Europ. J. Protistol. 37, 167–180 (2001)

http://www.urbanfischer.de/journals/ejp

European Journal of
PROTISTOLOGY

Stable for 15 million years: scanning electron microscope investigation of Miocene euglyphid thecamoebians from Germany, with description of the new genus *Scutiglypha*

W. Foissner[1,]* and W. Schiller[2]

[1]Universität Salzburg, Institut für Zoologie, Hellbrunnerstrasse 34, A - 5020 Salzburg, Austria;
Fax: +43 (0) 662 8044-5698
[2]Universität Frankfurt am Main, Geologisch-Paläontologisches Institut, Senckenberganlage 32–34, D - 60054 Frankfurt am Main, Germany; Fax: (069) 798-22958; E-Mail: W.Schiller@em.uni-frankfurt.de

Received: 20 November 2000; 28 March 2001. Accepted: 6 April 2001

Material of the Middle Miocene kieselgur deposit of Beuern (Germany, Vogelsberg), about 15 million years old limnic sediment of a volcanic crater lake was investigated with the scanning electron microscope without any previous preparations. The samples mainly consisted of diatom shells and sponge spiculae, but also contained well-preserved tests of euglyphid thecamoebians where, for the first time, individual test scales could be clearly seen. Two species, each represented by several specimens, were investigated in detail. Both had highly characteristic body and aperture scales, which were indistinguishable from those of extant *Euglypha crenulata* Wailes, 1912 and *E. scutigera* Penard, 1911. Thus, the fossil specimens were assigned to these extant species which, however, were referred to a new genus, *Scutiglypha*, characterised by scutiform, crenate body scales. Clearly, details of the scales remained stable over millions of generations, although testate amoebae are largely asexual. The mechanism responsible for this stability is unknown, but intrinsic ("internal") selection might override environmental ("external") selection, thus promoting evolutionary stability of main parts of the system.

Key words: *Euglypha*; Fossil thecamoebians; Internal selection; Kieselgur; Miocene; Testacea.

Introduction

The established pre-Quaternary finds of fossil thecamoebians are minimal and were excellently reviewed by Medioli et al. (1990). They listed and critically discussed seven reports published between 1930 and 1990. These contain 26 species, most of which are agglutinated forms and have thus been questioned for one reason or another. The earliest presumed thecamoebians are from the Namurian age (Upper Carboniferous), that is about 320 million years old. Since the review by Medioli et al. (1990), some new fossil testate amoebae have been reported. Wolf (1995) described *Arcella* sp. from about 300 M.Y. old Westphalian B (Upper Carboniferous) bituminous coals in Germany. *Pontigulasia*-like and *Cyphoderia*-like thecamoebians were discovered in Middle Cretaceous (~ 100 M.Y.B.P.) amber from Kansas and in 25–400 M.Y. old amber from the Dominican Republic (Waagoner 1996a, b). Boeuf and Gilbert (1997) provided excellent scanning electron micrographs of *Trinema lineare pliocenica* from about 3 M.Y. old pliocenic plant splinters moulded in volcanic ashes at Chilhac, France. Finally, Schönborn et al. (1999) described a new species, *Hyalosphenia baueri*, and two known species, *Cyclopyxis eurystoma* and *Centropyxis aculeata* var. *oblonga*, from 220 M.Y.

*corresponding author

0932-4739/01/37/02-167 $ 15.00/0

old Triassic amber from Bavaria. Very recently, Porter & Knoll (2000) reported exciting vase-shaped microfossils from marine, neoproterozoic deposits (about 750 M.Y.B.P.) of the Grand Canyon, USA. These fossils show details of morphology and taphonomy that collectively point to affinities with lobose and filose testate amoebae.

In the present paper, we report on two euglyphid testate amoebae from a Miocene lacustrine deposit. Both were briefly described in a preliminary report (Schiller 1997), which also contains a third species, probably a *Trinema*. More detailed investigations of the sediments provided further well-preserved specimens, which could be reasonably assigned to extant species, viz. *Euglypha crenulata* and *E. scutigera*, two rather rare but probably cosmopolitan freshwater thecamoebians. Our report is unique in that it shows the test-building scales with a clarity never seen before in thecamoebians older than 3 M.Y.

Material and methods

The material was collected in an abandoned kieselgur pit at the west-slope of the Vogelsberg near the village of Beuern, about 12 km NE of the town of Giessen, Germany (*R 3486830, H 5610680*). The kieselgur deposit was formed in a volcanic crater lake and the thickness of the sediments reached up to 12 m. According to the interbedded vertebrate fauna and the pollen flora, the lake sediments originated in the middle Miocene, that is, about 15 million years ago. See Schenk (1950), Eikamp (1978), Hottenrott (1988), and Schiller (2000) for details on geology, fauna, and flora. The microfossils mainly consist of diatoms, chrysophycean cysts, and spiculae of freshwater sponges.

Limnic kieselgur sediments usually represent stillwater deposits with distinct lamination. The sample material needs careful and very slow drying, otherwise the lamination will be interspersed by many fissures. Well dried and suitable material can be split with a scalpel parallel to the lamination. The scalpel should be used like a "crowbar" to separate the weakly lithified kieselgur layers without smearing the split surfaces. Depending on the bituminous content of the sediment, more or less thin-shaled layers can be produced. Oblique splitting will lead to terraced sample surfaces, showing several laminas allowing the examination of the sedimentation. The thickness of a single lamina reflects sedimentation at almost constant conditions of bioproductivity and may reach a few microns up to some millimetres.

The detection of well-preserved fossil thecamoebians embedded in a diatomite needs much time. But once proved, the probability of finding further remnants in the same layer is high. The surface of the layers can be pre-investigated by reflected light microscopy, however, this method will not guarantee detection of each remnant of thecamoebian tests.

For the present investigations, fifty samples presumed to contain thecamoebians were formatted and glued on specimen holders for scanning electron microscopy (SEM), and gold-sputtered. Only a few samples really contained thecamoebian remnants. Kieselgur samples tended to burst when examined with the SEM. Thus, only well-packed sediment specimens should be used.

For transmitted light microscopy, minute parts of the sediment were elutriated, spread on microscope slides and embedded in Naphrax, a mounting medium with high refractive index. However, no thecamoebian remnants could be found, possibly because the tests had disorganised and individual scales had too low a contrast.

Results

Scutiglypha nov. gen.

Diagnosis: Euglyphidae with scutiform, crenate body-scales.

Type species: *Euglypha crenulata* Wailes, 1912.

Etymology: Composite of the Latin noun "scutum" (shield) and the Greek noun "glyphe" (ornamented shell). Feminine gender.

Scutiglypha crenulata (Wailes, 1912) nov. comb.

1879 *Euglypha alveolata* – Leidy, Geol. Surv. Terr., 12: 207 (partim, Plate 35, Figs. 1, 5, 7–10).

1912 *Euglypha crenulata* Wailes, sp. nov., J. Linn. Soc., 32: 147.

1912 *Euglypha crenulata* var. *minor* Wailes, var. nov., J. Linn. Soc., 32: 148 (has to be considered as a subspecies, according to article 45.6.4. of the ICZN).

1917 *Euglypha australica* var. *elegans* Playfair, n. var., Proc. Linn. Soc. NSW, 42: 661 (has to be considered as a subspecies, according to article 45.6.4. of the ICZN).

1958 *Euglypha crenulata* var. *elongata* Thomas, n. var., Bull. Soc. Hist. nat. Afr. nord, 49: 88 (has to be considered as a subspecies, according to article 45.6.4. of the ICZN).

1962 *Euglypha crenulata* Wailes – Decloitre, Arch. Protistenk., 106: 62 (first reviser).

?1981 *Euglypha acanthophora* (Ehrenberg, 1841) – Ogden, Bull. Br. Mus. nat. Hist., 41: 138.

Synonymy: Synonymy is according to Playfair (1917) and Decloitre (1962). However, we do not consider it as definite because details of scale morphology are different. Thorough scanning electron microscopic investigations on various populations are required to explore the status of the subspecies.

Original description (Fig. 1a–d): Test large, not compressed, either elongate-oviform or with sides tapering from the hemispherical fundus in nearly straight lines to the aperture. Fundus furnished with 2–6 stout scale-spines arising at nearly equal distances from the apex. Aperture circular and bordered by two rows of finely denticulated scales, 12–14 in each row. The body-scales scutiform, with crenulated lower margins. Nucleus large, placed posteriorly; pseudopodia normal. Length 115–140 µm; diameter 67–77 µm. Aperture 25–30 µm. Length of spines 20–50 µm. Body-scales 10–12 µm long. Nucleus about 35 µm in diameter. Habitat: Submerged *Sphagnum*, lakes, ponds, etc. Distribution: New Jersey and Pennsylvania (Leidy); New York State, Long Island. Distinguishing features: Distinguished from *E. scutigera* and *E. armata* by its crenulated scutiform body-scales and its greater size; from *E. aspera* by its smooth test and usually smaller size. Remarks: The form of the spines is subject to the same variation as in *E. armata*: they may be either (a) short and truncate, (b) short and pointed, (c) long and straight, or (d) long and flexuous; they are sometimes incurved. Tests destitute of spines are occasionally found.

Original description of var. *minor* (Fig. 1e): Test similar to type, but smaller and usually destitute of spines. Length 80–100 µm; breadth 45–64 µm; aperture 16–20 µm. Habitat: Lakes and ponds. Distribution: Split Rock Lake, N.J., and Long Island, N.Y. Remarks: Is usually glabrous, but when provided with spines only distinguished from *E. armata* by the shape of the body-scales. The body-scales are somewhat different in shape from those of the type (Fig. 1e). When incorporated in the tests of *Nebela collaris* and *N. equicalceus* they are displayed with perfect distinctness, and can be observed even better than when artificially isolated. These scales are generally 11–12 µm in length, but the width varies according to the position they occupy on the test, those centrally placed being the widest.

The "variety" *elegans* has slightly different body-scales (Fig. 1f), is ellipsoidal, and has a size of 67–137 × 32–72 µm. The "variety" *elongata* is almost cylindrical, usually possesses spines, and has a size of 80–105 × 35–40 µm.

Description of the fossil *S. crenulata* (Fig. 2a–g, 6–13; Table 1): We found six specimens, of which

Table 1. Scale measurements (µm) on fossilised *Scutiglypha crenulata*.

Scales[1)]	Specimen 1	Specimen 2	Specimen 3[2)]	Specimen 4	Specimen 5	Specimen 6
Body-scales						
Length $\bar{x}$	7.9	10.0	6.4	8.0	8.3	8.1
M	8.0	10.7	6.5	7.9	8.2	8.0
SD	0.7	1.2	0.3	0.5	0.7	0.6
CV	8.4	11.5	4.8	5.7	8.5	7.5
Min	7.3	8.7	5.9	7.6	7.6	7.6
Max	9.0	10.7	6.5	8.5	9.4	8.8
n	7	3	4	3	6	3
Width $\bar{x}$	6.3	7.8	5.9	5.2	6.4	5.9
M	6.3	8.0	5.9	–	6.5	5.2
SD	0.3	1.0	0.0	–	0.8	1.2
CV	5.0	13.0	0.0	–	11.8	19.6
Min	6.0	6.7	5.9	4.1	5.3	5.2
Max	6.7	8.0	5.9	6.3	7.1	7.2
n	7	3	4	2	4	3
Aperture-scales	11 × 6.7	12.7 × 7.9	–	–	10 × 5	9.2 × 5.6
(length × width)	11.7× ?				10 × 5	

Measurements from SEM-micrographs and in µm. CV – coefficient of variation in %, M – median, Max – maximum, Min – minimum, n – number of scales measured, SD – standard deviation, – arithmetic mean.
[2)] Partially decomposed scales.

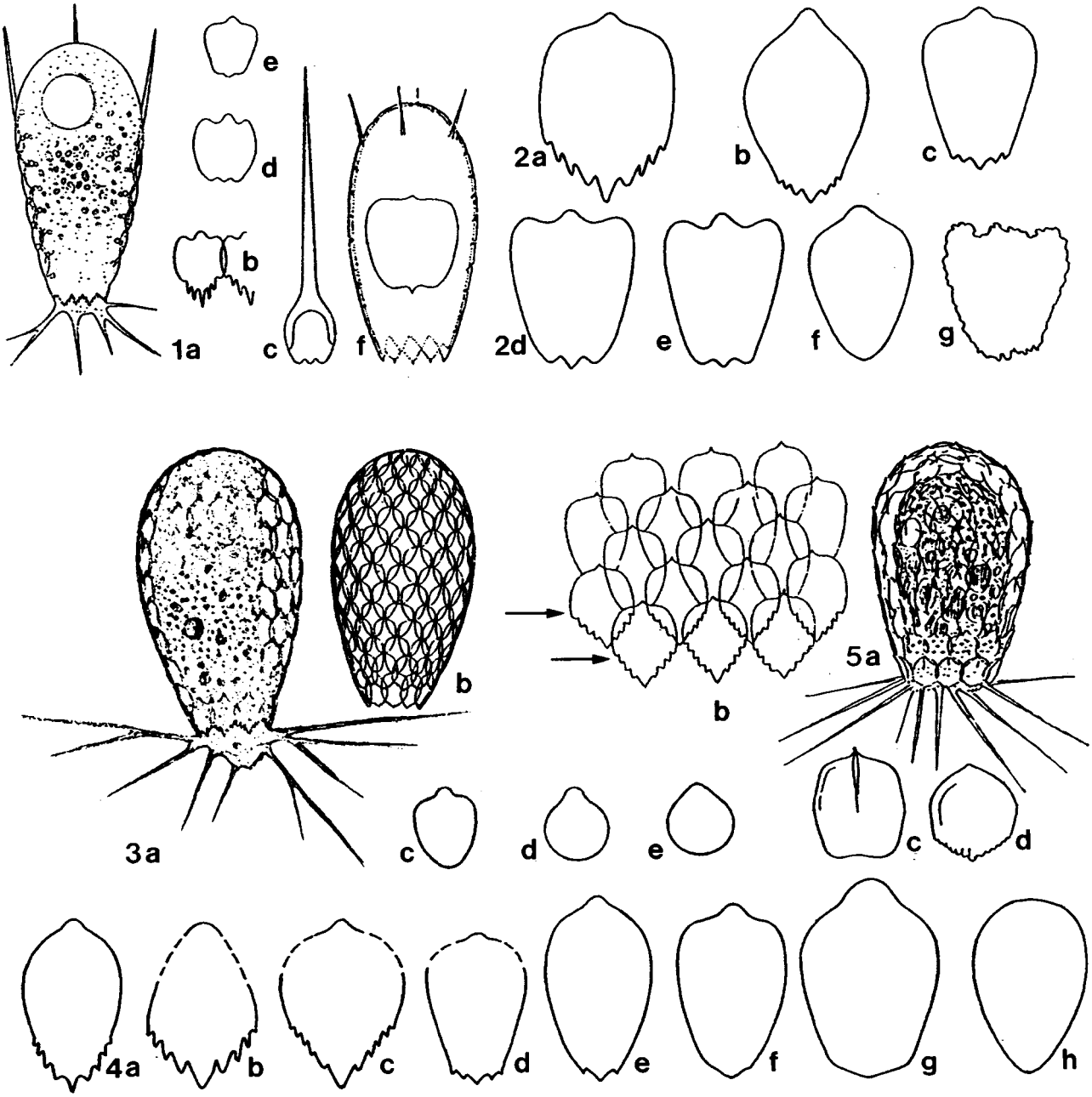

Fig. 1a–f. Original figures of recent *Scutiglypha crenulata* (a–d, from Wailes 1912), *S. crenulata minor* (e, from Wailes 1912), and *S. crenulata elegans* (f, from Playfair 1917). a: An active specimen, length 115–140 µm. b: Aperture-scale. c: Spine-scale, length 20–50 µm. d: Body-scale, length 10–12 µm. e: Body-scale of subspecies *minor*, length 11–12 µm. f: Test (length 67–137 µm) and scale (10–12 × 7–10 µm) of subspecies *elegans*. Fig. 2a–g. *Scutiglypha crenulata*, fossilised scales redrawn from SEM-micrographs. a–c: Aperture-scales in rows 1–3, size 9–13 × 5–8 µm. d, e: Body-scales, size 7.3–10 × 4.1–8 µm. f, g: Decaying body-scales, size about 6.5 µm. Fig. 3a–e. *Scutiglypha scutigera*, recent specimens from life (fromWailes 1915). a: An active specimen, length 75–90 µm. b: Original figure of test, length 77–88 µm (from Wailes and Penard 1911). c–e: Body-scales, 11–12 × 8–10 µm. Fig. 4a–h. *Scutiglypha scutigera*, fossilised scales redrawn from SEM-micrographs (stippled parts not seen). a–c: Aperture-scales from distalmost row, size 8–11.7 × 5.9–7.9 µm. d, e: Aperture-scales from second row, size as before. f–h: Body-scales, size 4.4–11.7 × 3–7.6 µm. Fig. 5a–d. *Scutiglypha aspera*, recent specimens from life (a, c, d, from Penard 1902; b, from Leidy 1879). a: An active specimen, length 150–170 µm. b: Part of test of North American population. Arrows mark the two rows of aperture-scales. c, d: Body-scale (length 18 µm) and aperture-scale.

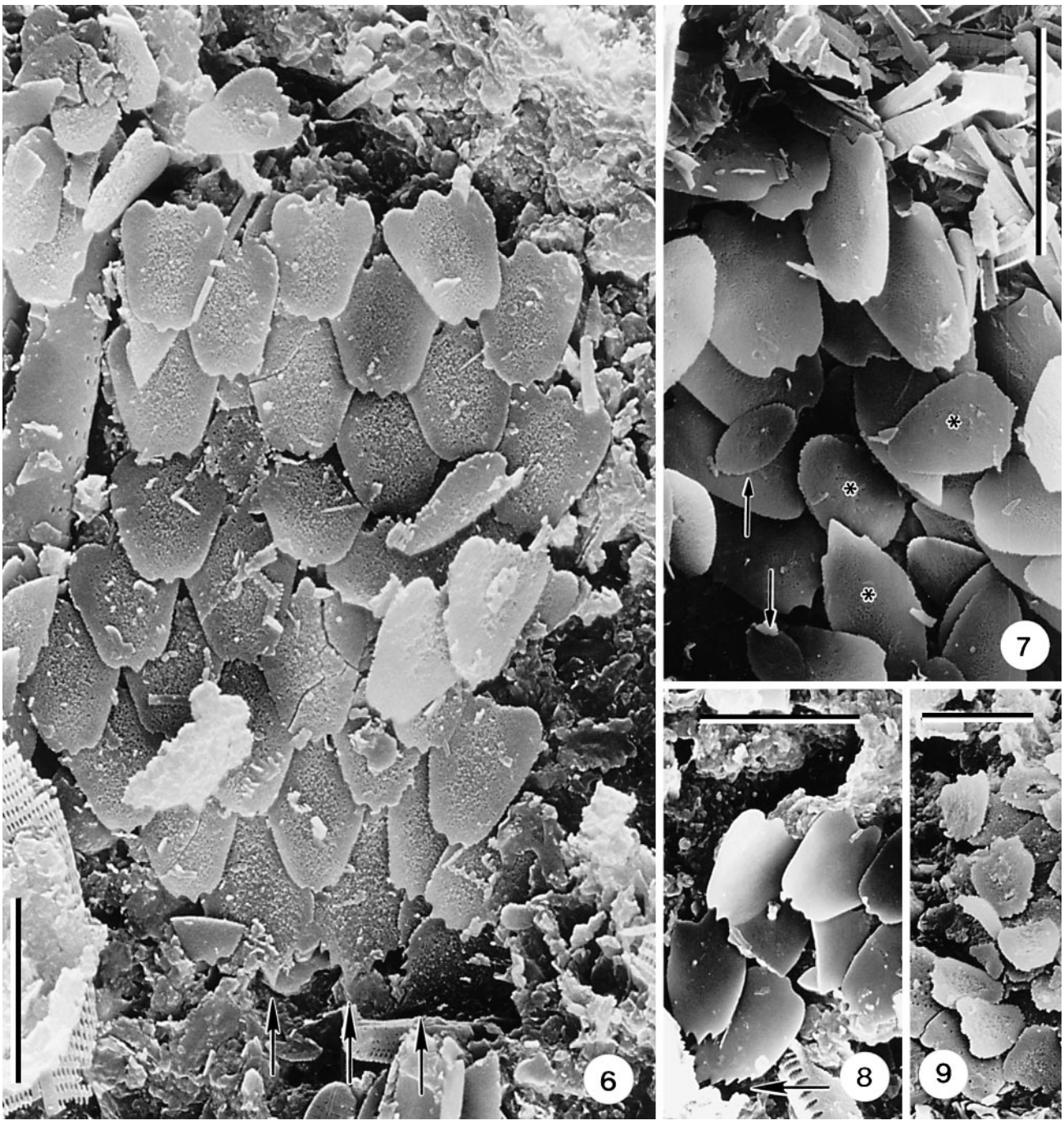

Fig. 6–9. *Scutiglypha crenulata*, Miocene fossilised specimens in the scanning electron microscope. 6: Specimen 1, which is very likely fully preserved in length and width, is composed of six or seven transverse rows of overlapping body-scales and a single row of aperture-scales. The body scales, which show mild signs of disintegration (rough surface), have the highly characteristic shape typical for this species (cp. figures 1a–f). The aperture-scales (arrows), which are slightly larger than the body-scales, have three minute lateral denticles per scale half and a prominent central tooth. 7, 9: Specimens 4 and 3 showing body scales (*) in various stages of disintegration. Apparently, disintegration commences at the scale margin causing the scales to become smaller (Table 1) and lose their crenelation. Possibly, the small, elliptical scales marked by an arrow represent a final stage of the disintegration process, or they are fundus scales. 8: Oral portion of specimen 6 showing a broad aperture-scale with four lateral denticles (arrow). Scale bars 10 µm.

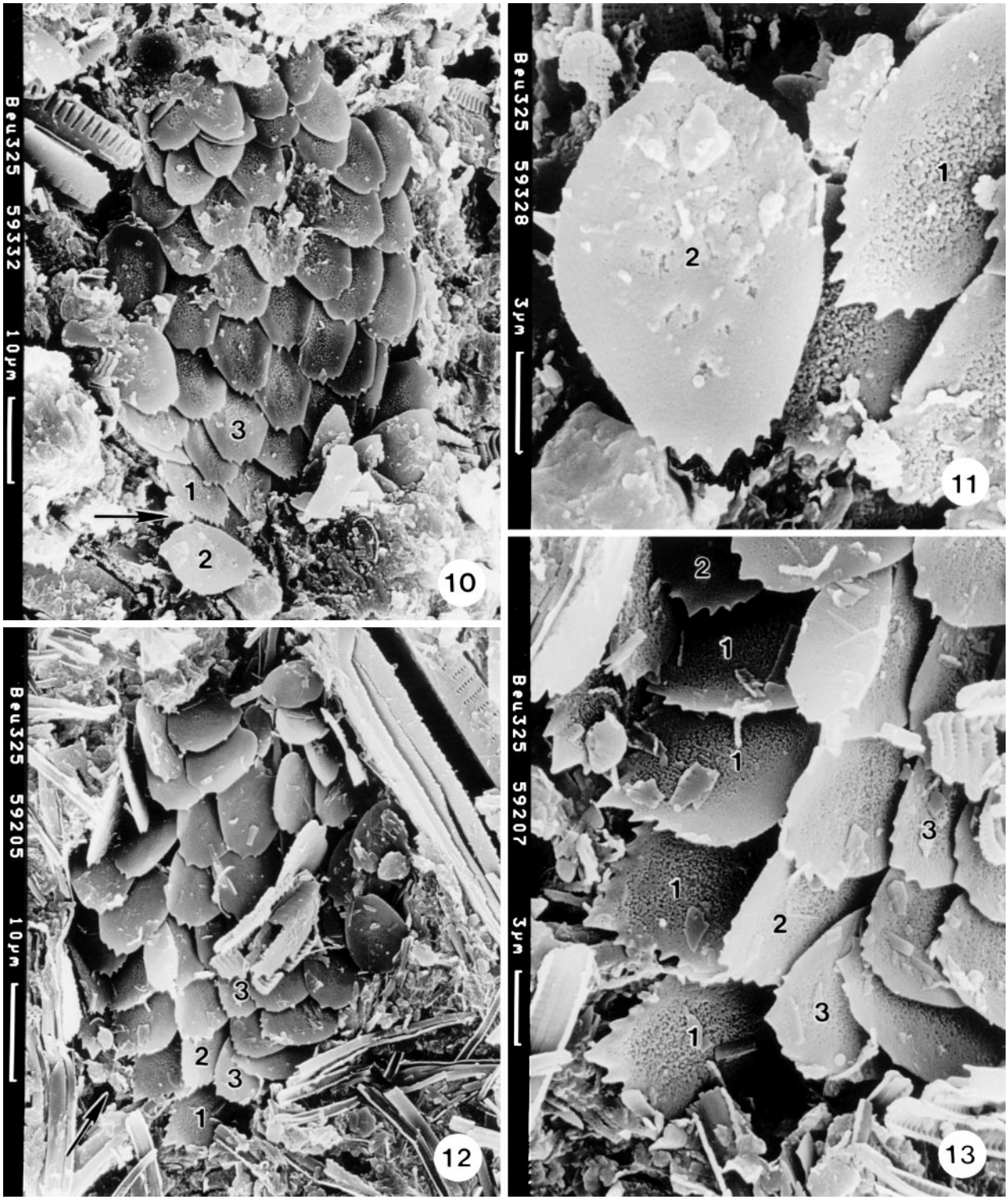

Fig. 10–13. *Scutiglypha crenulata*, Miocene fossilised specimens 2 and 5 in the SEM. Both tests are surrounded by siliceous remnants of other protists, mainly diatoms. Arrows mark regions shown at higher magnification in figures 11 and 13. Both specimens have three rows of aperture-scales (numbers 1–3) differing in the number of denticles from the body-scales and among themselves: four to five denticles per scale half in the first row (numbered 1), three denticles in the second row (2), two denticles in the third row (3), and one denticle in the following body-scales.

three look fairly complete, measuring about 46 × 33 µm, 47 × 35 µm, and 57 × 40 µm. The compressed, elliptical tests consist of 7–8 transverse rows of overlapping scales and are destitute of spines.

Body and apertural-scales are distinguishable in four of the six specimens. The body-scales are highly characteristic due to their trapeziform shape and neat crenulation of the oral and aboral margin. Invariably, the crenulation consists of two indentations orally and aborally, producing three processes, each usually more distinctly rounded aborally than orally, where they are often tooth-like (Fig. 2d, e, 6–8, 10, 12). The size of the scales is rather similar in all specimens (Table 1) and 8.4 × 6.4 µm on average (length: min 7.3, max 10.7, SD 1.0, CV 11.4, n 22; width: min 4.1, max 8.0, SD 1.0, CV 16.2, n 19).

The body-scales gradually change into aperture-scales by becoming larger (10.7 × 6.8 µm on average, n 6; Table 1) and more scutiform and crenate, that is, lose the aboral indentations and have more denticles (indentations) at the oral margin (from aboral to oral): one denticle per scale half in ordinary body-scales, two denticles in the third transverse scale row, three denticles in the second, and four to five in the first (apertural) scale row (Fig. 2a–c). This sequence is recognisable in two specimens (Fig. 10, 12, 13), while two other specimens possibly have only one row of aperture-scales (Fig. 6, 8). The denticulate margin of the distalmost aperture-scales is thickened (Fig. 13).

Most scales show signs of disintegration, that is, have a more or less rough surface and margin (Fig. 6, 7, 9, 11, 13). One of the six specimens is almost fully decayed showing that the scales dissolve from the margin to the centre, becoming smaller (Table 1) and scutiform or ellipsoidal, that is, gradually lose the characteristic crenulation (Fig. 7, 9). However, we cannot exclude that the small, elliptical platelets are fundus scales, which are usually smaller and simpler in *Euglypha* species.

Scutiglypha scutigera (Penard, 1911) nov. comb.

1911 *Euglypha scutigera* Penard, sp. nov., Proc. R. Ir. Acad., 31: 41.
1915 *Euglypha scutigera* Penard – Wailes, British Rhizopoda III: 7 (revision).
1962 *Euglypha scutigera* Penard – Decloitre, Arch. Protistenk., 106: 69 (revision).

Original description (Fig. 3a–e): Test, medium size, destitute of spines, oviform, circular in section. The circular aperture bordered by two rows of finely denticulate scales, ten or twelve in each row. The body-scales scutiform, imbricated, arranged in alternating longitudinal rows. Plasma and pseudopodia normal. Length 77–88 µm, width 46–51 µm, aperture 14–20 µm, scales 11 by 8 µm to 12 by 10 µm. Habitat: Aquatic plants and submerged moss. Distribution: North America, several Swiss localities, England. Remarks: *E. scutigera* is distinguished from *E. alveolata* and *E. armata* by the scutiform scales. It is probably the prototype of *E. aspera* Penard, a large deep-water form with the scutiform scales prolonged into thorn-like processes.

Description of the fossil *S. scutigera* (Fig. 4a–h, 14–20; Table 2): We found three specimens all looking well-preserved and fairly complete measuring 53 × 40 µm, 44 × 36 µm, and 56 × 38 µm. The strongly flattened, elliptical tests consist of 7–10 transverse rows of overlapping scales and are destitute of spines.

Body and apertural scales are distinguishable in each of the three specimens. The body-scales are highly characteristic due to their scutiform shape and the rounded aboral process, while the oral end is narrowly rounded or bluntly pointed; in the posterior pole region, the scales often lack the aboral process, that is, are obovate (Fig. 4f–h, 14, 17–19). Interestingly, in two specimens some scales are turned upside down within the ordinarily and rather regularly arranged scales (Fig. 14, 17, 18). The size of the scales is rather similar in specimens 1 and 3, while those of specimen 2 are considerably smaller (Table 2). On average, the scales measure 8.6 × 6.0 µm (length: min 4.4, max 11.7, SD 1.9, CV 22.1, n 27; width: min 3, max 7.6, SD 1.3, CV 21.7, n 27).

The body-scales gradually change into aperture-scales by becoming larger (10.2 × 6.7 µm on average, n 7; Table 2) and denticulate at the apertural margin (from aboral to oral): no distinct denticles in ordinary body-scales, one or two denticles per scale half in the second transverse scale row, and four to five denticles in the first (apertural) scale row. The denticulate margin is not thickened (Fig. 4a–e, 14, 15, 17–20).

The scales hardly show signs of disintegration but those of specimens 2 and 3 have many elliptical platelets, with a minute (< 1 µm) central hole, attached (Fig. 16–20). The platelets of specimen 2

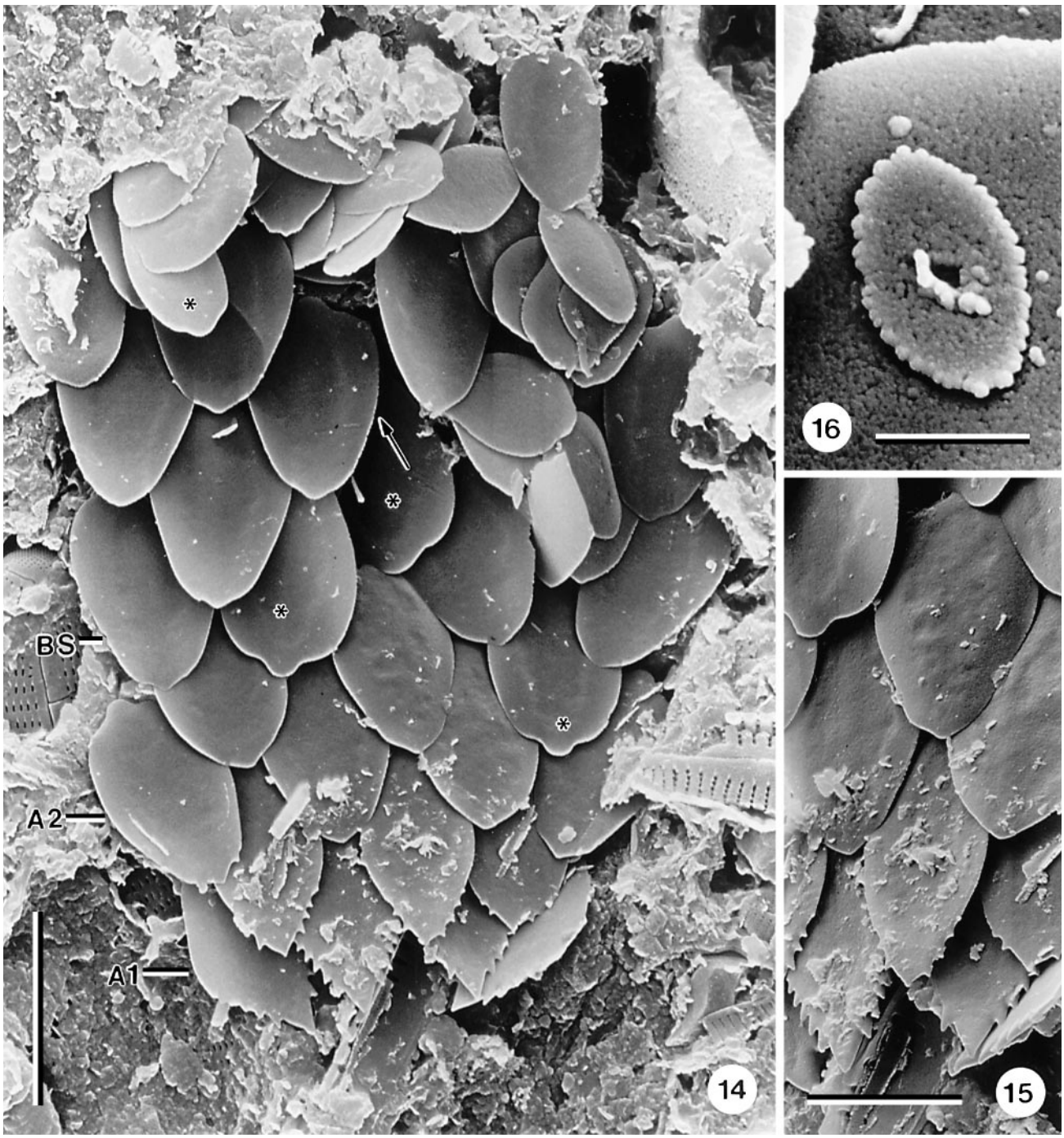

Fig. 14–16. *Scutiglypha scutigera*, Miocene fossilised specimens in the scanning electron microscope. 14, 15: Specimen 1, likely fully preserved in length and width, is composed of five or six transverse rows of body-scales (BS) and two rows of aperture-scales (A1, A2). Arrow marks a body-scale where the scutiform, name-giving shape is clearly recognisable. The first row of aperture-scales (A1) has four to five denticles per scale half, while the scales of the second row (A2) have only one or two. Asterisks mark upside-down scales. 16: Part of a body-scale with attached base-plate of an epizoic organism (for details, see next plate). Scale bars 10 µm (14), 6 µm (15), and 2 µm (16).

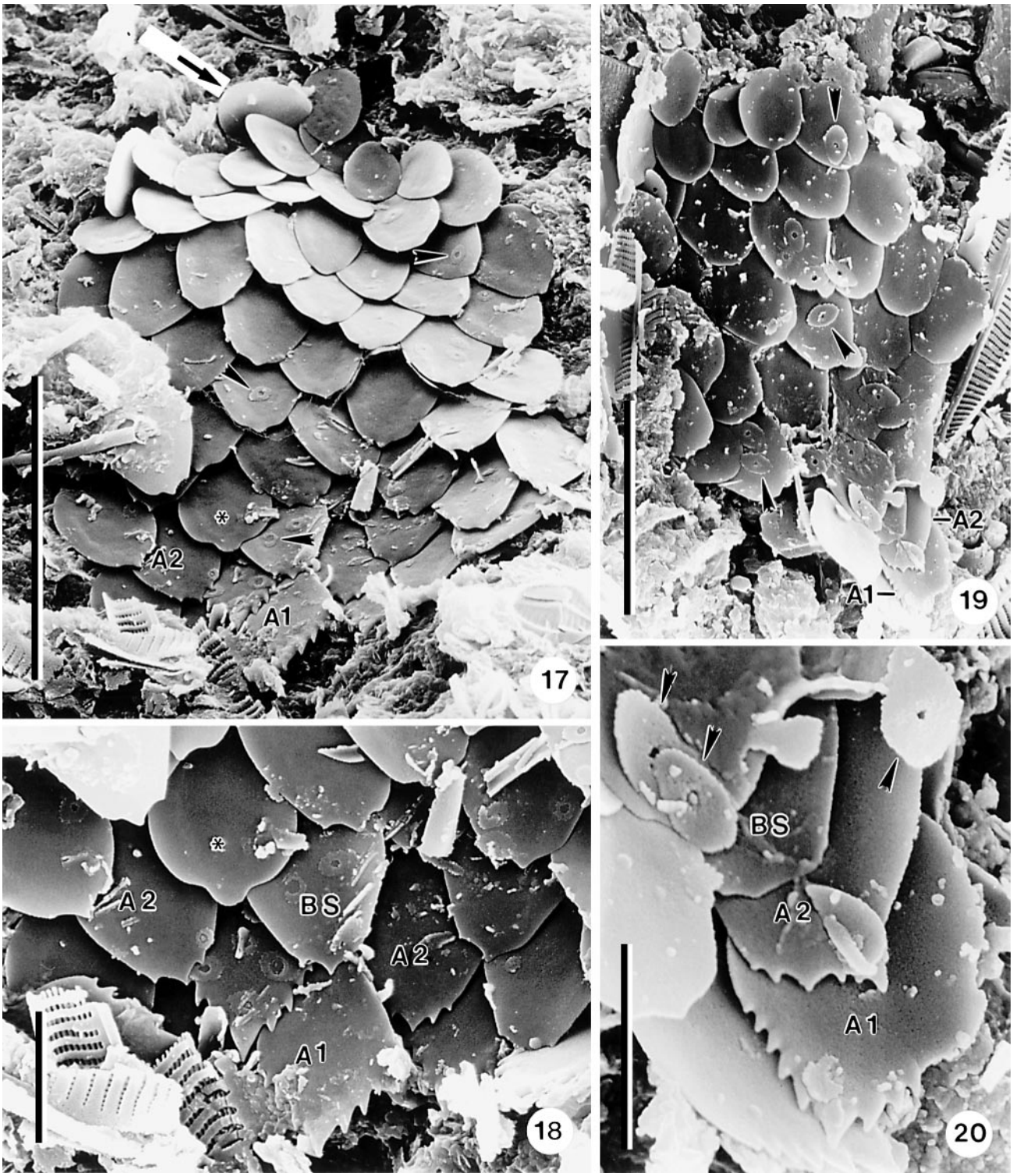

Fig. 17–20. *Scutiglypha scutigera*, Miocene fossilised specimens 2 (17, 18) and 3 (19, 20) in the SEM. The specimens, which appear slightly compacted longitudinally, are composed of two rows of aperture-scales (A1, A2) and seven or eight rows of body scales (BS). Arrow in figure 17 marks an obovate scale in the aboral pole area. Asterisk denotes a scale turned upside down. Arrowheads mark the elliptical base-plates of organisms attached to the testacean scales. Scale bars 20 µm (17, 19) and 6 µm (18, 20).

Table 2. Scale measurements (µm) on fossilised *Scutiglypha scutigera*.

Scales[1)]	Specimen 1	Specimen 2	Specimen 3	Attached platelets Specimen 2	Attached platelets Specimen 3
Body-scales					
Length $\bar{x}$	10.1	5.9	9.0	3.8	1.6
M	10.0	6.4	9.4	3.7	1.6
SD	1.1	0.8	1.0	0.4	0.3
CV	10.6	14.3	11.1	9.7	19.4
Min	8.3	4.4	7.6	3.5	1.1
Max	11.7	6.8	10.6	4.5	1.9
n	11	7	9	7	6
Width $\bar{x}$	6.3	4.9	6.6	2.4	1.1
M	6.7	4.4	7.1	2.6	1.1
SD	1.3	1.0	1.0	0.3	0.2
CV	21.1	19.8	14.5	14.4	17.2
Min	3.0	4.0	4.7	1.9	0.8
Max	7.3	6.0	7.6	2.8	1.4
n	11	7	9	7	6
Aperture-scales	11.3 × 7.3	8 × 6	10.6 × 7.9		
(length × width)	11.7 × 6.3		9.4 × 7.1		
	10.7 × 6.3		10.0 × 5.9		

[1)] Measurements from SEM-micrographs and in µm. CV – coefficient of variation in %, M – median, Max – maximum, Min – minimum, n – number of scales measured, SD – standard deviation, $\bar{x}$ – arithmetic mean.

have an average size of 3.8 × 2.4 µm, while those of specimen 3 measure only 1.6 × 1.1 µm (Table 2). The platelets have, compared to the testacean scales, a rough surface and are arranged without any order and in varying numbers (0 to 5) on the scale surface; furthermore, some appear firmly attached to the testacean scales, while others are almost detached.

Scutiglypha aspera (Penard, 1891) nov. comb. (Fig. 5a–d)

?1879 *Euglypha alveolata* – Leidy, Geol. Surv. Terr., 12: 207 (partim, Plate 35, Fig. 2–4, 6, 18).
1891 *Euglypha alveolata*, var. *aspera* Penard, var. nova, Archs Sci. phys. nat., 26: 144.
1899 *Euglypha aspera* sp. nov. Penard – Penard, Revue suisse Zool., 7: 75.
1902 *Euglypha aspera* Penard, 1899 – Penard, Faune Rhizopodique: 497 (redescription).
1962 *Euglypha aspera* Penard – Decloitre, Arch. Protistenk., 106: 59 (first reviser).

Remarks: The body-scales of this species have a slightly hexagonal outline with a minute thorn aborally and a slight crenulation orally (Fig. 5c). However, detailed data are not available, and Leidy's specimens might belong to another, new species because the scales are cordiform (Fig. 5b). Both Penard (1899, 1902) and Leidy (1879) mention two rows of apertural plates. Thus, there is no doubt that *E. aspera* belongs to *Scutiglypha*. Few reliable records are known, indicating that it is a rare species (Decloitre 1962).

Discussion

Fossil testate amoebae

Data on fossil thecamoebians are rare, possibly because palaeontologists misinterpreted, or did not devote much attention to them (Loeblich and Tappan 1964, Porter and Knoll 2000), but very likely also due to insufficient research methods. When we investigated conventional kieselgur preparations with the light microscope, we could not find testate amoebae, possibly because the tests disintegrated. Furthermore, volcanic glass particles, which are frequent in the kieselgur sediments of Beuern, may look very similar to testacean shell scales. However, testacean scales can easily be seen in recent lacustrine sediments (Douglas and Smol

1987). In the compacted fossil sediments, they were revealed only on direct investigation of undisturbed sediment samples with the scanning electron microscope. We are convinced that this simple technique will provide many more fossil protists when applied by more researchers on a variety of sediments. This is supported by very recent investigations, which showed *Euglypha* sp. and *Scutiglypha scutigera* in Oligocene and Eocene lake deposits (Schiller 1998, 1999).

Our scanning micrographs leave no doubt that the structures revealed are from testate amoebae because the scales are clearly recognisable and have a unique shape. Most other fossil thecamoebians have agglutinated tests, making them look like minute sand grains. Thus, most of these reports have been questioned for one reason or another (Medioli et al. 1990). Only the findings of Bradley (1931), Frenguelli (1933), and Boeuf and Gilbert (1997) were widely accepted because they show details characteristic for testate amoebae, viz., scales in geometric pattern in fossil *Quadrula*, *Tracheleuglypha*, and *Trinema*.

The new genus *Scutiglypha*

Euglypha belongs to the family Euglyphidae Wailes, 1915, which includes several widely known testate amoebae genera, such as *Euglypha*, *Trinema*, and *Corythion*. Main features are the filose pseudopodia and a rigid test composed of siliceous scales, often arranged in a geometrical pattern. Genera are distinguished mainly according to the location and shape of the pseudostome, and the structure of the aperture and body scales (Wailes 1915, Schönborn 1966).

Over a hundred distinct *Euglypha* morphospecies have been described, and taxonomists split the genus into two divisions with two sections each according to the structure of the spine-scales and the shape of the test and aperture in transverse view (Wailes 1915, Decloitre 1962, 1976, 1979). None of the "divisions" and "sections" have ever been ranked, possibly because transitions exist. Surprisingly, the shape of the body scales, which seems to be a very stable feature, was never used to split this large genus, very likely because most taxonomists described them as circular or elliptical, except for the three scutiform species treated in the present paper (Wailes 1915). This mistake even survived in the scanning electron microscopy area (Ogden and Hedley 1980), although the micrographs show two distinct types: circular to elliptical (in the type species, *E. tuberculata*) and elongate hexagonal (for instance, in *E. strigosa*). We suggest that the shape of the body-scales is as reliable a genus character as any of the other features used in testacean taxonomy. Thus, we refer the three species with scutiform, crenulated scales to a new genus, *Scutiglypha*. Likewise, the species with hexagonal body-scales should be referred to a new genus, however, only after a thorough reinvestigation of representative species, which is beyond the scope of this paper.

Certainly, *Scutiglypha* will evoke conflicts among testacean specialists because there may be some transistions to *Euglypha*. Meisterfeld (pers. comm.), for instance, identifies forms with crenated body-scales either as *E. acanthophora* or *E. crenulata*, depending on the distinctiveness of the crenulation, which is rather different in various populations. Likewise, Ogden (1981) mis(?)identified an *Euglypha* with crenated body-scales as *E. acanthophora*. On the other hand, *E. acanthophora* traditionally lacks crenated body-scales (Wailes 1915), and thus one may argue that Ogden's and Meisterfeld's taxa and transistions are varieties of *E. crenulata* or that *E. acanthophora* and *E. crenulata* are members of a sister species complex.

What is important in the present context is that there are undoubtedly *Euglypha* species with either ellipsoidal, hexagonal, or crenated body scales (Ogden and Hedley 1980, Ogden 1981, present paper). That transistion species exist, which cannot unambiguously be assigned, does not prohibit splitting the genus. Actually, transistions are found between most genera of the living world, especially when they are rich in species and well-investigated.

Another unexplored potential for splitting *Euglypha* are the aperture scales, which show several distinct types. Likely, gene sequence data will show that these clusters are distinct phylotypes separable at genus or subgenus level.

Identification of the fossil specimens

Identification of *Euglypha* species is mainly according to the size and shape of the test and its aperture, the shape of the body and apertural scales, and the presence/absence of spines. The shape of the test and aperture can not be reconstructed in the compressed fossil material and thus is not considered in the further discussion. Likewise, we can not entirely exclude the occurrence of spines, although it is likely that they are lacking,

considering the excellent preservation of the specimens. Accordingly, test size and shape of the body and apertural scales remain as diagnostic features. Unfortunately, none of the three extant "scutiform" species has been studied with the scanning electron microscope, making the comparison with our data difficult. Likewise, detailed morphometrics are lacking.

The size of our *S. crenulata* specimens is only half that of the extant species, although the subspecies *minor* has a lower limit of 67 µm, which is rather close to our largest specimen (57 µm). However, such a difference must not be over-weighted because the rather strongly overlapping scales indicate that the tests, or the scales, were somewhat compacted during fossilisation. Furthermore, the diminution of the scales during decay indicates that the specimens may have shrunk during fossilisation. Thus, the number of transverse rows formed by the scales is probably a more reliable feature, viz., 7–9 in our specimens and about 11–12 in the extant ones, as estimated from figures 1a, f. Assuming that a scale row measures about 10 µm, the lacking three to four rows would add 30–40 µm to test length, that is, our largest specimen would become 100 µm long, which is well within the size range of *S. crenulata minor*. Thus, the fossil specimens are indeed very likely smaller than the extant ones.

The shape and size of the body and apertural scales match well in the extant and fossil specimens (Fig. 1b, d, e, f, 6–8, 10–13). As the scale shape is highly characteristic for *S. crenulata*, our identification must be correct. The lack of spines and test size indicate that the fossil specimens are very near to *S. crenulata minor*.

The fossil *S. scutigera* specimens are at least one third smaller than the extant ones, which have, according to figures 3a, b, 9–11 transverse scale rows, while the fossil specimens have 7–10. This indicates, as explained in *S. crenulata* above, that the fossil specimens are slightly smaller than the extant ones and some diminution of the tests occurred during fossilisation. This is sustained by the size of the scales, which are considerably smaller in the fossil specimens (8.6 × 6 µm) than the extant ones (11–12 × 8–10 µm). In contrast, the shape of the scales, which is highly characteristic and thus the most important species feature, match perfectly in the extant and fossil specimens (Fig. 3a–e, 4a–h, 14–20). Thus, our identification is very likely correct, although the small size of the specimens might indicate a subspecies-level difference.

Two of the three fossil *S. scutigera* specimens have small scales, with a minute central hole, attached (Fig. 16, 17–20). We suggest that these are siliceous base-plates of sessile, possibly flagellated protists. The different size of these scales in each of the tests suggest that at least two species of the same or a similar genus colonised the tests.

The biotope

Both *S. crenulata* and *S. scutigera* were originally described from the Aufwuchs and bottom of aquatic habitats, such as lakes, ponds, and *Sphagnum* bogs. Later records suggested a global distribution (Decloitre 1962). This matches the fossil material, which is from a freshwater volcanic crater lake, as investigations on a wide variety of organisms showed (Schiller 1997, 2000). Indeed, the testacean tests are embedded in a mass of remnants from siliceous organisms, such as diatoms, cysts of chrysophytes, and spicules of freshwater sponges (Fig. 6, 10, 12–14, 17–19). However, we could not find testacean remnants in sediment from the silted-up zone. Whether this is due to insufficient fossilisation conditions or a preferred occurrence of the species in the central area of the lake needs further investigations, especially on the ecology taphonomy of extant populations. In this context, the study of Douglas and Smol (1987) must be mentioned. They investigated testacean scale occurrence in recent sediments of a wide variety of lakes worldwide. Although the authors did not try identification, their excellent micrographs show *Scutiglypha crenulata* scales in, at least, an Arctic lake and in lakes of Florida and Canada. Obviously, *S. crenulata* is much more common than indicated by the sparse records available.

Morphostasis in protists

We referred the fossil specimens to extant species, simply because we could not find sufficient differences for placing them in new taxa. Certainly, this decision may be questioned because of the size differences and the missing features mentioned above. On the other hand, the most important specific feature, the scale shape, is obviously very similar in the fossil and extant specimens. The pliocene *Trinema lineare pliocenica* differs in some minor features from the recent populations, suggesting separation at subspecies level (Boeuf and Gilbert 1997).

This means that the *Scutiglypha* species, and possibly testate amoebae in general (Deflandre 1953), hardly changed in over 15 million years. Even if we assume only one division per year, the organisms, although being largely asexual, stabilised their genetic material over at least 15 million generations! And there is evidence that also soft-bodied protists, such as various ciliates and flagellates, have hardly changed during the past 200 million years (Schönborn et al. 1999). As far as we know, no explanation is available for this stability, which might well explain the cosmopolitan distribution of many protists. Possibly, internal selection in the sense of Wagner and Schwenk (2000) stabilized protists at large, at least as concerns their overall appearance. There is evidence that genome composition is much more diverse than gross morphology (Nanney et al. 1998, Foissner and Berger 1999).

Acknowledgements: This study was supported by the Deutsche Forschungsgemeinschaft (DFG-project Ma 199/30-1) and the Austrian Science Foundation (FWF-project P-12367-BIO). The technical assistance of Dr. Eva Herzog and Mag. Eric Strobl is gratefully acknowledged.

References

Boeuf O. and Gilbert D. (1997): Présence de thécamoebiens du genre *Trinema*, au pliocène supérieur, découverte à Chilhac (Haute-Loire, France). C. r. hebd. Séanc. Acad. Sci., Paris 325, 623–627.

Bradley W.H. (1931): Origin and microfossils of the oil shale of the Green River Formation of Colorado and Utah. U.S. Geol. Surv., Professional Paper 168, 58 pp.

Decloitre L. (1962): Le genre *Euglypha* Dujardin. Arch. Protistenk. 106, 51–100.

Decloitre L. (1976): Le genre *Euglypha*. Arch. Protistenk. 118, 18–33.

Decloitre L. (1979): Mises à jour au 31.12.1978 des mises à jour au 31.12.1974 concernant les genres *Arcella*, *Centropyxis*, *Cyclopyxis*, *Euglypha* et *Nebela*. Arch Protistenk. 122, 387–397.

Deflandre G. (1953): Ordres des Testacealobosa (De Saedeleer, 1934), Testaceafilosa (De Saedeleer, 1934), Thalamia (Haeckel, 1862) ou Thécamoebiens (Auct.) (Rhizopoda Testacea). Traité de Zoologie 1(2), 97–148.

Douglas M. S. V. and Smol J. P. (1987): Siliceous protozoan plates in lake sediments. Hydrobiologia 154, 13–23.

Eikamp H. (1978): Pflanzen und Tiere aus dem Oberen Miozän – Die Kieselgur von Beuern. Mineralien Magazin 2, 94–102.

Foissner W. and Berger H. (1999): Identification and ontogenesis of the *nomen nudum* hypotrichs (Protozoa: Ciliophora) *Oxytricha nova* (= *Sterkiella nova* sp. n.) and *O. trifallax* (= *S. histriomuscorum*). Acta Protozool. 38, 215–248.

Frenguelli G. (1933): Tecamebiani e diatomee nel Miocene del Neuquén (Patagonia settentrionale). Bull. Soc. Geol. Ital. 52, 33–46.

Hottenrott M. (1988): Palynologie, Stratigraphie und Paläogeographie im Tertiär von Mittelhessen und Umgebung. Geol. Jb. Hessen 116, 113–168.

Leidy J. (1879): Fresh-water rhizopods of North America. U.S. Geol. Surv., Report 12, I–XI + 1–324 + XLVIII plates.

Loeblich A. R. jr. and Tappan H. (1964): Sarcodina, chiefly "Thecamoebians" and Foraminiferida. In: Moore R. C. (ed.): Treatise on invertebrate paleontology, Part C, Protista II: Vol. 1, p. i–xxxi + C1–C510a. University of Kansas Press, Kansas.

Medioli F. S., Scott D. B., Collins E. S. and McCarthy F. M. G. (1990): Fossil thecamoebians: present status and prospects for the future. In: Hemleben C. et al. (ed.): Paleoecology, biostratigraphy, paleooceanography and taxonomy of agglutinated Foraminifera, pp. 813–839. Kluwer Academic Publishers, Netherlands.

Nanney D. L., Park C., Preparata R. and Simon E. M. (1998): Comparison on sequence differences in a variable 23S r RNA domain among sets of cryptic species of ciliated protozoa. J. Euk. Microbiol. 45, 91–100.

Ogden C. G. (1981): Observations of clonal cultures of Euglyphidae (Rhizopoda, Protozoa). Bull. Br. Mus. nat. Hist. (Zool.) 41, 137–151.

Ogden C. G. and Hedley R. H. (1980): An atlas of freshwater testate amoebae. Oxford University Press, Oxford.

Penard E. (1891): Contributions l'étude des rhizopodes du Léman. Archs Sci. phys. nat. 26, 134–156.

Penard E. (1899): Les rhizopodes de faune profonde dans le Lac Léman. Revue suisse Zool. 7, 1–142 + 9 plates.

Penard E. (1902): Faune rhizopodique du bassin du Léman. Kündig, Genève.

Playfair G. I. (1917): Rhizopods of Sydney and Lismore. Proc. Linn. Soc. N.S.W. 42, 633–675.

Porter S. M. and Knoll A. H. (2000): Testate amoebae in the neoproterozoic era: evidence from vase-shaped microfossils in the Chuar Group, Grand Canyon. Paleobiology 26, 360–385.

Schenk E. (1950): Ein miozäner Vulkanbau bei Beuern nordöstlich von Gießen. Notizbl. hess. L.-Amt Bodenforsch. (VI)1, 260–268.

Schiller W. (1997): Kieselige Thekamöben aus der miozänen Kieselgur von Beuern/Vogelsberg im Vergleich mit rezentem Material von Borneo (Malaysia). Cour. Forsch.-Inst. Senckenberg 201, 385–392.

Schiller W. (1998): Kieselige Mikrofossilien aus dem Unter-Oligozän von Sieblos/Rhön. Geol. Abh. Hessen 104, 173–199.

Schiller W. (1999): Kieselige Thekamöben aus dem Mittel-Eozän des Eckfelder Maares in der Eifel. Mainzer naturwiss. Archiv 37, 55–62.

Schiller W. (2000): Miocene kieselgur from Beuern/Vogelsberg – a deposit of a volcanic crater lake. Terra Nostra – Schriften der Alfred-Wegener-Stiftung 2000/6, 460–464.

Schönborn W. (1966): Beschalte Amöben (Testacea). A. Ziemsen, Wittenberg, Lutherstadt.

Schönborn W., Dörfelt H., Foissner W., Krienitz L. and Schäfer U. (1999): A fossilized microcenosis in triassic amber. J. Euk. Microbiol. 46, 571–584.

Waggoner B. M. (1996a): The first fossil cyphoderiid testate amoeba, in Dominican Republic amber (Eocene-Oligocene). PaleoBios 17, 17–19.

Waggoner B. M. (1996b): Bacteria and protists from Middle Cretaceous amber of Ellsworth County, Kansas. PaleoBios 17, 20–26.

Wagner G. P. and Schwenk K. (2000): Evolutionarily stable configurations: functional integration and the evolution of phenotypic stability. In: Hecht M. K. et al. (ed.): Evolutionary biology, Vol. 31, 155–217. Kluwer Academic/Plenum Publishers, New York.

Wailes G. H. (1912): Freshwater Rhizopoda and Heliozoa from the states of New York, New Jersey, and Georgia, U.S.A.; with supplemental note on Seychelles species. J. Linn. Soc. 32, 121–161.

Wailes G. H. (1915): The British freshwater rhizopoda and heliozoa, Vol. III. Adlard and Son, London.

Wailes G. H. and Penard E. (1911): Rhizopoda. Proc. R. Ir. Acad. 31, 1–64.

Wolf M. (1995): Verkieste Amöben in Steinkohlen aus dem Ruhrgebiet – erster Nachweis von *Arcella* Ehrenberg im Paläozoikum. Paläont. Z. 69, 1–6.