepared slides using the above methods as early as 1987, and these slides are still well suited for microscopic studies.

## Mounting

For the final mounting, eriophyoidologists have also been applying different water based mixtures such as Heinze's medium (R. Petanovic, A. Skoracka, C.-Q. Wang, S.-G. Wei, pers. comm.), F-medium according to Keifer (1975) (C. Craemer, E. de Lillo, E. Denizhan, R. Monfreda, P. Natchev, G. Nuzzaci, R. Petanovic, S. Ozman-Sullivan, pers. comm.), modified Berlese's medium (J. W. Amrine Jr., A. Chandrapatya, M. Lewandowski, A. Skoracka, pers. comm.), and a stained Hoyer's medium (Faraji and Bakker 2008). The applied mounting procedure should always be reported in publications.

Most researchers add more or less mounting medium to alter the amount of pressure of the coverslip on the specimens. A few short fibers can be also mounted underneath at the borders of the coverslip to support it above the mite specimens, according to Keifer (1975). The diameter of the fibers can be chosen according to the thickness and width of the specimens to be mounted. Following Keifer's advice, de Lillo and Nuzzaci have selected three different fibers with different thicknesses cut into short lengths: fiberglass (about 10 µm in diameter), kapok (about 20 µm in diameter) and "wool" used for aquariums (about 40 µm in diameter). The main aim in using these fibers is to avoid excessive specimen flattening (Keifer 1975) which can alter feature proportions and the general shape of the mite. For example, ridges and furrows on the dorsal side of the opisthosoma, especially when they are slight, can be often "lost" during examination of flattened specimens. Moreover, the addition of fibers allows the coverslip to be moved more easily on the slide face by pushing one side of the coverslip with a pin or tweezers; of course, the coverslip must not be sealed and the medium must be fluid. In this way the mite can be rolled around its longitudinal axis, allowing observations and descriptions of a species from a single holotype specimen (Amrine and Manson 1996). However, even when fibers have not been added under the coverslip, if the mounting medium is still fluid and the slide has not been sealed yet, the coverslip can be moved changing the mite position, as reported by Nalepa (1906).

It is more difficult, and sometimes impossible, however, to study (draw and photograph) fine details (e.g., prodorsal shield features, female internal genitalia and the ray arrangement of particularly fine empodia presenting intricate details) on specimens that have not been flattened to some extent. It is probably most advisable to mount some specimens without and others with fibers. This will not always be feasible, depending on the specimens and time available. If important, and of consequence, it should also be mentioned in the description which method was used to obtain the particular morphological aspects described, because these can differ considerably, as in the example of the dorsal shield patterns in *Aceria angustifoliae* (Denizhan et al. 2008). It may even be arguable which method will render the best results for identification and comparative purposes. Both methods are difficult to standardize, and likely will vary depending on the mounting medium and amount used, the degree of clearing, and the skill of the worker.

Information on labeling and sealing methods is in Amrine and Manson (1996).

### Overcleared slides

In the case of overcleared specimens, slide sealing can be carefully removed by a large pin, scalpel, razor or by other means. Then the coverslip can be loosened using water and heat on a hotplate, and excess water removed. A drop of medium containing iodine stain (preferably using the same medium originally used to prepare the slide) can be placed to one side of the coverslip. An absorbent paper can be held against the other side of the coverslip while the slide is heated to boiling very briefly. In this way, the iodine stained medium is drawn over the mites and fills the coverslip, while the excess medium is removed by blotting. As result, the freshly stained mites can be observed, drawn and photographed. This may not work with very old slides, difficult media such as polyvinyl alcohol, or overly faded specimens; but if the slide is not usable, this technique is worth a try.

### Dismounting specimens from slides

Slides can be dismounted when specimens are undercleared or overcleared, or are at the coverslip edges, or when the researcher might need to view a different aspect of a mite, or would apply a different microscopic technique, as Nuzzaci and de Lillo did for the study by scanning electron microscopy of *Aceria caulobia* (Nalepa) and *P. avellanae* (Nuzzaci et al. 1991).

After removing the slide sealant, the water-based media can be made less viscous by addition of water and/or heating, such as in the procedures previously described, or by leaving the slide under a high humidity glass dome for a few hours at room temperature. These media are hygroscopic, absorbing water from the air at the coverslip margin and eventually becoming quite fluid, allowing the coverslip to slide freely, so that it can be propped up by insertion of a small pin from one side so that it can be lifted and removed with a forceps.

### Line drawings

Adequately descriptive line drawings of good quality are not easy to produce and appear to be one of the weak points in eriophyoid systematics. The importance of this part of a

description was highlighted by Keifer (1975) and Amrine and Manson (1996), but even stronger emphasis should be made.

Slide mounted eriophyoid specimens, including type material, usually deteriorate over time, and are eventually not adequate for study, and may ultimately be totally destroyed. This deterioration can be caused by several factors, including: the water-based mounting media which may dry out rather quickly, air may penetrate under the coverslip, and, under certain conditions, specimens may continue to be cleared by the chemicals in the mounting medium, and fade away. Other representations of the original type series, including digital images of slide mounted specimens and electron microscope images, should also be archived in addition to slide mounted specimens and they have a support value, as previously mentioned by Amrine and Manson (1996). In the absence of type material, however, the original description and, particularly the drawings, become critically important, usually being the only representation of the described species. In many cases, drawings presented in the original description become the nearest equivalent of a holotype.

Moreover, drawings are rather clearly understood by everyone, and they are useful for a primary comparison whatever language is utilized, and regardless of the interpretation of characters in the text description. Certainly, line drawings in the style of Keifer (Fig. 8) are permanent and universally understandable. They are the core of each description and must be the best basic representation of an eriophyoid species (Keifer 1975; Amrine and Manson 1996).

The adequacy and value of drawing depends largely on the skill and experience of the researcher and on the quality of the slide mounted specimens.

Edward Baker and Richard Newkirk (pers. comm.) observed Keifer working and both related that the researcher made his final drawings directly using a drawing tube (=camera lucida) device with extraordinary care and accuracy. In some cases, Keifer drew freehand from the eyepiece using no drawing aids. But Keifer's talent was certainly unique!

Because of the minute size of these mites and their features, when studying, drawing and describing them, the researcher should always use a good-quality, phase contrast light microscope equipped with an oil immersion 100× objective at a large numerical aperture (one of the best is a fluorite objective with 1.30 numerical aperture or higher), and a drawing tube. A zoom lens on the microscope or drawing tube is very helpful. A 2× objective can additionally be mounted on some drawing tubes to allow the enlargement of very small details to prevent their obstruction by the pencil's point. In addition Amrine employs a chemical apparatus clamp to hold a reversed binocular at the appropriate position and angle to form a small field visible through the drawing tube (Fig. 9). Very detailed structures such as of the empodia (Fig. 9), male genitalia, female genital apodemes, etc. can be drawn using this method.

The illustrator should try to prepare an image eventually on a lower plane than that of the microscope-base level: in this way the illustrator can sketch a larger preliminary draft, and the much larger drawing scale usually helps in reducing the visibility of mistakes and irregular lines when reduced for the final plate. The drawing plane should be illuminated by a table lamp adjustable for light direction and intensity in order to clearly and concurrently see the pencil marks superimposed below the cuticular structures of the mite, both with good contrast. It is advisable for the aspiring illustrator to be taught by an experienced researcher using these techniques if at all possible.

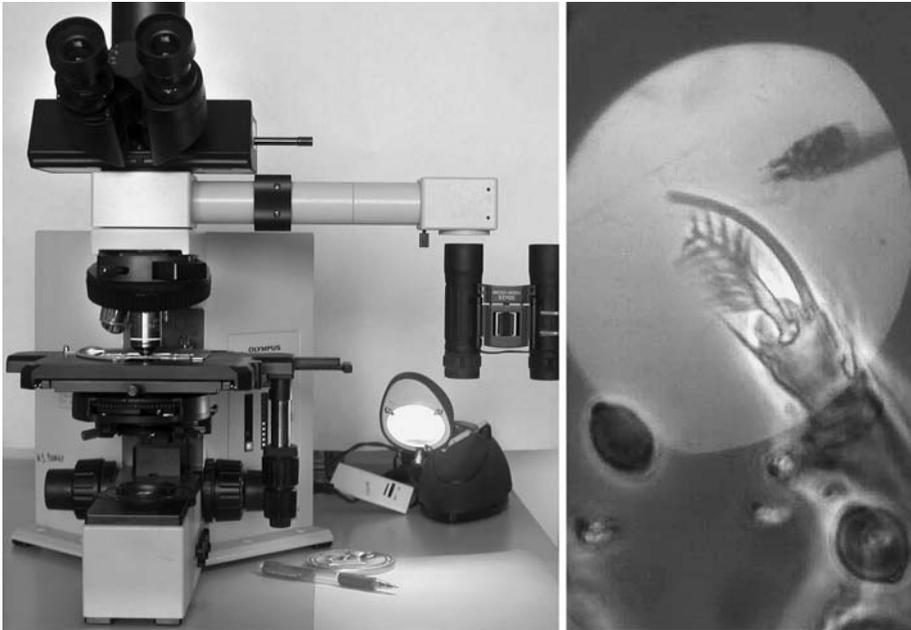
The microscope stage and the slide mounted specimen should be oriented on the horizontal plane in such a way as to make the outlines of the specimen well suited to the illustrator's drawing technique (usually, lines on a paper are more easily drawn when the



**Fig. 8** *Diptacus swensoni* Keifer: original inked line drawings by Keifer at the US National Museum of Natural History, Beltsville, Maryland, USA

hand moves from left below to right above for right-handed people). An initial focus plane on the slide is chosen, and the illustrator draws the visible cuticular details in that plane on the paper. The illustrator can then focus on a higher or lower plane and add more details to the original drawing. Progressively, plane by plane, the illustrator can portray all the morphological features critical to the identity of the mite.

During drawing, one can cover the tube opening by one's hand to reduce the external light noise on the slide and to increase the contrast of specimen details. One can additionally cover the field diaphragm by the hand to obscure the mite and see just the sketch through the tube. The repeated, fast alternate movement of the hands facilitates detection of the presence or absence of some details on the line drawing by alternately flashing the two superimposed images.



**Fig. 9** Apparatus for drawing fine details: inverted binocular placed under the drawing tube opening (on the left) to decrease the size of the pencil or pen relative to small structures, such as empodia (on the right)

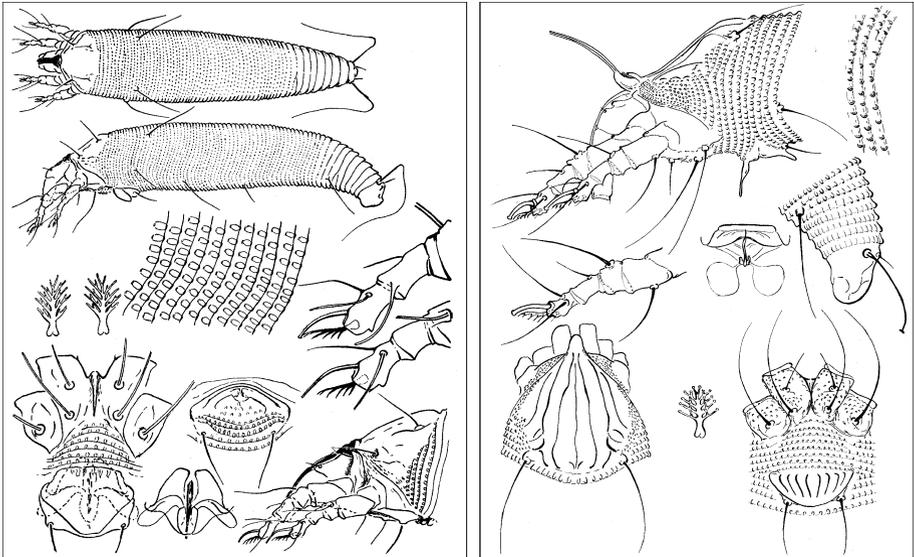
Descriptive drawings are usually semischematic, but they should be sufficiently detailed to portray the real morphology of the holotype specimen as closely as possible, and care should be taken not to “correct”, “exaggerate” or interpret the features too much, so that some information is altered (see Keifer’s recommendations in Fig. 1). The drawings should show the typical deviation from bilateral symmetry, and drawing one half, then copying the flip side of it to the other side becomes a fabrication, not science. Very often one specimen is not enough to get satisfactory information and additional specimens must be drawn, or even their features can be combined within one plate. This depends on the clearness, orientation and integrity of the specimen, contrast of the cuticle with the mounting media, and, of course, on the ability of the illustrator. This procedure also allows confirmation of some details.

In addition to the holotype specimen, other specimens should be studied to determine intraspecific variation in morphological features, at least in this one sample. Systematically important variations may be depicted in additional drawings if necessary.

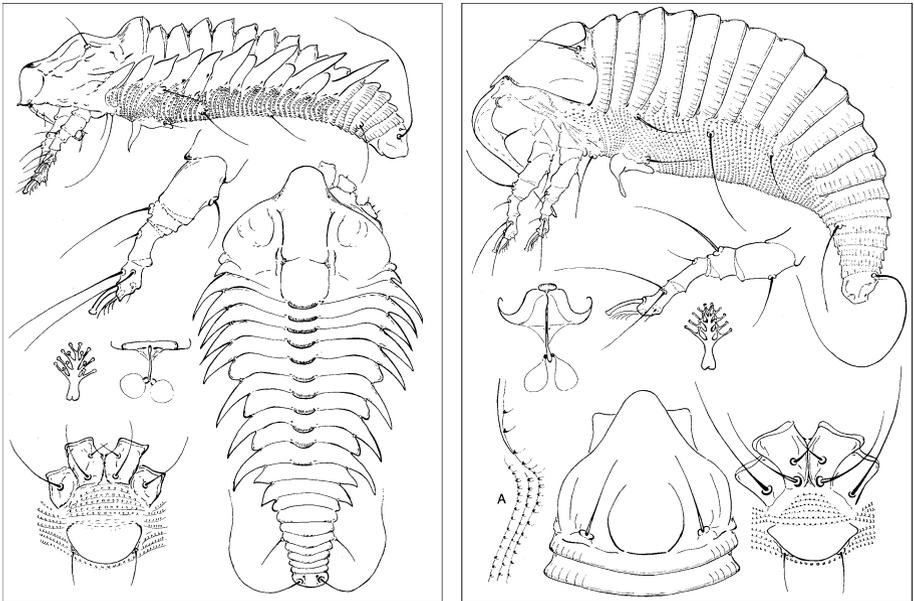
### **Content that should be included in eriophyoid descriptive drawings**

Often the content of eriophyoid plates can differ slightly depending on whether the depicted mite belongs to the Phytoptidae, Eriophyinae (Fig. 10), Phyllocoptinae or Dip-tilomiopidae (Fig. 11).

Amrine and Manson (1996) listed the most important body parts that should be illustrated by line drawings. Attempting to standardize the figure layout will make it



**Fig. 10** Line drawings of a phytoptid (*Phytoptus corniseminis* Keifer-redrawn; on the left) and an eriophyid mite (*Aceria ficus* [Cotte], drawing by E. de Lillo; on the right)



**Fig. 11** Line drawings of a phyllocoptid (*Tegonotus heptacanthus* [Nalepa]; on the left) and a diptilomiopid mite (*Rhynchaphytoptus ficifoliae* Keifer; on the right) (drawings by E. de Lillo)

easier to compare the depiction of different species with each other, and for finding particular details in a drawing. Additional information to enhance their list is provided below.

Scanning electron microscopy pictures can assist in the understanding and perception of all features included in a table, but should never be used to the exclusion of line drawings, light microscopy, or other illustrations.

### Prodorsal shield

The prodorsal shield must be depicted dorsally, including all ornamentations such as ridges, lines, granules, dots, cells, frontal lobe, anterior (*vi* and *ve*) and scapular (*sc*) setae and their tubercles, paying attention to the shape and size of these parts. The description of complex prodorsal shield ornamentation is almost always obscure if it is not accompanied by quality line drawings which are a fundamental requirement for a species' description, discrimination and identification although intraspecific variations may occur. Particular care should also be taken in depicting the frontal lobe margin and its shape. This lobe is frequently obscured by underlying gnathosomal and prodorsal elements, and may additionally be very thin and almost translucent, and its presence and margin may be very difficult to detect. It may help to study several specimens in this regard. Additionally, the illustrator must watch out for extremely tiny or obscure features like spines and extensions that may occur on the anterior edge of this lobe.

### Coxal area and genitalia

The coxal and genital regions have to be carefully drawn on the ventral view position of the specimen. When illustrating these structures, coxal ornamentation should be studied in detail (e.g., ornamentations on coxae I may differ from that on coxae II) and care should be taken to determine whether lines are internal apodemes (e.g., near the base of coxae II) or surface lines or ridges. The following information should also be depicted: presence, shape and position of coxal tubercles, coxal seta robustness and length, shape and length of the internal coxisternal apodeme (sternal line), number of coxi-genital semiannuli or other structures set between coxae II and female or male external genitalia, and number and shape of microtubercles on these semiannuli. Regarding the genital region, the following features should be depicted: female and male (if found) external genitalia, particularly including details of the female genital coverflap, whether the coverflap seems to be divided in more than one region (e.g., basal and distal region), the ornamentation on these regions, ornamentation just anterior of the coverflap and whether it is part of the area between coxae II. The length of setae *3a* should also be depicted accurately.

The genital apodemes, and softer parts like the spermathecae, are often difficult to observe and draw because of their size and their internal position and variability. Dissecting a clarified specimen at the level of the coxae or just posterior to the genital region is recommended, in order to have fewer disturbing elements (Keifer 1975). The length of the spermathecal tube and the size of the anterior part of the apodemes should be carefully observed, too.

### Legs

For legs in lateral view, the ornamentations (e.g., spines, ridges) along the segments and at the level of the articulations, size of solenidia and shape of its tip, presence, position, and length of all leg setae must be depicted.

Concerning the empodium, particular care is needed in distinguishing the central shaft shape (whether divided or simple), the number of rays, and eventually the shape of branch tips.

### Dorso-ventral view of the Diptilomiopidae

The large gnathosoma of the diptilomiopidae usually makes it difficult to have specimens oriented in true dorso-ventral position. In this case, the distal part of their large gnathosoma can be carefully removed or cut by a pin. Even though this is a tedious process, a bit of practice will allow the illustrator to get good results. A sharpened insect pin or fine dissecting scalpel should be used.

### Lateral view drawings

With regard to the anterior part of the body, the illustrator needs to represent the lateral view of the gnathosoma, legs, prodorsal shield with its frontal lobe and setae (when present), lateral seta, first annuli after the prodorsal shield, and genital area. When printed, the anterior part of the mite should preferably be oriented to the left of the drawing. Particular care must be paid to the gnathosomal details, including the presence and shape of the palp coxal seta (*ep*), dorsal palp genual seta (*d*) and subapical palp tarsal seta (*v*), shape and size of the prodorsal shield, and any other surface detail (e.g., ornamentation on the pedipalp segments).

The anal lobes and the annuli up to the setae *f* have to be drawn in lateral view taking care to depict microtubercles, setae and any other detail. The posterior part of the anal lobes should preferably be orientated to the right side of the drawing.

Moreover, a few annuli need to be drawn trying to elucidate the shape and the size of the microtubercles (elliptical, roundish, pointed, etc.), their distribution, orientation, size of dorsal and ventral semiannuli, and details of lateral lobes, if they are present. These should be portrayed in lateral view to show the possible contrast between dorsal and ventral opisthosomal arrangement. Usually, the area chosen to be depicted is between the annuli at the level of opisthosomal setae *c*<sub>2</sub> and *d*.

For species with large dorsal semiannuli (e.g., Phyllocoptinae and some Diptilomiopidae) it might be better to draw the whole specimen in dorsal and/or in lateral view; using particular care to point out ridges, furrows, and lateral lobes on the opisthosoma. If the illustrator draws the specimen in lateral view, the body should be horizontally placed in the plate and the anterior part should preferably be directed to the left. In case of the dorsal view, the body should be orientated in the vertical plane of the table and it should have the anterior part placed near the top of it.

### Preparation of the final plate

An initial draft with many notes and details is the result of the work at the drawing tube. The draft might be redrawn by pencil with special attention to the fine details. It should be compared at the microscope with mounted specimens without the light interference of the drawing tube in order to get more contrast and richness in details seen on the slide mounted specimen. Comparison of several specimens will allow you to note details of possible variation of key elements; this information can be presented in the description, or in some cases, a second drawing may be necessary.

After checking the initial draft, a smooth tracing paper can be put over the line drawing and final inking done on it, and not directly on the line drawing itself. For a better result, a large light table can be used for illuminating the draft and the final drawings to increase the line perception. Some researchers draw the final drawing by means of a technical pen (i.e., the well known rapidograph line pens, Rotring<sup>TM</sup> and Steadtler<sup>TM</sup> pens). Nuzzaci and de Lillo prefer metal nibs with pointed tips, of at least two sizes, mounted on a holder without an ink reservoir. The nib needs to be repeatedly refilled with a small amount of ink while drawing. Actually, nibs have the advantages of being easier to clean and to preserve than the tips of technical pens, and for making the size of the line variable by simply changing the pressure applied to the nib tip on the paper. Moreover, the dorsal, ventral and lateral parts of the nibs can be used according to the needed thickness of the line. Opaque black Indian ink is adequate and any mistaken and imperfect line can be removed from the tracing paper using a razor blade. Alternatively, mistakes can be removed from drawings after digitization using appropriate software applications.

The illustrator can initially prepare a large plate, with the drawings arranged on it, generally 42 cm wide and 58 cm high (an A2 sheet size). This large canvas allows the illustrator to reduce or mask defects such as those caused by a trembling hand, line imperfections, dirty marks, wrong lines, corrections, and so on. The large size of the drawing may be a problem for the printer. A scanner can be used to digitize the drawings (also single drawn body parts, if necessary), combine the plate, and to reduce their size keeping the high resolution required for printing. In preparing the final plate, the proportions of the single details should be carefully considered. Usually, the first leg and the microtubercle details are two times larger, and the empodium is four times larger than the other parts. Finally scale bars should be added, too.

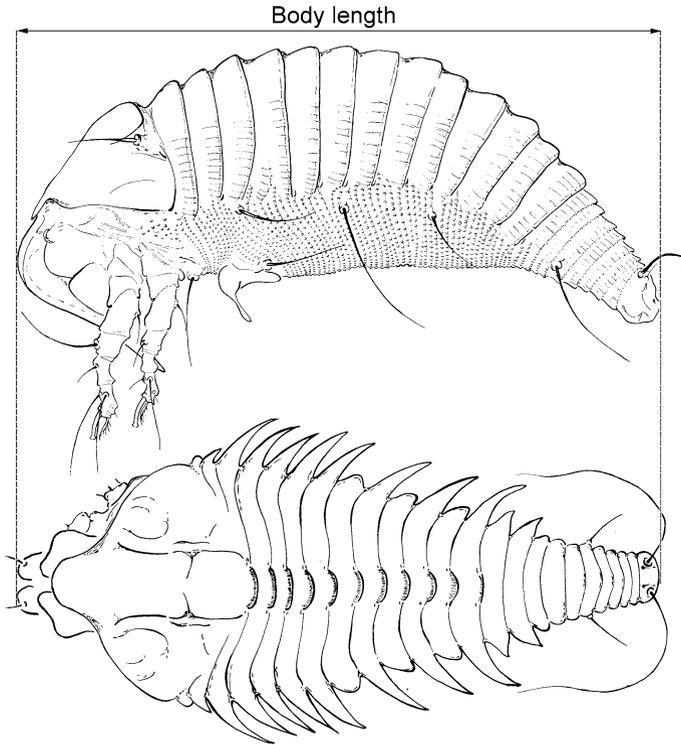
Line drawings can be also made on a computer (Li et al. 2006; Wei et al. 2007; Wang et al. 2007) using a digitizer tablet with a digital pen. Drawing digitally can replace the inking process of the initial pencil draft as described above, but first of all it still remains of utmost importance to accurately interpret critical detailed morphology. It should theoretically be possible to take digital images of the slide mounted specimen, and electronically draw directly on the image. However, this procedure is limited by the tiny and intricate morphology of Eriophyoidea and by the need of high magnifications with limited field depth. Images can be stacked, but it has not been tested whether the final image will be sufficiently detailed and contrasted to replace the carefully made initial pencil draft and final inked drawings.

## Measurements

Measurement instructions for typical descriptive features of Eriophyoidea were described by Amrine and Manson (1996). Figures demarcating the positions of features that may cause confusion or need better definition or standardization are surely more helpful and will overcome any linguistic misinterpretation (Figs. 12, 13, 14, 15).

Many interpretative doubts on the measurements of some features came out during the present authors' experiences and several of these have relevant importance, especially for phylogenetic studies on the Eriophyoidea. Most of them require a careful consideration about the standardization and correct definition of homologies between taxa.

However, each group, genus or species will present different problems and one precise solution will not fit all. Therefore, the author of a new mite must be flexible and able to



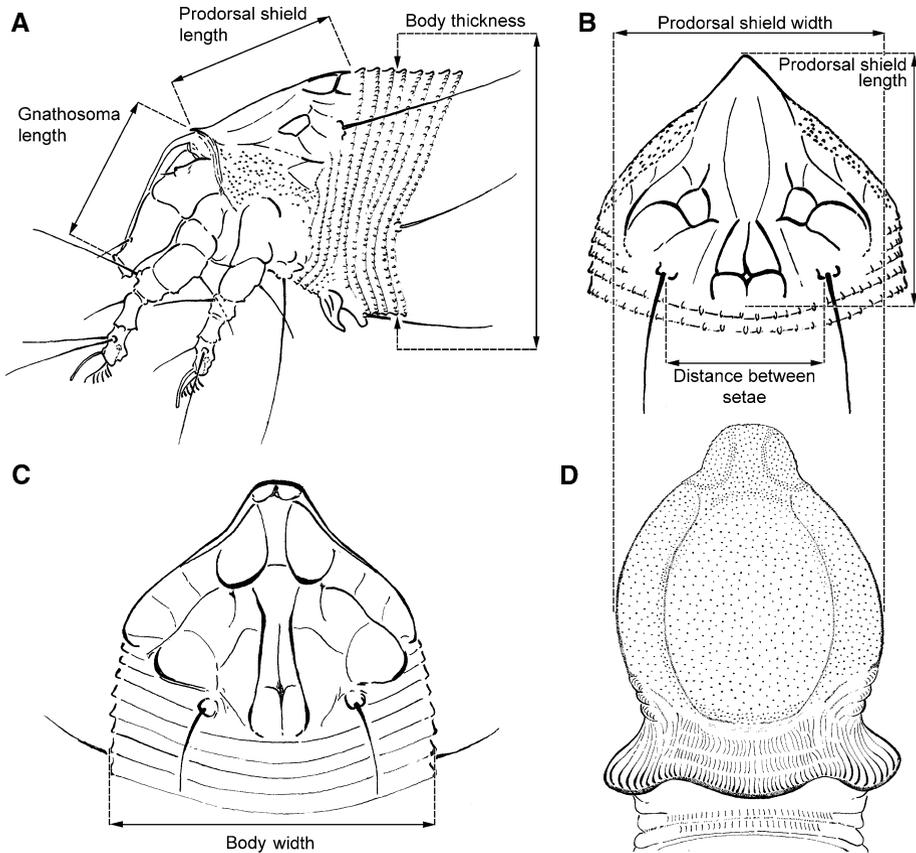
**Fig. 12** Body length: from the rear end of the anal lobe to the maximum extent of the gnathosoma (above for *Rhyncaphytoptus ficifoliae* Keifer) or from the rear end of the anal lobe to the tip of the pedipalps (below for *Tegonotus heptacanthus* [Nalepa]; de Lillo's original drawings)

adapt and see the unique characteristics of the specimen at hand. This means, also, that the description paper should give note to the views and ways the measurements were taken.

A stage micrometer slide is used to calibrate the eyepiece reticule. Then, to make measurements of the specimen, the calibrated reticule is simply superimposed over any image viewed through the light path of the microscope and the linear dimensions (length and width) of a specimen feature can be measured. When making many measurements, the researcher often finds tedious, tiring and time consuming all the little movements and rotations needed with the microscope stage and with the eyepiece for matching the reticule scale with the features to be measured, especially when the morphological details are very fine and short.

To alleviate the tedium, the stage micrometer slide is used to draw a scale paper strip through the drawing tube for each objective lens. Then, the operator can measure more details of the same specimen by just re-positioning the scale strip and without changing the position of the slide or of the eyepiece. This technique saves time and makes measurements much more convenient.

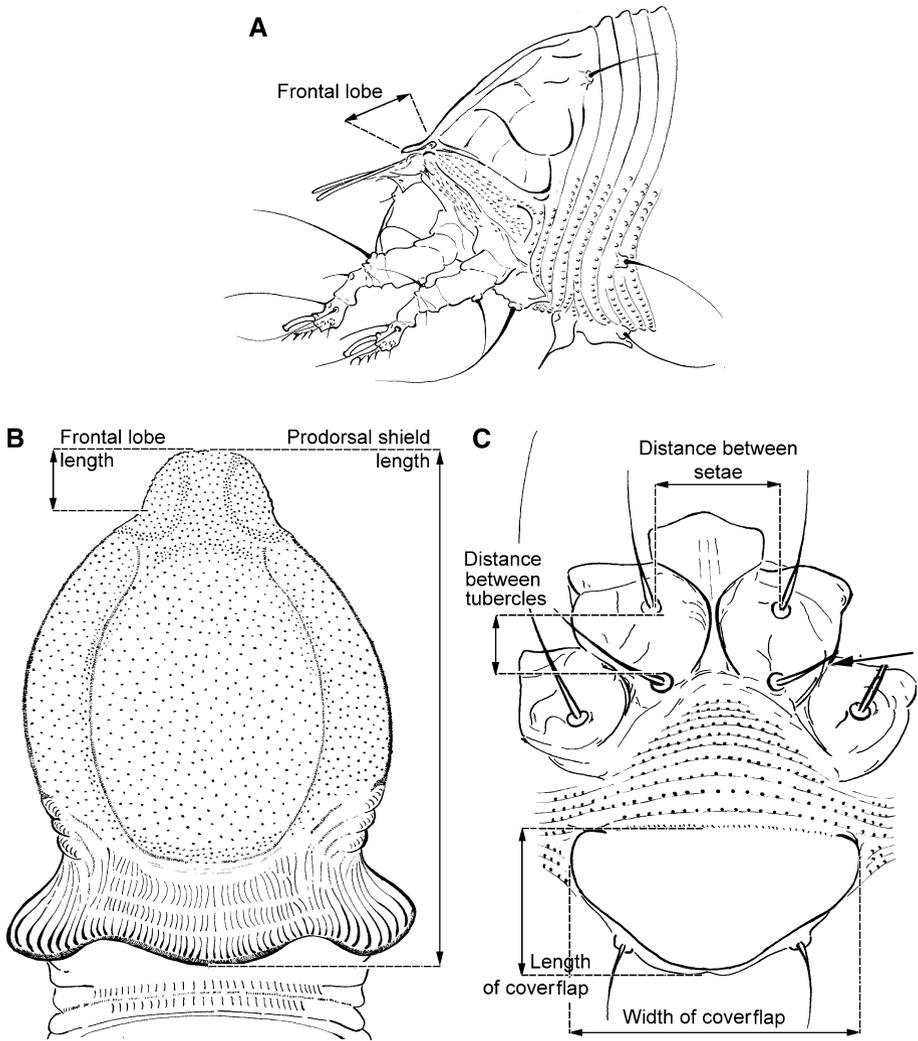
Measurements can also be taken with an electronic image analysis set up. A digital camera is mounted on the microscope, and images are captured and transferred to a computer. Then, they are viewed and manipulated on screen to various angles and levels, and details are measured using different techniques, depending on the digital image



**Fig. 13** **A, B** *Aceria novellae* Denizhan, Monfreda, Cobanoglu and de Lillo, **C** *Aculops pelekassi* (Keifer), **D** *Bariella famei* de Lillo. Gnathosoma length: from the proximal margin of the cheliceral bases to the midpoint of the pedipalp tips. Prodorsal shield length: from the most anterior margin (of the prodorsal shield or frontal lobe) to the anterior margin of the first complete annulus posterior to the shield. Prodorsal shield width: from side to side at the level of the first distinct lateral annulus or at the widest level if the shield margins are protruded. Body width: from side to side at the level of setae *c2* or widest dimension if *c2* is absent. Body thickness: from dorsal to ventral aspect of the opisthosoma at the level of setae *c2*, if present. Distance between setae *sc*: from the inside margins of the setal bases. Distance between tubercles of setae *sc*: from the inside margins of the tubercles (de Lillo's original drawings)

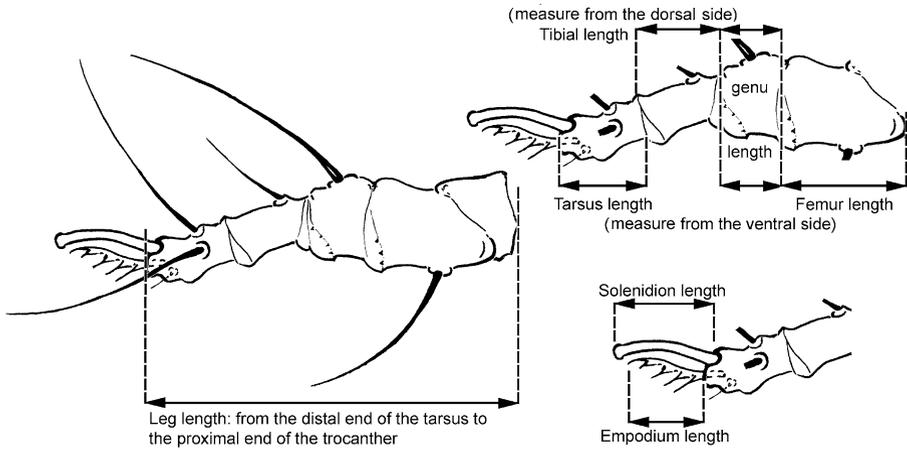
software application used. Care should be taken that the system has been correctly calibrated by comparing with a set of *manual* measurements, and that the digital images are clear and have sufficient contrast to show each minor detail to be measured completely (e.g., very fine setae). Craemer conducted more accurate measurements *on live/video* images (where the image can still be focused up and down on the screen), rather than on a captured images, which may not be able to capture the entire structure to be measured at once, even when using different stacking methods.

Concerning measurements of the holotype, de Lillo measures selected specimens of a population and chooses the holotype within this group as the specimen with closest measurements to the average value for the greatest number of details.



**Fig. 14** **A** *Aculops pelekassi* (Keifer), **B** *Bariella farnei* de Lillo, **C** *Diptacus gigantorhynchus* (Nalepa). Distance between coxal setae: from the inside margins of the setal bases. Distance between tubercles of coxal setae: from the inside margins of the tubercles. *Arrows* indicate the short apodeme exactly where the two legs come together, at their contact, or where they pivot; the apodeme is usually quite distinctive and the anterior edge of this apodeme is used as a reference point for measuring both leg I and leg II length in ventral-dorsal view. Prodorsal shield length: from the most anterior margin (of the prodorsal shield or frontal lobe) to the anterior margin of the first complete annulus posterior to the shield. Frontal lobe length: from the motivator (gnathosomal base) to the anterior edge of the frontal lobe. Genital coverflap width: from its lateral margins. Coverflap length: from the transverse line anteriorly placed to the rear line of the coverflap (de Lillo's original drawings)

Finally, when taking many measurements of many specimens, a spreadsheet such as Microsoft Office's Excel<sup>®</sup> is very convenient for taking averages and ranges of values for each characteristic; properly designed, the files can be printed as tables to support the description.



**Fig. 15** *Aceria ficus* (Cotte). Length of legs from the trochanter proximal margin to the distal margin of the tarsus excluding the empodium and solenidion. Femur and tarsus length: measured ventrally. Tibial length: measured dorsally. Genu length: measured dorsally or ventrally. Solenidion and empodium lengths: from the pigmented base where inserted to the distal tip (de Lillo's original drawings)

## Language

Every researcher is free to choose to publish eriophyoid taxonomic papers in the author's mother tongue. Fortunately, taxonomic articles published in English have been increasing considerably in the past decade, allowing for a wider and more convenient dissemination of information. Nevertheless, many older papers need to be translated because the drawings are often incomplete and cannot show data usually indicated in the morphometric description, and the English abstracts usually lack needed and detailed information. One particular language is not more important than another but, in the scientific environment, English is so widespread and most often understood that it is worth while to have an abstract which contains an English translation, taking care that it should be as complete as possible in order to be useful for identification purposes and morphological comparisons.

Considering the costs of having a paper translated, or of obtaining interlibrary loans, a shared web archive should be arranged and promoted within the copyright rules. The electronic information facilities of public institutions should be preferably used to ensure non-profit designations. This can allow researchers to upload/download translated and other papers in a sort of peer to peer network. Such a network would greatly facilitate sharing important biological and descriptive data, or key publications, and ultimately to promote greater advances in eriophyoid research. Many authorities provide digital copies of their key publications on their web sites, which is enormously helpful.

## Completeness of descriptions

Strong recommendations were given by Keifer (1975) and Amrine and Manson (1996) about the need to include knowledge on the host plant identification, mite habit and host plant relationships. Particular care should be taken in finding and collecting males; their morphology often helps to understand the female status as protogyne/deutogyne mites.

Often, in literature, many species are described based on a small population and/or without the male. Such descriptions often have very limited biological data and lack information about the intraspecific morphometric variability. A new species should ideally be described based on widely dispersed samples in order to avoid describing an accidental presence of a mite on an improper host.

Considering the general availability of GPS devices, collection localities should also include latitude and longitude data at least to the level of minutes.

The rules of the International Code of Zoological Nomenclature (ICZN 1999), currently on line at <http://www.iczn.org/iczn/index.jsp>, must always be followed for nomenclature decisions, name assignment and for gender agreement between genus and species name. Often, patronymic and locality names are assigned after their latinization. Researchers should try to avoid applying genus and species names characterized by a series of contiguous consonants or vowels in order to reduce typing mistakes when these names are listed in tables, indexes and catalogues, as sometimes happens.

Finally, in order to reduce confusion in eriophyoid systematics, species identity should be clearly established when biological observations are specifically carried out, and publications of unnamed or unidentified species should be avoided, such as recently happened for an *Acalitus* sp. of *Carpinus tschonokii* Maxim (Kawashima and Amano 2004).

## Concluding remarks

The information and recommendations given in this article may seem overly demanding and meticulous at first glance. However, these come from our collective and shared experience in studying Eriophyoidea for systematic purposes. It is not meant to be a text book recipe, but hopefully it will spur new ideas and techniques in attaining proper and exact descriptions that will add value and stability in eriophyoid systematics and reduce confusion which is currently prevalent in some groupings.

**Acknowledgments** The authors are strongly in debt to Mrs. Margherita Baldari (Bari, Italy), Dr. Giuseppe Bari (University of Bari, Italy), Dr. Angie Chandrapatya (Kasetsart University, Bangkok, Thailand), Dr. Danuta Knihinicki (ASCU, New South Wales, Australia), Dr. Mariusz Lewandowski (Warsaw University of Life Sciences, Poland), Dr. Rosita Monfreda (University of Bari, Italy), Professor Peter Natchev (Sofia, Bulgaria), Dr. Stefan Nesar (ARC-PPRI, Pretoria, South Africa), Professor Sebahat Ozman-Sullivan (OMU, Samsun, Turkey), Professor Radmila Petanović (University of Belgrade, Serbia), Mr. Giacomo Rondinone (University of Bari, Italy), Dr. Anna Skoracka (Adam Mickiewicz University, Poznan, Poland), Mrs. Terry Stasny (Morgantown, West Virginia, USA), Dr. Guo-Quan Wang (China Agricultural University, Beijing, China), Dr. Sui-Gai Wei (Guangxi University, Nanning, Guangxi, China) for their consistent contribution in testing and applying some technical solutions to collecting, preparing and studying eriophyoid mites. The authors would like to express their gratitude to Dr. Lincoln Smith (USDA-ARS Western Regional Research Center, Albany, California, USA) and the two anonymous reviewers for their contribution to improving the paper. The study was supported by the University of Bari (2008 and 2009).

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## APPENDIX M

**New characters from scanning electron microscopy improve Eriophyoidea descriptions, illustrated by the description of three new species of *Diptilomiopus* Nalepa, 1916 (Acari: Diptilomiopidae: Diptilomiopinae) from South Africa.**

**Charnie Craemer**

Article will be edited, formatted for and submitted to *Systematics and Biodiversity*.

**New characters from scanning electron microscopy improve Eriophyoidea descriptions, illustrated by the description of three new species of *Diptilomiopus* Nalepa, 1916 (Acari: Diptilomiopidae: Diptilomiopinae) from South Africa**

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**Abstract**

Three new *Diptilomiopus* species associated with indigenous trees from South Africa are described and illustrated. Two of the descriptions are significantly enhanced with morphological information from low temperature (cryo) scanning electron microscopic (LT-SEM) studies which provide novel characteristics. Morphology obtained from slide-mounted specimens, containing artefacts, is compared with SEM images. The new species are in the subfamily Diptilomiopinae of the family Diptilomiopidae: *Diptilomiopus faurius* **sp. nov.** from *Faurea rochetiana* (A. Rich.) Pic. Serm. (Proteaceae), and *Diptilomiopus apobrevis* **sp. nov.** and *Diptilomiopus apolongus* **sp. nov.** from *Apodytes dimidiata* E.Mey. ex Arn. (Icacinaceae). The species are vagrants on the leaf under-surfaces of their host plants, causing no apparent damage. The genus *Diptilomiopus* is re-diagnosed and re-described, and the generic assignments of some species currently in *Diptilomiopus*, are discussed. A key to the Diptilomiopidae species known from South Africa is provided.

**Key words:** Acari, Trombidiformes, Prostigmata, *Diptilomiopus*, plant-feeding mites, South Africa, taxonomy, morphology, scanning electron microscopy, new descriptive characters

## Introduction

Eriophyoidea are obligatory plant-feeding mites. They have a cosmopolitan distribution, and occur on a vast range of plant families of most groups of land-living multicellular plants, but the majority of species are generally regarded to be very host-specific (Oldfield, 1996). About 4 000 Eriophyoidea species are known worldwide (De Lillo & Skoracka, 2010). This probably represents only a tiny proportion of extant species. It is conservatively estimated that the world extant eriophyoid fauna may range from 35 000 to 50 000 species (Amrine *et al.*, 2003), and indicates that less than 8 to 11 % (depending on the country and area) may be known. In South Africa, and the remainder of the Afrotropical Region in particular, the diversity is largely unknown. The description of new eriophyoid species are thus of primary importance to address the short fall of the knowledge on the diversity of these mites.

The Eriophyoidea are morphologically distinct among mites. They are minute (on average 150–250  $\mu\text{m}$  long), with elongated, worm-like and annulated bodies, and are unique in having all instars of both sexes with two pairs of similarly developed legs anteriorly (Keifer, 1975a). Their minute, soft bodies and ultra-fine structures cause challenges for their morphological study and preparation for such study (De Lillo *et al.*, 2010). Eriophyoid morphology for systematic use is almost exclusively studied on cleared and slide-mounted specimens, which are not permanent (De Lillo *et al.*, 2010), using phase contrast light microscopy. Eriophyoid descriptions still largely follow the standard and format set by Keifer (Nuzzaci & De Lillo, 1996; De Lillo *et al.*, 2010). Amrine & Manson (1996) reviewed taxonomic characters and their use, and proposed what should be included in an eriophyoid species description. These standard descriptions are still the simplest and the most practical, and form the basis of the classification and identification of the Eriophyoidea.

Identification keys to the genera of the Eriophyoidea worldwide by Amrine (1996) and Amrine *et al.* (2003) are generally accepted, and identification, description and differentiation of eriophyoid taxa at all levels are more or less satisfactory. In practice, however, many descriptions and drawings do not achieve the required standard and in particular do not always convey exact taxonomically important detailed characteristics (Amrine & Manson, 1996; De Lillo *et al.*, 2010). Some of the factors causing it are problems with the quality and standardization of slide-mounted specimens (Amrine & Manson, 1996; De Lillo *et al.*, 2010), and the resultant standardization and quality of their description. Artefacts inherently caused by slide mounting and inefficient resolution of detailed morphology are contributing to the problem and may be detrimental to eriophyoid taxonomy. Additionally, some taxonomic characters are not well-defined and demarcated, and this presents problems for practical taxonomy and the determination of primary homologies for phylogenetic analyses. It is further evident that there are not enough morphological characters documented and available for phylogenetic analyses, and most of these were found to

be highly homoplasious by phylogenetic studies (Hong & Zhang, 1996a, b, 1997; C. Craemer, *in preparation*).

Many of these systematic problems can be rectified or improved on by the discovery of additional new systematically informative characters, apart from those characters that might be obtained from the discovery of new species. There is, however, not much such additional information available from slide-mounted specimens. The characters are already obtained from the entire body (Lindquist & Amrine, 1996; Amrine *et al.*, 2003), and most easily observable and taxonomically useful characters are already utilized. The degree of morphological diversity is also limited by the lack of observed ontogenetic diversity in characters (characters have the same states throughout all life stages), and the lack of distinctive characters in the male (Lindquist & Amrine, 1996). There is furthermore a need for the morphological study of eriophyoids in a more natural, artefact-free condition, to determine the types of artefacts caused by slide-mounting, and standardization of these for taxonomic practices.

Electron microscopy (EM), which facilitates higher resolution than light microscopy and renders three-dimensional images, is largely superior to light microscopy for studying the morphology of minute organisms with ultra-fine structures. Electron microscopy already contributed more information on the external and internal morphology of eriophyoid mites, and our understanding of and knowledge on their morphology have been improved (Nuzzaci & De Lillo, 1996). Scanning electron microscope (SEM) images are, however, sporadically, and not routinely, incorporated in eriophyoid taxonomic articles. They are moreover largely used to enhance and confirm descriptions of Eriophyoidea from slide-mounted specimens. When they are included in articles it is normally without particular comment or focus on them. The SEM-images probably contributed to the correctness and detail of the descriptions wherein they were used (e.g., Keifer *et al.*, 1982; Boczek & Nuzzaci, 1985; Schliesske, 1985; De Lillo, 1988; Chandrapatya & Boczek, 1991a,b; Boczek & Chandrapatya, 1992; Amrine *et al.*, 1994; De Lillo, 1994; Huang & Wang, 2004; Menon *et al.*, 2011), essentially no additional morphological or descriptive information were defined or discussed from them, except Menon *et al.* (2011) who described spines occurring on leg segments largely from SEM images.

Low-temperature SEM (cryo-SEM), with an integrated high vacuum freezing and sputter unit, seems to be the most successful in obtaining highly magnified, largely artefact-free images of eriophyoid mites, particularly minimizing shrinkage (Sutherland & Hallett, 1987; Duffner *et al.*, 1998; Wergin *et al.*, 2000; Achor *et al.*, 2001).

Three new *Diptilomiopus* spp. are described here.<sup>1</sup> The descriptions are more detailed than the norm for Eriophyoidea species descriptions, and the description of two of the species include

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<sup>1</sup> This is the second paper in a series of articles on the Diptilomiopidae Keifer, 1944. The previous article (Craemer *et al.*, 2005) entailed species recombinations and a new synonymy in *Diptilomiopus*.

characters described from SEM images, which are not visible or distinguishable in slide-mounted specimens. The SEM images are additionally compared with the slide-mounted specimens to determine the amount of artefacts and loss of information caused by slide-mounting.

Differentiation of the species, however, includes characters available from slide-mounted specimens. The morphometric information of the descriptions is provided statistically more structured than usually found in eriophyoid descriptions in an effort to improve the quality and usefulness of morphometric data in descriptions for phylogenetic analyses. It is now possible to utilize continuous morphometric data, without gap coding, in phylogenetic analyses in the program TNT<sup>®</sup> (Tree Analysis Using New Technology) (Goloboff *et al.*, 2008).

The new species are the first *Diptilomiopus* spp. (Diptilomiopidae: Diptilomiopinae) recorded from South Africa. The only record of the Diptilomiopidae from this country is *Africanus psydraxae* Meyer & Ueckermann, 1995, a genus and species described from *Psydrax livida* (Hiern) Bridson (Rubiaceae) (Meyer & Ueckermann, 1995).

*Diptilomiopus*, the type genus of the Diptilomiopidae, was described by Nalepa (1916) to accommodate the type species, *D. javanicus*, an inquiline in galls on the upper leaf surface of *Hemigraphis confinis* (Nees) T. Anders (Acanthaceae) in Java (Nalepa, 1916). Since 1916, and before the present study, 90 *Diptilomiopus* spp. have been described, with the majority from the Indomalayan Ecozone (Oriental Region) (Thailand – 31, India – 19, Taiwan – 16, China – 16, Philippines – 1, Japan – 1, Java – 1) (Hong & Zhang, 1997; Craemer *et al.*, 2005; Hong *et al.*, 2010), and one species from Australia (Keifer, 1969) in the Australasian Ecozone. Three species are known from the Afrotropical Ecozone (Sub-Saharan Region) and these are: *D. ficifolius* (Boczek & Oleczek, 1988) from *Ficus sur* Forssk. (= *F. capensis* Thunb.) (Moraceae) in Nigeria (Boczek & Oleczek, 1988); *D. holopteleae* Abou-Awad & El-Banhawy, 1992 from a *Holoptelea* Planch. sp. (Abou-Awad & El-Banhawy, 1992) (possibly *Holoptelea grandis* Mildbr.) (Ulmaceae) in Kenya; and *D. jevremovici* Keifer, 1960 from *Coffea arabica* L. (Rubiaceae) in the Democratic Republic of the Congo (Keifer, 1960). One species – *D. ficus* Attiah, 1967 from Egypt – is known from the Palearctic Ecozone. It, however, was collected on “fig trees” (Attiah, 1967), presumably the cultivated fig, *Ficus carica* L., which is believed to have originated in western Asia and later spread to the Mediterranean (Tous & Ferguson, 1996). *Diptilomiopus ficus* probably originated with its host in western Asia. It seems as if *Diptilomiopus* may have originated in the Indomalayan Ecozone, and may be restricted to the subtropical and tropical areas of this and the Australasian and Afrotropical Ecozones.

*Diptilomiopus* is re-diagnosed and re-described, based on a review of the genus. The classification of some *Diptilomiopus* spp. is discussed, and some of these are re-assigned to other genera. A key to the Diptilomiopidae species from South Africa is included.

## Materials and methods

Specimens were collected by hand from plant material using a stereo microscope mostly at 40x to 80x magnification, and were prepared and slide-mounted according to a modified version of Keifer's media and method (Keifer 1975a; De Lillo *et al.* 2010). The SEM images are of specimens prepared and studied with a modified version of the low-temperature (cryo) fixation technique described by Echlin *et al.* (1970), using a conventional JEOL JSM 840 SEM with a cryo-stage. The figures presented in this paper, consist of realistic line drawings of slide-mounted specimens, and SEM images of specimens studied by above technique.

The terminology and setal notation in the descriptions are largely based on Lindquist's (1996) system. In the text and figure captions (not tables), largely setae and solenidia are referred to by their alphabetic name in italics, without the term "seta" or "solenidion" before them. Only one side of the body is described and measured. Measurements and counts are rounded to the nearest integer, are in micrometers ( $\mu\text{m}$ ), and are presented in Tables 2–4.

All types of the new species are preserved as slide-mounted specimens. The type material is deposited in the National Collection of Arachnida: Acari, Biosystematics Building, Roodeplaat, ARC–Plant Protection Research Institute, P/Bag X134, Pretoria Queenswood, 0121 South Africa, and one slide, with paratypes, of each new species will be deposited in the Insect and Mite Collection, National Museum of Natural History (NMNH), Smithsonian Institution, Washington D.C., USA. The Mite Collection is housed at USDA, ARS, SEL, Beltsville, Maryland, USA. The scanning electron microscope images will be archived and kept in the collection in Pretoria in addition to the type material.

## Systematics

### Key to the Diptilomiopidae of South Africa

1. Seta *sc* present, but minute; *Ia* absent .....  
 ..... *Africus psydraxae* Meyer & Ueckermann, 1995
- Seta *sc* absent; *Ia* present ..... 2
2. Ridge m2 (dividing cells B1L and B1R) (Figs 1A,B; 3D,E) of the dorsal shield pattern present, and the prosternal apodeme anteriorly with a diamond-shaped part (Fig. 6A) .....  
 ..... *Diptilomiopus faurius* sp. nov.
- Ridge m2 (dividing cells B1L and B1R) (Figs 1A,B) of the dorsal shield pattern absent, or invisible in slide-mounted specimens, and the prosternal apodeme without a diamond-shaped part or prosternal apodeme effaced or absent ..... 3
3. Cells D1L and D1R at base of dorsal shield pattern (Figs 1A; 12A,D) present, number of opisthosomal microtubercles mid-ventrally about 12–18 / 20  $\mu\text{m}$ , approximate setal lengths: *d* 10–13, *e* 8–12, *3a* 6–9 (Table 1) ..... *Diptilomiopus apobrevis* sp. nov.

-- Cells D1L and D1R at base of dorsal shield pattern (Figs 1A; 21A,G) absent, number of opisthosomal microtubercles mid-ventrally about 8–13 / 20  $\mu\text{m}$ , approximate setal lengths: *d* 53–63, *e* 47–59, *3a* 10–13 (Table 1) ..... *Diptilomiopus apolongus* sp. nov.

**Table 1.** Measurements and counts of the three new *Diptilomiopus* spp., and *D. gilibertiae*.

	<i>faurius</i>			<i>apobrevis</i>			<i>apolongus</i>			<i>gilibertiae</i>		
	mean	min	max	mean	min	max	mean	min	max	mean	min	max
body length (pedipalpi included)	<b>272</b>	222	322	<b>222</b>	188	265	<b>280</b>	232	311	<b>251</b>	206	291
seta <i>v</i> (apico-ventral seta) length	<b>4</b>	3	4	<b>5</b>	5	6	<b>6</b>	5	6			
chelicerae length	<b>77</b>	70	82	<b>74</b>	68	77	<b>85</b>	81	98			
prodorsal shield length	<b>33</b>	28	37	<b>38</b>	32	41	<b>38</b>	35	42	<b>34</b>		
prodorsal shield width	<b>62</b>	56	69	<b>73</b>	65	80	<b>72</b>	63	80	<b>75</b>		
scapular tubercles distance apart	<b>24</b>	22	28	<b>27</b>	23	32	<b>28</b>	25	30	<b>27</b>		
number of ventral microtubercles / 20 $\mu\text{m}$	<b>10</b>	7	12	<b>15</b>	12	18	<b>10</b>	8	13			
seta <i>d</i> length	<b>48</b>	40	56	<b>12</b>	10	13	<b>58</b>	53	63	<b>12</b>		
seta <i>e</i> length	<b>40</b>	32	52	<b>10</b>	8	12	<b>53</b>	47	59	<b>8</b>		
setae <i>e</i> distance apart	<b>33</b>	27	38	<b>27</b>	22	31	<b>34</b>	30	36	<b>29</b>		
seta <i>f</i> length	<b>38</b>	35	41	<b>44</b>	40	48	<b>46</b>	43	48	<b>34</b>		
coxal seta <i>1a</i> (2nd coxal seta) length	<b>36</b>	29	40	<b>37</b>	28	46	<b>45</b>	36	55	<b>28</b>		
coxal seta <i>2a</i> (3rd coxal seta) length	<b>52</b>	50	59	<b>55</b>	47	61	<b>61</b>	53	68	<b>39</b>		
Leg I length (from base of trochanter)	<b>40</b>	37	43	<b>42</b>	37	45	<b>49</b>	45	57	<b>61</b>		
Leg I tarsus length (excluding extremities)	<b>13</b>	11	14	<b>11</b>	10	14	<b>15</b>	14	17	<b>12</b>		
Leg I empodium number of rays	<b>7</b>	7	8	<b>7</b>	7	8	<b>7</b>	7	8	<b>6</b>		
Leg II length (from base of trochanter)	<b>36</b>	33	39	<b>37</b>	34	42	<b>43</b>	39	47	<b>50</b>		
Leg II tarsus length (excluding extremities)	<b>11</b>	10	12	<b>11</b>	10	11	<b>14</b>	12	14	<b>10</b>		
Leg II seta <i>ft''</i> (lateral tarsal seta) length	<b>36</b>	32	39	<b>33</b>	32	35	<b>41</b>	39	45			
seta <i>3a</i> (genital seta) length	<b>11</b>	8	12	<b>7</b>	6	9	<b>12</b>	10	13	<b>6</b>		

### ***Diptilomiopus* Nalepa, 1916**

*Diptilomiopus* Nalepa, 1916: 283; Nalepa, 1918: 228–230; Keifer, 1938: 305; Keifer, 1975b: 521; Newkirk & Keifer, 1975: 585; Chakrabarti & Mondal, 1983: 299; Amrine & Stasny, 1994: 173; Boczek *et al.*, 1989: 25, 182 (plate 190 not of *D. javanicus*, but of *D. jevremovici*); Kuang, 1995: 168; Amrine, 1996: 111, 140; Hong & Zhang, 1996c: 71; Hong & Zhang, 1997: 321; Amrine *et al.*, 2003: 137, 195–196; Craemer *et al.*, 2005: 133–134.

*Sectipes* Keifer, 1962a: 18–19; Keifer, 1962b: 19, **syn.**; Newkirk & Keifer, 1975: 585.

*Vilaia* Chandrapatya & Boczek, 1991a: 427–428; Boczek & Chandrapatya, 1989: 139–140; Amrine & Stasny, 1994: 311–312; Amrine, 1996: 114, 141; Hong & Zhang, 1997: 321, **syn.**; Amrine *et al.*, 2003: 137, 215; Craemer *et al.*, 2005: 133–135.

Type species: *Diptilomiopus javanicus* Nalepa, 1916, by original designation.

**RE-DIAGNOSIS.** This diagnosis is a compilation and modification of the diagnoses by Nalepa (1916, 1917), Hong & Zhang (1997) and Amrine *et al.* (2003). *Diptilomiopus* has the typical diagnostic characteristics of the Diptilomiopidae and Diptilomiopinae, and is distinguished from other genera in the Diptilomiopinae by the following combination of character states: dorsal annuli slightly more than ventral annuli; legs I and II 5-segmented with the genu seemingly absent, probably fused with the femur, with *bv*, *l''*, and *l'* absent; coxisternal plates I with *1b* absent; opisthosoma with *c2* absent, and *d*, *e*, *f*, *h2*, *1a*, *2a* and *3a* present; *sc* absent.

**RE-DESCRIPTION.** This description is an expansion and modification of the genus descriptions by Nalepa (1916, 1917), Keifer (1938), but here including morphological variation within the genus, and new insight and information from a review and compilation of descriptive data of *Diptilomiopus* spp. worldwide. It does not include the family (Diptilomiopidae), subfamily (Diptilomiopinae) and diagnostic generic characteristics given above.

#### **FEMALE (typifies the genus).**

**Idiosoma** – fusiform to somewhat elongated fusiform (e.g., Figs 2; 11A,B); relatively thick in lateral and dorsal view, broadest at the prodorsum, tapering towards the rear end, sometimes sharply, with the rear end slanting downwards. Many species described to be light to dark amber (orangey) when alive.

**Opisthosoma** – evenly rounded; usually with a longitudinal middorsal ridge, which may be slight, flanked by a shallow trough on each side which sometimes, together, form a v-shape with narrow

end towards the rear, and each is frequently flanked on the outside by a very slight, barely discernable subdorsal ridge (e.g., Fig. 2D). These ridges and furrows fade towards the rear, until they are absent. All or some of these ridges and furrows are described as being absent in some species, but these cases need confirmation. Wax secretions not present, except in *D. artocarpae* (wax patches) and *D. melastomae* (covered with wax), but *D. artocarpae* do not belong in *Diptilomiopus* (see below).

Dorsal annuli entirely without microtubercles in most species, but in some species they are covered with microtubercles, which may occasionally be sparse or scattered, or may be restricted to certain areas, e.g., absent in a central band, which is flanked by microtubercles on each side, or only on rear dorsal annuli. Ventral annuli covered with microtubercles in all species.

Seta *h1* absent or present, when present always minute (e.g., Fig. 2C), the recorded absence in some species may be ambiguous, because its presence might have been missed due to the tiny size of the seta.

**Gnathosoma** – palp *d* present or absent, but presence or absence usually not recorded; *ep* and *v* probably always present, but are rarely recorded or described. Some ornamentation and structures may be present on palpi as illustrated in SEM images (e.g., Fig. 4) of the new species here described, but are most probably largely not visible in slide-mounted specimens, and were not previously described for *Diptilomiopus* spp.

**Prodorsal shield** – usually broadly oval, with width much broader than the length of the relatively short shield, with characteristic convex shape with declivitous rear area (e.g., Fig. 3A). It has been described as sub-triangular or diamond-shaped in some species, and the shape was sometimes used to differentiate between species. Evaluation and depiction of prodorsal shield shape is highly subjective, and may be ambiguous due to distortion of slide-mounted specimens.

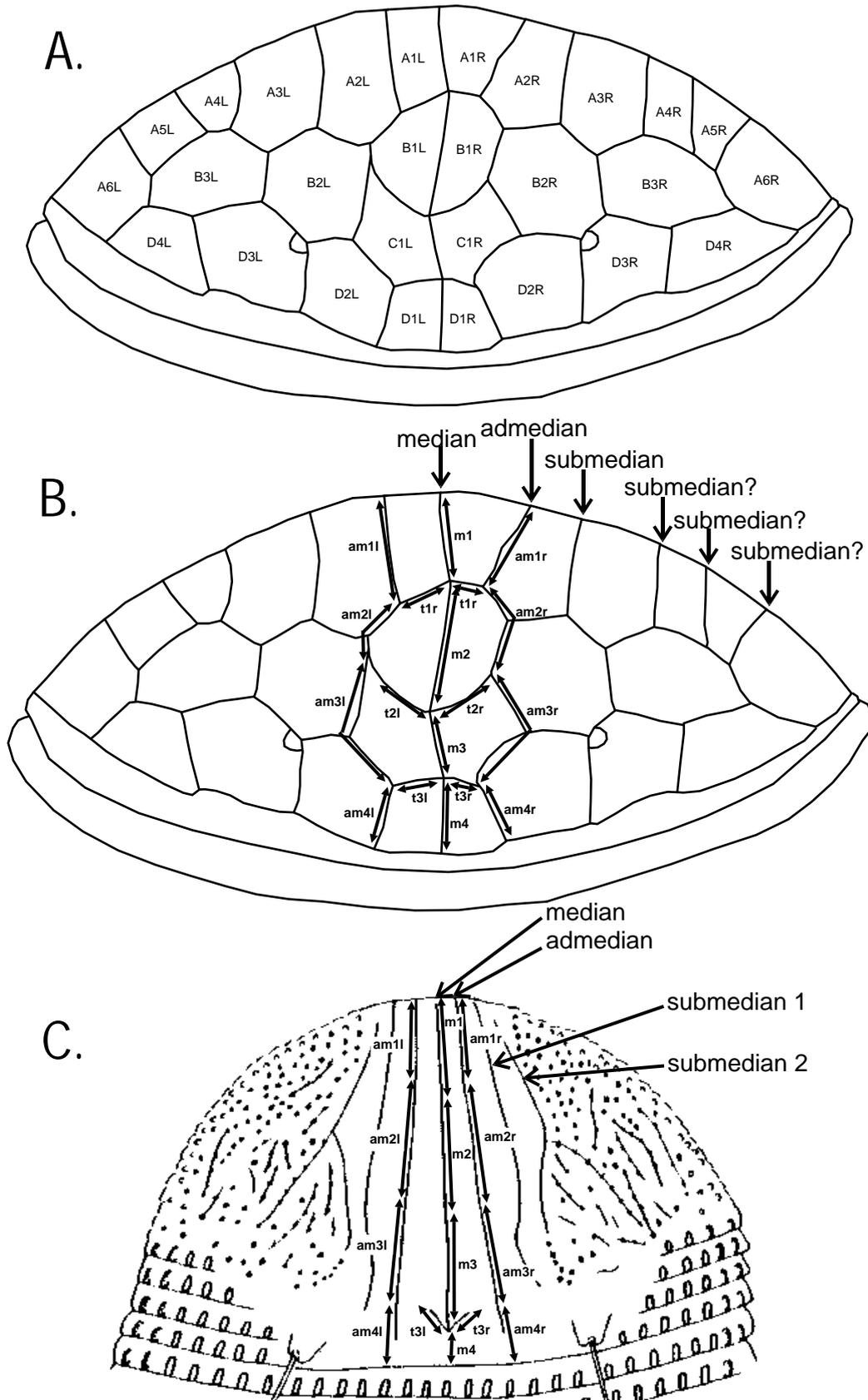
Prodorsal shield pattern prominent, consisting of ridges in a roughly typical cell-like pattern, of which a more complete version is schematically depicted in Fig. 1A. This complete typical expression of the ridge-pattern consists broadly of three rows of cells named as follows: 12 cells (A1L–A6L and A1R–A6R) in anterior row, six cells (B1L–B3L and B1R–B3R) in second row and two cells (C1L and C1R) in basal row; two open cell-like areas, D1L and D1R are formed at the base of the shield pattern on the declivitous basal part of the shield (Figs 1A; 3A,D,E) and may be regarded as a fourth cell row. Due to the compression of this area on the slides, if present, D1L and D1R are not always clearly present on the slide-mounted specimens (e.g., Fig. 12B). Alongside D1L and D1R three cell-like areas may be present, D4L, D3L, D2L and D2R, D3R, D4R, which sometimes seem like an extension of the basal row (C1L and C1R). More prominent variations of the dorsal shield ornamentation between species are: presence or absence of sections of the median line (e.g., ridge/line between cells A1L and A1R, B1L and B1R, C1L and C1R, and between D1L and D1R) or of the other transverse or longitudinal ridges, dividing or forming cells, as well as the

relative larger and smaller size or difference in shape of cells in comparison with the “complete” and “typical” shield pattern depicted in Fig. 1A. Sub-shield lateral area usually with granules.

Scapular setal tubercles present or absent – absence or presence of these tubercles was not described, and is still unknown, for some species, including the type species. Scapular tubercles when present and described as such, are relatively small, rounded and ahead of rear shield margin (e.g., Figs 3A–D). They may be obscured by the ridges on the shield, and recorded absence in species is ambiguous and should be confirmed.

A frontal lobe might be present in all or most species, but was recorded and depicted as absent in most species, because it is inconspicuous in slide-mounted specimens, as demonstrated by the new species described here (e.g., Figs 3D; 12B; 21A). In the new species, however, a transversely broad but longitudinally narrow frontal lobe with a slight, broad indentation of the anterior margin is unambiguously present when viewed with SEM (e.g., Figs 3A,B; 12A). The frontal lobe, when present, is probably thin and flexible and not overhanging the basal parts of the chelicerae, and is subjectively not classified as a frontal lobe when evaluated for use in descriptions and diagnoses of taxa, and the keys to genera and species in the Eriophyoidea. The presence or absence of the frontal lobe is used to differentiate supra-generic taxa.

**Legs** – tibia of average length in comparison with other Eriophyoidea genera, and usually shorter than, or sometimes equal in length to tarsus. It is longer than tarsus (but not twice the tarsal length) only in a few species: *D. hexogonus*, *D. holopteleae*, *D. integrifoliae*, *D. languasi*, and *D. lobbianus* (Huang, 2001; Abou-Awad & El-Banhawy, 1992; Mohanasundaram, 1981; Chandrapatya & Boczek, 1991b; Huang & Cheng, 2005, respectively). Shape and structures of tibiae I and II are well discernable in SEM images, and may entail ridges in the length, dorsally terminating in well developed spines (e.g., Fig. 7B); these are less discernable in slide-mounted specimens (e.g., Figs 7C,D). The shape of the tibia may be of systematic use in *Diptilomiopus* spp., but is not described.



**Figure 1. Prodorsal shield ornamentations.** A. Cell names of “*Diptilomiopus*”-like schematic prodorsal shield network; B. line or ridge names of “*Diptilomiopus*”-like schematic prodorsal shield network; C. line or ridge names of dorsal shield pattern of *Aceria barbertoni* Meyer & Ueckermann, 1992, reproduced from Meyer & Ueckermann (1992), with frontal lobe deleted to facilitate clearer labeling of lines.

Seta *ft*' on tarsus of leg II is present or absent, but presence or absence was rarely described. Both setae *ft* in legs I and II are usually simple and tapering, but in *D. eucalypti*, *D. ficifolius*, *D. musae* and *D. sandorici* these setae have a small sub-branch or spike near the bases of the setae (Chandrapatya & Boczek, 1991b; Boczek & Oleczek, 1988; Chandrapatya & Boczek, 1998, 1991a, respectively). In the diagnosis of *Vilaia*, a new genus described by Chandrapatya & Boczek (1991a), the tarsal setae are described “with short perpendicular spike at the base”. The tarsal setae of the designated type species of *Vilaia*, *Diptilomiopus pamithus* (= *Rhynacus pamithus*, = *Vilaia pamithus*) are not described in the text, however, and are depicted as simple (without spikes) (Boczek & Chandrapatya, 1989). *Vilaia* was designated a junior synonym of *Diptilomiopus* (Hong & Zhang, 1997), but might be a valid, monophyletic genus, supported by the possible synapomorphy, presence of spikes on the tarsal setae, but this should be recovered by phylogenetic analyses. Seta *u*' in legs I and II might be curved or sharply bent in some species (e.g., Figs 8A,B).

Typical ornamentation may be present on the leg surfaces, e.g., on the coxae, trochanters and femorogena, of all or some *Diptilomiopus* spp. as illustrated by the ridges and tubercles present on the legs of the new species. These ornamentations, which may have systematic value, are not described, though, because it may be invisible or inconspicuous in slide-mounted specimens, but are clearly discernable in some SEM images (e.g., Figs 7G,H).

Deeply divided empodium with rays of sub-branches which generally slant towards the front and against the central stems (e.g., Fig. 8C), ranging approximately from 4- to 9-rayed, except *D. stephanus* with 11–12 rays. Possibly due to some difficulty in counting the rays, their number is not reported for many species, and the range given here was partly determined from counting the rays in descriptive drawings.

Tarsal solenidion  $\omega$  always above and close to empodium.

**Coxae and genitalia** (Figs 7 & 8) – Coxisternal plates I and II may be smooth (unornamented) or ornamented, usually with rounded to short dash-like tubercles, usually almost covering coxisternal plates I entirely, and covering smaller areas of coxisternal plates II; inner margins of coxae I in most species touch and form a prosternal apodeme (sternal line), but sometimes the coxisternal plates are fused with the prosternal apodeme effaced, and in some species the coxisternal plates are separated, and inner margins present, but not forming a prosternal apodeme.

Setae *1a* and *2a* “normal” (simple and tapering), and *1a* positioned slightly or clearly ahead of an imaginary line through tubercles of *2a*, and never in line or behind *2a*.

Internal anterior genital apodeme extending forward, and spermathecae round or slightly ovalish with relatively short spermathecal tubes, as usually found in the Eriophyoidea (e.g., Fig. 14B); genital coverflap entirely smooth (unornamented), only ornamented on the basal area (e.g., Figs 6A,D), or entirely ornamented, sometimes with ornamentation clearly divided in a basal and distal area; ornamentation usually tubercles (granules), sometimes combined with dashes, when

ornamentation only on basal area, the ornamentation may loosely be divided into two separate subcircular areas (e.g., Fig. 6D); other types of ornamentation only present in a few species, e.g., one rank of longitudinal ridges in *D. artocarpae*, and semi-circular transverse lines in *D. ulmivagrans*. Genital coverflap ornamentation frequently vaguely and ambiguously described.

Based on this re-diagnosis and –description of *Diptilomiopus*, particularly the following species might not resort within the genus, and are dealt with or discussed, their eventual classificatory positions pending their relationships and the monophyly of the genus recovered by phylogenetic analyses:

1. ***Vimola artocarpae*** (Mohanasundaram, 1981) **comb. nov.**

*Diptilomiopus artocarpae* Mohanasundaram, 1981, Mohanasundaram (1981): 45–46.

Although the presence of the genu is not mentioned in the text description of *D. artocarpae*, legs I and II are depicted with six segments and the genu clearly present (Mohanasundaram, 1981), and *D. artocarpae* should thus not be in *Diptilomiopus*. There are additionally other characteristics of the species which differ from most *Diptilomiopus* spp., and which support its exclusion from *Diptilomiopus*: genu I with *l'* present; the dorsal shield pattern is different from the general cell-pattern (Fig. 1A) of other *Diptilomiopus* spp.; the tibia is twice the length of the tarsus, while the tibia is shorter, equal or only slightly longer than the tarsus in most *Diptilomiopus* spp.; and it has wax patches, while wax secretions are not present in *Diptilomiopus* spp., except in *D. melastomae*. The species keys out to *Vimola* Boczek, 1992 with the key to genera by Amrine *et al.* (2003).

2. ***Vimola thangaveli*** (Mohanasundaram, 1983) **comb. nov.**

*Diptilomiopus thangaveli* Mohanasundaram, 1983, Mohanasundaram (1983): 177–178.

The genu is present in legs I and II of *D. thangaveli* – depicted and mentioned in the differential diagnosis (Mohanasundaram, 1983), and genu I with *l'* present; and thus *D. thangaveli* does not belong to *Diptilomiopus*. The species keys out to *Vimola* Boczek, 1992 with the key to genera by Amrine *et al.* (2003).

3. ***Vimola ulmivagrans*** (Mohanasundaram, 1984) **comb. nov.**

*Diptilomiopus ulmivagrans* Mohanasundaram, 1984, Mohanasundaram (1984): 265, 282.

Although the presence of the genu is not mentioned in the text description of *D. artocarpae*, legs I and II are depicted with six segments and the genu clearly present and genu I with *l'* present (Mohanasundaram, 1984), and *D. ulmivagrans* thus do not belong in *Diptilomiopus*. The species keys out to *Vimola* Boczek, 1992 with the key to genera by Amrine *et al.* (2003).

4. *Diptilomiopus bengalensis* Chakrabarti & Mondal, 1979, Chakrabarti & Mondal (1979): 47–49.

The description of *D. bengalensis* by Chakrabarti & Mondal (1979) is ambiguous, but it clearly does not belong in *Diptilomiopus*. The presence of *bv* is ambiguous, because the length of *bv* is recorded for legs I and II, but these setae are absent in the descriptive drawings (Chakrabarti & Mondal, 1979). Although *l'* is absent, the genu is present, in legs I and II – described in the text and depicted (Chakrabarti & Mondal, 1979), and the species should thus not be in *Diptilomiopus*. This is supported by the dorsal shield pattern of *D. bengalensis* which is different from the general cell-pattern (Fig. 1A) of other *Diptilomiopus* spp. The species keys out to *Vimola* Boczek, 1992 with the key to genera by Amrine *et al.* (2003). The species keys out close to *Neolambella* Lin & Kuang, 1997, with the key to genera by Amrine *et al.* (2003), but *D. bengalensis* differs from *N. ligustri* Lin & Kuang, 1997 in the absence of *lb*, and the possible presence of *bv* in the former species. *Diptilomiopus bengalensis* may belong to a new genus, but this decision pending the correct description of the species, and results from phylogenetic analyses.

5. *Diptilomiopus ervatamiae* (Chandrapatya, 1991)

*Vilaia ervatamiae* Chandrapatya, 1991, Chandrapatya & Boczek (1991a): 430–431. *Diptilomiopus ervatamiae* keys out close to *Diptilomiopus* in the key by Amrine *et al.* (2003), but *lb* is present in the species, and it does not belong in *Diptilomiopus* as the genus is currently diagnosed, and might belong to a new genus, pending results of phylogenetic analyses. Seta *lb* is present in *D. ervatamiae*, and *la* is absent, but setal tubercle of *la* present (Chandrapatya & Boczek, 1991a). This is quite an unusual state in the Eriophyoidea, since *la* is rarely absent, in contrast to *lb*, and if absent, *lb* is also absent. The presence of *lb* and absence of *la* is autapomorphic for *D. ervatamiae* among *Diptilomiopus* spp. The probable exclusion of *D. ervatamiae* from *Diptilomiopus* is supported by the difference of the dorsal shield pattern of *D. bengalensis* from the general cell-pattern (Fig. 1A) of other *Diptilomiopus* spp.

6. *Diptilomiopus guajavae* Mohanasundaram, 1985, Mohanasundaram (1985): 23–25.

The text description and depiction of the legs of *D. guajavae* by Mohanasundaram (1985) are highly ambiguous, among other discrepancies in this description. The length of a tibiotarsus is recorded for legs I and II (Mohanasundaram, 1985). This description in comparison with the depiction of the legs by Mohanasundaram (1985) can be interpreted in four ways, if some corrections are presumed, with corresponding generic placement in brackets:

- Genu absent in legs I and II, *l''* absent (*Diptilomiopus*);
- Genu absent (tarsus and tibia present), *l''* present (possibly new genus, because *l''* is not present in *Diptilomiopus*);

- Tibiotarsus present and thus tibia absent, and genu present, in legs I and II, *l'* present on genu I (possibly new genus). It is highly unlikely that a tibiotarsus might be present in any species of the Diptilomiopinae.
- Tarsus, tibia and genu present as separate segments in legs I and II, *l'* present on genu I (*Vimola*).

The correct generic placement requires re-description of the species and results of phylogenetic analyses.

### **Species descriptions (exclude diagnostic generic characters described above)**

The new *Diptilomiopus* spp. from South Africa are differentiated from each other, and from *D. gilibertiae* Kadono, 1984 (Kadono, 1984), because the three new *Diptilomiopus* spp. from South Africa were found as a group which may be monophyletic, and *D. gilibertiae* was found to be a potential sister species of the South African species group, with preliminary parsimony analyses (C. Craemer, *in preparation*). The similarities between these four species include: broadly the same prodorsal shield ornamentation of ridges in a cell-like pattern; scapular setal tubercles present, small and rounded, positioned anterior of prodorsal shield rear margin; coxisternal plates I largely covered with granules, and coxisternal plates II with granules anterior of *2a*; the number of dorsal and ventral annuli about the same; palp *d*, and *ft'* on tarsus II, absent; minute *hl* present; female genital coverflap with granules on the basal area, and smooth distally; female internal genitalia broadly similar.

### ***Diptilomiopus faurius* sp. nov.**

#### DIFFERENTIAL DIAGNOSIS.

*Diptilomiopus faurius* sp. nov. is differentiated from the other new *Diptilomiopus* spp. from South Africa in the key to Diptilomiopidae of South Africa (above). Differences between this new species and *D. gilibertiae* include: dorsal annuli of *D. faurius* sp. nov. with microtubercles absent in a central band, flanked by microtubercles on each side, and the empodium with 7–8 rays; while the dorsal annuli are entirely covered with microtubercles in *D. gilibertiae*, and its empodium has 6 rays. Setae *d* and *e* (about 48 and 40  $\mu\text{m}$ , respectively) of *D. faurius* sp. nov. much longer than these in *D. gilibertiae* (about 12 and 8  $\mu\text{m}$ , respectively). A hump middorsally behind rear shield margin (Fig. 10B,D) in the nymph which might be unique to *D. faurius*.

Measurements and counts in Table 2.

**FEMALE** (Figs 2–5; 6A–F; 7; 8A–C)

**Idiosoma** – Fusiform to somewhat elongated fusiform (Figs 2A,B,D; 5B,E); light to dark amber (orangey) in life.

**Gnathosoma** (Fig. 4) – Shape and structures determined largely on SEM images: about basal half of palpcoxal base directed anteriorly in the same direction as the long axis of the body, where after palpi and gnathosomal stylets bend ventrad, perpendicularly to the long body axis, basal part with an approximately oval depression (Figs 4A,D,E) varying in size and depth; part of margin between palpcoxal base and trochanter-femur-genu interrupted by a smooth area (Fig. 4A); a conspicuous, rounded, dorsally somewhat flattened lobe-like structure enclosed by a ridge antero-laterally is situated just proximal of or at the distal margin of the trochanter-femur-genu (Figs 4A,C,E); a short ridge probably present dorsally on palptibia on the inner margin bordering the stylet sheath (Fig. 4D); palptibia with a convex hump distally on the ventral side (Figs 4A,B).

Infracapitular guides are conspicuous, apparently freely projecting processes originating at the level of the distal margin of the trochanter-femur-genu, interlocked or lying close together and appearing as one elongated triangular structure in the ventral view of the gnathosoma of slide-mounted specimens (Fig. 5B).

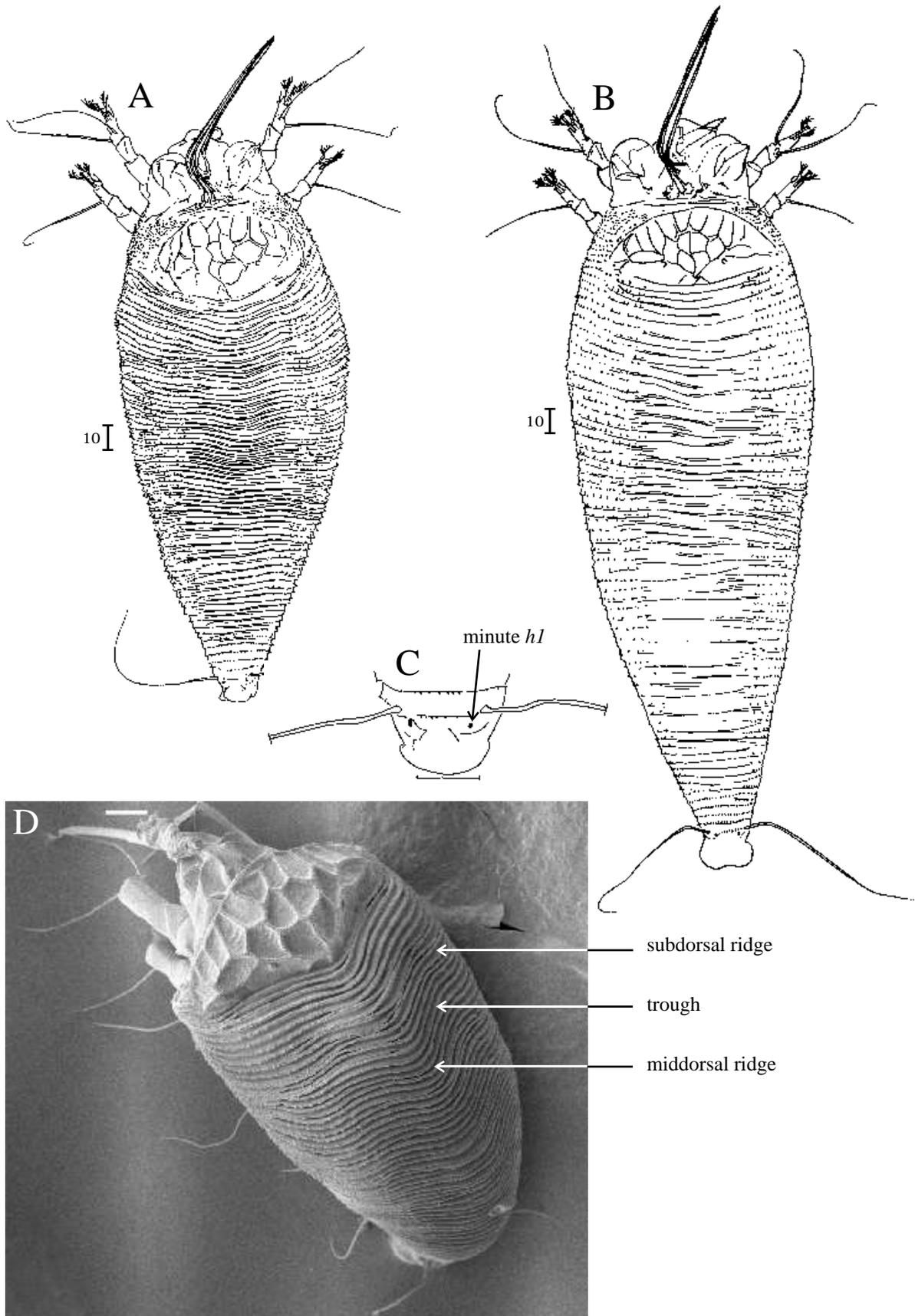
Palpi with palp *d* absent; *ep* present, lying or standing in a shallow groove, with a ridge on the outer and inner side (Figs 4A,D,E); *v* present, inserted on a small raised area ventrally and distally on the palptarsus (Fig. 4B).

Table 2. Measurements and counts *Diptilomiopus faurius* sp. nov.

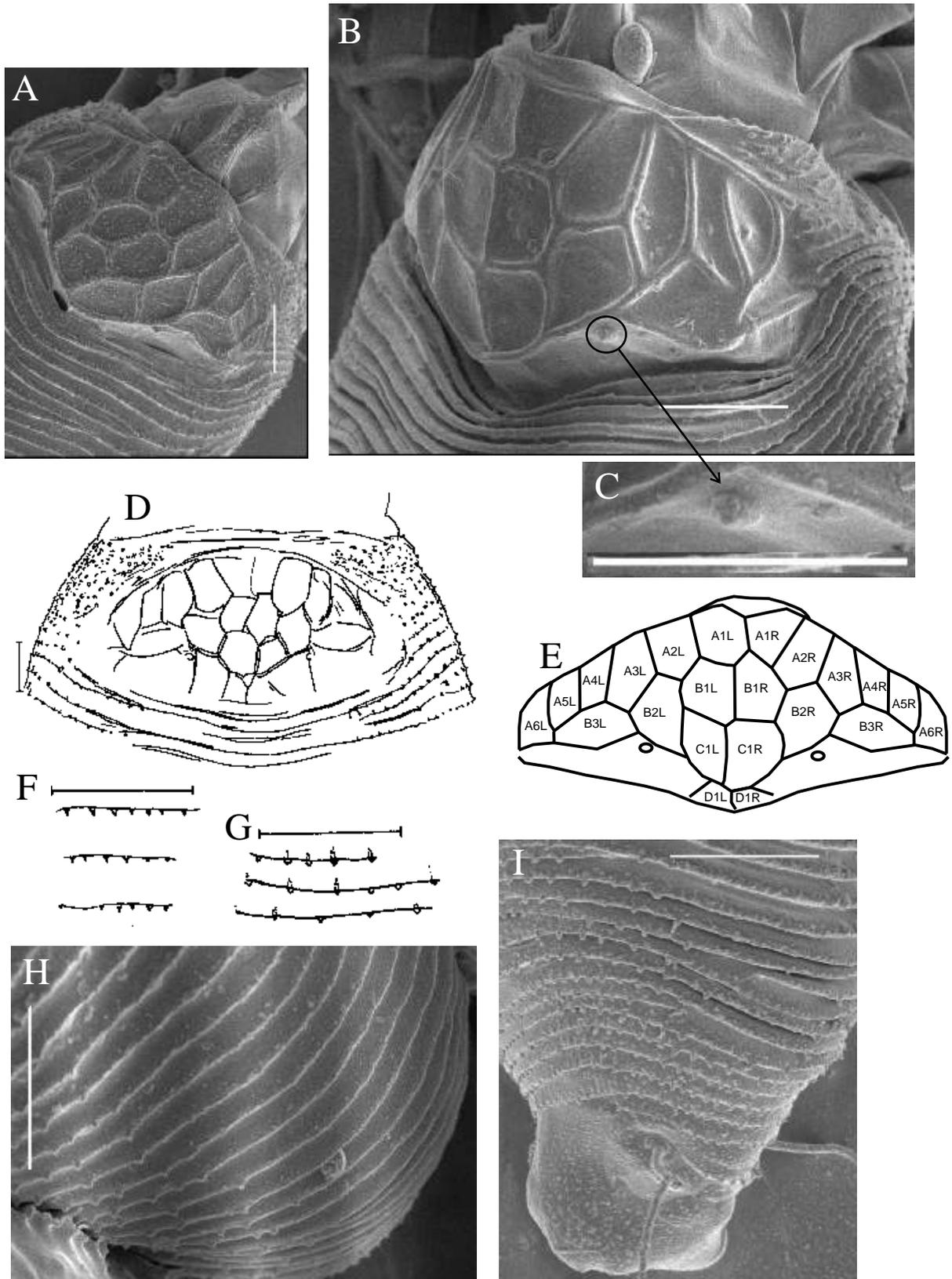
character	holotype ♀	measurements and counts of ♀ structures (dorsal/ventral view) (n=12) (rounded)						♀ lateral view (n=1)	♂ (n=1)	larva (n=1)	nymph (n=1)
		median	mean	SD	min	max	CV*				
<i>BODY SIZE</i>											
body length (pedipalpi included)	256	268	272	36	222	322	13.40		227	167	228
idiosomal length (gnathosoma excluded)	207	223	225	38	163	283	17.19	239	181	135	188
body width just behind prodorsum	86	81	82	4	78	90	5.45		67	58	67
body width at level of setae <i>f</i>	47	41	42	3	38	47	7.72		35	24	28
body thickness (just below genital flap)								89			
<i>GNATHOSOMA</i>											
length (dorsal) excluding stylets	49	49	51	7	42	64	13.04		44	29	45
length (lateral) excluding stylets								58			
seta <i>ep</i> (basal seta) length	3	3	3	0	3	3	0		3	2	2
seta <i>d</i> (antapical seta)	absent	absent						absent	absent	absent	absent
seta <i>v</i> (apico-ventral seta) length	4	4	4	0	3	4	10		4	3	3
chelicerae length	78	77	77	4	70	82	4.84	74	69	49	55

character	holotype ♀	measurements and counts of ♀ structures (dorsal/ventral view) (n=12) (rounded)						♀ lateral view (n=1)	♂ (n=1)	larva (n=1)	nymph (n=1)
		median	mean	SD	min	max	CV*				
<b>PRODORSAL SHIELD</b>											
length	36	33	33	3	28	37	9.17		29	28	31
width	63	62	62	4	56	69	6.90		55	33	49
<b>sc setal tubercles (dorsal tubercles)</b>											
distance apart	25	24	24	2	22	28	6.75		20	24	26
length	1	1	1	0	1	2	36.94		1	1	2
base width	2	2	2	0	1	2	15.38		2	1	2
distance ahead of rear shield margin	10	9	9	2	6	12	25.05		5	10	10
<b>OPISTHOSOMA</b>											
number of dorsal microtubercles / 20 µm	0 (smooth)								0	7	0
number of ventral microtubercles / 20 µm	11	11	10	1	7	12	14.15		11	8	9
<b>position seta d (first ventral seta)</b>											
position seta d (first ventral seta)	31	32	32	2	29	36	6.80		26	15	21
number of microtubercles between setae d	26	24	24	3	20	29	11.09		15	8	14
seta d length	53	49	48	5	40	56	10.13		35	25	37
setae d distance apart	51	53	52	5	45	58	8.94		40	30	43
<b>position setae e (second ventral setae)</b>											
position setae e (second ventral setae)	50	50	51	3	47	57	6.17		42	21	31
number of microtubercles between setae e	15	15	15	3	11	20	18.27		10	7	9
seta e length	37	40	40	5	32	52	13.43		39	15	35
setae e distance apart	32	32	33	4	27	38	11.39		28	22	30
<b>position setae f (third ventral setae)</b>											
position setae f (third ventral setae)	71	70	71	4	64	78	6.11		61	32	47
position setae f from rear	11	11	11	1	10	12	6.23		11	6	8
number of microtubercles between setae f	24	22	22	3	18	27	13.38		17	11	18
seta f length	40	38	38	2	35	41	5.58		37	19	27
setae f distance apart	35	33	33	2	30	37	6.73		29	21	24
<b>seta h2 (caudal seta) length</b>											
seta h2 (caudal seta) length	67	59	56	10	36	69	18.13		35	24	31
<b>seta h1 (accessory seta) length</b>											
seta h1 (accessory seta) length	2	1	1	0	1	2	37.70		1	1	1
<b>number of dorsal annuli lateral to shield</b>											
number of dorsal annuli lateral to shield	4	3	3	1	2	5	23.69		2?	0	4
<b>number of dorsal annuli</b>											
number of dorsal annuli	66	65	64	4	58	72	6.21		57	45	51
<b>number of annuli clearly forming central ridge</b>											
number of annuli clearly forming central ridge	36	35	35	6	19	42	17.65		30	0	7
<b>total number of dorsal annuli</b>											
total number of dorsal annuli	70	69	68	4	61	75	6.23		59	45	55
<b>total number of ventral annuli</b>											
total number of ventral annuli	81	79	81	5	74	89	5.74		71	37	54
<b>COXAL AREA</b>											
<b>sternal line length</b>											
sternal line length	7	7	7	2	4	9	25.65		8	absent	absent
<b>coxal seta 1a (2nd coxal seta) length</b>											
coxal seta 1a (2nd coxal seta) length	40	37	36	3	29	40	9.04		29	12	23
<b>coxal setae 1a distance apart</b>											
coxal setae 1a distance apart	10	10	10	1	9	11	8.03		9	6	7
<b>coxal seta 2a (3rd coxal seta) length</b>											
coxal seta 2a (3rd coxal seta) length	50	51	52	3	50	59	5.36		47	26	30

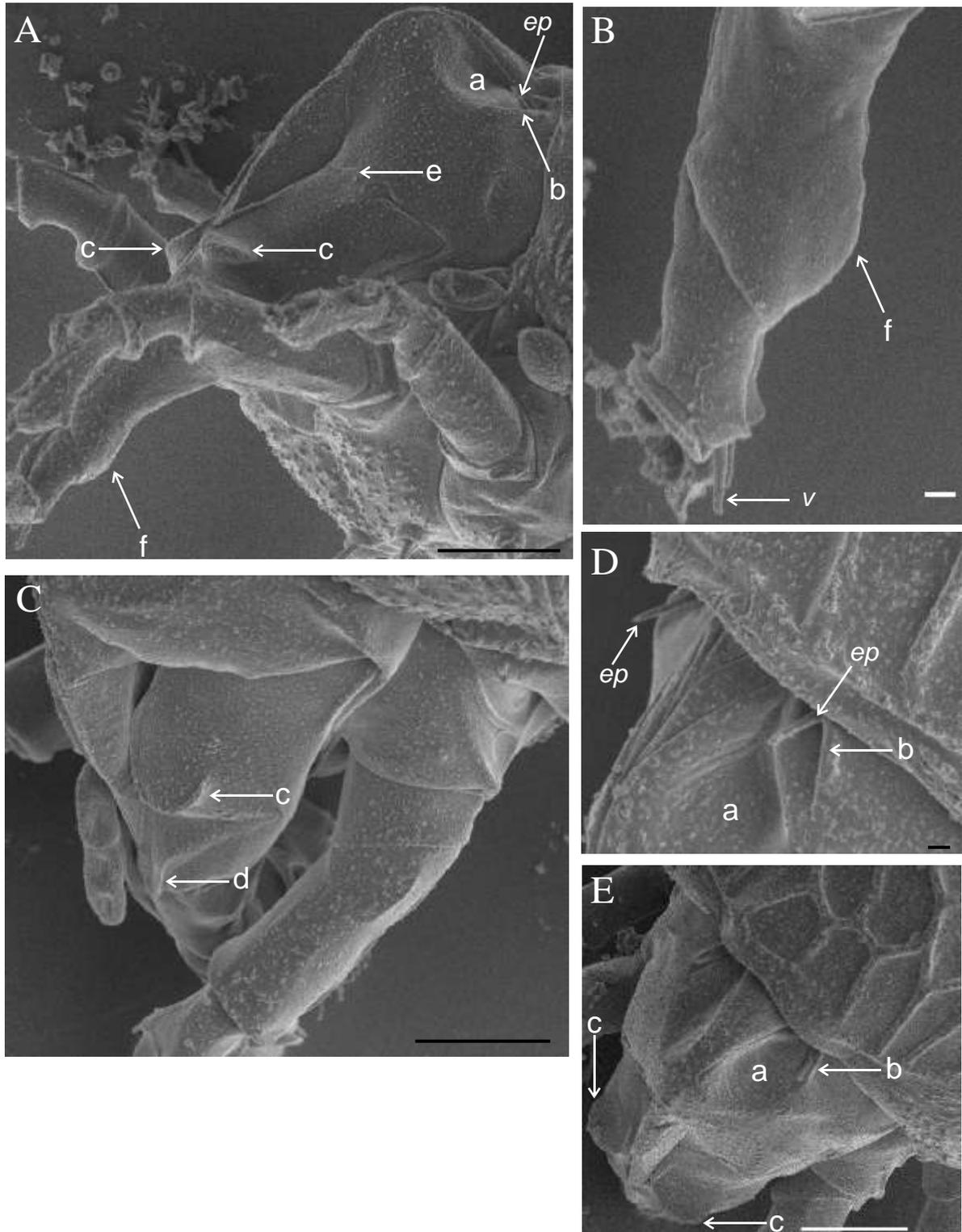
character	holotype ♀	measurements and counts of ♀ structures (dorsal/ventral view) (n=12) (rounded)						♀ lateral view (n=1)	♂ (n=1)	larva (n=1)	nymph (n=1)
		median	mean	SD	min	max	CV*				
coxal setae <i>2a</i> distance apart	32	31	31	2	27	34	7.47		28	18	24
Distance setae <i>1a</i> to <i>2a</i>	7	7	7	1	5	8	14.21		8	5	4
number of complete annuli in coxi-genital region	4	4	4	0	4	5	7.22		5	8	11
number of half annuli in coxi-genital region	2	2	2	0	2	3	14.15		2	0	0
total number of annuli in coxi-genital region	6	6	6	1	5	8	11.22		7	8	11
<i>LEGS I</i>											
length (including coxa) including extremities	77	73	73	2	70	77	3.04		65	45	57
length (including coxa) excluding extremities	70	65	65	2	62	70	3.67		58	40	52
length (from base of trochanter)	41	41	40	2	37	43	5.64	40	37	21	32
trochanter length (dorsal)	4	5	5	1	4	6	14.89	7	4	2	5
femur-genu length	16	17	17	2	15	19	9.62	17	17	7	12
tibia length	10	9	9	0	9	10	4.99	9	7	5	6
tarsus length (excluding extremities)	13	13	13	1	11	14	6.43	12	10	7	8
seta <i>ft''</i> (lateral tarsal seta) length	43	42	42	2	40	46	4.11		40	24	31
seta <i>ft'</i> (dorsal tarsal seta) length	41	41	40	2	38	44	4.48		37	23	20
seta <i>u'</i> (mesal seta) length	5	6	6	1	5	7	10.26		5	3	4
solenidion $\omega$ (tarsal solenidion) length	9	9	9	1	8	10	7.95	8	8	5	7
em (tarsal empodium) length	12	11	11	1	10	12	5.44	12	10	8	8
<i>LEGS II</i>											
length (including coxa) including extremities	61	59	59	2	55	62	3.89		54	35	45
length (including coxa) excluding extremities	52	51	50	2	45	53	4.34		46	31	41
length (from base of trochanter)	35	36	36	2	33	39	4.59	35	32	21	25
trochanter length (dorsal)	6	6	6	1	3	7	21.40	6	4	4	4
femur-genu length	16	15	14	2	11	16	13.32	13	13	8	8
tibia length	8	8	8	1	7	8	7.11	6	7	4	5
tarsus length (excluding extremities)	11	11	11	1	10	12	6.84	10	10	6	8
seta <i>ft''</i> (lateral tarsal seta) length	37	35	36	2	32	39	5.82		33	18	26
seta <i>ft'</i> (dorsal tarsal seta) length	absent								absent	absent	absent
seta <i>u'</i> (mesal seta) length	5	5	5	1	5	7	12.47		5	3	3
solenidion $\omega$ (tarsal solenidion) length	9	8	8	1	8	9	6.25		8	5	6
em (tarsal empodium) length	11	10	10	1	8	11	9.73	12	9	6	7
<i>EXTERNAL GENITALIA</i>											
genital coverflap width	31	31	31	1	29	33	3.50				
genital coverflap length	16	17	17	1	16	19	5.13				
male genitalia width									20		
male genital area length									17		
seta <i>3a</i> (genital seta) length	11	11	11	1	8	12	9.72		8	5	7
setae <i>3a</i> distance apart	22	22	22	1	20	24	6.37		19	7	12



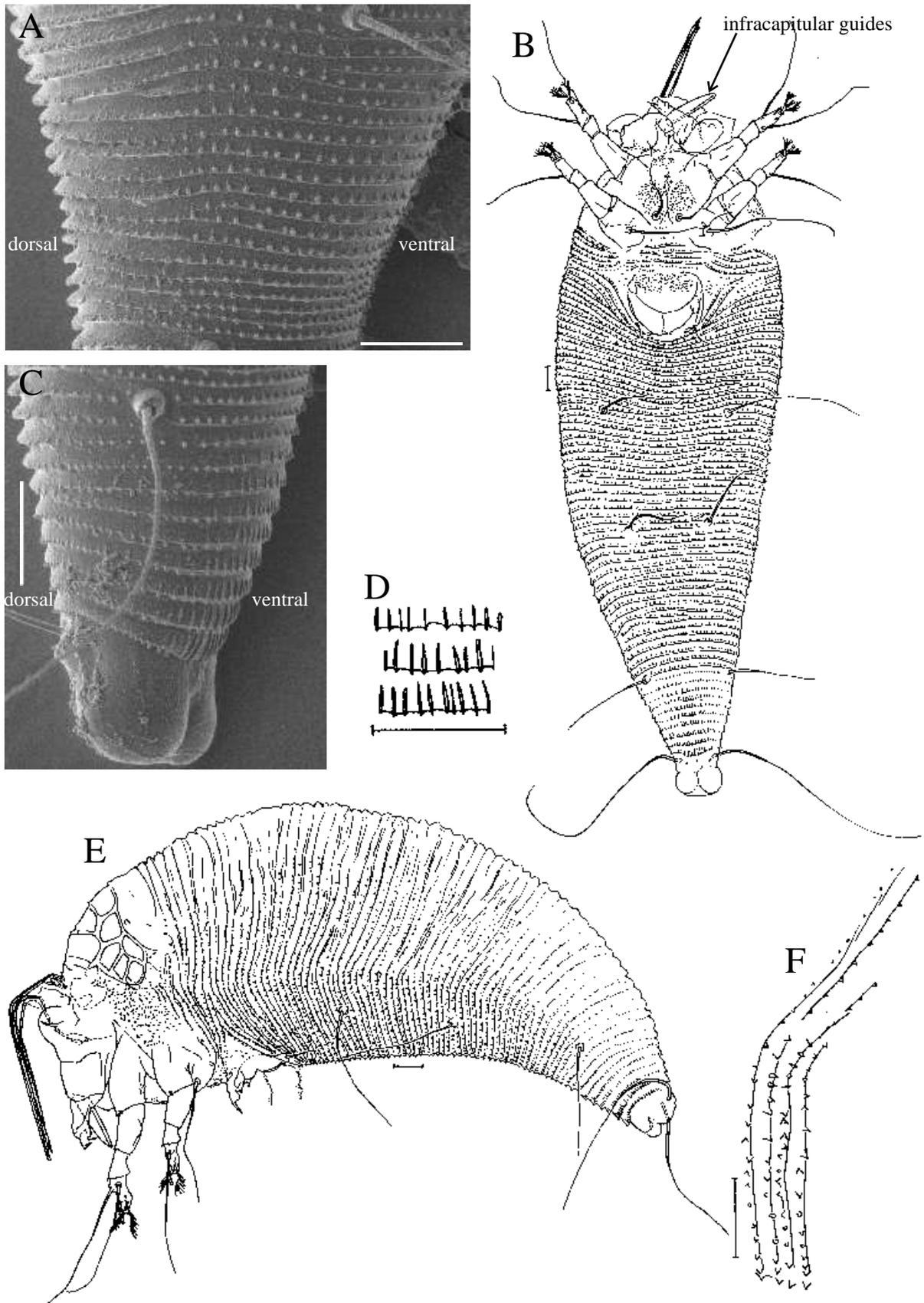
**Figure 2.** *Diptilomiopus faurius* sp. nov., female, dorsal views. **A.** smaller specimen; **B.** larger specimen probably more flattened and distorted than specimen A in this figure; **C.** enlarged rear (caudal) area; **D.** clearly with middorsal ridge flanked by a shallow trough and a subdorsal ridge on each side.



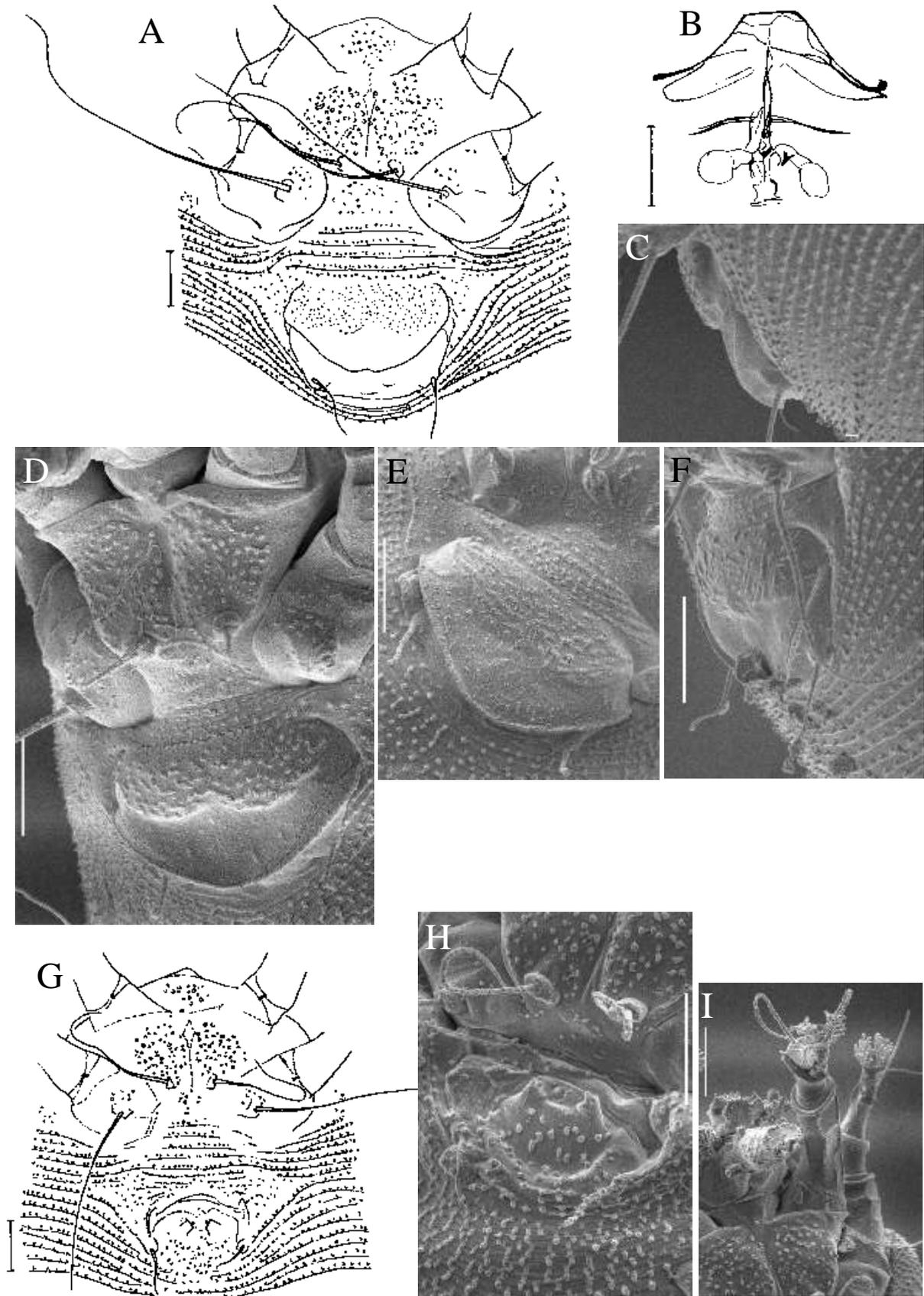
**Figure 3.** *Diptilomiopus faurius* sp. nov., female. **A, B, D.** dorsal view of prodorsum; **C.** enlargement of scapular tubercle; **E.** schematic, labeled version of prodorsal shield; opisthosomal microtubercles: **F.** middorsally just beyond setae *f* (caudal region); **G.** in lateral area of about middorsum; **H.** dorso-laterally; **I.** caudal area (oblique view).



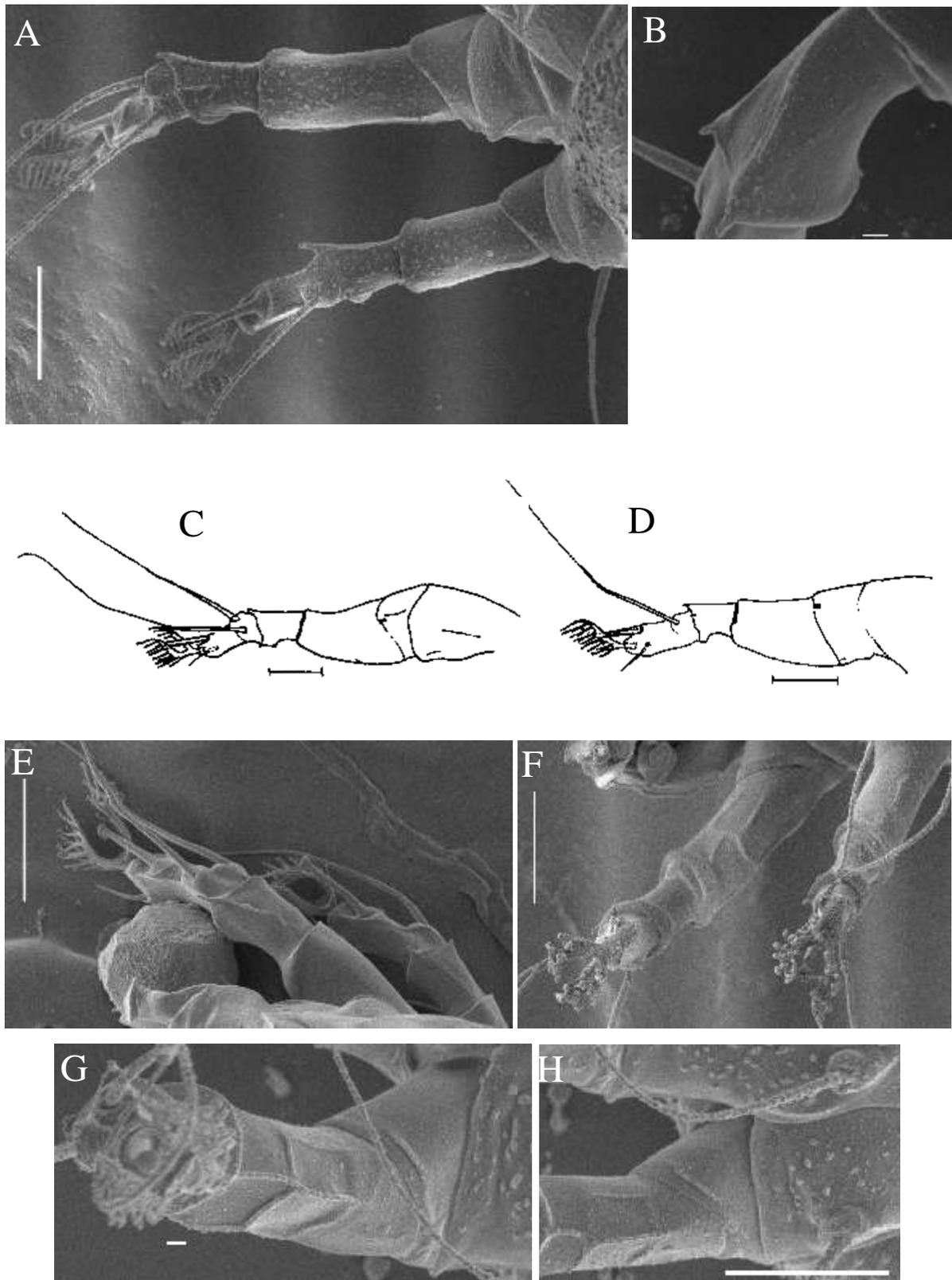
**Figure 4.** *Diptilomiopus faurius* sp. nov., female gnathosoma. **A.** lateral view; **B.** lateral view of tibia and tarsus; **C.** dorso-lateral view; **D.** dorso-lateral view of basal part of palpcoxal base; **E.** dorsal view of palpcoxa and trochanter-femur-genu. **a.** oval depression at base of palp coxa (varies between specimens in size and depth); **b.** ridge on outside of seta *ep*; **c.** round, flattened lobe, with ridged margin anteriorly, apically on trochanter-femur-genu; **d.** short ridge on inner margin of palp tibia bordering the stylet sheath; **e.** smooth area interrupting margin between palp coxal base and trochanter-femur-genu; **f.** convex bulged area ventrally and distally on palp tibia; *ep.* seta *ep* at the base of the palp coxa, lying in a groove; *v.* seta *v* inserted on a raised area distally on the palp tarsus.



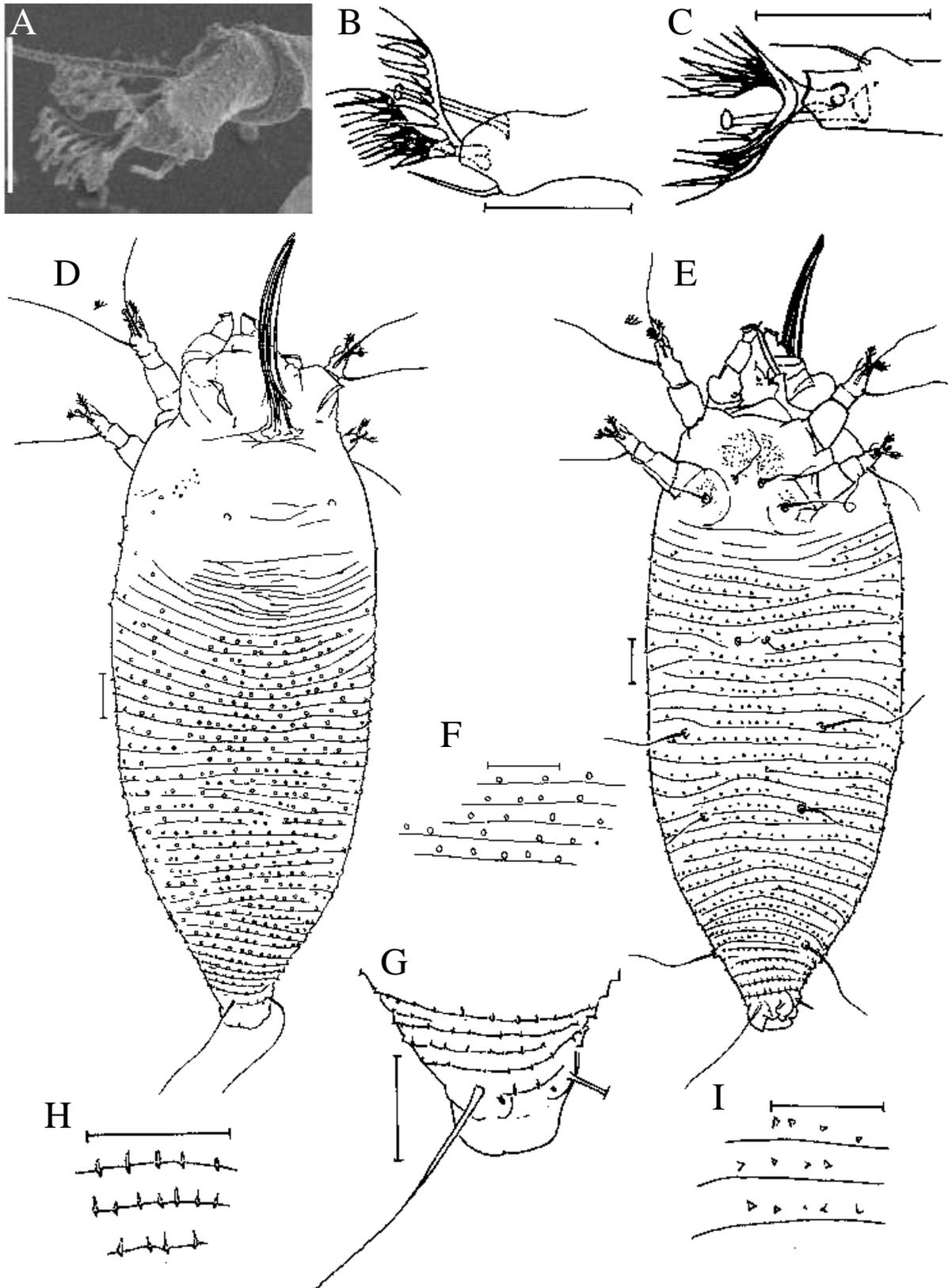
**Figure 5.** *Diptilomiopus faurius* sp. nov., female. **A.** opisthosomal microtubercles on the annuli between setae *e* and *f*; **B.** ventral view; **C.** opisthosomal microtubercles on the rear annuli; **D.** opisthosomal microtubercles on rear three caudal annuli; **E.** lateral view; **F.** opisthosomal microtubercles in lateral view at about the level of seta *d*.



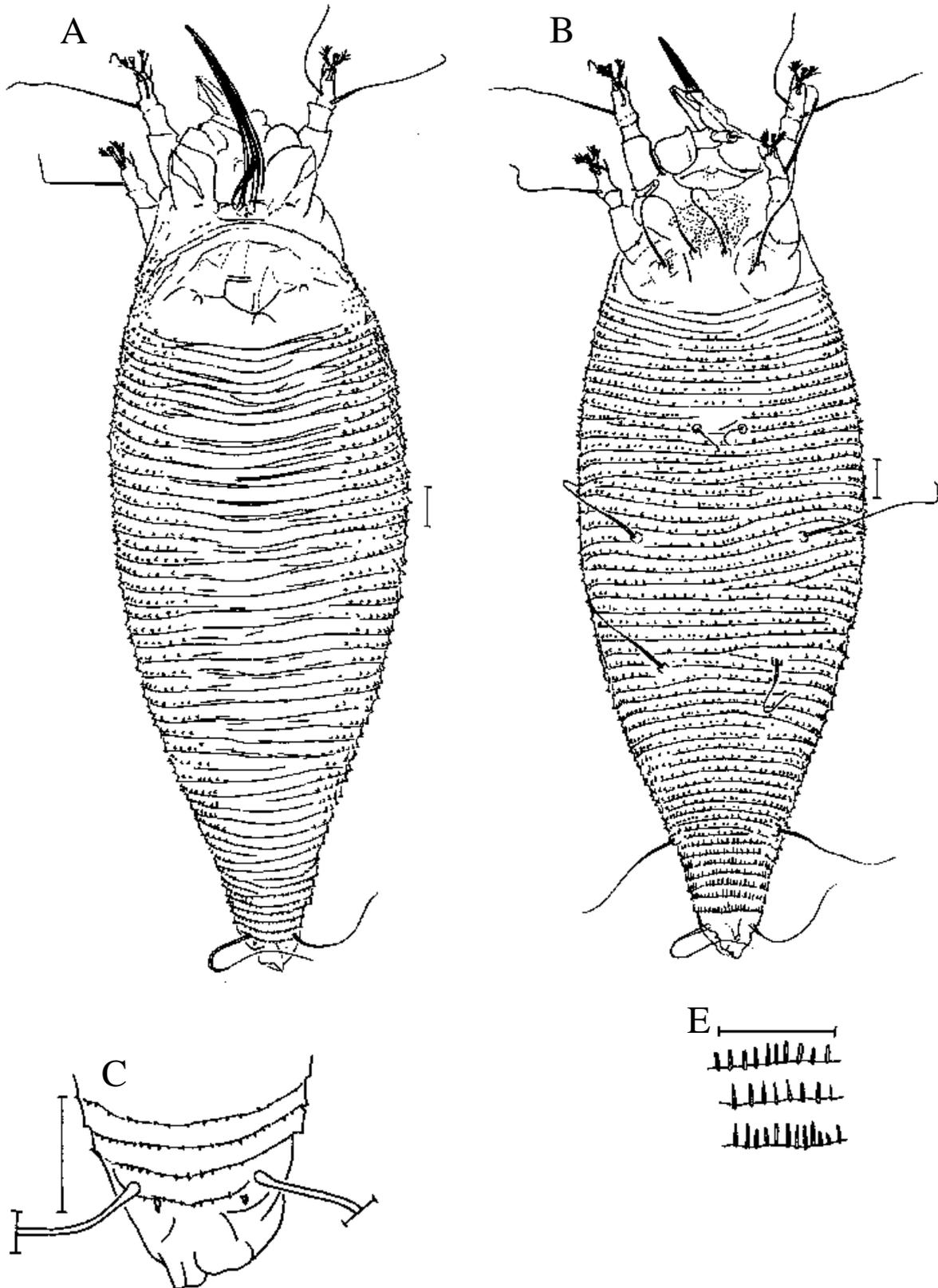
**Figure 6.** *Diptilomiopus faurius* sp. nov. Female. A, D. coxi-genital area; B. internal genitalia; C. external genitalia to show the three dimensional profile in lateral view; E, F. genital coverflap. Male. G, H. coxi-genital area; I. ventral view of legs, gnathosoma, and anterior part of coxi-sternal area.



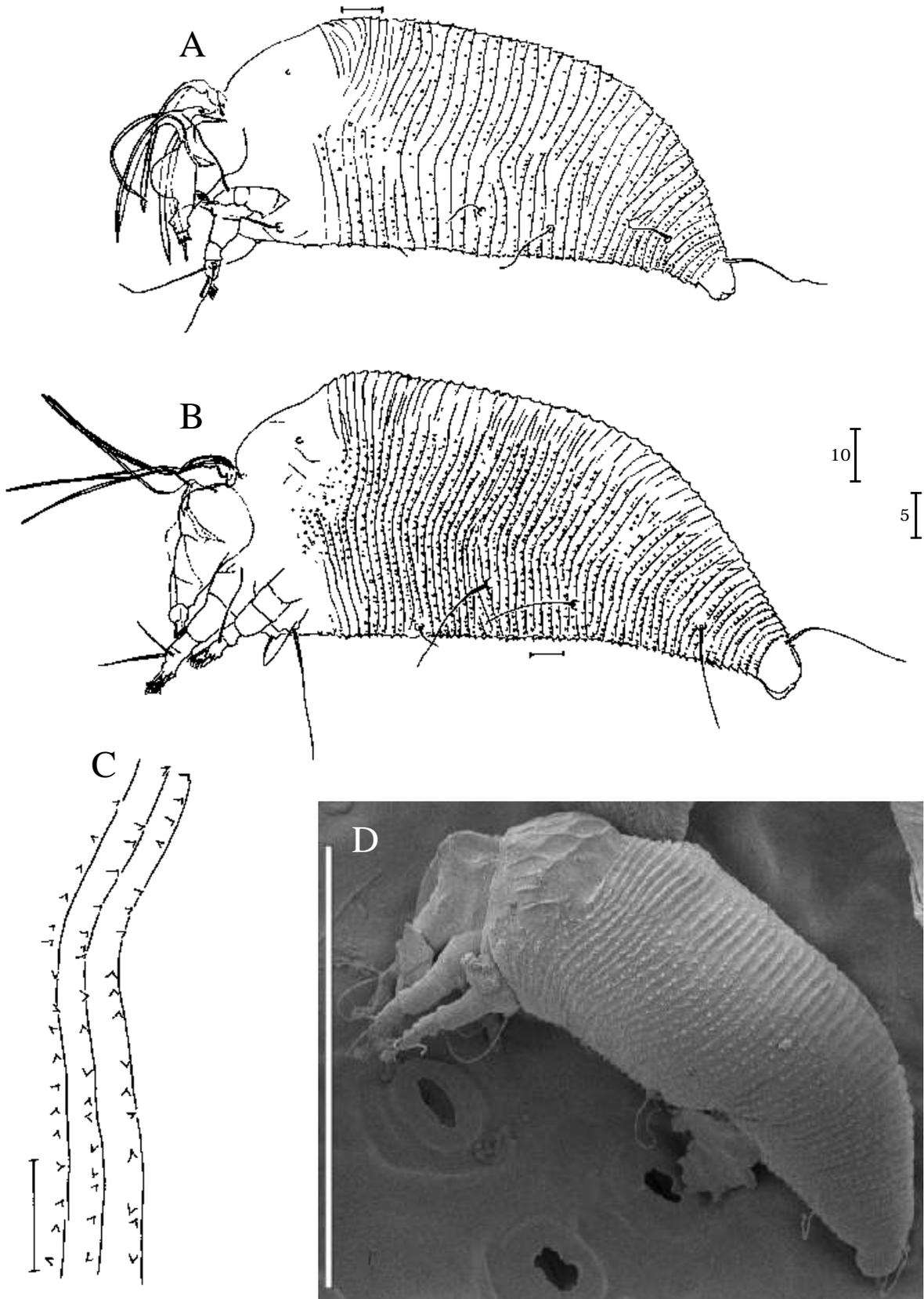
**Figure 7.** *Diptilomiopus faurius* sp. nov., female. **A.** lateral view of leg I (above) and II (below); **B.** oblique view of tibia; **C.** lateral adaxial view of leg I; **D.** lateral adaxial view of leg II. **E.** dorsal view of leg I (left) and II (right); **F.** ventral view of leg I (left) and II (right) to illustrate the pattern of ridges on femur-genu I and II, ridges more pronounced on femur-genu I than on femur-genu II; **G.** most complete ridge pattern on femur-genu I; **H.** ridge pattern on femur-genu II more pronounced in comparison with leg II in F of this figure.



**Figure 8. *Diptilomiopus faurius* sp. nov. Female empodium. A.** oblique view of tarsus of leg I; **B.** distal part of tarsus I with empodium, solenidion and seta *u'*, empodium seems to be 7-rayed, but may have an unbranched basal 8th ray; **C.** distal part of tarsus I, note the difficulty in counting the number of rays particularly when the empodium lies in a dorso-ventral position. **Larva. D.** dorsal view; **E.** ventral view; **F.** opisthosomal microtubercles in mid-dorsal area; **G.** dorsal view of caudum; **H.** opisthosomal microtubercles ventrally beyond setae *f*; **I.** opisthosomal microtubercles mid-ventral area; .



**Figure 9.** *Diptilomiopus faurius* sp. nov., nymph. **A.** dorsal view; **B.** ventral view; **C.** dorsal view of caudal region; **D.** opisthosomal microtubercles in lateral area of middorsal region on the level of setae *d*; **E.** opisthosomal microtubercles ventrally on last three caudal annuli; **F.** opisthosomal microtubercles ventrally between setae *d*.



**Figure 10.** *Diptilomiopus faurius* sp. nov., larva. **A.** lateral view, nymph. **B.** lateral view; **C.** opisthosomal microtubercles in area between setae *d* and *e* of specimen in lateral view; **D.** oblique view.

**Prodorsal shield** (Figs 3A–E) – broadly oval and short, with the characteristic convex shape with declivitous rear area, of other *Diptilomiopus* spp. (Fig. 3A). Shield ornamentation is formed by ridges in a cell-like pattern (Figs 3A,B,D) consisting broadly of three rows of cells numbered here as follows: 12 cells (A1L–A6L and A1R–A6R) in anterior row, six cells (B1L–B3L and B1R–B3R) in second row and two cells (C1L and C1R) in basal row; two open cell-like areas, D1L and D1R are formed at the base of the shield pattern on the declivitous basal part of the shield (Fig. 3E). Due to the compression of this area on the slides, D1L and D1R are not always clearly present on the slide-mounted specimens (Fig. 2A). Although all cells are typically present in all specimens, the shield pattern is not exactly symmetrical around the middle and cell shapes varies in different specimens (compare cells of Figs 3A,B,D). Sub-shield lateral area with granules somewhat arranged in lines (Fig. 3B), sometimes forming a loose circular pattern in an area more towards the legs and just above the coxae.

Frontal lobe present (Fig. 3A), but not overhanging the basal parts of the chelicerae which is necessary for regarding the frontal lobe as being present as required by the keys to genera and species in the Eriophyoidea, and additionally will probably be regarded by most authors as being absent, similar to other published *Diptilomiopus* spp. descriptions, because it is almost completely inconspicuous in slide-mounted specimens (Figs 2A,B; 3D; 5E). In SEM images a transversely broad but longitudinally narrow and probably thin and flexible frontal lobe with a slight, broad indentation of the anterior margin is unambiguously present (Figs 3A,B; 4E).

Scapular setal tubercles are present, relatively small, rounded and ahead of rear shield margin (Figs 2A,B,D; 3A–D; 5E). In some slide-mounted specimens it seemed as if there is a slight bump or slightly darker spot in the center of the tubercles. This could not be conclusively determined to be a bump on the tubercle, or distortion of the tubercle by the slide mounting process, or possibly a remnant of *sc*. Although indications of this “bump” are present in some SEM images (Fig. 3C), the exact nature of this feature cannot be conclusively determined here either, because resolution is too poor, and presence or possible presence of ice crystals obscures and confuses the detailed morphology. With evidence at hand and for practical reasons I regard *sc* absent.

**Opisthosoma** – Evenly rounded with a shallow, central ridge, flanked by shallow troughs and a barely discernable subdorsal ridge on either side, all fading towards the rear, absent from about annulus 44 onwards. In some of the slide-mounted specimens the ridges and troughs are barely visible (Fig. 2B), and the outer ridges are mostly undetectable, and in SEM imaged specimens they are more conspicuous to various degrees (Fig. 2D).

Ventral annuli on average about 13 annuli more than dorsal annuli (Table 2). Microtubercles on dorsal annuli: absent in a central band, flanked by microtubercles on each side (Figs 2A,B,D; 3H). Dorsolateral opisthosomal microtubercles, smaller, more sparsely spaced than on ventral annuli (Figs 5A,E), and situated on the rear annulus margins (Fig. 3G). In slide-mounted specimens these dorsal microtubercles appears more elongated with the proximal part beneath the dorsal surface, lightly coloured, with the apical point exposed on the rear annulus margin (Fig. 3G), the elongated submerged part becoming shorter and vaguer towards the rear. The last few rear dorsal annuli entirely covered with triangular, pointed microtubercles (Figs 3F,I). Opisthosomal microtubercles on ventral annuli pointed, about triangularly shaped in slide-mounted specimens, close to the rear edges of the annuli, becoming progressively elongated ridges from about the level of *f* (on the rear about 10 ventral annuli) (Figs 5A,C,D,F).

Seta *c2* absent; *d*, *e*, *f*, *h1* and *h2* present. Setae *d*, *e*, *f* and *h2* relatively long and finely tapered, *e* on average slightly shorter than *f*; *h1* very short, and may be interpreted as being minute (Figs 2C; 3I; 5B,E; Table 2).

**Legs** (Fig. 7) – 5-segmented, with genu absent, probably fused with the femur (Figs 7A,F).

Setal compliment of leg I (trochanter-[femur-genu]-tibia-tarsus): 0– [0–0] –0–3 (*bv*, *l''* and *l'* absent; *ft''*, *ft'* and *u'* present). Setal compliment of leg II (trochanter-[femur-genu]-tibia-tarsus): 0– [0–0] –0–2 (*bv*, *l''*, and *ft'* absent; *ft''* and *u'* present)

Setae-*ft* in both legs long and tapering, *u'* possibly curved or even with a sharp bent, apparent in some specimens (Fig. 8A), appearing about straight in others (Fig. 7C). This may be due to the angle in which the specimens were lying.

Outer surfaces of the coxae below the subshield lateral area ornamented with tubercles and with two ridges on this area of coxae I (Fig. 7A); ventral surface of trochanters I and II with a transverse ridge along the margin with the femorogenua (Figs 7G,H); femorogenua I and II ornamented with a pattern of ridges on the ventral surfaces (Figs 7F–H), usually more pronounced on femorogenu I (Fig. 7G), sometimes causing the illusion of a division of this segment; ventral ridge ornamentation on femorogenu II varying from nearly absent (Fig. 7F) to well developed (Fig. 7H). In slide-mounted specimens the ventral ornamentation on the femorogenua of both legs range from invisible to inconspicuous in most specimens, however discernable in some (Fig. 5B). Shape and structures of tibiae I and II are well discernable in SEM images with two ridges in the length dorsally terminating in well developed spines distally above the positions of *ft*-setae of tarsi I and II (*ft'* absent in tarsus II) (Figs 7A,B,E); shape and structure less discernable in slide-mounted specimens (Figs 2A,B; 7C,D) where the spines and one longitudinal line representing one of the ridges are sometimes visible.

Tarsal solenidion  $\omega$ , noticeably knobbed with a relatively large knob and slightly curved (Figs 7E; 8B,C). The knob is clearly separated from the shaft, but it is not apparent whether it is separated by a membrane, or whether it is just the fold of the knob creating an illusion of separation. Tarsal empodium *em* deeply and unambiguously divided (Figs 7A,C,E; 8A–C). The rays lie in an angle against the central stem of each branch of the divided empodium, especially at the base of the empodium, and the rays could not be counted accurately on the slide-mounted specimens or most of the SEM images, particularly in dorsal view, and has not been statistically dealt with. The empodia probably 7- or 8-rayed (when 8-rayed, the seventh but especially the eighth ray is inconspicuous and not always easy to discern) (Figs 7A,E; 8B,C).

**Coxae, coxigenital region and genitalia** (Figs 5B; 6A–F) – Suboral plate mostly smooth or with some granules, with convexly rounded central area (Figs 6A,D); coxisternal plates I and II with rounded to short dash-like granules: almost covering coxisternal plates I entirely, more sparsely on coxisternal plates II in a small area anterior to *2a* (Figs 6A,D); coxisternal plates I separated by a weak sternal apodeme for most of the inner margin, ending in a diamond-shaped area anteriorly (Fig. 6A).

Setae *1a* and *2a* “normal” (simple and tapering), relatively long and frequently convoluted, with well-developed setal tubercles; *1a* ahead of an imaginary line through tubercles of *2a*. Seta *3a* tapering and probably flexible (Figs 6A,D–F).

Internal genitalia depicted in Fig. 6B; external genital coverflap basally with a slightly raised area, vaguely in the shape of two continuous transverse round areas, covered with granules and dashes that varies in shape and clarity between specimens, distally smooth, with a shallow notch in the rear margin (Figs 6A,D–F).

#### **MALE** (Fig. 6G–I).

Morphology, including measurements and counts, similar to female [including ornamentation in coxisternal area, internal apodeme (Figs 6G–I), and rib-pattern ventrally on femorogenua (Fig. 6I)], except for genitalia (Figs 6G,H), albeit some setae are slightly shorter or in the short range of the female setal lengths (Table 2). Opisthosoma – 57 dorsal and 57 ventral annuli. Genitalia – scattered microtubercles just below the eugenital setae, similar to most other eriophyoid species; eugenital setae plainly present in slide-mounted specimens and in SEM images (Figs 6G,H).

Two immature stages could be identified (recorded to date all eriophyoid species studied in this regard with two immature stages):

**LARVA** (Figs 8D–I; 10A).

Specimens identified to be first immature stages (larvae) by absence of external genitalia, with the smallest size and irregularity of annuli just posterior of rear shield margin (Figs 8D; 10A). The prodorsum mostly smooth with single fold lines (Fig. 8D), single granules lateral on subshield lateral area (Fig. 8D). Scapular tubercles similar to those in adult (Figs 8D; 10A). The irregular annuli dorsally, posterior of the prodorsum smooth (Fig. 8D), the remainder of the dorsal annuli with small rounded microtubercles near the rear annulus margins (Figs 8D,F), becoming more pointed and sitting more on the rear annulus margins on the rear annuli beyond about the level of *f* (Figs 8D,G). Ventral annuli with triangularly shaped pointed microtubercles further away from the rear annulus margins than the dorsal microtubercles (Figs 8E,I) becoming elongated on ventral annuli beyond *f* (Figs 6E,H), some areas around ventral setae more sparsely microtuberculated (Fig. 6E). Setal compliments the same as in adult, however setae generally much shorter (Table 2); empodium *em* with less rays than in adult.

**NYMPH** (Figs 9; 10B–D).

Specimens identified to be second immature stages (nymphs) by the absence of external genitalia, their size, regularity of annuli just posterior of rear shield margin and position of scapular setal tubercles. Opisthosoma – 55 dorsal annuli and 45 ventral annuli. No external genitalia present, only slight interruption of annuli where genitalia will be positioned in adult (Fig. 9B). Dorsal annuli with smooth central band flanked by microtubercles laterally (similar to adult), microtubercles sharply pointed, about triangular and situated close to the rear annulus margin (Figs 9A; 10B–D); ventral annuli with microtubercles similar in shape and size to the dorsal microtubercles (Fig. 9B), but possibly slightly further away from rear annuli margins than those on the dorsal annuli (Fig. 10C); microtubercles on ventral annuli becoming more elongated until elongated ridges on about the 7 rear annuli (Figs 9B,E). A central dorsal hump posterior of the dorsal shield which is characteristic of the species, among the new species described here, clearly apparent in specimens studied (Figs 10B,D). Setal compliments the same as in adult, however setae generally shorter than in adult and longer than in larva (Table 2); tarsal empodium *em* possibly 4-rayed (difficult to count).

**ETYMOLOGY** – The species name is derived from the host genus name, *Faurea*.

**HOST PLANT** – *Faurea rochetiana* (A. Rich.) Pic. Serm. (= *Faurea speciosa* (Welw.) Welw.), (Proteaceae) (Palgrave, 2002). Common names: Broad-leaved Beechwood (English), *Breëblaarboekenhout* (Afrikaans). It usually grows as a small, leafy tree, 4–7 meters high, in

mixed deciduous woodland, on low open hills or in hilly grassland. The plant has medicinal value as the extract of the roots can be used to treat diarrhea and ear infections (Palgrave, 2002).

TYPE DATA – Hunderd-and-eight type specimens on 17 slides (AcY: 11/223) from type locality and host, *Faurea rochetiana*, Long Tom Pass, Limpopo, South Africa (25.07S, 30.35E), date unknown, S. Nesor: holotype female and 4 paratype specimens (unsexable specimen – probably a female, 2 males and 1 nymph) on 1 slide; the holotype (female) can be distinguished by being in a dorso-ventral position; and 103 paratype specimens (about 78 females, 16 males, 5 nymphs and 4 larvae) on 16 slides.

RELATION TO HOST – The mites occurred commonly but sometimes in sparse numbers mostly on the undersurfaces of the leaves amongst natural leaf hairs. No obvious symptoms could be attributed to the mites.

### ***Diptilomiopus apobrevis* sp. nov.**

#### DIFFERENTIAL DIAGNOSIS.

*Diptilomiopus apobrevis* sp. nov. is differentiated from the other new *Diptilomiopus* spp. from South Africa in the key to Diptilomiopidae of South Africa (above). Differences between this new species and *D. gilibertiae* include: prodorsal shield pattern of *D. apobrevis* with cells D1L and D1R present, these are absent in *D. gilibertiae*; dorsal annuli of *D. apobrevis* sp. nov. with microtubercles absent in a central band, flanked by microtubercles on each side, and the empodium with 7–8 rays; while the dorsal annuli are entirely covered with microtubercles in *D. gilibertiae*, and its empodium has 6 rays.

Measurements and counts in Table 3.

### **FEMALE** (Figs 11–16; 17A,B)

**Idiosoma** – Fusiform to somewhat elongated fusiform (Figs 11A,B); a mixture of *Diptilomiopus apobrevis* and *D. apolongus* live together on leaves, and were not distinguishable from each other before being mounted on slides and studied by SEM: in life dark amber (orangey to salmon) (seemingly mostly adults) to light amber (seemingly immatures and younger adults); legs and palpi colourless and translucent; chelicerae (and probably some of the other gnathosomal stylets as well) and tarsal setae *ft* dark-brown (almost black).

Table 3. Measurements and counts on *Diptilomiopus apobrevis* sp. nov. Measured and counted 12 female specimens in dorsal/ventral view, some measurements and counts could not be taken on some specimens. The maximum amount of specimens (n) were included, including the holotype female.

character	holotype ♀	measurements and counts of ♀ structures (dorsal/ventral view) (rounded)							♀ lateral view (n=1)	♂ (n=1)	larva (n=1)	nymph (n=1)
		n	median	mean	SD	min	max	CV*				
<b>BODY SIZE</b>												
body length (pedipalpi included)	221	10	216	222	30	188	265	13.56		197	121	175
idiosomal length (gnathosoma excluded)	190	10	186	187	26	149	223	14.07	212	165	101	140
body width just behind prodorsum	89	10	95	94	5	86	100	5.09		78	60	74
body width at level of setae <i>f</i>	44	9	45	46	3	42	52	6.67		38	25	35
body thickness (just below genital flap)									99			
<b>GNATHOSOMA</b>												
length (dorsal) excluding stylets	46	10	46	44	7	33	52	15.95		44	30	43
length (lateral) excluding stylets									54			
seta <i>ep</i> (basal seta) length	3	12	3	3	1	3	4	15.08	3	3	3	3
seta <i>d</i> (antapical seta)	absent		absent						absent	absent	absent	absent
seta <i>v</i> (apico-ventral seta) length	5	11	5	5	1	5	6	9.60	4	4	6	5
chelicerae length	75	12	74	74	3	68	77	3.75	69	58	40	58
<b>PRODORSAL SHIELD</b>												
length	32	11	39	38	3	32	41	9.22	37	29	25	33
width	66	8	74	73	7	65	80	9.27		62	45	60
<b>anterior shield lobe</b>												
shield lobe length		5	3	3	1	2	5	41.59		1		
shield lobe width		4	26	26	1	25	28	4.89		20		
<b>sc setal tubercles (dorsal tubercles)</b>												
distance apart	23	10	28	27	3	23	32	10.84		19	20	29
length	2	5	2	2	< 1	1	2	25.36		1		
base width	2	5	2	2	0	2	2	0		1		
distance ahead of rear shield margin	7	10	10	9	2	6	11	17.09		5	8	10
<b>OPISTHOSOMA</b>												
number of dorsal microtubercles / 20 µm	0	12	0 (smooth)							0	6-10	0
number of ventral microtubercles / 20 µm	18	10	14	15	2	12	18	16.68		11	7/10µm	7
width of smooth band behind prodorsal shield	49	10	50	51	7	41	65	13.07		41	n/a	entirely smooth
position seta <i>d</i> (first ventral seta)	32	10	31	31	2	28	34	7.44	32	23	13	17
number of microtubercles between setae <i>d</i>	24	10	25	25	3	21	29	11.61		17	9	7
seta <i>d</i> length	13	11	12	12	1	10	13	7.10	12	10	9	8
setae <i>d</i> distance apart	39	10	44	45	5	39	54	12.37		34	24	30
position setae <i>e</i> (second ventral setae)	49	10	49	48	2	45	52	5.07	52	35	17	25

character	holotype ♀	measurements and counts of ♀ structures (dorsal/ventral view) (rounded)							♀ lateral view (n=1)	♂ (n=1)	larva (n=1)	nymph (n=1)
		n	median	mean	SD	min	max	CV*				
number of microtubercles between setae <i>e</i>	16	11	16	16	3	13	22	17.27		12	7	6
seta <i>e</i> length	11	11	11	10	1	8	12	14.12	11	9	4	7
setae <i>e</i> distance apart	24	10	28	27	3	22	31	10.57		19	18	19
position setae <i>f</i> (third ventral setae)	69	10	69	69	3	66	73	3.91		50	27	40
position setae <i>f</i> from rear	10	12	11	11	1	10	12	6.84		10	8	8
number of microtubercles between setae <i>f</i>	22	9	24	24	3	19	28	11.65		20	11	12
seta <i>f</i> length	42	11	44	44	2	40	48	5.47		36	14	30
setae <i>f</i> distance apart	34	9	36	36	2	34	40	5.44		34	21	24
seta <i>h2</i> (caudal seta) length	64	8	59	57	9	45	70	16.83		55	18	> 25
seta <i>h1</i> (accessory seta) length	1	11	1	1	0	1	1	0		1	< 1	< 1
number of dorsal annuli lateral to shield	3	8	4	3	1	2	4	22.50		3	4	0
number of dorsal annuli	64	9	64	63	2	60	67	3.75		54	40	51
number of annuli forming central ridge	47	8	39	37	8	21	47	21.17		32	n/a	36
total number of dorsal annuli	67	10	67	66	3	63	70	3.88		57	44	51
total number of ventral annuli	78	10	78	79	3	77	84	3.64		59	32	47
<i>COXAL AREA</i>												
sternal line length	11	4	11	10	1	9	11	9.54		invisible	absent	absent
coxal seta <i>1a</i> (2nd coxal seta) length	32	12	37	37	5	28	46	14.87	43	26	12	22
coxal setae <i>1a</i> distance apart	11	10	13	13	1	11	14	7.83		10	8	9
coxal seta <i>2a</i> (3rd coxal seta) length	49	10	57	55	5	47	61	10.11		31	20	35
coxal setae <i>2a</i> distance apart	35	10	37	37	2	33	39	5.82		25	22	26
distance setae <i>1a</i> to <i>2a</i>	5	10	6	6	2	5	10	26.26		5	4	5
number of complete annuli in coxi-genital region	7	8	6	6	2	3	8	26.42		5	5	11
number of half annuli in coxi-genital region	0 or 3	4	1	1	1	0	2	120.32				none
total number of annuli in coxi-genital region	7	8	6	6	1	5	8	18.77		5	5	11
<i>LEGS I</i>												
length (including coxa) including extremities	73	10	73	75	3	72	80	4.28		56	40	59
length (including coxa) excluding extremities	64	10	68	67	3	64	72	4.17		50	35	53
length (from base of trochanter)	41	12	42	42	2	37	45	5.25	42	30	21	30
trochanter length (dorsal)	8	10	8.5	8.3	1	7	10	13.03	7	6	5	7
femur-genu length	18	11	19	19	1	16	21	7.35		13	9	14
tibia length	8	12	9	9	1	8	9	6.12		8	4	5
tarsus length (excluding extremities)	10	12	11	11	1	10	14	10.40		11	6	10
seta <i>fi''</i> (lateral tarsal seta) length	38	12	39	39	1	37	41	3.30		32	17	28
seta <i>fi'</i> (dorsal tarsal seta) length	36	11	37	37	2	33	39	5.02		32	17	28
seta <i>u'</i> (mesal seta) length	7	12	7	7	1	5	9	15.08		5	4	6
solenidion $\omega$ (tarsal solenidion) length	8	12	8	8	1	7	8	6.93		5	4	6
em (tarsal empodium) length	10	12	11	11	1	8	12	11.05		8	5	8

character	holotype ♀	measurements and counts of ♀ structures (dorsal/ventral view) (rounded)							♀ lateral view (n=1)	♂ (n=1)	larva (n=1)	nymph (n=1)	
		n	median	mean	SD	min	max	CV*					
<i>LEGS II</i>													
length (including coxa) including extremities	60	10	60	61	2	58	65	3.97		46	34	46	
length (including coxa) excluding extremities	52	10	53	53	2	51	57	4.04		41	30	38	
length (from base of trochanter)	35	12	37	37	3	34	42	7.01		30	20	25	
trochanter length (dorsal)	6	11	7	7	1	6	10	14.18		6	5	5	
femur-genu length	15	12	15	16	2	14	19	9.81		15	7	11	
seta <i>bv</i> (femoral seta)	absent	12	absent							absent	absent	absent	absent
seta <i>l''</i> (genu seta)	absent	12	absent							absent	absent	absent	absent
tibia length	7	11	7	7	< 1	7	8	6.56		6	4	5	
tarsus length (excluding extremities)	11	12	11	11	< 1	10	11	2.70		9	6	7	
seta <i>ft''</i> (lateral tarsal seta) length	34	12	34	33	1	32	35	3.04		27	13	24	
seta <i>ft'</i> (dorsal tarsal seta) length	absent	12	absent							absent	absent	absent	absent
seta <i>u'</i> (mesal seta) length	5	12	6	6	1	5	8	15.77		5	4	5	
solenidion $\omega$ (tarsal solenidion) length	7	12	7	7	< 1	7	8	6.37		5	4	6	
em (tarsal empodium) length	10	11	10	11	1	9	12	9.90		9	5	7	
<i>EXTERNAL GENITALIA</i>													
genital coverflap width	29	10	30	31	2	27	34	7.07			n/a	n/a	
genital coverflap length	20	10	17	17	2	15	20	11.32			n/a	n/a	
male genitalia width										22	n/a	n/a	
male genital area length										19	n/a	n/a	
seta <i>3a</i> (genital seta) length	8	11	7	7	1	6	9	11.22		6	3	4	
setae <i>3a</i> distance apart	20	10	21	22	2	20	24	7.83		14	9	13	

**Gnathosoma** (Figs 12E–H & 13A) – Shape and structures largely determined on SEM images: about basal third to half of palpcoxal base directed anteriorly in the same direction as the long axis of the body, where after palpi and gnathosomal stylets bend ventrad perpendicularly to the long body axis, basal part with a slight depression (Figs 12F,G), but not as pronounced as in *D. faurius*.

Infracapitular guides are conspicuous, apparently freely projecting processes originating at the level of the distal margin of the trochanter-femur-genu, interlocked or lying close together and appearing as one elongated triangular structure in the ventral view of the gnathosoma of slide-mounted specimens (Fig. 13A,B).

The cheliceral retainer depicted in Fig. 15B.

Palp with *d* absent; *ep* and *v* present.

**Prodorsal shield** (Fig. 12A–D) – broadly oval and short, with the characteristic convex shape with declivitous rear area, of other *Diptilomiopus* species (Fig. 12A). Shield pattern is formed by ridges in a cell-like pattern (Figs 12A,B) consisting broadly of three rows of cells numbered here as follows: 12 cells (A1L–A6L and A1R–A6R) in anterior row, five cells (B1L and B1R fused to form one cell, B2L–B3L and B2R–B3R) in second row and two cells (C1L and C1R) in basal row. The ridge (part of median line) dividing B1L and B1R from each other in some *Diptilomiopus* species (e.g. *D. faurea*) is invisible or absent in slide-mounted specimens (Fig. 12B), however vaguely present in SEM images (Fig. 12A). Two open cell-like areas, D1L and D1R are formed at the base of the shield pattern on the declivitous basal part of the shield (Fig. 12D). Due to the compression of this area on the slides, D1L and D1R are not always clearly visible in the slide-mounted specimens (Fig. 12B). Subshield lateral area with granules somewhat arranged in lines (Figs 12G).

Frontal lobe present, broad transversely but narrow longitudinally and probably thin and flexible, varying from clearly to vaguely visible in slide-mounted specimens (Fig. 12B), and could be identified especially after becoming aware thereof in SEM images (Figs 12A,F).

Scapular setal tubercles are present, relatively small, rounded and ahead of rear shield margin (Figs 12A,B). In some slide-mounted specimens it seems as if there is a slight bump or slightly darker spot in the center of the tubercles. This could not be conclusively determined to be a bump on the tubercle, or distortion of the tubercle by the slide mounting process, or possibly a remnant of *sc*. Although indications of this “bump” are present in some SEM images (Fig. 12C), the exact nature of this feature cannot be conclusively determined here either, because resolution is too poor, images of this aspect captured at too low magnification, and presence or possible presence of ice crystals obscures and confuses the detailed morphology. With evidence at hand and for practical reasons I regard *sc* absent.

**Opisthosoma** – Evenly rounded with a shallow, central ridge, flanked by shallow troughs and a barely discernable subdorsal ridge on either side, all fading towards the rear, absent on average from annulus 37 onwards. In most of the slide-mounted specimens the outer ridges and troughs are barely visible (Fig. 11B), while in SEM imaged specimens they are more conspicuous (Fig. 11A).

Ventral annuli on average 13 annuli more than dorsal annuli (Table 3). Microtubercles on dorsal annuli: absent in a central band, flanked by microtubercles on each side (Figs 11F,G). Dorsolateral opisthosomal microtubercles, smaller, obviously more sparsely spaced than on ventral annuli (Figs 11B; 13E), and situated on the rear annulus margins (Figs 11B; 13E). In slide-mounted specimens they are fine and small and some appears slightly elongated with the proximal part beneath the dorsal surface, lightly coloured, with the slightly darker apical point exposed on the rear annulus margin (Fig. 11E). About six rear dorsal annuli entirely covered with triangular, pointed microtubercles (Figs 11D). Opisthosomal microtubercles on ventral annuli pointed, about triangularly shaped in slide-mounted specimens, close to the rear edges of the annuli, becoming progressively elongated ridges from about the level of *f* (on the rear about 10 ventral annuli) (Figs 13,E,F). Ventral laterally, from about the mid-distance between *d* and *e*, up to *f*, bordering the boarder between the dorsal and ventral annuli, a band of the ventral opisthosomal microtubercles appears, particularly on the slide-mounted specimens, to be more elongated than the remainder of the ventral microtubercles (Fig. 15E).

Setae *c2* absent; *d*, *e*, *f*, *h1* and *h2* present. Setae *f* and *h2* relatively long and finely tapered, *d* and *e* unmistakably quite shorter than *f*; *h1* very short, and may be interpreted as being minute (Figs 13B; 11C & Table 3).

**Legs** (Figs 16A–F) – 5-segmented, with genu absent, probably fused with the femur (Figs 16A,B).

Setal compliment of legs I (trochanter– [femur-genu] –tibia–tarsus): 0– [0–0] –0–3 (*bv*, *l''* and *l'* absent; three tarsal setae present: *ft''*, *ft'* and *u'*).

Setal compliment of legs II (trochanter– [femur-genu] –tibia–tarsus): 0– [0–0] –0–2 (*bv*, *l''* and *ft'* absent; two tarsal setae present: *ft''* and *u'*).

Setae *ft* in both legs of all slide-mounted specimens are bluntly truncated in comparison with the tapering *ft* usually found in eriophyoid species described to date, and it seems unlikely that all these setae broke off. The shape of *ft* could not be confirmed in the SEM images where these setae sunk into the glue of the double sided sticky carbon tape used to mount the mites on the stub.

Particularly femorogenua I (Fig. 16B,C; 17A) ornamented with a pattern of ridges on the ventral surfaces. In slide-mounted specimens the ventral ornamentation on the femorogenua of legs I ranges from invisible to inconspicuous in most specimens, however discernable in some (Figs 16C).

Tarsal solenidion  $\omega$ , noticeably knobbed and virtually straight (Figs 16H–E). The knob is clearly separated from the shaft, but it is not apparent whether it is separated by a membrane, or whether it is just the fold of the knob base creating an illusion of separation. Tarsal empodium *em* deeply and unambiguously divided (Figs 16H,G,K,J). The rays lie in an angle against the central stem of each branch of the divided empodium, especially at the base of the empodium, and the rays could not be counted accurately on the slide-mounted specimens or on most of the SEM images, particularly in dorsal view, and has not been statistically dealt with. The empodia mostly unambiguously 7- , but some 8-rayed (sometimes the seventh but especially the eighth ray is not easily seen).

**Coxae, coxi-genital region and genitalia** (Figs 14A,C–F) – Suboral plate mostly smooth (Fig. 14A,D); coxisternal plates I and II with rounded to very short dash-like (oval) granules: almost covering coxisternal plates I entirely (Figs 14A,C,D), coxisternal plates II with a few fine tubercles in a small area anterior to *2a* (Figs 14A,C); coxisternal plates I seems separated on slide-mounted specimens and SEM images for most of the inner margin, however the inner sternal apodeme(s) (sternal line) is either absent or too vague to be discerned clearly in slide-mounted specimens (Fig. 14A) and appear as a depression or narrow furrow on the dorsal surface terminating in a triangular area anteriorly in the SEM images (Fig. 14D).

Seta *1b* absent; *1a* and *2a* present and “normal” (simple and tapering), relatively long and frequently convoluted, with well-developed setal tubercles; *1a* ahead of an imaginary line through tubercles of *2a* (Fig. 14C).

Internal genitalia depicted in Figs 14B; external genital coverflap basally with a slightly raised area, vaguely in the shape of two continuous transverse rounded areas, covered with granules and dashes that varies in shape and clarity between specimens, distally smooth (Figs 14C,E,F).

Seta *3a* present, tapering and probably flexible (Fig. 14C).

**MALE** (Figs 14G; 17C–F).

Morphology, including measurements and counts, similar to female, except for genitalia (Figs 14G; 17C–F), albeit some setae are slightly shorter or in the short range of the female setal lengths (Table 3). Genitalia – scattered microtubercles or granules just below the eugenital setae similar to many other eriophyoid species described to date; eugenital setae not clearly visible, and their presence could not be ascertained without doubt. Two half-circular ridges are present, one on each side, where eugenital setae are usually situated, and these are probably associated with the eugenital setae, obscuring them. Centrally in these ridges in slide-mounted specimens is an

indication of seta-like structures (Fig. 17F) and in the SEM image, particularly on the ridge in the right hand side of the image, a possible seta-like structure can be seen (Figs 17D,E).

Two immature stages could be identified (recorded to date all eriophyoid species studied in this regard with two immature stages):

#### **LARVA** (Fig. 18).

Specimens identified to be first immature stages (larvae) by absence of external genitalia, the smallest size and smooth area and some half, rounded irregular annuli just posterior of rear shield margin (Figs 18B,K). The prodorsal shield with a central cell that is probably the combined B1L and B1R, about radial lines from the central cells, the basal three forming probably C1L and C1R (Figs 18B,K); subshield lateral area smooth (Fig. 18H). Scapular setal tubercles present and smaller but similar to those in adults (Fig. 18A,B). Some of the irregular annuli dorsally posterior of the prodorsum smooth (Fig. 18K), the remainder of the dorsal annuli with small rounded, vague microtubercles near the rear annulus margins (Fig. 18E) almost invisible in SEM image (Fig. 18K), becoming more pointed and sitting more on the rear annulus margins on the rear annuli beyond about the level of *f* and particularly on the rear four annuli (Fig. 18F). Ventral annuli smooth laterally with microtubercles in a central band in the area margined by *3a*, *d*, *e*, and *f*, from *f* to the rear the ventral annuli are entirely microtuberculate; the ventral opisthosomal microtubercles are triangularly shaped and pointed, situated further away from the rear annulus margins than the dorsal microtubercles (Figs 18C,F,J) becoming elongated on ventral annuli beyond *f* (Fig. 18I), but particularly on the rear three annuli (Fig. 18G). All setal compliments the same as in adult, however setae generally much shorter (Table 3); empodial (*em*) possibly 4-rayed (difficult to count), however, definitely less rays than those in adult.

#### **NYMPH** (Figs 19; 20).

Specimens identified to be second immature stages (nymphs) by the absence of external genitalia, their size, regularity of annuli just posterior of rear shield margin and body shape. Opisthosoma – ... dorsal annuli and ... ventral annuli. No external genitalia present, only a discontinuance in annuli where genitalia will be in adult. A shallow middorsal ridge is visible in some specimens (Fig. 19A). First few dorsal annuli posterior of prodorsum entirely smooth, thereafter a lateral band of microtubercles on each side becoming broader towards the middorsum, until annuli are entirely microtuberculate from about 14<sup>th</sup> annulus from the rear (Figs 19A; 20A,C), microtubercles sharply pointed, about triangular and situated on or close to the rear annulus margin (Fig. 19C) becoming more densely spaced and slightly more elongated (in the slide-mounted specimens) towards the rear (Figs 19A,D); ventral annuli with microtuberculate with smooth areas in parts particularly around future genitalia, and ahead of *d* and *e* (Fig. 19B), microtubercles triangular and pointed,

slightly larger and more densely spaced than dorsal microtubercles (Fig. 19E) becoming more elongated until elongated ridges on about the 8 rear annuli (Figs 19B,F). All setal compliments the same as in adult, however setae generally shorter than in adult and longer than in larva (Table 3); tarsal empodium *em* probably 5-rayed (Fig. 20B) (difficult to count), however, definitely less rays than those in adult. Tarsal solenidion similar in shape as in adult, with a clear central longitudinal line in some (Fig. 20C).

**ETYMOLOGY** – The species name is a combination of the first syllable of the host genus name, *Apodytes*, and the Latin word *brevis*, which means short, referring to the short body setae in comparison with the other *Diptilomiopus* sp. found on this host.

**HOST PLANT** – *Apodytes dimidiata* E.Mey. ex Arn. (Icacinaceae) (Palgrave, 2002). Common names: White-pear (English), *Witpeer* (Afrikaans). It frequently is a small bushy tree 4–5 m tall, but can become 20 m tall in forests. It occurs in coastal evergreen bush, at the margins of medium-altitude evergreen forests, in riverine fringes and open woodland, and on grassy mountain slopes, often among rocks. It has medicinal value, and the wood is suitable for agricultural implements and furniture. It is an attractive tree used as garden ornamentals. (Palgrave, 2002).

**TYPE DATA** – Eighty-six type specimens on 20 slides (AcY: 11/222) from type locality and host, *A. dimidiata*, Botanical Garden, Nelspruit, Mpumalanga, South Africa (25.28S, 30.59E), date unknown, A. Witt with directions from S. Nesar (X03/130): holotype female and 3 paratype females on 1 slide, together with 1 female paratype of *Diptilomipus apolongus* sp. nov.; the holotype can be distinguished by ; and 82 paratype specimens (31 females, 13 males, 29 nymphs and 9 larvae) on 19 slides. Twenty-two paratype specimens of *D. apolongus* sp. nov. (8 females, 8 males, 3 nymphs and 3 larvae) are present on 11 of the 19 slides. Three paratype specimens on 2 of 10 slides (AcY: 11/224) from *Apodytes dimidiata* (planted for ornamental purposes), Baberton Road, Nelspruit, Mpumalanga, South Africa (25.28S, 30.59E), 30 June 1988, S. Nesar (NF1415, X88/148): 1 female, male and larva; with 45 *D. apolongus* type specimens, 5 specimens of a *cf. Tetra* sp. with 4-rayed empodia, and 9 specimens of another Phyllocoptinae species with 6-rayed empodia.

**RELATION TO HOST** – The mites occurred sparsely (many leaves devoid of them, and further about one to three specimens per leaf) on the underside of the leaves, in many cases close to or even on inner walls of turret galls probably caused by an insect. Mites also occurred on healthy looking leaves without any galls or other symptoms. No symptoms could be attributed to the mites.

## DESCRIPTION

### *Diptilomiopus apolongus* sp. nov.

#### DIFFERENTIAL DIAGNOSIS.

*Diptilomiopus apolongus* sp. nov. is differentiated from the other new *Diptilomiopus* spp. from South Africa in the key to Diptilomiopidae of South Africa (above). Differences between this new species and *D. gilibertiae* include: dorsal annuli of *D. apobrevis* sp. nov. with microtubercles absent in a central band, flanked by microtubercles on each side, and the empodium with 7–8 rays; while the dorsal annuli are entirely covered with microtubercles in *D. gilibertiae*, and its empodium has 6 rays.

Measurements and counts in Table 4. No SEM studies were done on this species, and it is described entirely from slide-mounted specimens, except colour.

### FEMALE (Figs 21; 22B-F)

**Idiosoma** – Fusiform to somewhat elongated fusiform (Fig. 21A,F); a mixture of *Diptilomiopus apobrevis* and *D. apolongus* living together on leaves, the two species were not distinguishable from each other before being mounted on slides and studied by SEM: colour in life dark amber (orangy to salmon) (seemingly mostly adults) to light amber (seemingly immatures and younger adults); legs and palpi colourless and translucent; chelicerae (and probably some of the other gnathosomal stylets as well) and fastigial tarsal setae dark-brown (almost black).

**Gnathosoma** (Figs 21A,F; 22F) – about basal third to half of palpcoxal base directed anteriorly in the same direction as the long axis of the body, where after palpi and gnathosomal stylets bend ventrad perpendicularly to the long body axis (Fig. 22F). Doesn't seem to have the pronounced depression on basal part as in *D. faurea*, because no obvious signs of such a depression similar to that seen on the outer edge of the basal part of the palpcoxal base present.

Infracapitular guides are conspicuous, apparently freely projecting processes originating at the level of the distal margin of the trochanter-femur-genu, interlocked or lying close together and appearing as one elongated triangular structure in the ventral view of the gnathosoma (Fig. 21F).

Palpi with palp *d* absent; *ep* and *v* present (Fig. 22F).

Table 4. Measurements and counts on *Diptilomiopus apolongus* sp. nov. Too few specimens in dorsal/ventral view to count and measure 12 specimens, thus included all usable mounted adult female specimens available, of which 8 specimens were more or less in a dorsal/ventral view, and 8 specimens in a lateral view. Some measurements may differ in lateral and dorsal view, and in the cases where the CV\* became larger with inclusion of lateral view specimens (e.g. body length), the data of only 8 specimens (dorsal view specimens) were included. ? = measurement/count unknown.

character	holotype ♀	measurements and counts of ♀ structures (dorsal/ventral view) (rounded)							♀ lateral view (n=1)	♂ (n=1)	larva (n=1)	nymph (n=1)	
		n	median	mean	SD	min	max	CV*					
<b>BODY SIZE</b>													
body length (pedipalpi included)	287	8	284	280	25	232	311	9.14		231	177	174	
idiosomal length (gnathosoma excluded)	225	8	222	226	26	195	261	11.51		202	146	148	
body width just behind prodorsum	94	7	91	92	4	87	98	4.05		76	68	78	
body width at level of setae <i>f</i>	48	5	48	47	2	44	49	5.28		35	29	35	
body thickness (just below genital flap)									95				
<b>GNATHOSOMA</b>													
length (dorsal) excluding stylets	50	6	57	56	7	45	65	13.31		32	31	44	
length (lateral) excluding stylets									71				
seta <i>ep</i> (basal seta) length	3	16	4	4	1	3	4	14.08		4	2	3	
seta <i>d</i> (antapical seta)	absent	16	absent						absent	absent	absent	absent	
seta <i>v</i> (apico-ventral seta) length	6	8	6	6	< 1	5	6	6.14		?	5	5	
chelicerae length	85	16	85	85	4	81	98	4.88		76	50	66	
<b>PRODORSAL SHIELD</b>													
length	35	8	39	38	2	35	42	5.68		34	31	33	
width	63	8	71	72	7	63	80	10.50		58	58	60	
<b>anterior shield lobe</b>													
shield lobe length	2	11	2	2	1	2	3	21.72		2		absent	
shield lobe width	15	6	27	25	7	15	34	29.18		23			
<b>sc setal tubercles (dorsal tubercles)</b>													
distance apart	26	8	28	28	2	25	30	6.82		24	24	30	
length	1	8	2	2	1	1	2	32.51		1	1	1	
base width	2	12	2	2	0	2	2	0		2	2	2	
distance ahead of rear shield margin	6	13	7	8	2	6	13	28.39		6	10	14	
<b>OPISTHOSOMA</b>													
<b>number of dorsal microtubercles / 20 µm</b>	0	8	0 (smooth)								0	6	0
number of ventral microtubercles / 20 µm	8	7	8	10	2	8	13	22.92		11	6	8	
width of smooth band behind prodorsal shield	40	8	42	42	3	39	47	7.04		37	n/a	38	
<b>position seta <i>d</i> (first ventral seta)</b>													
position seta <i>d</i> (first ventral seta)	29	15	31	31	2	28	35	7.25		23	12	20	
number of microtubercles between setae <i>d</i>	23	6	23	23	4	18	29	16.35		?	8	11	
seta <i>d</i> length	60	8	59	58	4	53	63	6.42		45	25	36	
setae <i>d</i> distance apart	56	6	55	53	4	49	57	6.70		?	35	40	

character	holotype ♀	measurements and counts of ♀ structures (dorsal/ventral view) (rounded)							♀ lateral view (n=1)	♂ (n=1)	larva (n=1)	nymph (n=1)
		n	median	mean	SD	min	max	CV*				
position setae <i>e</i> (second ventral setae)	48	14	50	49	3	44	54	6.32		37	16	29
number of microtubercles between setae <i>e</i>	14	6	15	15	1	13	16	8.43		11	6	9
seta <i>e</i> length	55	16	54	53	4	47	59	7.22		42	21	27
setae <i>e</i> distance apart	36	6	35	34	3	30	36	7.83		28	21	28
position setae <i>f</i> (third ventral setae)	66	15	68	68	4	59	73	6.49		53	25	41
position setae <i>f</i> from rear	12	16	12	12	1	10	13	5.66		13	6	9
number of microtubercles between setae <i>f</i>	17	5	21	22	5	17	27	21.31		?	8	16
seta <i>f</i> length	43	8	45	46	2	43	48	4.13		35	17	31
setae <i>f</i> distance apart	37	5	37	37	2	33	39	6.33		?	22	30
seta <i>h2</i> (caudal seta) length	100	12	86	84	11	59	100	13.69		?	34	53
seta <i>h1</i> (accessory seta) length	1	16	1	1	< 1	1	2	30.99		< 1	< 1	1
number of dorsal annuli lateral to shield	4	8	4	4	1	3	5	16.88		3	3	3
number of dorsal annuli	61	14	59	58	3	54	62	4.32		55	39	46
number of annuli forming central ridge	34	5	33	30	7	18	34	23.34		?	none	about 14
total number of dorsal annuli	65	14	62	62	3	57	66	4.49		58	42	49
total number of ventral annuli	78	15	79	79	5	68	84	5.98		65	30	49
<i>COXAL AREA</i>												
sternal line length	12	5	12	11	1	9	12	11.88		5	absent	absent
coxal seta <i>1a</i> (2nd coxal seta) length	36	8	46	45	6	36	55	14.35		41	15	21
coxal setae <i>1a</i> distance apart	12	8	12	11	2	9	14	16.63		10	7	8
coxal seta <i>2a</i> (3rd coxal seta) length	56	8	61	61	5	53	68	8.15		45	24	47
coxal setae <i>2a</i> distance apart	28	8	31	32	6	26	43	17.67		28	21	29
Distance setae <i>1a</i> to <i>2a</i>	8	8	8	8	2	6	11	23.62		6	5	7
number of complete annuli in coxi-genital region	4	6	5	5	1	4	7	19.77		5	about 7	about 10
number of half annuli in coxi-genital region	2	6	2	2	< 1	2	3	18.23		0?	none	none
total number of annuli in coxi-genital region	6	5	8	8	1	8	9	15.31		5	about 7	about 10
<i>LEGS I</i>												
length (including coxa) including extremities	76	8	85	84	8	74	92	9.79		73	48	67
length (including coxa) excluding extremities	67	8	76	75	9	65	86	12.03		65	44	58
length (from base of trochanter)	45	16	49	49	3	45	57	6.71		44	27	35
trochanter length (dorsal)	8	14	9	10	2	8	15	20.54		8	4	6
femur-genu length	20	16	21	21	1	18	24	6.29		19	12	15
tibia length	10	8	10	10	1	9	11	5.46		10	6	8
tarsus length (excluding extremities)	14	16	15	15	1	14	17	6.59		13	7	11
seta <i>ft''</i> (lateral tarsal seta) length	45	8	47	47	2	43	49	4.17		40	27	32
seta <i>ft'</i> (dorsal tarsal seta) length	40	16	41	41	1	39	44	3.03		35	25	28
seta <i>u'</i> (mesal seta) length	6	13	7	7	1	6	8	11.94		7	4	5
solenidion $\omega$ (tarsal solenidion) length	9	16	9	9	1	8	10	5.75		8	5	7
em (tarsal empodium) length	12	15	12	12	1	9	13	8.67		10	6	8

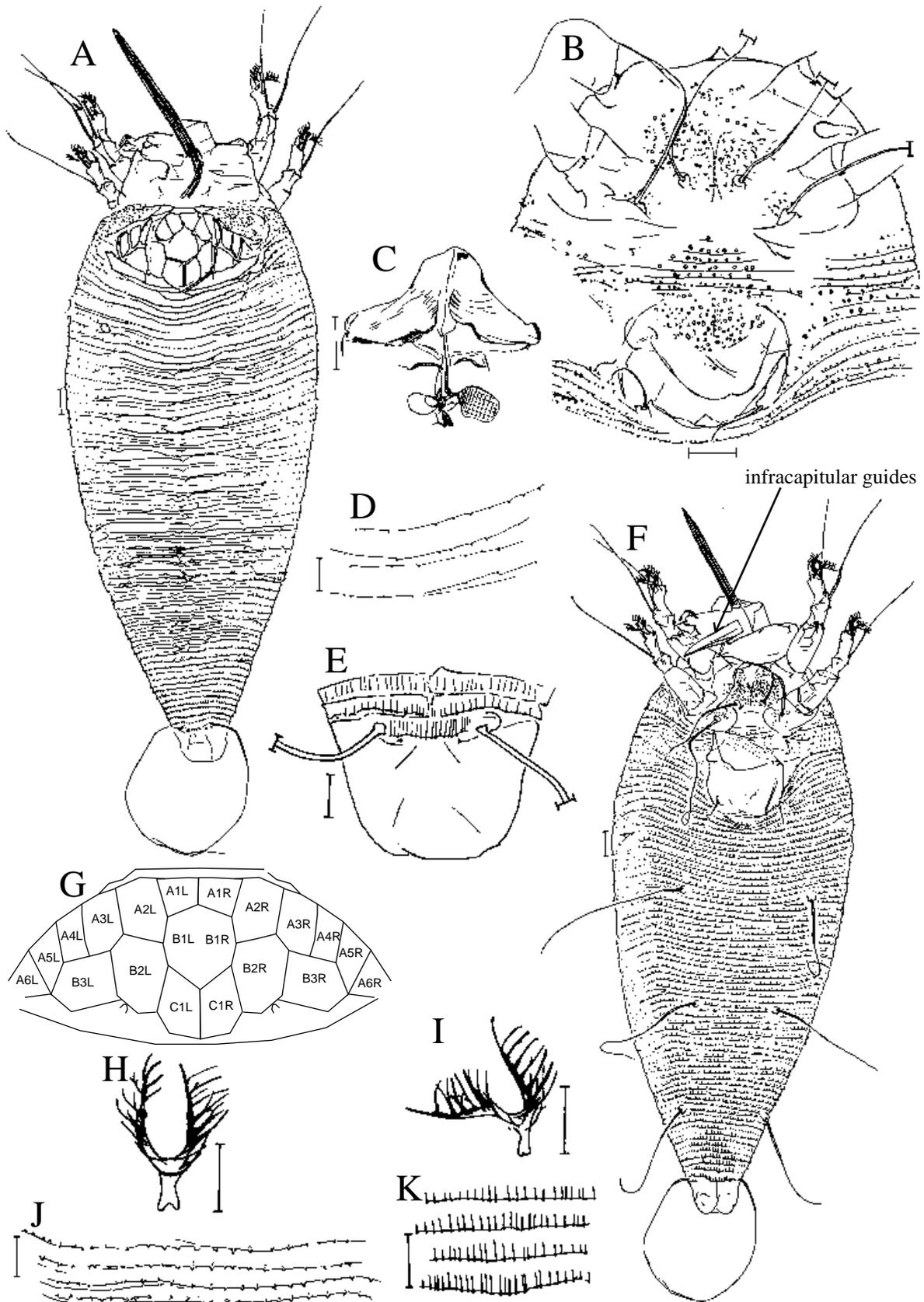
character	holotype ♀	measurements and counts of ♀ structures (dorsal/ventral view) (rounded)							♀ lateral view (n=1)	♂ (n=1)	larva (n=1)	nymph (n=1)	
		n	median	mean	SD	min	max	CV*					
<i>LEGS II</i>													
length (including coxa) including extremities	66	7	68	68	5	62	74	7.45		57	39	51	
length (including coxa) excluding extremities	56	7	58	59	5	54	65	7.94		50	35	45	
length (from base of trochanter)	42	14	43	43	2	39	47	5.19		37	25	33	
trochanter length (dorsal)	10	14	9	9	1	7	11	13.58		7	5	5	
femur-genu length	19	15	19	19	1	17	20	5.79		16	10	12	
tibia length	8	8	8	9	1	8	10	12.08		8	4	6	
tarsus length (excluding extremities)	12	8	14	14	1	12	14	5.25		11	6	9	
seta <i>ft''</i> (lateral tarsal seta) length	42	16	41	41	2	39	45	4.07		36	20	26	
seta <i>ft'</i> (dorsal tarsal seta) length	absent	16	absent							absent	absent	absent	absent
seta <i>u'</i> (mesal seta) length	5	8	7	7	1	5	7	11.87		5	3	4	
solenidion $\omega$ (tarsal solenidion) length	8	15	8	8	1	8	9	6.16		7	5	6	
em (tarsal empodium) length	11	8	11	11	< 1	10	11	4.40		9	6	9	
<i>EXTERNAL GENITALIA</i>													
genital coverflap width	33	8	36	36	4	30	43	11.82		n/a	n/a	n/a	
genital coverflap length	20	6	22	22	3	19	28	15.48		n/a	n/a	n/a	
male genitalia width	n/a									25	n/a	n/a	
male genital area length	n/a									20	n/a	n/a	
seta <i>3a</i> (genital seta) length	10	16	12	12	1	10	13	9.05		9	5	7	
setae <i>3a</i> distance apart	26	8	26	27	3	22	33	12.59		18	11	16	

Measurements or counts that may have been influenced by position of the specimen were: lengths – body, idiosomal, prodorsal shield, gnathosomal seta *v*, *sc* setal tubercle, tibia, and seta *ft''* of leg 1, tibia, tarsus, seta *u'* and empodium of leg 2, coxal setae *1a* and *2a*, opisthosomal setae *d* and *f*, and number of dorsal annuli lateral to shield.

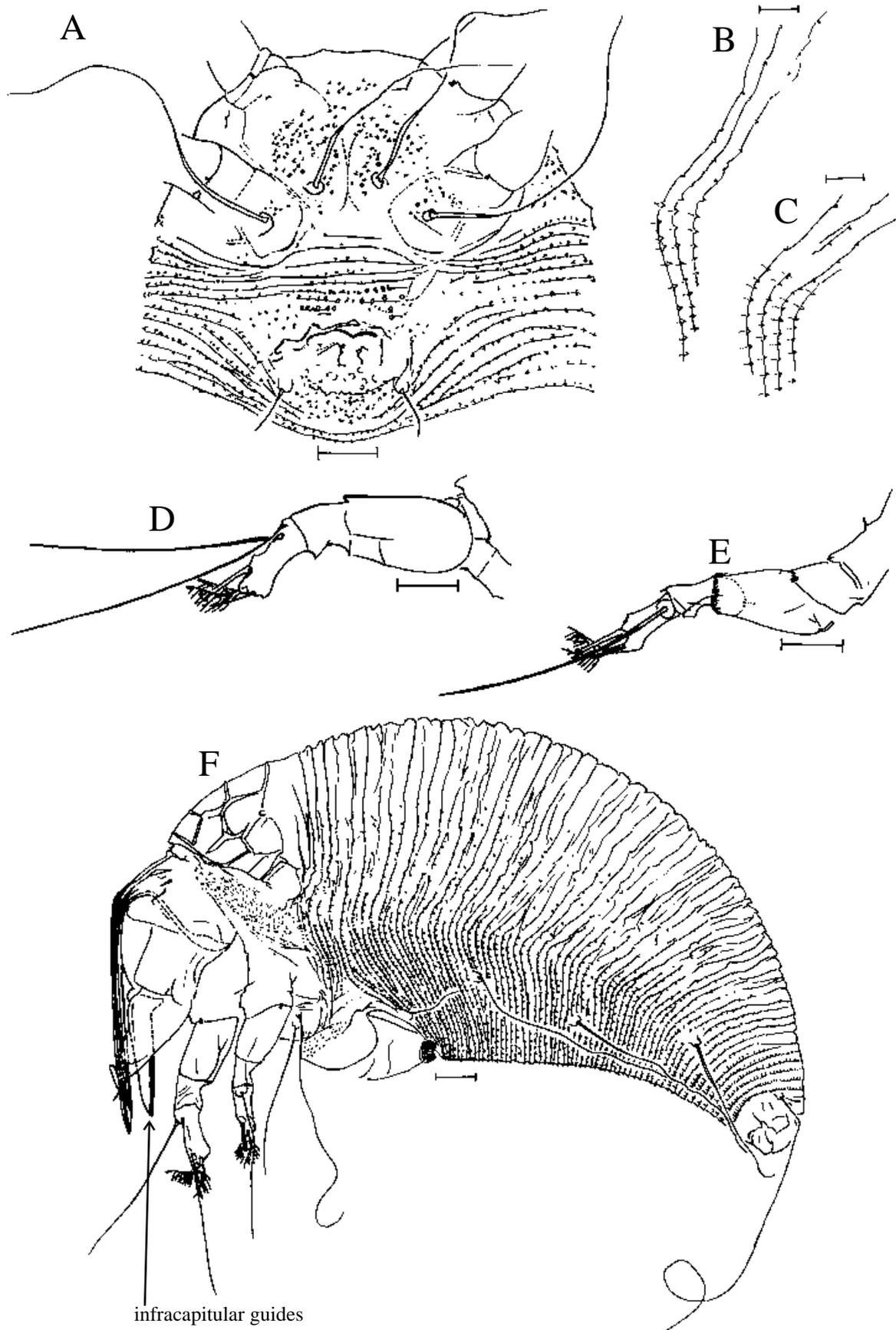
**Prodorsal shield** (Fig. 21A) – broadly oval and short, with the characteristic convex shape (Fig. 22F), presumably with declivitous rear area similar to some other *Diptilomiopus* spp. Shield pattern is formed by ridges in a cell-like pattern (Figs 21A,G). After studying several specimens it could be determined that the pattern consists broadly of three rows of cells numbered here as follows: 12 cells (A1L–A6L and A1R–A6R) in anterior row, with cells A6L and A6R incompletely formed, five cells (B1L and B1R fused to form one cell, B2L–B3L and B2R–B3R) in second row and two cells (C1L and C1R) in basal row. Subshield lateral area with granules and dashes arranged somewhat in lines (Fig. 22F).

Frontal lobe present, broad transversely but narrow longitudinally and probably thin and flexible, varying from clearly to vaguely visible (Figs 21A; 22F).

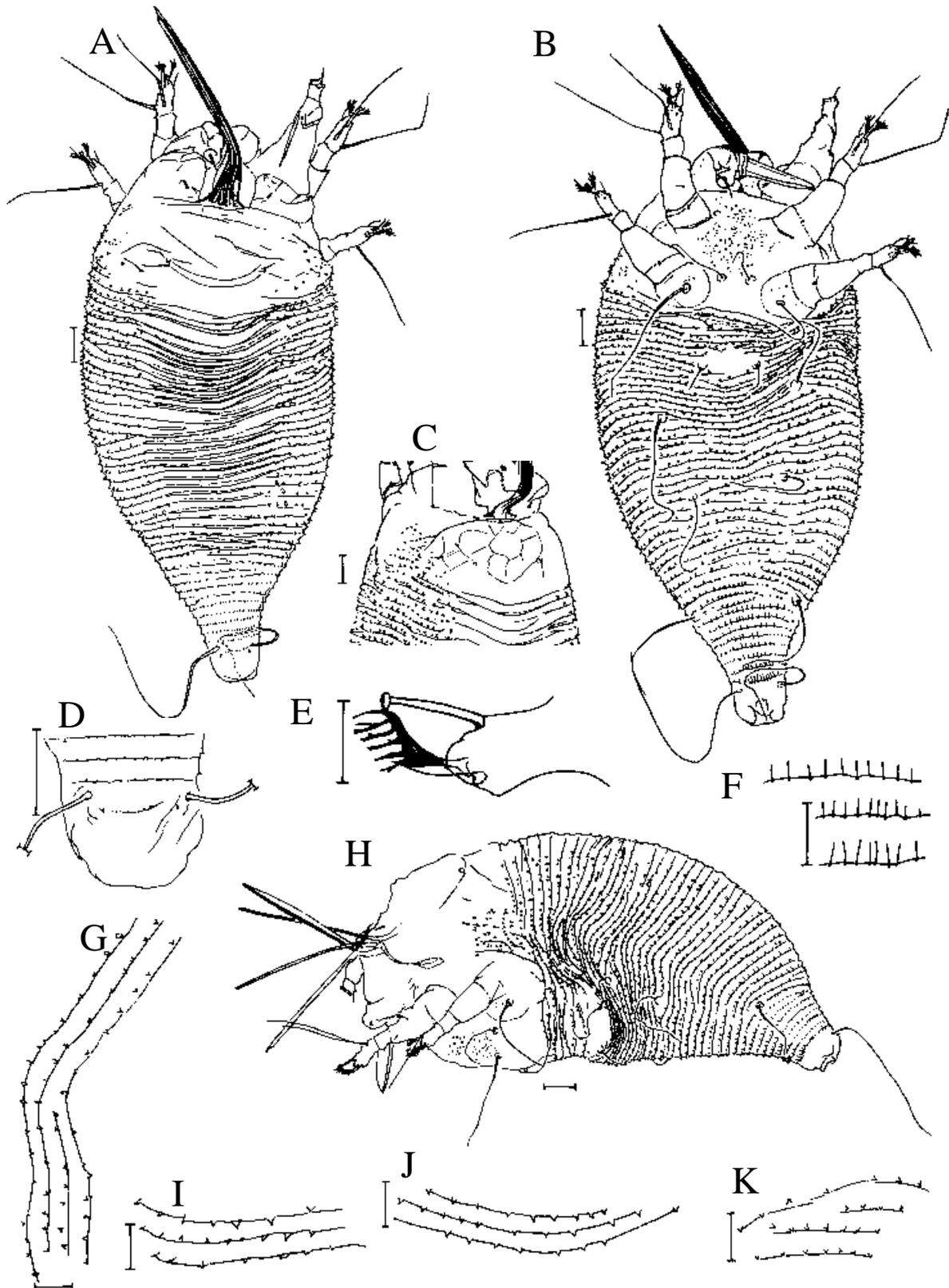
Scapular setal tubercles relatively small, rounded and ahead of rear shield margin (Figs 21A; 22F).



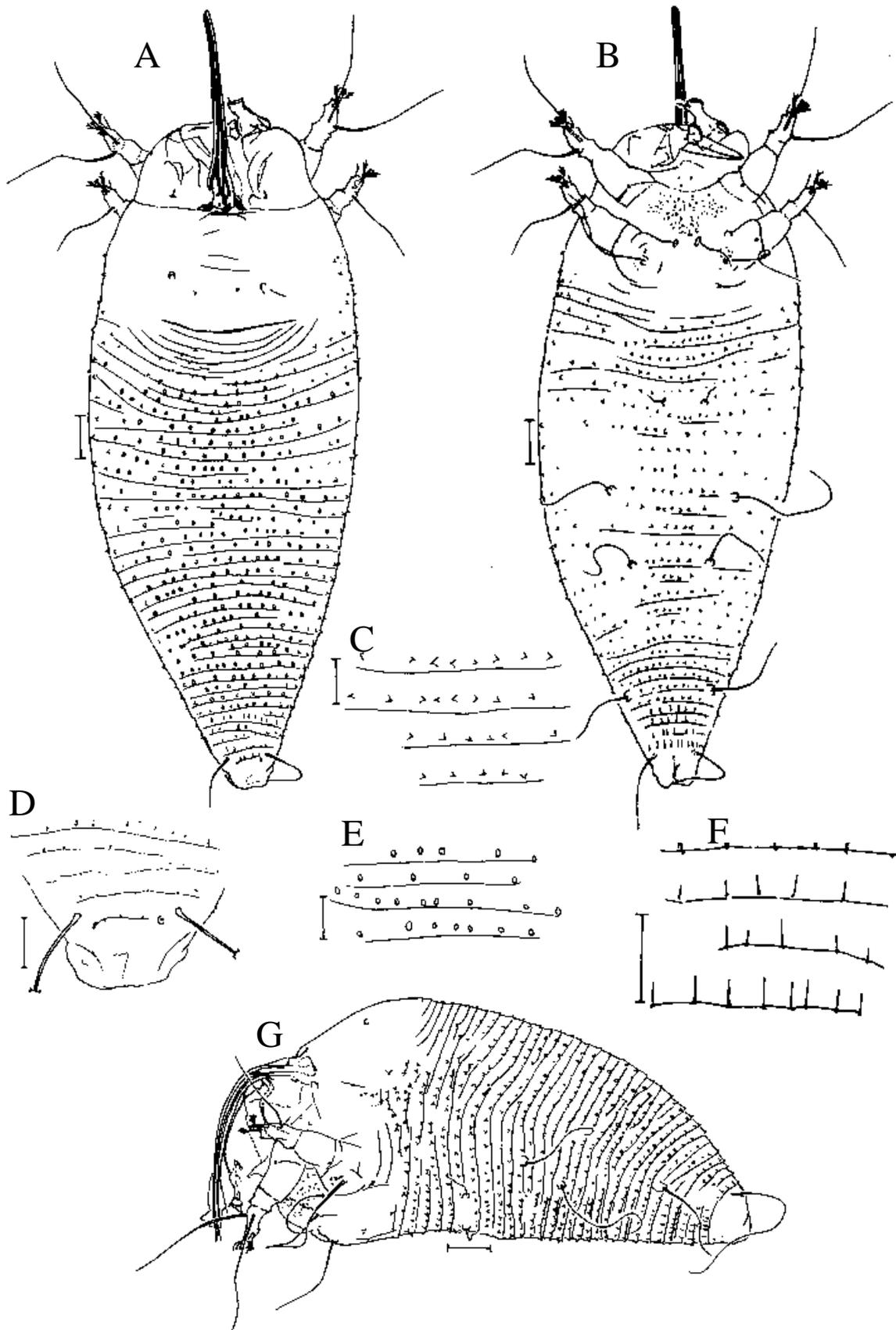
**Figure 21.** *Diptilomiopus apolungus* sp. nov., female. **A.** dorsal view; **B.** coxisternal region and genitalia; **C.** internal genitalia; **D.** opisthosomal microtubercles laterad of middorsal area just anterior of the level of seta *d*. **E.** dorsal view of rear caudal area; **F.** ventral view; **G.** labelled schemical drawing of cell network of ridges on prodorsal shield; **H.** empodium of leg I; **I.** empodium of leg II; **J.** opisthosomal microtubercles midventrally just posterior of seta *d*; **K.** opisthosomal microtubercles ventrally on rear four annuli.



**Figure 22.** *Diptilomiopus apolongus* sp. nov. Male. A. coxisternal area and genitalia. Female. B. opisthosomal microtubercles on lateral area just posterior of seta *d*; C. opisthosomal microtubercles on lateral area just posterior of seta *e*; D. leg I; E. leg II; F. lateral view.



**Figure 23.** *Diptilomiopus apolongus* sp. nov., nymph. **A.** dorsal view; **B.** ventral view; **C.** dorso-lateral view of anterior part of idiosoma; **D.** dorsal view of rear, caudal area; **E.** lateral view of anterior part of tarsus of leg I, including empodium, solenidion and *u'*; **F.** opisthosomal microtubercles ventrally on rear three caudal annuli; **G.** opisthosomal microtubercles laterally, arrow denotes border between dorsal annuli above arrow and ventral annuli below arrow; **H.** lateral view; opisthosomal microtubercles: **I.** lateral area of dorsum, at about level of seta *d*; **J.** midventrally just posterior of seta *d*; **K.** midventrally just anterior of seta *f*.



**Figure 24.** *Diptilomiopus apolongus* sp. nov., larva. **A.** dorsal view; **B.** ventral view; **C.** opisthosomal microtubercles midventrally at about the level of seta *d*; **D.** dorsal view of caudum; **E.** opisthosomal microtubercles middorsally in area at level of seta *d* towards *e*, microtubercles become smaller towards the rear; **F.** opisthosomal microtubercles ventrally on rear four caudal annuli; **G.** lateral view.

**Opisthosoma** – Evenly rounded with a shallow, middorsal longitudinal ridge (Fig. 21A) fading towards the rear. In some specimens the outer ridges and troughs are detectable, but in others they are for all practical purposes invisible.

Microtubercles on dorsal annuli: absent in a central band, flanked by microtubercles on each side, however, about 14 rear dorsal annuli entirely microtuberculate (Fig. 21A). Dorsolateral opisthosomal microtubercles obviously more sparsely spaced than on ventral annuli (Figs 21D,J), situated on the rear annulus margins, and are triangular, pointed, fine and small. From the first annulus that are entirely microtuberculate towards the rear, the microtubercles become gradually elongated with the proximal part beneath the dorsal surface, lightly coloured, with the slightly darker apical point exposed and on the rear annulus margin (Fig. 21E). Opisthosomal microtubercles on ventral annuli pointed, about triangularly shaped, close to the rear edges of the annuli, sometimes seemingly on the rear annulus margins, projecting over the rear annulus margin (Figs 21J; 22B,C), becoming progressively elongated ridges from about the level of *f* (on the rear about 12 ventral annuli) (Fig. 21K). Ventral laterally, from about *d* up to *f*, bordering the boarder between the dorsal and ventral annuli, a band of the ventral opisthosomal microtubercles appears to be more elongated than the remainder of the ventral microtubercles (Fig. 22C).

All opisthosomal setae relatively long and finely tapered (Figs 21F; 22F & Table 4), except *hl* that are very short, and may be interpreted as being minute (Fig. 21E & Table 4).

**Legs** (Figs 22D,E) – 5-segmented, with genu absent, probably fused with the femur.

Setal compliment of legs I (trochanter– [femur–genu] –tibia–tarsus): 0– [0–0] –0–3 (*bv*, *l''* and *l'* absent; three tarsal setae present: *ft''*, *ft'* and *u'*).

Setal compliment of legs II (trochanter– [femur–genu] –tibia–tarsus): 0– [0–0] –0–2 (*bv*, *l''* and *ft'* absent; two tarsal setae present: *ft''* and *u'*).

Setae *ft* in both legs strong, long and tapering.

Femorogenua I and II ornamented with a pattern of ridges on the ventral surfaces (Figs 22D,E), however the ornamentation on femorogenua is less developed and in some specimens seems almost to be absent.

Tarsal solenidion  $\omega$ , noticeably knobbed and virtually straight (Figs 22D,E). Tarsal empodium *em* deeply and unambiguously divided. The rays lie in an angle against the central stem, especially at the base, of each branch of the divided empodium, and could not be counted accurately, particularly in dorsal view, and has not been statistically dealt with. The empodia 7- or 8-rayed (sometimes the seventh but especially the eighth ray is not easily seen) (Figs 21H,I).

**Coxae, coxigenital region and genitalia** (Fig. 21B) – Suboral plate with granules similar to those on the coxisternal plates (Fig. 21B); coxisternal plates I and II with rounded to slightly pointed granules and dashes more to the outside of the plates: almost covering coxisternal plates I entirely, coxisternal plates II with a small patch of granules anterior to *2a* (Fig. 21B); the inner margins (apodemes) of coxisternal plates I light but clearly present, forming two shallow half circles, with central part broadly touching or uniting, forming a so called sternal line that can be categorized as simple and forked anteriorly and posteriorly (Fig. 21B).

Setae *1a* and *2a* present and “normal” (simple and tapering), relatively long, strong and frequently convoluted, with well-developed setal tubercles; *1a* ahead of an imaginary line through tubercles of *2a* (Fig. 21B).

Internal genitalia, particularly the anterior part, quite strongly present, with clear striae on the anterior apodeme and associated tissue (Fig. 21C); external genital coverflap basally covered with granules similar to those on coxisternal plates, distally smooth (Fig. 21B).

Seta *3a* tapering (Fig. 21F).

## **MALE.**

Morphology, including measurements and counts, similar to female, except for genitalia (Fig. 22A), albeit some setae are slightly shorter or in the short range of the female setal lengths (Table 4). Genitalia – scattered microtubercles or granules just below the eugenital setae similar to many other eriophyoid species described to date; granules close to eugenital setae large and rounded becoming smaller and more pointed lower down. Eugenital setae clearly present, with well defined setal tubercles (Fig. 22A)

Two immature stages could be identified (recorded to date all eriophyoid species studied in this regard with two immature stages):

## **LARVA** (Fig. 24).

Specimens identified to be first immature stages (larvae) by absence of external genitalia, the smallest size and rounded incomplete annuli just posterior of rear shield margin (Figs 24A,G). The prodorsal shield mostly smooth with a few lines that could be fold lines and two pointed, triangular granules or microtubercles one each on the inside of the dorsal tubercles (Figs 24A,G); subshield lateral area with a few pointed triangular granules or microtubercles in one small area (Figs 24A,G). Scapular setal tubercles present and smaller but similar to those in adults (Fig. 24A). The incomplete annuli dorsally posterior of the prodorsum smooth (Fig. 24A), the remainder of the dorsal annuli with small rounded microtubercles near the rear annulus margins, sometimes looking as if they may have a small protrusion (darker and possibly pointed on one side) (Fig. 24E), becoming smaller and pointed, and sitting more on the rear annulus margins on the rear

annuli beyond about the level of *f* and particularly on the rear four annuli (Fig. 24D). Ventral annuli entirely microtuberculate, but with smooth areas about on the outside and anteriorly of *d* and *e*; ventral opisthosomal microtubercles triangularly shaped and pointed, near the rear annulus margins (Figs 24B) becoming elongated on the rear four annuli (Figs 24B,F). All setal compliments the same as in adult, however setae generally much shorter (Table 4); empodial (*em*) with less rays than in adult.

#### **NYMPH** (Fig. 23).

Specimens identified to be second immature stages (nymphs) by the absence of external genitalia, their size, and complete and regular annuli just posterior of rear shield margin. Prodorsal shield varying from almost smooth, without ridges in cell pattern (Fig. 23A) to ridges in a more complete cell-like pattern towards the pattern seen in adults (Fig. 23C). Opisthosoma – No external genitalia present, only a discontinuance in annuli where genitalia will be in adult. A middorsal ridge present (Fig. 23A). Dorsal annuli with a middorsal smooth band, with a lateral band of microtubercles, annuli entirely microtuberculate from about 12<sup>th</sup> annulus from the rear (Fig. 23A), microtubercles sharply pointed, about triangular and situated on or close to the rear annulus margin (Figs 23G,I) becoming smaller towards the rear (Fig. 23D); ventral annuli entirely microtuberculate with microtubercles triangular and pointed similar to dorsolateral microtubercles (Figs 23J,K) becoming more elongated on about the 9 rear annuli (Fig. 23F). All setal compliments the same as in adult, however setae generally shorter than in adult and longer than in larva (Table 4); tarsal empodium *em* probably 6-rayed (Fig. 23E) (difficult to count in most specimens). Tarsal solenidion similar in shape as in adult (Fig. 23E).

**ETYMOLOGY** – The species name is a combination of the first syllable of the host genus name, *Apodytes*, and the Latin word *longus*, which means long, referring to the long body setae in comparison with the other *Diptilomiopus* sp. found on this host.

**HOST PLANT** – *Apodytes dimidiata* E.Mey. ex Arn. (Icacinaceae) (Palgrave, 2002). Common names: White-pear (English), *Witpeer* (Afrikaans). It frequently is a small bushy tree 4–5 m tall, but can become 20 m tall in forest. It occurs in coastal evergreen bush, at the margins of medium-altitude evergreen forest, in riverine fringes and open woodland, and on grassy mountain slopes, often among rocks. It has medicinal value, and the wood is suitable for agricultural implements and furniture. It is an attractive tree used as garden ornamentals. (Palgrave, 2002).

**TYPE DATA** – Forty-five type specimens on 10 slides (AcY: 11/224) from type locality and host, *A. dimidiata* (planted for ornamental purposes), Baberton Road, Nelspruit, Mpumalanga,

South Africa (25.28S, 30.59E), 30 June 1988, S. Nesor (NF1415, X88/148): holotype female and 4 paratype specimens (2 females and 2 nymphs) on 1 slide, together with a Phyllocoptinae specimen (6-rayed); the holotype can be distinguished by being the only *D. apolongus* sp. nov. female in a dorso-ventral position on this slide; and 40 paratype specimens (19 females, 7 males, 9 nymphs and 1 larva) on 9 slides. Three paratype specimens of *D. apobrevis* sp. nov. (1 female, male, and larva) are present on 2 of the 9 slides, and 5 specimens of a *cf. Tetra* sp. with 4-rayed empodia, and 8 specimens of another Phyllocoptinae species with 6-rayed empodia are dispersed on the 9 slides. Twenty-two paratype specimens on 12 of 20 slides (AcY: 11/222) from *A. dimidiata*, Botanical Garden, Nelspruit, Mpumalanga, South Africa (25.28S, 30.59E), date unknown, A. Witt with directions from S. Nesor (X03/130), together with 86 type specimens of *D. apobrevis* dispersed on the 20 slides.

RELATION TO HOST – The mites occurred sparsely (many leaves devoid of them, and further about one to three specimens per leaf) on the underside of the leaves, in many cases close to or even on inner walls of turret galls probably caused by an insect. Mites also occurred on healthy looking leaves without any galls or other symptoms.

## GENERAL DISCUSSION AND CONCLUSIONS

It is important to use the best techniques for slide-mounting of specimens to improve the morphological study of eriophyoid mites (De Lillo *et al.*, 2010). Apart from this, it is clear from the present study that many artefacts are present in slide-mounted specimens, and there are limitations inherent in light microscopy of these specimens that cannot be enhanced and rectified by improving slide-mounting techniques, and these inadequacies are already built into eriophyoid descriptions and classifications. Scanning electron microscope images reveal shape and structures which may be of systematic use, particularly for phylogenetic analyses, and which are not visible, discernable or are distorted in slide-mounted specimens. For example, the shape of the gnathosoma of the slide-mounted specimens is distorted, and furthermore, description of the morphology and even presence and length of gnathosomal setae are largely omitted in the published descriptions of other *Diptilomiopus* spp.

Information from SEM studies not only improved on and rectified information from light microscopic studies and resultant descriptions, but additionally provided a surprisingly large number of new structures that have not been previously reported, and can be of use in systematics. This increase in characters is essential for phylogenetic studies of the Eriophyoidea. Information from SEM studies additionally improves the information and clarity of morphological characters to such a degree that it will aid in the improvement of the identification and delimitation of characters and character states which are urgently required in eriophyoid systematics. For all these

reasons, the inclusion of SEM studies should not just be a mere enhancement of primary light microscopic studies for taxon descriptions. Morphology studied with SEM should be seriously and routinely incorporated into descriptions of taxa, and making it a requirement in some instances should be advocated. The inclusion of SEM studies is compulsory for the description of many nematode groups, largely implemented by peer review practices, and a description will hardly be accepted for publication if these are not included without acceptable reasons (M. Marais, *pers. comm.*). Numerous phylogenetic studies in spiders are also extensively incorporating information from SEM studies.

Thorough and precise descriptions of eriophyoids are extremely important when it is taken into account that slide-mounted specimens are not permanent, and that most type material is lost over time (Amrine & Manson, 1996; De Lillo *et al.*, 2010).

In reality, however, SEM facilities are not readily and widely available worldwide. Consequently, morphological information from SEM studies cannot be solely incorporated in the practical description, classification, differentiation and identification of eriophyoid mites, without concurrent and corroborating usable character states from slide-mounted specimens. This is similar to the situation with information from molecular studies.

The improvement of descriptions by including SEM studies is demonstrated and the SEM studies contributed additional novel characteristics which may be of use in systematics.

## ACKNOWLEDGEMENTS

Thank you, to Alan Hall who assisted with the SEM study, Stefan Nesor and A. Witt for collecting the mites. This study formed part of a study towards a PhD degree at the University of Pretoria, South Africa.

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