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Cucullanus sp. (Nematoda: Cucullanidae) Parasitizing the Thinlip Conger *Gnathophis mystax* (Delaroche, 1809) from the Tyrrhenian Coast of Calabria (Italy) †

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Abstract

Gnathophis mystax (Delaroche, 1809) is a benthic eel widely distributed in the Mediterranean and eastern Atlantic, whose parasitic fauna remains known. This study provides the first parasitological assessment of this species from the southern Tyrrhenian Sea (Calabria, Italy). A total of 41 specimens were examined. All recovered nematodes were identified as *Cucullanus* sp., with prevalence values of 66.7% and 70.0% in Vibo Valentia and Gioia Tauro, and mean intensities of 2.71 and 3.00. No significative differences in length and weight were found between localities, and no significative differences in the prevalence and abundance of *Cucullanus* between localities were found. These results represent the first record of *Cucullanus* sp. in *G. mystax*. Further studies are required to elucidate the taxonomy of this nematode in *Gnathophis mystax* and of the order Anguilliformes.

Keywords: Mediterranean biodiversity; parasite prevalence; host-parasite relationships; demersal ecology; marine nematodes; Tyrrhenian Sea

1. Introduction

Parasitic nematodes play a significant role in shaping host ecology, population dynamics, and health in marine ecosystems [1]. Among these, the genus *Cucullanus* (Cucullanidae) includes numerous species of gastrointestinal nematodes parasitizing teleost fishes in both marine and freshwater environments. Members of this genus are globally distributed and commonly occur in benthic and demersal fishes, often exhibiting marked host specificity and site fidelity [2,3].

Despite their prevalence, species of *Cucullanus* are often difficult to distinguish due to limited morphological differentiation and overlapping diagnostic traits, particularly when based solely on light microscopy [3]. Accurate identification frequently requires ultrastructural analyses such as scanning electron microscopy (SEM) and, increasingly, molecular approaches. In the Mediterranean Sea, several *Cucullanus* species have been described and reported from both commercial and non-commercial fish hosts [4,5], but their diversity and distribution remain poorly characterized, particularly in lesser-studied species.

Gnathophis mystax (Delaroche, 1809), commonly known as the thinlip conger, is a demersal eel of the family Congridae, distributed throughout the eastern Atlantic Ocean and the

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Mediterranean Sea. It inhabits muddy or sandy bottoms at depths ranging from 50 to 500 m and feeds primarily on small benthic invertebrates and fish [6]. Although occasionally captured in bottom trawl fisheries, the parasitic fauna of this species remains undocumented.

Investigating the parasitic communities of non-commercial fishes such as *G. mystax* contributes to a broader understanding of marine biodiversity, host–parasite interactions, and ecosystem health. In addition, parasites such as *Cucullanus* may serve as bioindicators of environmental conditions such as heavy metals (Fe, Zn, Cr, Mn, Pb, and Cu), as well as proxies for host diet and behavior [7–9]. The present study provides the first parasitological assessment of *G. mystax* from the southern Tyrrhenian Sea (Calabria, Italy), with particular focus on the presence, prevalence, and morphology of nematodes of the genus *Cucullanus*. By combining morphological analysis and epidemiological indices, this research contributes new data to the parasitic biodiversity of Mediterranean fishes and establishes a baseline for future taxonomic and ecological investigations.

2. Materials and Methods

2.1. Study Area

The Mediterranean Sea extends for approximately 3800 km from the Strait of Gibraltar to the coasts of Israel and Palestine Autonomy, with an average width of about 700 km and a maximum width of approximately 1600 km. Biogeographically, it encompasses areas with Mediterranean climates and diverse ecosystems. It is divided into four major regions, each with distinct faunal and floral characteristics. The western region reflects Central European influences, while the eastern region shows stronger affinities with Asian species [10]. The Mediterranean basin is unique due to its complex paleogeographic and paleoecological history, acting as a biogeographic crossroads among Europe, Africa, and Asia. Its biodiversity is particularly remarkable, lying at the interface of warm and cold seas and encompassing diverse continental and marine environments [11,12]. Within this system, the Tyrrhenian Sea constitutes the deepest part of the western Mediterranean basin, reaching depths of approximately 3800 m. It features an uneven seabed with volcanic ridges and seamounts such as Monte Masilii and Vavilov, rising up to 500 m. Abyssal plains are rare and limited in extent. From a hydrologically perspective, the Tyrrhenian Sea is a semi-enclosed basin with limited connections to adjacent seas: to the north via a 400 m depth channel with the Ligurian Sea; to the west via the shallow Strait of Bonifacio; and to the south via the Algerian–Tyrrhenian channel and the Sicily Channel, both characterized by complex bathymetric features [13].

2.2. Sample Collection

A total of 41 specimens of *G. mystax* were examined. Fish were collected along the Tyrrhenian coast of southern Calabria (Italy), specifically from the areas of Vibo Valentia (n = 21) and Gioia Tauro (n = 20) (Figure 1). Specimens were obtained from local commercial fishermen over the sampling period April and July 2012. Immediately after capture, fish were placed in polyethylene bags, stored on ice, and transported in thermal containers to the Parasitology Laboratory, Section of Zoology, Department of Ecology, University of Calabria. Upon arrival, specimens were frozen and subsequently defrosted at room temperature prior to examination. Each specimen was morphologically identified and individually labeled with a unique code, including information on date and sampling locality, to ensure traceability throughout the study.

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Figure 1. Sampling sites of *Gnathophis mystax* (Vibo Valentia and Gioia Tauro) along the Tyrrhenian coast of Calabria, Italian Peninsula.

2.3. Biometric Data Collection (Endoparasite Screening)

After thawing, each specimen of *G. mystax* was measured for the following biometric parameters: total length (TL), recorded in cm from the tip of the snout (mouth closed) to the end of the longest caudal fin lobe, and total weight (TW), measured in grams using a precision balance.

2.4. Parasitological Examination

2.4.1. External Examination

Each specimen underwent thorough external inspection, including the skin, fins, gills, mucus, and oral cavity, under a stereomicroscope to detect possible ectoparasites. Samples were subsequently labeled accordingly.

2.4.2. Parasite Isolation and Identification

Internal parasitological screening involved careful dissection and examination of internal organs. The gills were excised, placed in distilled water, and examined under a stereomicroscope. Each gill was scraped and rinsed; the rinse solution was diluted with 0.9% NaCl solution and re-examined. A midline incision was made to expose the internal organs. The digestive tract (esophagus and intestine) and associated organs (stomach, liver, spleen, pyloric caeca, swim bladder, kidneys, gall bladder, and heart) were separated, placed individually in Petri dishes containing distilled water, and examined under a stereomicroscope. All recovered parasites were isolated, counted when possible,

and preserved in 70% ethanol. Helminths were subsequently processed for morphological identification, photographed, and stored for further analysis.

2.5. Parasitological Preparations

Nematodes were fixed in 70% ethanol and cleared in Amman's lactophenol (Chemlab, Zedelgem, Belgium). For microscopic examination, specimens were mounted on glass slides in a drop of lactophenol (phenol 20%, glycerol 40%, distilled water 20%). Coverslips were carefully applied to avoid air bubbles, and excess mounting medium was removed using filter paper. Prepared slides were then examined under a Leica DMLB light microscope (Leica Microsystems, Wetzlar, Germany). Measurements are given in micrometers unless otherwise stated.

2.6. Data Analysis

Epidemiological Indices

Parasitic infestation levels were evaluated using the following indices [14,15]: prevalence (P), defined as the percentage of infected hosts among those examined and calculated as P (%) = (number of infected hosts/number of hosts examined) $\times$ 100; mean intensity (MI), defined as the average number of parasites per infected host and calculated as MI = number of parasites recovered/number of infected hosts; and mean abundance (MA), defined as the average number of parasites per examined host, including both infected and uninfected individuals, and calculated as MA = number of parasites recovered/number of hosts examined [15]. Statistical analyses were performed to evaluate differences in fish size and parasite infection between the two sampled localities (Gioia Tauro and Vibo Valentia). Fish total length and weight were compared between localities using independent-samples Student's *t*-tests. Prevalence of *Cucullanus* between localities was compared using Fisher's exact test. Abundance of *Cucullanus* was compared between localities using the non-parametric Mann–Whitney U test. Relationships between parasite abundance and fish biometric variables (length and weight) were assessed using Spearman's rank correlation coefficient. Statistical significance was set at $\alpha = 0.05$ for all analyses.

3. Results

In Vibo Valentia, the total length and weight of thinlip congers ranged from 42 to 70 cm (mean = 56 $\pm$ 8.94 cm) and 85 to 600 g (mean = 310.23 $\pm$ 165.72 g) (n = 21). In Gioia Tauro, the total length and weight of thinlip congers ranged from 42 to 75 cm (mean = 53.50 $\pm$ 10.55 cm) and 75 to 700 g (mean = 275.75 $\pm$ 185.61 g) (n = 20). There was no statistically significant difference in either fish length (t = 0.82, df = 39, $p = 0.418$) or fish weight (t = 0.63, df = 39, $p = 0.533$) between Vibo Valentia and Gioia Tauro.

A single taxa nematode belonging to the genus *Cucullanus* was found inhabiting the intestinal tract of *G. mystax*. Based on morphological features, the specimens recovered were confidently assigned to the genus *Cucullanus*. No more nematodes were detected (recovered trematodes are not presented in the present study), and no external parasites were found. However, nematode identification at the species level was not possible due to the high degree of morphological similarity among congeners [3] and the limited material in optimal conditions. *Cucullanus* species are known for their relatively uniform external features, which often complicate differentiation at the species level.

These nematodes exhibit marked sexual dimorphism. Males are generally smaller, possess a small fusiform sucker lacking a chitinous rim, and have 11 pairs of caudal papillae arranged in two longitudinal rows. Spicule length was 1162 µm and gubernaculum length was 229 µm in a single male measured (Figures 2 and 3). Females display elongated, sinuous ovaries, a vulval opening, bifurcated uteri, and oviducts oriented anteriorly. Eggs are ellipsoidal with smooth, thin shells.

Figure 2. Posterior end of male showing the spicules (scale bar = 500 µm). Recovered from *Gnathophis mystax* in the Tyrrhenian sea.

In Vibo Valentia, the prevalence, mean intensity, and mean abundance were 66.7% (14/21), 2.71 (±1.97) parasites per infected fish, and 1.80 (±2.06), respectively. The intensity of infection varied from 1 to 7 parasites per individual, and the total number of nematodes found was 38. In Gioia Tauro, the prevalence, mean intensity, and mean abundance were 70% (14/20), 3.00 (±1.89) parasites per infected fish, and 2.10 (±2.09), respectively. The intensity of infection varied from 1 to 7 parasites per individual, and the total number of nematodes found was 42. There was no was significant difference in *Cucullanus* prevalence between localities (Fisher's exact test, $p = 1.000$). *Cucullanus* abundance did not differ significantly between localities (Mann–Whitney U = 225, $p = 0.698$). Furthermore, parasite abundance was not significantly correlated with fish length (Spearman's $\varrho = 0.088$, $p = 0.583$) or fish weight (Spearman's $\varrho = 0.091$, $p = 0.573$).

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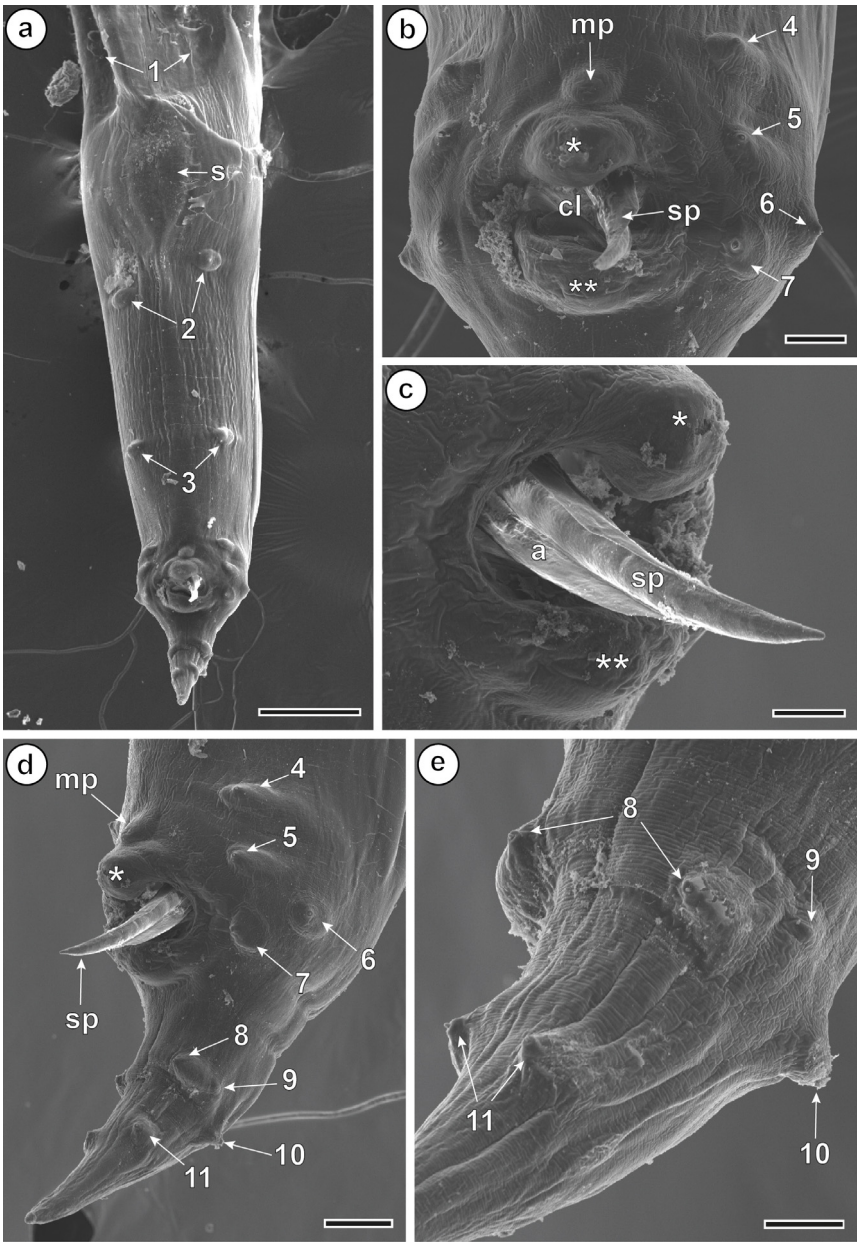

Figure 3. A male of *Cucullanus* sp. form *Gnathophis mystax*. (**a**) Ventral view of posterior end showing the three pairs on preanal papillae. (**b**) Detail of the cloaca showing four pairs of adanal papillae. (**c**) Detail of cloacal area and the spicule. (**d**) Detail of adanal and postanal papillae. (**e**) Detail of the postanal pairs of papillae at higher magnification. Scale bars: (**a**) = 200 µm; (**b**) = 30 µm; (**c**) = 20 µm; (**d**) = 50 µm; (**e**) = 20 µm; * = median outgrowth of anterior cloacal lip; ** = median outgrowth of posterior cloacal lip; 1–11 = pairs of caudal papillae; a = spicular ala; cl = cloaca; mp = median precloacal papilla-like formation; s = ventral sucker; sp = spicule.

4. Discussion

Species of the genus *Cucullanus* are widely distributed parasites of teleost fishes and sea turtles [2,16]. Due to the lack of species-level identification, no specific biogeographical or ecological information can be provided for the nematodes found in this study. Moreover, the life cycles of most *Cucullanus* species are poorly understood [17] and could not be determined here, though they are generally assumed to involve invertebrate intermediate hosts [18]. In the absence of species-level identification and larval stages, no conclusions could be drawn in the present study regarding either the life cycle or these intermediate hosts.

The present study represents the first known report of *Cucullanus* sp. in *G. mystax* from the southern Tyrrhenian coast of Calabria. In this study, morphological examination allowed for reliable assignment to the genus *Cucullanus*, although species-level identification was not possible due to the genus's high degree of morphological homogeneity [3].

Cucullanus hians was described in *Conger conger* belonging to the family Congridae as *G. mystax* in the Mediterranean Sea [4]. The spicule length was 1.26 mm, and the gubernaculum was 260 µm, which are within the range of our measurements. In the Atlantic Sea (Iberian Peninsula), also in *Conger conger*, the presence of *C. hians* and *C. longispiculum* has been reported, and the most important distinctive characteristic is the length of the male spicule: 0.80–1.80 mm in *C. hians* and 2.10–3.46 mm in *C. longispiculum* [19]. For related papillae, the original description of Campana-Rouget and Chabaud [4] includes eleven pairs of papillae, with four of them being postanal, as in our studied individuals. According to [20], Cucullanidae have ten pairs of papillae and one pair of specific papillae (phasmids). One of the four postanal papillae are considered phasmids. In the Atlantic Sea, in *C. conger*, there are four postanal papillae in *C. hians* but not in the phasmids [21]. A more detailed study including molecular data for representatives of the family Congridae will be necessary to determine if *C. hians* is distributed in different species of this host. For the above-mentioned reasons, despite our species possibly belonging to *C. hians*, we leave our individuals as unnominated species of *Cucullanus*. This taxonomic uncertainty was already evident in the validity of *Cucullanus hians* recovered from *Muraena helena*, a fish belonging to the order Anguilliformes, as do *C. conger* and *G. mystax* [22].

Due to the considerable taxonomic complexity of the genus *Cucullanus*, which includes more than 100 described species [23], further detailed morphological investigations, particularly using scanning electron microscopy (SEM), and molecular analyses are required for accurate species identification.

The prevalence of *Cucullanus* sp. was relatively high in both sampling sites, reaching 66.7% in specimens from Vibo Valentia and 70.0% in those from Gioia Tauro, indicating optimal conditions for the completion of the life cycle. However, comparisons remain limited due to the absence of previous parasitological studies on *G. mystax*, highlighting the novelty of the present findings. It will be of interest to determine how biotic and abiotic conditions could have influenced the presence of *Cucullanus* in this congrid by obtaining more data.

From a biogeographical and ecological perspective, the occurrence of *Cucullanus* sp. in *G. mystax*, a demersal species with benthic feeding habits, is not unexpected. Similar infections have been reported in other congrid eels in the Mediterranean Sea in an area close to our sampling area, such as those of the *Conger conger* in Sardinia island [24], with a prevalence of 46.2%, which occupy comparable ecological niches and have comparable feeding behaviors with *G. mystax*. Importantly, no zoonotic nematodes, such as members of the family Anisakidae, were detected. This finding indicates that the parasitic nematodes identified do not represent a known risk to human health under current European food safety regulations (Regulation EC No. 1881/2006).

5. Conclusions

In conclusion, this study provides the first parasitological assessment of *G. mystax* from the southern Tyrrhenian Sea, with particular emphasis on infection by *Cucullanus* sp. Despite the lack of species-level identification, the relatively high prevalence and consistent localization within the host contribute valuable information to our knowledge of the parasitic fauna of this species. *Cucullanus* were recorded at high prevalence in *G. mystax* at both sampling sites. Morphological identification allowed for identification at the genus level, but species determination is not achievable using light microscopy alone.

These findings establish a baseline for future parasitological and ecological investigations in *G. mystax* and highlight the need for further taxonomic investigations on *Cucullanus* in the Mediterranean region. Also, ecological studies undertaken to determine the biotic and abiotic factors influencing helminth fauna, covering recently collected localities, will be of interest. Additional research integrating morphometrical and molecular approaches will be essential to resolve species identity, clarify life cycles, and better understand the ecological factors influencing parasite transmission.

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