

Article



# First Investigation of the Marine Gastrotrich Fauna from the Waters of North Tunisia, with the Description of a New Species of *Halichaetonotus* (Gastrotricha, Chaetonotida) <sup>†</sup>

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Abstract: Gastrotrichs (hairy bellies) are microscopic, acoelomate worms that live in freshwater and marine environments and represent one of the significant components of the meiobenthic communities. There are over 520 marine species, and several of them are also found in the Mediterranean Sea. However, data on the gastrotrich fauna from Tunisia is negligible, with nearly zero annotated records. We have initiated a series of investigations along the Tunisian shores to address this gap. Herein, we report data from four collecting sites on the northern Tunisian coastline. Sandy samples from 2 m water depth yielded 21 species, 11 in the order Macrodasyida and 10 in Chaetonotida. One chaetonotidan new to science is formally described, while morphometric data and photomicrographs are provided for all the others. Incidentally, we report on the longest gastrotrich ever found. Information about the microhabitat's characteristics, including the water's salinity and temperature and granulometric parameters of the sediment, are also reported. Finally, we provide information on Tunisian species previously discovered by our team and involved in phylogenetic studies for which morphological data and details about their findings have never been published. We explicitly address and correct the errors in GenBank concerning the attribution of gene sequences for these species.

**Keywords:** biodiversity; biogeography; Gastrotricha; Mediterranean Sea; meiofauna; north Africa; taxonomy

# 1. Introduction

The phylum Gastrotricha consists of small, benthic metazoans that are closely related to Platyhelminthes flatworms [1]. They are found in both freshwater and marine habitats. The group is highly diverse, comprising approximately 890 formally recognized species, distributed across 72 genera, 18 families, and two orders [2,3]: Macrodasyida, primarily found in marine environments, often with a worm-like shape and no outer cuticular ornamentations, and Chaetonotida, mostly found in freshwater, characterized by a tenpin shape and spiny features [2]. Gastrotrichs are abundant in various aquatic environments, especially in sandy marine habitats [2,4–6], where they play a significant role in interstitial



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Copyright: © 2024 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (https://creativecommons.org/ licenses/by/4.0/). meiofaunal communities. They can also be found in inland water bodies, where they may exist as epibenthic, periphytic, and even semi-planktonic organisms [7,8]. The majority of gastrotrich species are microscopic, ranging from 80 to 3500 µm in body length. Chaetonotidans are usually smaller than Macrodasyidans [2,8]. Members of both orders are microphagous and detritivores, feeding on bacteria, fungi, microalgae, and protists, which they ingest mainly by suction due to their strongly muscularized (myoepithelial) pharynxes. In turn, gastrotrichs are preyed upon by large protozoans and carnivorous meio- and macrofauna [7,9,10]. Their defensive strategies may involve the cuticular armor made of scales and/or spines, as well as (repellent) exudates from numerous epidermal glands [11,12].

The classification of Gastrotricha has traditionally relied on morphological traits. However, recent advancements in methodologies such as high-resolution microscopy and gene sequencing have allowed for innovative systematics. This often involves an integrated approach using both morphological and genetic data, which has revitalized interest in the biodiversity, taxonomy, and phylogeny of the group (see Kånneby and Todaro [13]; Todaro et al. [14–17]; Leasi and Todaro [18]; Schuster et al. [19]; Fromm et al. [20]; Garraffoni et al. [21]; Bosco et al. [22]; Campos et al. [23]; Martinez et al. [24]; Kieneke and Todaro [25]; Kolicka [26,27]; Kolicka et al. [28]; Minowa and Garraffoni [29]; Rataj Križanová and Vďačný [30,31]; Salgado et al. [32]; Araújo [33]; Saponi et al. [34]).

Advanced methodologies have also significantly contributed to our knowledge of the neuro-muscular organization (see Hochberg and Litvaitis [35–38]; Leasi and Todaro [39]; Leasi et al. [40]; Rothe et al. [41,42]; Kieneke and Ostman [43]; Münter and Kieneke [44]), reproductive biology (see Guidi et al. [45–48]; Cesaretti et al. [49]), as well as the biogeography of Gastrotricha (see Curini-Galletti et al. [50,51]; Kieneke et al. [52]; Magpali et al. [53]; Macher et al. [54]).

Many of the above-reported studies have been conducted on gastrotrich species from the Mediterranean region, which is globally recognized as being the best-studied area, particularly in terms of marine organisms [55]. Todaro et al. [56] summarized information on the biodiversity and distribution of Gastrotricha in the Mediterranean and its connected seas in 2003. Later works [57–60] were included in 2010 in an informal document by Hummon [61], which also reports the author's unpublished findings from research conducted in the Eastern Mediterranean basin (Israel, Cyprus, and Greece). For those seeking additional data and more recent information, several other papers [16,17,24,25,49,50,62–67] are available.

Currently, based on the sources mentioned above, the Mediterranean and the Black Sea are home to 197 described marine Gastrotricha species, which account for approximately 38% of all known marine species worldwide (522 species, WoRMS [68]). The existing knowledge about the Mediterranean gastrotrich fauna is mostly based on research from northern countries such as Italy and France. However, the coasts of African nations have been less explored, with the exception of the Egyptian coast [56,61]. The Moroccan coast has not been studied, and the available information for Algeria is limited to a few sites in the western part of the country [69]. The Tunisian coasts have records of seven species, but these reports are not from focused, comprehensive faunistic surveys, being derived from a paper focusing on the interstitial fauna of a single beach [70] or from a phylogenetic study involving also species found in Tunisia [71].

A recent research program agreement between the University of Tunis El Manar (Tunisia) and the University of Modena and Reggio Emilia (Italy) aims to shed light on the Tunisian meiofauna biodiversity. As part of this agreement, a series of investigations have been initiated along the Tunisian shores to address the gap in knowledge of Tunisian marine gastrotrich biodiversity. In this report, we present the results of a faunistic survey conducted along the country's northern coastline. Additionally, we provide morphometric and other information on four Tunisian species (*Dactylopodola typhle* (Remane, 1927) [72], *Mesodasys littoralis* Remane, 1951 [73], *Tetranchyroderma esarabdophorum* Tongiorgi & Balsamo, 1984 [74], and *Xenotrichula intermedia* Remane, 1934 [75] that were previously collected by our team. These species have been involved in phylogenetic studies, but the details about their findings have never been published [71].

# 2. Materials and Methods

# 2.1. New Samples

On 16 September 2023, a sampling campaign was carried out by skin diving along the coasts of Bizerte, north of Tunisia. Samples of sandy material were collected at a depth of 2 m from four stations: Cap Angela (St1), Les Grottes (St2), Rimmel Beach (St3), and Rimmel Epave (St4) (Table 1, Figure 1). We started our sampling in the northern area for logistical reasons, as it is the most accessible region to us. The selected beaches reflect the physiographic characteristics of the northern coast of Tunisia and are located relatively close to one another, allowing us to conduct sampling within a single day. The limited number of sampling stations is designed to facilitate an in-depth in vivo study of the fauna within a reasonable timeframe (see below). At each station, sand was manually dredged from the top 10 cm layer of sediment using two 500 mL jars for each station. The collected material was transported to Modena, Italy the next day. In the laboratory, the sediment was analyzed for fauna within 10 days. Gastrotrichs were extracted from the sand using the narcotization decantation technique. In short, a spoonful of sand from a single jar was transferred each time to a beaker, and a 7% magnesium chloride solution was added to cover the sand. After 5 min, the supernatant was poured into 5 cm Petri dishes and scanned for specimens under a Wild M8 stereomicroscope [2]. Upon detection, gastrotrichs were carefully suctioned out using a handheld micropipette and whole mounted in a drop of MgCl<sub>2</sub> solution on a microscope slide. The morphological survey was conducted on living, (mostly) relaxed individuals using a light microscope equipped with differential interference contrast optics (Nikon Eclipse Ni-U, Tokyo, Japan) and a high-resolution digital camera (Nikon Fi3) for photographic recording. Measurements were taken from the photomicrographs using the Nikon NIS D v.4 software.

Salinity Temperature Date of Location Station Latitude Longitude (‰) (°C) Sampling 37°19′53″ N 09°46′10″ E St1 40 25 16 September 2023 Cap Angela 37°19'60" N 09°50'38" E Les Grottes St2 40 25 16 September 2023 37°15′34″ N 09°54'12" E **Rimel Beach** St3 40 25 16 September 2023 37°15′11″ N 09°56'35" E 25 **Rimel Epave** St4 4016 September 2023 36°16′46″ N 10°29'37" E 15 Bouficha а 38.5 13 March 2008 35°31′17″ N 11°02′37″ E 38.5 Mahdia b 15 13 March 2008

**Table 1.** Characteristics of the new and old sampling locations along the Tunisian coast. Stations, geographic coordinates, salinity, temperature, and date of sampling.



**Figure 1.** Maps of the Tunisian locations where marine Gastrotricha were sampled: (**A**) General map displaying Tunisia's position in the Mediterranean Sea area; (**B**) satellite map of Tunisia with a close-up on the four locations sampled in September 2023 (1—Cap Angela St1; 2—Les Grottes St2; 3—Rimel Beach St3, 4—Rimel Epave St4). In the same map (**B**), the yellow dot indicates the area around Tunis investigated by Westheide [70], while the cyan dots mark the locations from which the four species reported in Todaro et al. [71] were sampled (a—Bouficha; b—Mahdia). See text and Table 1 for further details.

# 2.2. Old Samples

Sampling campaign was conducted on 13 March 2008, using the procedure described above. Sandy samples containing *Xenotrichula intermedia* and *Tetranchyroderma esarab-dophorum* were collected from Mahdia (b), and those that yielded *Dactylopodola typhle* and *Mesodasys littoralis* were collected from Bouficha (a) (Table 1, Figure 1). The collected material was transported the next day to Modena and processed as described above except for the following differences: taxonomic survey and photographic recording were conducted under a Nikon Eclips 90i microscope equipped with DIC optics and a DS-5Mc Nikon digital camera, driven by the UCT-2U v.3 Nikon software.

## 2.3. Species Description

The species description adheres to the standards set by Hummon et al. [76]. The position of morphological traits along the body is given in percentage of unit (U) relative to the total body length, from front to rear. Abbreviations used in the text are as follows: PhIJ—pharyngo-intestinal junction; TbA—anterior adhesive tube; TbD—dorsal adhesive tube; TbDL—dorsolateral adhesive tube; TbL—lateral adhesive tube; TbP—posterior adhesive tube; TbV—ventral adhesive tube; TbVL—ventrolateral adhesive tubes; TL—total body length.

#### 2.4. Granulometric Analysis

The sediment's grain size distribution was analyzed using dry sieving as described in Todaro et al. [17,57]. The mean grain size, sorting coefficient, kurtosis, and skewness were

computed using a computer program [77] based on the equation developed by Seward-Thompson and Hail [78].

# 3. Results and Discussion

# 3.1. Sampling Stations

The water temperature and salinity of the sampling stations in 2023 were higher than those in 2008 (Table 1). This difference is clearly linked to the time of year when the sampling took place: early spring in 2008 and late summer in 2023.

#### 3.2. Granulometric Analysis

The sediment's granulometric characteristics varied slightly across the sampled stations. It mainly consisted of medium, moderately well-sorted, or sorted sand. The exception was the sediment at two locations, Rimel Beach (St3) and Mahdia (b), which was made up of fine sand (Table 2).

**Table 2.** Granulometric characteristics of the four stations sampled in 2023 (St1–St4) and two stations sampled in 2008 (a, b).

| Station | Mean Grain<br>Size (phi) | Size Class  | Sorting | Sorting Class             | Kurtosis | Skewness |
|---------|--------------------------|-------------|---------|---------------------------|----------|----------|
| St1     | 1.96                     | Medium sand | 0.61    | Moderately<br>well-sorted | 2.29     | -0.17    |
| St2     | 1.94                     | Medium sand | 0.62    | Moderately<br>well-sorted | 2.40     | -0.17    |
| St3     | 2.05                     | Fine sand   | 0.63    | Moderately<br>well-sorted | 2.68     | -0.54    |
| St4     | 1.71                     | Medium sand | 0.77    | Moderately sorted         | 2.58     | -0.24    |
| а       | 1.37                     | Medium sand | 0.72    | Moderately sorted         | 3.08     | -0.21    |
| b       | 2.33                     | Fine sand   | 0.83    | Moderately sorted         | 6.50     | -1.68    |

# 3.3. Gastrotrich Fauna

The faunistic analysis of the sandy samples collected in 2023 from the four locations along the north Tunisian coast (Bizerte) yielded 21 putative species and a total of 31 records (species x location) or a mean of  $7.8 \pm 2.2$  SD species per location (Table 3). Cap Angela (St1) displayed the highest species richness with eleven species (35% of total species), followed by Rimel Beach (St3) with eight species (26%). Rimel Epave (St4) and Les Grottes (St2) have the lowest species richness with seven (23%) and five (16%) species. It is noteworthy that there is a low overlap in fauna among the investigated stations (also see Tables S1 and S2). The maximum overlap observed is just three species that are shared between stations st1 and st3, which are characterized by distinctly different sediment types (st1 has medium sand, while st3 has fine sand). In this framework, it is somewhat paradoxical that station st3 is richer in Macrodasyida. This finding contrasts with most literature, which suggests that Chaetonotida typically prefer finer sediments (see [5,79]). Future studies, ideally involving higher sampling efforts and quantitative approaches, should aim to confirm or challenge our preliminary findings.

Eleven species in ten genera and six families belong to the order Macrodasyida, and ten species in six genera and two families belong to the order Chaetonotida (Table 3).

| Taxon   | St1 | St2 | St3 | St4 |
|---|-----|-----|-----|-----|
| MACRODASYIDA                                    |     |     |     |     |
| Cephalodasvidae                                 |     |     |     |     |
| Dolychodasys sp1                                | -   | -   | -   | +   |
| Dactylopodolidae                                |     |     |     |     |
| Dactylopodola typhle                            | -   | -   | +   | +   |
| Macrodasyidae                                   |     |     |     |     |
| Urodasys viviparus                              | -   | -   | -   | +   |
| Planodasyidae                                   |     |     |     |     |
| Megadasys sp1                                   | -   | -   | -   | +   |
| Thaumastodermatidae                             |     |     |     |     |
| Acanthodasys aculeatus                          | +   | -   | +   | -   |
| Diplodasys sanctimariae                         | -   | +   | -   | -   |
| Pseudostomella etrusca                          | -   | -   | +   | -   |
| Tetranchyroderma heterotubulatum                | -   | +   | +   | -   |
| Tetranchyroderma papii                          | +   | -   | -   | -   |
| Turbanellidae                                   |     |     |     |     |
| Paraturbanella teissieri                        | +   | -   | +   | -   |
| Turbanella bocqueti                             | -   | +   | -   | +   |
| Number of macrodasyidan species<br>by locations | 3   | 3   | 5   | 5   |
| CHAETONOTIDA                                    |     |     |     |     |
| Chaetonotidae                                   |     |     |     |     |
| Aspidiophorus mediterraneus                     | +   | -   | -   | -   |
| Aspidiophorus paramediterraneus                 | +   | -   | -   | +   |
| Chaetonotus dispar                              | +   | -   | -   | +   |
| Halichaetonotus bizertae sp. nov.               | +   | +   | -   | -   |
| Halichaetonotus thalassopais                    | +   | +   | -   | -   |
| Halichaetonotus euromarinus                     | +   | -   | -   | -   |
| Heterolepidoderma loricatum                     | +   | -   | +   | -   |
| Xenotrichulidae                                 |     |     |     |     |
| Draculiciteria tesselata                        | -   | -   | +   | -   |
| Heteroxenotrichula sp1                          | +   | -   | -   | -   |
| Heteroxenotrichula sp2                          | -   | -   | +   | -   |
| Number of chaetonotidan species<br>by locations | 8   | 2   | 3   | 2   |
| Total gastrotrich species<br>by locations       | 11  | 5   | 8   | 7   |

**Table 3.** Gastrotricha species found (+) at the four locations along the northern coast of Tunisia (Bizerte) investigated in September 2023.

Out of the 21 species found, sixteen are known and have been previously reported in the Mediterranean Sea, see [56,79]. One species of the genus *Halichaetonotus* is represented by specimens with a unique set of morphological traits, suggesting that it may be a new species. This new species is formally named and described below. To accurately determine whether the remaining four species are already classified or warrant descriptions as new species, more comprehensive data are essential. Therefore, they have been provisionally named *Dolichodasys* sp1, *Heteroxenotrichula* sp1, *Heteroxenotrichula* sp2, and *Megadasys* sp1 (Table 3).

Morphometric information along with photomicrographs as vouchers are provided for all recorded species under the taxonomic account section below.

#### 3.4. Taxonomic Account

Order Macrodasyida Remane, 1925 [80] [Rao & Clausen, 1970] [81] Family Cephalodasyidae Hummon & Todaro, 2010 [82] Genus *Dolichodasys* Gagne, 1977 [83] *Dolychodasys* sp1 Material recorded: A single specimen from Rimel Epave (St4). Description: The single extremely elongated specimen measures 2626

**Description:** The single, extremely elongated specimen measures 2626  $\mu$ m in TL (Figure 2). Unfortunately, the specimen was damaged during the mounting and observation;

therefore, not all the morphological traits could be recorded for complete identification. However, we were able to observe the following traits: two TbA, with one on each side (Figure 2C), the posterior end was not clearly lobed, and there were several TbP along its margin (four visible, seven estimated) (Figure 2B).



**Figure 2.** Photomicrographs of the adult specimen of *Dolichodasys* sp1 found at Rimel Epave: (**A**) habitus; (**B**) posterior end showing the posterior adhesive tubes (arrows); (**C**) anterior end, ventral view, showing one of the anterior adhesive tubes and the mouth. m-mouth; TbA—anterior adhesive tubes; TbP—posterior adhesive tubes. Differential interference contrast microscopy (Nomarski).

**Remarks:** Among macrodasyidan Gastrotricha, only specimens of two genera, *Megadasys* and *Dolichodasys*, may reach the total body length of the observed specimen. Although severely damaged, the few visible traits allow us to identify the studied gastrotrich as a species of *Dolichodasys*. In fact, the presence of a single TbA on each side, the posterior end being not clearly lobed, and very few TbP along its margin are diagnostic traits for *Dolichodasys* and, at the same time, exclude it from being a member of the genus *Megadasys*.

## Genus Mesodasys Remane, 1951 [73]

# Mesodasys littoralis Remane, 1951 [73]

Material recorded: Five specimens from Bouficha, collected in 2008.

**Description:** The following morphometric information is derived from an adult specimen reaching 980  $\mu$ m in TL (Figure 3). The body is strap-shaped with parallel sides that narrow posteriorly, then widen slightly towards the caudal lobe. The head is indistinct, carrying several sensorial cilia, some arranged in lateral clusters. Piston pits and eye spots are absent (Figure 3E). The TbA are arranged in three to four transverse rows ventrally behind the mouth, counting nine to ten pairs (Figure 3E). For TbVL, there were 43 per side, with 22 along the pharyngeal region; TbL were present but difficult to count; no TbD were seen; and for TbP, there were eight tubes at the edge of the caudal lobe (Figure 3B). The mouth, measuring 21.4  $\mu$ m in diameter, opens anteriorly and leads into a cup-like buccal cavity (31.6  $\times$  38  $\mu$ m); the pharynx is 352  $\mu$ m long and bears pores near the base (U38); the PhIJ is at U40; the intestine is sac-like, wider at the front than at the rear; and the anus opens ventrally at U84. This organism is a hermaphrodite, possessing paired testes that begin at the PhIJ (Figure 3D), with sperm ducts connecting to a small copulatory organ situated posteriorly (U79) (Figure 3C). The ovary is unpaired, and a developing egg, 86  $\times$  77  $\mu$ m, is located dorsally to the gut, at approximately half the length of the body.



**Figure 3.** Photomicrographs of *Mesodasys littoralis* found at Bouficha: (**A**) habitus; (**B**) close-up of posterior region, ventral view showing the adhesive tubes around the caudal lobe (arrows); (**C**) close-up of trunk region, internal view, showing the copulatory organ (arrow); (**D**) close-up of the internal view at the pharyngo-intestinal junction (arrowhead) showing the sperm-filled testes (arrow); (**E**) close-up of the anterior region, ventral view, showing the anterior adhesive tubes (arrows). cop—copulatory organ; m—mouth; ph—pharynx; PhIJ—pharyngo-intestinal junction. Differential interference contrast microscopy (Nomarski).

**Remarks:** *M. littoralis* was initially described from Cuma, Italy [73]. The species has repeatedly been found along the western shores of Italy [56,84,85], other Mediterranean coastlines [56,61,69], and along the North Sea and the Atlantic coasts of Europe [61]. However, despite the numerous records, comprehensive information regarding the anatomical traits of the specimens found is minimal. The morphometric characteristics of the Tunisian specimens align with literature data on the species. In particular, the large size of the current specimens is most similar to the size of the Italian specimens reported by Wilke [85] and those from Israel that were video-recorded by W.D. Hummon http://www.gastrotricha.unimore.it/moviegallery.htm" (accessed on 24 November 2024). It is essential to highlight that a specimen of *M. littoralis* from Bouficha was utilized by Todaro et al. [71] to obtain nucleotide sequences of the nuclear 18S and 28S rRNA genes. These sequences are available in GenBank under the following accession codes: JF357658.1 for 18S and JF357706.1 for 28S. A partial sequence of the mitochondrial COI gene was obtained from the same specimen (voucher MT45) and is available in GenBank under the accession code JF432044. However, due to a submission error, this sequence is incorrectly labeled as belonging to a different specimen (voucher MT50). The repository has not responded to requests for correction [86].

Family Dactylopodolidae Strand, 1929 [87]

Genus Dactylopodola Strand, 1929 [87]

Dactylopodola typhle (Remane, 1927) [72]

**Material recorded:** Three specimens, two from Rimel Beach (St3) and one from Rimel Epave (St4).

**Description:** All the specimens found were in the juvenile stage, meaning they did not have visible sperm or ova inside. The largest specimen was about 200  $\mu$ m in TL (Figure 4). It had a distinct pentagonal head, a noticeable neck constriction, and a bilobed caudum originating from a stalked posterior region. The adhesive apparatus consisted of a pair of TbA on each side located ventrally under the head, three pairs of TbL along the trunk region (arranged as 1 and 1 + 1 tubes on each side), and a pair of TbP on each side at the end of the bilobed caudum.





**Figure 4.** Photomicrographs of a juvenile of *Dactylopodola typhle* found at Rimel Beach: (**A**) habitus, dorsal view; (**B**) habitus, ventral view. C—bilobed caudum; TbA—anterior adhesive tubes; TbL—lateral adhesive tubes. Differential interference contrast microscopy (Nomarski).

**Remarks:** The identification of gastrotrich species should be based on adult specimens, as the metric and meristic characteristics vary during the ontogenetic development [2,14,88]. Consequently, the morphometric characteristics of the studied specimens alone could not allow a precise species determination; however, the reported presence of *D. typhle* at nearby Bouficha and the record of juveniles with matching morphology in a large population of *D. typhle* in Italy [89] reasonably suggest that the present specimens are members of the same taxon (i.e., *D. typhle*), especially considering that *D. typhle* has been recorded in several Mediterranean locations [61]. A specimen of *D. typhle* from Bouficha was used by Todaro et al. [71] to obtain nucleotide sequences for the nuclear 18S and 28S rRNA genes, as well as the Folmer region of the mitochondrial COI gene. These sequences, which are designated as voucher MT37, are available in GenBank under the following accession codes: JF357652.1, JF357700.1, and JF432037.1.

Family Macrodasyidae Remane, 1924 [90]

Genus Urodasys Remane, 1926 [91]

*Urodasys viviparus* Wilke, 1954 [85]

Material recorded: A single specimen from Rimel Epave (St4).

**Description:** The specimen that was examined measures 368  $\mu$ m in TL and has a maximum trunk width of 56  $\mu$ m. The tail is approximately 1550  $\mu$ m long (Figure 5). The pharynx measures 162  $\mu$ m in length. Along the trunk region, there is a pair of TbA and approximately five pairs of TbL. Additionally, one pair of TbD is observed in the mid-pharyngeal region and another in the posterior trunk region. Two relatively large, highly refractive glands are present in the anterior region just posterior to the piston-pit organs. Furthermore, the observed specimen contains an internal embryo at an early developing stage (egg cleavage) (Figure 5B).

**Remarks:** The extremely long tail is the distinctive characteristic of the genus *Urodasys* [92]. However, while most congeneric species are hermaphroditic, U. viviparus lacks testes and accessory reproductive structures [85] while retaining the paired ovaries. The maturing eggs are retained inside the body, where they develop by parthenogenesis, and the offspring are eventually delivered by rupture of the body wall (ovoviparity) [49,93,94]. *U. viviparus* has a cosmopolitan distribution (see Todaro and Rocha [95]; Hummon [61]; Hochberg et al. [93]) and in the Mediterranean Sea, it has been recorded in France, Croatia, and Greece and is widespread along the Italian coastlines [61,96–98]. Morphometrics of the *U. viviparus* specimen from Rimel Epave agrees with the literature data on the species [85,93,96].



**Figure 5.** Photomicrographs of *Urodasys viviparus* found at Rimel Epave: (**A**) habitus; (**B**) close-up of the posterior trunk region showing an early stage (egg cleavage) of the internal embryo. Em—embryo. Differential interference contrast microscopy (Nomarski).

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Family Planodasyidae Rao & Clausen, 1970 [81]
Genus Megadasys Schmidt, 1974 [99]
Megadasys sp1
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**Material recorded:** Four specimens, three adults, and a subadult from Rimel Epave (St4).

Description: The adult examined specimens range from 2773 µm to 3867 µm in TL (Figure 6). The body is naked and highly elongated, with an indistinct head and a rounded caudal lobe (70  $\mu$ m wide  $\times$  40  $\mu$ m long). The adhesive apparatus consists of four pairs of TbA as a continuation of the ventrolateral tubes, with at least twenty-four pairs of TbVL (Figure 6C); TbL and TbD are present but are difficult to count due to the twisting of the animals. There are at least 20 TbP distributed along the margin of the caudal lobe. Ventral locomotory cilia are arranged in two bands spanning almost the entire animal length. The mouth is terminal and 23–25 µm in diameter, leading into a shallow, almost cylindrical buccal cavity and then to a 555–830 µm long pharynx. The PhIJ is at about U22. The pharyngeal pores are located slightly away from the pharyngeal base at U17. The adults are simultaneous hermaphrodites; the paired testes originate well past the PhIJ, with the deferens filled with sperm and leading back along the intestine's sides, joining on the right side anterior to the mature egg (about U52) (Figure 6F,G). The paired ovaries have a mature egg centered at U60. The caudal organ is bullet-shaped, measuring 206  $\mu$ m long  $\times$  48  $\mu$ m wide, and is almost equally divided into an anterior glandular region and a posterior muscular, hollow portion. The frontal organ, per se, was not observed, but a bundle of allosperm (>230  $\mu$ m long) (Figure 6E) was visible on the right side, just posterior to the ovary (U65). The subadult measured about 1200 µm in length and showed only three TbA and eight TbP (Figure 6B).



**Figure 6.** Photomicrographs of *Megadasys* sp1, found at Rimel Epave: (**A**) habitus of the 3867 μm long specimen; (**B**) close-up of the caudal lobe of the subadult specimen, showing the TbP (arrow); (**C**) anterior region, ventral view, of an adult specimen showing the TbA (arrow); (**D**) another adult specimen showing the internal reproductive structures; (**E**) close-up of the bundle of allosperm (arrow); (**F**) close-up of the sperm-filled deferens (arrow); (**G**) close-up of the deferens confluence (arrow). as—allosperm; de—deferens; co—caudal organ; mo—mature ovum; ov—ovary; sd—deferens splice. Differential interference contrast microscopy (Nomarski).

**Remarks:** The genus *Megadasys* includes three species: *M. pacificus* Schmidt, 1974 [99]; *M. sterreri* (Boaden, 1974) [100], and *M. minor* Kisielewski, 1987 [101]. The last two species have also been found in the Mediterranean Sea [24,79]. Hummon [61] reports *M. pacificus* from the Egyptian coast of the Red Sea and along the Suez Canal region. The large size of our specimens rules out the possibility of them being *M. minor*. However, the differences in some characteristics with the other two species (see a lower number of TbA and TbP) do not conclusively place the Tunisian species into either category. It is also possible that the Tunisian specimens belong to the undescribed species reported from Israel by Hummon [61]. On the other hand, the notable difference in the overall length of the examined adults (greater than 1 mm) may suggest that the specimens belong to two separate species. Due to these uncertainties, we prefer to keep the status of the current specimens undetermined. We hope to obtain additional information on this species as our investigation along the Tunisia coasts continues. Meanwhile, we highlight that one of the current specimens exceeds the 3.5 mm length of *M. pacificus* by more than 350 µm, making it the longest gastrotrich ever recorded.

Family **Thaumastodermatidae Remane**, **1927** [102] Sub-family **Diplodasyinae Ruppert**, **1978** [103] Genus *Acanthodasys* **Remane**, **1927** [102] *Acanthodasys aculeatus* **Remane**, **1927** [102]

**Material recorded:** Two specimens, an adult from Cap Angela (St1) and a juvenile from Rimel Beach (St3).

**Description:** The fully grown specimen measures 637  $\mu$ m in TL, with a maximum trunk width of 112  $\mu$ m (Figure 7).



**Figure 7.** Photomicrographs of the adult specimen of *Acanthodasys aculeatus* found at Cap Angela: (A) habitus, ventral view; (B) habitus, dorsal view. e—egg; m—mouth; PhIJ—pharyngo-intestinal junction; TbVL—ventrolateral adhesive tubes; TbP—posterior adhesive tubes. Differential interference contrast microscopy (Nomarski).

The body is covered with uniancres, which are longer on the dorsal side (measuring 8 to 13  $\mu$ m) than on the ventral side (measuring 4 to 5  $\mu$ m). The adhesive apparatus is made up of three pairs of TbA located behind the mouth, seven pairs of TbVL located along the pharyngeal and trunk regions, at least three TbD along the mid-line of the posterior pharyngeal and anterior trunk region, and six TbP, two at the end of each caudal foot and one at the inner margin of the foot. The TbVL are the longest, ranging from 21 to 25  $\mu$ m. The frontal mouth, which is 58  $\mu$ m in diameter, leads to a 198  $\mu$ m long pharynx. The reproductive system matches the genus's structure, but the rosette pore and allosperm in front of the largest oocyte were absent in the examined specimen.

**Remarks:** The morphometric data of the examined specimen is consistent with the original description [102]. The TL falls within the range observed in previous studies (500–800  $\mu$ m) in the Mediterranean Sea [96,97]. In the Adriatic Sea, specimens have been recorded with a TL of 988  $\mu$ m [98].

# Genus Diplodasys Remane, 1927 [102]

#### Diplodasys sanctimariae Hummon & Todaro, 2009 [60]

Material recorded: A single sub-adult specimen found at Les Grottes (St2).

**Description:** The examined specimen measures 226  $\mu$ m in TL (Figure 8). It has a large ovoid anterior mouth with a rounded oral hood (Figure 8A). The dorsal side is covered with five columns of 22 flat scales that have numerous fine openings (Figure 8B), and there are 32 lateral spines on each side, from U13 to U92. The adhesive apparatus consists of 10 TbA arranged in two rows, 22 TbV on each side, and 12 TbP, two of which are located at the end of each caudal pedicle (Figure 8C).

**Remarks:** The species was first found at Santa Maria di Leuca, Italy, and later at Cyprus and the Red Sea [60]. The general morphology and cuticular covering match the original description of *D. sanctimariae*. The metrics of the examined specimen approach those of the sub-adult found in Cyprus [60].



**Figure 8.** Photomicrographs of *Diplodasys sanctimariae* found at Les Grottes: (**A**) habitus; (**B**) anterior end, dorsal view, showing the cuticular scales; (**C**) posterior end, ventral view, showing the posterior adhesive tubes (arrows). Differential interference contrast microscopy (Nomarski).

Sub-family Thaumastodermatinae Remane, 1927 [102]

Genus Pseudostomella Swedmark, 1956 [104]

Pseudostomella etrusca Hummon, Todaro & Tongiorgi, 1993 [76]

Material recorded: A single adult specimen found at Rimel Beach (St3).

**Description:** The specimen measures 343  $\mu$ m in TL (Figure 9). Its body features well-developed preoral palps and posterior caudal pedicles. The preoral palps curve ventromedially, with the dorsal border extending slightly beyond the ventral side. On the dorsal surface, there are five papillae arranged in a 2 + 1 + 2 pattern along the inner border of the palps, with the largest papillae positioned laterally and smaller ones medially (Figure 9B). On the ventral side, there are six to eight recognizable papillae, approximately the same size, symmetrically arranged towards the center (Figure 9C). The cuticular armature comprises relatively robust five-spined hooks (pentancres) covering the dorsal and ventrolateral aspects. The adhesive apparatus consists of four pairs of TbA on each side: three are located laterally, and one is positioned at the ventral border of the mouth (Figure 9C). Additionally, there are ten pairs of TbL along the trunk region on each side, three TbP (two at the ends of each caudal pedicle and one medially), and two TbDL, one on the trailing surface of each oral palp (Figure 9C) and one near the base of each caudal pedicle. The reproductive system is hermaphroditic. There is a single testis on the right side of the body, which leads to an extended structure that connects to a pear-shaped, glandular-muscular caudal organ, which is in turn connected to a vesicular frontal organ. Small, developing oocytes are present in the posterior trunk region.

**Remarks:** The species is endemic to the Mediterranean Sea and is widespread in both the western and eastern basins [61,76,105]. The morphometric data of the studied species agree with the original description, except for the lower number of TbL (10 pairs vs. 14 pairs). The difference may be an intraspecific variation due to the difficulty of recognizing smaller additional tubes in our specimen [76].



**Figure 9.** Photomicrographs of *Pseudostomella etrusca* found at Rimel Beach: (**A**) habitus, with indicated pre-oral palps and the caudal pedicles; (**B**) anterior region, dorsal view, showing the cuticular scales and the dorsal papillae of the palps; (**C**) anterior region, ventral view; showing the anterior and dorsolateral adhesive tubes and the ventral papillae on the palps. cp—caudal pedicles; dpp—dorsal papillae of the palps; TbA—anterior adhesive tube; TbDL—dorsolateral adhesive tube; vpp—ventral papillae of the palps Differential interference contrast microscopy (Nomarski).

# Genus Tetranchyroderma Remane, 1926 [91]

# Tetranchyroderma esarabdophorum Tongiorgi & Balsamo, 1984 [74]

**Material recorded:** Two specimens, a subadult and a juvenile from Mahdia collected in 2008.

**Description:** The subadult specimen measures 252 µm in total length (Figure 10). The body is covered by pentancres and features three pairs of rod-like tubes on its dorsal side, which give the species its name. One pair is situated in the pharyngeal region (U28), while the other two pairs are located along the trunk (U47 and U64) (Figure 10A). There are four pairs of epidermal glands. The head has dorsal rod-like tentacles, sometimes referred to as antennae, and lateral knob-like sensory organs (Figure 10B). The caudal region consists of two short caudal pedicles. The adhesive apparatus includes, on each side, three TbA: a medial one located behind the mouth rim and two lateral ones. Additionally, there are 11 TbVL; the first is found in the anterior pharyngeal region (U20) (Figure 10C) and the others extend along the trunk. There is one TbDL in the posterior trunk region (U89) and four TbP, with three at the end of each caudal pedicle and one tube along the inner margin of the pedicle.

**Remarks:** *T. esarabdophorum* was initially described from the northern Adriatic Sea, specifically at Pesaro [74]. It has since been reported in various locations throughout eastern and western Italian coastlines [79] and is also known from the western Mediterranean Sea [56,61]. Unlike our subadult specimen, adult *T. esarabdophorum* can reach a total length (TL) of up to 350 µm and typically possess four pairs of TbA and up to 22 pairs of ventral tube appendages (TbVL). Nonetheless, the distinct pentancrous covering, the head furnished of rod- and knob-like tentacles, and the three pairs of dorsal rod-like tubes clearly indicate that the specimens from Madhia belong to *T. esarabdophorum*. Notably, a specimen collected in 2008 at Madhia has yielded nucleotide sequences for the nuclear 18S and 28S rRNA genes, as well as the mitochondrial COI gene [71]. These sequences, designated as



voucher MT07, are available in GenBank under the following accession codes: JF357627.1, JF357675.1, and JF432022.1.

**Figure 10.** Photomicrographs of a subadult specimen of *Tetranchyroderma esarabdophorum* found at Mahdia in 2008: (**A**) habitus, dorsal view, showing the head rod-like tentacles (arrowhead) and the dorsal rod-like tubes (arrows); (**B**) anterior region, dorsal view, showing the head knob-like tentacles (arrow); (**C**) anterior region, ventral view, showing the TbA (arrows). eg—epidermal glands; TbDL—dorsolateral adhesive tubes; TbVL—ventrolateral adhesive tubes. Differential interference contrast microscopy (Nomarski).

#### Tetranchyroderma heterotubulatum Hummon, Todaro & Tongiorgi, 1993 [76]

**Material recorded:** Four specimens, two from Les Grottes (St2) and two from Rimel Beach (St3).

**Description:** The following morphometric information is derived from the largest specimen found at Les Grottes, which measures 323 µm in TL (Figure 11). The head is bilobed and features a subterminal mouth that is 33 µm wide. The pharynx measures 93 µm in length, with pharyngeal pores located near its base. The pharyngeal intestinal junction (PhIJ) is situated at U36. Approximately 11 pairs of epidermal glands are scattered along the sides of the body, which are covered with robust tetrancres. The adhesive apparatus includes three pairs of TbA (Figure 11B), eight pairs of TbVL, inserted from U42 to U78, two pairs of TbD at U55 and U98 (Figure 11A), and five pairs of TbP. The latter comprises two tubes at the end of each caudal pedicle, two along the medial side of the pedicle, and one on its lateral side (Figure 11C). The glandular-muscular caudal organ is pear-shaped, while the vesicular frontal organ is roughly spherical and contains a bundle of sperm inside (Figure 11D).

**Remarks:** The species exhibits an amphiatlantic distribution. Originally described in Italy, it has also been found in northern Europe and along the Atlantic coast of the USA [61]. In the Mediterranean Sea, it is relatively common in both the western and eastern basins [56,106]. The metric and meristic data of specimens from Tunisia fall within the range of morphometric information known for the species (see Hummon et al. [76]).



**Figure 11.** Photomicrographs of an adult specimen of *Tetranchyroderma heterotubulatum* found at Les Grottes: (**A**) habitus, with indicated the dorsal adhesive tubes; (**B**) anterior region, ventral view, showing the anterior adhesive tube; (**C**) posterior region, ventral view showing the ventro-lateral and posterior adhesive tubes; (**D**) posterior region showing the frontal- and the caudal organ. co—caudal organ; cp—caudal pedicule; fo—frontal organ; TbA—anterior adhesive tube; TbD—dorsal adhesive tube; TbP—posterior adhive tube; TbVL—ventrolateral adhesive tube. Differential interference contrast microscopy (Nomarski).

# Tetranchyroderma papii Gerlach, 1953 [107]

Material recorded: A single juvenile specimen from Cap Angela (St1).

**Description:** The specimen examined is a juvenile measuring 219  $\mu$ m in TL (Figure 12). Its body is covered with pentancres and shows four pairs of epidermal glands. The head features a pair of rod-shaped tentacles on the dorsal side, each measuring approximately 11  $\mu$ m in length, along with a pair of elongated spatiform tentacles on either side of the mouth measuring 26.5  $\mu$ m in length. The adhesive apparatus consists of six TbA, which are located ventrally behind the mouth rim, along with three pairs of TbVL along the mid trunk, a pair of TbDL located in the posterior trunk region, eight TbP, three tubes arranged at the end of each caudal pedicle, and two tubes in between the pedicles (Figure 12B). A pair of lateral cirrata tubes are along the pharyngeal region between the first two epidermal glands (Figure 12B).

**Remarks:** The morphometric characteristics of the examined specimens do not allow for precise determination at the species level; however, identification can still be inferred. A combination of rod-like and spatiform tentacles is observed in only three species of *Tetranchyroderma*: *T. papii*, *T. bunti* (Thane-Fenchel, 1970) [108], and *T. parapapii* (Hummon, 2009) [109]. *T. bunti* features a cuticular armature composed of tetrances and is primarily distributed along the Atlantic and Gulf coasts of the USA [61,110]. *T. parapapii*, described as the North American counterpart of *T. papii*, shares a covering of pentances. However, *T. parapapii* is found only along the Atlantic coast of the USA, from Maine to South Carolina, whereas *T. papii* is distributed throughout the Mediterranean Sea, with an additional record from southern Portugal [110]. Given this information, it is reasonable to identify the studied species as *T. papii*, particularly since *T. papii* has previously been reported in Tunisia by Westheide [70]. In a wider taxonomic context, the morphometric information reported may aid studies that illuminate the ontogenetic morphometric variation occurring in species of the genus *Tetranchyroderma* and beyond.



**Figure 12.** Photomicrographs of the juvenile specimen of *Tetranchyroderma papii* found at Cap Angela: (A) habitus, dorsal view, showing the cuticular covering made up of pentancres, the head tentacles, and the epidermal glands; (B) habitus, ventral view, showing the head tentacles and the adhesive apparatus. cp—caudal pedicles; ct—cirrata tube; eg—epidermal gland; rt—rod-like tentacle; st—spatiform tentacle; TbA—anterior adhesive tubes; TbDL—dorso-lateral adhesive tube; TbP—posterior adhesive tube; TbVL—ventro-lateral adhesive tube. Differential interference contrast microscopy (Nomarski).

Family **Turbanellidae Remane**, **1926** [102] Genus *Paraturbanella* **Remane**, **1927** [102] *Paraturbanella teissieri* **Swedmark**, **1954** [111]

**Material recorded:** Nine specimens, seven from Cap Angela (St1) and two from Rimel Beach (St3).

**Description:** The following description is based mainly on an adult specimen 504  $\mu$ m in TL and 58  $\mu$ m in width, found at Rimel Beach (Figure 13). The body is ribbon-shaped, with a recognizable head and a noticeable cone between the slender caudal lobes. The head is 35  $\mu$ m wide and is characterized by a marked anterior hexagonal portion that includes the anterior mouth (10  $\mu$ m in diameter) and the heavily cuticularized buccal cavity (19 × 13  $\mu$ m). Lateral sensory pits and ventral papillae are present, past the perioral swellings (Figure 13B,D). The pharynx is 139  $\mu$ m in length and shows the pharyngeal pores near the base (U31); PhIJ is at U35. The intestine is straight and not clearly regionalized; the anus opens ventrally at U91. The adhesive apparatus consists of TbA, with six per side originating from a fleshy base, with the inner tube being the smallest (6  $\mu$ m vs. 9  $\mu$ m) (Figure 13E), and TbP, with six per side occurring along the inner and distal margin of each caudal lobe. The medial cone is 11  $\mu$ m long. The paired accessory adhesive tubes (dohrni/Seitenfüsschen) are located at U16, the longest tube is 32  $\mu$ m, and the shortest is 18  $\mu$ m (Figure 13C). The hermaphroditic reproductive system features paired testes that extend posteriorly from U36; the sperm ducts curve toward the front at U55 and empty into

the exterior through the male pore located at U39. The ovaries are also paired, with oocytes maturing from the posterior to the anterior. In most specimens a large egg, measuring approximately  $129 \times 45 \mu m$ , is visible in the mid-gut region centered at U57. The vesicular frontal organ is located dorsal to the intestine, centered at around U63; in some specimens, it contained bunches of agglutinated sperm along with secretory material.



**Figure 13.** Photomicrographs of an adult specimen of *Paraturbanella teissieri* found at Rimel Beach: (**A**) habitus; (**B**) close-up of the anterior region showing the piston pit; (**C**) posterior region showing the posterior adhesive tubes and the caudal cone; (**D**) anterior region, ventral view showing the ventral papillae; (**E**) anterior region, ventral view showing the anterior adhesive tubes. cc—caudal cone; ps—piston pit; TbA—anterior adhesive tubes; TbP—posterior adhesive tubes; vp—ventral papillae. Differential interference contrast microscopy (Nomarski).

**Remarks:** Initially described in northern Europe, *Paraturbanella teissieri* has been regarded as a senior synonym of *P. microptera* Wilke, 1954 [85], which was described from the Gulf of Naples in Italy [101,112]. In the Mediterranean Sea, this species is widely distributed [56]. The anatomical characteristics of the Tunisian specimens align with those documented by Tongiorgi [113] and Balsamo et al. [114] for specimens found in the Italian seas.

# Genus Turbanella Schultze, 1853 [115]

## Turbanella bocqueti Kaplan, 1958 [116] sensu Boaden, 1974 [100]

**Material recorded:** Two specimens, an adult from Les Grottes (St2) and a juvenile from Rimel Epave (St4).

**Description:** The studied adult specimen measures circa 1025  $\mu$ m in TL and 110  $\mu$ m in width (Figure 14). The body is vermiform, with a clearly defined head, trunk widest in the midgut region, tapering gradually to the caudal base; the caudum is bilobed, bearing a small median cone. The head is furnished with lateral, cone-like tentacles (18  $\mu$ m long) and is separated from the rest of the body by a marked neck constriction. The mouth opens anteriorly, leading into a sallow buccal cavity and then to a muscular pharynx (230  $\mu$ m in length) bearing pores near the base (U19). The PhIJ is at U22; the intestine is straight with refringent material in the anterior half; the ventral anus opens at U94. The adhesive apparatus includes, on each side, 11 TbA, originating from a fleshy base (Figure 14C); the tubes are all the same size (8  $\mu$ m) except the innermost one, which is shorter (5  $\mu$ m); a total of 32 TbL are visible along the body side (14–18  $\mu$ m), and there are 11 TbP (6–10  $\mu$ m) inserting along the inner and distal margin of the caudal lobe (Figure 14B). TbD are present but difficult to count. The reproductive apparatus is hermaphrodite, but only the male organs were seen in detail in the examined specimen. The paired testes originate past the



mid-trunk (U56) and extend posteriorly to U69, where sperm ducts curve toward the front and join anteriorly on the midline at U29.

**Figure 14.** Photomicrographs of the adult specimen of *Turbanella bocqueti* found at les Grottes: (A) habitus; (B) close-up of the posterior region, ventral view, showing the posterior adhesive tubes; (C) close-up of the anterior region, ventral view, showing the anterior adhesive tubes. TbA—anterior adhesive tubes; TbP—posterior adhesive tubes. Differential interference contrast microscopy (Nomarski).

**Remarks:** *Turbanella bocqueti* was first described in Northern Europe [100,116,117] and is also found in the Mediterranean Sea, as well as along the Atlantic and Gulf coasts of the USA [61]. In the Mediterranean Sea, it is widely distributed in both the western and eastern basins [56]. The size and general morphology of the Tunisian population align with data reported for both North European and Mediterranean populations [100,113]. However, there is a notable difference in the location of the testes between populations. In the Tunisian population, the testes originate along the mid-trunk, while in the North European population, they are situated near the pharyngo-intestinal junction [117]. Conversely, the position of the male pore is similar in both populations. Since the arrangement of the reproductive system has phylogenetic significance [16,45,118], the highlighted difference, if confirmed, could suggest the existence of a species complex. Future studies should also focus on examining the female component of the reproductive system, as there is currently no information regarding for example the frontal organ of this species.

Order CHAETONOTIDA Remane, 1925 [76] [Rao & Clausen, 1970] [77] Sub-order Paucitubulatina d'Hondt, 1971 [119]

Family Chaetonotidae Gosse, 1864 [120] sensu Leasi & Todaro, 2008 [18]

Sub-family Chaetonotinae Kisielewski, 1991 [121]

Genus Aspidiophorus Voigt, 1903 [122]

Aspidiophorus mediterraneus Remane, 1927 [123]

**Material recorded:** Two specimens, an adult and a subadult from Cap Angela (St1). **Description:** The adult examined specimen is about 170 μm in TL (Figure 15); it has a unilobed head and well-recognizable furca.

The head features the cephalion and hypostomion (2  $\mu$ m long  $\times$  7.5  $\mu$ m wide) (Figure 15H). The furca is 23  $\mu$ m long, including the terminal adhesive tubes (10–12  $\mu$ m). The widths at the head/neck/trunk and caudal base are 31  $\mu$ m/37  $\mu$ m/40  $\mu$ m and 22  $\mu$ m measured at U05/U26/U61 and U91, respectively. The cuticular armature comprises pedunculated, ovoid scales (3.7  $\times$  2  $\mu$ m) bearing a noticeable median keel throughout the length (Figure 15E). Scales are arranged in 43 longitudinal columns (23 dorsal, 10 + 10 ventral); the median column comprises about 65–70 scales. The sensory cilia (10–24  $\mu$ m long) are arranged in two tufts on the dorsolateral side of the head and as a paired element on the

dorsolateral sides along the pharyngeal region (U22) and on the posterior trunk region (U86); the latter two pairs originate from small papillae (Figure 15B–D). Locomotory cilia are arranged into two bands wider under the head and neck region, narrowing while approaching the intestinal region but never joining (Figure 15G). The mouth (5.5  $\mu$ m in diameter) is subterminal; the pharynx is 38.6  $\mu$ m in length, showing a noticeable posterior swelling (10  $\mu$ m wide); the PhIJ is at U24; the intestine is straight; the anus opens ventrally at U88.



**Figure 15.** Photomicrographs of *Aspidiophorus mediterraneus* found at Cap Angela: (**A**) habitus of the adult specimen; (**B**–**G**) subadult specimen; (**B**) habitus, dorsal view; (**C**) close-up of the neck region showing the sensory cilia (arrowhead); (**D**) close-up of the posterior trunk region showing the sensorial cilia (arrowheads); (**E**) close-up of the dorsal scales; (**F**) habitus, showing the internal anatomy; (**G**) habitus, ventral view; (**H**) details of the perioral region, showing the mouth and hypostomion (arrowhead). cb—ciliary band; f—furca; hsc—head sensorial cilia; i—intestine; m—mouth; ph—pharynx; phb—pharynx bulb; PhIJ—pharyngo-intestinal junction; psc—posterior sensorial cilia. Differential interference contrast microscopy (Nomarski).

**Remarks:** *A. mediterraneus* was first described by Remane [123] from samples collected in the Gulf of Naples, Italy. Currently, this species is known from several regions around the world [61], including various Mediterranean locations such as Marseille [104], Naples [85], the coasts of Tuscany [124], and many other Italian locations [79]. The morphometric characteristics of the specimens found in Tunisia align with those reported for specimens from the Tuscany coasts by Luporini et al. [124]. However, the number of longitudinal columns observed in the Tunisian specimens (43 columns) differs from those found on Elba Island in Italy (60 columns, Balsamo et al. [125]) and from specimens collected in North America (20 columns, Hummon [126]). These notable differences suggest that further investigations at the molecular genetic level are needed to explore potential phenomena of cryptic speciation within this taxon.

#### Aspidiophorus paramediterraneus Hummon, 1974 [126]

**Material recorded:** Three specimens, one from Cap Angela (St1) and two from Rimel Epave (St4).

**Description:** The adult specimen from Rimel Epave is 298  $\mu$ m in TL (Figure 16); it features a single-lobed head and a relatively short furca. The head bears a small dorsal cephalion and obvious ventral hypostomion (5 long  $\times$  13  $\mu$ m wide) (Figure 16E); the furca is 34  $\mu$ m long, including the terminal adhesive tubes (14  $\mu$ m). The widths at head/neck/trunk and caudal base are 35  $\mu$ m/54  $\mu$ m/66  $\mu$ m and 32  $\mu$ m measured at U10/U24/U58 and U88, respectively. The cuticular armature comprises pedunculated, elliptic scales (about 4.5 long  $\times$  2.5  $\mu$ m wide) bearing a noticeable median keel throughout their length (Figure 16B). Scales are arranged in 62 longitudinal columns (40 dorsal, 11 + 11 ventral); the median column comprises about 120 scales. The sensory cilia  $(15-21 \,\mu m \log)$  are arranged in two tufts on the dorsolateral side of the head and as a paired element on the dorsal side of the posterior trunk region (Figure 16A). Locomotory cilia are arranged ventrally in two bands that narrow in an antero-posterior direction and that join anteriorly to mid-trunk to form a single band (Figure 16D). The mouth is subterminal (10 µm in diameter), the pharynx is 66 µm in length and features a noticeable posterior bulb (17 µm wide); the PhIJ is at U26, the intestine is straight and slightly larger in front; the anus opens ventrally at U86, surrounded by a pair of elongated, keeled perianal scales (9  $\mu$ m long  $\times$  2  $\mu$ m wide) (Figure 16F). An egg occupies most of the trunk region (Figure 16C).

**Remarks:** *A. paramediterraneus* was first described from Beaufort, North Carolina, USA by Hummon [126]. Moreover, it is noted from several areas around the world, in the Mediterranean Sea, it has been recorded from Cyprus, Greece, Egypt, France, and Italy [61]. The Tunisian examined specimen matches the original description in terms of body shape and size, the shape of the pedunculated scales, and the arrangement of the ciliary band. However, it possesses a much higher number of columns of scales and scale per column. The cuticular covering (62 longitudinal columns, and 120 scales in the median column) corresponds to the Italian specimen described from Tuscany Archipelago (65 longitudinal columns, 33 dorsal) [125]. The difference in the number of columns and scales per column between the Mediterranean and the American populations may be due to the different methods used for their computation [77]. Nonetheless, such differences in the morphological patterns found in species from geographically distant locations need to go through molecular genetic analyses to test speciation patterns.



**Figure 16.** Photomicrographs of *Aspidiophorus paramediterraneus* found at Rimel Epave: (**A**) habitus, dorsal view; (**B**) close-up of the dorsal scales; (**C**) close-up of the posterior sensorial cilia (arrow); (**D**) habitus, ventral view; (**E**) habitus, internal view; (**F**) close-up of the perioral area showing the hypostomion (arrowhead); (**G**) close-up of perianal scales (arrows). cb—ciliary bands; e—egg; f—furca; i—intestine; m-mouth; ph—pharynx; phb—pharynx bulb; PhIJ—pharyngo-intestinal junction; sc—sensory cilia; scb—single ciliary band. Differential interference contrast microscopy (Nomarski).

# Genus *Chaetonotus* Ehrenberg, 1830 [127] *Chaetonotus dispar* Wilke, 1954 [85]

**Material recorded:** Two specimens, one adult from Cap Angela (St1) and a juvenile from Rimel Epave (St4).

**Description:** The adult specimen from Cap Angela is 134  $\mu$ m in LT (Figure 17); it features a tri-lobed head and a relatively short furca. The head bears dorsal cephalion and small ventrolateral pleurae; the hypostomion is not seen; the furca is 27  $\mu$ m in length, including the terminal adhesive tubes (17  $\mu$ m long). The widths at the head/neck/trunk and furcal base are 24.5  $\mu$ m/16.7  $\mu$ m/30  $\mu$ m and 16.5  $\mu$ m measured at U09/U30/U60 and U84, respectively. The cuticular armature comprises 11 longitudinal columns (7 dorsal, 2 + 2 ventrolateral) of separated three-lobed scales, with each carrying a robust spine increasing in length from front to the rear; the median column comprises about 17–18 scales; spines of the dorsal columns bear two accessory points at about 3/4 of their length and a notched tip (Figure 17A); ventrolateral spines have only the two accessory points along their lengths. The ventral locomotory cilia are arranged in two longitudinal bands that are wider in the front but never merge. The interciliary field is covered with tiny, round, keeled scales arranged in 4–6 longitudinal columns from U35 to U72. The ventral terminal scales are elongated and furnished with a keel that continues in a short, notched spine

(Figure 17C). On the ventral side of the furcal base, there are two pairs of small trilobate, keeled scales, each carrying a spine similar to the ventrolateral ones; the spines of the medial pair protrude from the furcal indentation. The subterminal mouth is 6.5  $\mu$ m in diameter and leads into a 32.5  $\mu$ m long pharynx, which shows gentle swelling at both ends; the PhIJ is at U27; the intestine (70  $\mu$ m in length) is straight and lacks evident differentiation; the anus opens ventrally at U81 (Figure 17B).



**Figure 17.** Photomicrographs of *Chaetonotus dispar* found at Cap Angela: (**A**) habitus, dorsal view, arrowhead show the two accessory points along the spines; (**B**) habitus, internal anatomy; (**C**) habitus, ventral view. icf—interciliary field, ifs—intrafurcal spines; lc—locomotor cilia; m—mouth; ph—pharynx, PhIJ—pharyngo-intestinal junction; vls—ventrolateral spine. Differential interference contrast microscopy (Nomarski).

**Remarks:** *C. dispar* is a cosmopolitan species that was first described from the Gulf of Naples [61,85] and has been frequently reported in the Mediterranean Sea [56]. The morphometric data of the specimen from Cap Angela is consistent with the original description, and with data reported for the specimens from the Tuscan Archipelago [125] and those from the US Virgin Islands [128].

Genus *Halichaetonotus* Remane, 1936 [129] *Halichaetonotus bizertae* sp. nov.

## (Figures 18–21)

http://zoobank.org/urn:lsid:zoobank.org:act:357EC41C-3D17-4870-A462-867242603119 Etymology: *"bizertae"* refers to Bizerte, the city where the species was first found.



**Figure 18.** Schematic drawings of *Halichaetonotus bizertae* sp. nov. (**A**) dorsal view showing scales and spines pattern; (**B**) ventral view, showing also the pharynx and intestine (dotted lines).

**Diagnosis:** Small-sized *Halichaetonotus*,  $127 \pm 2.2 \mu m$  in TL; the head, neck, trunk, and furcal appendages are well-defined. The head is slightly five-lobed with a frontal cephalion and lateral pleurae but lacks the hypostomion; there is a medium-long furca  $(25.3 \pm 1.7 \,\mu\text{m})$  projecting from the posterior of the trunk. The body is covered dorsally by seven longitudinal columns of alternating, keeled scales. The median dorsal column is composed of 18-20 scales; the scales are shield-shaped on the head and trunk and round on the neck; in general, the keel extends beyond the edge of the scales as a spiny process. In the posterior trunk region, there are two round, double-keeled scales carrying sensorial bristles and a pair of scales with spines protruding into the furcal indentation; there are keeled, spiny scales on the dorsal and ventral base of the furca. Laterally and ventrolaterally, there are 2 + 2 columns of hydrofoil scales with spine-bearing lamellae of varying length; ventrally, there are 2 + 2 additional columns of small, round scales; locomotory cilia are arranged in two longitudinal bands; the interciliary field is naked, ending with two pair of perianal, elliptical keeled scales of similar size, with each having a keel extending in a spiny process. The mouth is of medium size (6.4  $\pm$  0.2  $\mu$ m in diameter), opening into a 29.9  $\pm$  0.5 long pharynx, showing swellings at both ends; the anterior bulb is provided



with pharyngeal teeth; the intestine is sack-like with a terminal ventral anus. All specimens are parthenogenetic, sometimes with a single large egg in the dorsal to mid-intestine region.

**Figure 19.** Photomicrographs of the holotype of *Halichaetonotus bizertae* sp. nov. found at Les Grottes: (**A**) habitus, showing the intrafurcal spines (arrows); (**B**) close-up of the trunk hydrofoil scales (**C**) close-up of the neck hydrofoil scales; (**D**) habitus, ventral view, showing the hydrofoil scales of the ventrolateral columns (arrow) and of the lateral columns (arrowhead), (**E**) posterior region, ventral view, showing the first pair of the terminal scales (arrows); (**F**) posterior region, ventral view, showing the second pair of terminal scales (arrows). m-mouth; f—furca. (**A**) bright field microscopy; (**B**–**F**) Differential interference contrast microscopy (Nomarski).

**Type locality:** Tunisia, Bizerte: Les Grottes  $(37^{\circ}19'60'' \text{ N}; 09^{\circ}50'38'' \text{ E};$  Figures 19 and 20). The specimens were found in medium, moderately well-sorted sand. The characteristics of the site are shown in (Table 2). Additional site: Cap Angela  $(37^{\circ}19'53'' \text{ N}; 09^{\circ}46'10'' \text{ E})$ .

**Type material:** Holotype: The adult specimen shown in Figures 19 and 20, no longer extant, was collected by AS on 16 September 2023 (International Code of Zoological Nomenclature 19, Articles 73.1.1 and 73.1.4; also see the recommendation 73 G–J of Declaration 45—Addition of Recommendations to Article 73) [130,131]. Additional studied specimens: Two specimens from Cap Angela (St1), showing maturing eggs inside. All specimens were examined in vivo and were destroyed during the microscopical survey.



**Description:** The description is mainly based on the holotypic adult specimen measuring a length of 129  $\mu$ m in TL (Figures 19 and 20).

**Figure 20.** Photomicrographs of *Halichaetonotus bizertae* sp. nov. holotype found at Les Grottes. (**A**) habitus, dorsal view; (**B**) close-up of the head scales; (**C**) close-up of the neck scales; (**D**) close-up of the trunk scales; (**E**) habitus, showing the internal anatomy. ce—cephalion; i—intestine; ph—pharynx; PhIJ—pharyngo-intestinal junction; pht—pharyngeal teeth. Differential interference contrast microscopy (Nomarski).



**Figure 21.** Photomicrographs of *Halichaetonotus bizertae* sp. nov., another adult specimen found at Cap, Angela. (**A**) habitus, showing the internal anatomy; (**B**) habitus, ventral view. cb—ciliary bands; ce—cephalion; f—furca; hysc—hydrofoil scale; i—intestine; m—mouth; ph—pharynx; PhIJ—pharyngo-intestinal junction; pht—pharyngeal teeth; sc—sensorial cilia. Differential interference contrast microscopy (Nomarski).

The body is tenpin-shaped, featuring a marginally penta-lobed head consisting of two pairs of loose tufts of sensorial cilia, a shallow cephalion (10  $\mu$ m wide), and small pleurae but lacks a hypostomion and eye spots; the neck constriction is well-defined, and the trunk is sac-like, terminating in a furcate caudum. The body widths at the head/neck/trunk and furcal base are 19  $\mu$ m/15.5  $\mu$ m/25  $\mu$ m and 14  $\mu$ m measured at U01/U25/U36 and U78, respectively. The caudal appendages are of medium length (27  $\mu$ m), with paired laterally divergent adhesive tubes (15  $\mu$ m in length) and a slightly inflated base (6  $\mu$ m) covered by scales (Figures 18 and 19A,D).

Cuticular armature: The cuticular armature extends over the entire body surface, except for the ventral interciliary field. The head, neck, and trunk covered dorsally and lateroventrally by alternating columns (seven dorsal, 1 + 1 lateral hydrofoil, and 1 + 1ventrolateral hydrofoil, 2 + 2 ventral) of 18-20 keeled scales, barely overlapping. On the dorsal side (Figures 18A and 20A-D), the head and trunk scales are shield-shaped  $(3 \times 5 \,\mu\text{m}$  on the head, Figure 20B;  $5 \times 8 \,\mu\text{m}$  on the trunk Figure 20D); on the neck, the scales have round heads (3  $\mu$ m in diameter, Figure 20C). In general, the keel on the dorsal scales extends beyond the edge of scales as a spiny process; the spines are about 5  $\mu$ m long on the head, 3  $\mu$ m on the neck, and up to 10  $\mu$ m on the trunk (Figures 18A and 20B–D). On the posterior trunk region, there are two round, double-keeled scales (about 4  $\mu$ m in diameter), each anchoring a sensorial bristle at U78, and a couple of scales (4  $\times$  3  $\mu$ m) bearing spines (5 µm long) protruding into the furcal indentation, along with a pair of spiny scales on the furcal branches (Figures 18A and 19A). The lateral and ventrolateral hydrofoil scales are similar in shape and size to the dorsal scales. They have spines that bear flattened lamellae, most of which taper into a pointed process. The lamellae-bearing spines of the lateral scales are more protracted than related ventrolateral ones (up to 10 µm vs. up to  $5 \mu$ m), while the lamellae of a column are longest at the mid-trunk (Figures 18 and 19B–D). On the ventral side, there are two columns of rounded keeled scales with a short spine (3-4 µm long) on each side between the ciliary band and the hydrofoil scales; the innermost

column consists of scales smaller than the outer one; on the posterior ventral region, up to five keeled scales, 3–4  $\mu$ m long, cover the fleshy portion of each furcal branch (Figures 18B and 19E,F). The interciliary field appears naked except for two pairs of elliptical, keeled scales in the perianal region; the scales of the anterior pair are slightly larger (7 × 4  $\mu$ m) than the posterior ones (6.0 × 3  $\mu$ m); the keel of these scales continues in a short spine process (Figures 18B and 19E,F).

**Ventral ciliation:** Paired longitudinal bands extend from U03 to approximately U73. These bands are broadly club-shaped at the front but narrow significantly toward the posterior pharyngeal region. They come close together just behind the mouth but remain separate along their entire length. The individual cilia measure between 9 and 12  $\mu$ m in length (Figures 18B, 19B and 21B).

**Digestive tract:** The subterminal mouth is of medium size (7  $\mu$ m in diameter), projecting very slightly ventrally and leading progressively into a 30  $\mu$ m long pharynx featuring swellings at both ends; the anterior bulb is slightly larger (11  $\mu$ m vs. 9  $\mu$ m in diameter) and is provided with pharyngeal teeth; the pharyngeal-intestinal junction is at U26; the intestine is straight, narrowing posteriorly; the anus opens ventrally at U77 (Figures 18B, 20E and 21A).

**Reproductive tract:** All the examined specimens were in the parthenogenetic phase (i.e., no spermatozoa were observed); the holotype had a mature egg ( $41.5 \times 21 \mu m$ ) located dorsally to the intestine, filling almost the trunk region.

**Variability and remarks.** The general appearance and characteristics of the other two specimens studied are very similar to those of the holotype (Figure 21 and Table 4).

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| Pharynx length       30       30       29         Furca length       27       26       23         Adhesive tubes length       15       15       12         Head width       25       23       19         Neck width       15       15       14         Trunk width       30       25       23         Furcal base width       13       14       14         Anterior pharynx width       11       10       10         Mid pharynx width       6       6       6         Posterior pharynx width       9       9       9         Head scale length       5       5       5         Head scale length       5       -       -         Neck scale length       3       4       4         Head spine length       3       3       4         Neck scale width       3       3       4         Neck scale width       5       4       -         Trunk scale width       4 <t< td=""><td>Total length</td><td>129</td><td>128</td><td>124</td></t<> | Total length                       | 129      | 128 | 124 |  |
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| Terminal interciliary scale length777Terminal interciliary scale width444Spine length888Cephalion length112Cephalion width1088Mouth diameter766Bristle scale length454Bristle scale width343Egg length-42-Egg width-21-   | Trunk spine length                 | 10       | 8   | -   |  |
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| Cephalion length112Cephalion width1088Mouth diameter766Bristle scale length454Bristle scale width343Egg length-42-Egg width-21-   | Spine length                       | 8        | 8   | 8   |  |
| Cephalion width1088Mouth diameter766Bristle scale length454Bristle scale width343Egg length-42-Egg width-21-  | Cephalion length                   | 1        | 1   | 2   |  |
| Mouth diameter766Bristle scale length454Bristle scale width343Egg length-42-Egg width-21-   | Cephalion width                    | 10       | 8   | 8   |  |
| Bristle scale length454Bristle scale width343Egg length-42-Egg width-21-  | Mouth diameter                     | 7        | 6   | 6   |  |
| Bristle scale width343Egg length-42-Egg width-21-   | Bristle scale length               | 4        | 5   | 4   |  |
| Egg length-42-Egg width-21-   | Bristle scale width                | 3        | 4   | 3   |  |
| Egg width - 21 -  | Egg length                         | -        | 42  | -   |  |
|   | Egg width                          | -        | 21  | -   |  |

**Table 4.** *Halichaetonotus bizertae* sp. nov. main taxonomic characters and measurements (in  $\mu$ m) of the specimens found. Specimen 1 is the holotype.

Negligible differences were observed among the specimens regarding the following major traits: (i) total body length ranging from 124 to 129  $\mu$ m; (ii) pharynx length fluctuating between 29 and 30  $\mu$ m; (iii) furca length spanning from 23 to 27  $\mu$ m; and (iv) adhesive tube length varying from 12 to 15  $\mu$ m. The measurements of these traits are generally correlated with the size of the animals (Table 4).

**Taxonomic affinities:** The marine genus *Halichaetonotus* comprises 31 known species, showcasing a significant diversity in their cuticular coverings. However, the specimens found in Tunisia share certain characteristics with five other species: they possess a naked intraciliary field and the keel on the dorsal scale that extends into a spiny process. The species sharing these traits are *H. bataceus* Evans, 1992 [132], *H. etrolomus* Hummon, Balsamo & Todaro, 1992 [133], *H. genatus* Balsamo, Fregni & Tongiorgi, 1995 [97], *H. sanctaeluciae* Todaro, Dal Zotto, Bownes & Perissinotto, 2011 [134], and *H. euromarinus* Hummon & Todaro, 2010 [82]. The specimens we are studying can be easily distinguished from *H. bataceus*, *H. etrolomus*, and *H. genatus* by the presence of small round scales on the ventral side, located between the ciliary bands and the hydrofoil scales, a feature that the latter three species lack. In contrast, the Tunisian specimens share this trait with *H. sanctaeluciae* and *H. euromarinus*. Additionally, all three are characterized by having two pairs of large scales in the perianal area.

Despite their similarities, these three species can be easily distinguished by various prominent morphological features. For instance, *H. euromarinus* has only ventrolateral columns of hydrofoil scales, while *H. sanctaeluciae* and the Tunisian specimens have lateral columns as well. Furthermore, the specimens being studied can be differentiated from *H. sanctaeluciae* because they do not possess the three dorsal posterior scales, one median and two laterals, which have long, robust spines extending beyond the end of the trunk. In contrast, the Tunisian specimens have a couple of scales in the posterior region that feature spines protruding into the furcal indentation, which are absent in *H. sanctaeluciae*. Based on these distinctions, it is clear that the specimens under study are unique enough to be classified as a new species, which we propose to name *Halichaetonotus bizertae* sp. nov.

*Halichaetonotus euromarinus* Hummon & Todaro, 2010 [82] Syn. *Halichaetonotus spinosus* Mock, 1979 [135]

Material recorded: A single specimen from Cap Angela (St1).

**Description:** The specimen studied measures 133  $\mu$ m in TL and features a roughly rounded head with a distinct furca (Figure 22).



**Figure 22.** Photomicrographs of *Halichaetonotus euromarinus* found at Cap Angela: (**A**) habitus, dorsolateral view; (**B**) insert showing the dorsal scales bearing the keels extending in a spiny process; (**C**) habitus, ventrolateral view; (**D**) posterior region, ventral view showing the two pairs of perianal scales (arrows). hsc—hydrofoil scales. Differential interference contrast microscopy (Nomarski).

The head includes a frontal cephalion and small, ventrolateral pleurae, while the hypostomion is not visible. The furca measures 21 µm in length, including the terminal adhesive tubes, which are 14  $\mu$ m long. The widths at the head/neck/trunk and furcal base are 27  $\mu$ m (16.3  $\mu$ m/34.4  $\mu$ m/and 15  $\mu$ m, respectively, measured at U12/U30/U58 and U82. The cuticular armature consists of nine dorsal columns of slightly overlapping, keeled scales. The scales on the head and trunk regions are shield-shaped, measuring approximately 8  $\mu$ m by 4  $\mu$ m on the trunk, while those on the neck are roughly round. In all cases, the keel extends into a short spiny process (Figure 22B). The dorsal medial column contains 17-18 scales, with the posterior-most scale's spiny process protruding from the furcal indentation. Additionally, each side features two ventrolateral columns of scales. The lateral column contains scales similar to those on the dorsal side but has much higher keels that bulge upward and backward, lacking the spiny process found on the dorsal scales. The more ventral column consists of roughly circular scales that bear spinous lamellae (hydrofoil scales) (Figure 22C). In addition, on each side, between the ciliary band and the hydrofoil scales, there are three to four columns of small, rounded spined scales. The ventral locomotory cilia are arranged in two longitudinal bands that are wider at the front but never merge. The interciliary field is bare, and in the perianal area, there are two pairs of elliptical, weakly keeled scales, which are flanked on each side by three to four smaller scales (Figure 22D). The subterminal mouth has a diameter of  $5.5 \,\mu m$ and leads into a 27 µm long pharynx, which displays conspicuous swellings at both ends. The anterior bulb contains pharyngeal teeth; the PhIJ is located at U24. The intestine is straight and broader in the anterior half, while the anus opens ventrally at U82.

**Remarks:** *Halichaetonotus euromarinus* has an amphi-Atlantic distribution; described initially from the North Sea [135], it has been reported from the Atlantic and Gulf coasts of the USA [5,61]. The occurrence of the species in the Mediterranean Sea was reported first by Todaro and Balsamo [136], who found it in Sicily (see also Balsamo et al. [125]). Since then, the species has been found frequently in western and eastern basins [56]. The Tunisian specimens match the original description of the species.

# Halichaetonotus thalassopais Hummon, Balsamo & Todaro, 1992 [133]

**Material recorded:** Seven specimens, five from Cap Angela (St1) and two from Les Grottes (St2).

**Description:** The following information is based mainly on an adult specimen from Cap Angela measuring 114  $\mu$ m in TL (Figure 23). The specimen shows a tri-lobed head and a relatively short furca. The head bears dorsal cephalion, small ventrolateral pleurae, and a sub-rectangular hypostomion; the furca is about 20 µm in length, including the terminal adhesive tubes (11  $\mu$ m long). The widths at the head/neck/trunk and furcal base are  $20 \ \mu m/15 \ \mu m/36 \ \mu m$  and  $14 \ \mu m$  measured at U12/U36/U65 and U87, respectively. The cuticular armature consists of 15 longitudinal columns (nine dorsal, 2 + 2 ventrolateral, and 1 + 1 ventral) of overlapping ovoidal, keeled scales (Figure 23B); the dorsal medial column contains 17 scales; the ventrolateral scales have higher keels that rise more as the columns progress ventrally, bulging upward and backward, protruding past the posterior end of the scale; the scales of the ventral columns bear delicate spinous lamellae (hydrofoils scales). Ventral locomotory cilia are organized in paired longitudinal rows, each forming a broad patch anteriorly that meets medially behind the hypostomion. The interciliary field is bare. On the posterior ventral surface, there are six or seven pairs of ovoid keeled scales; the perianal scales are the largest and the only ones with a keel that extends posteriorly as a spiny process (Figure 23D). The mouth is subterminal, about 6  $\mu$ m in diameter, leading into a 35 µm long pharynx showing strong anterior and posterior swellings (9 µm in diameter). The anterior bulb is armed with robust pharyngeal teeth; the PhIJ is at U29 (Figure 23C); the intestine is straight and lacks evident differentiation; the anus opens ventrally at U82. A mature egg occupies most of the trunk region (Figure 23A).



**Figure 23.** Photomicrographs of *Halichaetonotus thalassopais* found at Cap Angela: (**A**) habitus; (**B**) close-up of the dorsal scales; (**C**) anterior region, internal view, showing the pharynx; (**D**) posterior region of different specimen, showing the high keel of the ventro-lateral scales and the lamellae of the hydrofoil scales. e—egg; hsc—hydrofoil scales; ph—pharynx; pht—pharyngeal teeth; vlk—keels of the ventrolateral scales; vtsc—ventral scales of the posterior region. Differential interference contrast microscopy (Nomarski).

**Remarks:** *Halichaetonotus thalassopais* was first described in Tuscany, Italy [133]. Since then, it has been reported in several other locations across the Italian peninsula, as well as in Greece and along the Mediterranean coasts of Israel and Egypt [56,61,79]. The metric and meristic characteristics of the specimens from Tunisia align with the original description [133]. We are presenting microphotographs of this species here for the first time.

# Genus Heterolepidoderma Remane, 1927 [72] Heterolepidoderma loricatum Schrom, 1972 [137]

**Material recorded:** Two adult specimens, one from Cap Angela (St1) and one from Rimel Beach (St3).

**Description:** The following information is primarily based on an adult specimen from Rimel Beach, measuring 110  $\mu$ m in TL (Figure 24). The body is relatively stout and features a roughly trilobed head and distinct furca. The head includes a frontal cephalion, noticeable ventrolateral pleurae, and a broad hypostomion. The furca measures 18  $\mu$ m in length, including the 11  $\mu$ m long terminal adhesive tubes. The widths at the head/neck/trunk and furcal base are 22  $\mu$ m/21  $\mu$ m/31  $\mu$ m, and 20  $\mu$ m, respectively, measured at U08/U23/U65/and U86. The cuticular armature consists of keeled scales arranged in 13 longitudinal, imbricated columns, with 9 columns covering the dorsal side. There are 18 scales in the medial column (Figure 24A). The scales in the ventral-most column on each side feature spines with lamellae (hydrofoil scales), rather than just a keel. Ventral locomotory cilia are arranged in two longitudinal bands that are broader at the

front but do not merge. The interciliary field is covered by rounded keeled scales from U37 to U88, and there are three terminal pairs of elliptical, keeled scales (Figure 24B). The mouth, which is 4  $\mu$ m in diameter, opens subterminally and leads to a 28  $\mu$ m long pharynx that has swellings at both ends. The anterior bulb is armed with pharyngeal teeth; the PhIJ is located at U33. The intestine is straight and slightly broader in the anterior portion, with the anus opening ventrally at U86 (Figure 24C). The specimen is in the parthenogenetic phase and shows a large egg that fills most of the trunk.



**Figure 24.** Photomicrographs of *Heterolepidoderma loricatum* found at Cap Angela: (**A**) habitus, dorsal view; (**B**) habitus, ventral view, showing among others, the hydrofoil scales and the terminal scales (arrows); (**C**) habitus, internal anatomy. c—cilia; i—intestine; hsc—hydrofoil scales; hy—hypostomion; icsc—scales of the interciliary field; m—mouth; ph—pharynx; pht—pharyngeal teeth. Differential interference contrast microscopy (Nomarski).

**Remarks:** *Heterolepidorma loricatum* is an amphiatlantic species [5]. It was originally described from the northern Adriatic Sea [137] and has since been recorded in several other locations in Italy and the Mediterranean Sea (see Balsamo et al. [138]; Todaro et al. [56]). The morphometric data of *H. loricatum* from Rimel Beach align with the published information on this species. Specimens that possess hydrofoil scales have frequently been observed, leading Todaro [77] to suggest that these features may have been overlooked by Schrom [137] during the species' initial description due to their elusive nature.

Family Xenotrichulidae Remane, 1927 [139] Sub-family Draculiciterinae Ruppert, 1979 [140] Genus *Draculiciteria* Hummon, 1974 [141]

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# Draculiciteria tesselata (Renaud-Mornant, 1968) [142] Synonym Polymerurus tesselatus Renaud-Mornant, 1968 [142] Material recorded: a single subadult specimen from Rimel Beach (St3).

**Description:** The specimen studied measures 155  $\mu$ m in TL (Figure 25) and features a distinct, roughly pentagonal head and relatively long furca (47  $\mu$ m in length). The terminal, lateral spines at the furcal base are 8  $\mu$ m long. The widths at the head/neck/trunk and furcal base are 33.5  $\mu$ m/17  $\mu$ m/30.5  $\mu$ m and 15.5  $\mu$ m measured at U08/U22/U55 and U75, respectively. Due to the specimen's poor condition, the details of the cuticular ornamentations, such as shape and arrangement, could not be detected. The mouth is 7  $\mu$ m in diameter and leads to a 52  $\mu$ m long cylindrical pharynx; the PhIJ is at U35; the intestine is straight and lacks evident differentiation; the anus opens ventrally at U70.



**Figure 25.** Photomicrograph of *Draculiciteria tessalata* found at Rimel Beach. tlsp—terminal lateral spines. Differential interference contrast microscopy (Nomarski).

**Remarks:** *Draculicitera tesselata* is a cosmopolitan species. Described initially as *Polymerurus tesselatus* from Ischia Island, Italy [142] (see also Hummon [141]), it is known to occur in different areas of the World (see Hummon [61]). In the Mediterranean Sea, it is relatively common [56]. The general morphology of the Tunisian specimens aligns with literature data on the species; the metric data are in line with those of the subadult specimens recorded along the Italian coastlines [79].

# Sub-family Xenotrichulinae Remane, 1927 [139] Genus *Heteroxenotrichula* Wilke, 1954 [85] Heteroxenotrichula sp1

Material recorded: A single adult specimen from Cap Angela (St1).

**Description:** The specimen surveyed is 188  $\mu$ m in TL (Figure 26) and features a tenpinshaped body, which has a distinct, trapezoidal head and a pedunculated, V-shaped furca. The head carries two pairs of dorsal sensory cirri and one pair of relatively short lateral tentacles/auricles (9  $\mu$ m); the furca is 53  $\mu$ m in length (measured from the indentation to the tip), including the 15  $\mu$ m long adhesive tubes. The widths of the heads at the auricles/brain commissure/neck/trunk and furcal base are 26  $\mu$ m/30  $\mu$ m/37  $\mu$ m/51  $\mu$ m and 18  $\mu$ m measured at U4.5/U10.5/U21/U48 and U68, respectively. The body is covered dorsolaterally with nine longitudinal columns (seven dorsal, 1 + 1 lateral) of overlapping scales (Figure 26A); dorsal columns made up of *squamosa*-type scale; lateral columns composed of hydrofoil scales (Figure 26B). The anterior locomotory cirri are of two sizes, the smaller confined under the head to U11, the larger distributed between U11 and U30; the paired tuft of posterior cirri (trunk cirri) is located at U46 (Figure 26B); each tuft is made up of three cirri of equal size. The mouth is about 3  $\mu$ m in diameter, leading to a 35  $\mu$ m long pharynx featuring an anterior swelling armed with pharyngeal teeth; the PhIJ is at U21; the intestine is straight without evident differentiation; the anus opens ventrally at



U69. The reproductive apparatus is hermaphrodite; the male pore opens ventrally at U23, and the mature egg is centered at U45.

**Figure 26.** Photomicrographs of *Heteroxenotrichula* sp1 found at Cap Angela: (**A**) habitus, dorsal view; (**B**) habitus, ventral view. a—auricules; ahsc—anterior head sensory cirri; tlc—trunk locomotory cirri; dlhsc—dorsolateral head sensory cirri; e—egg; f—furca; fp—furca peduncle; lac—large anterior cirri; m—mouth; mp—male pore; sac—small anterior cirri; sp—spermatozoa. Differential interference contrast microscopy (Nomarski).

**Remarks:** Most of its morphometric characteristics approach the Tunisian specimens to the cosmopolitan *H. squamosa* Wilke, 1954 [85], which is widely distributed also in Mediterranean [56,61,79,140,143]. However, the V-shaped furca and especially the much shorter distal adhesive tube compared to the proximal, scaled portion suggest it may belong to a different, undescribed species. Given the uncertainties, we prefer to keep the status of the current specimen as undetermined. We hope to obtain more information about this species as our investigation along the coasts of Tunisia continues.

## Heteroxenotrichula sp2

Material recorded: A single adult specimen from Rimel Beach (St3).

**Description:** The surveyed specimen is 308  $\mu$ m in TL (Figure 27) and features an elongate tenpin-shaped body furnished with a distinct, trapezoidal head and a slightly pedunculated, U-shaped furca. The head carries two pairs of dorsal sensorial cirri and one pair of relatively short lateral tentacles/auricles (15  $\mu$ m); the furca is 57  $\mu$ m in length (measured from the indentation to the tip), including the 25  $\mu$ m long adhesive tubes. The widths of the heads at the auricles/head at the brain commissure/neck/trunk and furcal base are 36  $\mu$ m/43  $\mu$ m/57  $\mu$ m/71  $\mu$ m and 27  $\mu$ m measured at U2.5/U9/U23/U58 and U79, respectively. The body is covered dorsolaterally with nine (seven dorsal, 1 + 1 lateral) longitudinal columns of overlapping scales; dorsal columns made up of *squamosa*-type scale, lateral columns composed of hydrofoil scales. The anterior locomotory cirri are of two sizes, the smaller confined under the head to U9.5 and the more oversized inserted between U9.5 and U23; the paired tuft of posterior cirri (trunk cirri) is located at U47; each tuft is made up of three cirri of equal size. The mouth is about 4  $\mu$ m in diameter, leading to a 60  $\mu$ m long pharynx featuring an anterior swelling showing pharyngeal teeth; the PhIJ is

at U21; the intestine is straight without evident differentiation; the anus opens ventrally at U78. The reproductive apparatus is probably hermaphrodite; however, only sparse spermatozoa were seen.



**Figure 27.** Photomicrographs of *Heteroxenotrichula* sp2 found at Cap Angela: habitus, ventral view. Differential interference contrast microscopy (Nomarski).

**Remarks:** The distinctive shape and large size of the Tunisian specimen set it apart from the 11 species of *Heteroxenotrichula* that have been described to date [68,140,144,145]. However, the morphometric data collected from this single specimen are insufficient to provide a modern description of a new species. Therefore, we prefer to classify the current specimen as undetermined. We look forward to gathering more information about this species as our investigation along the Tunisian coasts progresses.

# Genus Xenotrichula Remane, 1927 [139]

Xenotrichula intermedia Remane, 1934 [75]

**Material recorded:** Two specimens, an adult and a subadult collected at Mahdia in 2008.

**Description:** The adult specimen is 208  $\mu$ m in TL (Figure 28). The body is tenpinshaped and includes a unilobed head and U-shaped furca. The head bears two pairs of dorsal sensorial cirri and lack of auricles; the furca is 30  $\mu$ m in length including the 15  $\mu$ m long adhesive tubes. The width at head/neck/trunk and caudal furca are 36  $\mu$ m/30  $\mu$ m/52  $\mu$ m and 23  $\mu$ m at U6/U24/U54 and U86, respectively. The cuticular covering is composed of *intermedia*-type stalked scales. On the dorsal side, scales are organized in 17 columns with 55 scales in the median column (Figure 28A). The anterior mouth is 4  $\mu$ m in diameter and leads into a 47  $\mu$ m long cylindrical pharynx; the PhIJ is at U25; the intestine is straight, narrowing in the rear; the anus opens ventrally at about U74 (Figure 28B). The reproductive apparatus is hermaphrodite.

**Remarks:** *X. intermedia* was first described from the North Sea [75] and has since been reported in various oceans and connected seas [61]. However, there is increasing evidence suggesting that populations located far apart are actually distinct, cryptic species, which can be identified through genetic analysis and/or muscle architecture [39,44,146]. The morphometric data for the Tunisian specimen aligns with findings reported for Italian populations. It is important to note that a specimen of *X. intermedia* from Mahdia was used by Todaro et al. [71] to obtain nucleotide sequences of the nuclear 18S and 28S rRNA genes, which are accessible in GenBank under the following accession codes: JF357664.1 (18S) and JF357712.1 (28S). Additionally, from the same specimen (voucher MT71), a partial sequence of the mitochondrial COI gene was obtained, available in GenBank under the accession code JF432048.1. However, due to an error during submission, this sequence is mistakenly



labeled as belonging to a different specimen (voucher MT82). So far, the repository has not responded to requests to correct this error [86].

**Figure 28.** Photomicrographs of *Xenotrichula intermedia* found at Mahdia: (**A**) habitus, dorsal view; (**B**) habitus, ventral view. an—anus; i—intestine; ph—pharynx; PhIJ—pharyngo-intestinal junction; sc—sensorial cirri. Differential interference contrast microscopy (Nomarski).

## 4. Conclusions

Gastrotrichs are a vital part of marine meiobenthos found in sandy habitats [4,147]. This study provides, for the first time, a faunistic overview, though not comprehensive, of this group along the northern coast of Tunisia. In a larger framework, the research expands the known marine gastrotrich species in Tunisia from 6 (5 Macrodasyida and 1 Chaetonotida) to 24 species (14 Macrodasyida and 10 Chaetonotida). The average number of species per location is 7.8  $\pm$  2.5 and aligns with the data for Italy, where the average is  $8.5 \pm 2.5$  species per site [79]. This is notable considering the vicinity of the sampled locations and the small quantity of sandy material collected from each location. As anticipated, most of the species identified in this research were already known from the Mediterranean Sea. However, the discovery of Halichaetonotus bizertae sp. nov. and likely at least two other new species (Heteroxenotrichula sp1 and sp2) indicates that our understanding of Mediterranean gastrotrichs is still incomplete. This suggests a pressing need for further research, particularly along the southern shores of the *Mare Nostrum*. Our research underscores a critical gap in the literature: the scarcity of morphometric data and photographs, even for the most commonly reported species. This shortfall greatly limits our ability to compare distant populations and increases the risk of misidentification. In today's scientific landscape, where molecular genetic data forms the backbone of many analyses, it is imperative that we prioritize thorough morphological vouchering of species. Such efforts will enhance accuracy and reliability in our research, paving the way for more robust scientific conclusions.

**Supplementary Materials:** The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/d17010017/s1, Table S1. Number of species shared among the

four Tunisian stations sampled in 2023; Table S2. Pairwise Dice-Sørensen similarity coefficient [1,2] calculated for the four Tunisian stations sampled in 2023. References [148,149] are cited in the supplementary materials.

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**Data Availability Statement:** The data that support this study are available in the article and in the Supplementary Materials.

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