

## *Korotnevelia hemistylelepis* N. Sp. and *Korotnevelia monacantholepis* N. Sp. (Paramoebidae), Two New Scale-covered Mesohaline Amoebae

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**ABSTRACT.** Two new species of *Korotnevelia* Goodkov, 1988, *Korotnevelia hemistylelepis* n. sp. and *Korotnevelia monacantholepis* n. sp., are described from mesohaline ecosystems. The amoebae are characterized on the basis of light and electron microscopy with special emphasis on the structure of the basket scales, which have species-specific architecture. The two new species are the second and third ones recovered from environments other than freshwater. In terms of scale morphology they most closely resemble a freshwater species, *Korotnevelia bulla* (Schaeffer, 1926) Goodkov, 1988. Two genus names, *Dactylamoeba* Korotnev, 1880 and *Korotnevelia* Goodkov, 1988, are in current use. The latter name is preferred, pending rediscovery and characterization of *Dactylamoeba elongata* Korotnev, 1880, the type species of the genus. *Korotnevelia* species can be divided into three groups on the basis of scale morphology, suggesting that the genus may not be monophyletic. A key to species is provided.

**Key Words.** Amoeba morphology, *Dactylamoeba*, gymnamoebae, *Mayorella*, *Neoparamoeba*, *Paramoeba*, protistan taxonomy, scale structure, Vexilliferidae.

THE genus *Korotnevelia* Goodkov, 1988, encompasses free-living *Mayorella*-like amoebae of the family Paramoebidae. They are characterized by the absence of parasomes and the presence of a surface coat consisting of one or two types of discrete, non-mineralized scales (Goodkov 1988; Smirnov 1996–97, 1999). Four species have been recognized, all but one of which are from freshwater environments (Smirnov 1999). Species diversity, taxonomic boundaries, and phylogenetic relationships are not well understood. For example, species with several different scale types are included in the genus. The genus name is itself controversial, since both *Korotnevelia* and *Dactylamoeba* Korotnev, 1880, have been used in recent publications to refer to the same organisms (Butler and Rogerson 1997; Page 1983, 1988, 1991; Smirnov 1996–1997, 1999).

A better understanding of all paramoebids is needed, not least because parasome-containing marine species are associated with diseases of commercially-important crustaceans (Johnson 1977; Sawyer 1976; Spencer et al. 2000; Sprague, Beckett, and Sawyer 1969), echinoderms (Jones 1985), and fishes (Dyková et al. 1998; Dyková, Figueras, and Peric 2000; Kent, Sawyer, and Hedrick 1988; Roubal, Lester, and Foster 1989).

In this paper, two new scale-covered species of amoebae, *Korotnevelia hemistylelepis* O'Kelly, Peglar & Nerad, n. sp., from the Pocomoke River, Maryland, and *Korotnevelia monacantholepis* O'Kelly, Peglar & Nerad, n. sp., from an aquarium maintained at North Carolina State University, Raleigh, are described at both the light and electron microscopical levels. The taxonomic status and nomenclatural history of the genus *Korotnevelia* are reviewed, and a key to the species is provided.

### MATERIALS AND METHODS

**Isolation and cultivation.** *Korotnevelia hemistylelepis* n. sp. was collected from water samples in July 1998, at the mouth of the Pocomoke River, Chesapeake Bay, Maryland, USA (37° 44.287' N, 75° 51.575' W). A clonal culture was established and maintained in T-75 tissue culture flasks containing 30 ml of a 1:1 mixture of ATCC medium 1405 (Nerad 1993) and ATCC medium 802 (Nerad 1993) supplemented with *Klebsiella pneumoniae* subsp. *pneumoniae* (ATCC 700831) as a food source. Stock cultures were maintained at 20–25 °C.

*Korotnevelia monacantholepis* n. sp. was recovered from a mesohaline marine aquarium maintained in the laboratory of Dr. Edward J. Noga at the Department of Veterinary Science,

North Carolina State University, Raleigh, North Carolina, USA, in June 2000. The amoebae were established in clonal culture in ATCC medium 1405 diluted to 17‰ salinity with distilled water. The strain was maintained in T-75 tissue culture flasks containing 30 ml of medium, fed periodically with *Rhodomonas* sp. CCMP768, and maintained at 20–25 °C. Some cultures were offered *Pfiesteria piscidida* (CCMP 1834) or *Tetrahymena malaccensis* (ATCC 205024), instead of *Rhodomonas*. No other food sources were tested.

Cryopreservation experiments were conducted on both strains, using procedures described by Nerad and Daggett (1992).

**Light microscopy.** Light microscopical observations were made on live cells using the following equipment: 1) a Zeiss Axioskop compound microscope equipped with an Optronics DEI-470 CCD camera; 2) a Zeiss Axioplan compound microscope equipped with a Zeiss AxioCam digital camera; 3) a Zeiss Televal 31 inverted microscope equipped with a 35 mm Pentax camera. Images were captured either electronically or on 800 ASA Kodak color film. Measurements of *K. hemistylelepis* cells were made from images captured with the AxioCam camera, using Zeiss Axiovision version 2.0.5.3 software. Measurements of *K. monacantholepis* cells were made using a calibrated ocular micrometer fitted to the inverted microscope.

**Transmission electron microscopy.** Cells were fixed, embedded, serially sectioned, stained, and examined using procedures slightly modified from those described in detail elsewhere (O'Kelly 1997; O'Kelly and Patterson 1996). Briefly, exponential-phase cells were fixed in a cacodylate-buffered glutaraldehyde-osmium cocktail for 15 min at room temperature (ca. 20 °C). Fixed cells were washed with distilled water, dehydrated in a graded acetone series and embedded in Eponbed-Araldite resin (Electron Microscopy Sciences, Fort Washington, PA, USA). Blocks were trimmed by hand, serially sectioned with a diamond knife, mounted on naked 600-mesh or formvar-and-carbon-coated 100-mesh grids, stained with uranyl acetate and lead citrate, and examined with a Zeiss EM 902 transmission electron microscope operated at 80 kV.

Whole-mount preparations of scales were made by allowing cells in growth medium to settle for 30 min onto formvar-and-carbon-coated 100-mesh grids. The grids were then exposed to vapor of osmium tetroxide for 60 min, drained and dried briefly, washed in distilled water, floated on saturated uranyl acetate in 50% ethanol for one minute, drained and dried without washing, and examined.

**Internet data dissemination.** Updated summaries on the

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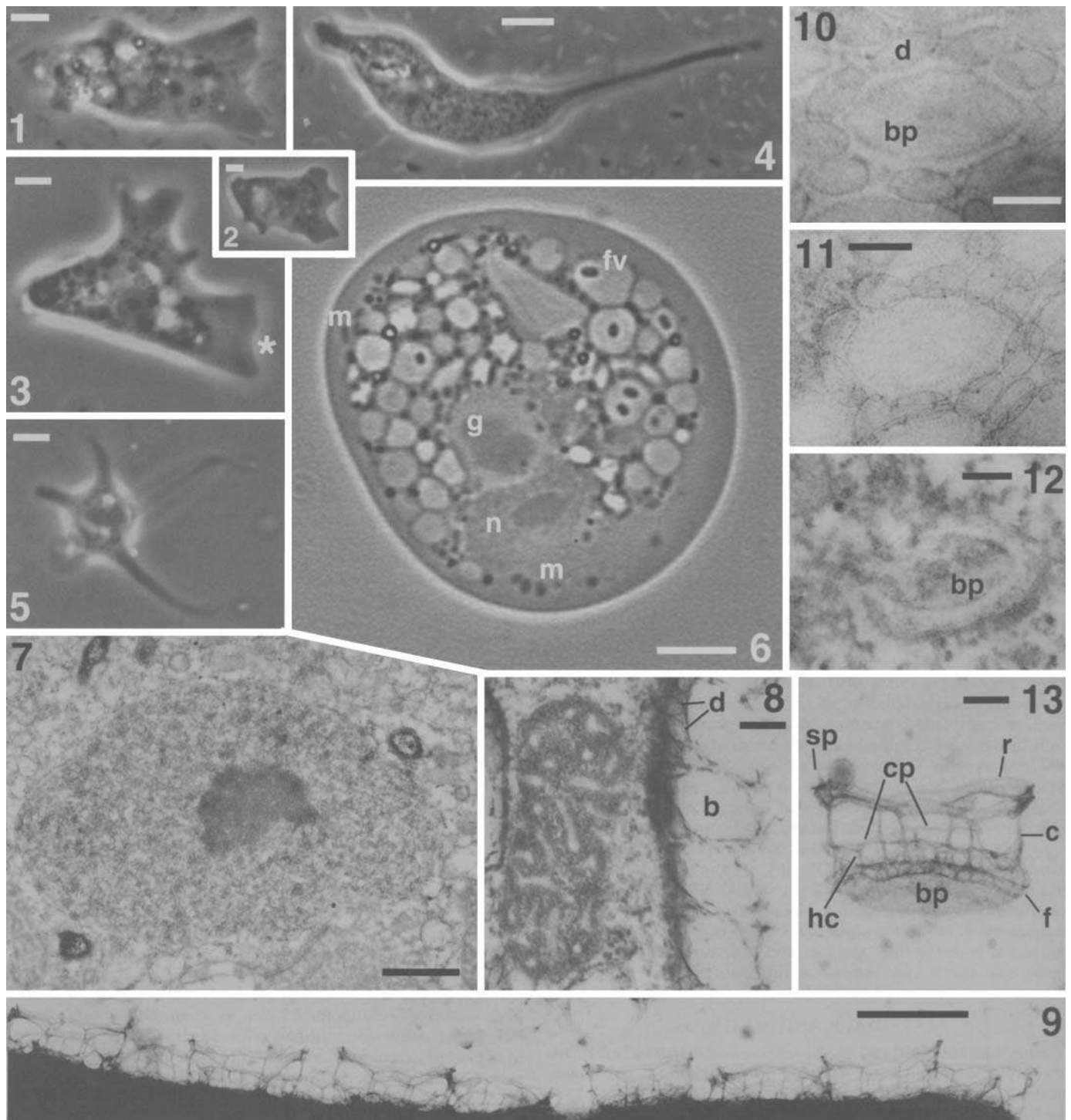


Fig. 1–13. Light (Fig. 1–6) and transmission electron (Fig. 7–13) micrographs of the amoeba *Korotnevella hemistylelepis* n. sp. Fig. 1–4. Habit of locomotive amoeba. Fig. 1–3. Amoebae with attached pseudopodia. Fig. 1. Initial stage of dactylopodium formation; amoeba with divided hyaline front. Scale bar = 5  $\mu$ m. Fig. 2. Formation of two subpseudopodia on each arm of the hyaline front. Scale bar = 5  $\mu$ m. Fig. 3. One pseudopodium is established in the direction of forward motion (asterisk); the other is moving towards the posterior end of the cell. Scale bar = 5  $\mu$ m. Fig. 4. Attached amoeba in heavily fed culture, with single long unattached pseudopodium. Scale bar = 5  $\mu$ m. Fig. 5. Floating form. Scale bar = 5  $\mu$ m. Fig. 6. Quiescent amoeba, flattened under cover-slip pressure, showing nucleus (n) associated with a single large Golgi body (g), numerous mitochondria (m), and food vacuoles (fv). Scale bar = 5  $\mu$ m. Fig. 7. Nucleus with simple nucleolus. Scale bar = 1  $\mu$ m. Fig. 8. Mitochondrial profile, showing tubular cristae. Dish-shaped (d) and basket (b) scales are presented in oblique tangential section of the cell surface. Scale bar = 250 nm. Fig. 9. Whole mount of pseudopodium, showing disposition of basket scales. Scale bar = 500 nm. Fig. 10. Semi-thin (250 nm) section showing arrangement of dish-shaped scales (d) around the basal plate (bp) of a basket scale. The margins of the dish scales and the basket scale basal plate do not overlap. Scale bar = 100 nm. Fig. 11. Thin (80 nm) section; the flange surrounding the basket scale basal plate overlaps the dish scales. Scale bar = 100 nm. Fig. 12. Basal plate of a basket scale (bp) in a *trans-*

morphology, taxonomy, and biology of *Korotnevela* are available from the Protist Image Data Web site (<http://megasun.bch.umontreal.ca/protists/>).

## RESULTS

**The morphology of *Korotnevela hemistylepis*.** Locomotive cells advanced from a hyaline front that bifurcated, giving the cell a Y-shaped appearance (Fig. 1). Each arm of the advancing front commonly had two small conical marginal subpseudopodia (Fig. 2). One arm of the Y determined the direction of movement while the other moved posteriorly and could be resorbed (Fig. 3). In some cases the arm moving posteriorly was not resorbed but changed into a bifurcated hyaline zone that determined a new direction of movement (Fig. 3). When this happened, the direction of movement was usually shifted approximately 90°. In cultures with high bacterial densities, attached cells often produced a single, long, unattached finger-like pseudopodium (dactylopodium; Page 1981), as long as or longer than the main body mass. These dactylopodia could freely move horizontally or vertically (Fig. 4). Differentiated uroidal structures were absent.

Rayed floating forms typically had 3–8 thin, radiate pseudopodia 1–4 times longer than the central body mass which measured about 7 µm in diam. (Fig. 5). Old cultures contained rayed forms with usually 3 very long, extremely thin pseudopodia.

Live cells on microscope slides rapidly contracted when exposed to light; diagnostic features of pseudopodial morphology and locomotive behavior were lost after only a few seconds of illumination. Under coverslip pressure, cells became flattened and rounded, and locomotion ceased. It was only in this state that the single large Golgi body could be observed (Fig. 6).

Locomotive amoebae were 15.5–48.5 µm long, exclusive of free pseudopodia (mean 26.3 ± 6.7 µm, n = 25), and 3.1–13.0 µm wide (mean 7.3 ± 2.53 µm, n = 25). The length/width ratio was highly variable, with a mean of 3.6 ± 2.6.

The cells were uninucleate (Fig. 6). The nucleus generally contained a single, centrally located, roughly spherical nucleolus with a regular margin and lacking apparent inclusions (Fig. 6, 7). On some occasions the nucleolus was divided into two unequal pieces. Mean nuclear diam. was 3.6 ± 0.6 µm (range 2.8–4.8 µm, n = 13), mean nucleolar diam. was 1.6 ± 0.3 µm (range 1.1–2.2 µm, n = 13). A single large Golgi body was adjacent to the nucleus (Fig. 6). Bacilliform mitochondria were numerous (Fig. 6), and had tubular cristae (Fig. 8). Numerous food vacuoles contained bacteria (Fig. 6).

Two types of non-mineralized scales, visible only with electron microscopy, covered the entire cell surface (Fig. 8–11). Scale elements also appeared in *trans*-Golgi vesicles (Fig. 12). The smaller, dish-shaped scales were ellipsoidal in outline, with a discernable rim and no conspicuous ornamentation. The long axis of these scales generally measured 100 nm (Fig. 8, 10, 11). They were distributed around the periphery of the larger basket scales (Fig. 10, 11).

Basket scale structure was more complex. An ellipsoidal, unornamented basal plate with pointed apices was appressed to the cell surface (Fig. 10, 11, 13). It was typically 250–350 nm long and 130–170 nm wide (Fig. 10, 11, 13), and did not overlap the smaller scales (Fig. 10). Around the entire margin of the basal plate was a perforated flange, approximately 20 nm

wide (Fig. 13). This flange sometimes extended over a small portion of the dish scales (Fig. 11). From the basal plate margin, at the junction of the basal plate and the flange, six vertical columns arose, one at each apex and two additional ones on each side (Fig. 13). The columns were 100–200 nm long, longer at the apices and shorter along the sides. They supported a narrow perforated rim, around 20 nm wide and slightly protruding from the column supports at each end (Fig. 13). These protrusions also incorporated a single spinule, 10–20 nm long (Fig. 13). At about 40 nm above the basal plate, a single circumferential crosspiece connected the vertical columns. The crosspiece was also supported by six half-columns that arose from the basal plate and were spaced equidistantly from the full-length vertical columns (Fig. 13). No other surface structures were observed.

No life history stages other than attached and floating gymnamoebae were observed.

**The morphology of *Korotnevela monacantholepis*.** Well-fed amoebae were often more or less circular in outline with dactylopodia emerging from a narrow hyaline zone and almost covering the entire perimeter of the cell. There were also unattached dactylopodia rising upward from the cell surface (Fig. 14). Starved locomotive amoebae were variable in shape. The cells could appear to have an almost truncate circular outline with dactylopodia covering the entire curved hyaline margin or sometimes an irregular triangular appearance with the dactylopodia covering the anterior half of the body. At other times the cells could be completely irregular with dactylopodia appearing to emerge all along the perimeter of the cell (Fig. 15). Floating forms were spherical, with typically ten or more long, thin pseudopodia radiating in all directions from the cell body (Fig. 16). Differentiated uroidal structures were absent.

Locomotive amoebae were 75.0–160 µm long (mean 106 µ ± 15.3 µm, n = 25), and 50.0–90.0 µm wide (mean 61.4 ± 11.2 µm, n = 25). The mean length/width ratio was 1.7 ± 1.4.

The cells were uninucleate (Fig. 14, 15, 17, 18). The nucleus usually contained a single, centrally located, ellipsoidal nucleolus (Fig. 17). Sometimes two nucleoli of unequal size were present (Fig. 18). Nucleoli had irregular margins and numerous translucent canaliculi (Fig. 17–19). Mean nuclear length was 12 ± 2.1 µm (range 9.0–15 µm, n = 11), and width 10 ± 2.7 µm (range 6.0–15 µm, n = 11). Mean nucleolar length (in nuclei with single nucleoli) was 7.0 ± 2.8 µm (range 4.0–12 µm, n = 11), and width 4.2 ± 0.91 µm (range 3.0–6.0 µm, n = 11). Food vacuoles contained remnants of protistan food items and were often highly refractile (Fig. 14, 15).

Two types of non-mineralized scales, visible only with electron microscopy, covered the entire cell surface (Fig. 20, 21). Scale elements also appeared in *trans*-Golgi vesicles (Fig. 12). The smaller, dish-shaped scales were ellipsoidal in outline with rounded apices (Fig. 21, 22). Both the rim and the floor were minutely perforate (Fig. 22). The long axis of these scales generally measured 200–250 nm (Fig. 21, 22). They were distributed around the periphery of the larger basket scales (Fig. 21).

The basal plate of basket scales was appressed to the cell surface. The basal plate had pointed apices, and was unornamented except for a fine mark along most of the long axis (Fig. 21). It was typically 500–600 nm long and 200–250 nm wide (Fig. 21, 23), and did not overlap the smaller scales (Fig. 21).

Golgi vesicle. Scale bar = 100 nm. Fig. 13. Lateral view of basket scale, showing the basal plate (bp) with its minutely fenestrated flange (f), the six full columns (c), the intermediate crosspiece (cp) with its six supporting half-columns (hc), the minutely fenestrated rim (r), and the spinules (sp) at the scale apices. Whole mount. Scale bar = 100 nm.

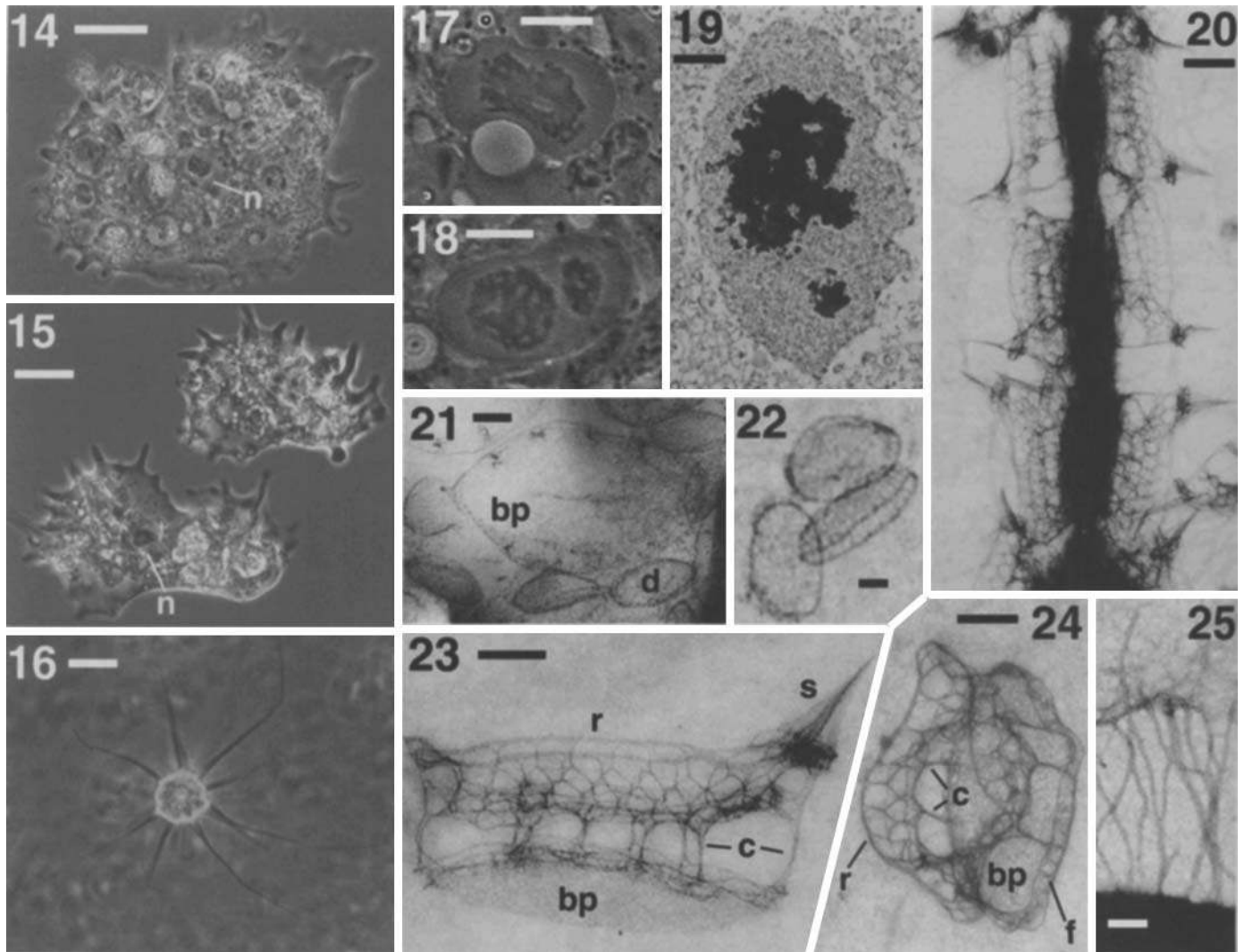


Fig. 14–25. Light (Fig. 14–18) and transmission electron (Fig. 19–25) micrographs of *Korotnevelva monacantholepis* n. sp. Fig. 14, 15. Habit of locomotive amoeba, showing disposition of dactylopodia. The single nucleus (n) is visible. Fig. 14. Well-fed amoeba with both attached and unattached dactylopodia. Scale bar = 20  $\mu$ m. Fig. 15. Two starved cells, showing variation in cell shape and disposition of mostly attached dactylopodia. Scale bar = 25  $\mu$ m. Fig. 16. Floating form. Scale bar = 50  $\mu$ m. Fig. 17. Nucleus with a single nucleolus. Scale bar = 5  $\mu$ m. Fig. 18. Nucleus with two nucleoli of unequal size. Scale bar = 5  $\mu$ m. Fig. 19. Nucleolus with irregular outline and perforations. Scale bar = 1  $\mu$ m. Fig. 20. Whole mount showing disposition of basket scales along a pseudopodium of a floating form. Scale bar = 250 nm. Fig. 21. Tangential section of cell surface, showing arrangement of dish-shaped and basket scales. Scale bar = 100 nm. Fig. 22. Dish-shaped scales. Whole mount. Scale bar = 50 nm. Fig. 23. Lateral view of spine bearing scale, showing basal plate (bp), ten vertical columns (c), broad rim with two rows of latticework (r), and spine (s). Whole mount. Scale bar = 100 nm. Fig. 24. Dorsal view of spineless scale (the basal plate appressed to the grid surface), showing the fenestrated flange to the basal plate (f) in addition to the other features shown in Fig. 23. Whole mount. Scale bar = 250 nm. Fig. 25. Fine filaments associated with the cell surface, whole mount. Scale bar = 100 nm.

Around the entire margin of the basal plate was a perforated flange, around 25 nm wide (Fig. 24). From the basal plate margin, at the junction of the basal plate and the flange, eight (Fig. 24) or ten (Fig. 23) vertical columns arose, one at each apex and the additional ones on each side. The columns were 50–100 nm long, longer at the apices and shorter along the sides. They supported a broad rim, 100–150 nm wide and composed of a bilayered lattice (Fig. 23, 24). The columns were always attached only to the base of the rim (Fig. 23, 24). The rim protruded slightly from the column supports at each end (Fig. 23, 24). From one end of most (Fig. 20, 23) but not all (Fig. 24) scales, a conspicuous spine  $\sim$  150 nm long appeared. Ad-

jacent scales in situ could have the spine located at either end of the scale (Fig. 20).

Fine filaments, of  $\sim$  5 nm diam. and up to 1  $\mu$ m in length, were frequently encountered (Fig. 25).

Live cells on microscope slides rapidly contracted when exposed to light; diagnostic features of pseudopodial morphology and locomotive behavior were lost after only a few seconds of illumination.

No life history stages other than attached and floating gymnamoebae were observed.

Cultured cells of *K. monacantholepis* engulfed and thrived on all three protistan species offered as food.

Table 1. The valid and proposed species of *Korotnevela*.

Species name/ Authority	Basionym	Habitat	Type locality	Additional records	Large scales	Small scales
<b>Group 1: described species</b>						
<i>Korotnevela stella</i> (Schaeffer, 1926) Goodkov, 1988 <sup>a</sup>	<i>Astramoeba stella</i> Schaeffer, 1926	freshwater	Lonsdale Marshes, Tennessee, USA	England, Russia	basket	present
<i>Korotnevela riparia</i> (Page, 1972) Goodkov, 1988 <sup>b</sup>	<i>Mayorella riparia</i> Page, 1972	freshwater	England	not known	basket	present
<i>Korotnevela bulla</i> (Schaeffer, 1926) Goodkov, 1988	<i>Mayorella bulla</i> Schaeffer, 1926	freshwater	Lonsdale Marshes, Tennessee, USA	England, Russia	basket	present
<b>Group 1: new and undescribed species</b>						
<i>Korotnevela</i> n. sp. ined.	" <i>Mayorella</i> sp. 1" Pennick and Goodfellow, 1975	marine	Gorleston on Sea, England	only known from type locality	basket	unknown
<i>Korotnevela hemistylepis</i> n. sp.		marine	Pocomoke River, Chesapeake Bay, Maryland, USA	only known from type locality	basket	present
<i>Korotnevela monacantholepis</i> n. sp.		marine	marine aquarium, Raleigh, North Carolina, USA	only known from type locality	basket	present
<b>Group 2: described species</b>						
<i>Korotnevela nivo</i> Smirnov, 1996–1997 <sup>c</sup>		marine	Nivå Bay, Denmark	England; Sargasso Sea; Long Island Sound	crown <sup>d</sup>	absent
<b>Group 3: described species</b>						
<i>Korotnevela diskophora</i> Smirnov, 1999 <sup>e</sup>		freshwater	Lake Leshevoe, Russia	England	disk	absent

<sup>a</sup> The type species when *K. riparia* is placed in synonymy with *K. stella*.

<sup>b</sup> Synonymized with *K. stella* (Page 1988, 1991, as *Dactylamoeba stella* (Schaeffer, 1926) Page, 1988). The type species when recognized as distinct from *K. stella* (Goodkov 1988).

<sup>c</sup> Includes "*Mayorella* sp. 2" of Pennick and Goodfellow (1975) and the Sargasso Sea amoeba described but not named by Andersen (1977) (Smirnov 1999).

<sup>d</sup> Scales identical with those of *Paramoeba eilhardi* Schaudinn, 1896 (Smirnov 1996–97, 1999).

<sup>e</sup> Includes "*Mayorella* sp. 3" of Pennick and Goodfellow (1975) (Smirnov 1999).

**Cryopreservation.** Cells of *K. hemistylepis* were cryopreserved in their growth medium using standard cooling and thawing regimens and 9% DMSO (v/v) as a cryoprotective agent. It has not yet been possible to cryopreserve cells of *K. monacantholepis* by conventional methods.

## DISCUSSION

**Scale structure determination.** The use of uranyl acetate as a positive stain for non-mineralized protistan scales is frequent, especially for species in the green algal class Prasinophyceae (e.g. McFadden, Hill, and Wetherbee 1986; Moestrup and Ettl 1979; Pennick, Clark, and Cann 1976). It has not, apparently, been employed in previous studies of scale structure in gymnamoebae. The procedure is easier and faster than shadowcasting, and can reveal fine details of scale structure.

**The species of *Korotnevela*.** Three groups of species can be recognized in *Korotnevela* as circumscribed by Goodkov (Goodkov 1988) and Smirnov (Smirnov 1996–97, 1999) (Table 1). Species in the first group have large, ornate, basket-shaped and smaller dish-shaped scales. This group contains *Korotnevela stella* (type species) and *Korotnevela bulla*, both of which are from freshwaters. "*Mayorella* species 1" of Pennick and Goodfellow (1975) has basket scales of this type and therefore probably also belongs here. The second group, represented only by *Korotnevela nivo*, is characterized by simpler crown scales, with an unornamented rim supported by plain columns, and the absence of smaller scales. The single species of the third group, *Korotnevela diskophora*, has a single layer of oval, disk-shaped, overlapping scales with a central boss.

*Korotnevela hemistylepis* and *K. monacantholepis* both belong to the first group. Moreover, both are more similar to *K. bulla* than to the others in the group as they have basket scales with distinctive vertical columns connecting the rim to the base (Page 1981, as *Mayorella bulla*). Such columns are lacking from the basket scales of *K. stella* (Pennick and Goodfellow 1975, as *M. riparia*; Page 1981, as *M. stella*) and "*Mayorella* species 1" (Pennick and Goodfellow 1975).

*Korotnevela monacantholepis*, in fact, closely resembles *K. bulla*, as described by Page (1981, as *M. bulla*), in many respects. Both are large amoebae with similar dimensions in the locomotive form and similarly numerous, long, narrow pseudopodia in the floating form. Both consume eukaryotes rather than bacteria, and both will accept *Tetrahymena*. The full range of food preferences has not been explored for either species, but *K. monacantholepis* at least does not seem to be particularly selective. Both have basket scales with conspicuous spines, the only *Korotnevela* species to have this characteristic.

However, the basket scales of *K. bulla* usually have two spines, one at each end, while those of *K. monacantholepis* have one or none, never two. The rim latticework of *K. bulla* scales tends to have quadrangular elements, while the elements of the *K. monacantholepis* rim lattice tend to be triangular or pentagonal, seldom quadrangular. The lateral columns of *K. bulla* basket scales may attach to the middle of the rim as well as to the base, while those of *K. monacantholepis* attach only to the base of the rim. The *K. bulla* basket scales have consistently eight vertical columns, two apical and six lateral, while the *K. monacantholepis* basket scales commonly have ten columns.

Nuclei of *K. monacantholepis* usually have a single central nucleolus, while those of *K. bulla* usually have multiple nucleoli. Finally, *K. monacantholepis* is a mesohaline amoeba, whereas *K. bulla* is known only from freshwaters.

The differences in scale structure (spines, rim geometry, column number), nuclear morphology, and habitat indicate that *K. monacantholepis* and *K. bulla* are distinct but sibling species. Molecular sequence data are expected to support this conclusion, since such data tend to corroborate species boundaries based on scale morphology in other protist groups (e.g. Caron et al. 1999; Daughjerg, Moestrup, and Arctander 1994).

*Korotnevela hemistylepis*, by contrast, is clearly distinguishable from other members of the genus. Its cells are smaller than those of other Group 1 species (in the genus as a whole, only *K. diskophora* is smaller), with few dactylopodia expressed in the locomotive amoeba. It is bacterivorous, not surprising given its small size. Its basket-shaped scales have three unique elements: the narrow, fenestrated rim; the circumferential crosspiece linking the six full-length vertical columns; the six half-columns that support the crosspiece.

Fine filaments are present in most Group 1 *Korotnevela* species (Page 1981; Pennick and Goodfellow 1975), including *K. monacantholepis*. They have not been seen in *K. hemistylepis*, but this absence is not considered to be a significant character. The filaments, being fine, may be overlooked. Moreover, the character may be inconstant; strains assigned to the species *Paramoeba pemaquidensis* Page, 1970 and *Paramoeba aestuarina* Page, 1970 are known to vary in their production of fine filaments (Cann and Page 1982; Dykova, Figueras, and Peric 2000, as *Neoparamoeba*).

**What is the correct genus name, *Korotnevela* or *Dactylamoeba*?** To answer this question, a synopsis of relevant taxonomic and nomenclatural events is necessary. When it became clear that amoebae conforming to the light microscopical circumscription of *Mayorella* Schaeffer, 1926 could be divided into two groups, one bearing a surface coat of discrete scales, the other a thick cuticle, Page (1981) retained *Mayorella* for the scale-bearing species, and referred the cuticulate species to *Hollandella* Page, 1981. At that time, *Mayorella bigemma* (Schaeffer, 1918) Schaeffer, 1926, the type species of the genus, had not been examined by electron microscopy, and Page (1981) opined that it could not be cuticulate. Also at that time, Page (1981) noted that *Dactylamoeba* Korotnev, 1880, was indistinguishable on the basis of published light microscopical characters from *Mayorella*. To avoid replacing a well-known name with an obscure but older one, Page (1981, 1982) proposed to conserve *Mayorella* over *Dactylamoeba*. This proposal was accepted (Melville 1985).

Meanwhile, *M. bigemma* was examined with the electron microscope and reported to be cuticulate after all (Page 1983). Subsequently, Page (1983, 1987, 1988, 1991) treated cuticulate species as *Mayorella* (rendering *Hollandella* superfluous), and applied *Dactylamoeba* to the scale-bearing species—guessing that the type and only species of the genus, *Dactylamoeba elongata* Korotnev, 1880, which has not been observed since its description (Page 1982), has scales.

Goodkov (1988), followed by Smirnov (1996–97, 1999), argued that the characteristics of *D. elongata* cannot be accurately assessed on the basis of the data presented by Korotnev (1880), and that no additional taxa should be added to this genus until the type species is rediscovered and examined with modern methods. Goodkov (1988) proposed *Korotnevela* for scaly *Mayorella*-like species, leaving *Dactylamoeba* monotypic and *incertae sedis*.

The approach of Goodkov (1988) is the sounder because it does not require divination of the diagnostic features of *D. elon-*

*gata*. Should *D. elongata* be rediscovered and prove to be a scaly species, then, barring conservation, *Dactylamoeba* would be correctly applied to species now known as *Korotnevela* (or, to some of them; see the next section), and *Korotnevela* would disappear. Should *D. elongata* be a cuticulate species, then *Dactylamoeba* would disappear (*Mayorella* being conserved against it) and *Korotnevela* would be correctly applied to scaly species.

The correct authority citation for the genus is *Korotnevela* Goodkov, 1988, not *Korotnevela* (Page, 1981) Goodkov, 1988 (Goodkov 1988) nor *Korotnevela* (Schaeffer, 1926) Goodkov, 1988 (Smirnov 1999). Neither Page nor Schaeffer created the name *Korotnevela* at any taxonomic rank.

**Is *Korotnevela* monophyletic?** Since knowledge of the diversity of gymnamoebae in general, and scale-covered gymnamoebae in particular, is inadequate, the inclusion of dactylopodiate, scale-bearing, parasome-free species in a single genus, *Korotnevela*, has had a certain conservative logic. However, with the addition of *K. hemistylepis* and *K. monacantholepis* to Group 1 of the genus, the “outlier” status of *K. nivo* and *K. diskophora* (Group 2 and Group 3 in Table 1) is accentuated. Both of the outlier species have just one scale type, and the morphology of these scales has little in common with those of Group 1 *Korotnevela*. No other features of *K. nivo* and *K. diskophora* are diagnostic for *Korotnevela*. For example, dactylopodia and the single, large, perinuclear Golgi body are characteristics common to most *Mayorella*-like amoebae, not just species of *Korotnevela*.

Moreover, as has been noted several times (summarized by Smirnov 1996–97), the scales of the parasome-free *K. nivo* are scarcely distinguishable from the scales (Kästchen; Grell and Benwitz 1966, 1970) of the parasome-containing *Paramoeba eilhardi*, the type species of its genus. Pennick and Goodfellow (1975) indicated that the crown scales of their “*Mayorella* species 2”, which Smirnov (1996–97, 1999) allied with *K. nivo*, have a double rim, while those of *P. eilhardi* have a single rim. The character seems to have been deduced from a single shadowcast image (Plate 5d in Pennick and Goodfellow 1975) and requires confirmation. The only other feature used by Smirnov (1996–97) to separate *K. nivo* from *P. eilhardi* is cell size, with *K. nivo* being smaller. However, as Smirnov (1996–97) pointed out, the locomotive cell sizes for the parasome-free *K. nivo*-like amoebae observed by Grell and Benwitz (1970) and Anderson (1977) are more nearly comparable to those reported for *P. eilhardi*. Furthermore, the great range in cell size observed among subclones from a single clonal isolate of *K. stella* (Page 1981, as *M. stella*) indicates the great care with which cell size must be used as a taxonomic character among paramoebids.

If scale morphology is a good predictor of taxonomic and phylogenetic affinity, then upon further examination (using, for example, molecular sequence data), Group 1 *Korotnevela* should form a clade. *Korotnevela diskophora* should prove to be an outlier, not closely related to other known species. *Paramoeba eilhardi* and *K. nivo* should form a clade exclusive of other species in both *Paramoeba* and *Korotnevela*. Such a finding would impel reconsideration of both the biology of the parasome and its use as a taxonomic character. The parasome may well be a parasite or symbiont as suggested by Hollande (1980), and if so, it—or they, if there are multiple species—may facultatively inhabit different species of amoebae. Page (1987, 1991) emphasized surface coat characters, and de-emphasized the parasome, when he moved glycostyle-bearing species of *Paramoeba* to the genus *Neoparamoeba* and the family Vexilliferidae. Among the questions now are whether *Korotnevela nivo* is merely a parasome-free *Paramoeba eilhardi*, and wheth-



er the *Neoparamoeba* species are merely parasome-infested species of *Vexillifera*.

### Diagnoses

#### *Korotnevela hemistylepis* O'Kelly, Peglar & Nerad, n. sp.

Uninucleate, small, bacterivorous mesohaline gymnamoebae. Locomotive cells advance from a hyaline front that bifurcates giving the cell a Y-shaped appearance, each arm of the Y commonly having two small conical marginal pseudopodia. Differentiated uroidal structures absent. Floating forms with 7- $\mu$ m diam. spherical cell body, typically with 3–8 thin radiate pseudopodia 1–4 times longer than the central body mass. Locomotive amoebae 15.5–48.5  $\mu$ m long and 3.1–13.0  $\mu$ m wide, exclusive of unattached dactylopodia. Nucleus with single centrally positioned nucleolus with a regular margin and no obvious inclusions. Nuclear diameter 2.8–4.8  $\mu$ m, nucleolar diam. 1.1–2.2  $\mu$ m. A single large Golgi body next to the nucleus. Mitochondria numerous, bacillariform, with tubular cristae. Non-mineralized scales of two types covering the entire cell surface. Smaller scales dish-shaped, ellipsoidal, 100 nm in the long dimension, unornamented, arrayed about the periphery of the basal plates of the larger scales. Larger scales basket-shaped, with an ellipsoidal base 250–350 nm in the long dimension, a narrow fenestrated rim supported by six full columns, and six half-columns supporting a circumferential crosspiece below the rim that connects the full columns to each other. Only locomotive and floating forms are known. Differs from other *Korotnevela* species with two scale layers by its smaller cell size, small number of pseudopodia in both locomotive and floating forms, and characters of the basket scale, including the narrow rim, the circumferential crosspiece, and the six half-columns.

**Type locality.** Pocomoke River estuary, Maryland, USA (37° 44.287' N, 75° 51.575' W).

**Holotype.** Cryopreserved living material, conserved at the American Type Culture Collection (ATCC) as strain 50804. Resin-embedded cells derived from strain 50804 conserved at the Natural History Museum, Smithsonian Institution.

**Etymology.** *hemistyleos*, Greek “half columns”, referring to the six half-length uprights supporting the intermediate crosspiece in the basket-shaped scale; *lepis*, Greek “scale”.

#### *Korotnevela monacantholepis* O'Kelly, Peglar & Nerad, n. sp.

Uninucleate, protistivorous mesohaline gymnamoebae of moderate size. Locomotive forms varying greatly in appearance. Well-fed cells nearly circular in outline, with numerous short, dactylopodia arranged around the periphery of the cell and emerging from a thin hyaline front. Starved cells with more variable shapes ranging from truncate circular to somewhat triangular in outline. In these forms the dactylopodia cover as much as 50% of the perimeter of the anterior half of the cell and emerge from a hyaline zone that is much more obvious than that found in well-fed cells. Differentiated uroidal structures absent. Floating forms with spherical cell body and ten or more long narrow pseudopodia deployed in all directions from the cell body and 5 or more times as long as the body mass. Locomotive amoebae 75.0–160  $\mu$ m long and 50.0–90.0  $\mu$ m wide. Nucleus usually with single centrally positioned nucleolus, more rarely with two nucleoli of unequal size. Nucleoli with irregular margins and numerous translucent channels. Nuclear length 9.0–15  $\mu$ m, width 6.0–15  $\mu$ m; nucleoli, in nuclei with single nucleoli, 4.0–12  $\mu$ m long, 3.0–6.0  $\mu$ m wide. Non-mineralized scales of two types covering the entire cell surface. Smaller scales dish-shaped, ellipsoidal, 200–250 nm in the long dimension, uniformly and minutely perforated, arrayed about

the periphery of the basal plates of the larger scales and separating them from one another. Larger scales basket-shaped, with an ellipsoidal base 500–600 nm in the long dimension, and a broad rim consisting of a two-tiered lattice and supported by 8 or 10 full columns attached to the base of the rim. Most scales with a straight, stout spine emerging from the rim at one scale apex; others lack spines. Only locomotive and floating forms are known. Similar to *Korotnevela bulla* (Schaeffer, 1926) Goodkov, 1988, but it: has one spine, or none, on the basket scale (not two); has 8 or 10 vertical columns on the basket scale (not 8 only); has quadrangular rim lattice elements on the basket scale (not triangular or pentangular); has nuclei with single nucleoli (not multiple nucleoli); inhabits mesohaline environments (not freshwaters).

**Type locality.** Mesohaline aquarium, North Carolina State University at Raleigh, USA.

**Holotype.** Living material, conserved at the American Type Culture Collection (ATCC) as strain 50819. Resin-embedded cells derived from strain 50819 conserved at the Natural History Museum, Smithsonian Institution.

**Etymology.** *monacanthos*, Greek “one spine”, referring to the spine at one end of many of the basket-shaped scales; *lepis*, Greek “scale”.

Key to species of scale-bearing dactylopodiata amoebae (genera *Korotnevela* and *Paramoeba* p.p.)

1. Cells with only one type of scale; scales not basket-shaped ..... 2.
1. Basket-shaped scales present; cells usually bearing two types of scales ..... 4.
2. Scales oval or disk-shaped, with a central spine, overlapping; freshwater; locomotive amoebae to 25  $\mu$ m long ..... *K. diskophora* Smirnov, 1999
2. Scales crown-shaped, with 6–14 vertical columns supporting an unornamented rim ..... 3.
3. Parasome present ..... *Paramoeba eilhardi* Schaudinn, 1896
3. Parasome absent ..... *K. nivo* Smirnov, 1996–97
4. Basket scales with six, full vertical columns supporting the rim and six half-columns supporting an intermediate crosspiece; rim narrow, minutely fenestrate; locomotive amoebae rarely more than 35  $\mu$ m in length; marine ..... *K. hemistylepis* n. sp.
4. Basket scales lacking half columns and with more ornate rims; locomotive amoebae generally longer than 40  $\mu$ m ..... 5.
5. Basket scales with obvious vertical columns arising from the basal plate, supporting a broad rim, and usually bearing at least one conspicuous spine ..... 6.
5. Basket scales without obvious vertical columns and spines ..... 7.
6. Basket scales with one spine or none; columns attached only to the basal crosspiece of the rim; rim lattice elements triangular or pentangular; nucleolus generally intact; marine and brackish water ..... *K. monacantholepis* n. sp.
6. Basket scales with two spines, one at each end of the rim; columns sometimes attached to the middle crosspiece of the rim; rim lattice elements quadrangular; nucleolus fragmented; freshwater ..... *K. bulla* (Schaeffer, 1926) Goodkov, 1988
7. Rim margin convex; small scales unknown; marine ..... *K. sp.* (“*Mayorella* sp. 1” of Pennick and Goodfellow 1975)
7. Rim margin concave; lattice element suspended just above the scale base; freshwater ..... *K. stella* (Schaeffer, 1926) Goodkov, 1988 [= *K. riparia* (Page, 1972) Goodkov, 1988]

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