

22. Nemertina

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The phylum Nemertina is comprised of non-segmented worms whose anatomy includes: the diagnostic rhynchocoel housing a protrusible proboscis, a closed blood-vascular system lined by endothelium, a regionated gut with anal pore, a fully ciliated and glandular epidermis, a nervous system with four cerebral ganglia and a pair of lateral nerve cords, and serial sacculate gonads. There are about 900 described species of nemertines. They are primarily marine and found in all major marine habitats, but they also are known from freshwater and terrestrial habitats. The size of adult worms ranges from 1 mm to many meters in length and 0.15 mm to a few centimeters in width.

Classification

The phylum consists of two classes, the Anopla and Enopla, the names reflecting respectively the absence or presence of specialized proboscis armature, the stylets. Anopla have a ventral mouth and all known meiofaunal Enopla have an anterior terminal pore that serves as mouth and proboscis pore. According to the classification of Iwata (1972) the Anopla consists of the orders Archi-, Palaeo- and Heteronemertina, whereas the Enopla includes only the order Hoplonemertina.

Enopla.--The hoplonemertean genus *Otocyphlonemertes* has a world-wide distribution, but there are major gaps in the geographic records (Kirsteuer, 1977). Members of the genus lack ocelli and are characterized by the presence of a pair of statocysts located dorsally on the posterior extensions of the ventral ganglia. Müller (1968) comments on 14 species known to that time and provides a key for them (see also Norenburg, 1988). Kirsteuer (1977) provides the most recent and extensive account on the principal features used in taxonomy of this group.

The remaining genera of interstitial hoplonemertines (Figure 22.1d-f) are each known from only one or a few species. We know little about which characteristics will prove to be significant to the taxonomy of these species or genera. *Annulonemertes minusculus*, and *Arenonemertes minutus*, both from northern Europe, have smooth

stylets and are recognized by pseudosegmentation, annular constrictions, of the body wall in the intestinal region (Berg, 1985; Friedrich, 1949). Related species have been encountered in the Gulf of Maine and in Puget Sound (unpublished observations). These worms, as a group, are among the smallest nemertines known, adults often being less than 2 mm long and 150 μ m in diameter. *Arenonemertes microps* is known only from near Kiel; it is 2-3 mm long, has four ocelli, the rhynchocoel and proboscis extend to the caudal terminus, and the stylets are smooth (Friedrich, 1933). A probable congener of *Prostomatella arenicola* has been encountered interstitially in shallow water off Florida (unpublished observations). *Otonemertes marcus* is a small species (2 mm long) with a pair of ocelli, a pair of statocysts, and distinct mid-body pigmentation (Corrêa, 1958); little else is known about it.

Anopla.--Anoplans (Figure 22.1a-c) may be recognized by their non-regionated, fully protrusible proboscis that lacks discrete stylets. Interstitial archinemertines of the genera *Cephalothrix* (Figure 22.1a) and *Procephalothrix* may be recognized by the position of the mouth at the anterior end of the gut and far posterior to the cephalic tip (as much as 1 mm in a worm 7-10 mm long). The mouth of palaeonemertines, such as *Carinina arenaria* (Figure 22.1b), and heteronemertines is relatively more anterior, located just posterior to the cerebral ganglia. The archinemertines uniformly lack cerebral organs (glandular sensory pits, associated posteriorly with the cerebral ganglia of other anoplans), whereas they are well developed in *C. arenaria* and an undescribed, interstitial heteronemertine (Figure 22.1c) (unpublished observations). *Carinina arenaria*, in addition, is reported to possess statocysts posteriorly in the ventral ganglia (Hylbom, 1957). The species of interstitial *Cephalothrix* are characterized by a proboscis whose epithelium contains prominent cirri (long ciliary tufts), cells with a cluster of rhabdite-like inclusions, and cells with a pseudocnide (an urn with an eversible thread) (Gerner, 1969). All of these genera require the same detailed observations *in vivo* as do the hoplonemertines, but, because of the frequent lack of ocelli, discrete

proboscis armature, and statocysts (except *Carinina arenaria*), differences between species, genera, and even orders of anoplans tend to be much more subtle and often require histological study.

Habitats and Ecological Notes

Macrofaunal species within each order have similar habitat preferences (Kirsteuer, 1971; Norenburg, 1985). The archinemertines are mostly of small diameter and live in soft mud or in interstitial spaces. Palaeo- and heteronemertines show tendencies to dramatic increases in size and in body-wall musculature and complexity, which correspond with increased burrowing ability compared to archinemertines (Norenburg, 1985). The hoplonemertines generally are small, relatively less muscular and tend to be epibenthic, thereby increasing the importance of cilia in locomotion (Norenburg, 1985). Similar segregation of these higher taxa is evident in the meiofauna.

Meiofaunal palaeonemertines, such as *Carinina coei*, *Hubrechtella dubia*, and *Tubulanus pellucidus*, live at or in the surface of muddy and silty marine bottoms (Coe, 1943; Hylbom, 1957; unpublished observations). The other three orders are represented in the mud fauna, but with lesser taxonomic diversity (Kirsteuer, 1963) and few would qualify as meiofauna except as juveniles (often abundant) (unpublished observations). Kirsteuer (1971) notes that the mud-dwelling nemertines "...have, with the exception of some statocyst-bearing palaeonemertines, no developed morphological features which would make them quite distinct..." from their relatives in other marine habitats. In contrast, the nemertine fauna of the interstitial spaces of coarse marine sediments is morphologically distinct and hoplonemertines predominate in taxonomic diversity (Kirsteuer, 1971). The remainder of this review concerns those nemertines that may be considered interstitial.

Interstitial nemertines generally are less than 0.5 mm in diameter and may be up to 50 mm in length, but many are less than 0.3 mm in diameter and 10 mm in length. Kirsteuer (1971) points out that the marine interstitial nemertine fauna is relatively non-diverse systematically and shows clear morphological adaptations to the interstitial environment. About thirty species of interstitial nemertines have been described. Of the three orders, the hoplonemertines predominate in taxonomic diversity, with 23 species in five genera. They include, since the last review (Kirsteuer, 1971), a newly described genus, *Annulonemertes* Berg, 1985, and newly described species of *Otocyphlonemertes* (Mock and Schmidt, 1975). The literature references at least another 7 new, but undescribed, species of *Otocyphlonemertes* (Kirsteuer, 1977; Mock and

Schmidt, 1975). In addition, a new hoplonemertine genus (Figure 22.1d), as well as new species in the genera *Annulonemertes* (Figure 22.1f), *Prostomatella*, and *Otocyphlonemertes* have been encountered in New England, Puget Sound, Florida, and Pacific Panama (Norenburg, 1988, unpublished observations). The described archinemertines are represented by two genera, *Cephalothrix* (Figure 22.1a) with 5 species and *Procephalothrix* with 1 species. *Carinina arenaria* (Figure 22.1b) is the only described interstitial palaeonemertine. Recently, the first truly interstitial heteronemertine (Figure 22.1c) was discovered in Puget Sound (Norenburg, 1988).

Information on the natural history of interstitial nemertines is scarce. They move principally by ciliary gliding, without displacing the substratum (Corrêa, 1949; Kirsteuer, 1971). Mock (1981a) was unable to substantiate Friedrich's (1935) observation that *Prostomatella arenicola* (Figure 22.1e) moves by nematode-like writhing. Interstitial nemertines are either carnivorous predators or scavengers (Corrêa, 1949; Mock, 1978, 1981a). Somewhat more is known of distribution and reproductive biology.

Most interstitial nemertines are recorded from intertidal and shallow subtidal clean, relatively coarse sand and shell hash, i.e., high-energy beaches or subtidal areas subject to considerable current action, where coarse sand and shell fragments accumulate. Interstitial nemertines are rarely found in areas with high loads of organic particulates or silt. Some anoplans tolerate greater amounts of silt than do hoplonemertines (personal observations). This is consistent with the generalized habit of their macrofaunal relatives (Kirsteuer, 1963; Norenburg, 1985). Kirsteuer (1986) notes that information concerning patterns of distribution of interstitial nemertines is sketchy and must be regarded with caution because surveys have often focused on a limited portion of the potential habitat of a species. Corrêa (1949) and Mock and Schmidt (1975) provide quantitative data for some *Otocyphlonemertes*. Corrêa (1949) indicates a distribution peak somewhat above mean low water level (unspecified species), while *O. fila* (sp. A of Kirsteuer, 1977), in studies by Mock and Schmidt (1975), appears to have a distribution peak just below mean low water. Other species have considerably broader distributions; e.g., *O. pallida* extends from the intertidal to depths of 10 m (Müller, 1968).

All interstitial nemertines described thus far are gonochoric. Gonads may be more or less regularly disposed among intestinal diverticula (when present) or they may be scattered about the intestine. Interstitial nemertines most commonly have only one ovum per ovary, less frequently 2-3 ova per ovary, evident. The number of mature ova per individual is a function of animal and ova size and ranges from

less than 10 for the smallest species to more than 100 for the largest. Sperm morphology for representatives of each of the interstitial nemertine genera includes an elongate head and solitary flagellum. External fertilization is assumed, with the possible exception of *Cephalothrix germanica* (Gerner, 1969). Ova of interstitial hoplonemertines are relatively large, at 150–200 μm in diameter. They probably undergo direct development with lecithotrophy, as is common for other hoplonemertines and would be predicted with interstitial habit (Swedmark, 1964). Mock (1981b) has confirmed this for a species of *Otocyphlonemertes*. The mode of development for interstitial anoplans is of special interest, as macrofaunal cephalothricids usually have a simple, planktotrophic larval phase and macrofaunal heteronemertines generally have a specialized, often planktotrophic, larval phase.

Methods of Collection and Extraction

Most methods for the collection of other soft-bodied meiobenthic organisms are satisfactory for the collection of nemertines.

Meiofaunal nemertines must be sorted live. A simple method for extracting them in good condition from sand samples is to place sand in a pail to a depth of 5–8 cm and cover this with seawater to about 20 cm, stir the sand gently with a rotational lifting motion of the hand, about five times, so that less dense material (including the worms) separates from the sand as it falls back to the bottom. As the sand settles, but before the water stops spinning, quickly decant the water through a sieve (6–8 cm diameter) with a mesh size of 63–125 μm . Use a wash-bottle to quickly wash the contents of the sieve into a finger-bowl. Repeat the decanting procedure 3–4 times or until no more worms are obtained, then repeat the procedure with MgCl_2 solution (approximately 7.5%).

Other procedures (Chapter 9) may be preferable in some instances, e.g., where organic particulates or fine silt are a problem. Many nemertines may be kept for days to several months in their original substratum if stored in Whirlpak plastic bags in running seawater or stored in buckets over which a thin flow of seawater is maintained (there must be no macrofauna in these samples). Meiofaunal nemertines are best sorted live using a dissecting microscope at low magnification (10–15x). Most can be recognized by their long, relatively cylindrical shape as they glide smoothly through their surroundings (unlike the agitated movement of many otoplanid turbellarians). Hoplonemertines may often be recognized by an epidermal furrow that encircles the body in the vicinity of the cerebral ganglia.

Nemertodermatid turbellarians may be mistaken

for the longer nemertines, even after long experience. Some nemertines (<10 mm) may be confused with kalyptorhynch and similar turbellarians, but with practice may be distinguished by their behavior and more cylindrical shape. Extraordinary efforts, such as wholemounts of individual worms, would be required to enable one to distinguish preserved meiofaunal nemertines from similar, preserved turbellarians.

Methods of Preparation for Taxonomic Study

Detailed observations of living specimens (preferably 10 or more) are essential for taxonomic work. Norenburg (1988) provides a key that is intended for use with living specimens and includes most of the described species and several undescribed species. With the dissecting microscope one records color of integument, gut, and cerebral ganglia, as well as information on size, shape and behavior of the worm while actively gliding, when "at rest," and when contracted. This information includes: shape of the cephalic region and its proportions relative to the cerebral ganglia; position and shape of the mouth for anoplans; shape of the caudal terminus and presence or absence of a caudal cirrus (tail-like extension of some anoplans) or adhesive plate (cannot always be detected in the living worm); and, when possible, relative extent of the rhynchocoel and proboscis. Some of these observations will require amplification with the compound light microscope.

Although nemertines in general have few external distinguishing features, interstitial nemertines have the advantage of small size and relative transparency, enabling one to make detailed observations of internal anatomy from living specimens (Figure 22.1). This is accomplished by placing a worm in a drop of seawater on a glass slide and floating or supporting, with bits of wax or clay, a coverslip on this drop so that the worm may still move (Kirsteuer, 1967). The coverslip is then carefully lowered, by drawing off water or pressing on it, so that the worm is somewhat flattened and locomotion is restricted. If one works rapidly and monitors evaporation the worm usually can be recovered intact.

Observations that need to be made with the compound microscope depend somewhat on the taxon in question and should be documented with sketches and with photomicrographs, if possible. In all cases it is desirable to obtain at least a good dorsal view to document the distribution of sensory cirri (also called sensory or ciliary bristles). Sometimes these bristles are numerous and readily evident, but in some species they may be lacking or difficult to observe until the worm rolls into the proper position. It is essential to determine whether cerebral organs

are present, their form and position; size and shape of the cerebral ganglia; position and structure of ocelli and/or statocysts; extent of rhynchocoel; structure and length of proboscis and position of its posterior insertion; characteristics of stylet apparatus and other proboscideal elements such as rhabdite-like barbs and pseudocnides; and general construction of the gut. If at all possible, observations on an everted proboscis should be obtained. The proboscis may be everted voluntarily or under increased pressure from the coverslip. Sometimes dabbing the side of the coverslip with an irritant such as dilute acetic acid may provoke eversion of the proboscis. If an everted proboscis is obtained it should be carefully described and then preserved with the rest of the specimen.

Internal anatomy based on histology is an essential component of all nemertine taxonomy, but the subject is beyond the scope of this chapter.

Differences between any of the species of interstitial genera may be very subtle and, in general, considerable experience is required for taxonomic work at the specific level. All taxonomic descriptions of nemertines also require histological work. To facilitate the latter, specimens must be individually anesthetized and fixed. Kirsteuer (1967) emphasizes the importance of adequate narcotizing. Anesthetizing specimens in 7.5% MgCl₂ in distilled water is usually satisfactory. A few species react badly to this, evidenced by blistering or sloughing off of the epidermis or fragmenting of the worm. Other anesthetics that may be tried include urethane, chloral hydrate (both of which may be added to seawater as crystals or used in prepared solutions), propylene phenoxetol, or Lidocaine. As soon as the worm is suitably narcotized, within 1-3 minutes for most interstitial species (it may continue to glide by latent ciliary activity), it is pipetted with a minimum of fluid onto a glass slide so that it, or at least the anterior region, is relatively straight. Fixative is then immediately, but carefully, pipetted along the worm. When the fixative noticeably begins to take effect, gently flush the fluid back and forth so that the specimen will not adhere to the slide. After 1-2 minutes flush the worm into a vial of fixative. A good histological fixative, such as Hollande's cupri-picri-formol-acetic fluid, for light-microscopy, or a glutaraldehyde-based electron microscopy fixative (Chapter 10) should be used.

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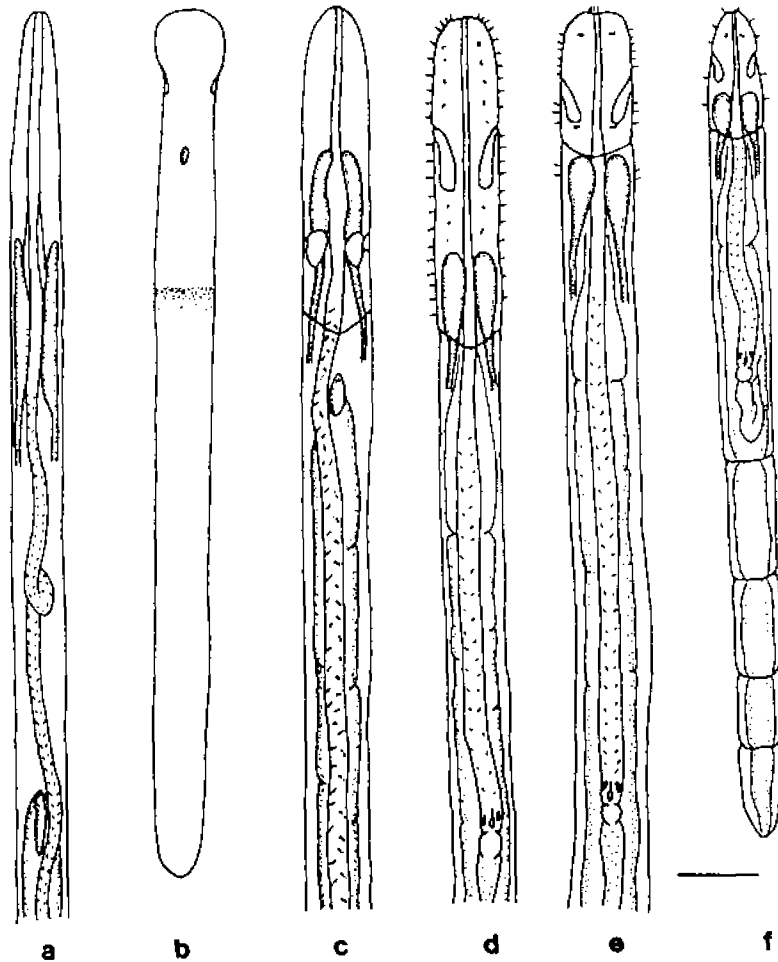


Figure 22.1.—Nemertina: Diagrams of representative interstitial nemertines, all except b, showing internal features observable in living specimens. cerebral organs (not present in a), cerebral ganglia, proboscis, and gut. a, Archinemertina: *Cephalothrix* sp.; b, Palaeonemertina: *Carinina arenaria* (after Hylbom, 1957); c, Heteronemertina sp. (Puget Sound specimen); d-f, Hoplonemertina, Monostilifera: d, Hoplonemertina sp. (New England specimen); e, *Prastomatella arenicola* (after Mock, 1981a); f, *Annulonemertes* sp. (Puget Sound specimen). (Scale = 250 μ m.)

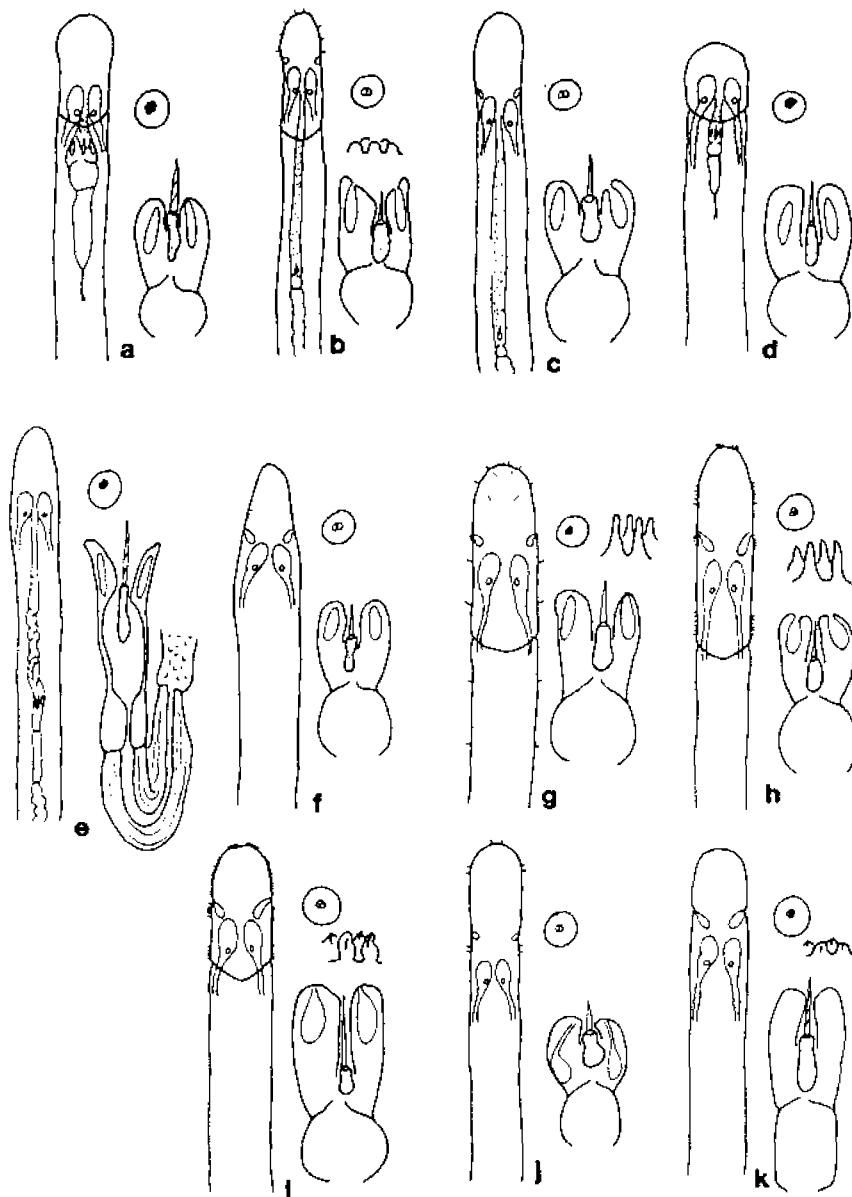


Figure 22.2.—Nemertina (continued): Diagrams depicting diversity and various characteristics observable in living specimens of *Ototyphlonemertes*. a, *O. americana* (after Gerner, 1969); b, *O. antipai* (after Müller, 1968); c, *O. aurantiaca* (after Gerner, 1969); d, *O. brevis* (after Corrêa, 1948); e, *O. macintoshi* (after Bürger, 1895); f, *O. brunnea* (after Bürger, 1895); g, *O. cirrula* (after Mock and Schmidt, 1975); h, *O. pallida* (after Mock, 1978); i, *O. erneba* (after Corrêa, 1950; Kirsteuer, 1977); j, *O. evelinae* (after Corrêa, 1958); k, *O. fila* (after Corrêa, 1953). (Diagrams not to scale.)