Introduction

Sipunculans, commonly known as peanut worms, are a group of exclusively marine benthic organisms. They are protostome, coelomate, unsegmented bilaterally symmetric invertebrates. Since the mid-twentieth century Sipuncula was considered an independent phylum (Hyman 1959; Stephen 1964; Clark 1969; Stephen & Edmonds 1972), but nowadays this status is unclear and recent genetic studies would place the group within the phylum Annelida in a clade yet to be determined (e.g. Struck et al. 2007, 2011; Dordel et al. 2010). Sipunculans are likely to be placed in their own class in Annelida due to their distinctive features; e.g. lack of segmentation, U-shaped digestive tube and a sipunculan-specific larva (pelagosphera). From this perspective, in the present work no particular taxonomic rank has been assigned them.

Nowadays, the sipunculans remain a group of little known and scarcely studied animals. This is mainly due to a lack of specialists (no more than 20 worldwide) and difficulties in species identification, since a detailed dissection is often required to clarify the details of their internal anatomy. Dissection is a difficult technique that requires skills and training, since most of the specimens are just a few mm in length. Moreover there is a lack of specific teaching materials. Although a few monographs aid in identification (Saiz-Salinas 1993; Cutler 1994; Pancucci-Papadopoulou et al. 1999), the information included is insufficiently illustrated, which is always a major problem. There are thus large gaps in the knowledge of this taxon. Unfortunately, most sipunculans collected in macrobenthic studies are not identified further than Phylum level, except for a few common species. This guide provides the advice and guidance needed to establish a proper identification of the different taxa of this group present in the Mediterranean Sea, as well as useful ecological and zoogeographical data.
According to the fossil record (Hickman 2008), Sipuncula is a group with a historically low diversity. Its range of temperature tolerance (from -1.9°C to 29°C) and depth (Murina 1973, 1984; Cutler 1979) are very wide, so its distribution covers all the world’s oceans and the whole bathymetric range, from intertidal zone to abyssal bottoms, excepting brackish areas (Murina 1971, 1975, 1977, 1984; Cutler 1975, 1977a, 1977b, 1994; Cutler & Cutler 1980, 1987; Pancucci-Papadopoulou et al. 1999). Species can be collected from the shoreline to deep bottoms, since most are eurybathic and generally present a wide range of tolerance to depth (Fig. 1). However, some show preferences for deeper bottoms (e.g. some species of the genus Nephasoma) and others are commonly found in shallower areas (e.g. some species belonging to genera Phascolosoma or Aspidosiphon). It is also known that nearly 75% of the species are present in shallow waters (<200 m), almost half in the euphotic zone of the continental shelf (Murina 1984).

Regarding their latitudinal distribution, sipunculans are distributed from the equator to both geographic poles (82°N-77°S) (Murina 1984), with some species living at both poles (Murina 1975; Amor 1993). Distribution of most of the species is confined to tropical waters, reaching 73% of the total in the case of shallow water, with Southeast Asia as the area with the highest diversity (Murina 1984). Despite their small number of species and generally a low and highly variable abundance in littoral soft bottoms (Ferrero-Vicente 2014), sipunculans are able to extensively colonize the sea floor. Notable exceptions are brackish areas, evidence of their exclusively marine benthic character and their low or null osmoregulatory capacity, which prevents their development in areas with high salinity fluctuations. Sipunculans avoid low salinities, this being a significant factor influencing their horizontal distribution range (Murina 1984). An exception in European low salinity waters is the record of Nephasoma diaphanes diaphanes in the Black Sea by Jakubova (1948). Sipunculans are far less sensitive to salinities over 35 psu; the Red Sea with its rich and abundant...
Sipuncula fauna may serve as an example of their natural habitat, characterized by maximal salinities of 40 to 44‰ (Murina 1984).

Most sipunculan species have been traditionally considered to be cosmopolitan, due to their worldwide distribution. However, recent studies suggest high levels of genetic differentiation between individuals of distant populations (Kawauchi & Giribet 2010, 2013; Schulze et al. 2012), which could be leading to processes of differentiation and speciation.

Sipunculans occupy most marine habitats, although their distribution range and environmental preferences are little known. They are present in most typical marine habitats: burrowing into soft substrata (from mud to coarse sand or gravel) as well as in holes bored into coral reefs, limestone rock, coralligenous beds, detritus, between the pores of volcanic rock; also as associated

![Fig. 1: Vertical known distribution for all Mediterranean sipunculans (depth range restricted to Mediterranean waters).](image-url)
fauna with seagrasses, seaweeds, sponges and corals, taking refuge in empty shells and tubes, or almost anywhere they are protected (e.g. Hyman 1959; Murina 1984; Cutler 1994; Açı̇k 2008a, 2011; Adrianov & Maiorova 2010; Singh et al. 2013, Ferrero-Vicente 2014; Ferrero-Vicente et al. 2016).

In the case of the Mediterranean Sea, there are 37 known species and subspecies of peanut worms, considering only those with valid status in the WoRMS database (World Register of Marine Species; access date: February 2016). Although not a high number, this represents almost a quarter of the total sipunculan species (~150 species in Cutler 1994).

None of these 37 species is endemic to the Mediterranean, despite the presence there of many other endemic invertebrate taxa (Cutler 1994; Boudouresque 2004; Coll et al. 2010). Absence of endemism is common within Sipuncula (Cutler 1994; Kędra & Murina 2007), which gives us an idea of the cosmopolitan distribution of this taxonomic group. Despite a revision of the zoogeography of peanut worms in the Mediterranean (Pancucci-Papadopoulou et al. 1999), even in such highly studied areas the species distribution and processes influencing it are still barely known, especially in deep water (Açı̇k 2008a; Coll et al. 2010; Saiz et al. 2014; Ferrero-Vicente 2014). In Mediterranean coastal waters, knowledge of their species distribution and ecology has undergone remarkable development during the last 10 years, mostly due to numerous studies in both the eastern basin (Açı̇k et al. 2005; Açı̇k 2007-2014) and the western (Ferrero-Vicente 2014, Ferrero-Vicente et al. 2011-2016).

Sipunculans can be found in almost all coastal habitats of the western Mediterranean, whether soft substrata (mud, sand or gravel) or hard such as limestone reefs, within coral skeletons such as Oculina patagonica or Cladocora caespitosa, or inhabiting mollusc reefs of the species Dendropoma lebeche (Ferrero-Vicente et al. 2016). Additionally, inside the rhizome of Posidonia oceanica, they may live in the sediment trapped between photophilic rhizoids of algae, which sometimes show high abundances of Golfingia vulgaris (Ferrero-Vicente, pers. observ.). They can also
appear within clusters of mussels (particularly of the genus *Phascolosoma*) inside sponges, coralline bottoms and virtually any type of habitat, although their abundances are generally low or concentrated in aggregations. They may however achieve relatively high abundances in coral skeletons of *Oculina patagonica* (Ferrero-Vicente *et al.* 2016; Ferrero-Vicente, pers. observ.).

In the eastern Mediterranean, sipunculans inhabit a variety of habitats such as soft substrata: mud, sand, muddy sand, sandy mud, muddy sand with *Caulerpa prolifera*, muddy sand with *C. cylindracea*, muddy sand with *Flabellia petiolata*, algae: *Amphiroa rigida*, *Ellisolandia elongata*, *Cystoseira crinita*, *C. elegans*, *C. spinosa*, *Halopteris scoparia*, *Jania rubens*, *Padina pavonica*, *Sphacelaria cirrosa*, *Sargassum hornsuchi*, rocks, the mussel *Brachidontes pharaonis*, *Spondylus gaederopus*, *Pinna nobilis*, sponges (*Sarcotragus* sp.), seagrasses: *Posidonia oceanica*, *Zostera marina* (Açik *et al.* 2005; Açik 2007-2011). Most specimens of *Aspidosiphon muelleri* and *Apionsoma misakianum* were found within empty shells of molluscs *Bittium reticulatum*, *Vexillum eburnus*, *Antalis inaequicostata*, *A. panorma* and *Nassarius pygmaeus* and tubes of serpulid polychaetes *Vermiliopsis* sp. and *Serpula* sp. (Açik 2007a; 2011). Zavodnik & Murina (1976) reported *A. muelleri* within shells of *Turritella cingulata*, *Murioidea blainvillei*, *Antalis dentalis* and *Antalis vulgaris* in the north Adriatic Sea. Koukouras *et al.* (1985) also found it within shells of *Turitella communis* in the north Aegean Sea.

### Alien species

The Mediterranean Sea has been greatly under the influence of alien species, mainly introduced to the region via the Suez Canal and shipping (Çinar *et al.* 2011). A total of 955 alien species have been reported from the region up to date (Zenetos *et al.* 2010). Six alien sipunculan species belonging to three families were listed in the Mediterranean Sea by Zenetos *et al.* (2010) and Çinar *et al.* (2011). Among them, four (*Apionsoma misakianum, Phascolosoma scolops,*
Aspidosiphon mexicanus, and A. elegans) have become established in the region and two (Apionsoma trichocephalus and Phascolion convestitum) are casual. More recently, Açik (2011) gave the first record of Nephasoma eremita in the Mediterranean Sea. This species might have been introduced to the region via ballast water of ships. In the western basin, Ferrero-Vicente et al. (2012) reported the first record of Phascolion caupo and questioned whether the species came from the Atlantic Ocean or is an autochthonous species frequently identified as the ubiquitous P. strombus.

Morphology

This section briefly describes the main anatomical characteristics of sipunculans, especially those with major relevance to species identification. Their body is divided in two main sections: the trunk, and the introvert or retractable proboscis, which can range from half to several times the trunk in length. It can be retracted into the trunk by contraction of the retractor muscles. The introvert ends in a tentacular crown where the mouth is located (Fig. 2). These tentacles are more developed or less so, depending on the taxonomic group. Hooks are present throughout the introvert, either scattered or forming rings, especially in its distal part. Observed under the compound microscope, these are extremely important as taxonomic characters, as are the numerous papillae (of various shapes and sizes) and other skin bodies often situated on the integument of most species.

The internal anatomy of sipunculans is characterized by the arrangement of the digestive tract, which is unique and distinctive of sipunculans. The intestine forms a U-shaped twisted loop. The gastrointestinal tract begins in the distal part of the proboscis, where the mouth is located, and ends at the anus, near the base of the introvert. The digestive tract is attached to the body wall by several fastening muscles. From one to four retractor muscles are present (important taxonomic character), extending from the body wall (the insertion point may also be a discriminative
Fig. 2: General anatomy of a sipunculan worm.
character) and inserted into the base of the esophagus, behind the cerebral ganglia. The presence of villi on the contractile vessel is diagnostic for some species (genus *Thysanocardia*). There may be one (genera *Phascolion* and *Onchnesoma*) or two nephridia located ventro-laterally to the anterior part of the trunk, although it is a character that shows intra-specific variability. The nervous system consists of a subepidermal nerve cord that runs along the ventral part of the animal coming from a bilobed cerebral ganglion. The peripheral nervous system is based on lateral nerves departing irregularly from the ventral cord.

Some species belonging to the order Aspidosiphoniformes present a hardened structure forming a shield on the front of the trunk, which functions like the mechanical operculum of a gastropod, protecting individuals who seek refuge in crevices or empty shells. Another shield structure also appears at the back of the trunk, which might play an anchoring role or help rock-boring species make their burrows. Members of the genus *Phascolion* present a dense aggregation of papillae in the anal area, which could also function as an operculum, to avoid predation when these animals dwell in shells or tubes discarded by other animals (Cutler 1994, Ferrero-Vicente et al. 2013b).

### Ecology

With few exceptions, four major ecological groups can be distinguished regarding their mobility, habitat and feeding behavior:

1. **Burrowers;** actively moving worms which indiscriminately swallow the fine particles of the substratum. They do not burrow continuously as earthworms do and once the tunnel has been created these species tend to remain protected in it. This group comprises most of the large sand-dwelling species in the class Sipunculidae.

2. **Semimobile worms hiding in empty shells and tubes** (Fig. 3). They usually show reduced mobility, anchoring the
proboscis to the sediment and pulling the occupied shell/tube by compressing the retractor muscles. They collect detritus from the sediment surface by extending their proboscis around the surrounding sediment. This group comprises many *Phascolion* and *Aspidosiphon* species in the case of Mediterranean sipunculans. Their presence in the sediment is linked to the availability of shells/tubes (Ferrero-Vicente et al. 2013b).

3. Free-living, semimobile, waiting sestonophages (epifauna and cryptofauna) that feed on very fine particles of suspended organic matter. Here are included species with a highly developed tentacular crown (e.g. in the genus *Themiste* and some in *Thysanocardia*).

4. Sessile, endolithic forms scraping detritus off the substratum with papillae and hooks of the introvert (cryptofauna). Within this group are included many species of the genera *Aspidosiphon* and *Phascolosoma*, with presence in rocky substrata and coral skeletons in the Mediterranean.

**Sensitivity to environmental changes**

Sipunculans have not been used as specific indicator species of environmental changes or deterioration. There are almost no studies on the effect of environmental stress on sipunculans or the utility of these organisms as bioindicator species. Indeed, Ferrero-Vicente (2014) points out that sipunculans are not very useful animals to be used in environmental studies on soft bottoms. This is basically because they do not fulfill the major characteristics of good indicators, despite most of their species presenting a worldwide cosmopolitan distribution. To start with, knowledge of sipunculan ecology and species distribution is scarce and background data are almost inexistent in many areas. Species identification is difficult for non-specialists since it often requires laborious dissection of the specimens. They also have low fidelity and specificity, conditioned by very high natural variability (Ferrero-Vicente, 2014).