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"Nymphes plissées" structure of the cuticle of juveniles of some oribatid mites (Acari: Oribatida)

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Abstract. The cuticular structure of juveniles of several oribatids of different families with wrinkled cuticles were compared: *Hermannia gibba*, *Tectocepheus velatus*, *Scutovertex minutus*, *Achipteria coleoptrata* and *Eupelops occultus*. Both the surface and internal structures of the "plissée" were studied. Light microscopy revealed several patterns in mites studied with Masson's triple stain and these results were supported by TEM. Although the "plissée" looks similar at the body surface, the structure and ultrastructure differ among groups. Some types of wrinkling is supported by small muscles, probably for changing body shape. Differences in the structure of the cuticle of the prosoma and opisthosoma were observed. The differences in the wrinkling in the cuticle in diferent lines is associated with change in the body shape in response to different moisture conditions.

INTRODUCTION

Several authors have investigated the cuticular structure of mites (Oribatida: Michael, 1884; Grandjean, 1951; Woodring & Cook, 1962; Bäumler, 1970; Tarba & Semenova, 1976; Pugh et al., 1987; Smrž 1989; Iordansky & Stein-Margolina, 1993; Ixodida: Balashov, 1979; Hackman, 1982). These studies are summarized in general texbooks of acarology (Krantz, 1978; Evans, 1992). Alberti et al. (1981) compared the cuticular ultrastructure of most of the important mite groups. These authors describe differences in cerotegument as well as cuticular structure in mite groups. Gas chromatography and mass spectrometry were used to analyse of the fatty acids in the integument (cerotegument and cuticle) by of four species of oribatids including *Steganacarus* (= *Tropacarus*) *carinatus* Raspoting & Krisper (1998). The general internal microanatomy and ultrastructure of mites including the cuticular structure are described and discussed in the review of Alberti & Coons (1999).

Generally, several integumental layers can be distinguished when stained whith Masson's triple stain and examined using light microscopy (Smrž, 1989, 1992a, 1994, 1995) – cerotegument = rough lipoid (Krantz, 1978) layer on the cuticular surface, frequently incorporating mineral or litter particles. It can be separated or removed from the cuticle, e.g., by lactic acid or by simple abrasion. Some types of cerotegument are destroyed during embedding for the histological sectioning. Cerotegument is not stained by Masson's triple stain.

– epicuticle = thin outermost cuticular layer, hyaline, translucent, without any internal division or structure under light microscopy. It is not stained by Masson's triple stain, but easy to distinguish from the other layers.

The underlying layer can be homogeneous or divided into several sublayers. These are termed exocuticle and endocuticle following the usual arthropod cuticular terminology or, together, as procuticle. But some mites exhibit several types of "exocuticle" or "endocuticle" which cannot be homologized with the exocuticle or endocuticle of other arthropods. This appears to be common in mites. This phenomenon was discussed by Alberti (Alberti et al., 1981; Alberti & Coons, 1999). He proposed the more general term "layers of procuticle" based on the very rich and diverse range of objects and types of cuticles observed. This structural diversity appears to be better illustrated by this terminology. In this paper, I use the terms for layers and sublayers proposed by Alberti (Alberti et al., 1981; Alberti & Coons, 1999), with the conventional, arthropodological terminology is indicated in brackets for orientation and comparison. The main types of procuticular sublayers are those previously described for oribatids studied under a light microscope after staining with Masson's triple stain (Smrž 1989, 1992a, 1994, 1995):

– exocuticle = amber-coloured layer, not stained by Masson's triple stain. It may lack an internal structure or have lamellae or striae perpendicular to the cuticle surface.

– endocuticle = any internal structure, very conspicuously a carmine colour after staining with Masson's triple stain.

As mentioned below, there are several other types.

Of course, more details can be distinguished under TEM. Such observations support the above, although they are some differences especially in the striation or lamellar structures. Moreover, the cerotegument survived much better tha preparation for TEM than for histological examination. The different electron densities of the layers, however, correspond generally to differences in staining by Masson. These differences may result from the different of preparation, e.g. fixatives. As mentioned above, this supports the more general terminology – procuticle sublayers.

The juveniles of various oribatid families have a conspicuously wrinkled or folded cuticle ("nymphes plissées" sensu Grandjean, 1953). This phenomenon occurs in different unrelated groups (e.g. Hermanniidae, Tectocepheidae, Scutoverticidae, Pelopidae (= Phenopelopidae auct.), Achipteriidae). This study compares the cuticular structure of the juveniles of some members of the above mentioned families.

MATERIAL AND METHODS

The juveniles of *Hermannia gibba* (C.L. Koch), *Tectocepheus velatus* (Michae), *Scutovertex minutus* (C.L. Koch), *Achipteria coleoptrata* (Linnaeus) and *Eupelops occultus* (C.L. Koch) were collected on moist filter paper in Berlese-Tullgren funnels and then prepared:

The specimens for light microscopy (LM) were fixed in Bouin-Dubosque-Brasil fluid modified for oribatids (Smrž, 1989), embedded in Histoplast (Serva), sectioned using a MSE rotation microtome (thickness 5 µm), stained with Masson's triple stain and observed under a Provis AX 70 microscope (Olympus) and the images edited in Microimage 3.1 image analysis (Olympus).

Those for TEM were fixed in cacodylate-buffered glutaraldehyde (4%) postfixed in 1% osmium tetroxide, embedded in Spurr medium and sectioned using an Ultracut ultramicrotome (Reichert). Sections were stained in uranyl acetate and lead citrate and observed under a transmission electron microscope (TEM) Philips EM 300.

RESULTS

Hermannia gibba (Figs 1, 2)

On the prosoma, the cuticle consists of an epicuticle and a red stained procuticle with the lamellae parallel with the body surface. Both layers form pad of the superficial ridges and tubercles of the integument.

The cuticle on the opisthosoma can be divided into two regions – dorsal and ventral. Both consist of a epicuticle and a thick red stained procuticle. The dorsal surface, however, is covered by pectinate projections, especially on the anterior opisthosoma (Figs 3, 4) which was confirmed by TEM (Fig. 5). The wrinkling on the ventral opisthosomal region is moderate and formed by tubercles. The lamellar procuticle is of endocuticular type overlain with a thick epicuticle which is covered by the cerotegument (Figs 6, 7). The cuticle of the whole body is wrinkled only externally.

The cuticle of the legs has a by smooth epicuticle and procuticle consisting of two layers: amber-coloured ("exocuticle") and red stained ("endocuticle") (Fig. 8).

Tectocepheus velatus (Fig. 9)

The prosomal cuticle consists of an epicuticle and a thick procuticle of the endocuticular type without remarkable wrinkling (Figs 10, 11). The opisthosomal cuticle is conspicuously wrinkled over most of the dorsal region. This wrinkling results from the nearly regular sinusoidal folding of the epicuticle and procuticle of endocuticular type (Fiss 11, 12). The underlying muscles originate on the anterior part of this folding and are inserted on the most posterior part (Figs 11, 12). These muscles are of the same length as the folded area. The sinusoidal pattern, i.e. gradual folding of the cuticle is visible on the outer as well as the inner surface, and confirmed by TEM. The latter also revealed the mushroom-like cerotegument (Fig. 13). Mineral particles from soil can cover these folds (Fig. 10). The ventral part of the opisthosoma resembles the prosoma, it is nearly smooth, with small tubercles. The structure of the cuticle is the same as on the dorsal surface.

The leg cuticle resembles that described for *Hermannia gibba.*

Scutovertex minutus (Fig. 14)

The prosomal cuticle has no conspicuous wrinkles and consists of a thin epicuticle and procuticle of endocuticular type (red stained). Its surface is nearly flat (Figs 15, 16). The opisthosomal notogaster is similar to that of *Tectocepheus*, i.e. epicuticle and underlying procuticle of endocuticular type are both folded (Fig. 17) as in *T.velatus*, including the presence of muscles. They appear to be, however, shorter, in the anterior region and terminate in the folded region (Fig. 15). The ventral surface is moderately folded, but in this case due to superficial waves and tubercles whereas the inner surface is flat (Fig. 18).

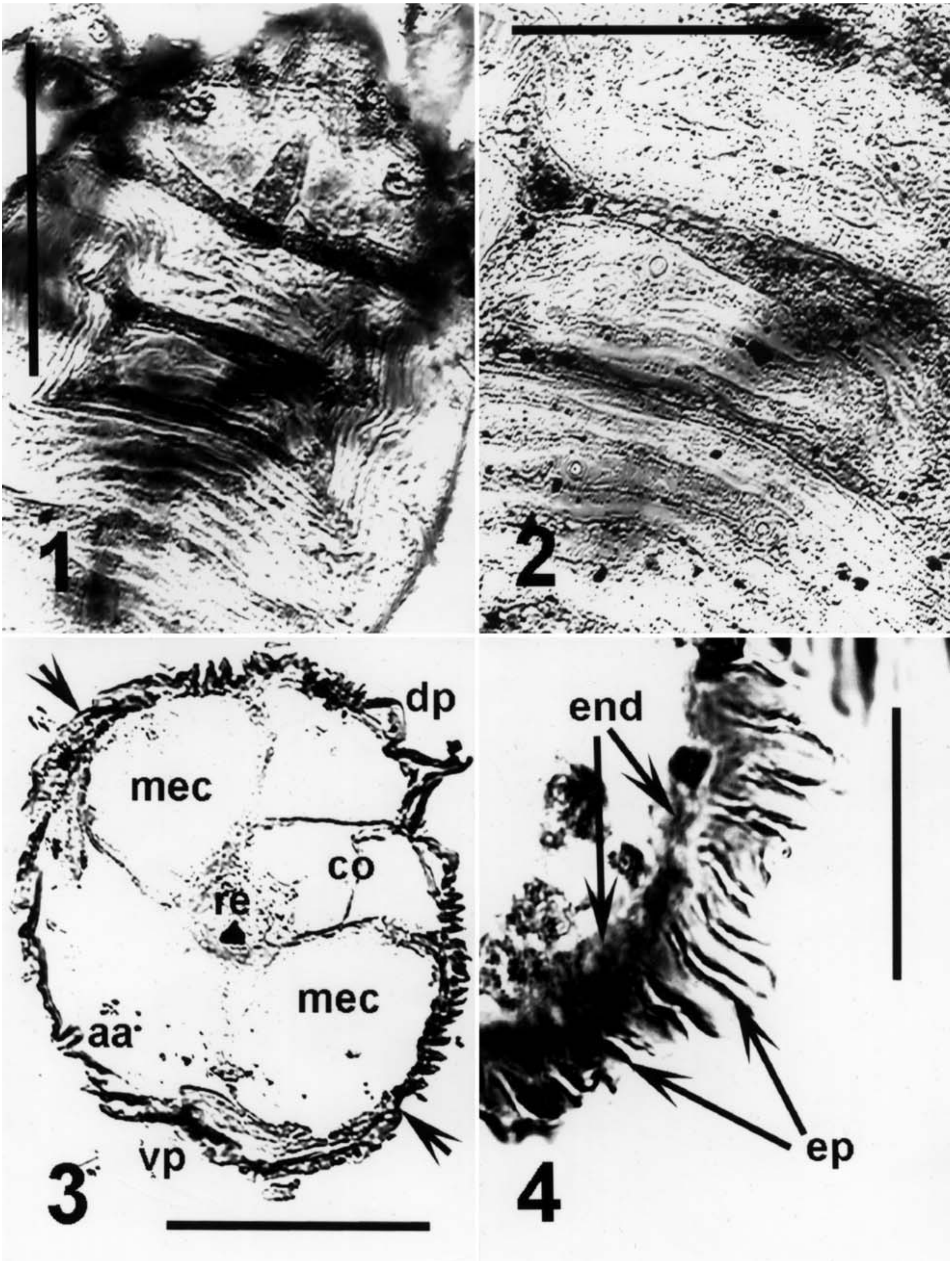
The leg cuticle resembles that described for *Hermannia gibba.*

Achipteria coleoptrata (Fig. 19)

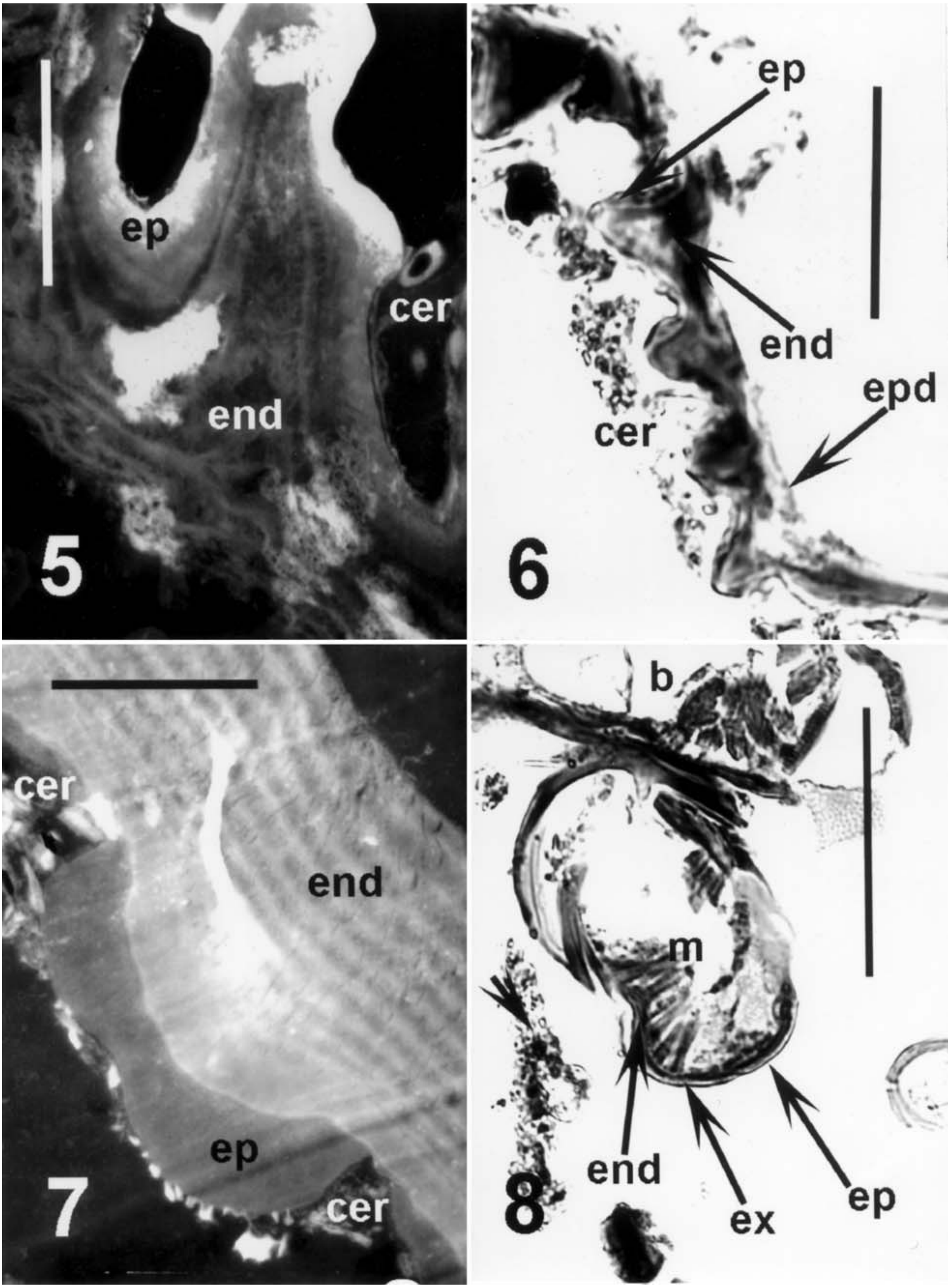
The prosomal cuticle consists of an epicuticle and thick procuticle of the endocuticular type. Its surface is nearly flat (Fig. 20). On the opisthosoma, wrinkling is present not only on the whole dorsal surface, but also on the anterior ventral surface, whereas the posterior venter is nearly flat, with moderate tubercles. The conspicuous wrinkling, especially on the anterior dorsal surface differs from that in *T. velatus* or *S. minutus.* In *A. coleoptrata*, the folds or waves are pointed, not gradually folded. The inner surface of the cuticle has a complementary pattern to that on the other surface, hence, no gradual, sinusoidal waves (Figs 21, 22). The uppermost layer – the epicuticle, is covered with a thick cerotegument, with the occasional incorporated mineral particles. The leg cuticle consists of an epicuticle and two sublayers of procuticle: amber coloured exocuticule type and a thin red stained "endocuticular" sublayer. The "exocuticle" forms the waves and tubercles that give leg cuticle its rough surface, which is covered by a conspicuous cerotegument (Fig. 23).

Eupelops occultus (Fig. 24)

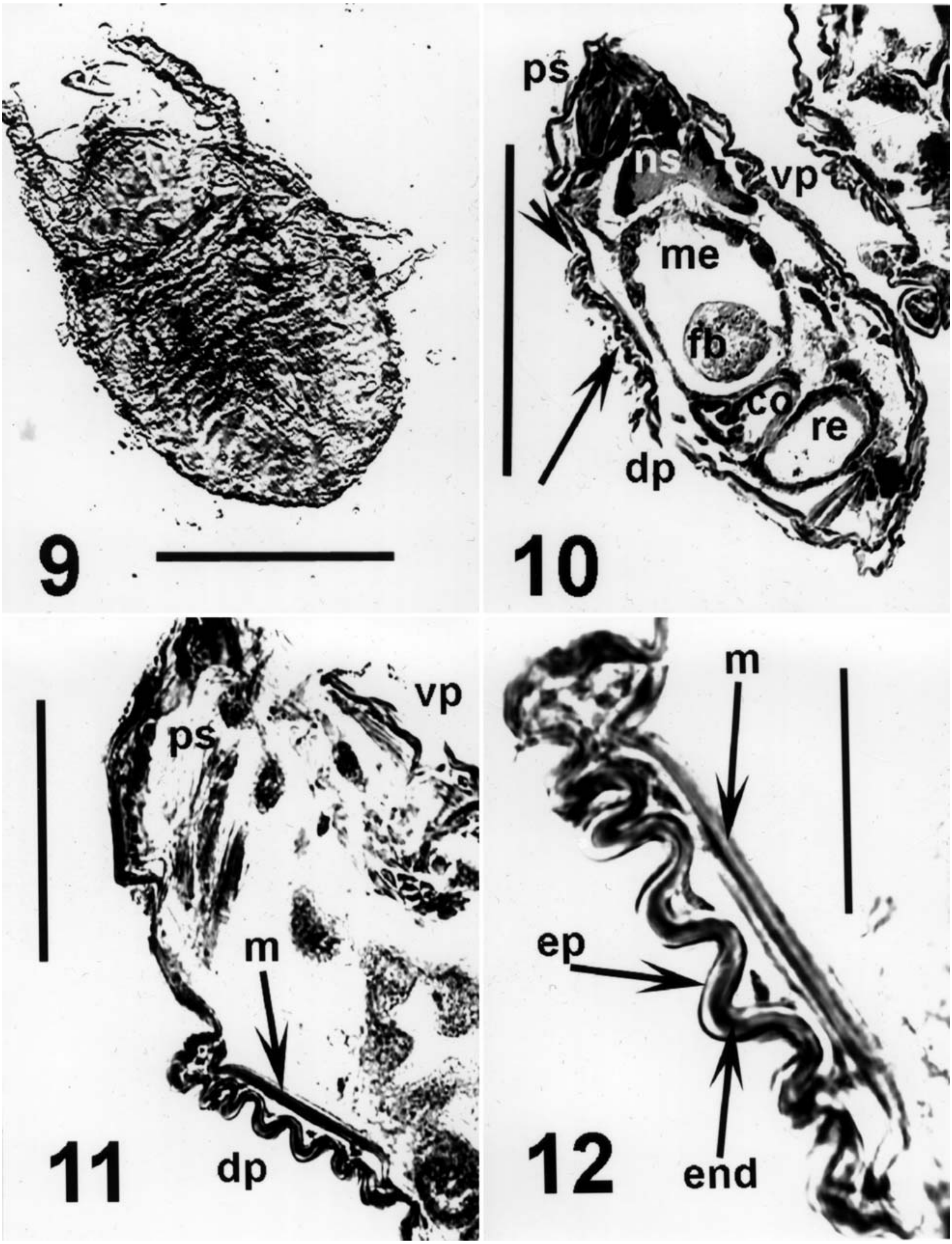
The prosomal cuticle consists of an epicuticle and procuticle of the endocuticular type. Its surface is flat without any conspicuous wrinkling (Figs 25, 26). A thin epicuticle and thick procuticle of the endocuticular type are present on the opisthosoma,. Conspicuous wrinkling of the cuticle occurs in the anterior region of the notogaster (Figs 24, 25, 26, 27). This is followed by a nearly flat posterior dorsal cuticle (Fig. 24). Again, the muscle originates on the anterior part of this folding and are inserted on half of this folding.cuticle field (Fig. 25). Thus the muscle is shorter than the folds. On the ventral surface, there are only small tubercles. The innermost cuticular layer, however, is flat (Figs 25, 28).



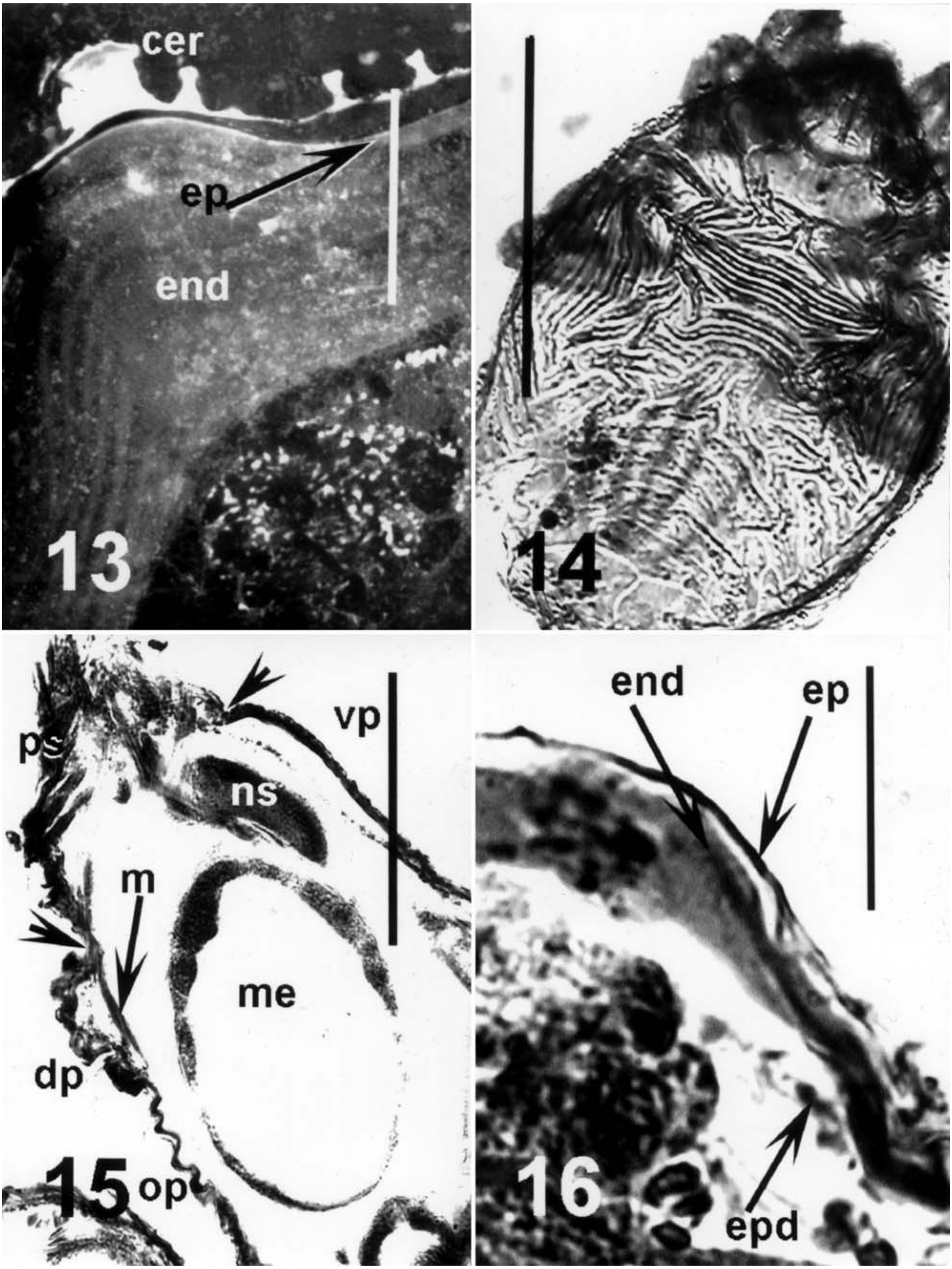
Figs 1–4: *Hermannia gibba*, tritonymph, light microscopy. 1 – intact specimen in lactic acid, dorsal view, 2 – the same specimen, detail of the dorsal surface, 3 – cross section of a whole mite, arrowheads point to the line between the dorsal and ventral parts, 4 – cross section of the same specimen, detail of the dorsal surface. Intact specimens (1, 2), Masson's triple stain (3, 4). Scales: 200 µm (1), 100 µm (2), 50 µm (3), 20 µm (4). Abbreviations used: aa – anal atrium, co – colon, dp – dorsal part, end – endocuticle, ep – epicuticle, mec – mesenteral caeca, re – rectum, vp – ventral part.



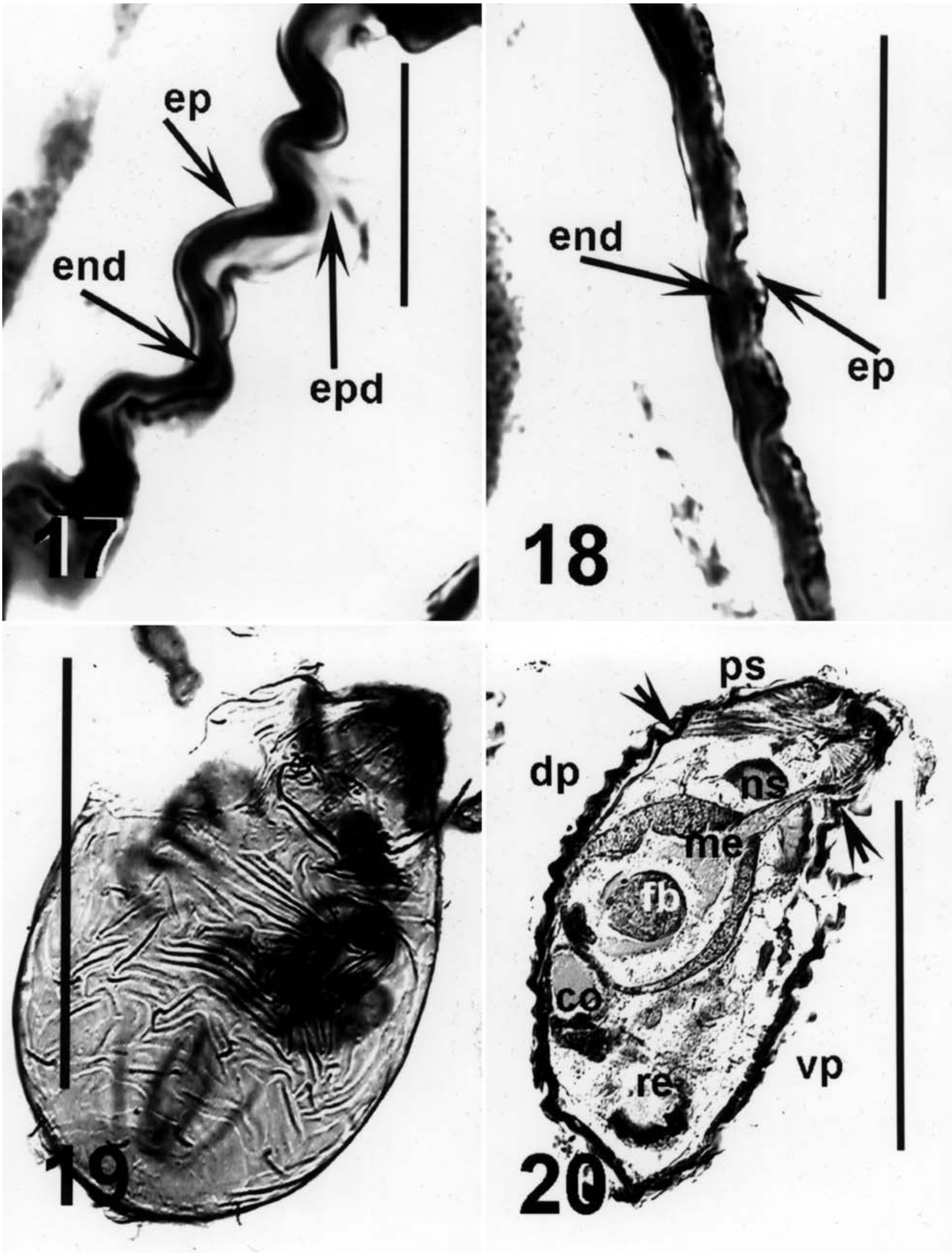
Figs 5–8: *Hermannia gibba*, tritonymph. 5 – cross section of dorsal cuticle, TEM, 6 – sagittal section of ventral cuticle, light microscopy, 7 – sagittal section of ventral cuticle, TEM, 8 – cross section of a leg, light microscopy. Masson's triple stain (6, 8), TEM (5, 7). Scales: 20 µm (6, 8), 2 µm (5, 7). Abbreviations used: b – body cavity, cer – cerotegument, end – endocuticle, ep – epicuticle, epd – epidermis, ex – exocuticle, m – muscles.



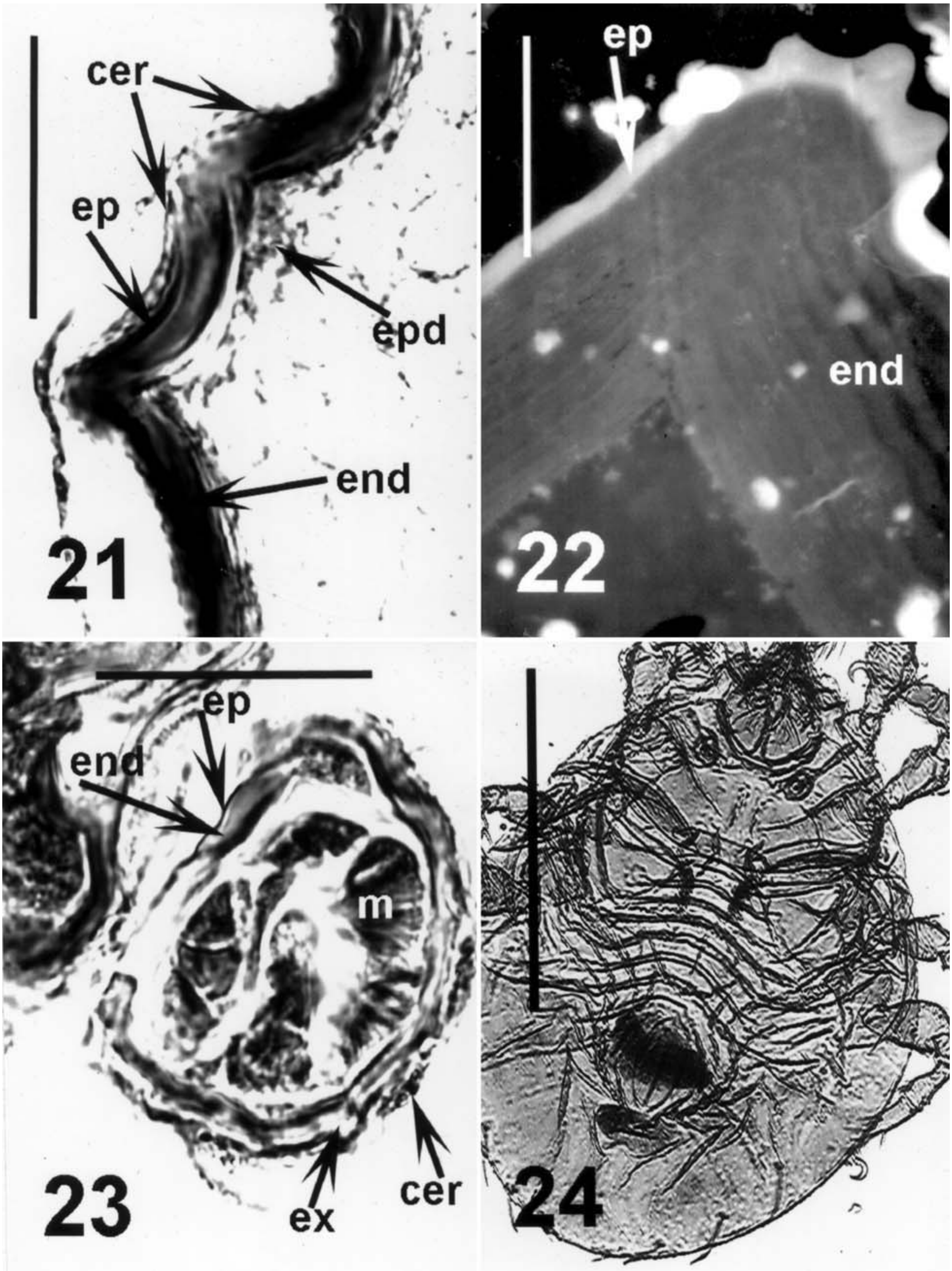
Figs 9–12: *Tectocepheus velatus* tritonymph, light microscopy. 9 – intact specimen in lactic acid, dorsal view, 10 – sagittal section of a whole mite, arrowhead points to the sejugal suture, arrow points to the coating of by mineral soil particles, 11 – parasagittal section of the anterior part, 12 – the detail of the anterior part of notogaster of the same specimen. Intact specimen (9), Masson's triple stain (10–12). Scales: 300 µm (9, 10), 50 µm (11), 20 µm (12). Abbreviations used: co – colon, dp – dorsal part, end – endocuticle, ep – epicuticle, fb – food bolus, m – muscles, me – mesenteron, ns – nervous ganglion, ps – prosoma, re – rectum, vp – ventral part.



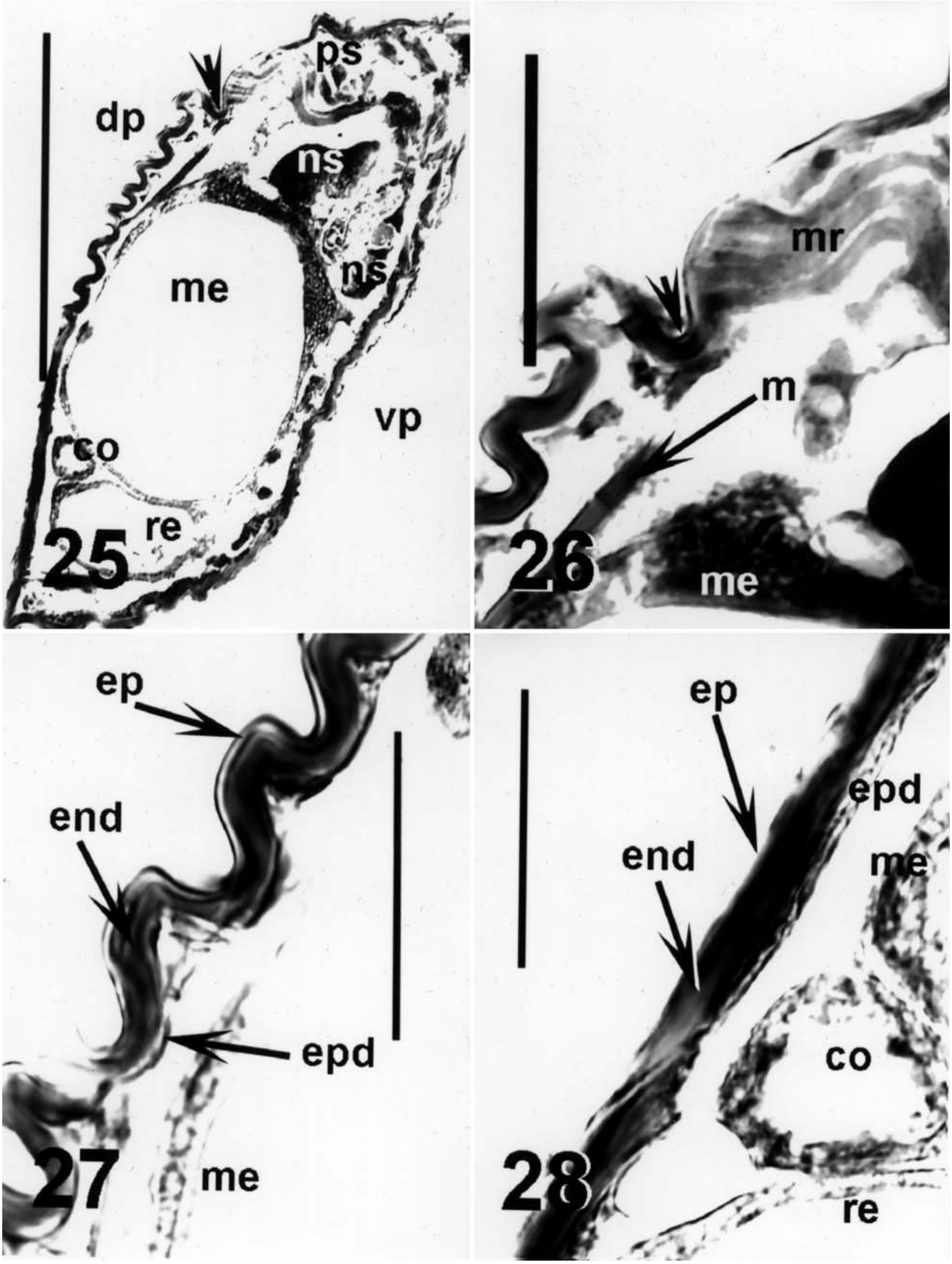
Figs 13–16. 13 – *Tectocepheus velatus*, tritonymph – TEM, sagittal section of the notogastral cuticle, 14 – *Scutovertex minutus*, tritonymph, intact specimen in lactic acid, dorsal view, 15 – parasagittal section of the same species, parasagittal section, arrowheads point to the dorso- and ventrosejugal sutures, 16 – parasagittal section of the same species, detail of prosomal cuticle. TEM (13), intact specimen in lactic acid (14), Masson's triple stain (15, 16). Scales: 200 µm (14), 20 µm (15), 5 µm (16), 1 µm (13). Abbreviations used: cer – cerotegument, dp – dorsal part, end – endocuticle, ep – epicuticle, epd – epidermis, m – muscles, me – mesenteron, ns – nervous ganglion, op – opisthosoma, ps – prosoma, vp – ventral part.



Figs 17–20. 17, 18 – *S. minutus*, tritonymph: 17 – sagittal section, of anterior part of notogaster, 18 – sagittal section of ventral part, 19, 20 – *Achipteria coleoptrata*, tritonymph: 19 – intact specimen in lactic acid, dorsal view, 20 – sagittal section of whole mite arrowhweads point to the dorso- and ventrosejugal suture. Masson's triple stain (17, 18, 20), intact specimen (19). Scales: 200 µm (19, 20), 10 µm (17, 18). Abbreviations used: co – colon, dp – dorsal part, end – endocuticle, ep – epicuticle, epd – epidermis, fb – food bolus, me – mesenteron, ns – nervous ganglion, ps – prosoma, re – rectum, vp – ventral part.



Figs 21–24. 21–23 – *Achipteria coleoptrata*, tritonymph. 21 – sagittal section of notogastral cuticle, 22 – sagittal section of the dorsal cuticle, TEM, 23 – cross section of leg, light microscopy 24 – *Eupelops occultus*, tritonymph, intact specimen in lactic acid, dorsal view. Masson's triple stain (21, 23), TEM (22), intact specimen in lactic acid (24). Scales: 200 µm (24), 20 µm (23), 10 µm (21), 2 µm (22). Abbreviations used: cer – cerotegument, end – endocuticle, ep – epicuticle, epd – epidermis, ex – exocuticle, m – muscles, me – mesenteron.



Figs 25–28: *Eupelops occultus*, parasagittal section of tritonymph. 25 – whole mite, arrowhead points to dorsosejugal suture, 26 – the same specimen showing, details of the contact between the prosoma and opisthosoma, arrowhead points to the dorsosejugal suture, 27 – the same specimen showing detail of anterior part of notogaster, 28 – the same specimen showing detail of posterior part of notogaster. Masson's triple stain. Scales: 200 µm (25), 50 µm (26), 10 µm (27, 28). Abbreviations used: co – colon, dp – dorsal part, end – endocuticle, ep – epicuticle, epd – epidermis, m – muscles, me – mesenteron, mr – cheliceral retractor muscle, ns – nervous ganglion, ps – prosoma, re – rectum, vp – ventral part.

The leg cuticle resembles that described for *Hermannia gibba.*

DISCUSSION

The "plissées" of juveniles occur in several oribatid families. This is based on a reviews of this cuticular structure. This study revealed two types of wrinkling in oribatids: (1) "Waves" only on the external surface of the cuticle (including epicuticle), whereas the inner surface of tha cuticle (procuticle) is flat (*Hermannia gibba*). (2) Whole cuticle is wrinkled, especially on the notogaster. The ventral surface is moderately wrinkled due to the presence of superficial tubercles, or nearly flat (*T. velatus*, *S. minutus*, *A. coleoptrata*, *E. occultus).*

This type of cuticle has evolved in several distantly related groups. A completely wrinkled cuticle evolved especially in juveniles dwelling in exposed, extreme environments where abiotic factors fluctuate, mainly moisture. *Scutovertex minutus* inhabits moss growing epilithicaly or epiphyticaly and both adults and juveniles are able to survive extremely moist conditions (Smrž, 1992a, b, 1994). Their water content fluctuates widely, hence, changes in body volume occur, which appear to be facilitated by the flexible wrinkled cuticle of the opisthosoma, especially dorsally and dorso-laterally. A similar structure occurs in *Tectocepheus velatus*, a ubiquitous species in very disturbed biotopes [including agroecosystems – Smrž & Jungová (1989), moss on buildings, etc.]. On the other hand, *Achipteria coleoptrata* exhibits a somewhat different type of wrinkling although it is also classified as "plissée". The wrinkling of the surface of the opisthosoma (cf. *S. minutus*, *T. velatus*, *E. occultus*) is of the "sharp type" without gradually rounded folding on both sides. This type of cuticle seems to be less flexible. This is confirmed by the absence of muscles under the folding. The allochthonous material in the thick cerotegument indicates the function of this wrinkling: the rough surface covered with detritus reduces water loss from the body. This wrinkling is associated with the moisture content of the soil. All juveniles are "dwellers" or "divers" (sensu Smrž, 1996). That is, they cannot escape innundation or dessication , but stay and even continue grazing and moving under water or in dry substrates. The function of the integument differs in the juvenile mites studied – it mechanically protects the body by picking up soil particles etc. (*H. gibba*, *A. coleoptrata*).

It has a physiological role in enabling the volume and shape of the body to change, when water content of their body fluctuates. This phenomenon is accompanied by guanine crystal deposition or expulsion (under dry or moist conditions respectively) and increased activity of the opisthosomal glands as reported in *S. minutus* by Smrž (1992a, 1994, 2002) This mite inhabits biotopes affected by great fluctuations in moisture (e.g. epilithic moss). *T. velatus* is found in the same biotopes as *E. occultus*, epiphytic algae or lichens (Strenzke, 1952; Smrž & Kocourková, 1999).

The cuticle of the legs appears to mainly serve – as the origin of muscles. Therefore, this cuticle is similarly uniform, in all the mites studied. Moreover, the amber coloured sublayer of the procuticle ("exocuticle") is associated with the presence of strong locomotory muscles. The rough external surface (*A. coleoptrata*) appears to function as a barrier to water loss. Such surfaces may be covered by detritus giving additoned protection against water loss.

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