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Apical Feeding in the Karyorelictids (Protozoa, Ciliophora) *Sultanophrys arabica* and *Tracheloraphis* sp.

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ABSTRACT. *Sultanophrys arabica* and *Tracheloraphis* sp., two interstitial karyorelictid ciliates, were cultivated in sealed 100–200 ml glass bottles half-filled with filtered interstitial water to which some millilitres of the natural organism community and a couple of wheat grains were added. Removing sand grains and sealing the bottles were crucial to achieve a low oxygen tension milieu, which was maintained by the algae contained in the community. This cultivation method provided, for the first time, rich cultures with many feeding, dividing, and conjugating cells. Both species were omnivorous and fed through the apical end, where a well-developed oral apparatus is present. Apical feeding was documented by micrographs of living specimens and by scanning electron microscopy of preserved cells.

Key Words. Interstitial ciliates, Saudi Arabian Gulf coast, scanning electron microscopy.

ALTHOUGH being at the base of the ciliate tree (Hammerschmidt et al. 1996; Lynn and Small 1997), trachelocercid ciliates have not been studied extensively. Even their mode of feeding is controversial since Lenk, Small and Gunderson (1984) and Lenk, Hollander and Small (1989) proposed that it occurs through the glabrous stripe, a non-ciliated area, which extends the whole body length in the middle third of the left side and is framed by specialised cilia. Previously, apical feeding was assumed, but never directly observed, because of oral apparatus-like structures at the anterior cell end (Dragesco 1960; Fenchel 1968; Kahl 1930). More recently, complex oral structures were found in many trachelocercids, but feeding specimens were never observed (Foissner 1996, 1997; Foissner and Dragesco 1996a, b). The proposal by Lenk, Small and Gunderson (1984) and Lenk, Hollander and Small (1989), which was based on *Tracheloraphis* fed with yolk from hard-boiled chicken eggs, elegantly explains a baffling observation made by several authors: many trachelocercids contain in the food vacuoles large metazoan prey, such as rotifers and nauplii, although their apical end is comparatively small (diameter ~ 20–50 μm) and devoid of conspicuous oral structures.

Part of the controversy arises because no one has succeeded in obtaining well-growing cultures of trachelocercids, although Lenk, Small and Gunderson (1984) and Miyake (1997) reported having cultivated some species. However, neither report mentions dividing cells, and thus it is unclear whether permanent cultures were established. We were fortunate in obtaining cultures from several trachelocercids, especially *Sultanophrys arabica* Foissner and AL-Rasheid (1999a), which divided so readily that we could study its morphogenesis in detail (Foissner and AL-Rasheid 1999b). The present paper reports on cultivation and feeding of *S. arabica* and *Tracheloraphis* sp., and confirms, like Miyake (1997), the traditional view that trachelocercids feed with the apical end, where a typical oral infraciliature is present (Foissner 1996, 1997; Foissner and Dragesco 1996a, b).

MATERIALS AND METHODS

Material and observation methods. The ciliates were collected at the Saudi coast of the Arabian Gulf near the coastal oasis of Al Qatif in the village of Safwa, which is situated half the way between Dammam Harbor and the industrial port of Ras Tannurah (50° 06′ E, 26° 39′ N). The site contains scattered shallow to moderately deep ponds of brackish to saline waters (salinity 15–28‰). The salt-tolerant tall reed (*Phragmites communis*) and black mangrove (*Avicennia marina*) are the dominant plants surrounding the rims of the ponds. Sediment samples with many ciliates were collected and transported to Riyadh, where the ciliates were cultivated as described below.

Two species were studied: *Sultanophrys arabica* Foissner and AL-Rasheid (1999a) and *Tracheloraphis* sp. (possibly *T. geopetiti* or *T. gracilis*, as described by Dragesco 1960). Observations on the feeding process were performed on live cells with dissecting and compound microscopes. Preserved specimens were studied in protargol slides and in the scanning electron microscope (Foissner 1991). Unfortunately, most specimens lost their prey either when isolated with micropipettes or during the procedures involved in the preparation for scanning electron microscopy. Furthermore, feeding usually ceased when specimens were transferred from the cultures to a small drop of water for photography. Thus, documentation of the process was difficult.

Cultivation. The technic is community-based, as many attempts to start cultures with single cells and defined prey failed. The following protocol was found to provide flourishing cultures in about half of the trials. However, the system was fragile: flourishing cultures, which were transferred from the Riyadh to the Salzburg laboratory, soon ceased feeding and division and could not be stimulated to grow again. Possibly, our method works best (or only!) with microaerophilic species. We have not yet tried to cultivate species from clean sands. The steps in the method are as follows:

1. Collect several samples of marine interstitial waters and sediments from the top few centimeters of an intertidal area.
2. Collect some of the interstitial water and filter through a glass filter to get rid of most of the sand grains.
3. Put 50 or 100 ml of cleaned water into each of several 100 or 200-ml glass bottles with tightly sealed caps. Do not use plastic bottles as these never provided good cultures.
4. Subdivide the sediment samples into several glass Petri dishes and rotate them under a dissecting microscope to move the sand into the middle. Then pick up trachelocercids and other organisms with a wide-mouthed pipette from the clear supernatant. Transfer about 10 ml of the organism community to the glass bottles half-filled with filtered interstitial water. Cultures should be started with many specimens and contain the whole interstitial organism community, but sand grains should be removed.
5. Wheat or rice grains and dried cereal or hay leaves should be added to enhance bacterial growth for the prey organisms. Two or three grains are added every 5 to 16 days. The old grains should not be removed, but left to be digested completely. This will form some kind of organic fluff around which trachelocercids like to feed.
6. Culture bottles must now be tightly sealed and kept at

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room temperature away from direct sunlight. Incubation trials with fixed temperatures and light intervals did not work. After two to six days, cells become more active and commence to feed on large prey. They become dark due to many food vacuoles. After this fattening period, cells prefer to feed on small prey and possibly also on organic debris produced by the wheat grains. They become lighter and slower and eventually divide. Conjugation occurs when most of the large prey is depleted. Cells do not feed during division and conjugation.

7. Keeping bottles sealed during cultivation is crucial, indicating that the species prefer a low oxygen tension milieu. However, they do not sustain anaerobity for longer periods. Oxygen is provided by the algae contained in the community. Cultures thrived best when large numbers of *Condylostoma* were present.

RESULTS

Brief description of *Sultanophrys arabica*. Consult Foissner and AL-Rasheid (1999a) for a detailed description of the species, and Foissner and Dragesco (1996a) for terminology. In vivo, *S. arabica* is about 800 μm long, 70 μm wide, and 2:1 flattened (Fig. 1, 20). On the right side are about 34 meridional ciliary rows, while the left is barren, except for a row of bristles that borders the barren area, the so-called glabrous stripe (Fig. 1–7, 20). *Sultanophrys arabica* can contract up to half the body length, and the glabrous stripe then shows conspicuous, transverse tuberosities (Fig. 3, 7, 10). There are about 31 macronuclear nodules in midline of cell (Fig. 1). Cells appear dark at low magnification due to innumerable brilliant brown cortical granules, some of which form a distinct ring in the oral bulge just above the circumoral ciliary row. The flattened apical (oral) end, the head, is about 40 × 40 × 20 μm and black due to many cytoplasmic granules of different size and shape (Fig. 1, 4, 9, 11, 20). The head contains distinct oral structures consisting of a circumoral kinety and, in the midline of the left side, a brosse composed of three minute ciliary rows (Fig. 1, 2, 7, 20). About 15 μm long fibres (nematodesmal bundles) originate from the circumoral basal body pairs and form an inconspicuous funnel. The apical surface is hemispherically indented to form a distinct oral cavity (Fig. 3, 7, 20).

Tracheloraphis sp. is similar to *S. arabica,* but is more slender, has a distinct tail, and the four macronuclear nodules form a single group (capsule).

Feeding. Both species showed similar feeding behaviour and were omnivorous. However, often a certain type of food was preferred in the individual cultures, possibly depending on prey abundance. The following food items were observed in 34 protargol-impregnated specimens from a single culture of *Tracheloraphis* sp. (Fig. 22): bluegreen cyanobacteria (3%), colourless, filamentous bacteria (5.8%), various diatoms (65%; 30–130 μm, usually around 40 μm long), flagellates (8.8%; up to 40 μm), various ciliates (5.8%), rotifers (8.8%; about 100 μm long), nematodes (56%; 100–700 μm, usually 100–200 μm long), gastrotrichs (5.8%; around 100 μm long), nauplii (3%; about 150 μm), starch from the added wheat grains (3%), and unidentified material (18%). A similar food spectrum was observed in live and prepared *S. arabica* (Fig. 8–19). However, a detailed quantification was impossible in the silver slides because the cytoplasm was almost unstained in well-impregnated specimens and too dark in poorly-impregnated cells. Most specimens contained several of the items mentioned. Diatoms were found singly in the prey cytoplasm or clustered to large food vacuoles containing up to 25 specimens. Ciliate prey included large *Frontonia* sp., *Condylostoma* sp., *Holosticha* sp., small scuticociliates, hypotrichs (*Euplotes*), and even other trachelocercids.

Sultanophrys arabica and *Tracheloraphis* sp. fed while gliding on the sediment surface or the bottom of the culture dish. When sensing a large, slender, moving item, such as a nematode or a *Condylostoma,* they increased gliding speed to reach it. As soon as the head touched the narrowed prey end, a small portion was ingested. Then the oral bulge expanded to engulf the prey. The ciliates then looked very much like a small snake swallowing a large bird egg, when the prey was voluminous (Fig. 17). When a small portion of the prey has been ingested, the trachelocercids stopped moving and contracted rather strongly, as evident from the tuberose glabrous stripe (Fig. 8). Even if the prey kept moving and tried to escape, the predator hardly moved. It seems that the internal part of the neck worked like a "glue" to hold the prey during the expansion of the oral bulge. Indeed, when cells were disrupted in this early feeding stage, the ingested prey portion was already embedded in viscous, granular cytoplasm, indicating that digestion had begun during ingestion (Fig. 16). The dark accumulation of granules in the oral area (head) and the ring of brilliant brown granules in the oral bulge did not change during feeding (Fig. 9, 11, 12, 16).

Feeding invariably occurred through the apical end in both species (Fig. 8–19). Depending on the kind of food, the oral cavity opened more or less widely. When prey was large and spreading, for instance nauplii, the oral area may even be damaged, especially at the fragile brosse site (Fig. 2, 7), giving the impression of subapical feeding (Fig. 15). When prey was lost during cytological preparation, the oral opening remained trumpet-like and expanded (cp. Fig. 20, 21). Finally, the prey was surrounded by a large vacuole, which swelled the glabrous stripe and more or less deformed the predator (Fig. 3, 9).

Feeding on large prey items was a long process while filamentous bacteria, diatoms, and small ciliates were ingested within a few minutes. Swallowing an about 300 μm long *Condylostoma* took about 10 min; rotifers and long nematodes took up to 40 min to be fully ingested. Usually, prey became immobile when ingestion began, but some nematodes were still moving when completely ingested and may then heavily deform the predator (Fig. 9).

DISCUSSION

Cultivation. Several authors mentioned that they were unable to establish cultures of trachelocercids. For instance, Raikov (1958) performed his classical study on the karyorelictean nuclear apparatus with freshly collected field material; Raikov and his students were possibly the only ones who have ever seen dividing trachelocercids. Dragesco (1960, and pers. commun.), a specialist on the group, could never cultivate any species; and Foissner and Dragesco (1996b) mentioned that they did not find a single dividing cell among more than 1,000 well-impregnated specimens. The senior author (W. F) tried to cultivate some of the common species occurring at the French Atlantic coast. However, all trials failed though specimens survived for weeks in the sampling jar and in subcultures set up in Petri dishes with various food items and oxygen concentrations.

There are, however, also reports of successful cultivations, but it has to be emphasised that none stated having observed dividing specimens, indicating that the cultures only maintained the original inoculation, as described above. Borror (1973), for instance, "maintained cultures of *Tracheloraphis haloetes* in the original culture dish at 82 parts per thousand salinity with no additional nutrient other than the blue-green algae present in the culture," but could not establish subcultures. Lenk, Small and Gunderson (1984) cultivated a *Tracheloraphis* sp. on filtered, natural sea water enriched with small amounts of freshly

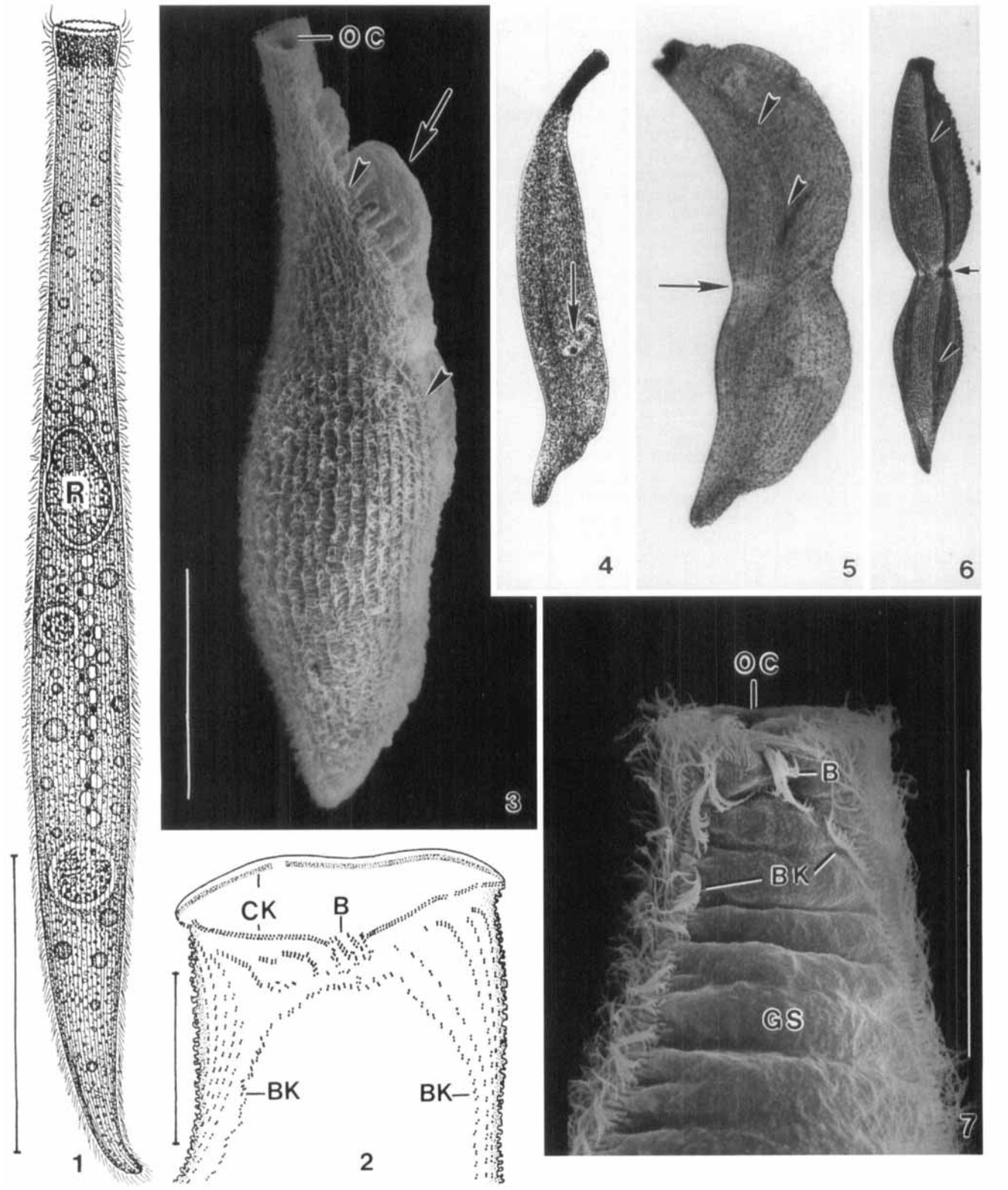

Fig. **1–7**. *Sultanophrys arabica* from live (Fig. 1, 4–6), after protargol impregnation (Fig. 2), and in the scanning electron microscope (Fig. 3, 7). **1**: Right side view of a fully extended specimen with an ingested rotifer (R). Bar 200 μm. **2, 7**: Left side views showing main oral structures: circumoral kinety (CK), brosse (B) and oral cavity (OC). The glabrous stripe (GS) is bordered by the bristle kinety (BK) and strongly folded in contracted (prepared) specimens. Bars 20 μm, respectively, 40 μm. **3**: Lateral view of a contracted specimen whose glabrous stripe has a large hump (arrow) probably containing a food item ingested through the oral cavity (OC). Arrowheads mark border between ciliated right and glabrous left side. Bar 100 μm. **4**: Partially contracted specimen with a small food vacuole (arrow). **5, 6**: Lateral views of a middle and a late divider showing division furrow (arrows) and border (arrowheads) of ciliated right and glabrous left side. The newly formed oral apparatus is marked by an accumulation of refractile and thus black granules (Fig. 6, arrow).

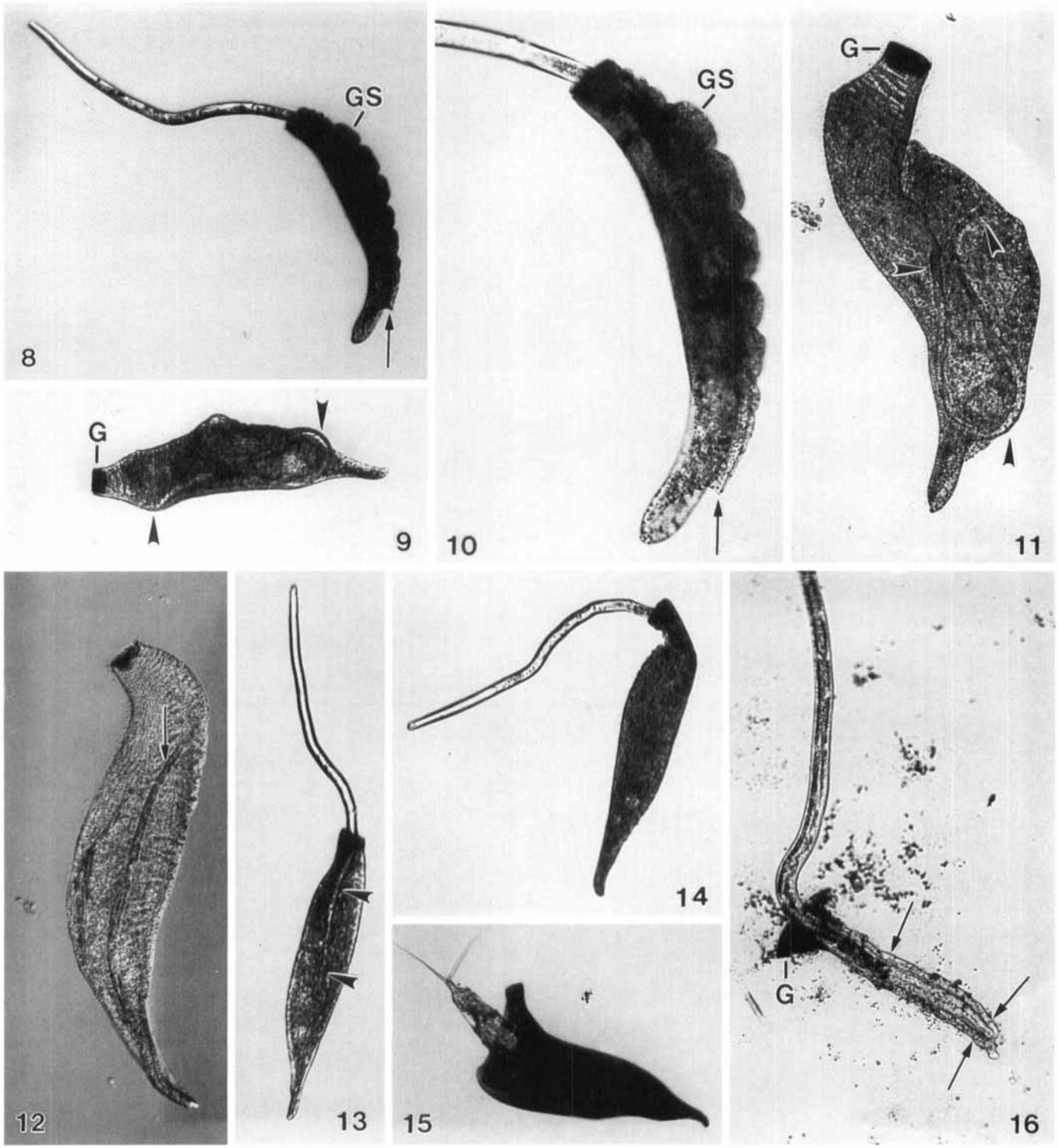

Fig. **8–16**. *Sultanophrys arabica*, micrographs of live, feeding specimens from cultures as described in Materials and Methods. Feeding invariably occurs through the anterior end. **8–11**: A single specimen ingesting a long nematode (arrowheads) from tail to head. The tail tip (arrows) is already near the posterior end of the ciliate and stretches the glabrous stripe (GS). The nematode is curled up inside the ciliate, which becomes strongly deformed (Fig. 9). The head contains a mass of highly refractile and thus black granules (G), which remain unchanged during ingestion. **12**: When a nematode (arrow) has been ingested, the ciliate becomes broader and, typically, sigmoidally curved. **13, 14**: Two other specimens each ingesting a nematode, the posterior third of which is recognisable within the ciliate (Fig. 13, arrowheads). The head is slightly widened trumpet-like in the specimen shown in Figure 14. **15**: Ingestion of large, wide food items, such as rotifers and copepods, may rupture the left part of the oral bulge and simulate subapical feeding through the glabrous stripe. **16**: Remnants from a feeding, squashed ciliate. The dark granule accumulation (G) from the ciliate's head still surrounds the prey. The ingested anterior part of the nematode is granular and surrounded by very viscous cytoplasm (arrows).

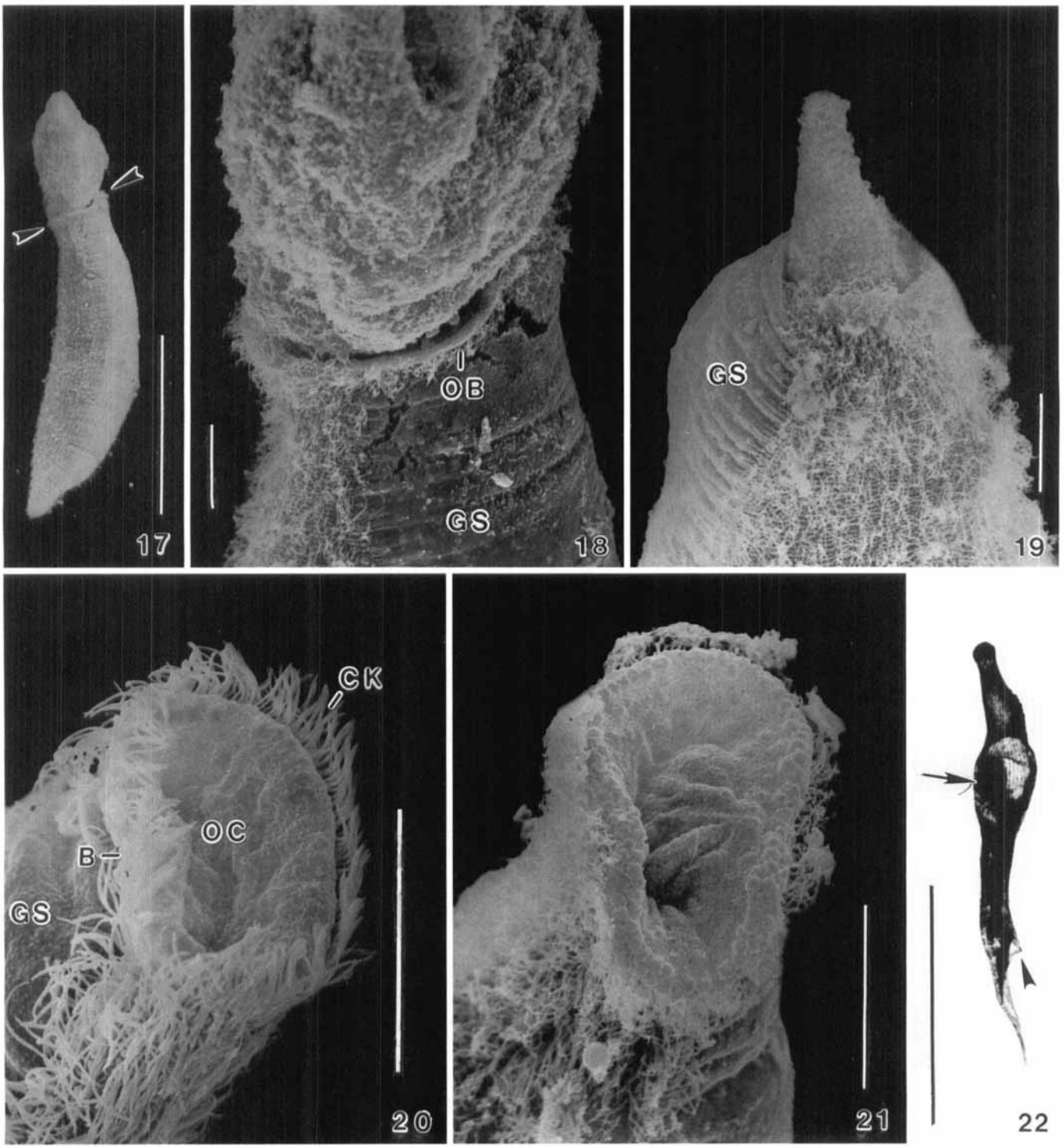

Fig. **17–22**. *Sultanophrys arabica* (17–21) and *Tracheloraphis* sp. (Fig. 22) in the scanning electron microscope (Fig. 17–21) and after protargol impregnation (Fig. 22). **17, 18**: Overview and detail of a specimen ingesting a rotifer through the anterior end. Note the widely opened mouth (arrowheads) surrounded by the narrow oral bulge (OB). **19**: Detail of a specimen finishing ingestion of a ciliate, very likely another trachelocercid. Feeding occurs through the apical end and does not involve the glabrous stripe (GS). **20, 21**: Oral views of a non-feeding and a feeding specimen, which lost prey during preparation. In the feeding specimen (Fig. 21), the oral cavity (OC) is distinctly larger and deeper than in the non-feeding cell. **22**: This specimen contains a gigantic food vacuole (arrow) with a large ciliate (very likely *Frontonia* or *Condylostoma*). Furthermore, it contains a small nematode (arrowhead), which expands the cortex (cp. Fig. 8, 10). Bars 200 μm (Fig. 17, 22) and 20 μm (Fig. 18–21).

boiled egg yolk. The micrographs provided, show ingested yolk particles. Miyake (1997) did not provide details on cultivation, but mentioned that trachelocercids were placed in artificial sea water where they ingested wheat and rice starch grains, dried beer yeast, and dying or dead cells of *Colpidium* and *Sathrophilus*.

Our community-based cultivation method is far from being perfect because neither the medium nor the food is defined. Certainly, this is a serious shortcoming, especially for ecological studies. However, it was at least possible to obtain sufficient dividers and conjugants for detailed studies on ontogenesis and nuclear processes (Foissner and AL-Rasheid 1999b). Division (Fig. 5, 6) and conjugation were often so massive in our cultures that hundreds of specimens could be collected within an hour. Nouzarede (1977) used a similar method for the cultivation of karyorelictid geleiids.

Feeding. Our data show two trachelocercids from different genera (*Sultanophrys, Tracheloraphis*) feeding through the apical end, where a rather complex oral infraciliature is present (Fig. 2, 7, 20). This is in accordance with the traditional view (Dragesco 1960; Kahl 1930) and recent observations (Miyake 1997). Accordingly, the reports by Lenk, Small and Gunderson (1984) and Lenk, Hollander and Small (1989) that *Tracheloraphis* sp. takes up food items by the glabrous (unciliated) stripe on the left body surface may not be correct. Indeed, the micrographs provided by Lenk, Small and Gunderson (1984) and Lenk, Hollander and Small (1989) show food particles only either on the surface or within the cell; an opening in the glabrous stripe is not recognisable. Possibly, Lenk, Small and Gunderson (1984) and Lenk, Hollander and Small (1989) were influenced by the very incomplete data available at that time on the oral infraciliature of trachelocercids and by Small's (1984) hypothesis on the origin of the ciliate oral architecture from an undifferentiated ventral area. Of course, it cannot be entirely excluded that the observations by Lenk, Small and Gunderson (1984) and Lenk, Hollander and Small (1989), which were included in recent reviews (e.g. Verni and Gualtieri 1997), are correct, i.e. that trachelocercids have various modes of feeding. This is, however, unlikely considering the structural homogeneity of the group (Foissner and Dragesco 1996b).

Compared with other ciliates, feeding of trachelocercids highly resembles that of haptorid gymnostomes and prostomatids, most of which have, like trachelocercids, an apical oral apparatus, which can be opened widely to ingest large items (Kuhlmann, Patterson and Hausmann 1980; Verni and Gualtieri 1997). In haptorids and prostomes, however, the prey is often lysed outside the cell, possibly by toxicysts (Corliss 1979). Oral extrusomes have also been reported in some trachelocercids (Dragesco 1960), but firm proof is lacking. The conspicuous, extrusome-like granules around the oral opening of *S. arabica* remained unchanged during food uptake and the prey was ingested in largely undisrupted condition (Fig. 8, 14, 15, 16, 19).

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