First record of Marphysa chirigota (Annelida: Eunicidae) in the Mediterranean Sea (Gulf of Tunis)

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First record of *Marphysa chirigota* (Annelida: Eunicidae) in the Mediterranean Sea (Gulf of Tunis)

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Abstract

The genus *Marphysa* (Annelida: Eunicidae) is represented by only three species, *Marphysa sanguinea*, *Marphysa aegypti* and *Marphysa birgeri*, in the Mediterranean Sea. Combining morphological, molecular data (16S rRNA and cytochrome c oxidase subunit I mitochondrial loci) and environmental information, we present the first Mediterranean report of *Marphysa chirigota*, based on the specimens collected at Radès Station (Gulf of Tunis, W Mediterranean). The current information on species distribution in *Marphysa* strongly supports that *M. sanguinea* inhabits hard bottoms and has a restricted distribution close to its type location (south English coast and nearby NE European Atlantic). Radès Station specimens and all those reported as *M. sanguinea* along the Tunisian coast were found in shallow water soft bottoms. Therefore, we suggest that the presence of *M. sanguinea* in Tunisia seems doubtful, and all species reports of *Marphysa* from Tunisia might correspond to *M. chirigota*.

Keywords: Eunicids; Polychaetes; First report; Environment; Distribution; Mediterranean Tunisian coast; integrated taxonomy; DNA barcode.

Introduction

The family Eunicidae (Annelida) includes 453 species grouped in eleven extant and one extinct genera (Read & Faucauld, 2020; Zanol et al., 2021). Many of them have a large number of synonyms, while some [e.g., *Marphysa sanguinea* (Montagu, 1813)] have been traditionally considered cosmopolitan (e.g., Hutchings & Karageorgopoulos, 2003; Lavesque et al., 2019; Read & Faucauld, 2020). Among polychaetes, either numerous synonymies or cosmopolitanism often indicate the need for integrative taxonomic revisions. By combining molecular, morphological and geographical evidence, these reviews often describe new species (or recover previously synonymized ones) showing locally restricted geographical distributions (Hutchings & Kupriyanova, 2018). Eunicids are not an exception, and an excellent example occurs in *Marphysa Quatrefages*, 1865, one of the most speciose genera of the family, with 74 species (Zanol et al., 2021). *Marphysa sanguinea*, the genus type species, was described from England’s south coast and later redescribed as inhabiting only in nearby areas (Hutchings & Karageorgopoulos, 2003; Hutchings et al., 2012; Lavesque et al., 2019). The species is now known to have a locally restricted distribution in the NE Atlantic coasts, from the Southern Bight, the Celtic Sea and the North Sea to the north, and from somewhere between Arcachon (France) and Cádiz (Spain) to the south (Martin et al., 2020). All other reports worldwide must be considered doubtful. All populations that have currently been checked revealed to belong to different species having restricted biogeographical distributions, while at least eight species previously synonymized with *M. sanguinea* have been reinstalled (Lewis & Karageorgopoulos, 2008; Molina-Acevedo & Carrera-Parra, 2015; Lavesque et al., 2017; Elgetany et al., 2018; Glasby et al., 2019; Hutchings et al., 2020; Kara et al., 2020; Martin et al., 2020; Molina-Acevedo & Idris, 2020). All together form the so-called “sanguinea” group (Martin et al., 2020), which may consist of at least 24 different species (Molina-Acevedo & Idris, 2020).

The NE Atlantic (including the Mediterranean) has also focused on numerous recent studies on the “sanguinea” group. As a result, six species have been reported from the region (Lavesque et al., 2017; Elgetany et al., 2018; Lavesque et al., 2019; Martin et al., 2020). *Marphysa sanguinea*, *Marphysa chirigota* Martin, Gil and Za-
Tunisia, our paper aimed: a) to document the first report of *M. sanguinea* in the Mediterranean, b) to discuss the validity of the previous reports of *M. sanguinea* in Tunisian waters, and c) to propose possible alternatives explaining the actual distribution of *M. chirigota*.

**Molecular analyses**

Consensus sequences for each individual and gene were obtained from forward and reverse sequences and edited using Geneious vs. R8 (Kearse et al., 2012). They were aligned with the GenBank sequences in Mesquite using Muscle (Rozewicki et al., 2017). COI sequences were translated into amino acids with the code for in-
vertebrate mitochondrial genes to check for stop codons and exclude pseudogenes' presence. The final alignment included 660 bp for COI and 959 bp for 16S rDNA. Un-corrected pairwise distances were calculated with PAUP* v.4.0a161. Additional sequences belonging to other species of Marphysa were obtained from GenBank, together with other genera of Eunicidae, of which those of Leodice rubra Grube (1856), Eunice cf. violaceomaculata Ehlers, 1887 and Palola viridis Gray in (Stair, 1847) from Gen- eBank were used as outgroups. The maximum likelihood (ML) and Bayesian inference (BI) analyses were performed separately for each gene's data set. The best-fit model of nucleotide substitution for each gene was estimated with the software package Iq-Tree 1.6.12 (Chernomor et al., 2016) using the Akaike information criterion (AIC). TIM2 +F+I+G4 was the best fitting evolutionary model for the 16S dataset and TIM3 +I+G for the COI database. ML analyses were performed using the software raxmlGUI 2.0 (Edler et al., 2021), optimizing the best fit model for each dataset and choosing the option ML + thorough bootstrap + consensus the version RAxML-NG. Two hundred bootstraps pseudoreplicates generated support values for the ML analyses. BI analyses were run in MrBayes 3.2.7a (Ronquist & Huelsenbeck, 2003) as implemented in CIPRES Science Gateway V 3.3 (Miller et al., 2012), with two independent runs, starting from random trees, with four chains running simultaneously (two cold and two heated). Chains were run for 10^7 generations, sampled every 1,000 generations, and 25% of the generations were discarded as burn-in. Tracer v. 1.7.1 was used to check the convergence of runs was reached with adequate sample size (ESS) values over 200 (Rambaut et al., 2018). Trees were visualized in Figtree v 1.4.2 (Rambaut, 2006).

**Fig. 1:** A. Known distribution of Marphysa chirogota (yellow spot: Bay of Cádiz; white spot: Bay of Tunis) and the currently accepted Mediterranean species of Marphysa (red spot: M. birgori; green spot: M. aegypt). B. Location of Radès Station (C: white square) at the Bay of Tunis. C. Location of collecting site (white spot) at Radès Station. D. Landscape view of the collecting site. A-C: photos from Google Earth (images: © 2020 Landsat/Copernicus, TerraMetrics, Maxar Technologies; data: SIO, NOAA, U. S. Navy, NGA, GEBCO).
Table 1. Summary of main morphological characters and measurements in Atlantic (Bay of Cádiz) and Mediterranean (Bay of Tunis) specimens of *Marphysa chirigota* and *M. aegypti*.

| Character                      | *M. chirigota* Bay of Cádiz | *M. chirigota* Bay of Tunis | *M. aegypti* |
|-------------------------------|-----------------------------|-----------------------------|--------------|
| Chaetiger number              | 370                         | >430                        | 293          |
| Body length (mm)              | 265                         | 225 - >420                  | 143          |
| Body width (mm)               | 7.9                         | 7.7 - 8.9                   | 9            |
| Chaetiger length vs. width    | up to 13                    | up to 18                    | up to 7      |
| Antennae central / lateral (up to chaetiger) | 1 / 3                       | 2 / 3                       | 3 / 4        |
| Palps (up to chaetiger)       | 1                           | 1                           | 1            |
| Pseudocelis absence           | absent                      | absent                      | present      |
| Mx I                          | 1+1, brown with dark tips   | 1+1, brown with dark tips   | 1+1; dark, with white tips |
| Mx II                         | 4/5+5                       | 3/4+4                       | 4+4          |
| Mx III                        | 6+0                         | 5/6+0                       | 5+0          |
| Mx IV                         | 4/5+7                       | 4+7                         | 4+6          |
| Mx V                          | 1+1                         | 1+1                         | 2+1          |
| Notopodial cirri              | triangular; longer (anterior), as long as (median), shorter (posterior) and longer (posterior-most) than chaetal lobes | triangular; longer (anterior), as long as (median), shorter (posterior) and longer (posterior-most) than chaetal lobes | digitiform; longer than chaetal lobes along whole body |
| Branchiae                     | Chaetigers 25/30 to 330     | Chaetigers 31/34 to 390     | Chaetigers 29 to 245 |
| Branchial filaments           | up to 6                     | up to 6                     | up to 6      |
| Maximum number from chaetiger | 55-75                       | 60-70                       | 88           |
| Neuropodial aciculae          | up to 6, golden brown       | up to 4, golden brown       | up to 3, black |
| Subacicular hook              | 1-2, unidentate, from chaetiger 30-45 | 1-2, unidentate, from chaetigers 31 | 1-2, unidentate, from chaetigers 38-48 |
| Pectinate chaetae Type 1      | isodont (with external teeth markedly differing in length), symmetrical | isodont (with external teeth markedly differing in length), symmetrical | isodont (with external teeth markedly differing in length), symmetrical |
| Shape                         | ≈25                         | 20-30                       | ≈19          |
| Number of teeth               |                             |                             |              |
| Pectinate chaetae Type 2      | isodont, asymmetrical       | isodont, asymmetrical       | isodont, asymmetrical |
| Shape                         | > 25                        | 20-30                       | ≈15          |
| Number of teeth               |                             |                             |              |
| Pectinate chaetae Type 3      | isodont, asymmetrical       | isodont, asymmetrical       | isodont, asymmetrical |
| Shape                         | slightly filiform           | slightly filiform           | pointed      |
| Teeth tips                    |                             |                             |              |
| Number of teeth               | 13–16                       | 10–16                       | 9            |
| Pectinate chaetae Type 4      | anodont, asymmetrical       | anodont, asymmetrical       | anodont, asymmetrical |
| Shape                         | 4–5                         | 4–5                         | 2            |
| Number of chaetae             |                             |                             |              |
| Number of teeth               | 4–7                         | 4–7                         | 5 – 6        |
| Teeth length vs. width        | 2.5                         | 3                           | 4            |
| Tip width (mm)                | ≈45                         | ≈45                         | ≈25          |
Table 2. Primers and parameters used for the PCR analyses.

| Gene | Primers | Sequence (5’-3’) | PCR Parameters | Reference |
|------|---------|------------------|----------------|-----------|
| COI  | ACOIAF  | CWA ATC AYA AAG ATA TTG GAAC | 94° for 3-5 min, 35 cycles*(94°C for 1 min, 53°C for 1 min, 72°C for 2 min), 72°C for 7 min. | Zanol et al. (2010) |
|      | COIEU-R | TCD GGR TGD CCA AAR AAT CA |                |           |
| 16S  | Mar_16SF | GTGAGCTGATCTTTACTTG | 95°C for 5 min, 35 cycles* (94°C for 1 min + 42°C for 1 min + 72°C for 1 min), 72°C for 5 min. | Martin et al. (2020) |
|      | Mar_16SF | GCTCTGGAGGA AGATTAGTC |                |           |

Table 3. List of the GenBank accession numbers of the sequences used in the phylogenetic reconstructions; n.a.: not available.

| Species                  | COI                      | 16S RDNA                  | Type locality          | Collecting locality          | References |
|--------------------------|--------------------------|---------------------------|------------------------|-----------------------------|------------|
| *Marphysa aegypti*       | MF196969-71              | n.a.                      | Suez canal             | Suez canal                  | Elgetany et al. (2018) |
| *Marphysa bifurcata*     | KX172177-78              | n.a.                      | Point Peron, Western Australia | Australia                  | Zanol et al. (2016) |
| *Marphysa brevitentaculata* | GQ497548                | GQ478158                  | Tobago, West Indies    | Mexico                      | Martin et al. (2020) |
| *Marphysa Californica*    | GQ497552                | GQ478162                  | San Diego, California, USA | California, USA             | Martin et al. (2020) |
| *Marphysa chirigota*     | MN816442-44, MW221034, MW221035; MW221036 | MN813670-72; MW219694 | Bay of Cádiz, Iberian Peninsula | Bay of Tunis, Tunisia       | Martin et al. (2020); This study |
| *Marphysa corallina*     | KX172410; KX823389; KX823371; KX823306; KX823300; KX823271 | n.a.                      | Hawaii                 | KwaZulu-Natal, Eastern Cape, South Africa | Kara et al. (2020); Martin et al. (2020) |
| *Marphysa fauchaldi*     | KX172165                | n.a.                      | off Elizabeth River, Darwin, Australia | Australia                  | Zanol et al. (2016) |
| *Marphysa gaditana*      | MN816441                | MN813673-74               | Bay of Cádiz, Iberian Peninsula | Bay of Cádiz, Iberian Peninsula | Martin et al. (2020) |
| *Marphysa haemasmata*    | MN067877                |                           | Cape of Good Hope, South Africa | Kommetjie, South Africa    | Kara et al. (2020) |
| *Marphysa hongkongensis* | MH598525                | MH598527-28               | Plover Cove, Hong Kong | China                       | Martin et al. (2020) |
| *Marphysa iloiloensis*   | MN133418; MN106281; MN106279 | n.a.                      | Buyu-an, Philippines   | Philippines                 | Martin et al. (2020) |
| *Marphysa kristiani*     | KX172158; KX172155; KX172153; KX172152; KX172151; KX172150; KX172148; KX172147; KX172146; KX172145; KX172144; KX172143; KX172143 | n.a.                      | Stingray Bay, New South Wales, Australia | Australia                  | Zanol et al. (2016) |

Continued
Table 3 continued

| Species                  | COI            | 16S RDNA | Type locality | Collecting locality | References         |
|--------------------------|----------------|----------|---------------|---------------------|--------------------|
| *Marphysa mossambica*    | KX172164; JX559751 | JX559747 | Mossimboa, Mozambique | Philippines, Mozambique | Zanol et al. (2016) |
| *Marphysa mulawa*        | KX172176; KX172175; KX172173; KX172172; KX172171; KX172170; KX172168; KX172167; KX172166 | n.a. | Fisherman’s Island, Australia | Australia | Martin et al. (2020) |
| *Marphysa pseudosessilosa* | KY605406 | n.a. | Careel Bay, Australia | Australia | Zanol et al. (2017) |
| *Marphysa regalis*       | GQ497562 | GQ478165 | Bermuda | Brazil | Zanol et al. (2016) |
| *Marphysa sanguinea*     | MN106284; MN106283; MN106282; MK541904; MK590582; MK950851; GQ497547; MK967470 | GQ478157; AY38883; KF733802; NC_023124 | Polperro, Cornwall, England | England, France | Zanol et al. (2016); Lavesque et al. (2019) |
| *Marphysa sanguinea/gaditana* | KR916870; KR916873; KR916872; KR916871; KP255196; KP254890; KP254743; KP254644; KP254643; KP254537; KP254503; KP254223 | n.a. | European and USA North East Atlantic | Martin et al. (2020); Lobo et al. (2016), Leray and Knowlton (2015) |
| *Marphysa sherlockae*    | MT840349–MT840351 | | Durban, South Africa | Strand, South Africa | Kara et al. (2020) |
| *Marphysa tripectinata*  | MN106278; MN106277; MN106274 | n.a. | Behai, China | China | Liu et al. (2018) |
| *Marphysa victori*       | MG384996-99 | MG385001; MG385000 | Arcachon Bay, France | France | Zanol et al. (2016) |
| *Marphysa viridis*       | GQ497553 | GQ478163 | Boca Grande Key, USA | Brazil | Zanol et al. (2010) |
| *Marphysa sp.*           | NC023124 | | | Florida, USA, China | Li et al. (2016) |
| *Palola viridis*         | GQ497556 | GQ478167 | Samoa, Polynesia | Kosrae, Micronesia | Zanol et al. (2010) |
| *Paucibranchia bellii*   | KT307661 | | Chausey Island, France | Spain | Ayalagas et al. (2016) |
| *Paucibranchia disjuncta* | GQ497549 | | Los Angeles County, USA | California, USA | Zanol et al. (2010) |
| *Eunice cf. violaceomaculata* | GQ497542 | GQ478148 | Florida, Caribbean Sea | Carrie Bow Cay, Belize | Zanol et al. (2010) |
| *Leodice rubra*          | GQ497528 | GQ478132 | Saint Thomas, Virgin Islands | Ceará, Brazil | Zanol et al. (2010) |

*Genus updated according to Zanol et al. (2014), species as *Eunice rubra* in GenBank.*
Results

Systematics

Order Eunicida Dales, 1962
Family Eunicidae Berthold, 1827
Genus *Marphysa* Quatrefages, 1865

*Marphysa chirigota* Martin, Gil and Zanol, 2020 in Martin *et al.* (2020)
Figs. 2-4

*Marphysa chirigota* Martin *et al.* (2020): 17-25, figs. 3C, 3D, 5C, 5D, 7B-7D, 9C, 9D, 11-13 and 14A-14D.

Material examined

MNCN 16.01/18933, January 14, 2020, Radès Station, Gulf of Tunis, 36.804722° N, 10.294444° E, coll. M. Chaibi from muddy sand, 2 m depth, 3 specimens; MNCN 16.01/18934, July 19 2020, Radès Station, Gulf of Tunis, 36.804917° N, 28.7778° E, coll. M. Chaibi from muddy sand, 2 m depth, 14 specimens.

Extended diagnosis

Based on specimen MNCN 16.01/18933, except for mandibles, which are based on specimen MNCN 16.01/18934–1; measurement ranges and variability are indicated in Table 1. Body long, similarly wide, tapering at posterior end, with a round cross-section in anterior and middle regions, flattening posteriorly. Prostomium darker in center and lighter toward distal end, with a pattern of brown and whitish patches (Fig. 2A-B). One median and two lateral antennae, folding back to middle of chaetiger 3; two palps, folding back until beginning of chaetiger 1 (Fig. 2A-B). One pair of dark brown eyes, lateral to basis of lateral antennae (Fig. 2B). Calcareous cutting plates longer than sclerotized matrix, 0.56 long per 0.57 wide, overall thick, with thin translucent borders, broadly rhomboidal; mandible carriers 4.32 long per 0.87 of maximum width (Fig. 3A). Maxillary carriers 1.46 mm long (Fig. 3B). Maxillary formula: MxI = 1 + 1 (3.66 mm long), MxII = 3/3 + 4 (2.79 mm long), MxIII = 5/6 + 0 (1.01 mm long), MxIV = 4 + 7 (0.84 mm long), MxV = 1 + 1 (0.39 mm long). MxMx VI absent (Fig. 3B-C). Branchial filaments whitish, starting at chaetiger 31, with a maximum of four filaments, starting at chaetiger 65, filaments 5-8 times longer than notopodial cirri and at least three times longer than branchial stems (Fig. 2D-E). Notopodial cirri triangular, tapering (almost three times longer than wide at base), decreasing in length towards posterior end, more extended than post-chaetal lobes in anterior chaetigers long as in median chaetigers and shorter in posterior ones (Fig. 2D-E). Ventral cirri thumb-shaped with roughly roundtips and inflated bases from chaetiger 5 to posterior body end (Fig. 2D-E). Notopodial

![Fig. 2: Marphysa chirigota MNCN 16.01/18933. A. Anterior anterior end, dorsal view. B. Anterior end, lateral view. C. Posterior end, showing the position of long dorsal (lac) and short ventral (sac) anal cirri in the pygidium (p). D. Midbody branchial parapodium, posterior view; E. Midbody branchial parapodium, anterior view; F. Detail of the location of aciculae (a) and subacicular hook (sh). D-E: Chaetiger 65.](chart)
**Fig. 3:** *Marphysa chirigota* MNCN 16.01/18934–1. A. Dissected mandible. B. Dissected maxillae. C. Detail of left maxillae III to V.

**Fig. 4:** *Marphysa chirigota* MNCN 16.01/18933. Mid-body branchial parapodium (chaetiger 65). A. Capillary notochaeta. B. Compound spiniger neurochaeta. C. Subacicular hook. D. Tips of aciculae. E. Type 2 pectinate chaetae; F. Type 3 pectinate chaeta; G. Type 4 pectinate chaetae. Scale bar is the same for all images.
aciculae pale yellow, inconspicuous. Neuropodial aciculae golden brown, 2-3 per parapodia, with blunt tips protruding from acicular lobe (Fig. 2F, 4D). Chaetae in two distinct bundles: supracircular with limbate (Fig. 4A) and pectinate chaetae (Fig. 4E-G) at anterior edge, and subaciculae with compound spiniger chaetae (Fig. 3B) and one solid and golden subacicula hook, always unidentate (Fig. 2F, 4C), starting at chaetiger 31. Pectinate chaetae four types, in all chaetigers, except for chaetigers 1-4. Type 1 present on anterior parapodia (thin, flat to slightly curved, lightly serrated, with evenly tapering fine teeth, isodont with external teeth markedly differing in length, with ca. 20-30 teeth). Type 2 present alone on the half posterior body (thin, flat to slightly curved, lightly serrated isodont asymmetrical with ca. 20-30 evenly tapering fine teeth; Fig. 4E). Type 3 (thick, flat to little curved chaetae, marked asymmetrical, isodont, with 10-16 coarse and long teeth, of variable length on different chaetae, Fig. 4F) and Type 4 (thick, large, non-curved, asymmetrical, anodont, with 4-7 thick, almost triangular teeth, tapering to filiform ends, 3-5 times longer than wider; Fig. 4G) appearing from around midbody up to posterior-most parapodia. Two pairs of pygidial cirri (Fig. 2C).

Remarks

Marphysa chirigota belongs to the species of the sanguinea-group having unidentate subacicula hooks. It can be distinguished from the species with all subacicula hooks unidentate in having: (1) subacicula hooks from chaetiger 30-45 vs. 46 in Marphysa durbanensis Day, 1934, 71 in Marphysa bulla Liu, Hutchings & Kupriyanova, 2018, 255 in Marphysa nobilis Treadwell, 1917 and 170 in Marphysa triplicata Liu, Hutchings & Sun, 2017; (2) two types of isodont pectinate chaetae vs. one isodont and one anodont pectinate chaetae in Marphysa aransensis Treadwell, 1939; (3) first branchiae before chaetigers 25-30, vs. at 35 in Marphysa furcellata Crossland, 1903, Marphysa iloiloensis Glasby, Mandario, Burghardt, Kupriyanova, Gunton & Hutchings, 2019, and Marphysa mangeri Augener, 1918, and after 30 in Marphysa macintoshi Crossland, 1903 and M. tamurai Okada, 1934; (4) pectinate chaetae from first chaetigers vs. only in the posterior body region in Marphysa parishii Baird, 1869; (5) 4/5 + 5 (maxilla II) and 5/5 + 7 (maxilla IV) vs. 6 + 6 and 8 + 9 in Marphysa acicularum brevibranchiata Treadwell, 1921, and (6) 1 + 1 (maxilla V) and up to six golden brown neuropodial aciculae vs. 2 + 1 (maxilla V) and three black aciculae in M. aegypti.

The specimens of M. chirigota are almost identical to those from the Bay of Cádiz. The main differences are linked to the fact that some Tunisian worms were bigger. This likely influenced some characteristics (e.g., body width, chaetiger length vs. body width), while others, apparently size-dependent (e.g., starting of branchiae, extension of branchial segments, starting of subacicula hooks) did not vary significantly (Table 2). Overall, M. chirigota most closely resembles M. aegypti in body size and in having one (sometimes two) unidentate subacicula hooks but differs in numerous morphological characters (Table 2). Our results confirm that, despite the numerous differences, distinguishing the two species requires carefully observing key characters, as already stated by Martin et al. (2020).

Distribution

Atlantic Ocean: Bay of Cádiz (Iberian Peninsula), probably present in Portugal; W Mediterranean: Gulf of Tunis (Tunisia).

Habitat

Soft substratum with mud and sand. All collected specimens were non-ripe adults.

Molecular analysis

Overall, our phylogenetic reconstructions were congruent with those of Martin et al. (2020) and Kara et al. (2020), with well-supported clades corresponding to the currently accepted species of Marphysa. The phylogenetic trees based on both 16S rDNA and COI showed that the Tunisian specimens formed a single clade with the sequences obtained from the specimens of M. chirigota from Bay of Cádiz, with both BI and ML analyses providing consistent topologies (Figs 5 and 6). This clade was well-supported in the 16S tree (1 pp, 100% bs) and had moderate support in the COI tree (0.95 pp, 78% bs). The maximum within-clade distance between the Tunisian specimens and M. chirigota was 0.15% for COI and 0.14% for 16S rDNA. The closest relationship with other species was with M. aegypti: However, there was enough distance (2.8-3.8% COI uncorrected p-distances) to consider them as different taxa (Martin et al., 2020).

Discussion

By combining morphological observations and molecular analysis, we are here confirming that the specimens of Marphysa from Radès Station belonged to M. chirigota. Although it may also be present in Portugal, this species is currently known only from the Bay of Cádiz in the Atlantic coasts of the Iberian Peninsula, living on shallow intertidal muddy sands (Martin et al., 2020). In addition to the morphology and genetic features, the type of habitat also seemed to be informative to distinguish among the species of the “sanguinea” group (Martin et al., 2020), with M. sanguinea appearing to be restricted to live on hard substrata (Hutchings & Karageorgopoulos, 2003; Jumars et al., 2015; Lavesque et al., 2019). Taking this into account, we considered all previous Tunisian reports of M. sanguinea (Ben Amor, 1984; Ayari et al., 2009; El Barhoumi et al., 2013; Mdaini et al., 2019). Despite the target polychaete species cannot be checked
due to the absence of voucher specimens, we strongly suggest that they may correspond to *M. chirigota* instead of *M. sanguinea* due to: 1) their currently known distributions (Lavesque et al., 2019; Martin et al., 2020), 2) the geographical proximity of all Tunisian locations, and 3) the fact that all Tunisian environments corresponded to soft bottoms.

Our finding of *M. chirigota* in the Radès Station of the Gulf of Tunis (1) certifies its presence in the coasts of Tunisia, and (2) represents its first report for the country and for a Mediterranean location. However, it must be taken into account that the Radès Station is a highly industrialized area, with a well-developed petrol industry and heavily navigated waters. As there is only one previously confirmed, very recent record, which indeed was in an Atlantic location (Martin et al., 2020), we cannot entirely discard the possibility of the species being introduced in Radès Station. However, our data allow us to suggest that it is an Atlanto-Mediterranean species previously misidentified as *M. sanguinea*.

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**Fig. 5:** Bayesian inference (BI) tree based on the COI dataset (96 sequences, 660bp). BI and Maximum likelihood (ML) statistic supports are indicated on the nodes (BI/ML < 0.5 pp or 50% bs not shown). * at nodes corresponds to support = 1 pp and >99% bs. Codes before species names: GenBank accession numbers; inds: number of sequenced individuals; *Marphysa* Tunis: specimens from this study.
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