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A New Flagship Peritrich (Ciliophora, Peritrichida) from the River Rhine, Germany: *Apocarchesium arndti* n. sp.

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ABSTRACT. We discovered a free-living peritrich ciliate with outstanding features in the River Rhine. Its morphology and 18S rRNA gene sequence were studied with standard methods. *Apocarchesium arndti* n. sp. has several peculiarities. (i) There are ordinary zooids, macrozooids, and microzooids, which form a hemispherical rosette on a discoidal base, the stalk dish, locking the ~ 18 μm wide and up to 2 mm long, spirally contracting colony stalk. (ii) The stalk myoneme is connected only to the microzooids. (iii) A rosette contains up to 50 zooids not connected to each other but individually attached to the stalk dish with the scopula. (iv) The ordinary zooids are epistylidid, trumpet-shaped (~ 6:1 length:width), about 180 × 30 μm in size, and have an ellipsoidal macronucleus subapically between oral cavity and dorsal side. (v) The myoneme system of the zooids, which can contract individually, forms a tube-like structure in the narrow posterior half of the cell. (vi) The silverline pattern belongs to the transverse-striate type. (vii) The oral apparatus is of usual structure, with kinety 1 of peniculus 3 distinctly shortened proximally. (viii) The 18S rRNA places *A. arndti* n. sp. as a distinct lineage near *Vorticella* and *Carchesium*. These data are used to provide an improved diagnosis of the genus *Apocarchesium*. Features (i)–(iii) and the molecular data indicate that *Apocarchesium* could be the type genus of a new peritrich family.

Key Words. Biodiversity, biogeography, colonial peritrichs, molecular phylogeny, myoneme system.

THE bell-shaped infusorians have fascinated the founders of protistology, such as Müller (1786), Ehrenberg (1838), and Stokes (1888), because of their neat appearance, the sessile habit, the distinct contractility, and the ability to form large colonies. This early research was collated by Kahl (1935), whose review stimulated a bloom of peritrich studies in Germany (for a brief review, see Biegel 1954) and Hungary (Stiller 1971) between 1935 and 1970. In the 1960s and 1970s of the past century, Lom (1964) and Foissner and Schiffmann (1974, 1975) established silver impregnation in peritrich taxonomy, showing new details of their oral structures and silverline pattern. This caused a split of several genera (e.g. *Vorticella/Pseudovorticella*) and a series of generic reviews (e.g. Clamp 1982, 1991; Foissner 1975, 1977; Warren 1986, 1987, 1988), which, however, could not supersede the comprehensive reviews of Kahl (1935) and Stiller (1971). The current research concentrates on describing and redescribing new and insufficiently known species (e.g. Clamp and Coats 2000; Ji et al. 2005a, b; Sun et al. 2006; Sun, Song, and Xu 2007), reviews at genus level (Jankowski 2007; Lynn 2008), and molecular phylogenetic analyses (Foissner et al. 2009; Li et al. 2008a, b; Martin-Cereceda et al. 2007; Williams and Clamp 2007).

New peritrich genera and species are continuously described, showing that we are a long way from completing describing their diversity (Jankowski 2007; Ji et al. 2009; Sun et al. 2006, 2007). Thus, we were not too surprised to discover a new type of peritrichs in the River Rhine, Germany, during ecological studies (Norf and Foissner 2009). However, during our investigation of this ciliate, a new genus and new species, *Apocarchesium rosettum*, has been described from the ancient Lake Biwa in Japan (Ji and Kusuoka 2009). Closer investigations revealed that the Lake Biwa and the River Rhine ciliate possibly belong to the same genus but represent highly different species, adding an interesting biogeographical aspect to the matter. Nevertheless, the description of *A. rosettum* did not show all the outstanding features of this genus. Thus, we provide a very detailed description of the River Rhine ciliate, and suggest the possibility of a family rank for this kind of peritrichs.

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MATERIALS AND METHODS

Material and morphological methods. *Apocarchesium arndti* n. sp. was discovered in biofilms of the Cologne Ecological Rhine Station (Germany; 50°54′25″N, 6°58′43″E); for details, see ''Occurrence and ecology.'' Colonies were picked up under a dissecting microscope in October 2008 and sent to Salzburg, Austria for morphological analysis. Other colonies were fixed in 80% ethanol, Bouin's solution, and in 3% (v/v) glutaraldehyde. Unfortunately, material for the morphological investigations was collected only at the end of the ecological studies and was thus sparse. Later, the species occurred in very low numbers or not at all. Cultivation attempts failed. Thus, ontogenetic data cannot be provided.

Living cells were studied using a high-power oil immersion objective and differential interference contrast microscopy. Silver impregnation and scanning electron microscopy (SEM) were performed as described in Foissner (1991). Counts and measurements on silvered specimens were conducted at a magnification of 1,000X. In vivo measurements were performed at magnifications of 40–1,000X. Illustrations of live specimens were based on free-hand sketches and micrographs, while those of prepared cells were made with a drawing device.

As concerns the morphological terminology, we follow mainly Corliss (1979) and Lynn (2008). Two new terms, rosette and stalk dish, are explained in ''Discussion.''

DNA isolation and sequencing. Colonies of *A. arndti* n. sp. were collected from field samples and starved for 48 h before the genomic DNA was extracted following a modified cetyl trimethyl ammonium bromide protocol (Wylezich 2004). The small subunit (SSU) rRNA was amplified by polymerase chain reaction (PCR), using the 18s1F and 18sR universal eukaryotic primers (see Wylezich et al. 2002). The amplification protocol consisted of one preliminary heating step of 2 min at 95 °C, followed by 20 cycles of 30 s at 95 °C, 30 s at 52 °C, 45 s at 72 °C, and a final extension step of 6 min at 72 °C. The PCR products were cleaned using the NucleoSpin Extract II (Macherey-Nagel GmbH, Düren, Germany) DNA purification kit. Both strands of the SSU rDNA were sequenced with the two amplification primers and four additional sequencing primers (590F, 1280F, 600R, and 1300R; see Wylezich et al. 2002). Cycle sequencing reactions were performed using the BigDye Terminator Cycle Sequencing Kit v3.1 (Applied Biosystems, Langen, Germany). The fragments were purified via AutoSeq G-50 columns (Amersham Biosciences, Braunschweig, Germany) and sequenced on an ABI 3730 DNA sequencer

(Applied Biosystems, Foster City, CA). All cycling and sequencing steps were performed following the manufacturer's instructions.

Phylogenetic analyses. Sequences used for determining the phylogenetic position of *A. arndti* n. sp. were retrieved from the SILVA rRNA database project (Pruesse et al. 2007). The SSU rRNA sequence of *A. arndti* was aligned to these sequences with T-COFFEE v6.18 (Notredame, Higgins, and Heringa 2000), using the Myers and Miller pair-wise alignment algorithm with no gap penalties. The alignment was then manually refined considering conserved regions of the SSU rRNA. The alignment may be obtained from the authors on request. Maximum likelihood (ML) was computed with PhyML v2.4.4 (Guindon and Gascuel 2003), using the GTR+Γ+*I* nucleotide substitution model as determined by MrAIC v1.4.3 (Nylander 2004). Maximum parsimony (MP) was computed with PAUP v4.0b10 (Swofford 2002), using the tree-bisection-reconnection branch-swapping algorithm and random species addition ($n = 10$). Data for both ML and MP analyses were bootstrap resampled 1,000 times. The ML and MP trees were collapsed using the 50% cutoff value, and a merged tree was formatted with the MEGA v4.0 software (Tamura et al. 2007) because they showed similar topologies. Uncorrected genetic distances (*p* distances; Nei and Kumar 2000) between *A. arndti* and related peritrichs were calculated with MEGA v4.0.

RESULTS

Description of *Apocarchesium arndti* n. sp. (Tables 1–3 and Fig. 1–58, 61, 63, 65). *Apocarchesium* colonies consist of three parts: the zooids, the stalk dish, and the stalk.

Colonies (Table 2 and Fig. 1, 8, 14, 17–20, 23–25, 32–36, 39, 53, 54, 57). The colonies of *A. arndti* n. sp. are 1–2 mm high and composed of about 30–50 zooids (Table 2). They are attached by an inconspicuous, roundish disc to organic and inorganic mud particles, occasionally also to the loricae of *Stentor* and micrometazoans (Fig. 14, 17, 25, 53). Fully contracted colonies are hemispherical and about 150–200 μm wide (Fig. 19, 25, 34).

Table 1. Species sampling and GenBank accession numbers used in this study.

Species	GB #
***Apocarchesium arndti* n. sp.**	GQ221940
Astylozoon enriquesi	AY049000
Campanella umbrellaria	AF401524
Carchesium polypinum	AF401522
Epicarchesium abrae	DQ190462
Epistylis galea	AF401527
Epistylis hentscheli	AF335513
Opercularia microdiscum	AF401525
Ophrydium versatiles	AF401526
Opisthonecta henneguyi	X56531
Opisthonecta minima	EF417834
Pseudovorticella paracratera	DQ662847
Pseudovorticella punctata	DQ190466
Pseudovorticella sinensis	DQ845295
Tetrahymena bergeri	AF364039
Tetrahymena corlissi	U17356
Tetrahymena rostrata	AF364042
Vorticella campanula	DQ662849
Vorticella convallaria	DQ868348
Vorticella fusca	DQ190468
Vorticella microstoma	DQ868347
Zoothamnium alternans	DQ868352
Zoothamnium niveum	DQ868350
Zoothamnium pelagicum	DQ868351

The newly sequenced taxon is bold.

At first glance, the zooids seem to form a sphere (Fig. 17). On more detailed investigation, they are arranged in a hemispherical, umbel-like manner with the zooids attached to the distal surface, and margin of the stalk dish, a disc-shaped structure on the distal end of the stalk (Fig. 1, 20, 34, 36, 39). In vivo, the stalk dish is about 30 μm in diameter, 5 μm high, and hyaline, becoming distinct mainly due to the scopulas of the marginally attached zooids (Fig. 23, 24). In protargol preparations, the stalk dish is hyaline and without a definite fine structure (Fig. 8, 54, 57), while SEM reveals it as a slightly convex, finely reticular, spongious structure (Fig. 35).

The stalk of *A. arndti* colonies is about 18 μm wide and contracts spirally, forming an average of nine revolutions when fully contracted; it can contract at any site, forming one to several spiral turns (Table 2 and Fig. 1, 8, 14, 17–19, 25, 32, 33, 53, 57). The stalk has a vorticellid structure, i.e. both the stalk envelope and the myoneme are elliptical in transverse section (Fig. 32, 33). The stalk envelope is structureless in vivo and in SEM preparations and very conspicuous when contracted, becoming 40–80 μm wide (Fig. 8, 18, 19, 25, 32, 33, 53, 54). The stalk myoneme becomes thinner posteriorly and does not penetrate the stalk dish. Thus, it is not branched anteriorly and not connected with the zooids (Fig. 34, 35, 53, 54, 57), except for the microzooids described below. The myoneme is surrounded by a membrane and one side is covered with many pale, about 1 μm-sized granules, possibly mitochondria (Fig. 32). A second type of stalk granule, which occurs in only half of the specimens, is about 3 μm across and impregnates deeply with protargol (Fig. 57).

Ordinary zooids (Table 2 and Fig. 2–7, 12, 14, 17–22, 24–31, 34, 36–52, 56, 57). The zooids are so firmly attached to the stalk dish that they hardly can be separated by strong shaking of the sample or with a needle. This makes the preparations difficult, especially silver nitrate impregnation. In vivo, the zooids are 150–210 × 25–35 μm in size, on average about 180 × 30 μm, thus having a length:width ratio of about 6:1 at the width level of the oral bulge and of about 20:1 at the width level of the posterior end (Table 2). Thus, the zooids are conspicuously slender and trumpet-shaped, with the posterior half stalk-like and narrowed. They are slightly asymmetric and straight or indistinctly curved when fully extended (Fig. 3, 14, 17–19, 52). The zooids are very flexible and contract accordion-like in the posterior body half, where deep furrows arise; fully contracted zooids are about half as long as extended ones and are not globular, as in many other peritrichs, but clavate or broadly conical (Fig. 3, 18, 19, 21, 22, 25, 27–30, 34).

One of the most conspicuous peculiarities of *A. arndti* n. sp. is the location of the macronucleus: subapical between the oral cavity and the dorsal side of the cell (Fig. 3, 7, 26, 42). Compared with the size of the ciliate, the macronucleus is small (i.e. 10–20 × 5–10 μm in vivo). Further, it is rather hyaline and thus difficult to recognize. Usually, the macronucleus is ellipsoidal; rarely it is distinctly reniform. It contains a rather large, central nucleolus, and rarely there are two nucleoli, one each near the ends of the nucleus. The nucleoli are compact, spongious, or ring-shaped (Table 2 and Fig. 3–5, 7, 12, 21, 22, 24, 42). The micronucleus did not impregnate. The contractile vacuole is underneath the oral bulge on the ventral wall of the oral cavity (Fig. 3, 26, 42). Unfortunately, we did not observe the site of the cytoproct. The cytoplasm is granular and rather hyaline, especially in the narrow posterior body half. All specimens contain few to many strongly refractive, membrane-bound crystals 2–3 μm in size. The food vacuoles are 5–10 μm across and contain bacteria (Fig. 3, 20, 31, 42, 52, 56). There is no accumulation of refractive granules in the posterior third of the body as, for instance, in many *Epistylis* species.

The cortex is very flexible and appears smooth in the anterior body half, while rugged in the posterior half due to distinct trans-

Table **2.** Morphometric data on *Apocarchesium arndti* n. sp.

Characteristics[a]	Method	Mean	*M*	SD	SE	CV	Min.	Max.	*n*
Colony, length (including zooids)	IV	1,444.4	1,500	240.4	80.1	16.6	1,000	1,800	9
Colony, number of specimens[b]	IV	43.3	40	—	—	—	30	50	9
Colony stalk, width	IV	17.7	18	2.1	0.7	11.7	15	20	9
Colony stalk, number of turns	PR	8.9	9	0.8	0.2	9.2	7	10	20
Colony (stalk) dish, diameter	PR	28.4	28	3.4	0.8	11.9	23	33	19
Zooids, body length	IV	178.0	180	19.2	4.2	10.8	150	208	21
Zooids, body width at oral bulge	IV	30.7	30	2.2	0.5	7.3	25	35	21
Zooids, scopula diameter	PR	5.1	5	0.7	0.2	12.9	4	6	19
Zooids, total number of silverlines[b]	SN	94.9	95	—	—	—	85	103	9
Zooids, macronucleus length	PR	10.8	11	2.1	0.4	19.1	8	15	21
Zooids, macronucleus width	PR	5.9	6	0.9	0.2	15.1	5	8	21
Macrozooids, body length (pre-mature)[c]	IV	84.4	80	8.9	3.0	10.5	70	100	9
Macrozooids, body width (pre-mature)[c]	IV	74.8	80	13.4	4.5	18.0	50	90	9
Macrozooids, body length (pre-mature)[c]	PR	62.1	60	7.5	2.4	12.0	55	78	10
Macrozooids, body width (pre-mature)[c]	PR	55.5	55	5.3	1.7	9.6	47	63	10
Macrozooids (pre-mature), scopula length[c]	PR	15.5	16	2.2	0.8	14.2	12	19	8
Macrozooids (pre-mature), scopula width[c]	PR	7.5	8	1.8	0.6	23.6	4	10	8
Microzooids, length	PR	18.2	18	2.7	0.6	14.9	15	23	18
Microzooids, width	PR	13.2	13	0.7	0.2	5.5	12	14	18

[a]Data based on randomly selected specimens from environmental material. Measurements in μm.
[b]Rough values.
[c]Specimens attached to colony, but with developing aboral ciliary wreath.
CV, coefficient of variation in %; IV, in vivo; *M*, median; max., maximum; min., minimum; *n*, number of specimens investigated; PR, protargol impregnation; SD, standard deviation; SE, standard error of mean; SN, silver nitrate impregnation.

verse ridges up to 3 μm apart (Fig. 3, 38). As mentioned above, the zooids are highly contractile and firmly attached to the stalk dish. Thus, details of the silverline pattern could be not studied. There are pellicular pores and about 95 transverse silverlines, about half of which extend between the anterior body end and the anlage of the aboral ciliary wreath (Table 2 and Fig. 40, 41). ''Thick silverlines,'' each composed of several very narrowly spaced ones, are formed due to the strong body contraction when the cells are air-dried for silver nitrate impregnation (Fig. 41). The anlage of the aboral ciliary wreath (''telotroch'') is not recognizable in vivo. In protargol preparations, where the zooids are strongly contracted, the anlage is near mid-body and consists of a ring of slightly irregularly arranged mono- or dikinetids (Fig. 7, 12, 29, 30).

Another remarkable feature of *A. arndti* n. sp. is the zooid's myoneme system, which forms a conspicuous tube in the narrow posterior half of the body, seemingly doubling the cortex (Fig. 3, 31). Further, the myoneme system of the individual zooids is neither connected among each other nor with the stalk myoneme of the colony. Thus, the zooids can contract individually (Fig. 31, 34, 36, 57). The myonemes commence above the scopula and extend anteriorly to be anchored along the external part of the adoral ciliary spiral (Fig. 3, 7, 12, 29–31). The oral bulge contains a narrow, unbranched myoneme not connected with the peristomial disc (data not shown). Depending on the fixative and the impregnation conditions, the myonemes appear fibrillar or fibro-granular, thicker or thinner, or do not impregnate at all.

The scopula is about 5 μm across and distinct in vivo (Fig. 3, 31), in silver preparations (Fig. 7, 12, 21, 22, 29, 36, 39, 40), and in the SEM (Fig. 37). The scopula organelles are about 1.5-μm-long rods, which are very densely spaced along the scopula margin and rather loosely spaced on the scopula surface (Fig. 37, 39). Altogether, each zooid has about 50 scopula organelles extending into the spongious stalk dish. This might explain, why they are anchored so strongly.

The oral apparatus is of ordinary size and structure (Fig. 3, 20, 26, 42, 52). However, we shall describe and illustrate it in considerable detail because it is slightly different from that of *A. rosettum* and of great significance for distinguishing these two species. The oral bulge, which represents the widest site of the

Table **3.** *p*-distances between *Apocarchesium arndti* and vorticellid ciliates in percent (pairwise deletion option set).

Species	1	2	3	4	5	6	7	8	9	10	11
Apocarchesium arndti (1)	0.0										
Carchesium polypinum (2)	4.8	0.0									
Epicarchesium abrae (3)	3.9	4.3	0.0								
Ophrydium versatilis (4)	3.3	3.6	3.2	0.0							
Pseudovorticella sinensis (5)	5.4	6.0	4.3	3.9	0.0						
Pseudovorticella paracratera (6)	5.0	5.2	3.4	3.6	1.6	0.0					
Pseudovorticella punctata (7)	4.6	4.9	3.2	3.4	0.3	2.5	0.0				
Vorticella campanula (8)	3.6	3.5	3.4	2.6	4.1	3.8	3.9	0.0			
Vorticella convallaria (9)	3.0	3.7	2.7	2.2	3.9	3.5	3.3	1.3	0.0		
Vorticella fusca (10)	3.1	3.6	2.9	2.0	3.7	3.4	3.2	1.1	0.4	0.0	
Vorticella microstoma (11)	4.6	4.8	5.3	5.3	6.0	5.1	5.0	5.0	4.9	4.8	0.0

All sequences aside from *A. arndti* were retrieved from the SILVA rRNA database project.

zooids, is 3–4 μm thick, corresponding to the width of the cell. The peristomial disc, which is flat to slightly convex, is not stalked but projects distinctly and obliquely from body proper in feeding specimens. The oral cavity is spacious and extends obliquely to the dorsal side and the second quarter of the cell. The oral cilia are 15–20 μm long and are arranged in a helically extending, dikinetidal undulating membrane (haplokinety) commencing slightly behind the adoral polykinety, which appears to consist of many short, oblique rows (Fig. 3, 6, 7, 20, 26, 42, 43, 46, 47, 50, 51). The adoral ciliary spiral performs about 1.25 turns ($\sim 450^\circ$) around the peristomial disc before it plunges into the oral cavity, where it performs a further turn. As usual, the polykinety spreads into three peniculi (buccal membranelles), each composed of three basal body rows, in the proximal third of the oral cavity (Fig. 2, 24, 44–51). Peniculus 1, which is an extension of the external polykinety, is hook-like curved proximally, where it meets the two long rows of peniculus 3. Thus, five basal body rows are recognizable at the proximal end of the oral ciliature (Fig. 2, 49). Peniculus 2 ends subterminally between peniculi 1 and 2; its basal body row 3 spreads near the distal end of the peniculus, where the basal bodies become slightly disordered. The three short basal body rows of peniculus 3 are spread fan-like distally and arranged in a special way (Fig. 2, 49): row 1 ends furthest subterminally at level of peniculus 2; row 2 ends near level of peniculus 1; and row 3 ends subterminally and is distinctly shortened distally. This pattern has been observed in eight specimens and is thus very likely highly constant.

Macrozooids (Table 2 and Fig. 1, 10, 11, 13, 20, 25, 31, 35, 52, 56–58). Usually, there are two, rarely one or three macrozooids attached to the margin of the stalk dish. They are not connected with the stalk of the colony. We could not clarify whether the macrozooids develop independently or from ordinary zooids.

The developing macrozooids are barrel-shaped, becoming globular and about 80 μm across during maturation (Table 2). Thus, they are rather conspicuous, especially in contracted colonies (Fig. 1, 20, 25, 31, 35, 52, 56, 57). Further differences between ordinary zooids and macrozooids include: (i) the macronucleus is centrally located, larger, and horseshoe-shaped; (ii) the scopula and the scopular organelles strongly increase in size and number, respectively, becoming elliptical and about 16×8 μm in developing macrozooids (Fig. 11, 55), while roundish and about 15 μm across in mature ones (Table 2 and Fig. 13, 58); (iii) the myonemes are thicker and more numerous, forming 50–100 strands extending from the scopula to the adoral ciliary spiral; (iv) the anlage of the aboral ciliary wreath develops to be very narrowly spaced, circa 5-μm-long kineties with about 10 cilia each; and (v) the ciliature in the oral cavity is more or less distinctly reduced.

Microzooids (Table 2 and Fig. 1, 8, 9, 53, 54, 56, 57). Usually, there are two, rarely one or three microzooids attached to the anterior end of the stalk of the colony (i.e. underneath the stalk dish). Actually, the microzooids are connected with the stalk myoneme via an about 15-μm-long branch that originates from the stalk myoneme (Fig. 1, 8, 9, 53, 54, 56, 57). Obviously, the microzooids develop independently (i.e. not from ordinary zooids).

In vivo, the microzooids are ellipsoidal to globular and about 20×15 μm in size (Table 2). Near the posterior end, there is a distinct, bowl-shaped bulge whose proximal surface contains a ring-shaped accumulation of scopula organelles. The macronucleus, which is in the body centre, is smoothly to wrinkled globular and very hyaline, except for minute accumulations of argyrophilic material (nucleoli?). In mid-body, there is the anlage for an aboral ciliary wreath. The myoneme fibres of the stalk of the colony extend into and to the anterior third of the microzooid. The oral structures are strongly reduced (Fig. 8, 9, 54, 56, 57).

Phylogenetic analysis (Table 3 and Fig. 59). The SSU rRNA sequence of *A. arndti* n. sp. is 1,634-bp long, has GC contents of 42.23%, and is available under GenBank accession number GQ221940. In all analyses, *A. arndti* n. sp. branches within a group containing peritrichs with a contractile stalk (e.g. *Vorticella*, *Carchesium*) and secondarily (possibly!) stalkless peritrichs, such as *Astylozoon* and *Opisthonecta* (Fig. 59). However, bootstrap support of this cluster is fairly low (i.e. 81% for ML and 74% for MP). Within the cluster, *A. arndti* n. sp. is associated with vorticellids and ophrydiids with low bootstrap support (76/89%).

The genetic (*p*) distances between *A. arndti* n. sp. and its supposed relatives are moderate (Table 3): 4.8% to *Carchesium polypinum*, 3.9% to *Epicarchesium abrae*, 3.3% to *Ophrydium versatile*, 4.6–5.4% to *Pseudovorticella* spp., and 3–4.6% to *Vorticella* sp.

Occurrence and ecology. The following data are from casual observations during ecological investigations and experiments in the years 2006, 2007, and 2008. As yet, we observed *A. arndti* n. sp. only at the Cologne Ecological Rhine Station, Germany, mainly in late autumn and early spring at water temperatures between 6 °C and 22 °C. Highest abundances (up to four colonies with a total of about 120 zooids/cm^2) occurred at low flow velocities (of 0.005–1 ms^{-1}) and on soft sediments; sometimes, colonies were attached to dwelling tubes of *Stentor* spp. and micrometazoans, for instance, very young specimens of *Chironomus* sp. Lower abundances (≤ 2 colonies) occurred at high flow velocities (1–3.5 ms^{-1}) and on hard substrates. This is surprising because the very firm attachment of the zooids suggests *A. arndti* n. sp. as an inhabitant of fast flowing, turbulent waters.

DISCUSSION

Colony types in peritrich ciliates. We introduced the terms ''rosette'' and ''stalk dish.'' This needs to be discussed because both play a major role in the classification of *Apocarchesium*.

There are several types of colonial peritrichs but all have the zooids connected by a common stalk, even *Carchesium* and *Carchesium*-like genera, where the myoneme is interrupted at the stalk branches (for reviews, see Jankowski 2007; Kahl 1935; Stiller 1971). Thus, *Apocarchesium* is a great exception: it makes colonies but the stalkless zooids sit on a small plate, the stalk dish, and are not connected with the contractile colony stalk (Fig. 1, 61). This is what we call a ''rosette.'' A similar type of rosette occurs in some peritrichs with an acontractile colony stalk (e.g. in *Pyxidium canthocampti* Penard, 1922 and in *Orbopercularia matthesi* Lust, 1950): ''The colonies are composed of many individuals on a short, branched stalk. They sit on a broad plate which is the end of a long, unbranched stalk'' (Lust 1950, translated by the authors). A variation of this pattern occurs in *Systylis hoffi* Bresslau, 1919: this species has an acontractile, branched stalk with the zooids making a hemispherical bunch on the broadened stalk ends (Fig. 16, 62). Another type of colony can be produced by solitary peritrichs, when they attach on a substrate close together (Fig. 60; Foissner, Berger, and Kohmann 1992). Such groupings are often called ''pseudocolonies.''

Zooids, macrozooids, and microzooids. In the old literature, the ordinary zooids of macrozooid-forming peritrichs are often called ''microzooids'' (e.g. Bresslau 1919). Unfortunately, Ji and Kusuoka (2009) used this outdated terminology in the description of *A. rosettum*. This would create a bewildering situation in *A. arndti* n. sp., which has three kinds of zooids: (ordinary) zooids, macrozooids, and microzooids (Fig. 1). Thus, we suggest to follow the clear terminology of Corliss (1979) and Lynn (2008): ''Zooid, a term generally restricted to mean only the body proper of an attached sessile form (e.g. the bell of many peritrichs), minus the stalk; the term also refers to the individual members of a col-

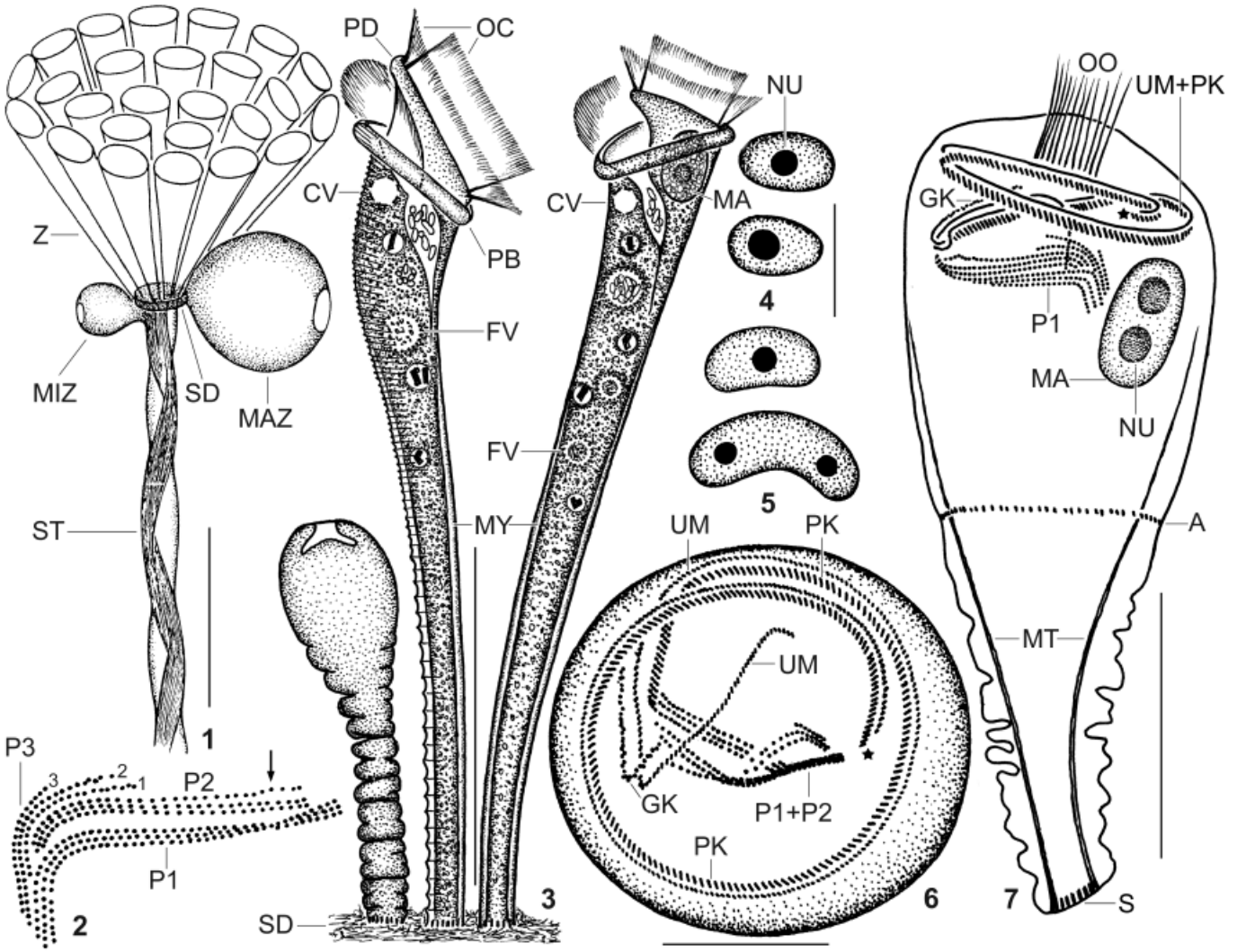
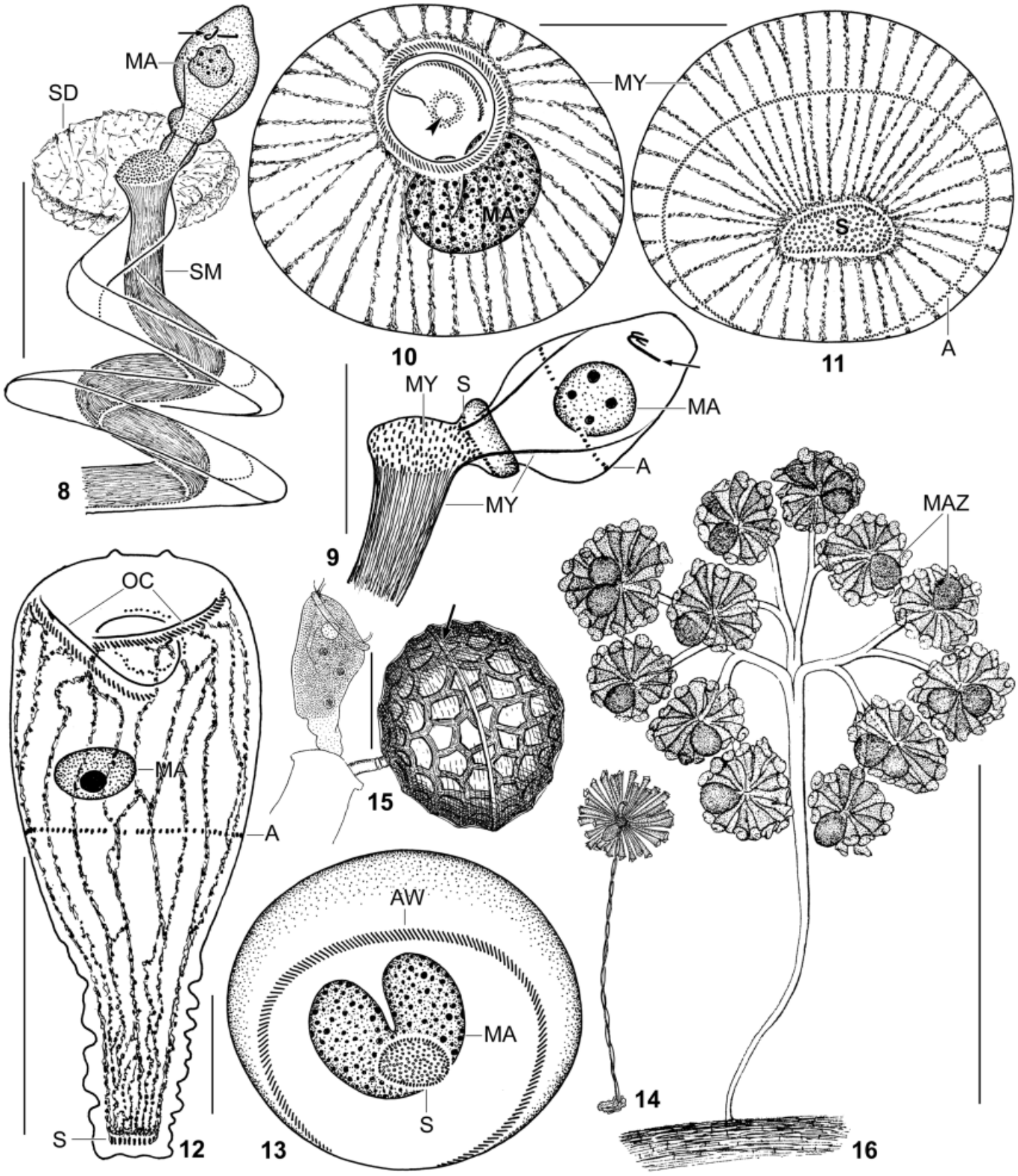


Fig. **1–7.** *Apocarchesium arndti* from life (1, 3) and after protargol impregnation (2, 4–7). **1.** Scheme of distal portion of a colony, showing the general organization and the three types of zooids: ordinary zooids, macrozooids, and microzooids. **2.** Proximal portion of oral ciliary pattern. The arrow marks a small irregularity in the arrangement of the cilia at the distal end of peniculus 2. Note numbering of ciliary rows of peniculus 3. **3.** A contracted and two extended specimens (~ 180 μm) attached to the stalk dish, showing the organization of *A. arndti* zooids. Note the curious location of the macronucleus and the ''doubled'' cortex caused by the myoneme tube. **4, 5.** Variability of shape and size of macronucleus. **6.** Frontal view of oral ciliary pattern with begin of adoral ciliary spiral marked by an asterisk. **7.** Lateral view showing the oral and somatic ciliary pattern, the macronucleus, and the myoneme tube. The begin of the adoral ciliary spiral is marked by an asterisk. A, anlage of aboral ciliary wreath; CV, contractile vacuole; FV, food vacuoles; GK, germinal kinety; MA, macronucleus; MT, myoneme tube; MAZ, macrozooid; MIZ, microzooid; NU, nucleolus; OC, oral ciliary spiral; OO, oral opening; PB, peristomial bulge; PD, peristomial disc; PK, polykinety; P1, 2, 3, oral peniculi (adoral membranelles); SD, stalk dish; ST, stalk; UM, undulating membrane (haplokinety); Z, ordinary zooids. Scale bars 10 μm (Fig. 4–6), 20 μm (Fig. 8), 70 μm (Fig. 3), and 100 μm (Fig. 1).

Fig. **8–16.** *Apocarchesium arndti* (8–14) and *Systylis hoffi* (15,16; from Bresslau 1919) from life (14–16) and after protargol impregnation (8–13). **8, 9.** The minute microzooids are easily overlooked and are connected to the stalk myoneme, in contrast to the ordinary zooids and the macrozooids, which are attached to the stalk dish (see also Fig. 1, 34–36). The arrows mark the strongly reduced oral ciliature. **10, 11.** Anterior and posterior polar view of a developing macrozooid, showing, inter alia, the anlage of the aboral ciliary wreath, the ellipsoidal scopula, the myoneme system, and the oral opening (arrowhead). **12.** Ventral view showing the myoneme system. **13.** Posterior polar view of a mature macrozooid, showing the aboral ciliary wreath, the roundish scopula, and the reniform macronucleus. **14, 16.** Comparison of *A. arndti* and *S. hoffi*; drawn to scale, showing that *A. arndti* is about half as high as *S. hoffi*. **15.** A zooid and a resting cyst of *S. hoffi* attached to the stalk end. The cyst is facetted and has an equatorial suture (arrow), where the wall opens during excystment. A, anlage of aboral ciliary wreath; AW, aboral ciliary wreath; MA, macronucleus; MAZ, macrozooids; MY, myonemes; OC, oral ciliature; S, scopula; SD, stalk dish; SM, stalk myoneme. Scale bars 15 μm (Fig. 9), 25 μm (Fig. 8, 12), 30 μm (Fig. 10, 11, 13), 100 μm (Fig. 15), and 1,500 μm (Fig. 14, 15).



ony (free or attached), but usually (again) only of the arboroid colony so typical of peritrichs; micro- and macrozooids are distinguishable by size and exhibition of certain functional differences (e. g. in *Zoothamnium* only macrozooids are capable of starting new colonies).''

Comparison of *Apocarchesium arndti* n. sp. with *Apocarchesium rosettum* Ji and Kusuoka, 2009. Both species are very distinct differing, inter alia, in body size and shape, in the shape and location of the macronucleus, and the structure of peniculus 3 (Fig. 63–66). The absence vs. presence of an epistomial mem-

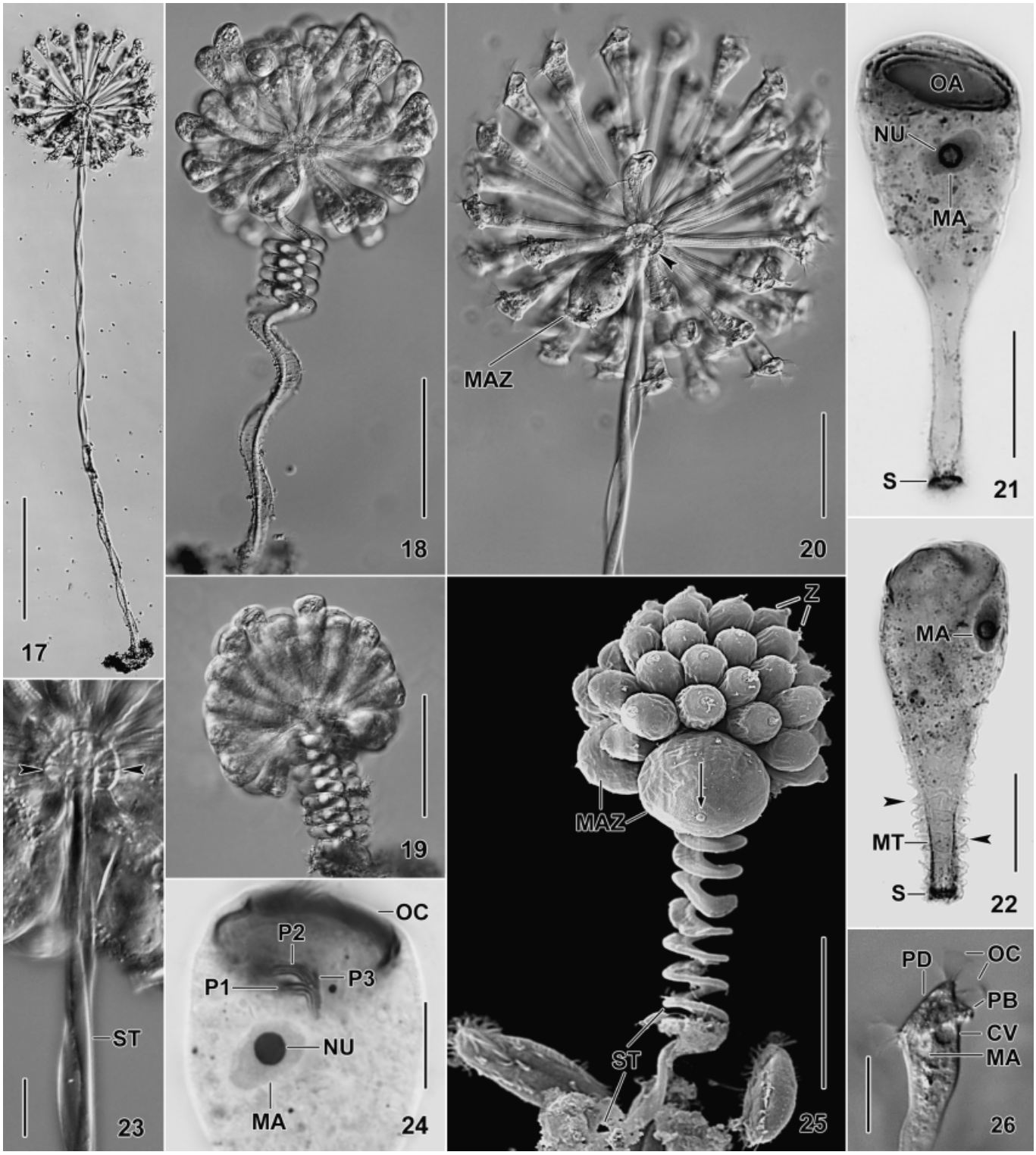


Fig. **17–26.** *Apocarchesium arndti* from life (17–20, 23, 26), after protargol impregnation (21, 22, 24), and in the scanning electron microscope (25). **17–19.** Extended, partially contracted, and fully contracted colony. Even fully contracted zooids are not globular but elongate conical or clavate (19). **20, 23.** The very slender zooids are attached to the minute stalk dish (arrowheads). **21, 22, 24.** Usually, the macronucleus is ellipsoidal and possesses a single, prominent nucleolus. The posterior half of the contracted zooids is strongly wrinkled (arrowheads). **25.** A contracted colony with two macrozooids and 27 visible ordinary zooids. Arrow marks oral opening. **26.** Anterior region of a zooid showing the oral apparatus and the ventrally located contractile vacuole. CV, contractile vacuole; MA, macronucleus; MAZ, macrozooids; MT, myoneme tube; NU, nucleolus; OA, oral apparatus; OC, oral ciliary spiral; PB, peristomial bulge; PD, peristomial disc; P1–3, oral peniculi; S, scopula; ST, stalk; Z, zooids. Scale bars 300 μm (Fig. 17), 100 μm (Fig. 18–20, 25), 30 μm (Fig. 23, 26), 20 μm (Fig. 21, 22), and 10 μm (Fig. 24).

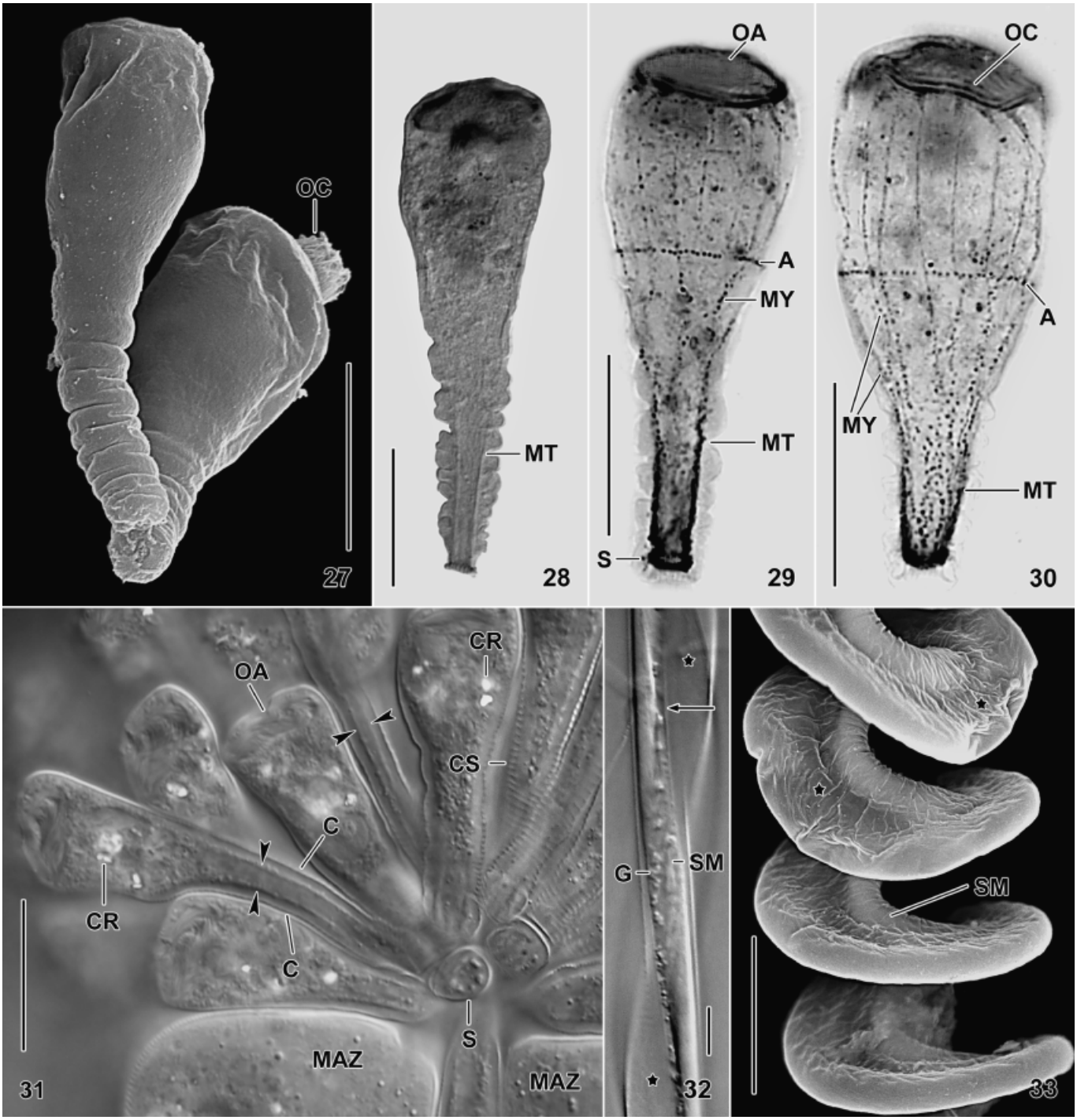


Fig. **27–33.** *Apocarchesium arndti* from life (31, 32), after protargol impregnation (28–30), and in the scanning electron microscope (27, 33). **27, 28.** Contracted zooids are club-shaped or elongate conical and have a strongly wrinkled posterior half. **29, 30.** Myoneme system. In the posterior half, the myonemes form a conspicuous tube (see also Fig. 28, 31). The anlage (A) of the aboral ciliary wreath is in mid-body, indicating that the stalk-like narrowed posterior half is a main apomorphy. **31.** Proximal portion of a colony. The myonemes form a conspicuous tube in the posterior zooid half (arrowheads), causing that the cell's periphery (cortex) becomes separated and appears doubled. **32, 33.** The colony stalk is about 18 μm wide, contracts spirally (33), and is composed of the stalk envelope (asterisks) and the stalk myoneme (SM) surrounded by a membrane (arrow). A, anlage of aboral ciliary wreath; C, cortex; CR, crystals; CS, transverse cortical striation; G, stalk granules, possibly mitochondria; MAZ, macrozooids; MT, myoneme tube; MY, myonemes; OA, oral apparatus; OC, oral ciliary spiral; S, scopula; SM, stalk myoneme. Scale bars 20 μm (Fig. 27–31, 33) and 10 μm (Fig. 32).

brane is a further distinct but problematic feature. This short kinety, which consists of only four to eight basal bodies, is often difficult to recognize because the oral apparatus is contracted in the preparations. Thus, few solid data are available, and we cannot completely exclude its presence in *A. arndti*, despite the clear preparations (Fig. 46–51). Possibly, the epistomial membrane is a

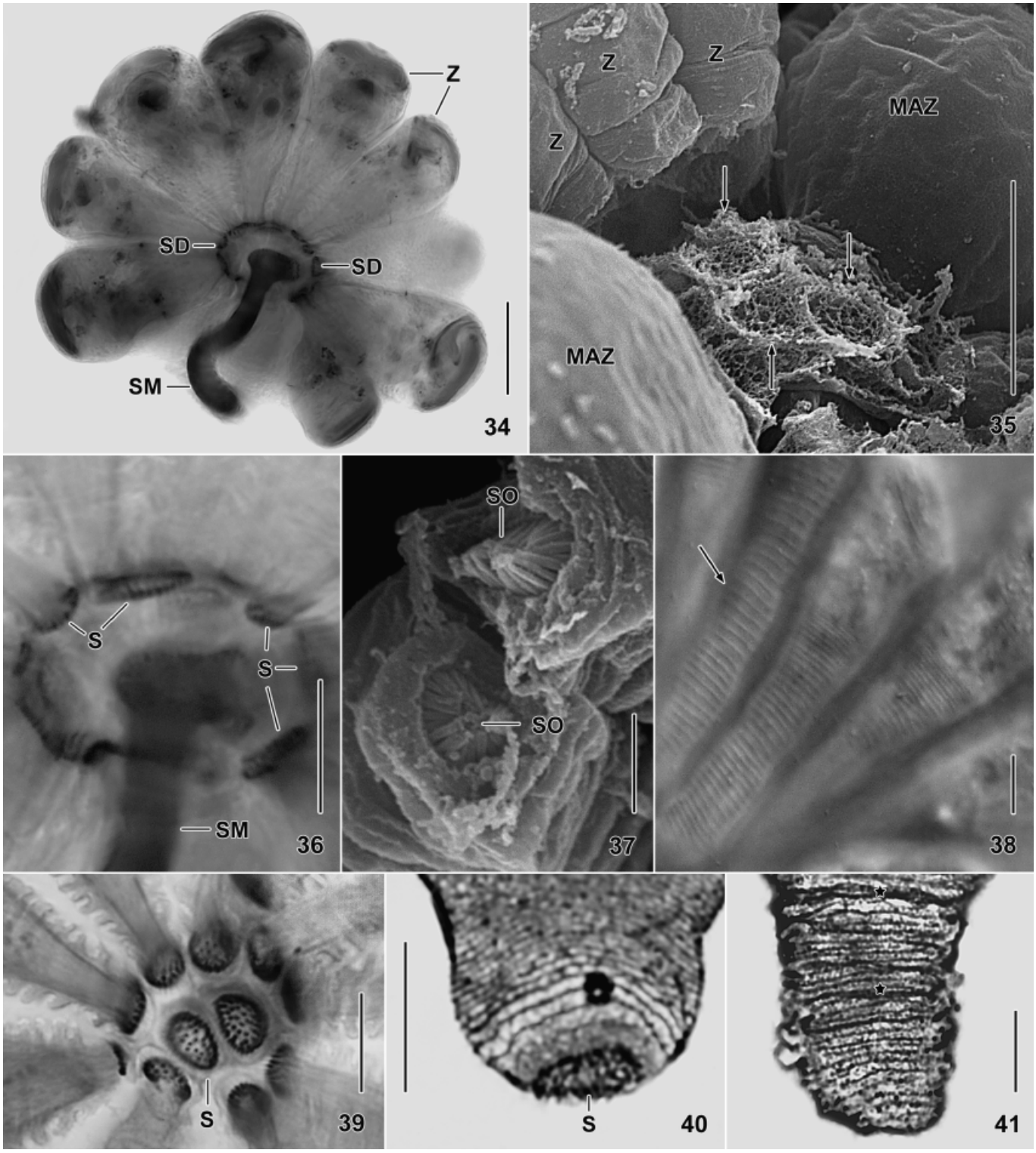


Fig. **34–41.** *Apocarchesium arndti* from life (38), after silver nitrate (40, 41) and protargol (34, 36, 39) impregnation, and in the scanning electron microscope (35, 37). **34, 36, 39.** The zooids of *A. arndti* are stalkless and thus directly attached with the scopula (S) to the margin and surface of a discoidal structure, the stalk dish (SD). **35.** Distal surface of stalk dish with zooids removed in dish centre (arrows). The stalk dish consists of spongious material, forming concave areas where the zooids were attached. **37.** Showing the bristle-shaped scopula organelles, possibly highly modified cilia. **38.** The cortex of the zooids is transversely striated. The striation becomes up to 3 μm wide when the stalk-like posterior third is fully extended (arrow). **40, 41.** Silverline pattern in posterior region. Asterisks mark thick silverlines produced by body contractions and thus composed of several silverlines. A, anlage of aboral ciliary wreath; MAZ, macrozooids; S, scopula; SD, stalk dish; SM, stalk myoneme, SO, scopula organelles; Z, ordinary zooids. Scale bars 20 μm (Fig. 34), 10 μm (Fig. 35, 36, 38, 39, 40, 41), and 2 μm (Fig. 37).

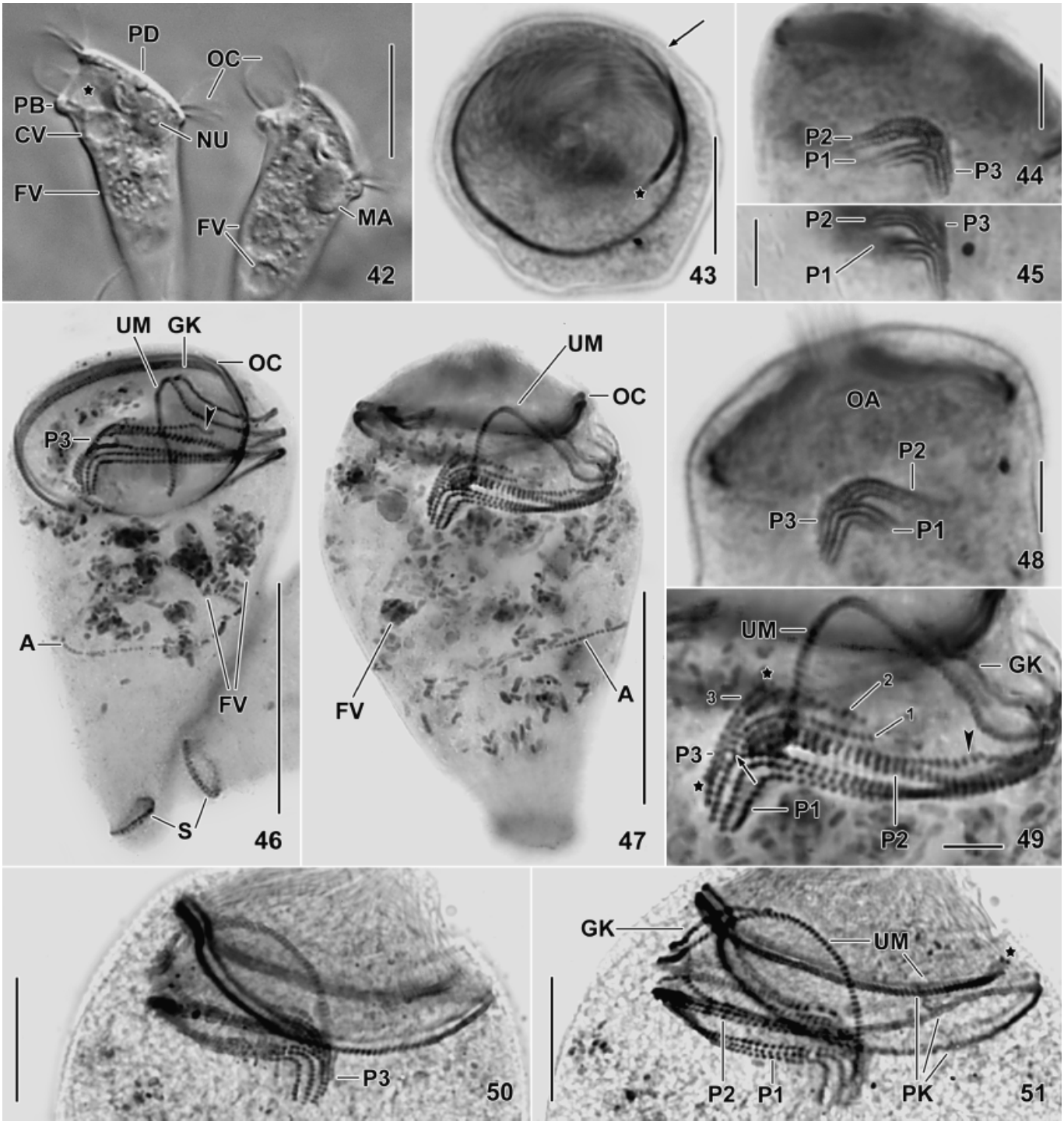


Fig. **42–51.** *Apocarchesium arndti*, oral structures from life (42) and after protargol impregnation (43–51). **42.** Anterior portion of two zooids, showing the oral apparatus and the unusual location of the macronucleus between peristomial disc and dorsal wall of oral cavity (asterisk). **43.** Frontal view showing begin (asterisk) and end (arrow) of external portion of adoral ciliary spiral. **44, 45, 48.** Proximal area of adoral peniculi. **46, 47, 49.** Total views (46, 47) and detail from Fig. 47 (49), showing location and structure of the oral apparatus (see also scheme, Fig. 2). Peniculus 2 is shortened proximally, where peniculi 1 and 3 merge, and basal body row three separates from rows 2 and 1 near distal end of the peniculus (arrowheads). Peniculus 3 consists of three basal body rows: row 3, marked by asterisks, is shortened anteriorly and posteriorly; row 2 is longest but slightly shortened anteriorly and posteriorly; and row 1 is distinctly shortened posteriorly (end marked by arrow). **50, 51.** Oral apparatus at two focal planes. Asterisk marks begin of adoral ciliary spiral. A, anlage of aboral ciliary wreath; CV, contractile vacuole; FV, food vacuoles; GK, germinal kinety; MA, macronucleus; NU, nucleolus; OA, oral apparatus; OC, oral ciliary spiral; PB, peristomial bulge; PD, peristomial disc; PK, polykinety; P1–3, peniculi; S, scopula; UM, undulating membrane (haplokinety). Scale bars 30 μm (Fig. 42, 46, 47), 10 μm (Fig. 43, 50, 51), and 5 μm (Fig. 44, 45, 48, 49).

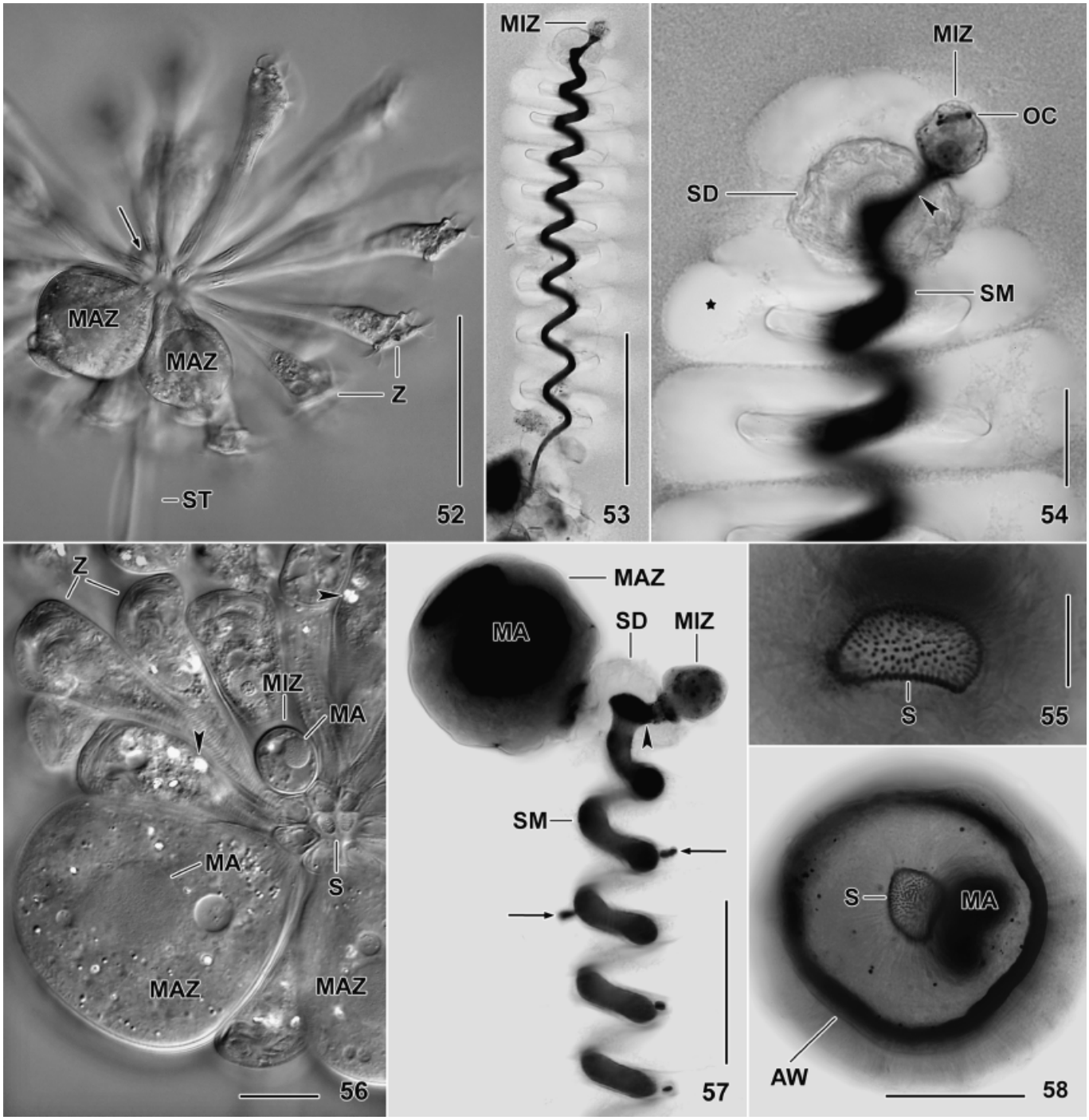


Fig. **52–58.** *Apocarchesium arndti*, macro- and microzooids from life (52, 56) and after protargol impregnation (53–55, 57, 58). **52.** Posterior view of a colony, showing two macrozooids attached to the margin of the stalk dish (arrow). Note the very slender ordinary zooids, a main difference to the campanulate zooids of *Apocarchesium rosettum*. **53, 54, 57.** Overviews and detail (54 from 53) of silver-impregnated macro- and microzooids. In contrast to the zooids and macrozooids, the microzooids are connected with the stalk myoneme (arrowheads) but have reduced almost completely the oral ciliature (54). Note the mighty stalk with the stalk envelope marked by an asterisk in Fig. 54. Some specimens have attached comparatively large, strongly argyrophilic granules to the stalk myoneme (57, arrows). **56.** Colony showing ordinary zooids, two macrozooids, and a microzooid. Arrowheads mark cytoplasmic crystals. **55, 58.** Posterior polar views showing a developing (55) and a mature (58) macrozooid. The scopula becomes large and elliptical during macrozooid development (55). Note the conspicuous aboral ciliary wreath of a mature macrozooid (58). AW, aboral ciliary wreath; MA, macronucleus; MAZ, macrozooids; MIZ microzooids; OC, oral ciliary spiral; S, scopula; SD, stalk dish; SM, stalk myoneme; ST, stalk; Z, ordinary zooids. Scale bars 100 μm (Fig. 52, 53), 40 μm (Fig. 57), 30 μm (Fig. 56, 58), 20 μm (Fig. 54), and 10 μm (Fig. 55).

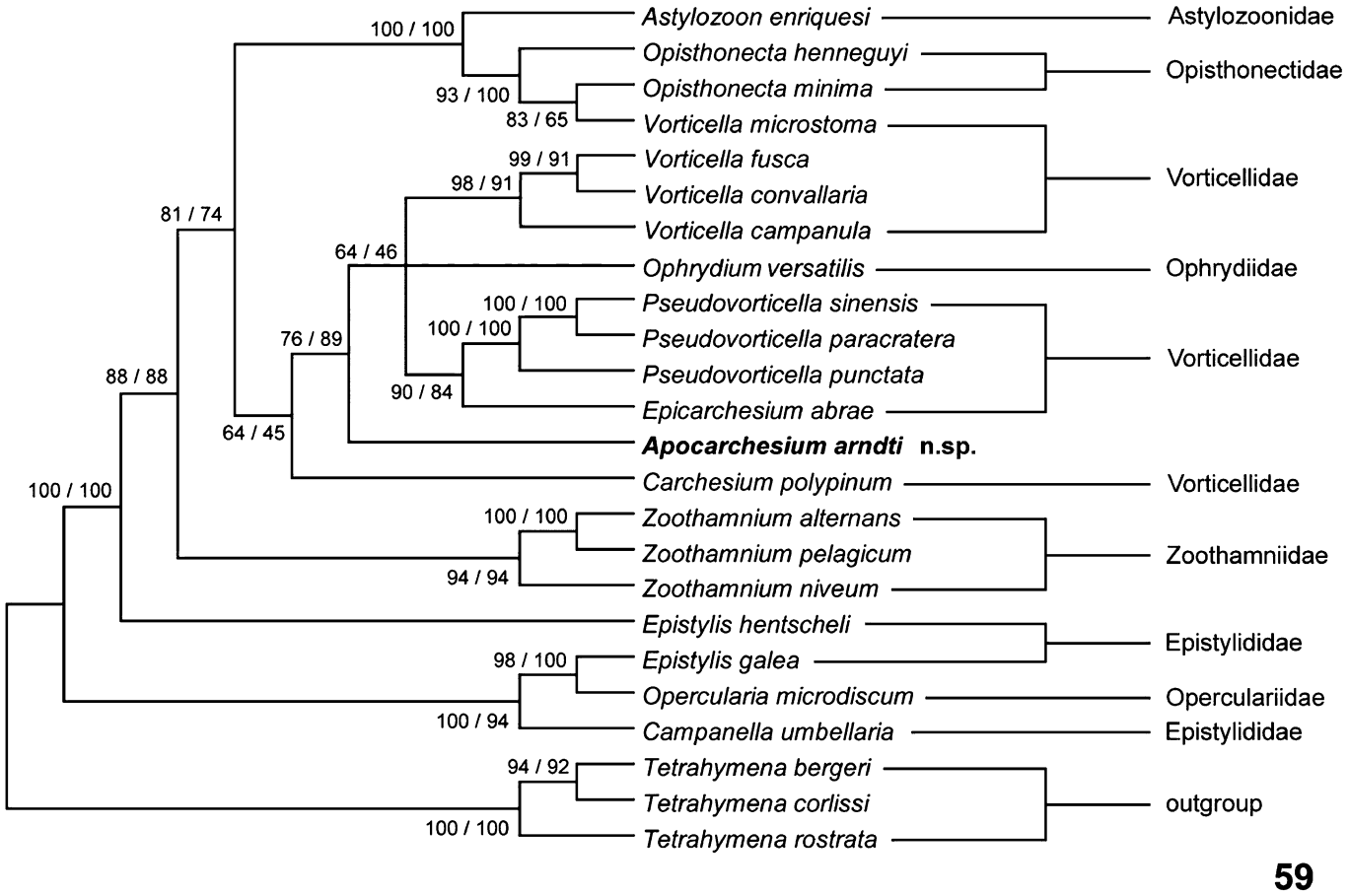


Fig. **59.** Consensus tree, constructed from two phylogenetic trees and with *Tetrahymena* spp. as outgroup, showing the phylogenetic position of *Apocarchesium arndti* within the peritrich order Sessilida. At the branches, the maximum likelihood values are followed by the maximum parsimony support values for 1,000 bootstrap replicates.

general feature of peritrichs and the absence caused by methodological difficulties. However, the epistomial membrane of *A. rosettum* is also doubtful for the following reasons: (i) it is not shown by a micrograph but only in a line drawing and (ii) it is depicted to consist of dikinetids, while all other well-documented species have it composed of monokinetids (e.g. Foissner 2000; Foissner, Agatha, and Berger 2002; Ji et al. 2009).

Are *Apocarchesium arndti* n. sp. and *Apocarchesium rosettum* Ji and Kusuoka, 2009 congeneric?. Generic separation is a matter of taste and agreement in all organisms. In peritrichs, frequently used generic features are, e.g. the shape of the macronucleus (strand-like vs. globular), the silverline pattern (transverse-striate vs. reticular), and the habit (solitary vs. colonial).

Apocarchesium rosettum seemingly differs from *A. arndti* n. sp. by four main features, each, in our opinion, sufficient for a generic distinction: the stalk dish (absent vs. present), the colony shape (globular vs. hemispherical), the epistomial membrane (present vs. absent; but see previous section), and the occurrence of ''true'' microzooids. However, micrograph 2B in the paper of Ji and Kusuoka (2009) reveals a stalk dish (cf. with Fig. 20, 23 in our paper), and thus the colony shape of *A. rosettum* is very likely also hemispherical. Therefore, only the microzooids and the supposed lack of an epistomial membrane remain as main differences. However, microzooids are usually produced only for sexual reproduction, and the population studied by Ji and Kusuoka (2009) might have been in another stage of the life cycle. All these data are summarized in the emended diagnosis of *Apocarchesium*.

Beside the features discussed above, there are others that argue for a generic separation of *A. rosettum* and *A. arndti* n. sp. These include the shape of the body and the macronucleus and the general organization of the zooids: those of *A. rosettum* appear vorticellid, while the zooids of *A. arndti* n. sp. look like those of slender epistylidids, such as *Epistylis plicatilis* (for a brief review of this species, see Foissner et al. 1992). There is no other aloricate peritrich—if *Ophrydium* spp. are excluded—that is as slender as *A. arndti* n. sp., and there is no other peritrich with such a curious location of the macronucleus. Thus, *A. arndti* n. sp. is an outstanding species. Indeed, if the same measure is applied as in other peritrichs, *A. arndti* n. sp. represents a distinct genus, especially because of the macronucleus shape, a feature that has been used to separate *Orbopercularia* from *Opercularia* (Lust 1950) and *Orborhabdostyla* from *Rhabdostyla* (Foissner et al. 2009). However, in *A. arndti* n. sp. we interpret the macronucleus shape not as a specific evolutionary trait but as caused by the very slender body shape. Spatial constraints would also explain another peculiarity of *A. arndti* n. sp., viz., the tube-like arrangement of the myonemes in the narrow posterior body half.

Thus, we do not introduce a new genus but provide an improved generic characterization (see ''Taxonomic summary''), possibly useful when further species are discovered and a sequence becomes available for *A. rosettum*.

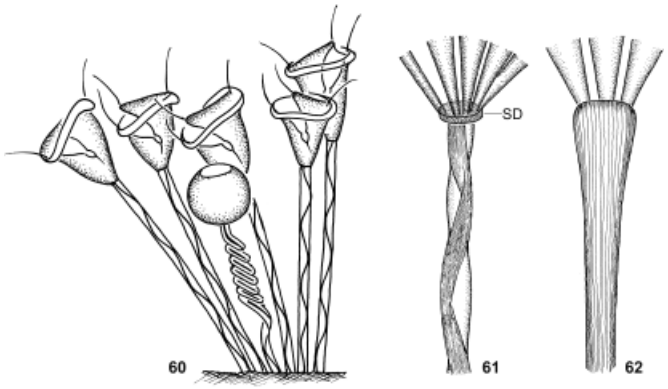


Fig. **60–62.** Three kinds of colonies. **60.** Solitary specimens of *Vorticella* spp. form a pseudocolony when attaching close together. **61.** The zooids of *Apocarchesium* form a rosette, i.e. sit on a stalk dish (SD) and are not connected to the stalk myoneme. **62.** Several to many zooids sit on the end of a broadened, acontractile stalk, e.g. in *Systylis*.

Family classification. Ji and Kusuoka (2009) classified *Apocarchesium* in the Vorticellidae, specifically, near *Carchesium*, but mentioned relationships to the Zoothamnidae because of the ability of *A. rosettum* to form macrozooids. In our opinion, *Apocarchesium* could represent a distinct family, considering its unique features, especially, the stalk dish, which causes stalkless zooids, and the ability to produce microzooids and macrozooids, a feature absent from its nearest neighbours in gene phylogenies (i.e. *Carchesium*, *Epicarchesium*, *Vorticella*, and *Pseudovorticella*). Further, the SSU rDNA does not exclude family position. However, our suggestion was strongly opposed by two of the three reviewers. The most stunning feature of *Apocarchesium* is the stalk dish, which is either a new invention, the vestige of a lorica, or a precursor of a lorica. The vestige hypothesis is supported by stalk dish-like differentiations in some loricate peritrichs, such as *Cothurnia* (Kahl 1935) and *Rovinjella* (Matthes 1972). However, the sole loricate peritrich sequenced, *Vaginicola crystallina*, appears near the base in the molecular trees (Li et al. 2008a), and thus far away from *Apocarchesium*. Accordingly, the stalk dish is very likely a new invention. The stalk dish separates the colony stalk from the ordinary zooids and the macrozooids; only the microzooids are connected to the stalk. Possibly, this connection keeps the colony stalk alive, providing it with materials needed.

Biogeographic aspects. *Apocarchesium* spp. is of high interest for protist diversity and distribution considerations because of the late discovery of flagship species in widely separated habitats.

Apocarchesium rosettum occurs in the coastal biofilm of Lake Biwa, an ~ 4 million years old freshwater lake in Japan, containing many endemics (Foissner, Kusuoka, and Shimano 2008b; Ji and Kusuoka 2009; Nishino and Watanabe 2000). In contrast, *A. arndti* n. sp. lives in the biofilm of the River Rhine, Germany. Both regions belong to the Palaearctic but are separated by a distance of about 9,000 km. If protist species were distributed globally, as proposed by Finlay (2001), one would expect to find the same species in Germany and Japan, in spite of the somewhat different habitats in which *A. rosettum* and *A. arndti* n. sp. were discovered. Nevertheless, both are biofilm dwellers and *A. arndti* was found more frequently at low flow velocities. Further, Central Europe, and especially Germany and Hungary, are the best studied areas globally for peritrich diversity, including large lakes, such as Lake Plön and Lake Balaton (for reviews, see Biegel 1954; Sommer 1951; Stiller 1971). Thus, we interpret the findings as an indication for a biogeographic differentiation of protist communities (for a review, see Foissner 2006).

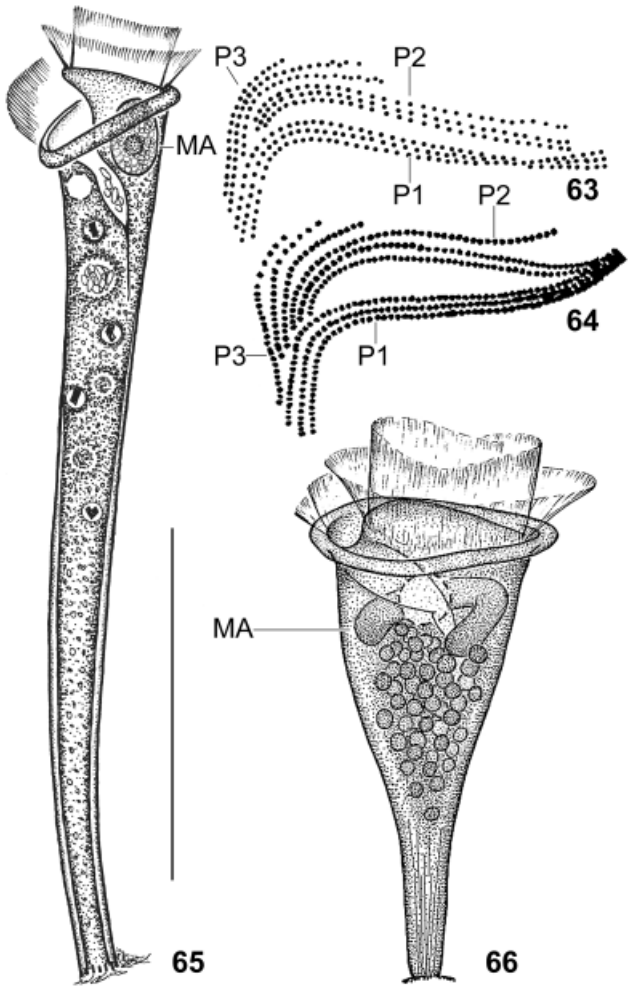


Fig. **63–66.** Comparison of zooids (65, 66) and proximal oral structures (63, 64) in *Apocarchesium arndti* (63, 65) and *Apocarchesium rosettum* (64, 66; from Ji and Kusuoka 2009). Zooids drawn to scale, bar 70 μm; oral structures semischematic and thus without bar. MA, macronucleus; P1–3, peniculi (adoral membranelles).

Both, *A. rosettum* and *A. arndti* n. sp. are flagship species in a biogeographical context (Foissner 2006): they are large and have a conspicuous shape. Thus, their late discovery is an impressive example of our ignorance of protist diversity. If such prominent species escaped researchers for 200 years, then one can imagine that there must be thousands of undiscovered inconspicuous species. Thus, and for many other reasons (Foissner 2006, 2008; Foissner, Chao, and Katz 2008a), our estimate of the number of free-living ciliate species is one order of magnitude higher (i.e. 30,000 vs. 3,000) than that of Finlay (2001).

TAXONOMIC SUMMARY

Class Oligohymenophorea de Puytorac, Batisse, Bohatier, Corliss, Deroux, Didier, Dragesco, Fryd-Versavel, Grain, Groliére, Hovasse, Iftode, Laval, Roque, Savoie & Tuffrau, 1974
Subclass Peritrichia Stein, 1859
Order Sessilida Kahl, 1933
Suborder Vorticellina Fromentel, 1875
Family Vorticellidae (?)
Genus *Apocarchesium* Ji & Kusuoka, 2009

Emended diagnosis. Colonial peritrichs with single, helically contracting stalk possessing an expanded distal tip, the stalk dish, from which stalkless zooids arise to form a hemispherical rosette. Macrozooids always present in addition to ordinary, vegetative zooids, and both types not connected to myoneme of colony stalk. Microzooids connected to colony myoneme. Transverse-striate silverline pattern.

Type species (by original designation). *Apocarchesium rosettum* Ji and Kusuoka, 2009.

Apocarchesium arndti n. sp.

Diagnosis. Colonies up to 2 mm high and with up to 50 zooids. Ordinary zooids epistylidid and trumpet-shaped, in vivo about 180 × 30 μm in size. Macronucleus subapical between oral cavity and dorsal side of cell, ellipsoidal to reniform. Contractile vacuole on ventral wall of oral cavity. Myoneme system tube-like in posterior body half, seemingly doubling the cortex. About 95 silverlines in total. Adoral ciliary spiral as usual, performs about 1.25 revolutions on peristomial disc; row 1 of peniculus 3 distinctly shortened proximally. Macro- and microzooids broadly ellipsoidal, in vivo about 80 and 20 μm in size, respectively.

Type locality. River Rhine in the town of Cologne, Germany, 50°54′25″N, 6°58′43″E.

Type material. One holotype slide with protargol-impregnated specimens and 14 paratype slides with silver nitrate- or protargol-impregnated specimens have been deposited in the Biologiezentrum of the Oberösterreichische Landesmuseum in Linz (LI), Austria. Relevant specimens have been marked by black ink circles on the coverslip. The sequence of the SSU rRNA gene has been deposited in GenBank, Accession Number: GQ221940.

Dedication. We dedicate this new species to Prof. Dr. Hartmut Arndt, University of Cologne, a remarkable protist ecologist, who founded the Ecological River Rhine Station, where the new species was discovered.

ACKNOWLEDGMENTS

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