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

Marwa CHAIBI[1], Chiara ROMANO[2], Atf AZZOUNA[1] and Daniel MARTIN[2]

[1] University of Tunis El Manar, Faculty of Sciences of Tunis, LR18US41 Biology,
Physiology and Ecology of Aquatic Organisms 2092, Tunis, Tunisia
[2] Center for Advanced Studies of Blanes (CEAB-CSIC),
14 Accés a la Cala Sant Francesc street, 17300 Blanes, Girona, Catalonia, Spain

Corresponding author: marwachaibi89@gmail.com

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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 & Fauchald, 2020; Zanol *et al.*, 2021). Many of them have a large number of synonymies, while some [e.g., *Marphysa sanguinea* (Montagu, 1813)] have been traditionally considered cosmopolitan (e.g., Hutchings & Karageorgopoulos, 2003; Lavesque *et al.*, 2019; Read & Fauchald, 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 Zanol, 2020 in Martin *et al.* (2020), and *Marphysa birgeri* Molina-Acevedo and Idris, 2020 are currently considered as native, while *Marphysa victori* Lavesque, Daffe, Bonifácio & Hutchings, 2017, *Marphysa aegypti* Elgetany, El-Ghobashy, Ghoneim and Struck, 2018, and *Marphysa gaditana* Martin, Gil and Zanol, 2020 in Martin *et al.* (2020) are (or maybe) non-native.

Marphysa victori was described from the Bay of Arcachon (the Bay of Biscay, NE Atlantic coast of France) and later found in E Asia, known as *Marphysa bulla* Liu, & Kupriyanova (2018). Molecular and morphological evidence proved that (i) *M. victori* and *M. bulla* were the same species, (ii) *M. victory* had priority and (iii) the French population might be alien, most likely introduced (probably from China or Japan) together with specimens of *Crassostrea gigas* Thunberg, 1793 imported for aquaculture (Lavesque *et al.*, 2020).

Marphysa aegypti was initially described from the Red Sea. The Mediterranean specimens (from Alexandria, Mediterranean coasts of Egypt) were considered Lessepsian migrants, introduced from the Red Sea to the Mediterranean via the Suez Canal (Elgetany *et al.*, 2018).

Marphysa gaditana was reported to occur at both sides of the N Atlantic, from Cap de la Hague (France), the Sado Estuary (Portugal) and the Bay of Cádiz (Iberian Peninsula) in the eastern side, and in Florida and Virginia (USA) in the western side. This strongly suggested that it might be non-native at some of these locations, although it was impossible to assess from where it has been introduced (Martin *et al.*, 2020).

Nowadays, *Marphysa* currently comprises 81 species (Kara *et al.*, 2020; Read & Fauchald, 2020; Molina-Acevedo & Idris, 2021). They are typically free-living, tubicolous or burrowing worms inhabiting from soft sediments to rocky grounds in warm and temperate waters (Jumars *et al.*, 2015; Zanol *et al.*, 2016). Moreover, some of them are of the highest commercial interest and, labeled as "*M. sanguinea*", are harvested and internationally distributed mainly as fish baits (Olive, 1994; Cole *et al.*, 2018; Font *et al.*, 2018). Indeed, this may lead to a high risk of introductions, stressing the relevance of knowing how many species are being currently traded under "*M. sanguinea*" (Martin *et al.*, 2020). In Tunisian waters, for instance, *M. sanguinea* was reported from Zembra Island (Ben Amor, 1984; Ayari *et al.*, 2009) and appeared to be also present in the Lagoon of Tunis (El Barhoumi *et al.*, 2013; Mdaini *et al.*, 2019), where it was used as bait for sport and commercial fishing and was considered as one of the most important economic resources (El Barhoumi *et al.*, 2013).

While *M. sanguinea* has not been confirmed in Mediterranean coasts, two species belonging to the "*sanguinea*" group had valid Mediterranean reports to date, *M. aegypti* and *M. birgori*. Based on morphological and molecular analyses of specimens collected in the Bay of Tunis, our paper aimed: a) to document the first report of *M. chirigota* in the Mediterranean, b) to discuss the validity of the previous reports of *M. sanguinea* in Tunisian coasts, and c) to propose possible alternatives explaining the actual distribution of *M. chirigota*.

Material and Methods

Collection, preservation and morphological analyses

Marphysa chirigota was collected in Radès Station, Gulf of Tunis, 36.804722° N, 10.294444° E (Fig. 1) by hand digging at 2 m depth, on January 14, 2020, and July 19, 2020. All specimens were preserved in 95% ethanol and deposited with the Museo Nacional de Ciencias Naturale of Madrid (MNCN) collections.

Light microscopy photos were taken with a CMEX 5 digital camera connected to a ZEISS Stemi CS-2000-C stereomicroscope (body and parapodia) and an SP100 KAF1400 and with an SP100 KAF1400 digital camera connected to a Zeiss Axioplan compound microscope (chaetae). Key morphological structures allowing to validate the species identification (Table 1) were either described or measured based on direct observations and/or on digital images captured with the ISListen software, version 5.4(1) © by Tucsen Photonics Co. Ltd.). Measurements were done with the Analysis routine and the Rule tool in Photoshop version 21.2.4 © by Adobe.

DNA extraction, amplification and sequencing

Small body fragments from posterior segments (excluding posterior-most parapodia and pygidial region) were cut from specimens fixed in ethanol (70-90%) to extract total DNA using DNAeasy Tissue Kit (Qiagen) following the manufacturer's protocol. Fragments of two mitochondrial genes, 16S rDNA (800-900 bp) and Cytochrome c oxidase I, COI (600-800 bp), were amplified using primers and PCR parameters listed in Table 2. PCR reactions took place in 25 µL total reaction volume. For COI, the PCR mix contained 0.15 µL BioTaq DNA Polymerase (5 U/ µL, Bioline), 2 µL DNA template, 2.5 µL reaction buffer, 2 µL $MgCl_2$ (50 mM), 1 µL Bovine serum albumin (10 mg/ml), 2 µL dNTPs (10 µM), 1 µL each primer (10 µM), and 13.35 µL milliQ water. For 16S rDNA, the solution contained 0.15 µL BioTaq DNA Polymerase (5 U/ µL, Bioline), 1 µL DNA template, 2,5 µL reaction buffer, 0.5 µL $MgCl_2$ (50 mM), 1 µL dNTPs (10 µM), 0.8 µL each primer (10 µM), and 18.25 µL milliQ water. Agarose gel electrophoresis was used to visualize PCR products to confirm fragment amplification. Successful amplifications were purified using ExoSAP-IT Express (USB) and sequenced in both directions (forward and reverse) by Macrogen, Inc. (Seoul, Korea). The obtained sequences were deposited in GenBank (Table 3).

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-

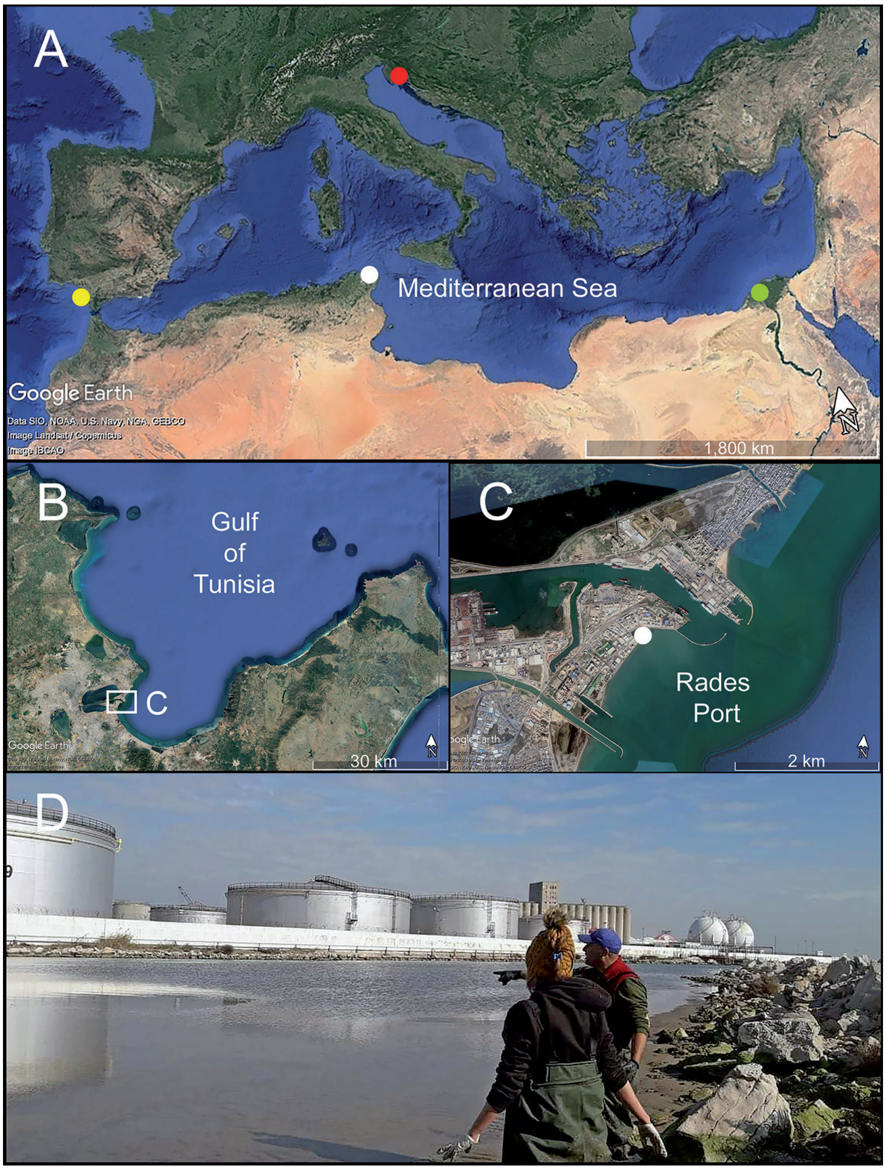

Fig. 1: A. Known distribution of *Marphysa chirogota* (yellow spot: Bay of Cádiz; white spot: Bay of Tunis) and the currently accepted Mediterranean species of *Marphysa* (red spot: *M. birgori*; green spot: *M. aegypti*). B. Location of Radès Station (C: white square) at the Bay of Tunis. C. Location 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).

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. Uncorrected 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 GeneBank 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).

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*.

	M. chirigota **Bay of Cadiz**	***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 lenght *vs*. width	up to 13	up to 18	up to 7
Antenae central / lateral (up to chaetiger)	1 / 3	2 / 3	3 / 4
Palps (up to chaetiger)	1	1	1
Peduncle	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			
Shape	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
Number of teeth	≈25	20-30	≈19
Pectinate chaetae Type 2			
Shape	isodont, asymmetrical	isodont, asymmetrical	isodont, asymmetrical
Number of teeth	> 25	20-30	≈15
Pectinate chaetae Type 3			
Shape	isodont, asymmetrical	isodont, asymmetrical	isodont, asymmetrical
Teeth tips	slightly filiform	slightly filiform	pointed
Number of teeth	13–16	10–16	9
Pectinate chaetae Type 4			
Shape	anodont, asymmetrical	anodont, asymmetrical	anodont, asymmetrical
Number of chaetae	4–5	4–5	2
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	GTGAGCTGATCTTTACTTGC	95℃ for 5 min, 35 cycles* (94℃ for 1 min + 42℃ for 1 min + 72℃ for 1 min), 72℃ 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	KT823410; KT823389; KT823371; KT823306; KT823300; KT823271	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 haemasoma	MN067877		Cape of Good Hope, South Africa	Kommetjie, South Africa	Kara *et al.* (2020)
Marphysa hongkongensa	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; KX172156; KX172155; KX172153; KX172152; KX172151; KX172150; KX172148; KX172147; KX172146; KX172145; KX172144; KX172143; KX17214, KX172159-62	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, Australia	Zanol *et al.* (2016)
Marphysa mullawa	KX172176; KX172175; KX172173; KX172172; KX172171; KX172170; KX172168; KX172167; KX172166	n.a.	Fisherman's Island, Australia	Australia	Martin *et al.* (2020)
Marphysa pseudosessiloa	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; MK950852; MK950851; GQ497547; MK967470	GQ478157; AY83883; 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	Aylagas *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 basis), 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

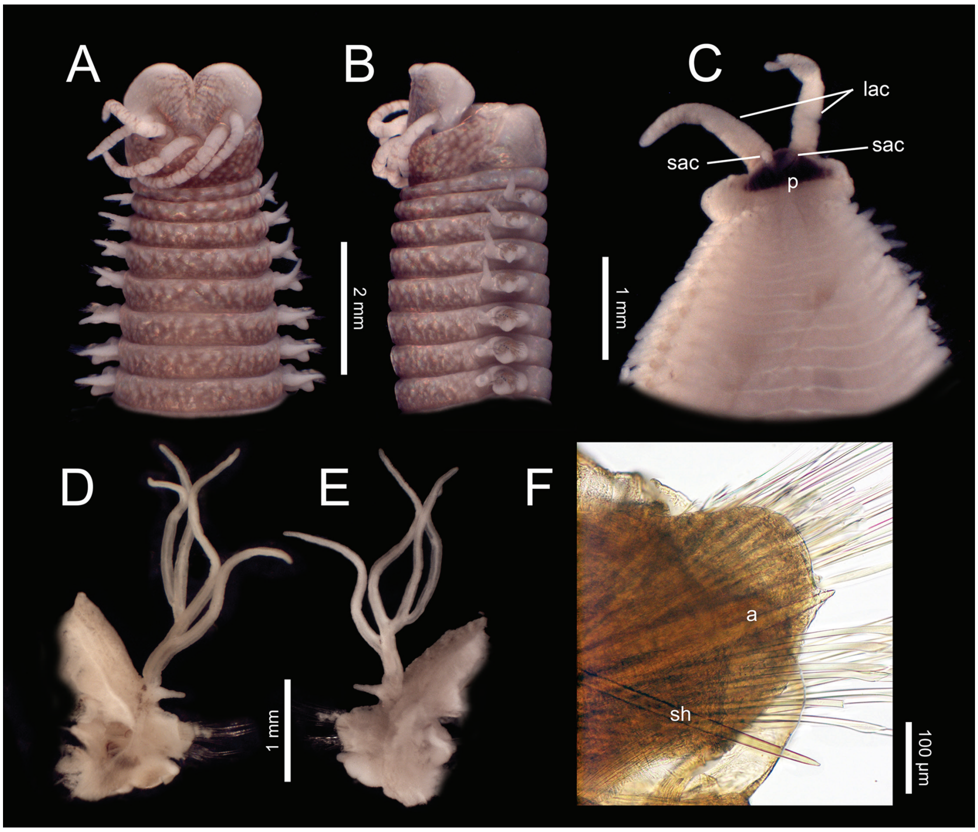

Fig. 2: *Marphysa chrigota* MNCN 16.01/18933. A. Anterior anterior end, dorsal view. B. Anterior end, lateral view. C. Posterior end, showing the position of long dorsal (lac) and short ventral (sac) anal cirri in the pygidium (p). D. Midbody branchial parapodium, posterior view; E. Midbody branchial parapodium, anterior view; F. Detail of the location of aciculae (a) and subacicular hook (sh). D-E: Chaetigier 65.

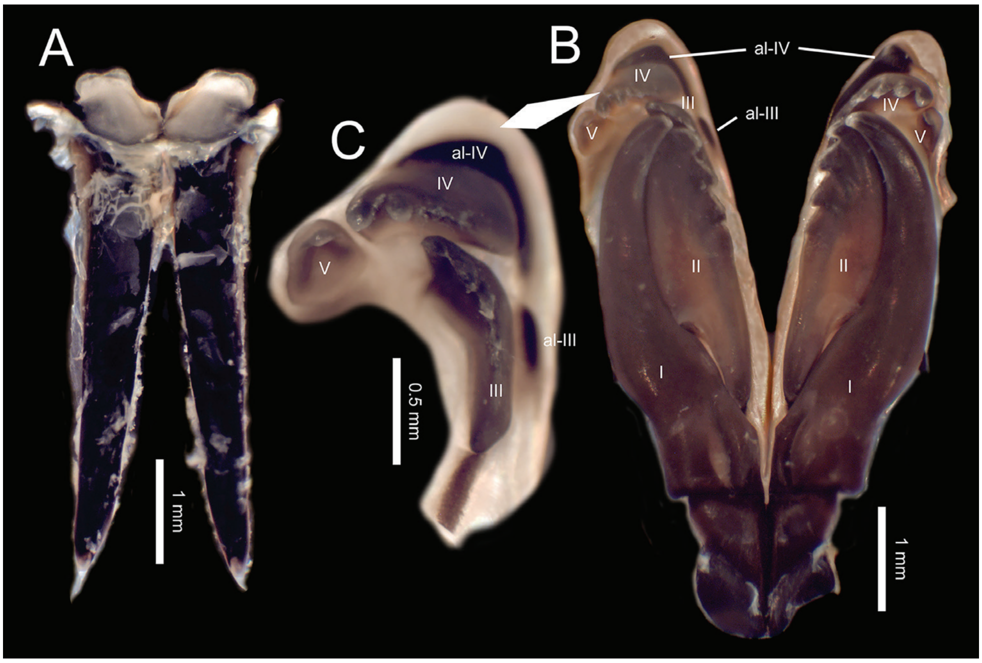

Fig. 3: *Marphysa chirigota* MNCN 16.01/18934–1. A. Dissected mandible. B. Dissected maxillae. C. Detail of left maxillae III to V.

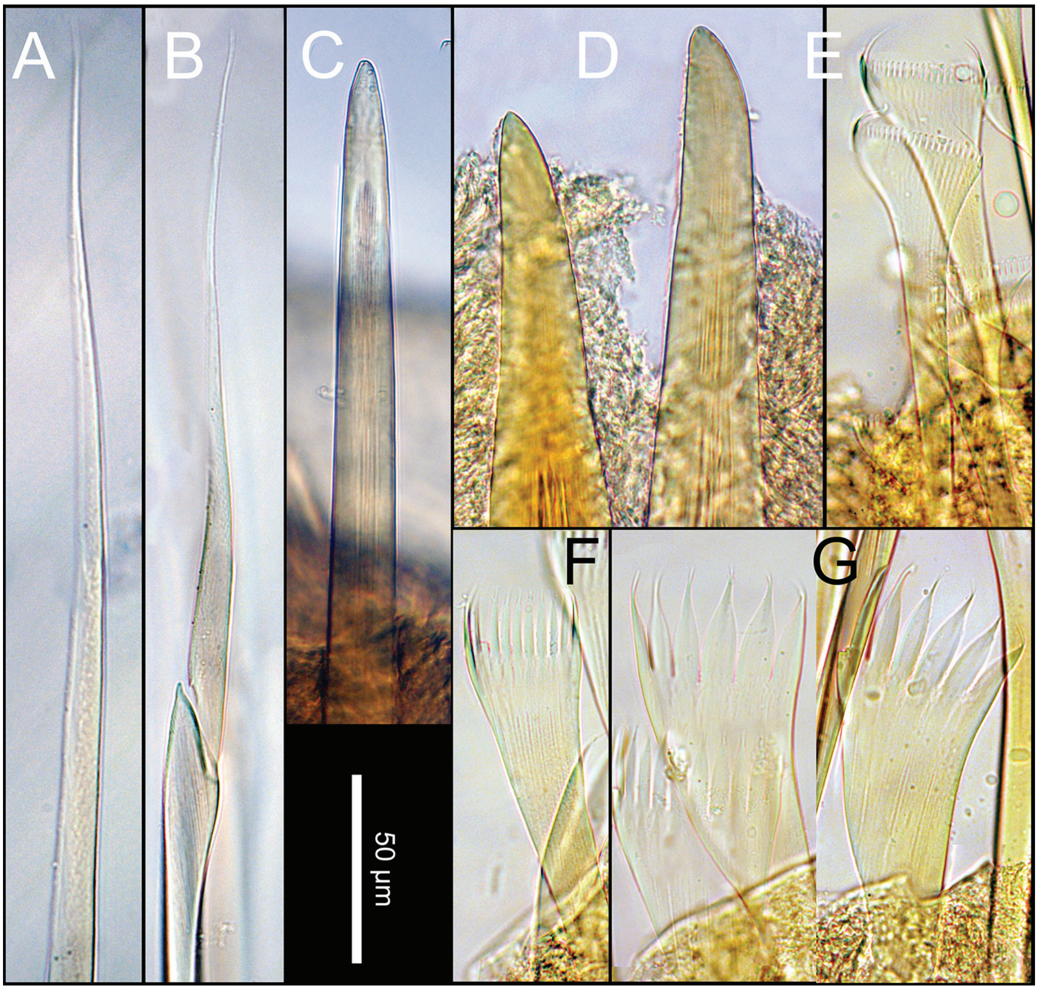

Fig. 4: *Marphysa chirigota* MNCN 16.01/18933. Mid-body branchial parapodium (chaetiger 65). A. Capillary notochaeta. B. Compound spiniger neurochaeta. C. Subacicular hook. D. Tips of aciculae. E. Type 2 pectinate chaetae; F. Type 3 pectinate chaeta; G. Type 4 pectinate chaetae. Scale bar is the same 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: supracicular with limbate (Fig. 4A) and pectinate chaetae (Fig. 4E-G) at anterior edge, and subacicular with compound spiniger chaetae (Fig. 3B) and one solid and golden subacicular 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 anterior 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, markedly 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 subacicular hooks. It can be distinguished from the species with all subacicular hooks unidentate in having: (1) subacicular 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 tripectinata* 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* Okuda, 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 acicula 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 likley 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 subacicular 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 subacicular 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

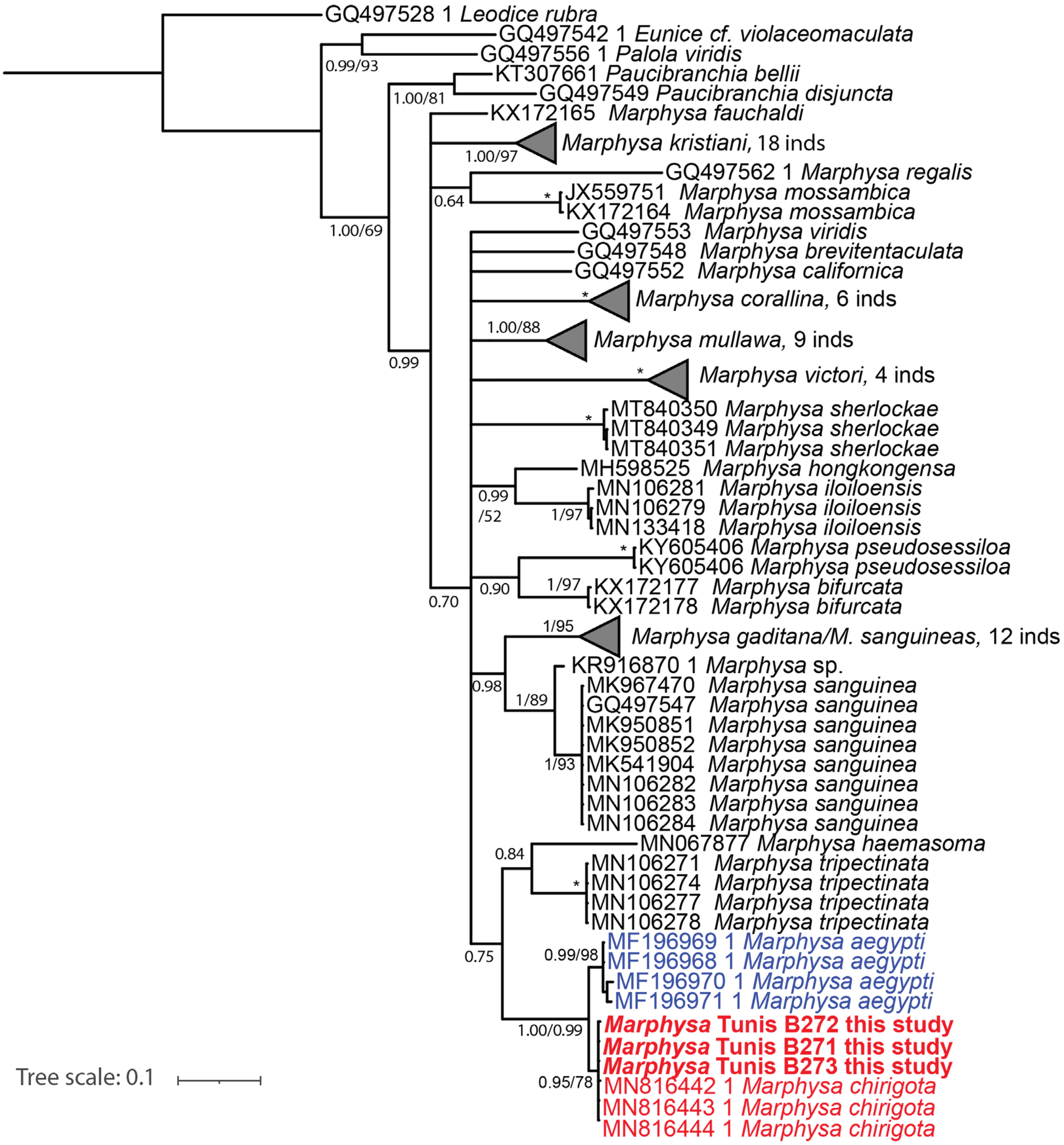

Fig. 5: Bayesian inference (BI) tree based on the COI dataset (96 sequences, 660bp). BI and Maximum likelihood (ML) statistic supports are indicated on the nodes (BI/ML < 0.5 pp or 50% bs not shown). * at nodes corresponds to support = 1 pp and >99% bs. Codes before species names: GenBank accession numbers;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

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*.

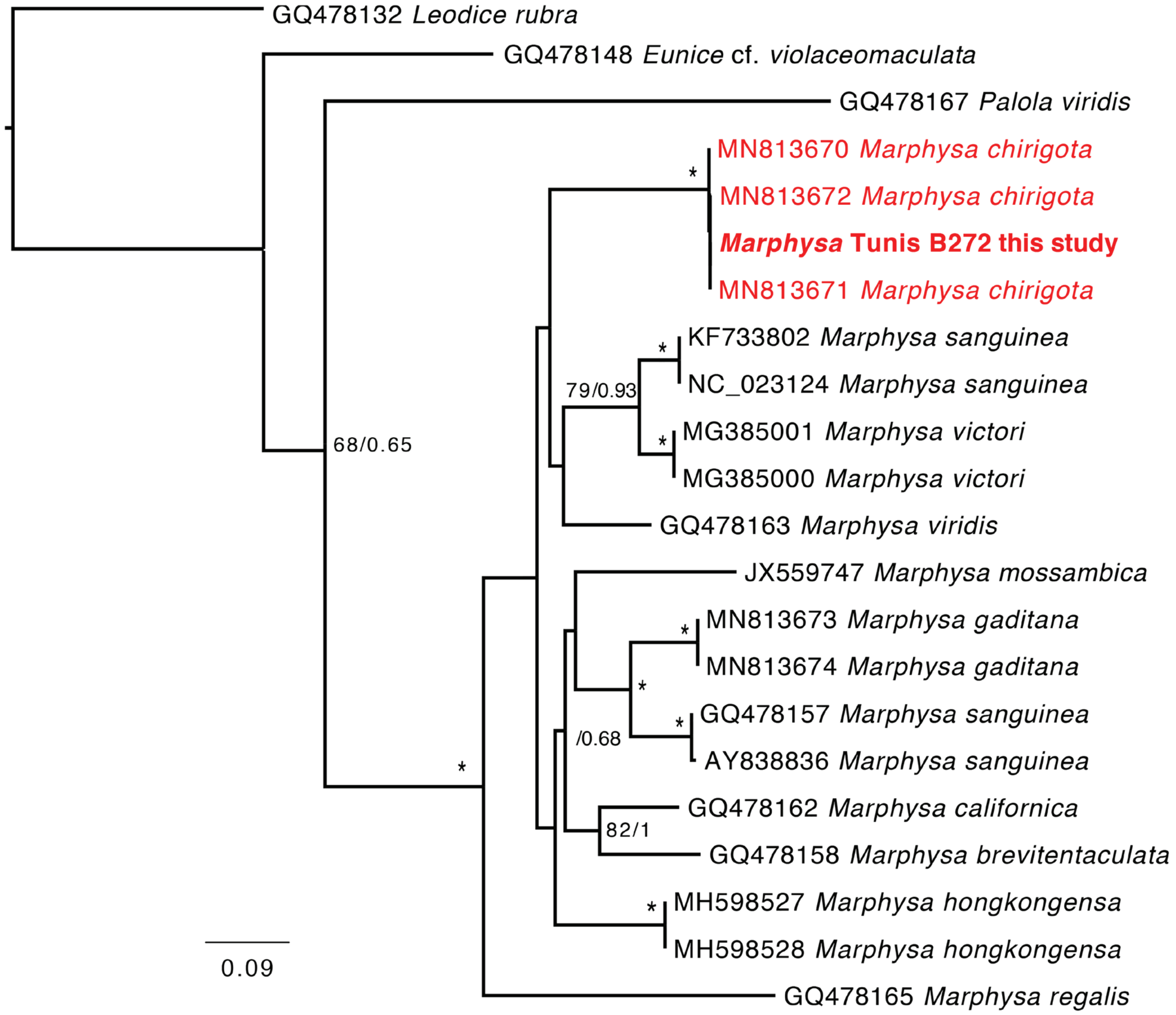

Fig. 6: Bayesian inference (BI) tree based on the 16S rDNA dataset (22 sequences, 959 bp). BI and Maximum likelihood (ML) supports are indicated on nodes (BI/ML < 0.5 pp or 50% bs not shown). *: support > 0.98 pp and 98% bs; Codes before species names: GenBank accession numbers.

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