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Study of the trophi of *Testudinella* Bory de St. Vincent and *Pompholyx* Gosse (Rotifera: Testudinellidae) by scanning electron microscopy

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Abstract

The fine morphology of the trophi of *Pompholyx sulcata* and nine species of *Testudinella* (Rotifera, Monogononta, Flosculariacea, Testudinellidae) was studied by scanning electron microscopy. The number of unci teeth and arched rami scleropili, and the shape of the major unci teeth and fulcrum are considered to be reliable additional characters for identification.

Introduction

In Testudinellidae (Rotifera, Monogononta, Flosculariacea) three genera are recognized, *viz. Anchitestudinella*, *Testudinella*, and *Pompholyx*. To date, the taxonomy of the species exclusively relies (e.g., Kutikova, 1970; Koste, 1978) on the shape of the lorica, the shape and position of the foot opening, and the position of the lateral antennae. As has been shown for other Flosculariacea, e.g., *Filinia* (Sanoamuang, 1993, 2002), *Floscularia* (Segers, 1997), and Conochilidae (Segers & Wallace, 2001), trophi are fairly species-specific, and their study by scanning electron microscopy (SEM) is a helpful or even the most reliable tool for identification. Apart from the little documented information by Markevich (1989) on the trophi of *Pompholyx complanata* and *Testudinella patina*, a frontal view of the trophi of *Testudinella elliptica* shown in De Smet (1998), and a caudal view attributed to *T. truncata* figured in Sørensen (2002), extensive studies dealing with the detailed morphology of the trophi of Testudinellidae using SEM are lacking. I, therefore initiated a comparative study on the trophi morphology of the commonly found species belonging to the genera *Testudinella* and *Pompholyx*.

Materials and methods

Pompholyx sulcata and nine species of *Testudinella* were investigated (as shown in Table 1). Trophi were isolated using NaOCl, followed by repeated washing in distilled water (De Smet, 1998). The dried trophi were sputter coated with gold and examined using a Philips SEM 515 microscope, operated at 20 kV.

The terminology of the different sclerite elements and structures introduced by Markevich (1989), and Markevich & Kutikova (1989), is only followed partially.

Results

General description

The trophi of Testudinellidae belong to the malleoramate type, characteristic of the order Flosculariacea (see Remane, 1929; de Beauchamp, 1965; Nogrady et al., 1993). They are almost symmetrical and composed of paired manubria, rami and unci, and an unpaired fulcrum. The manubria are elongate crescentic elements without

Table 1. List of taxa examined

Taxon	Origin
Pompholyx sulcata (Hudson, 1885)	Belgium: Merksem, Fort; Willebroek, Lacourtvijver
Testudinella caeca (Parsons, 1892)	Belgium: Ekeren, Oude Landen
T. clypeata (Müller, 1786)	The Netherlands: Veerse Meer
T. elliptica (Ehrenberg, 1834)	Belgium: Ekeren, Oude Landen; Willebroek, Lacourtvijver; Opgrimbie, Zijpbeek
T. incisa (Ternetz, 1892)	Belgium: Begijnendijk, De Putten; Genk, Het Wik; Gent, Vinderhoutse bos
T. mucronata (Gosse, 1886)	Belgium: Landen, Beemden; Beringen-Koersel, Hemelbrug
T. parva (Ternetz, 1892)	Belgium: Kampenhout, Torfbroek; Begijnendijk, De Putten
T. patina (Hermann, 1783)	Alaska, Nome; Belgium: Brasschaat, Zeurt; St. Jan-Eremo, Roeselarekreek; Canada: Victoria Isl., Cambridge Bay; Congo: Bas-Congo, Muema & Kivu, Rubare; Galápagos: Santa Cruz; Greenland: Kangerlussuaq; Kenya: Lake Victoria; Morocco: Zeida-Midelt
T. truncata (Gosse, 1886)	Belgium: Ekeren, Muisbroek; Opgrimbie, Zijpbeek
T. sp. n	France: Mediterranean, Bay of Hyères

cauda, bordering the lateral edges of the unci. The unci are plate-shaped, composed of a great many differentiated and webbed teeth. The rami are elongate-triangular, and almost completely overlain by the unci; their inner edge and caudal part is provided with numerous rod-shaped sclerite bodies. The fulcrum is short and plate-shaped.

Manubria

The crescent manubria are composed of three superimposed chambers, the dorsal, median and ventral chamber (e.g., Fig. 2: dc, mc, vc), separated from each other by transverse walls, and opening caudally. The dorsal and ventral extremities of the manubria are more or less strongly tapering to inwardly directed projections. These projections are connected by tiny ligaments to the tip and base of the rami respectively (e.g., Fig. 7: l).

Unci

The unci plates consist of transversely placed, well-differentiated and more or less strongly webbed teeth, firmly connected to the inner edge of the frontal side of the manubria. The teeth of both unci plates are interlocking in the closed trophi. The size of the teeth shifts gradually from the large proximal teeth towards the smaller distal teeth. All species studied to date display three pairs of distinctly larger proximal teeth. Each uncus tooth shows a more or less strongly clubbed head, and a long shaft. The head of the major teeth is continuous with the shaft. The head of the smaller teeth is continuous with the shaft also (e.g., Fig. 3), or more or less strongly kinked towards the rami (e.g., Figs. 1 and 5). The shaft appears almost triangular in cross section, and shows a longitudinal ridge frontally, the sutura externa; a longitudinal seam is often distinct caudally (Figs. 16, 18). Structural differences between the proximal and distal teeth are usually small within the different species, but pronounced differences in morphology of the three major teeth are obvious between taxa. For example, the heads of the proximal teeth can be slender and weakly offset, and provided with two small lateral knobs at their base (e.g., *P. sulcata*, *T. elliptica*: Figs. 23 and 25), or stout and distinctly offset without small knobs (e.g., *T. clypeata*, *T.* sp. n.: Figs. 26 and 19). The shafts of the proximal teeth are more or less loosely connected (e.g., *P. sulcata*, *T. elliptica*: Figs. 23 and 25), or a large section of the shafts distally from the weakly offset heads, appears strongly fused into a plate (e.g., *T. caeca*, *T. incisa:* Figs. 9 and 24). A lateral semi-circular expansion at the free margin of the most proximal teeth is present in *T. caeca, T. incisa* and *T. parva* (Figs. 24, 9 and 13).

The subunci (Fig. 20: su) consist of a narrow rim, composed of small and fused scleropili, firmly attached to the uncinal plate at the height of the base of the heads of the teeth.

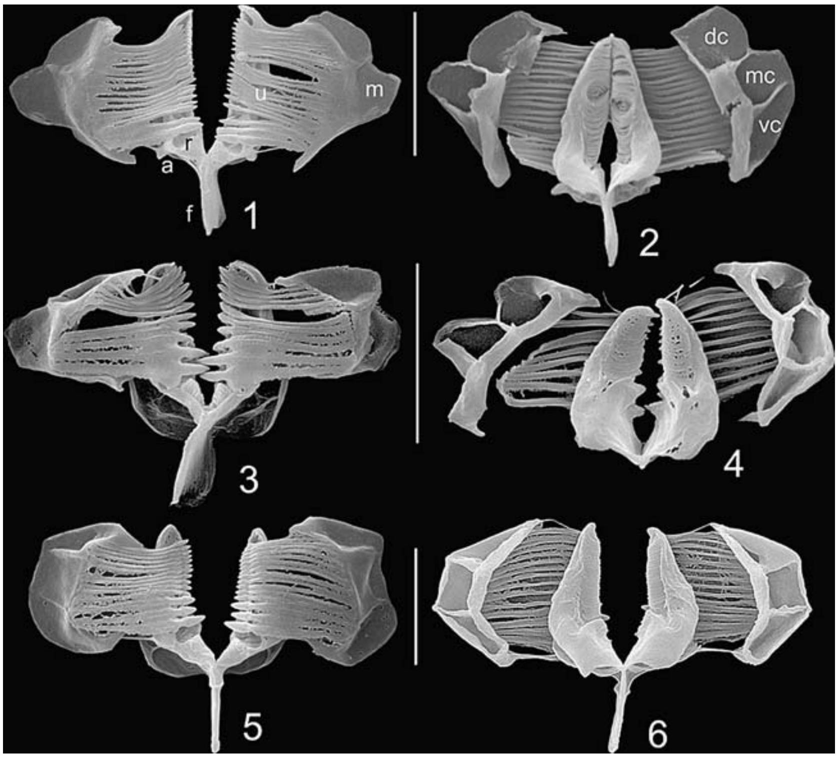

Figure 1–6. *Pompholyx sulcata* and *Testudinella* spp., SEM of trophi. 1, 2. *Pompholyx sulcata*. 3, 4. *T. caeca*. 5, 6. *T. clypeata*. 1, 3, 5. Frontal view; 2, 4, 6. caudal view. Scale bars: 10 μm. a, alula; dc, dorsal chamber; f, fulcrum; m, manubrium; mc, median chamber; r, ramus; u, uncus; vc, ventral chamber.

The number of unci teeth is rather constant in the different species (Table 2). It varies (right/left) from 9–11/9–11 (*T. caeca*) to 16–17/16–17 (*T. elliptica*) in *Testudinella*, and 17–20/18–21 in *Pompholyx sulcata*. Both symmetrical and asymmetrical unci configurations occur; asymmetrical ones predominate. Intra- and interpopulation variation in tooth number of each of the unci plates is fairly low, and not more than four teeth. The frequency distribution of unci teeth in a population of *P. sulcata* (N = 20; Fig. 27) shows a tendency for skewness: positive skew for the right uncus plate (mode 18 teeth), and negative skew for the left one (mode 20 teeth). Intrapopulation variation in *T. clypeata* (N = 29; Fig. 28) shows no pronounced maximum of frequency distribution in teeth number of the right uncus plate: almost all observations are more or less equally distributed over 14, 15 and 16 teeth. On the contrary, the left uncus plate shows a normal distribution of frequency of teeth number, with a mode of 15 teeth. The frequency histogram of *T. patina* (N = 33; Fig. 28) is based on specimens from different localities. No difference was found concerning the teeth number and the major biogeographical zones. A similar pattern as for *T. clypeata* was observed: the bulk of the frequencies for the right uncus plate is formed by more than one tooth, *viz*. 14 and 15 teeth, whereas in the left plate there is one considerable peak at 15 teeth. A relatively high frequency, both right and left, was also noted for tooth number 12, that could not be related to the morphology of the lorica nor to the origin of the specimens.

Rami

The rami are more or less elongate-triangular in frontal/caudal view. In lateral view the proximal enlarged part forms an obtuse angle with the distal part, and points ventro-caudally. The lateral proximal tips of the frontal walls are drawn out in

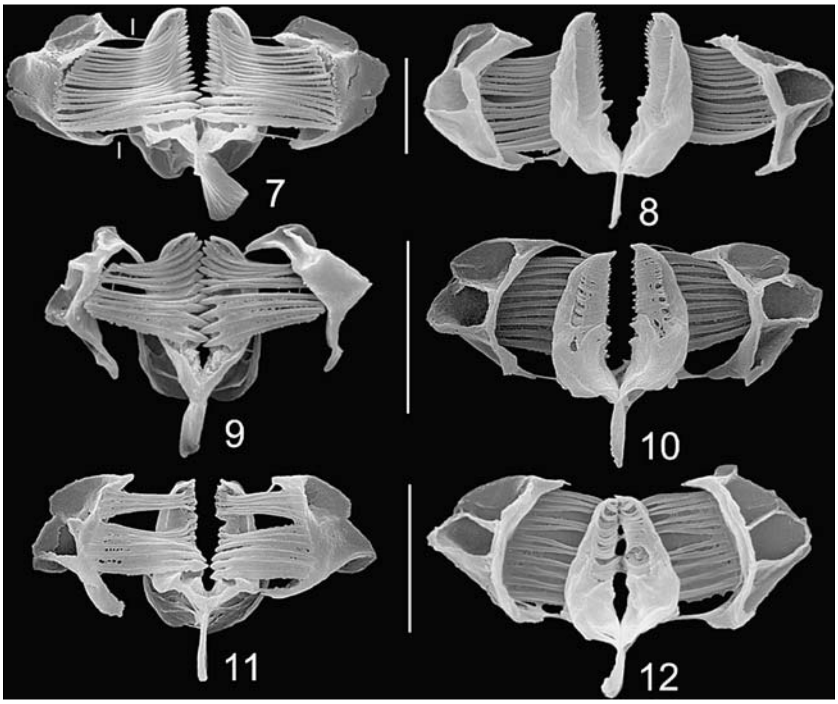

Figure 7–12. Testudinella spp., SEM of trophi. 7, 8. *T. elliptica*. 9, 10. *T. incisa*. 11, 12. *T. mucronata*. 7, 9, 11. Frontal view; 8, 10, 12. caudal view. Scale bars: 10 μm. l, ligament.

symmetrical alulae of variable length (e.g., Fig. 1: a); alulae can be absent also. The proximal part is largely hollow and shows a large lateral opening, continuing caudally. In ventral view, a shallow and oblique transverse septum (Figs. 30–32: s) can be seen, inserted on the inner side of the frontal wall and running dorsally, from the alula or caudolateral edge towards the trophi axis. The sections ventrally and dorsally from the septum probably represent the subbasal and basal rami chambers respectively (Figs. 30–32: sc, bc). The wall ventrally to the septum bears short sclerite bodies, which are probably part of the ramus apophysis.

In caudal view the inner margins of the proximal part of the rami bear asymmetrical and interlocking median apophyses, clearly composed of fused sclerite bodies (e.g., Fig. 35: ma). These apophyses are developed to a different degree in the different taxa studied. Openings in the proximal parts, probably median basifenestrae, are only found occasionally (e.g., *T. parva*, *T. truncata*: Figs. 14 and 18). The inner margins of the distal rami sections bear numerous transversely placed elongate and arched sclerite bodies, the arched rami scleropili (e.g., Fig. 33: rs), that can be more or less strongly webbed (e.g., *T. caeca*, *T. parva*: Figs. 4 and 14). The number of arched sclerite bodies varies according to the taxon (as shown in Table 2). In *Testudinella* the lowest number was noted in *T. caeca* (approximately 11/10), and the highest in *T. elliptica* (~46–66/50); in *P. sulcata* the numbers were 35–40/32–35.

In frontal view the rami show a proximal part bearing basal apophyses (e.g., Fig. 23: ba), and a gutter-shaped distal part terminating in more or less strongly hook-shaped rostella (e.g., Fig. 36: ro). The proximal margins may bear a more or less pronounced rim (e.g., Figs. 3, 9 and 13). The basal apophyses are more or less strongly developed, and consist of a varying number of more or less strongly fused rod-shaped sclerite bodies. The outer edge of the gutter-shaped section bears a fringe of fused sclerite bodies, the crista, at its inner side (e.g., Fig. 36: c). Near the inner margin of the gutter-shaped part several rows of shorter, more or less straight rami scleropili (e.g., Fig. 36: rs) are fairly loosely attached on the frontal wall of the gutter. They are probably grouped forming

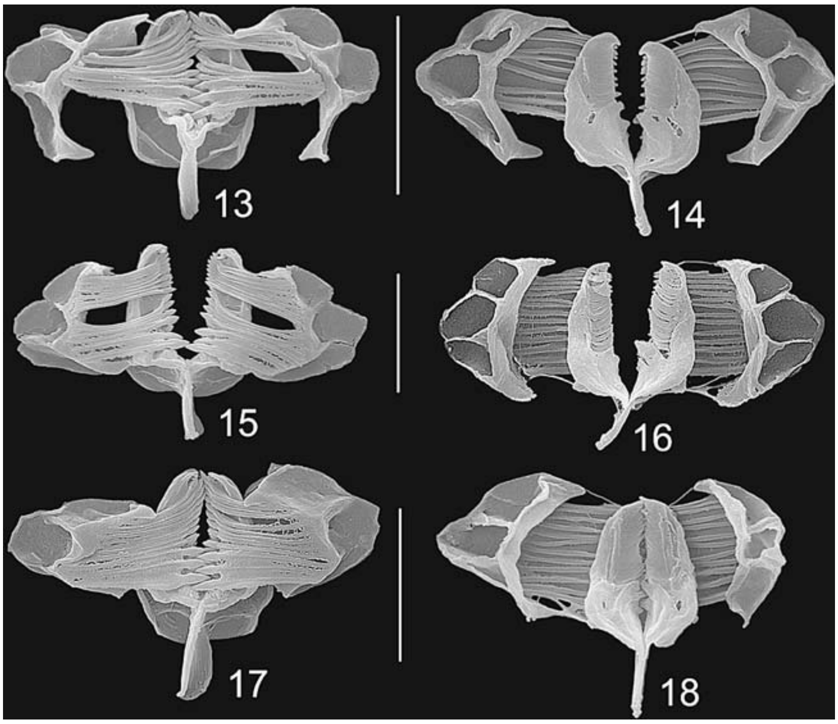

Figure 13–18. Testudinella spp., SEM of trophi. 13, 14. *T. parva*. 15, 16. *T. patina*. 17, 18. *T. truncata*. 13, 15, 17. Frontal view ; 14, 16, 18. caudal view. Scale bars: 10 μm.

some molar surface (e.g., *T. elliptica*, Fig. 38). Their distal end may be rounded (e.g., *T. elliptica*, Fig. 38), or acute (e.g., *P. sulcata*, Fig. 36; *T. truncata*, Fig. 40). The rami scleropili show weakly developed seams.

Fulcrum

The fulcrum is placed in the extension of the oblique proximal rami part, pointing ventro-caudally (Figs. 29 and 30). It is short, more or less trapezoid in lateral view, and uniformly thin with the exception of the frontal margin that can be thickened more or less (e.g., *T. incisa*, *T. truncata*). The fulcrum is apparently composed of a double layer of long and appressed sclerite bodies. Frontally a great number of these sclerite bodies are not involved in the formation of the junction with the rami. They can be appressed (e.g., *P. sulcata*, *T. elliptica*, Figs. 23, 25), or bordering a distinct opening proximally (*T. clypeata*, *T.* sp. n., Figs. 19, 22 and 26). A basal plate or hook is absent.

Discussion

Amongst the distinct challenges in rotifer research, reviewed by Wallace (2002), are taxonomic training and study, and phylogeny. The importance of detailed descriptions of the generally species-specific trophi based on SEM in taxonomic (e.g. Sanoamuang, 1993, 2002; Segers, 1995; De Smet, 1996, 1997; Segers & Wallace, 2001) and phylogenetic studies (e.g. Markevich, 1989; Segers & Wallace, 2001; Sørensen, 2002) has been established irrefutably. However, data from many taxa are completely lacking or based on light microscopy only. The results presented above for *Pompholyx sulcata* and nine species of *Testudinella* are the first to describe SEM trophi morphology of Testudinellidae in an extensive and detailed way. It is obvious that the overall similarity of the structure of the trophi in the Testudinellidae studied is very great. No fundamental differences are apparent between the genera *Pompholyx* and *Testudinella*, which in combination with the other

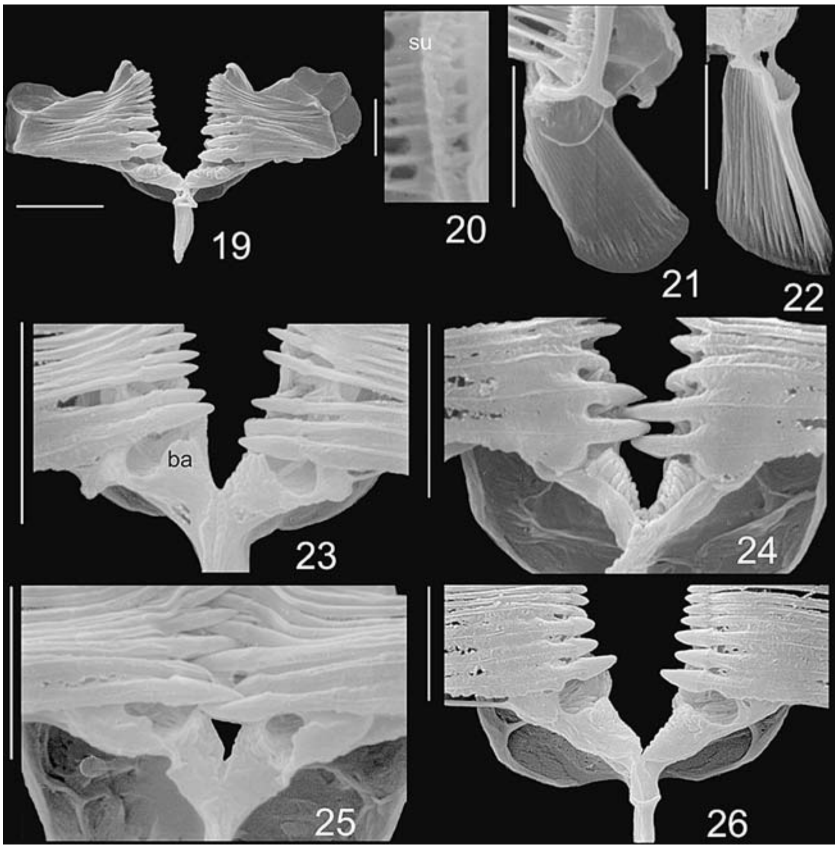

Figure 19–26. *Pompholyx sulcata* and *Testudinella* spp., SEM of trophi. 19. *Testudinella* sp. n., frontal view. 20. *P. sulcata*, detail subuncus. 21. *P. sulcata*, fulcrum, left lateral view. 22. *T. clypeata*, fulcrum, right lateral view. 23–24. Detail unci and rami, frontal view: 23. *P. sulcata*, 24. *T. caeca*, 25. *T. elliptica*, 26. *T. clypeata*. Scale bars: 19: 10 μm; 20: 1 μm; 21– 26: 5 μm. ba, basal apophysis; su, subuncus.

Table 2. Number of unci teeth and arched rami scleropili (right/left)

Taxon	Unci teeth	Arched rami scleropili
Pompholyx sulcata	17–20/18–21 (20)*	~35–40/32–35 (5)*
Testudinella caeca	9–11/9–11 (7)	~11/10 (2)
T. clypeata	14–16/14–16 (25)	~25–26/28–30 (5)
T. elliptica	16–17/16–17 (6)	~46–66/50 (3)
T. incisa	11/9–10 (8)	~18–25/20–25 (3)
T. mucronata	11–12/11–12 (8)	~15/20 (5)
T. parva	10/10 (8)	~19/18 (3)
T. patina	12–16/12–16 (26)	~30–31/31–*32* (4)
T. truncata	12/12 (5)	~25/22 (4)
T. sp. n.	10/10 (3)	–

*Number of specimens bracketed.

external morphological features, confirm them as monophyletic taxa.

To date, taxonomy of the genus *Testudinella* is exclusively based on features of the lorica (e.g. outline and cross-section, position of lateral antennae and foot opening), that are known to vary independently of each other, resulting in a large number of combinations (Ruttner-Kolisko, 1974; Koste, 1978). This led to the description of approximately 80 species and subspecies, of which several were synonymized or considered formae (e.g. Koste, 1978). In a recent publication, Segers (2002a) recognizes a tentative number of 40 valid species. The results of the SEM prove that trophi morphology in *Testudinella* is highly species-specific, and will be a most valuable feature for identification and revision of the family. Among

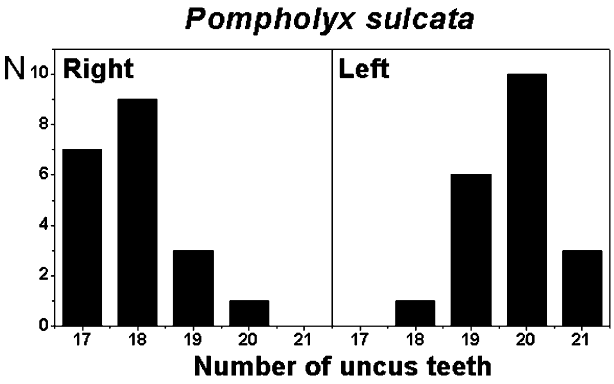

Figure 27. Frequency distributions of right and left uncus teeth in a population of *Pompholyx sulcata* (Fort Merksem, Belgium, 3 July 1985).

the characters considered to be most reliable and manageable for identification are: number of unci teeth and arched rami scleropili, shape of the major unci teeth, presence/absence of lateral expansion on the first major teeth, presence/absence of a proximal opening in the fulcrum, and presence/absence of alulae.

A detailed comparison of the trophi of Testudinellidae with these of the other families of Flosculariacea is difficult, since published records are still few (e.g., Sanoamuang, 1993, 2002: *Filinia*; Segers, 1997: *Floscularia*; Melone et al., 1998: *Sinantherina*; Segers & Wallace, 2001: Conochilidae, *Lacinularia*, *Ptygura*; Sørensen, 2002: *Floscularia*;

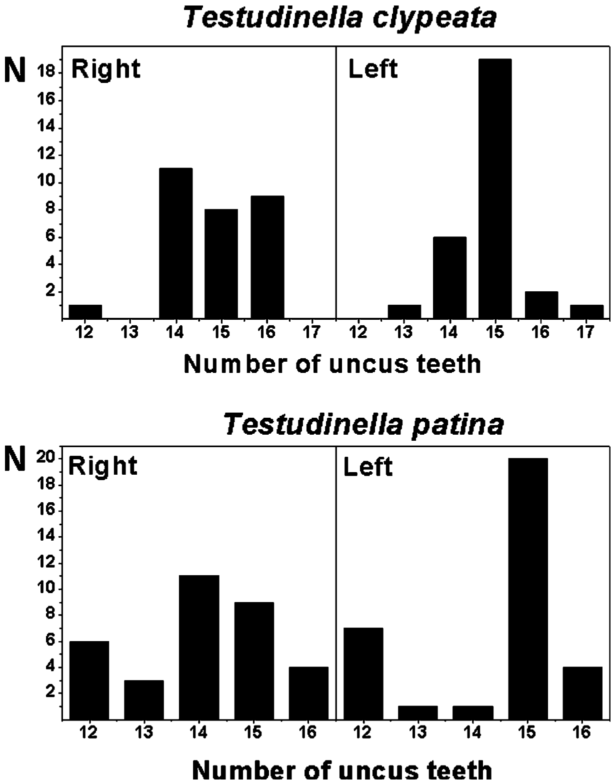

Figure 28. Frequency distributions of right and left uncus teeth in a population of *Testudinella clypeata* (Veerse Meer, The Netherlands, 8 September 2002), and in *T. patina* originating from different geographical localities.

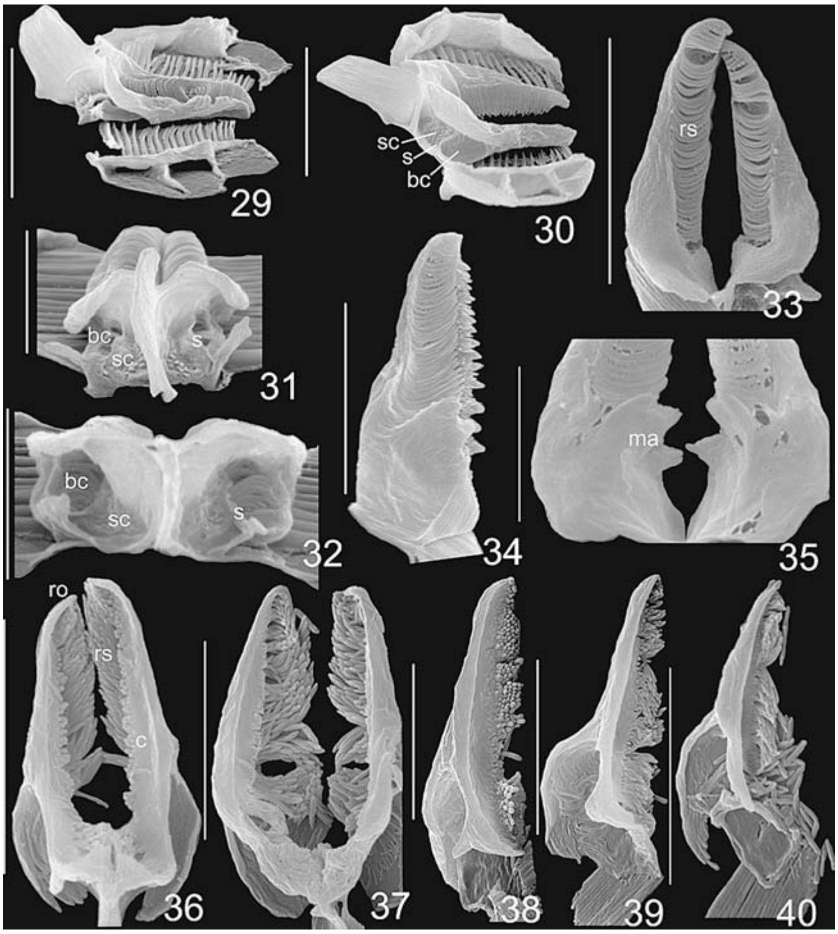

Figure 29–40. Pompholyx sulcata and *Testudinella* spp., SEM of trophi. 29. *P. sulcata*, caudo-lateral view. 30. *T. clypeata*, caudo-lateral view. 31. *P. sulcata*, rami caudo-ventral view. 32. *T. incisa*, rami caudo-ventral view. 33. *P. sulcata*, rami caudal view. 34. *T. patina*, ramus caudal view. 35. *T. caeca*, median apophyses, caudal view. 36–40. Rami, frontal view: 36. *P. sulcata*, 37. *T. clypeata*, 38. *T. elliptica*, 39. *T. patina*, 40. *T. truncata*. Scale bars: 29–31, 33, 34, 36–40: 10 μm; 32, 35: 5 μm. c, crista; bc, basal chamber; ma, median apophyses; ro, rostellum; rs, rami scleropili; s, septum; sc, subbasal chamber.

Segers, 2002b: *Trochosphaera*, *Horaella*). At this stage generalizations should be interpreted with caution, e.g., proximal expansions on the first major teeth reported (Segers & Wallace, 2001) to be present only in *Horaella brehmi* and *Filinia brachiata* (Trochosphaeridae), have now been demonstrated in *Testudinella* likewise. Within Flosculariacea trophi are fairly homogeneous. Supposed taxonomically significant differences in trophi structure between Testudinellidae and the other families concern the pseudoalulae, the shape of the fulcrum, and asymmetry of the unci. To date pseudoalulae have been reported in Trochosphaeridae only. Fulcra of Conochilidae, Hexarthridae and Flosculariidae usually bear a basal plate or hook, which is absent in Testudinellidae, and a pronounced asymmetry of the unci plates is characteristic for Conochilidae.

The fulcrum is an unpaired structure present in Monogononta and Seisonidea, but absent in Bdelloidea (de Beauchamp, 1965; Nogrady et al., 1993). Controversy exists on the origin of the fulcrum. According to Markevich (1985) and Markevich & Kutikova (1989) it is derived from scleropili on the inner margin of the rami. In an alternative hypothesis Segers & Melone (1998) suggest that the fulcrum is the median unpaired part of the original set of elements in rotifer trophi, which implies that the fulcrum has been lost in Bdelloidea. In Testudinellidae the fulcrum seems to arise by fusion of two sheet-like sets of sclerite bodies, which I suggest are derived from paired lateral elements (Segers & Melone, 1998), homologous to the elements responsible for the formation of the distal group of minor unci teeth in Bdelloidea.

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References

de Beauchamp, P., 1965. Classe des Rotifères. In P. -P. Grassé, Traité de Zoologie. T IV(3): 1225–1379. Masson et Cie. Ed., Paris.

De Smet, W. H., 1996. Rotifera 4. The Proalidae (Monogononta). In Nogrady, T. & H. J. F. Dumont (eds) Guides to the Identification of the Microinvertebrates of the Continental Waters of the World 9. SPB Academic Publishing bv, Amsterdam: 102 pp.

De Smet, W. H., 1997. Dicranophoridae (Monogononta) in Rotifera 5. The Dicranophoridae and the Ituridae (Monogononta). In Nogrady, T. & H. J. F. Dumont (eds) Guides to the Identification of the Microinvertebrates of the Continental Waters of the World 12. SPB Academic Publishing bv, Amsterdam: 1–325.

De Smet, W. H., 1998. Preparation of rotifer trophi for light and scanning electron microscopy. Hydrobiologia 387/388: 117–121.

Koste, W., 1978. Rotatoria. Die Rädertiere Mitteleuropas. Ein Bestimmungswerk, begründet von Max Voigt. Überordnung Monogononta. 2nd edn. I. Textband, 673 pp, II. Tafelband, 234 Taf., Gebrüder Borntraeger: Berlin, Stuttgart.

Kutikova, L. A., 1970. Kolovratki fauny SSSR (Rotatoria). Opredeliteli Faune SSSR 1049, 744 pp, Akademiya Nauk SSSR, Leningrad. (in Russian).

Markevich, G. I., 1985. Main trends of idioadaptive evolution of rotifers. Jaws. In Kutikova L. A. (ed), Proceedings of the Second All-Union Symposium on Rotifers, Leningrad, October 18–20, 1983. Akademiya Nauk SSSR, Zoologiceskaya Institut, 17–37.

Markevich, G. I., 1989. Morphology and principal organization of the sclerite system of the rotifer mastax. In Biologiya, Sistematika I Funksionalnya Morfologiya Presnovodnyk Zhivotnyk. Trudy Instituta Biologii Vnutrennikh Vod. Akademiya nauk S.S.S.R. 56: 27–82. (in Russian).

Markevich, G. I. & L. A. Kutikova, 1989. Mastax morphology under SEM and its usefulness in reconstructing rotifer phylogeny & systematics. Hydrobiologia 186/187: 285–289.

Melone, G., C. Ricci & H. Segers, 1998. The trophi of Bdelloidea (Rotifera): a comparative study across the class. Canadian Journal of Zoology 76: 1755–1765.

Nogrady, T., R. L. Wallace & T. W. Snell, 1993. Rotifera 1. In Nogrady, T. & H. J. F. Dumont (eds), Biology, Ecology and Systematics. Guides to the Identification of the Microinvertebrates of the Continental waters of the World. SPB Academic Publishing, The Hague, 142 pp.

Remane, A., 1929–1933. Rotatoria. In Bronn's Klassen und Ordnungen des Tier-Reichs, Bd. 4, Abt. II/1. Akademische Verlagsgesellschaft m.b.H., Berlin, 577 pp.

Ruttner-Kolisko, A., 1974. Plankton rotifers. Biology and Taxonomy. Die Binnengewässer 26: 99–234.

Sanoamuang, L., 1993. Comparative studies on scanning electron microscopy of trophi of the genus *Filinia* Bory De St. Vincent (Rotifera). Hydrobiologia 264: 115–128.

Sanoamuang, L., 2002. Genus *Filinia* Bory de St. Vincent, 1824. In Nogrady, T., H. Segers & H. J. F. Dumont (eds), Rotifera 6. Asplanchnidae, Gastropodidae, Lindiidae, Microcodidae, Synchaetidae, Trochosphaeridae and *Filinia*. Guides to the Identification of the Microinvertebrates of the Continental Waters of the World, 224–257.

Segers, H., 1995. A reappraisal of the Scaridiidae (Rotifera, Monogononta). Zoologica Scripta 24: 91–100.

Segers, H., 1997. Contribution to a revision of *Floscularia* Cuvier, 1798 (Rotifera: Monogononta): notes on some Neotropical taxa. Hydrobiologia 354: 165–175.

Segers, H., 2002a. The nomenclature of the Rotifera: annotated checklist of valid family- and genus-group names. Journal of Natural History 36: 631–640.

Segers, H., 2002b. Family Trochosphaeridae Harring, 1913. In Nogrady, T., H. Segers & H. J. F. Dumont (eds), Rotifera 6. Guides to the Identification of the Microinvertebrates of the Continental waters of the World, 214–223.

Segers, H. & G. Melone, 1998. A comparative study of trophi morphology in Seisonidea (Rotifera). Journal of Zoology, London 244: 201–207.

Segers, H. H. & R. L. Wallace, 2001. Phylogeny and classification of the Conochilidae (Rotifera, Monogononta, Flosculariacea). Zoologica Scripta 30: 37–48.

Sørensen, M. V., 2002. On the evolution and morphology of the rotiferan trophi, with a cladistic analysis of Rotifera. Journal of Zoological Systematics and Evolutionary Research 40: 129–154.

Wallace, R. L., 2002. Rotifers: exquisite metazoans. Integrative and Comparative Biology 42: 660–667.