

# Gastrotricha

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## I. INTRODUCTION

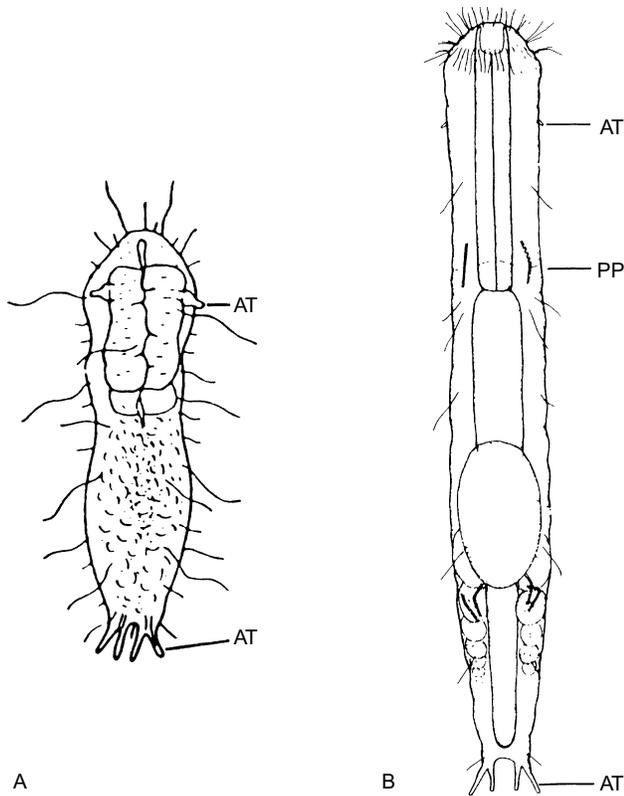
Gastrotrichs are among the most abundant and poorly known of the freshwater invertebrates. They are nearly ubiquitous in the benthos and periphyton of freshwater habitats, with densities typically in the range of 100,000–1,000,000 m<sup>-2</sup>. Nonetheless, the remarkable life cycle of freshwater gastrotrichs was worked out only recently, and we know almost nothing about how the distribution and abundance of these animals are controlled in nature. The impact of freshwater gastrotrichs on their food resources or freshwater ecosystems has not yet been investigated. Twelve genera and fewer than 100 species of freshwater gastrotrichs are now known from North America. Because the North American gastrotrich fauna has received so little study, these numbers understate the real diversity of the fauna.

Formerly placed in the obsolete phyla Aschelminthes or Nematelminthes, gastrotrichs usually are now considered to constitute a phylum of their own. The evolutionary placement of gastrotrichs is unclear; some authors<sup>[64,104]</sup>

believe that they are most closely related to nematodes, kinorhynch, loriciferans, nematomorphs, and priaulids (i.e., Cycloneuralia<sup>[73]</sup>), while others<sup>[18,27]</sup> think that gastrotrichs are more closely related to rotifers and gnathostomulids (i.e., Gnathifera<sup>[94]</sup>) and less closely to turbellarians. The gastrotrichs do not fit well into either of these schemes. Important general references on gastrotrichs include Remane<sup>[82]</sup>, Hyman<sup>[50]</sup>, Voigt<sup>[103]</sup>, d'Hondt<sup>[33]</sup>, Hummon<sup>[44]</sup>, Ruppert<sup>[89,90]</sup>, Schwank<sup>[95]</sup>, Kisielewski<sup>[58,60]</sup>, and Balsamo and Todaro<sup>[5]</sup>. The phylum contains two orders: Macrodasysida, which consists almost entirely of marine species, and Chaetonotida, containing marine, freshwater, and semiterrestrial species. Unless noted otherwise, the information in this chapter refers to freshwater members of Chaetonotida.

Macrodasysidans usually are distinguished from chaetonotidans by the presence of pharyngeal pores and more than two pairs of adhesive tubules (Fig. 7.1). Macrodasysidans are common in marine and estuarine sands but are barely represented in freshwaters. Two species of freshwater gastrotrichs have been placed in the Macrodasysida.

Ruttner-Kolisko<sup>[91]</sup> described an aberrant gastrotrich, *Marinellina flagellata* (Fig. 7.1a), from the hyporheic zone of an Austrian river. Unfortunately, she was able to find only two specimens, both of them apparently immature. Because *Marinellina* has a pair of anterior lateral structures that Ruttner-Kolisko interpreted as adhesive tubules, she placed this species in the Macrodasysida. Remane<sup>[83]</sup> rejected the assignment of *Marinellina* to the macrodasysidans, and placed it instead in the chaetonotidan family Dichaeturidae. Kisielewski<sup>[57]</sup> reaffirmed Ruttner-Kolisko's original placement of the species. An animal similar to *Marinellina* was discovered in the hyporheic zone of another Austrian stream<sup>[93]</sup>, but until it is studied critically, the systematic placement of these enigmatic Austrian gastrotrichs will remain unclear.



**FIGURE 7.1** Freshwater macrodasyidan gastrotrichs: (A) *Marinellina flagellata*; (B) *Redudasys fornerise*. AT = adhesive tube, PP = pharyngeal pore. From Ruttner-Kolisko<sup>[91]</sup> and Kisielewski<sup>[57]</sup>.

Kisielewski<sup>[57]</sup> discovered an unquestionable macrodasyidan, *Redudasys fornerise* (Fig. 7.1b), from the psammon of a Brazilian reservoir. Additional freshwater macrodasyidans probably will be found when appropriate habitats (psammon, hyporheic zone) are explored. The distribution, biology, and evolutionary relationships of any such species will be of great interest.

## II. ANATOMY AND PHYSIOLOGY

The following brief account of gastrotrich anatomy is summarized chiefly from Remane<sup>[82]</sup>, Hyman<sup>[50]</sup>, Hummon<sup>[44]</sup>, Boaden<sup>[9]</sup>, and Ruppert<sup>[90]</sup>, which should be consulted for greater detail.

### A. External Morphology

Gastrotrichs are colorless animals, spindle- or tenpin-shaped, and ventrally flattened (Fig. 7.2). Freshwater gastrotrichs are 50–800  $\mu\text{m}$  long. Conspicuous external features include a more or less distinct head, which bears sensory cilia, and a cuticle, which in most species is ornamented with spines or scales of various shapes. In the most common freshwater family, the Chaetonotidae (as well as in

the rare Dichaeturidae and Proichthyidiidae), the posterior end of the body is formed into a furca, which contains distal adhesive tubes that allow the animal to attach itself tenaciously to surfaces. In other families, these structures are absent, but the posterior end of the body may bear long spines or sensory bristles. The ventral side of the animal bears longitudinal columns or patches of cilia, which provide the forward-gliding locomotion of the animal.

### B. Organ System Function

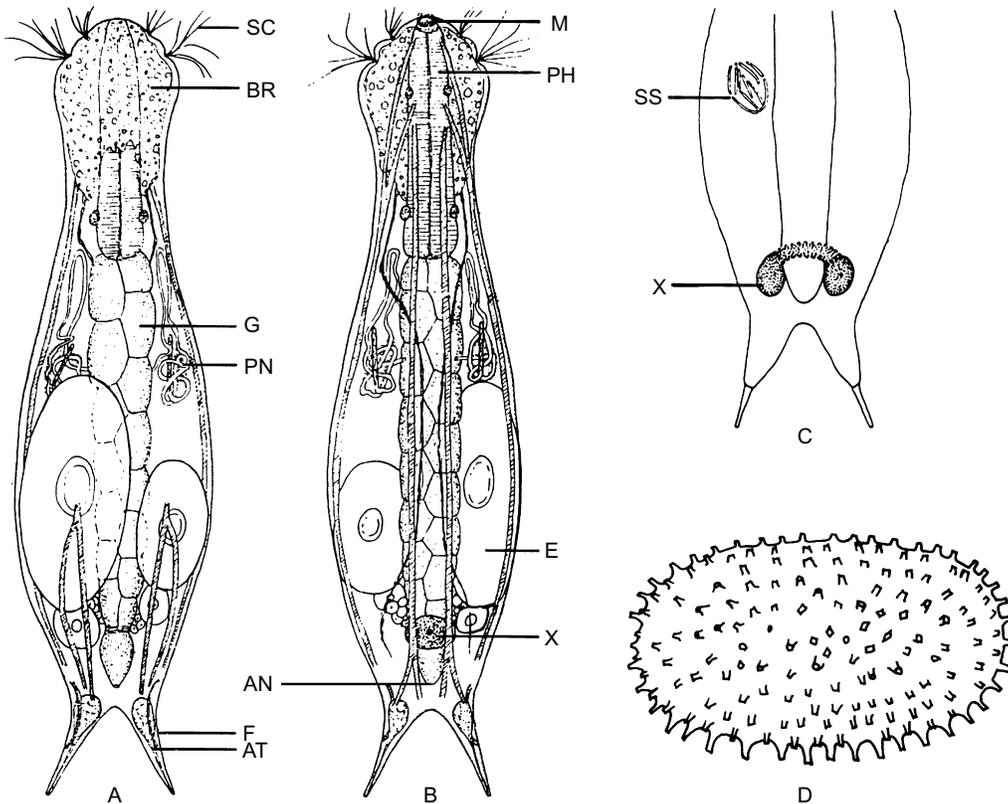
The digestive system begins with a subterminal mouth, which may be surrounded by a ring of short bristles. Between the mouth and the pharynx lies a cuticular buccal capsule, which is often somewhat protrusible. The muscular pharynx is similar to the nematode pharynx, with a triradial, Y-shaped lumen. Often, there are anterior and posterior swellings, but these lack the valves characteristic of the pharyngeal bulbs of nematodes. Posterior to the pharynx is an undifferentiated gut, which empties into the anus.

The paired reproductive organs lie lateral to the gut in the posterior half of the body. In young animals, large, developing, parthenogenetic eggs are present. As there are no oviducts, the egg is released through a rupture in the ventral body wall. Older animals become hermaphrodites (see below) and bear both sperm sacs and developing sexual (i.e., meiotic) eggs lateral to the gut. A medial organ of unknown function, the X-organ, lies posterior to the gonads near the anus.

The muscular system includes a series of individual muscle bands (not layers) in four orientations: circular, helicoidal, dorsoventral, and longitudinal<sup>[31]</sup>. The pharynx is tightly wrapped in circular muscle fibers and a few helicoidal bands. Somatic and visceral circular muscles are generally absent from the trunk. Helicoidal muscles continue onto the intestine and wrap several longitudinal muscles that extend from the mouth to the posterior end, inserting in the caudal furca or close to the body midline. Several species possess a pair of branched dorsal longitudinal muscles that are free of the helicoidal bands and maintain the position of developing parthenogenetic eggs. No muscles are associated with any reproductive structures. The muscular system of freshwater species is generally reduced relative to that of macrodasyidans and primitive marine chaetonotidans.

The brain is bilobed, straddling the pharynx. Sensory organs include long cilia and bristles, which presumably are tactile. Balsamo<sup>[1]</sup> demonstrated that *Lepidodermella squamata* is photosensitive, although it does not appear to have distinct multicellular photoreceptors. Gray and Johnson<sup>[28]</sup> found evidence of a tactile chemical sense in a marine macrodasyidan; it seems probable that freshwater chaetonotidans possess similar chemosensory abilities.

The excretory system consists of a pair of protonephridia<sup>[11]</sup> in the anterior midbody, which empty through pores on the ventral body surface. There is no circulatory or respiratory system *per se*.



**FIGURE 7.2** Schematic illustration of a typical chaetonotidan gastrotrich showing: (A) dorsal view; (B) ventral view; (C) posterior end of a hermaphrodite, showing sexual organs; and (D) egg, after deposition, AT = adhesive tubes, AN = anus, BR = brain, E = egg, F = furca, G = gut, M = mouth, PH = pharynx, PN = protonephridium, SC = sensory cilia, SS = sperm sac, X = X-organ. Modified from Remane<sup>[82]</sup>, Voigt<sup>[103]</sup>, and Kisielewska<sup>[52]</sup>.

### III. ECOLOGY AND EVOLUTION

#### A. Diversity and Distribution

Gastrotrichs are widely distributed in freshwaters in surface sediments and among vegetation. Some species of the Neogosseidae and Dasydytidae are good swimmers, and are occasionally reported from the plankton of shallow, weedy lakes<sup>[29,49,58,107]</sup>. However, no gastrotrich has become as truly planktonic as the daphnid cladocerans or ploimate rotifers.

Gastrotrichs have been found in a wide range of freshwater and semiterrestrial habitats. They are abundant in most lakes, ponds, and wetlands (Tables 7.1 and 7.2). Kisielewski<sup>[54,55]</sup> showed that gastrotrich density and species richness are positively correlated with the productivity of the habitat, and several workers have found that gastrotrich density is highest in highly organic sediments. Apparently, most gastrotrichs live very near the sediment surface in lakes (Fig. 7.3). Gastrotrichs are also abundant in sand and gravel bars of unpolluted streams, where they may be very abundant and penetrate deep into the sediments<sup>[45,48,92]</sup> (Fig. 7.4). Apparently, they are scarce in ground waters other than the hyporheic zone<sup>[84]</sup>. Their rarity in underground waters is surprising, because many marine gastrotrichs are interstitial in habit<sup>[33,84]</sup> and

because the small size and bacterial diet of gastrotrichs would seem to preadapt them to the groundwater habitat.

Gastrotrichs are among the few animals commonly found in anaerobic environments<sup>[17,66,98]</sup>, remaining abundant even during extended periods (months) of anoxia. The physiological basis of the anaerobiosis of freshwater gastrotrichs has not yet been studied. It seems likely that some freshwater gastrotrichs possess a sulfide detoxification mechanism similar to that demonstrated for marine gastrotrichs<sup>[79,80]</sup> and freshwater nematodes<sup>[74,75]</sup> to deal with the elevated concentrations of H<sub>2</sub>S that often accompany extended anoxia.

Little is known of the factors that control the distribution of individual species of gastrotrichs in freshwater. Arguing largely from analogy with marine work<sup>[33]</sup>, we might expect factors of primary importance to include the granulometry, stability<sup>[92]</sup>, packing and organic content of the sediment, the amount of dissolved oxygen<sup>[107]</sup>, and the density and composition of communities of microbes and predators. Also, culture work suggests that the inorganic chemistry of the water and the presence of anthropogenic contaminants can exert a strong influence on gastrotrich populations<sup>[24,40,42]</sup>.

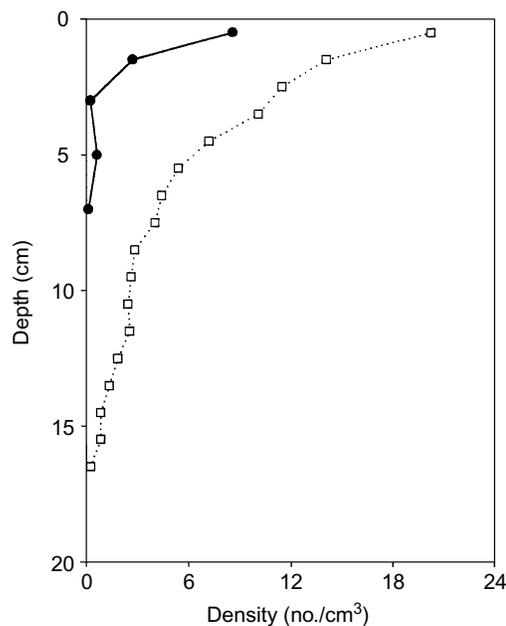
Many genera of freshwater gastrotrichs are known to have intercontinental or cosmopolitan distributions. Exceptions include several genera so far known only from Brazil (the macrodasyidan *Redudasys*, the chaetonotidans

**TABLE 7.1** Number of species of gastrotrichs found in some freshwater habitats.

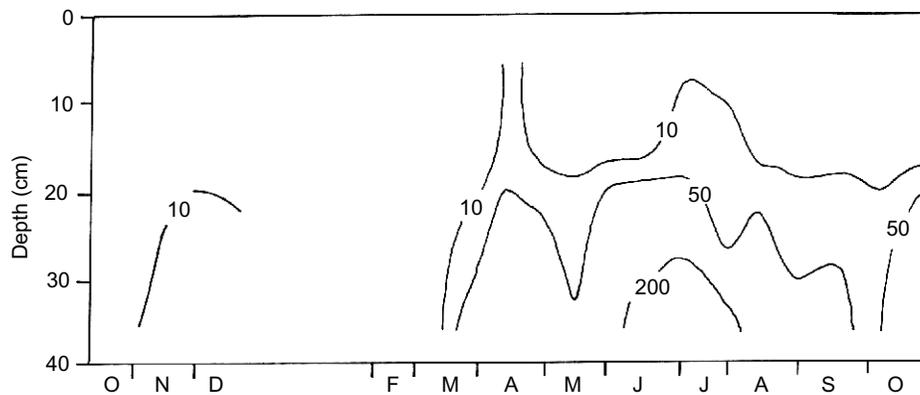
| Habitat                             | Total | <i>Chaetonotus</i> | Other<br>Chaetonotidae | Dasydytidae | Neogosseidae | Source     |
|-------------------------------------|-------|--------------------|------------------------|-------------|--------------|------------|
| European lakes <sup>a</sup>         | 18    | 12                 | 5                      | <1          | 0            | [2, 7, 81] |
| Mirror Lake, NH                     | 20–32 | 8–20               | 12                     | 0           | 0            | [98]       |
| Ponds, Poland <sup>b</sup>          | 21    | 10                 | 7                      | 3           | <1           | [55, 68]   |
| Peat bogs, Poland <sup>c</sup>      | 27    | 16                 | 9                      | 2           | 0            | [54]       |
| Bog pools, Poland <sup>d</sup>      | 24    | 12                 | 8                      | 4           | 0            | [53]       |
| <i>Phragmites</i> mats<br>(Romania) | 28    | 16                 | 7                      | 5           | 0            | [87]       |
| Ponds, Brazil <sup>e</sup>          | 38    | 14                 | 12                     | 10          | 2            | [58]       |
| Rivers, Brazil <sup>e</sup>         | 22    | 5                  | 12                     | 4           | 1            | [58]       |

<sup>a</sup>Mean of four lakes.<sup>b</sup>Mean of seven ponds.<sup>c</sup>Mean of four bogs.<sup>d</sup>Mean of two pools.<sup>e</sup>Sum of several collecting sites.**TABLE 7.2** Density and biomass of gastrotrichs in some freshwater habitats<sup>a</sup>.

| Site                         | Density<br>(No./m <sup>2</sup> ) | Biomass<br>(mg<br>DM/m <sup>2</sup> ) | Source |
|------------------------------|----------------------------------|---------------------------------------|--------|
| Lake Bikcze,<br>Poland       | 1,160,000 <sup>a</sup>           | 23 <sup>a,b</sup>                     | [71]   |
| Lake Brzeziczno,<br>Poland   | 920,000 <sup>a</sup>             | 30 <sup>a,b</sup>                     | [71]   |
| Lake Piaseczno,<br>Poland    | 910,000 <sup>c</sup>             | 23 <sup>b,c</sup>                     | [71]   |
| Mirror Lake, NH              | 130,000 <sup>d</sup>             | 1 <sup>d</sup>                        | [98]   |
| Lake Suviana,<br>Italy       | 57,000 <sup>e</sup>              | –                                     | [65]   |
| Lake Erie, OH                | 50,000 <sup>f</sup>              | –                                     | [23]   |
| Three small<br>ponds, Poland | 1,600,000–<br>2,600,000          | 25–78 <sup>b</sup>                    | [71]   |
| Mississippi River,<br>MN     | 130,000–<br>230,000 <sup>g</sup> | –                                     | [45]   |

<sup>a</sup>Littoral zone.<sup>b</sup>Converted from wet mass by multiplying by 0.15.<sup>c</sup>Mean of three stations.<sup>d</sup>Lakewide mean.<sup>e</sup>Mean of two deepwater stations.<sup>f</sup>Beaches.<sup>g</sup>Sand bars.**FIGURE 7.3** Vertical distribution of gastrotrichs within the sediments of Lake Brzeziczno, Poland (dashed line) and Mirror Lake, New Hampshire (solid line) as a function of depth from the sediment surface<sup>[70,98]</sup>.

*Arenotus* and *Undula*, and the dasydytid *Ornamentula*), the rare *Dichaetura* and *Marinellina* known only from Europe, and the proichthyidiids *Proichthyidium* and *Proichthyioides*, which have been found only at their type localities in Argentina and Japan, respectively. The geographic distribution of individual species is not well



**FIGURE 7.4** Density of gastrotrichs (number per L of interstitial water collected) in the hyporheic sediments of the Oberer Seebach, Austria. Depth defined here as the distance below the sediment-water interface. Modified from Schmid-Araya<sup>[92]</sup>.

known, because of the primitive state of the species-level taxonomy of freshwater gastrotrichs and the paucity of field studies throughout most of the world. Even in North America, only Arkansas<sup>[20]</sup>, Illinois<sup>[35,85,86]</sup>, northern Indiana<sup>[78]</sup>, Michigan<sup>[12,13,15]</sup>, and eastern Ohio<sup>[19]</sup> have received even cursory surveys of freshwater gastrotrichs. Thus, the 76 species that Schwank<sup>[95]</sup> reported from North America probably are a small fraction of North America's freshwater gastrotrich fauna. Some species have been reported to occur over broad ranges, including in some cases more than one continent<sup>[58]</sup>. Until further studies are made, such reported intercontinental distributions should be regarded with caution<sup>[25,100]</sup>, especially because "conspecifics" collected from different continents often exhibit marked morphological differences from one another<sup>[22,86]</sup>.

*Chaetonotus* typically dominates gastrotrich faunas everywhere in freshwater, both in terms of numbers of species and numbers of individuals (Table 7.1). Other genera of the Chaetonotidae are common in all kinds of freshwaters, but are usually less abundant than *Chaetonotus*. The Dasydytidae and Neogosseidae are less widespread than the Chaetonotidae, usually living in weedy, productive waters, where they may however become numerically abundant<sup>[8,54,55,68]</sup>. Dasydytids and neogosseids also are more abundant and speciose in the tropics than in temperate waters<sup>[58]</sup> (Table 7.1). The very rarely seen Dichaeturidae and Proichthyidiidae have been collected from cisterns, underground waters, tree holes, and among moss<sup>[82,91,99]</sup>.

## B. Reproduction and Life History

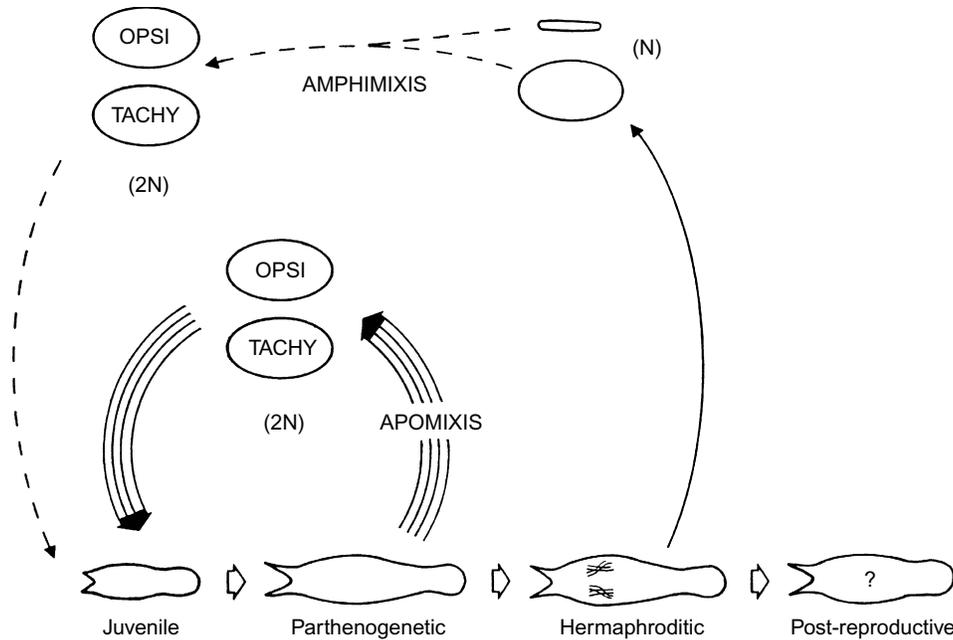
Until recently, populations of freshwater gastrotrichs were thought to consist entirely of parthenogenetic females<sup>[50,77]</sup>. However, more detailed recent studies have dispelled this notion, and have revealed a remarkable life cycle among freshwater gastrotrichs<sup>[36–39,62,105,106]</sup> (Fig. 7.5).

Newly hatched gastrotrichs are relatively large (approximately two-thirds the length of adults<sup>[14]</sup>) and already contain

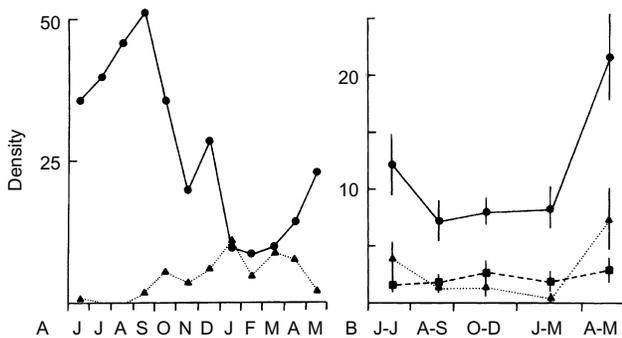
developing parthenogenetic eggs. These eggs develop rapidly under favorable conditions. At 20°C, the first egg may be laid within a day after the mother hatches. Typically, a total of four parthenogenetic eggs are laid over a 4-day period. Apparently, parthenogenesis is apomictic, so that offspring are genetically identical to their mother.

There are two kinds of parthenogenetic eggs. The more common kind, the tachyblastic egg, develops immediately and hatches quickly (within a day of being laid at 20°C). Occasionally, the final parthenogenetic egg laid by a female is not a tachyblastic egg, but a resting, or opsiblastic, egg. Opsiblastic eggs are thick-shelled, a little larger than tachyblastic eggs, and are very resistant to freezing and drying<sup>[14]</sup>. The factors that induce the production of opsiblastic eggs are not well known, although such eggs are often produced by animals in crowded cultures. Opsiblastic eggs are almost always the final egg produced by an animal, even if the total number of eggs is fewer than four.

Following the production of parthenogenetic eggs, animals develop into hermaphrodites<sup>[41,46,47]</sup> (Fig. 7.2c). During this time, sperm and meiotic sexual eggs are produced, and the X-body grows. These changes occur slowly, over the period of a week after the last parthenogenetic egg is laid. No one has yet observed sperm transfer or fertilization, although Levy and Weiss<sup>[62,63]</sup> reported finding a third kind of egg (the *plaque-bearing egg*) in cultures of *Lepidodermella squamata*. They suggested that plaque-bearing eggs may be the product of sexual reproduction. Because the sperm are few in number (32 or 64 per animal) and nonmotile, fertilization probably is internal. Animals reared in isolation do not appear to produce fertilized sexual eggs, so cross-fertilization probably is the rule. The absence of ducts associated with the male or female reproductive system makes it difficult to suggest a mechanism of sperm transfer (although M.R. Hummon<sup>[39]</sup> described one bizarre possibility). Probably the enigmatic X-organ is involved. Much remains to be learned about the post-parthenogenetic sexual phase and its importance in nature.



**FIGURE 7.5** Schematic diagram of the proposed generalized life cycle for freshwater chaetonotidan gastrotrichs, based predominately on study of *Lepidodermella squamata*. Dashed lines show hypothetical events that have not yet been demonstrated. From Levy<sup>[62]</sup>, after ideas presented by Levy and Weiss<sup>[63]</sup>, with permission of the authors. OPSI = opsiblastic egg, TACHY = tachyblastic egg.



**FIGURE 7.6** Seasonal trends in gastrotrich abundance. (A) Density (number/cm<sup>3</sup>) of two species of dasydytids in the surface sediments of a boggy pool ("Complex B") in Poland: *Setopus dubius* (●) and *Dasydytes ornatus* (Δ). From data of Kisielowska<sup>[53]</sup>. (B) Density (number/cm<sup>2</sup>) of all gastrotrichs (●), an unidentified species (probably *Heterolepidoderma*) (■), and *Lepidodermella triloba* (Δ) on the gytja sediments of Mirror Lake, New Hampshire<sup>[98]</sup>. Plotted points are means  $\pm$  s.e. ( $n = 16$ ).

The life cycle just described is unique among invertebrates and offers considerable ecological flexibility to gastrotrich populations. The initial parthenogenetic phase allows for explosive population growth under favorable conditions: workers have commonly reported growth rates ( $r$ ) of 0.1–0.5 per day in laboratory cultures<sup>[24,39,40,42]</sup>. Production of parthenogenetic resting eggs (opsiblastic eggs) buffers the population against unfavorable conditions and presumably allows for dispersal among habitats. Finally, the subsequent sexual phase introduces genetic recombination. Sexual reproduction is most likely to occur in populations in which

rates of mortality are low enough to allow some gastrotrichs to reach the age required for sexual development.

### C. Ecological Interactions

Gastrotrichs feed on bacteria, algae, protozoans, detritus, and small inorganic particles. Bacteria probably are of primary importance. Bennett<sup>[6]</sup> demonstrated that *Lepidodermella squamata* readily digested bacteria and found that this gastrotrich would not survive in laboratory cultures in the absence of bacteria. He reported that *L. squamata* could digest the green alga *Chlorella* as well, but suggested that algae were of secondary importance in gastrotrich diets. Gray and Johnson<sup>[28]</sup> showed that the marine macrodasyidan *Turbanella hyalina* could choose among various strains of natural bacteria, apparently on the basis of a tactile chemical sense. Thus, the quality as well as quantity of bacterial populations was important to the gastrotrichs. Freshwater gastrotrichs are most likely capable of similar fine discrimination among bacteria and other prey.

Reported predators of gastrotrichs include heliozoan and sarcodine amoebae, cnidarians, and tanypodine midges<sup>[10,14,67]</sup>, but many other benthic predators presumably feed on gastrotrichs. Nothing is known about the importance of predation in regulating populations of freshwater gastrotrichs or about the quantitative importance of gastrotrichs as a food item for various predators.

We have no direct information on what regulates gastrotrich populations in nature. Gastrotrichs are capable of enormous population growth (10–50% per day) in

laboratory cultures. If potential growth rates are anywhere near this high in nature, as seems likely in some circumstances, then there must be an equally high counterbalancing mortality, perhaps from predation. There are no detailed studies of the population dynamics of freshwater gastrotrichs in nature. The few quantitative studies of the seasonal dynamics of freshwater gastrotrich populations (Figs. 7.4 and 7.6) have shown that population densities are usually (but not always) lowest during the winter<sup>[68,92,98]</sup>. We do not know what drives these seasonal dynamics, but seasonal changes in water temperature, food supply, and predation pressures are obvious possibilities.

Gastrotrichs, along with nematodes and rotifers, are among the most abundant animals in the freshwater benthos, often having densities on the order of 10–100 cm<sup>-2</sup> (=100,000–1,000,000 m<sup>-2</sup>) (Table 7.2). However, because there have been no direct measurements of the roles of gastrotrichs in freshwater ecosystems, their importance can only be guessed at. There is only a single, tentative estimate of gastrotrich metabolism in freshwater: Strayer<sup>[98]</sup> estimated secondary production and respiration of gastrotrichs in Mirror Lake, New Hampshire, each to be 50–100 mg dry mass m<sup>-2</sup> yr<sup>-1</sup>, which is less than 1% of total production or respiration of the zoobenthic community. This estimate suggests that gastrotrich metabolism, and processes such as nutrient regeneration that are correlated with metabolism, are of minor importance in freshwater ecosystems. It would be imprudent, however, to dismiss gastrotrichs as quantitatively unimportant to ecosystem functioning without actually measuring gastrotrich activities under defined conditions. The extraordinarily high rates of population turnover and potentially highly selective feeding behavior of gastrotrichs suggest that they may exert a considerable influence on the composition of natural bacterial communities.

## D. Evolutionary Relationships

Three major groups of gastrotrichs are widely recognized: the order Macrotrichida and the suborders Multitubulatina and Paucitubulatina of the order Chaetonotida. Hochberg and Litvaitis<sup>[30]</sup> produced the most comprehensive study assessing phylogenetic relationships of gastrotrichs using morphological and anatomical analyses. They showed that the phylum is monophyletic with respect to nematodes and gnathostomulids and that the Macrotrichida is separate from the Chaetonotida; however, they also concluded that the Chaetonotidae is polyphyletic.

An alternative phylogenetic analysis, based on 18S rRNA, is developing rapidly. The study of Todaro et al.<sup>[101]</sup> is the most diverse to date, having used partial and complete 18S rRNA sequences from 43 species (28 Macrotrichida, 15 Chaetonotida), with 34 genera represented (26 macrotrichidan, 8 chaetonotidan), expanding the outgroup

analysis to complete sequences for 53 metazoan taxa, representing 26 phyla. This analysis grouped gastrotrichs loosely with the micrognathozoans, rotifers, ciliophorans, and gnathostomulids. The phylum Gastrotricha was accepted as monophyletic, but the Macrotrichida and Chaetonotida, as currently recognized, were paraphyletic. Although the Paucitubulatina was monophyletic, some of its genera (e.g., *Chaetonotus*) appear not to constitute natural groups. Yet to be treated in these studies are the four non-chaetonotidan families that caused confusion among the paucitubulatin Chaetonotida in the morphological study of Hochberg and Litvaitis<sup>[30]</sup>.

While Boaden<sup>[9]</sup> suggested that modern Macrotrichida and Paucitubulatina are descended from a *Neodasydys*-like ancestor, we believe, on the basis of digestive tract ciliation, striated musculature, and other characters, that the ancestral gastrotrich was more likely similar to the dactylopodolid macrotrichidans.

There is a paucity of basic morphological, biochemical, and zoogeographical information about most gastrotrich species. Most species have not even been described, let alone studied. The correct systematic placement of some genera (e.g., *Dichaetura*, *Marinellina*) will require much additional study. In North America, perhaps 75–90% of the probable diversity of freshwater gastrotrichs species are undescribed. Furthermore, there is growing concern<sup>[54,57–59,82,88,95]</sup> that new species having characters intermediate between traditionally defined genera of the Chaetonotidae may blur distinctions between genera such as *Chaetonotus*, *Heterolepidoderma*, and *Lepidodermella*. Some characters traditionally used to define gastrotrich genera (e.g., adhesive tubes, cuticle ornamentation) are subject to convergent evolution<sup>[4,58]</sup>, and so might not reflect common lines of descent. Finally, it seems likely that *Ichthydium*, which is defined by an absence of cuticular ornamentation, is polyphyletic. In response to these concerns, new genera of chaetonotids have been erected and existing genera redefined<sup>[58,95]</sup>, but the classification of this group will need to be redone as more complete information becomes available.

## IV. COLLECTING, REARING, AND PREPARATION FOR IDENTIFICATION

Gastrotrichs may be collected by taking samples of sediments or vegetation. For quantitative work on sediment-dwelling species, small diameter (2–5 cm) cores are preferable. For plant-dwelling forms, quantitative samples probably could be obtained by modifying sampling methods developed for macroinvertebrates<sup>[21,51]</sup> to use very fine mesh and small sample volumes or subsampling.

Because living animals are preferable to preserved animals for many purposes, it often is desirable to extract the animals from the sample prior to preservation. If the sample must be preserved immediately, narcotize the animals

with 1% MgCl<sub>2</sub> for 10 min, then fix them in 10% formalin with Rose Bengal<sup>[43,45]</sup>.

It is difficult to extract or count the gastrotrichs from a sample. Sometimes gastrotrichs are handpicked and counted under a dissecting microscope<sup>[23,43,45,98]</sup>, but this procedure is tedious. Density gradient centrifugation<sup>[72,96]</sup> should be useful in extracting gastrotrichs from sediment, but has not yet been tested on freshwater species. A modified Baermann funnel has been useful for extracting chaetonotidans, but not dasydytids, from pond sediments<sup>[69]</sup>. Sieves should be avoided or used cautiously, because gastrotrichs are too small to be retained quantitatively on even very fine mesh sieves. For example, Hummon<sup>[43]</sup> found that a 37- $\mu$ m mesh sieve retained only 31% of the gastrotrichs from samples taken in the upper Mississippi River. For quantitative work, it is important to check the efficiency of whatever extraction or counting method is used, because gastrotrichs are so small and easily overlooked<sup>[98]</sup>.

Living gastrotrichs are preferable to dead gastrotrichs for taxonomic work. Living gastrotrichs often are too active for critical observations to be made, so they must be slowed down by gently squeezing the animal (either with a rotocompressor<sup>[97]</sup> or by removing some of the water from beneath a cover slip with a tissue), by placing it in a viscous medium such as methylcellulose, or by narcotizing it. Cocaine was the traditional narcotic of choice<sup>[16]</sup>, but it is now difficult to obtain for laboratory use. d'Hondt<sup>[32]</sup> recommended using MS 222 (tricaine methanesulfonate), and we have had very good success narcotizing gastrotrichs by bleeding 1.8% neosynephrine (available at pharmacies) or 1.5% MgCl<sub>2</sub> under the cover slip. Animals may be killed with formalin or fumes of osmium tetroxide<sup>[15]</sup> following narcotization. Osmium tetroxide is a superior fixative, but is dangerous and should be used with extreme care. It is sometimes necessary to examine individual scales, which can be isolated from an animal by bleeding 2% acetic acid under the cover

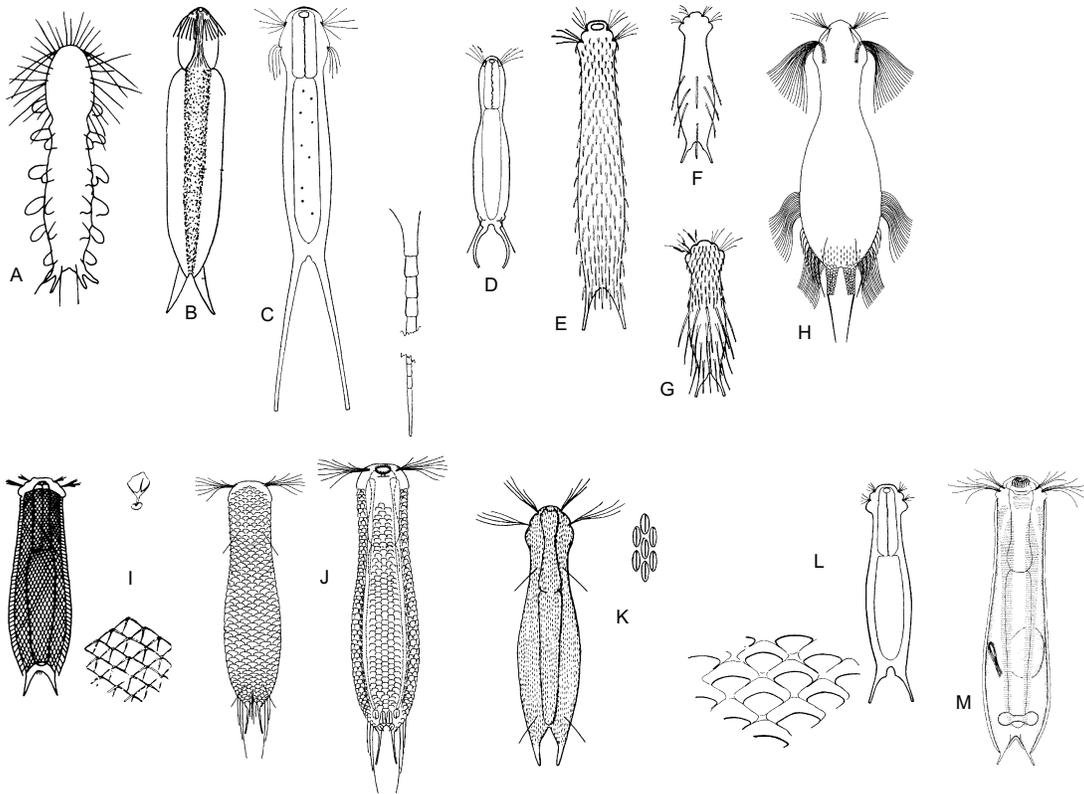
slip. For serious taxonomic work, videomicroscopy of living animals may provide better permanent documentation of species characters than killed specimens or slides.

Gastrotrichs have been cultured<sup>[6,14,42,76,102]</sup> on 0.1% malted milk, raw egg yolk, wheat grain infusion, baked lettuce infusion, and baker's yeast. Animals should be acclimated gradually to culture media when collected from the wild<sup>[14]</sup>. Hummon<sup>[42]</sup> described a procedure for starting individual cultures of known-age animals from eggs that may be especially useful for bioassay work.

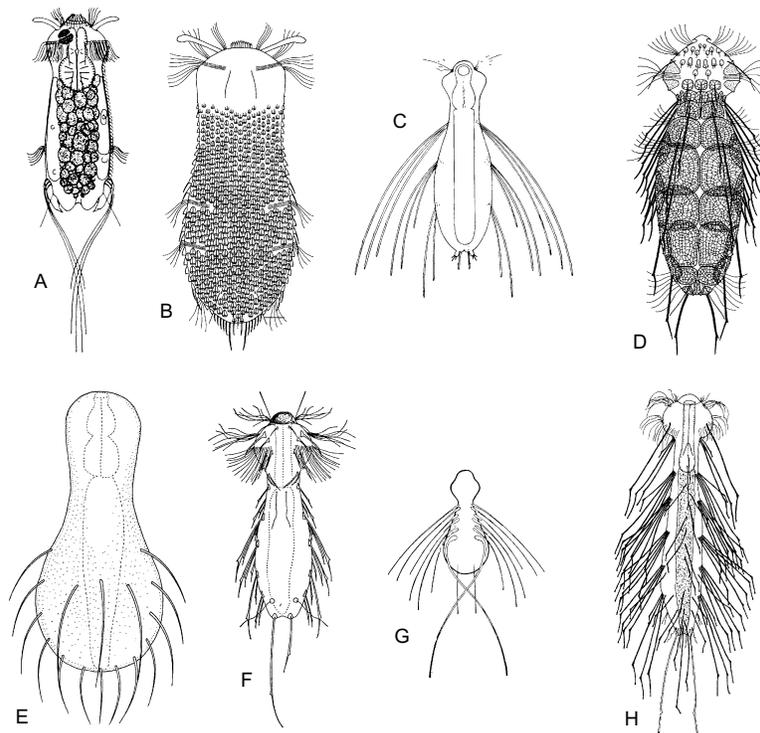
## V. TAXONOMIC KEY TO GASTROTRICHA

It is relatively easy to identify most North American freshwater gastrotrichs to genus and very difficult to identify them to species. Species identification requires a keen eye, careful observation, a cooperative gastrotrich, and some luck, because most of the freshwater gastrotrichs of North America undoubtedly are undescribed. The following works are helpful in species identification: Brunson<sup>[15,16]</sup>, who keyed and illustrated species then known from North American freshwaters; Robbins<sup>[85,86]</sup>, who provided additional information and drawings of North American species; d'Hondt<sup>[34]</sup>, who published a key to the species of *Lepidodermella* and defined three subgenera of *Ichthydium*; Kisielewski<sup>[54]</sup>, who made a critical evaluation of the morphological characters that must be measured to describe (or identify) a species; Kisielewski<sup>[56]</sup>, who gave a recent treatment of *Aspidiophorus*; Schwank<sup>[95]</sup>, who provided illustrated keys (in German) for all known freshwater gastrotrichs worldwide; Kisielewski<sup>[58]</sup>, who described many Brazilian species and addressed several important issues in gastrotrich systematics; and Balsamo and Todaro<sup>[5]</sup>, who provided a key to the freshwater genera of the world; and Balsamo et al.<sup>[108]</sup> who discussed gastrotrich taxonomy and presented a list of all known freshwater species.

- |          |   |  |
|----------|---|--|
| 1a.      | Animal with at least three pairs of adhesive tubules (one anterior and two posterior) and a pair of pharyngeal pores, body strap-shaped (Fig. 7.1a,b); an almost entirely marine group not yet reported from North American freshwaters .....   | order Macrodasysida                          |
| 1b.      | Animal lacking adhesive tubules (Fig. 7.8a-h) or with one pair (very rarely two pairs) of adhesive tubules posteriorly (Fig. 7.7a-m), and no pharyngeal pores, body strap-shaped or tenpin-shaped; common and widespread in freshwater .....  | order Chaetonotida ..... 2                   |
| 2a (1b). | Posterior end of body usually with furca and adhesive tubules; body usually strap-shaped or tenpin-shaped (Fig. 7.7a-m) .....   | 3  |
| 2b.      | Posterior end of body without furca or adhesive tubules, although sometimes bearing spines or pegs; body usually tenpin- or bottle-shaped (Fig. 7.8a-h) .....   | 14   |
| 3a (2a). | Furca doubly branched; scales and spines sparse or absent (Fig. 7.7a); a rare genus not yet reported from North America .....   | family Dichaeturidae ..... <i>Dichaetura</i> |
| 3b.      | Furca singly branched; scales and spines present or absent (Fig. 7.7b-m); common and widespread .....   | 4  |
| 4a (3b). | Branches of furca heavy, sickle-shaped, curved, and tapered, not distinctly divided into a cone-shaped basal part and a distal duct; head with long cilia that are not arranged in bundles; head plates absent (Fig. 7.7b); a rare family not yet reported from North America .family Proichthydiidae ..... | 5  |
| 4b.      | Branches of furca usually with a cone-shaped base and a distal adhesive duct; body often with numerous spines or scales; head with cilia that are arranged in bundles; cephalic plates present (Fig. 7.7c-m); common and widespread .....   | family Chaetonotidae ..... 6                 |
| 5a (4a). | Head bearing a row of cilia shorter than the head (Fig. 7.7b) .....   | <i>Proichthydium</i>                         |
| 5b.      | Cilia on the head much longer than the head .....   | <i>Proichthydioides</i>                      |



**FIGURE 7.7** Genera of freshwater Dichaeturidae, Proichthyidiidae, and Chaetonotidae: (A) *Dichaetura*; (B) *Proichthyidium*; (C) *Polymerurus*, showing detail of ringed branches of furca; (D) *Ichthyidium*; (E–G) *Chaetonotus*, showing examples of spination; (H) *Undula*; (I) *Aspidiophorus*, with detail of coat of scales and a single scale; (J) *Lepidochaetus*, in dorsal (left) and ventral (right) views; (K) *Heterolepidoderma*, with detail of scales; (L) *Lepidodermella*, with detail of scales; and (M) *Arenotus*<sup>[15,50,57,58,82,85,103]</sup>.



**FIGURE 7.8** Genera of freshwater Neogosseidae and Dasydytidae: (A) *Neogossea*; (B) *Kijanebalola*; (C) *Stylochaeta*; (D) *Ornamentula*; (E) *Anacanthoderma*; (F) *Setopus*; (G) *Haltidytes*; and (H) *Dasydytes*<sup>[3,15,58,61,95]</sup>.

|            |  |                           |
|------------|--|---------------------------|
| 6a (4b).   | Branches of furca ringed and often very long; body often large and without a distinct neck (Fig. 7.7c) .....   | <i>Polymerurus</i>        |
| 6b.        | Branches of furca not ringed (Fig. 7.7d-m) .....   | 7                         |
| 7a (6b).   | Dorsal surface of body without spines or scales (except for dorsal sensory bristles or a few scales at the base of the furca (Fig. 7.7d) .....   | 8                         |
| 7b.        | Dorsal surface of body with numerous scales or spines (Fig. 7.7e-l) .....  | 9                         |
| 8a (7a).   | Cuticle very thick and smooth, distinct from the epidermis, entirely without scales; mouth with large mouth ring and strong pharyngeal teeth (Fig. 7.7m); known only from Brazil .....   | <i>Arenotus</i>           |
| 8b.        | Cuticle not especially thick, sometimes with scales near the base of the furca or the bases of cuticular bristles, or with minute cuticular structures; mouth ring small and without pharyngeal teeth (Fig. 7.7d); common and widespread ..... | <i>Ichthydium</i>         |
| 9a (7b).   | Spines or spined scales present and often numerous (Fig. 7.7e,f,g,j) .....   | 10                        |
| 9b.        | Spines absent (occasionally a few thin spines are present at the base of the furca) (Fig. 7.7h,i,k,l) .....  | 11                        |
| 10a. (9a)  | Ventral scales different from dorsal scales; spines of various types (Fig. 7.7e-g); common and widespread .....  | <i>Chaetonotus</i>        |
| 10b.       | Ventral scales similar to dorsal scales; posterior part of body with several long spines that reach beyond the end of the furca (Fig. 7.7j); not yet reported from North America .....   | <i>Lepidochaetus</i>      |
| 11a (9b).  | Furca without adhesive tubes; body markedly tenpin-shaped, with groups of long cilia on the head and posterior part of the body (Fig. 7.7h); a semiplanktonic genus known only from Brazil .....   | <i>Undula</i>             |
| 11b.       | Furca with adhesive tubes; body strap-shaped or tenpin-shaped; without groups of long cilia on head and posterior body (Fig. 7.7i,k,l) .....   | 12                        |
| 12a (11b). | Dorsal surface of body covered with stalked scales (Fig. 7.7i) .....   | <i>Aspidiophorus</i>      |
| 12b.       | Scales on dorsal surface of body unstalked (Fig. 7.7k,l) .....   | 13                        |
| 13a (12b). | Scales elongate, with longitudinal keels (Fig. 7.7k) .....   | <i>Heterolepidoderma</i>  |
| 13b.       | Scales not keeled (Fig. 7.7l) .....  | <i>Lepidodermella</i>     |
| 14a (2b).  | Head with club-shaped tentacles (Fig. 7.8a,b) .....  | family Neogosseidae ..... |
| 14b.       | Head without club-shaped tentacles (Fig. 7.8c-h) .....   | family Dasydytidae .....  |
| 15a (14a). | Posterior end of body with two groups of long spines (Fig. 7.8a); elements of mouth-ring jointed .....   | <i>Neogossea</i>          |
| 15b.       | Posterior end of body with single medial group of spines (Fig. 7.8b); elements of mouth-ring unjointed .....   | <i>Kijanebalola</i>       |
| 16a (14b). | Posterior end of body with pair of peg-like protuberances (Fig. 7.8c) .....  | <i>Stylochaeta</i>        |
| 16b.       | Posterior end of body without peg-like protuberances (Fig. 7.8d-h) .....   | 17                        |
| 17a (16b). | Body enclosed in a "lorica" of large, thick, ornamented scales (Fig. 7.8d); known only from Brazil .....   | <i>Ornamentula</i>        |
| 17b.       | Scales absent or small and inconspicuous (Fig. 7.8e-h) .....   | 18                        |
| 18a (17b). | Head much narrower than body and scarcely wider than neck; lateral spines absent or identical to dorsal spines; pharynx with two bulbs (Fig. 7.8e); not yet reported from North America .....  | <i>Anacanthoderma</i>     |
| 18b.       | Head distinctly wider than neck; body with long lateral spines; pharynx with one bulb or no bulb (Fig. 7.8f-h) .....   | 19                        |
| 19a (18b). | Some lateral spines movable and sharply bent basally; posterior end of body rounded, without rear spines (Fig. 7.8g) .....   | <i>Haltidytes</i>         |
| 19b.       | All spines fixed, straight or bent distally; posterior end of body usually with spines (Fig. 7.8f,h) .....   | 20                        |
| 20a (19b). | Lateral spines with 1-3 lateral denticles and often terminally bifurcated; spines of uniform thickness from their base to the last lateral denticle; pharynx usually with a distinct posterior bulb (Fig. 7.8h) .....                          | <i>Dasydytes</i>          |
| 20b.       | Lateral spines tapered, with at most one weak lateral denticle and never terminally bifurcated; pharynx without posterior bulb (Fig. 7.8f) .....   | <i>Setopus</i>            |

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