The elephant in the room: first record of invasive gregarious species of serpulids (calcareous tube annelids) in Majorca (western Mediterranean)

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Summary: Although there are several nuisance species of serpulids reported worldwide, the present study shows that some of them have been overlooked even in geographic areas that are considered well studied. We report for the first time in Majorca the invasive species *Ficopomatus enigmaticus* and the first records for the Balearic Islands of the also non-native species *Hydroides dianthus*, *H. dirampha*, *H. elegans* and *H. nigra*. The most abundant species were *F. enigmaticus*, found at higher densities in Portixol (with up to 280 ind. m^{-2}), and *H. elegans*, found at highest densities in the port of Palma (with up to 270 ind. m^{-2}). Species have been identified after morphological examination and corroborated by mitochondrial DNA sequence data: cytochrome c oxidase subunit 1 (COI) and cytochrome b (Cytb). Molecular data show that the same haplotypes have been found in distant and disjoint biogeographic areas worldwide, which is only explicable by unintentional translocation of specimens through vectors (i.e. ship hulls or ballast water). Species delimitation analyses support previous findings that species complexes are common in both *Ficopomatus* and *Hydroides*. In fact, only among the Majorcan samples were we able to detect three species of the *F. enigmaticus* species complex, two of the *H. elegans* species complex and two of the *H. dianthus* species complex. The genetic distances between members of the *F. enigmaticus* species complex are 8.7% to 16.7% in COI sequences. The species of *Hydroides* hold 5.4% to 47.6 % genetic divergence between species of the same complex.

Keywords: non-indigenous; *Hydroides*, *Ficopomatus*; Serpulidae; polychaetes; COI; Cytb; integrative taxonomy.

El elefante en la habitación: primera cita de serpúlidos gregarios invasores (anélidos de tubo calcáreo) en Mallorca (Mediterráneo occidental)

Resumen: Hay constancia de especies alóctonas de serpúlidos reportadas en el mundo. Pero el presente estudio muestra que algunas de ellas han sido pasadas por alto incluso en áreas geográficas consideradas bien estudiadas. En este trabajo se cita por primera vez en Mallorca la especie invasora *Ficopomatus enigmaticus* y se registra también por primera vez para las Islas Baleares las también especies exóticas *Hydroides dianthus*, *H. dirampha*, *H. elegans* e *H. nigra*. Las especies más abundantes fueron *F. enigmaticus*, encontrada en mayores densidades en Portixol (con hasta 280 ind. m^{-2}) y *H. elegans*, encontrada en mayores densidades en el puerto de Palma (con hasta 270 ind. m^{-2}). Las especies han sido identificadas después su estudio morfológico y corroboradas por las secuencias de ADN mitocondrial: subunidad 1 de la citocromo c oxidasa (COI) y citocromo b (Cytb). Los datos moleculares muestran que se han encontrado los mismos haplotipos en áreas biogeográficas distantes e inconexas de a nivel mundial, lo que solo se explica por la translocación involuntaria de ejemplares a través de vectores (es decir, cascos de barcos o agua de lastre). Los análisis de delimitación de especies respaldan resultados previos acerca de la existencia de complejos de especies en ambos géneros *Ficopomatus* e *Hydroides*. De hecho, sólo entre las muestras mallorquinas pudimos detectar tres especies del complejo de especies *F. enigmaticus*, dos del complejo de especies de *H. elegans* y dos del complejo de especies de *H. dianthus*. Las distancias genéticas entre los miembros del complejo de especies de *F. enigmaticus* son del 8.7 al 16.7% en las secuencias COI. Las especies de *Hydroides* tienen un 5.4-47.6% de divergencia genética entre especies del mismo complejo.

Palabras clave: alóctonas; *Hydroides*; *Ficopomatus*; Serpulidae; poliquetos; COI; Cytb; taxonomía integrativa.

INTRODUCTION

According to the International Union for Conservation of Nature, biological invasions and destruction of habitat are the most important causes of biodiversity loss. Biological invasions refer to the introduction (accidental or intentional), establishment and expansion of species outside their natural geographic range (ISSG 2011). Non-native species are regarded as a major threat to marine biodiversity and a contributor to environmental change (Bax et al. 2003, Molnar et al. 2008, Katsanevakis et al. 2014). However, introductions have increased radically in recent years due to numerous human-driven activities such as aquaculture, marine traffic and interconnection of hydrogeographic basins. Such introductions, especially if the organisms establish themselves and become invasive, may cause important negative environmental impacts with economic and social implications. Displacement and extinction of local species, hybridization and genetic contamination, alteration of community' structures and complex ecological interaction networks, introduction of parasites and pathogens, obstruction of canals, infrastructure damage and losses in mariculture and facilitation of native species (Rodriguez 2006) are some of the processes that have already been documented as consequences of invasive species (Bax et al. 2003, Zenetos et al. 2005, Molnar et al. 2008).

The family Serpulidae Rafinesque, 1815 are marine, benthic sedentary annelids living in the calcareous tubes they build—a diagnostic feature shared by all members of this family. Serpulids are an important component of the encrusting fauna in benthic environments and can play an important role as ecosystem engineers (Toonen and Pawlik 2001, Wright and Gribben 2017). Non-native serpulid species can therefore cause severe impacts in newly colonized habitats as they can potentially aggregate, forming large biogenic reefs that change the habitat structure (Crooks 2002, Heiman and Micheli 2010, Pernet et al. 2016), reduce food availability for other species (Bruschetti et al. 2009, Pan and Marcoval 2013) and have an effect on sedimentation processes that can change the ecological dynamics (Davies et al. 1989, Schwindt et al. 2001, 2004). Serpulids can also have a more direct impact on human activities by damaging ships or anthropogenic structures in harbours (Ulman et al. 2019) and clogging sewage systems and cooling water in take pipes for power plants (Zibrowius 2002, Read and Gordon 1991, Peria and Pernet 2019).

Ficopomatus enigmaticus (Fauvel, 1923) is a nuisance biofouling organism and a highly invasive species that has colonized estuaries and ports around the planet, where it can build reefs up to several metres in diameter (Fauvel 1923, Dittmann et al. 2009, Styan et al. 2017). It is also the only annelid registered in the Spanish Catalogue of Exotic Invasive Species (http://invasiber.org/). Although its geographical origin is unclear, the most agreed hypothesis is that it spread from Australia or the Indo-Pacific (Dittmann et al. 2009, Styan et al. 2017). The first specimens were discovered in the canals of northern France, were they were probably introduced during World War I, attached to the hulls of warships (Fauvel 1923). The first accurate report for the Iberian Peninsula was in Galicia, northwestern Spain (Rioja 1923), very soon after the species description. Decades later, it was reported in several localities along the Mediterranean coastline and in Turkey, Greece, Italy and Albania (e.g. Ergen 1976, Ambrogi 2000, Shumka et al. 2014). On the Levantine coast of the Iberian Peninsula, *F. enigmaticus* has been reported from Catalonia, Valencia and Murcia. In the Balearic Islands, it has been found in the Albufera of Menorca forming large reefs (Martínez-Taberner et al. 1993, Fornós et al. 1997) but has never been reported from Majorca.

Seven species of the genus *Hydroides*, *H. dianthus* (Verrill, 1873), *H. dirampha* Mörch, 1863, *H. brachyacantha* Rioja, 1941, *H. elegans* (Haswell, 1883), *H. heterocera* (Grube, 1868), *H. minax* (Grube, 1878) and *H. operculata* (Treadwell, 1929), have also been reported as invasive and their presence is well documented for the eastern Mediterranean and for the Levantine coast of the Iberian Peninsula (Çinar 2006, Gil 2011, Alcázar and San Martín 2016, although some of them should be assessed with molecular data). Confirmation of whether a species within the *H. brachyacantha* and *H. operculata* that has already been already assessed or a different one within complexes (Sun et al. 2016, 2017b) is present in Mediterranean waters is still needed, as this was not part of the scope of this study. *Hydroides elegans* was originally described from Australia and has been reported in many ports and bays all over the world (Gil 2011). *Hydroides dirampha* described from the Antillean Islands and probably native to the Caribbean (Gil 2011) is a common species in fouling communities of tropical and temperate seas. *Hydroides dianthus*, described from New England, is also commonly reported in fouling communities of the Atlantic and the Mediterranean coastal environments (Sun et al. 2017a).

Three species were originally described from the Mediterranean: *Hydroides pseudouncinata* Zibrowius, 1968, *Hydroides nigra* Zibrowius, 1971 and *Hydroides stoichadon* Zibrowius, 1971. The former has been commonly reported in several localities of the Mediterranean, including the Balearic Islands and the northeast Atlantic, mainly under the name *Hydroides uncinata* (Philippi, 1884) (Alcázar and San Martín 2016). *Hydroides nigra* is considered endemic to the Mediterranean, and has only been reported twice along the Spanish coastline, in Murcia (San Martín and Vieitez 1984) and Majorca (Sun et al. 2017b). *Hydroides stoichadon* has occasionally been reported in southern France, Italy and Spain (Alcázar and San Martín 1996). Except for *H. nigra* and *H. pseudouncinata*, none of the other *Hydroides* species cited above have yet been reported from the Balearic Islands.

It was not until 2009 that an effort to resolve the species complexes within Serpulidae was made (e.g. Halt et al. 2009, Smith et al. 2012, Willette et al. 2015, and several others thereafter). Among these studies, genetic analyses of *F. enigmaticus* using Cytochrome b

(Cytb) revealed high genetic diversity in the group and possible cryptic species (Styan et al 2017, Yee et al. 2019, Oliva et al. 2020). Similar patterns were found in some of the species of the genus *Hydroides* using the markers cytochrome c oxidase subunit 1 (COI) and Cytb (Sun et al. 2016, 2017a). Those findings highlight the importance of DNA-based methods for assessing non-indigenous species.

We present the first record in Majorca of the invasive species *F. enigmaticus*, which is already considered invasive, and the first record for the Balearic Islands of the species *H. dianthus*, *H. dirampha* and *H. elegans*. We also provide results after genetic analyses of DNA sequences and insights about their introduction events and pathways. We refer to the metaphor of the "elephant in the room" in the title, with the aim of highlighting how major issues such as the presence of large populations of marine invasive species have been overlooked in apparently well-known areas such as harbours.

MATERIALS AND METHODS

Study area and sampling design

Majorca is the largest island in the Balearic archipelago, located in the western Mediterranean. Its geographical situation has made it a strategic point for trade and exchange with the rest of the Mediterranean since the Phoenician colonies in the third century BC (Aubet 2001), and since the mid-20th century tourism has played a fundamental role in its economy. The archipelago is considered one of the main tourist destinations in Europe, and more than 800 cruise ships and 44000 merchant' ships dock annually in its four main international ports (Palma, Alcudia, Ibiza and Mahón). In addition, Majorca's 30 marinas have over 28000 private docks for pleasure boats (APB 2017). All this maritime traffic has likely caused the involuntary dispersion of adult specimens attached to ship hulls (Zibrowius 1991) or larvae in ballast waters (Hewitt et al. 2009).

Table 1. – Stations and habitats where *Ficopomatus* and *Hydroides* species were found in this study.

| Station Number	Location	Species Found	Depth (m)	Habitat
PAR-01	Port of Palma 39°34'01.5"N; 2°38'38.6"E	*H. dianthus*	0.5	
PAR-02	Puerto de Palma 39°33'55.7"N; 2°37'54.7"E	*F. enigmaticus* *H. dianthus* *H. elegans*	1	Concrete dock
PAR-06	Port of Palma 39°33'10.4"N; 2°37'55.4"E	*H. elegans*	0.5	
PAR-12	Puerto de Palma 39°33'55.7"N; 2°37'54.7"E	*F. enigmaticus* *H. elegans* *H. dirampha*	1	Concrete dock
POR-01	Portitxol 39°33'35.6"N; 2°40'05.1"E	*H. dianthus*	1	
POR-02	Portitxol 39°33'40.3"N; 2°40'06.6"E	*F. enigmaticus*	0.5	Concrete dock covered with cirripeds
POR-03	Portitxol 39°33'42.5"N; 2°40'10.2"E	*F. enigmaticus*	0.5	Concrete dock covered with cirripeds
POR-04	Portitxol 39°33'39.7"N; 2°40'08.3"E	*F. enigmaticus* *H. dianthus*	0.3	Concrete pier with brown algae and sponges
POR-12	Portitxol 39°33'40.3"N; 2°40'06.6"E	*F. enigmaticus*	0.5	Concrete dock
POR-13	Portitxol 39°33'42.5"N; 2°40'10.2"E	*F. enigmaticus* *H. dianthus* *H. elegans*	0.5	Concrete dock covered with cirripeds
POR-14	Portitxol 39°33'39.7"N; 2°40'08.3"E	*F. enigmaticus* *H. dianthus* *H. nigra*	0.3	Concrete dock with brown algae and sponges
PAD-03	Puerto de Palma 39°33'57.7"N; 2°37'45.5"E	*F. enigmaticus*	2	Muddy sediment
PABA-14	Puerto de Palma 39°33'24.0"N; 2°37'32.9"E	*F. enigmaticus*	1	Fouling plate
PALC-01	Port of Alcudia 39°50'20.6"N; 3°08'03.9"E	*H. elegans*	0-1	Fouling plate
PALC-02	Port of Alcudia 39°50'21.2"N; 3°08'01.7"E	*H. dianthus* *H. elegans*	0-1	Fouling plate
PALC-03	Port of Alcudia 39°50'22.1"N; 3°07'57.9"E	*H. dianthus*	0-1	Fouling plate
PALC-04	Port of Alcudia 39°50'13.1"N; 3°08'06.0"E	*H. dianthus*	0-1	Fouling plate
PALC-05	Port of Alcudia 39°50'14.0"N; 3°08'06.2"E	*H. dianthus* *H. dirampha*	0-1	Fouling plate
PALC-06	Port of Alcudia 39°50'16.1"N; 3°07'57.6"E	*H. dianthus* *H. elegans*	0-1	Fouling plate
PALC-07	Port of Alcudia 39°50'21.6"N; 3°08'12.6"E	*H. dianthus*	0-1	Fouling plate
PSOL-01	Port of Sóller 39°47'47.8"N; 2°41'43.4"E	*H. dirampha*	0-1	Fouling plate
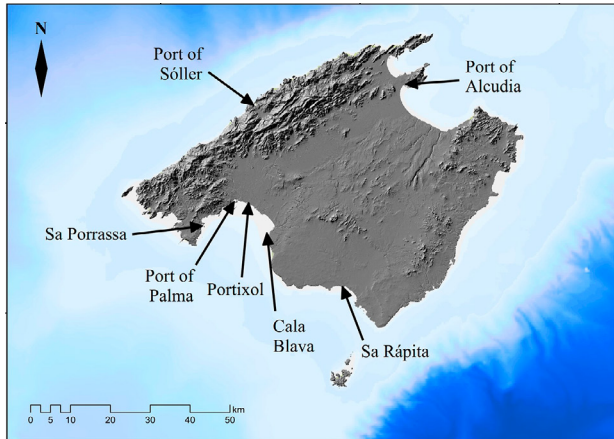
Fig. 1. – Map of the localities sampled around the island of Majorca.

Seven localities were sampled around the island between July 2017 and July 2019 (Fig.1), including the international ports of Palma and Alcudia and the leisure or fishing ports of Sóller, Sa Rápita and Portitxol. In addition, two localities in the bay of Palma but outside the harbour environments were also selected: Sa Porrassa and Cala Blava, in the Marine Reserve of the Bay of Palma. Several samples were taken at each locality, maximizing habitat diversity (exposure, orientation, traffic, type of substrate, etc., Table 1). Modified qualitative rapid assessment surveys (similar to Ulman et al. 2017) were undertaken with a rectangular scraper equipped with a 1 mm diameter mesh fabric at one end and a 2 m stick at the other. At each station, artificial substrates were scraped off on the shore to determine the absence or presence of targeted species. Finally, 3 to 5 L sediment samples were collected with a van Veen grab from both anthropogenic and natural environments.

Quantitative samples were also taken by scuba divers (Fig. 2A, B), who scraped a standard surface of 30×30 cm on hard substrates (both artificial and natural) at depths of 0.5 to 7 m (Fig. 2A, B). In addition, 33×33 cm fouling plates were placed at depths of between 0.5 and 5 m for 3 to 6 months in order to study settlement and colonization processes (Fig. 2D). The surfaces were also scraped after this time.

Fig. 2. – A, B, Photographs showing field work and sample collection from harbour environments (port of Palma). C, *Ficopomatus enigmaticus* aggregation collected from shallow water (1 m deep) in Portixol. D, *Hydroides* species attached to a fouling plate after four months of being deployed, at port of Palma.

Target species from all samples were sorted in the laboratory and stored at 4°C to 6°C in 96% ethanol. Additional specimens of *H. norvegica*, fixed and preserved in 96% ethanol, were obtained from Norway for genetic comparison with the morphologically similar *H. elegans*.

The aggregation or colonies present at each locality were considered as populations. A total of 52 specimens of *Hydroides* and 21 specimens of *Ficopomatus* were selected from the collected samples for molecular work.

Morphological studies

All specimens were examined with a stereo and compound light microscope and identified to morphospecies following original and updated descriptions (e.g. Zibrowius 1971, Fauvel 1923, Bastida-Zavala and ten Hove 2002). For scanning electron microscopy (SEM), specimens were dehydrated in a series of mixtures of absolute ethanol and hexamethyldisilazane (HMDS) with the following ratios 3:1, 2:2, 1:3, and 1:1, 1:3, and then into pure HMDS. The prepared samples were mounted on holders, sputter-coated with gold (10 nm thickness) and examined with a HITACHI S-3400N scanning electron microscope at the University of the Balearic Islands. Vouchers were deposited at the Museo Nacional de Ciencias Naturales (MNCN, Madrid; Table 2).

Molecular data

A small portion, 1 to 2 mm, of each specimen's thorax or a few radioles were taken for molecular work. DNA was extracted from *Ficopomatus* specimens using the Quick-gDNA Miniprep Kit (Zymo) according to the manufacturer's instructions. DNA was extracted from *Hydroides* specimens using QuickExtract (Epicentre). Cytochrome c oxydase subunit 1 (COI) and Cytochrome b (Cytb) were amplified by PCR. The amplification reaction of COI for *Ficopomatus* contained 10.8 μl of water, 1.2 μl of 50 mM $MgCl_2$, 2 μl of buffer 10×, 0.4 μl of Biotaq 5 U μl^{-1}, 2 μl of dNTPs mix at 10 mM, 0.8 μl of each primer jgL-CO1490/jgHCO2198 (Geller et al. 2013) at 10 mM and 2 μl of DNA (4-20 ng μl^{-1}). PCR reactions for amplification of Cytb and COI for *Hydroides* contained 4.5 μl of water, 1 μl of each primer jgLCO1490/jgH-CO2198, Hydro-COIF/Hydro-COIR or Cytb424F/cobr825, 7.5 μl of MyTaq Red Mix (Bioline) and 1 μl of DNA (4-20 ng μl^{-1}). Primer sequences and cycling conditions are given in Table 3. PCR products were run on a 1% agarose gel containing ethydium bromide for 30 min at 80 V and visualized with UV light. Amplified PCR fragments were of around 660 bp, while Cytb fragments were of around 430 bp. Successful PCR products were cleaned using microCLEAN for PCR clean-up (Microzone) or ethanol/sodium acetate precipitation. For some samples, cycle sequencing was performed on both strands by Eurofins Genomics

Table 2. – Information about the specimens used for the phylogenetic analyses, species names, vouchers, collection information and GenBank accession numbers.

Species	Voucher	DNA Code	COI GenBank	Cytb GenBank	Station	Collection Date	Latitude	Longitude	Depth
Ficopomatus enigmaticus	MNCN 16.01/18765	FIC01	MT044486	MT215015	POR-02	26-Jul-17	39°33'40.3"N	2°40'06.6"E	0.5 m
Ficopomatus enigmaticus	MNCN 16.01/18766	FIC02	MT044489	MT215014	POR-02	26-Jul-17	39°33'40.3"N	2°40'06.6"E	0.5 m
Ficopomatus enigmaticus	MNCN 16.01/18767	FIC04	MT044492	NA	POR-03	26-Jul-17	39°33'42.5"N	2°40'10.2"E	0.5 m
Ficopomatus enigmaticus	MNCN 16.01/18768	FIC05	MT044491	NA	POR-04	26-Jul-17	39°33'39.7"N	2°40'08.3"E	0.3 m
Ficopomatus enigmaticus	MNCN 16.01/18769	FIC12	MT044494	NA	POR-13	28-Nov-17	39°33'42.5"N	2°40'10.2"E	0.5 m
Ficopomatus enigmaticus	MNCN 16.01/18770	FIC15	MT044488	NA	POR-14	28-Nov-17	39°33'39.7"N	2°40'08.3"E	0.3 m
Ficopomatus enigmaticus	MNCN 16.01/18771	FIC16	MT044495	NA	PAR-12	28-Nov-17	39°33'55.7"N	2°37'54.7"E	1 m
Ficopomatus enigmaticus	MNCN 16.01/18772	FIC17	MT044490	NA	PAR-12	28-Nov-17	39°33'55.7"N	2°37'54.7"E	1 m
Ficopomatus enigmaticus	MNCN 16.01/18773	FIC20	MT044487	NA	PAR-12	28-Nov-17	39°33'55.7"N	2°37'54.7"E	1 m
Ficopomatus enigmaticus	MNCN 16.01/18774	FIC21	MT044493	NA	POR-13	28-Nov-17	39°33'42.5"N	2°40'10.2"E	0.5 m
Hydroides nigra	MNCN 16.01/18775	HYD11	NA	MT215009	PAR-06	26-Jul-17	39°33'10.4"N	2°37'55.4"E	0.5 m
Hydroides dianthus	MNCN 16.01/18776	HYD14	NA	MT215010	PALC-02	26-Jul-17	39°50'21.2"N	3°08'01.7"E	0.5m
Hydroides elegans		HYD22	NA	MT215012	PARBAL-15	28-Nov-17	39°33'15.5"N	2°37'33.7"E	
Hydroides elegans	MNCN 16.01/18777	HYD23	NA	MT215008	PAR-02	26-Jul-17	39°33'55.7"N	2°37'54.7"E	1 m
Hydroides dianthus	MNCN 16.01/18778	HYD26	NA	MT215011	PAR-01	26-Jul-17	39°34'01.5"N	2°38'38.6"E	0.5 m
Hydroides norvegica		HYD30	NA	MT215013	BER-FASCD2	10-Sep-18	60.40017	5.30842	12 m
Hydroides dirampha	MNCN 16.01/18779	PAR06i05	MT044496	NA	PAR-06	26-Jul-17	39°33'10.4"N	2°37'55.4"E	0.5 m
Hydroides dianthus	MNCN 16.01/18780	POR02i09	MT044497	NA	POR-02	26-Jul-17	39°33'40.3"N	2°40'06.6"E	0.5 m
Hydroides elegans	MNCN 16.01/18781	PAR02i05	MT044498	NA	PAR-02	26-Jul-17	39°33'55.7"N	2°37'54.7"E	1 m
Hydroides elegans	MNCN 16.01/18782	PAR02i04	MT044499	NA	PAR-02	26-Jul-17	39°33'55.7"N	2°37'54.7"E	1 m
Hydroides elegans	MNCN 16.01/18783	PAD01i03	MT044500	NA	PAD-01	26-Jul-17	39°34'01.5"N	2°38'38.6"E	2 m
Hydroides elegans	MNCN 16.01/18784	PAR02i08	MT044501	NA	PAR-02	26-Jul-17	39°33'55.7"N	2°37'54.7"E	1 m

DNA Sequencing Department (Ebersberg, Germany). The rest of the samples were terminated using BigDye Terminator v3.1 (ThermoFisher) and sequenced on an ABI3130 sequencer. Forward and reverse reads were merged into consensus sequences and edited using Geneious v.7 (Kearse et al. 2012).

Additionally, 57 COI and 42 Cytb sequences of *F. enigmaticus* and *Hydroides* spp. were downloaded from GenBank (Benson et al. 2008, see Supplementary Material).

Phylogenetic and species delimitation analysis and genetic distances

Sequences were aligned with MAFFT 7 online version (Katoh and Standley 2013) and alignments were checked in Aliview 1.25 (Larsson 2014). Flanking regions with missing data were removed using Gblocks 0.91b (Castresana 2000) with the softest parameters (allow for smaller final blocks, gap positions within the final blocks and less strict flanking positions).

Best-fitting models and partition schema for each marker were selected using PartionFinder 2.1.1 (Lanfear et al. 2016, Guindon et al. 2010) with the Bayesian information criterion. The number of variable and parsimony-informative sites was calculated with MEGA X 10.0.5 (Kumar et al. 2018). Two datasets were created for phylogenetic analyses: one containing unique COI sequences and one containing unique Cytb sequences.

Bayesian inference (BI) analyses were performed on both datasets to obtain ultrametric trees compatible with downstream species delimitation analyses. BI analyses were conducted using BEAST2 (Bouckaert et al. 2014) for both datasets (COI and the concatenated dataset) using the nucleotide substitution model mentioned above. A strict clock was assumed for both datasets. A Yule model was used as tree prior with a default Γ distribution as birth rate prior. A lognormal distribution with M=1.0 and S=1.25 was used for the kappa parameter prior (Drummond and Bouckaert 2015). All analyses were run with a chain length of 50000000. Convergence of each run and parameter was checked using Tracer 1.7.1 (Rambaut et al. 2018), i.e. ESS > 200. A maximum clade credibility tree was obtained with Treeannotator (Bouckaert et al. 2014) after discarding 25% of the trees as burnin. All phylogenetic analyses were performed on Cypres Science Gateway (Miller et al. 2010). Trees were visualized and edited using FigTree 1.4.4 (Rambaut 2014) and later in LibreOffice Draw 5.1.6.2.

The general mixed Yule-coalescent model (GMYC) (Pons et al. 2006, Fujisawa and Barraclough 2013) and the multi-rate Poisson tree process (mPTP) (Kapli et al. 2017) were used to delimit the number of molecular species in the datasets. GMYC and mPTP 0.2.4 were used on both BI trees with a single threshold. The methods were applied separately on the *Ficopomatus* and *Hydroides* groups for the Cytb datasets. GMYC was implemented in R (R Core Team 2015) with the packages ape 5.3 (Paradis and Schliep 2018), MASS 7.3-45 Venables and Ripley 2002), Paran 1.5.2 (Dinno 2018) and splits 1.0-19 (Ezard et al. 2017). mPTP was applied through its webserver (https://mptp.h-its.org).

Nucleotide divergence (K2P) over sequence pairs within and between the well supported lineages after the phylogenetic analyses and species delimitation analyses were estimated in MEGA X 10.0.5 (Kumar et al. 2018). Paired positions containing gaps and missing data were removed.

RESULTS

Morphological analyses and records

The presence of *F. enigmaticus* was confirmed in two of the seven sampled areas: the port of Palma and Portixol (Fig. 2C). In the port of Palma, it was only found on one of the deployed fouling plates. Scrapings in Portixol recovered aggregations of at least 280 individuals/m^2 at some sites, especially those closed to the torrent mouth, right at the end of the port. Four species of *Hydroides* were identified morphologically from the ports of Palma, Portixol, Alcudia and Sóller: *H. dianthus*, *H. elegans*, *H. dirampha* and *H. nigra* (Figs. 2D, 3). These five serpulid species are recorded for the first time in Majorca. Juvenile or damaged

Table 3. – Primers used in this study with their respective cycles.

Marker	Primers	Source	Sequence	Cycle
COI	jgLCO1490	(Geller et al. 2013)	TITCIACIAAYCAYAARGAYATTGG	4 min 95°C; 34x: 40 s 94°C, 40 s 48°C, 60 s 72°C; 6 min 72°C
	jgHCO2198	(Geller et al. 2013)	TAIACYTCIGGRTGICCRAARAAYCA	
	Hydro-COIF	(Sun et al. 2012)	TCWRTWRTKACDGTKCATGCTA	5 min 95°C; 35x 40 s 94°C, 40 s 48°C, 60 s 72°C; 6 min 72°C
	Hydro-COIR	(Sun et al. 2012)	CMRYAGGWTSAAARAACCTAGTA	
Cytb	Cytb 424F	(Boore and Brown 2000)	GGWTAYGTWYTWCCWTGRGGWCARAT	4 min 95°C; 35x 40 s 94°C, 40 s 50°C, 60 s 72°C; 6 min 72°C
	cobr825	(Burnette et al. 2005)	AARTAYCAYTCYGGYTTRATRTG	

specimens without an operculum could not be identified to the species level. The most common and abundant species was *H. elegans*, which was found in the port of Palma at densities of up to 270 individuals/m². No *Hydroides* specimens were found in the ports of Sa Rápita and Cala Blava and only one specimen of one species, *H. nigra*, was collected at the Islet of Sa Porrassa (donated to Sun et al. 2017b). Specimens identified as *H. norvegica* from Norway were compared morphologically with *H. elegans* to confirm that the main morphological differences are in the collar chaetae, with subdistal spines in the latter absent in the former. The wide variation observed in the opercular morphology of members of both species indicates that this is a less reliable character for species discrimination.

Phylogenetic and species delimitation analyses

Despite our efforts to amplify and sequence both markers, only 10 COI and two Cytb sequences of *F. enigmaticus* were obtained (GenBank accession numbers (AC): MT044486-MT044495, MT215014-MT215015; Table 2), as well as six COI and five Cytb sequences of *Hydroides* (AC: MT044496-MT044501, MT215008-MT215013; Table 2). The COI dataset contained 68 sequences and was 379 bp long, with 228 variable sites, 212 of them parsimony-informative. The Cytb dataset contained 50 sequences and was 264 bp long, with 190 variable sites, 175 of them parsimony-informative.

Specimens of *F. enigmaticus* split into three distinct and supported clades, and species delimitation analyses (GMYC and mPTP) confirmed the presence of three molecular species of the *F. enigmaticus* species complex in Majorca. For two species, *F. enigmaticus* Clade 4 and 5 (Fig. 4), only COI sequences are available and do not match any existing records. Therefore, these species could be new cryptic species within the *F. enigmaticus* complex, or else could match any of the clades previously assessed in Australia (Styan et al. 2017). For the third species, *F. enigmaticus* Clade 1 (Fig 4, 5), both COI and Cytb sequences are available, and this species has previously been recorded from distant bioregions such as Australia, New Zealand, California and Portugal and northern Spain (e.g. Styan et al. 2017, Yee et al. 2019, Oliva et al. 2020). K2P distances within these species range from 0.3% to 4.7%, and K2P distances between them range from 10.5% to 24.7% (Tables 4, 5).

Among specimens identified as *Hydroides elegans*, two species are recovered after phylogenetic and species delimitation analyses. For one species, *H. elegans* Clade E2 (Fig 4), only COI sequences are available, and do not match any existing records. Therefore, this species appears to be a new cryptic species within the *H. elegans* species complex. For the second species, *H. elegans* Clade E1 (Figs 4, 5), both COI and Cytb sequences are available (albeit from different specimens). This species has previously been recorded from distant bioregions such as
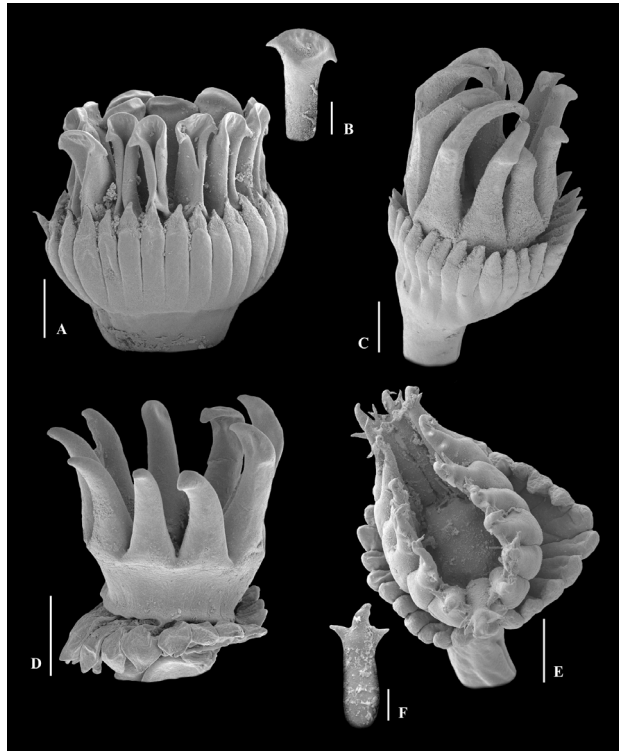

Fig. 3. – SEM pictures of the operculum of the four species of *Hydroides* recorded. A, *H. dirampha*, scale bar 200 μm; B, detail of operculum of *H. dirampha*, scale bar 100 μm; C, *H. nigra*, scale bar 200 μm; D, *H. dianthus*, scale bar 200 μm; E, *H. elegans*, scale bar 100 μm; F, detail of operculum of *H. elegans*, scale bar 50 μm.

Australia, California and China. K2P distances within these species range from 0.07% to 1.4%, and K2P distances from other *Hydroides* species range from 9.2% to 46.3% (Tables 4, 5).

Among the specimens identified as *Hydroides dirampha*, one species was recovered with one COI sequence available (Fig. 4). This species has previously been recorded from distant bioregions such as Australia and Panama. K2P distances from other *Hydroides* species range from 21.9% to 31.3% (Tables 4, 5).

Among the specimens identified as *Hydroides dianthus*, two species were recovered. For one species, *H. diantus* Clade A (Fig. 4), one COI sequence is available. This species has been previously identified from the Mediterranean, as well as from distant bioregions such as China, Brazil and the east coast of the US. For the other species, *H. dianthus* Clade B (Fig. 5), one Cytb sequences was available. This species has previously been recorded from distant bioregions such as Ukraine and Texas. K2P distances within these species range from 0.08% to 1.7%, and K2P distances from other *Hydroides* species range from 6.7% to 34.2% (Tables 4, 5).

One Cytb sequence was obtained from the specimen identified as *H. nigra*, which does not cluster with any available sequences, including that of another specimen identified as *H. nigra* from Majorca (Fig. 5). K2P distances from other *Hydroides* species

Table 4. – Divergences measured in COI fragment using the K2P model (lower left corner) and p-distance (upper right corner). In grey cells, intraspecific genetic variation (K2P). Ambiguous positions removed for each sequence pair.

COI	*F. enigmaticus* Clade 1	*F. enigmaticus* Clade 4	*F. enigmaticus* Clade 5	*H. dirampha*	*H. dianthus* Clade A	*H. dianthus* Clade B	*H. elegans* Clade E1	*H. elegans* Clade E2	*H. elegans / ezoensis*	*H. nigra*	*H. triversiculosa*	*H. basispinosa / gradata*	*H. inornata / operculata*	*H. operculata*	*H. pseudouncinata*	*H. crucigera*	*H. brachyacantha*	*H. recurvispina / dolabrus*	*H. panamensis*	*H. sanctaecrucis*	*H. fusicola*
Ficopomatus enigmaticus Clade 1	0.02	0.11	0.16	0.58	0.61	0.59	0.56	0.51	0.58	0.61	0.63	0.56	0.52	0.57	0.62	0.63	0.60	0.60	0.59	0.58	0.59
Ficopomatus enigmaticus Clade 4	0.11	0.01	0.15	0.51	0.58	0.58	0.51	0.49	0.55	0.58	0.54	0.56	0.51	0.55	0.59	0.51	0.55	0.54	0.54	0.56	0.50
Ficopomatus enigmaticus Clade 5	0.16	0.15	n	0.55	0.56	0.57	0.53	0.49	0.55	0.55	0.52	0.52	0.52	0.51	0.57	0.50	0.57	0.53	0.57	0.57	0.48
Hydroides dirampha	0.58	0.51	0.55	n	0.25	0.27	0.31	0.29	0.29	0.31	0.29	0.30	0.28	0.29	0.30	0.24	0.22	0.25	0.23	0.23	0.28
Hydroides dianthus Clade A	0.61	0.58	0.56	0.25	0.01	0.07	0.31	0.30	0.24	0.32	0.32	0.31	0.28	0.30	0.28	0.24	0.19	0.17	0.20	0.15	0.25
Hydroides dianthus Clade B	0.59	0.58	0.57	0.27	0.07	0.01	0.29	0.30	0.26	0.32	0.31	0.31	0.30	0.33	0.26	0.24	0.23	0.18	0.21	0.14	0.28
Hydroides elegans Clade E1	0.56	0.51	0.53	0.31	0.31	0.29	0.01	0.09	0.30	0.32	0.30	0.29	0.29	0.31	0.35	0.36	0.29	0.32	0.28	0.31	0.31
Hydroides elegans Clade E2	0.51	0.49	0.49	0.29	0.30	0.30	0.09	0.01	0.28	0.32	0.32	0.30	0.26	0.27	0.32	0.31	0.26	0.30	0.26	0.27	0.28
Hydroides elegans / ezoensis	0.58	0.55	0.55	0.29	0.24	0.26	0.30	0.28	0.00	0.29	0.30	0.24	0.25	0.28	0.30	0.27	0.26	0.29	0.31	0.28	0.11
Hydroides nigra	0.61	0.58	0.55	0.31	0.32	0.32	0.32	0.32	0.29	n	0.30	0.27	0.26	0.29	0.20	0.28	0.30	0.30	0.29	0.30	0.30
Hydroides triversiculosa	0.63	0.54	0.52	0.29	0.32	0.31	0.30	0.30	0.30	0.30	n	0.34	0.35	0.35	0.29	0.27	0.28	0.31	0.31	0.29	0.29
Hydroides basispinosa / gradata	0.56	0.56	0.52	0.30	0.31	0.31	0.29	0.26	0.24	0.27	0.34	0.00	0.15	0.20	0.26	0.26	0.30	0.31	0.29	0.30	0.26
Hydroides inornata / operculata	0.52	0.51	0.52	0.28	0.28	0.30	0.31	0.27	0.25	0.26	0.35	0.15	0.00	0.10	0.25	0.24	0.24	0.26	0.26	0.25	0.27
Hydroides operculata	0.57	0.55	0.51	0.29	0.30	0.33	0.35	0.32	0.28	0.29	0.35	0.20	0.10	n	0.25	0.25	0.26	0.28	0.29	0.27	0.27
Hydroides pseudouncinata	0.62	0.59	0.57	0.30	0.28	0.26	0.36	0.31	0.30	0.20	0.29	0.26	0.25	0.25	0.01	0.23	0.21	0.24	0.25	0.23	0.31
Hydroides crucigera	0.63	0.51	0.50	0.24	0.24	0.24	0.29	0.26	0.27	0.28	0.27	0.26	0.24	0.25	0.23	n	0.22	0.23	0.21	0.22	0.26
Hydroides brachyacantha	0.60	0.55	0.57	0.22	0.19	0.23	0.32	0.30	0.26	0.30	0.28	0.30	0.24	0.26	0.21	0.22	n	0.18	0.18	0.16	0.26
Hydroides recurvispina / dolabrus	0.60	0.54	0.53	0.25	0.17	0.18	0.28	0.26	0.29	0.30	0.31	0.31	0.26	0.28	0.24	0.23	0.18	0.00	0.14	0.15	0.27
Hydroides panamensis	0.59	0.54	0.57	0.23	0.20	0.21	0.31	0.27	0.31	0.29	0.31	0.29	0.26	0.29	0.25	0.21	0.18	0.14	0.03	0.18	0.29
Hydroides sanctaecrucis	0.58	0.56	0.57	0.23	0.15	0.14	0.30	0.29	0.28	0.30	0.29	0.30	0.25	0.27	0.23	0.22	0.16	0.15	0.18	0.01	0.27
Hydroides fusicola	0.59	0.50	0.48	0.28	0.25	0.28	0.31	0.28	0.11	0.30	0.29	0.26	0.27	0.27	0.31	0.26	0.26	0.27	0.29	0.27	n

Table 5. – Divergences measured in Cytb fragment using the K2P model (lower left corner) and p-distance (upper right corner). In grey cells, intraspecific genetic variation (K2P). Ambiguous positions removed for each sequence pair.

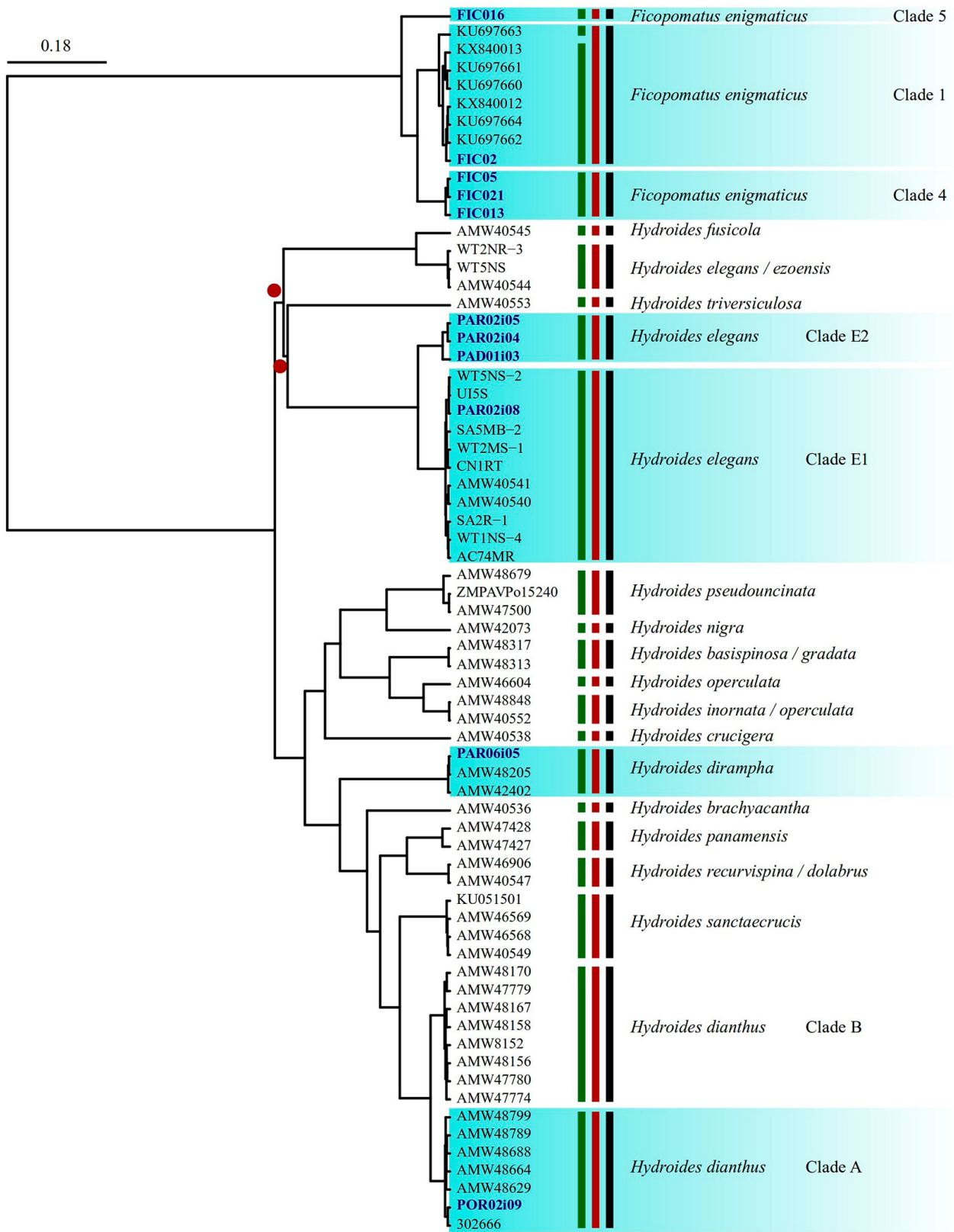| Cytb	*F. enigmaticus* Clade 1	*F. enigmaticus* Clade 2b	*F. enigmaticus* Clade 2a	*H. elegans* Clade E1	*H. norvegica*	*H. dianthus* Clade B	*H. dirampha*	*H. cf nigra*	*H. nigra*	*H. pseudouncinnata*	*H. tuberculata*	*H. triversiculosa*	*H. minax*	*H. nikae*	*H. operculata*	*H. panamensis*	*H. dolabrus*	*H. sanctaecrucis*	*H. brachyacantha a*	*H. brachyacantha b*	*H. crucigera*	*H. fusicola*	*H. ezoensis*	*H. novaepommeraniae*
Ficopomatus enigmaticus Clade 1	0.03	0.25	0.21	0.64	0.71	0.61	0.65	0.63	0.65	0.72	0.62	0.64	0.65	0.74	0.63	0.59	0.57	0.60	0.63	0.64	0.74	0.69	0.68	0.72
Ficopomatus enigmaticus Clade 2b	0.25	0.00	0.19	0.72	0.70	0.64	0.67	0.66	0.68	0.77	0.68	0.65	0.66	0.73	0.68	0.63	0.63	0.60	0.67	0.69	0.76	0.72	0.78	0.70
Ficopomatus enigmaticus Clade 2a	0.21	0.19	0.05	0.70	0.66	0.65	0.65	0.70	0.65	0.76	0.65	0.57	0.65	0.65	0.65	0.64	0.63	0.62	0.67	0.67	0.69	0.70	0.69	0.69
Hydroides elegans Clade E1	0.64	0.72	0.70	0.01	0.26	0.44	0.38	0.46	0.43	0.42	0.40	0.40	0.37	0.41	0.43	0.37	0.44	0.40	0.38	0.35	0.43	0.43	0.40	0.46
Hydroides norvegica	0.71	0.70	0.66	0.26	0.04	0.41	0.42	0.45	0.47	0.43	0.43	0.36	0.38	0.39	0.44	0.36	0.36	0.37	0.39	0.36	0.42	0.36	0.41	0.43
Hydroides dianthus Clade B	0.61	0.64	0.65	0.44	0.41	0.02	0.25	0.26	0.31	0.31	0.28	0.28	0.28	0.29	0.22	0.18	0.23	0.19	0.19	0.24	0.21	0.28	0.27	0.34
Hydroides dirampha	0.65	0.67	0.65	0.38	0.42	0.25	n	0.22	0.26	0.26	0.27	0.23	0.26	0.28	0.23	0.17	0.19	0.22	0.22	0.19	0.25	0.34	0.26	0.30
Hydroides cf nigra	0.63	0.66	0.70	0.46	0.45	0.26	0.22	n	0.32	0.34	0.34	0.31	0.29	0.35	0.26	0.24	0.25	0.22	0.23	0.22	0.25	0.36	0.36	0.29
Hydroides nigra	0.65	0.68	0.65	0.43	0.47	0.31	0.26	0.32	n	0.22	0.34	0.31	0.35	0.29	0.27	0.29	0.34	0.31	0.31	0.30	0.31	0.42	0.35	0.38
Hydroides pseudouncinnata	0.72	0.77	0.76	0.42	0.43	0.31	0.26	0.34	0.22	n	0.36	0.30	0.29	0.29	0.27	0.27	0.29	0.27	0.32	0.30	0.27	0.37	0.29	0.36
Hydroides tuberculata	0.62	0.68	0.65	0.40	0.43	0.28	0.27	0.34	0.34	0.36	0.19	0.30	0.25	0.26	0.28	0.25	0.25	0.26	0.27	0.29	0.29	0.30	0.27	0.30
Hydroides triversiculosa	0.64	0.65	0.57	0.40	0.36	0.28	0.23	0.31	0.31	0.30	0.30	n	0.17	0.27	0.25	0.22	0.26	0.27	0.26	0.23	0.25	0.29	0.25	0.24
Hydroides minax	0.65	0.66	0.65	0.37	0.38	0.28	0.26	0.29	0.35	0.29	0.25	0.17	n	0.26	0.24	0.20	0.27	0.25	0.22	0.23	0.26	0.29	0.28	0.23
Hydroides nikae	0.74	0.73	0.65	0.41	0.39	0.29	0.28	0.35	0.29	0.29	0.26	0.27	0.26	0.00	0.26	0.22	0.27	0.29	0.27	0.28	0.27	0.32	0.29	0.33
Hydroides operculata	0.63	0.68	0.65	0.43	0.44	0.22	0.23	0.26	0.27	0.27	0.28	0.25	0.24	0.26	n	0.16	0.24	0.21	0.18	0.21	0.27	0.34	0.32	0.29
Hydroides panamensis	0.59	0.63	0.64	0.37	0.36	0.18	0.17	0.24	0.29	0.27	0.25	0.22	0.20	0.22	0.16	n	0.10	0.13	0.15	0.16	0.20	0.26	0.24	0.29
Hydroides dolabrus	0.57	0.63	0.63	0.44	0.36	0.23	0.19	0.25	0.34	0.29	0.25	0.26	0.27	0.27	0.24	0.10	n	0.17	0.19	0.23	0.24	0.28	0.24	0.30
Hydroides sanctaecrucis	0.60	0.60	0.62	0.40	0.37	0.19	0.22	0.22	0.31	0.27	0.26	0.27	0.25	0.29	0.21	0.13	0.17	n	0.17	0.21	0.21	0.24	0.25	0.28
Hydroides brachyacantha a	0.63	0.67	0.67	0.38	0.39	0.19	0.22	0.23	0.31	0.32	0.27	0.26	0.22	0.27	0.18	0.15	0.19	0.17	0.00	0.19	0.21	0.29	0.28	0.31
Hydroides brachyacantha b	0.64	0.69	0.67	0.35	0.36	0.24	0.19	0.22	0.30	0.30	0.29	0.23	0.23	0.28	0.21	0.16	0.23	0.21	0.19	0.00	0.22	0.29	0.26	0.32
Hydroides crucigera	0.74	0.76	0.69	0.43	0.42	0.21	0.25	0.25	0.31	0.27	0.29	0.25	0.26	0.27	0.27	0.20	0.24	0.21	0.21	0.22	n	0.30	0.27	0.35
Hydroides fusicola	0.69	0.72	0.70	0.43	0.36	0.28	0.34	0.36	0.42	0.37	0.30	0.29	0.29	0.32	0.34	0.26	0.28	0.24	0.29	0.29	0.30	n	0.14	0.37
Hydroides ezoensis	0.68	0.78	0.69	0.40	0.41	0.27	0.26	0.36	0.35	0.29	0.27	0.25	0.28	0.29	0.32	0.24	0.24	0.25	0.28	0.26	0.27	0.14	n	0.35
Hydroides novaepommeraniae	0.72	0.70	0.69	0.46	0.43	0.34	0.30	0.29	0.38	0.36	0.30	0.24	0.23	0.33	0.29	0.29	0.30	0.28	0.31	0.32	0.35	0.37	0.35	n

Fig. 4. – BI COI tree. Species found in Majorca are highlighted in blue. Red dots above or under branches indicate bootstrap values under 70. Specimens sequenced for this study are in bold and dark blue. GMYC entities are indicated by green lines. mPTP entities are indicated by red lines. *Ficopomatus* clades are named as in Styan et al. (2017). *Hydroides dianthus* clades are named as in Sun et al. (2017b).

range from 21.7% to 46.3% (Table 5). This result may indicate that either of the two specimens identified as *H. nigra* from Majorca actually belongs to a different species, with morphological features similar to the diagnostic features of this species.

DISCUSSION

Molecular data for assessing species diversity and invasive status

Assessing cryptic annelid diversity after analyses of DNA sequences is now a common procedure (Nygren 2014). Previous analyses of Cytb sequences showed that *F. enigmaticus* was in fact a species complex (Styan et al. 2017, Yee et al. 2019), gathering at least two cryptic species (understood as morphologically identical but separately evolving metapopulation lineages).

Clade 1 is currently reported as widely distributed (e.g. California, Australia and Majorca) (Styan et al. 2017, Yee et al. 2019, present study). In the case of two of the lineages found in the present study, Clade 4 and Clade 5, there are no previous records of these species elsewhere, and they are for now only known from Majorca. Considering that the *F. enigmaticus* species complex is not originally from Mediterranean waters, they have probably been introduced, and it is also expected that members of these clades can be found in other regions worldwide. It is interesting to note that Clade 2 reported by Styan et al. (2017) and Yee et al. (2019) is recovered as two separate species in the present analyses. While we did not include all the data available for this clade in the species delimitation analyses, this suggests that molecular species delimitation is not straightforward in this group.

The situation is similar with species of *Hydroides* found in Majorcan waters. Both *H. operculata* and *H. dianthus* have previously been shown to be species complexes (Sun et al. 2017a,b). *Hydroides dianthus* Clade A has already been reported from the eastern and southern USA, Brazil, east China, Japan, Turkey and Italy. Its distribution area now includes Majorca. Although *H. dianthus* was originally from the USA (Verrill 1873), it is argued that it may originate from the Mediterranean (Sun et al. 2017a). If this is confirmed, although the species has not been reported in the Balearic Islands before, it would involve reconsidering their status as invasive in Mediterranean localities. The situation is different for *Hydroides* cf *dianthus* Clade B, which has been recorded from only two places, Texas (Caribbean) and Ukraine (Black Sea), and never before in the same region as *Hydroides dianthus* clade A. It was suggested that this species has been introduced via the Mediterranean from an American population to the Black Sea, and our record provides more evidence in support of this hypothesis. However, previous studies of *H.* cf. *dianthus* Clade B were done after analyses of COI sequences, and our record uses a Cytb sequence, which limits further interpretations. (The link of the identity of sequences from these two markers was made possible by later sequencing of a voucher for more global phylogenetic purposes - see Sun et al. 2018.)

Genetic data has a great potential for detecting invasive species (Muñoz-Colmenero et al. 2018), determining the source of the invasion and understanding the routes taken by the species (Geller et al. 2010, Yee et al. 2019). When encountering cryptic diversity, it is also particularly important to distinguish between the potentially invasive and native lineages. Though it was one of the aims of the present study, despite the recent progress in the COI DNA barcoding of *Hydroides* species (Sun et al. 2012), we encountered many difficulties in sequencing COI and Cytb from *Hydroides* and *Ficopomatus*. While the data we obtained are sufficient to detect the presence of the species complexes and identify the species present in Majorcan waters, they are not sufficient to properly assess their population structure or the sources of the invasion.

Distribution of the *Ficopomatus enigmaticus* species complex

The presence of *F. enigmaticus* (*sensu lato*) has been observed in the ports of Palma and Portitxol and near the mouth of torrents. These environments are characterized by low hydrodynamism (expect for after a large rainfall), eutrophic waters and changes in salinity. This kind of habitat has been observed previously in other studies dealing with this species (Yee et al. 2019) and conforms to the ecology of the optimal habitat for the proliferation of *F. enigmaticus* (*sensu lato*), which consists of enclosed environments with murky waters. The wide ranges of tolerance to salinity and temperature give members of this species complex the ability to establish themselves in these variant environments, unlike other serpulid species (Dittmann et al. 2009).

During the summer, there was a greater proliferation and density of *F. enigmaticus* populations, as previously observed in other studies (Vuillemin 1958, Dixon 1981). According to the literature, the minimum temperature to reproduce ranges between 14°C and 18°C, which in the Balearic Islands corresponds to the month of May. The main episode of settlement and growth is between spring and summer, which correlates with an increase in biomass and carbonated production (Fornós et al. 1997). Knowing that complete maturation takes four months (Obenat and Pezzani, 1994), large aggregations may appear during the summer. The addition of the intensification of the international maritime traffic at this period increases the potential for dispersion.

Distribution of the *Hydroides* species

Species diversity showed an uneven presence and abundance between the locations sampled (Table 1). The most common species were *H. dianthus* and *H. elegans*, which were present at more sites within the ports of Palma, Portixol and Alcudia. *Hidroides dirampha* occurred in the ports of Palma, Alcudia and Sóller, and *H. nigra* only in Portixol (Table 1). Accordingly, the port environments with the greatest species diversity were in descending order the ports of Palma, Alcudia, Portixol and Sóller. There seems to be some correlation between the ports with the highest volume of maritime traffic (Palma, Alcudia and Portixol) and the greatest diversity of invasive species, which would conform to the expected re-
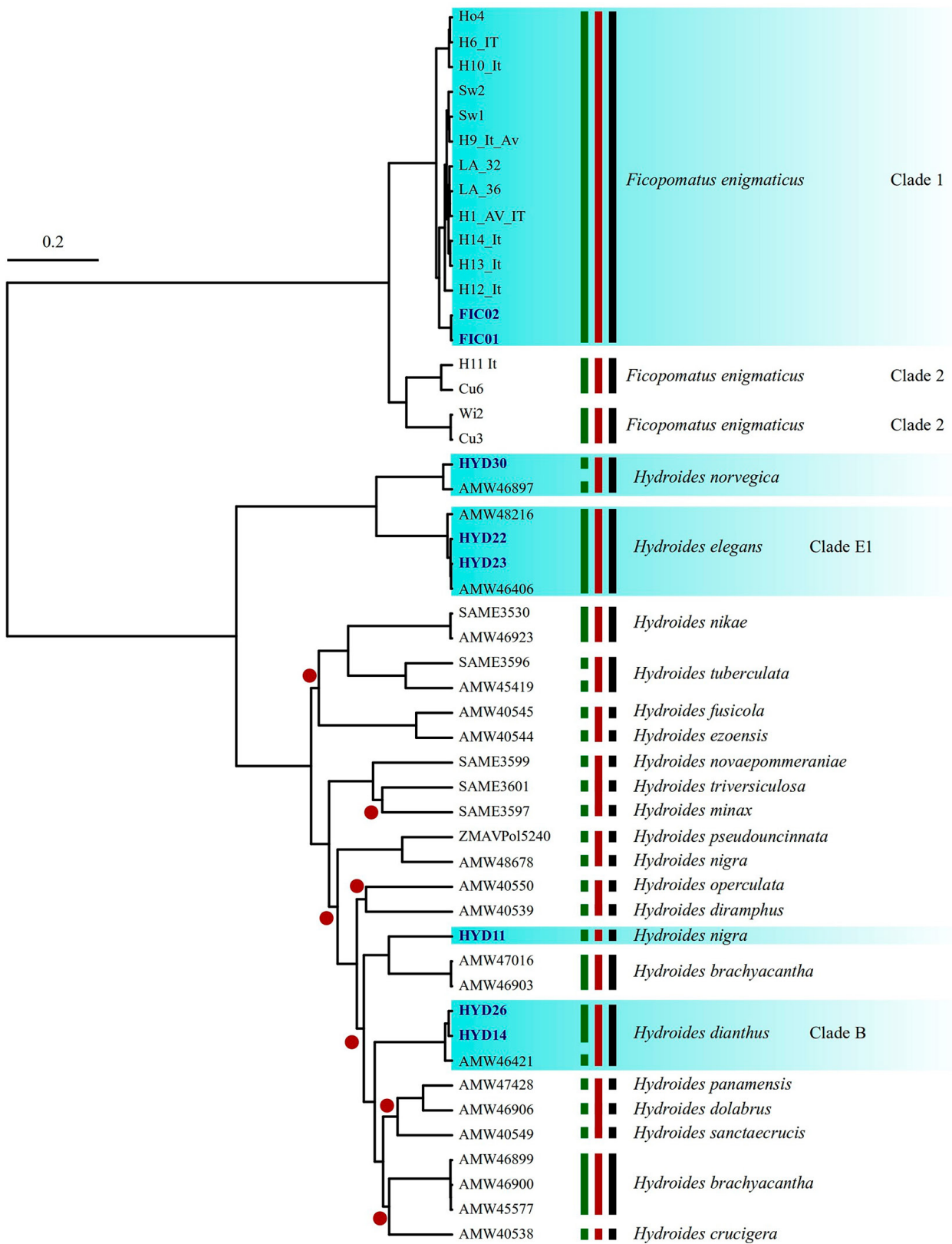
Fig. 5. – BI Cytb tree. Species found in Majorca are highlighted in blue. Red dots above or under branches indicate bootstrap values under 70. Specimens sequenced for this study are in bold and dark blue. GMYC entities are indicated by green lines. mPTP entities are indicated by red lines. *Ficopomatus* clades are named as in Styan et al. (2017). *Hydroides dianthus* clades are named as in Sun et al. (2017b).

sults, because a greater flow of maritime transport could favour the translocation of these species associated with fouling communities embedded on the hulls of the boats (e.g. Zibrowius 1971, ten Hove and Kupriyanova 2009, Çinar 2012). In the present study, the preference of *Hydroides* for artificial substrates is verified (as in Pawlik 1992, Kupriyanova et al. 2001), with four species being present in the ports of Palma and Portixol, while none were recorded on rocky substrates in nearby anthropized areas, such as Cala Blava, and only one specimen in Sa Porrasa Islet (Sun et al. 2017b).

CONCLUSION

We report for the first time the presence of the Serpulidae species *Ficopomatus enigmaticus*, *Hydroides dianthus*, *H. dirampha*, *H. elegans* and *H. nigra* from Majorca. From these species, at least *F. enigmaticus*, *H. dianthus* and *H. elegans* are actually species complexes, within which at least one of the species is cosmopolitan and known as invasive. Three other species within these complexes are for now only known to Majorca and their possible status as invasive is unknown. A better knowledge of the introduction events of these species can be gained by sequencing more specimens from the archipelago.

ACKNOWLEDGEMENTS

REFERENCES

Alcázar J., San Martín G. 2016. Serpúlidos (Annelida, Serpulidae) colectados en la campaña oceanográfica "Fauna II" y catálogo actualizado de especies íbero-baleares de la familia Serpulidae. Graellsia 72: e053.

Ambrogi A.O. 2000. Biotic invasions in a Mediterranean lagoon. Biol. Invasions. 2: 165-176.
https://doi.org/10.1023/A:1010004926405

Aubet M.E. 2001. The Phoenicians and the West: politics, colonies and trade. Cambridge Univ. Press, 448 pp.

Autoridad Portuaria de Baleares (APB). 2017. Memoria Anual, Port de Baleares, 229 pp.

Bastida-Zavala J. R., ten Hove H.A. 2002. Revision of *Hydroides* Gunnerus, 1768 (Polychaeta: Serpulidae) from the Western Atlantic Region. Beaufortia 52: 103-178.

Bax N., Williamson A., Aguero M., et al. 2003. Marine invasive alien species: a threat to global biodiversity. Mar. Policy 27: 313-323.

Benson D.A., Karsh-Mizrachi I., Lipman D.J., et al. 2008. GenBank. Nucleic Acids Res. 36: 25-30.
https://doi.org/10.1093/nar/gkm929

Boore J.L., Brown W.M. 2000. Mitochondrial genomes of *Galathealinum*, *Helobdella*, and *Platynereis*: sequence and gene arrangement comparisons indicate that Pogonophora is not a phylum and Annelida and Arthropoda are not sister taxa. Mol. Biol. Evol. 17: 87-106.
https://doi.org/10.1093/oxfordjournals.molbev.a026241

Bouckaert R., Heled J., Kühnert D., et al. 2014. BEAST 2: A Software Platform for Bayesian Evolutionary Analysis. PLoS Comput. Biol. 10: e1003537.
https://doi.org/10.1371/journal.pcbi.1003537

Bruschetti M., Bazterrica C., Luppi T., et al. 2009. An invasive intertidal reef-forming polychaete affect habitat use and feeding behavior of migratory and locals birds in a SW Atlantic coastal lagoon. J. Exp. Mar. Biol. Ecol. 375: 76-83.
https://doi.org/10.1016/j.jembe.2009.05.008

Burnette A.B., Struck T.H., Halanych K.M. 2005. Holopelagic *Poeobius meseres* ('Poeobiidae', Annelida) is derived from benthic flabelligerid worms. Biol. Bull-US 208: 213-220.
https://doi.org/10.2307/3593153

Castresana J. 2000. Selection of conserved blocks from multiple alignments for their use in phylogenetic analysis. Mol. Biol. Evol. 17: 540-552.
https://doi.org/10.1093/oxfordjournals.molbev.a026334

Çinar M. E. 2006. Serpulid species (Polychaeta: Serpulidae) from the Levantine coast of Turkey (eastern Mediterranean), with special emphasis on alien species. Aquat. Invasions 1: 223-240.
https://doi.org/10.3391/ai.2006.1.4.6

Çinar M. E. 2012. Alien polychaete species worldwide: current status and their impacts J. Mar. Biolog. Assoc. U.K. 93: 1257-1278.
https://doi.org/10.1017/S0025315412001646

Crooks J. A. 2002. Characterizing ecosystem-level consequences of biological invasions: the role of ecosystem engineers. Oikos 97: 153-166.
https://doi.org/10.1034/j.1600-0706.2002.970201.x

Davies B.R., Stuart V., De Villiers M. 1989. The filtration activity of a serpulid polychaete population (*Ficopomatus enigmaticus* (Fauvel)) and its effects on water quality in a coastal marina. Estuar. Coast. Shelf. Sci. 29: 613-620.
https://doi.org/10.1016/0272-7714(89)90014-0

Dinno A. 2018. paran: Horn's Test of Principal Components/Factors. R package version 1.5.2.
https://CRAN.R-project.org/package=paran

Dittmann S., Rolston A., Benger S.N., et al. 2009. Habitat requirements, distribution and colonisation of the tubeworm *Ficopomatus enigmaticus* in the Lower Lakes and Coorong. Report for the South Australian Murray-Darling Basin Natural Resources Management Board, Adelaide, 99 pp.

Dixon D.R. 1981. Reproductive biology of the serpulid *Ficopomatus* (*Mercierella*) *enigmaticus* in the Thames Estuary, SE England. J. Mar. Biol. Assoc. UK. 61: 805-815.
https://doi.org/10.1017/S0025315400048220

Drummond A.J., Bouckaert R.R. 2015. Bayesian Evolutionary Analysis with BEAST. Cambridge Univ. Press.
https://doi.org/10.1017/CBO9781139095112

Ergen Z. 1976. Investigations on the taxonomy and ecology of Polychaeta from Izmir Bay and its adjacent areas. Sci. Rep. Fac. Sci. Ege Univ. 209: 1-73.

Ezard T., Fujisawa T., Barraclough T. 2017. splits: SPecies' LImits by Threshold Statistics. R package version 1.0-19/r52
https://R-Forge.R-project.org/projects/splits/

Fauvel P. 1923. Un nouveau serpulien d'eau saumatre *Mercierella enigmatica* n. sp. Bull. Soc. Zool. Fr. 46: 424-430.

Fornós J.J., Forteza V., Martínez-Taberner A. 1997. Modern polychaete reefs in western Mediterranean lagoons: *Ficopomatus enigmaticus* (Fauvel) in the Albufera of Menorca, Balearic islands. Palaeogeogr. Palaeoclimatol. Palaeoecol. 128: 175-186.
https://doi.org/10.1016/S0031-0182(96)00045-4

Fujisawa T., Barraclough T.G. 2013. Delimiting species using single-locus data and the Generalized Mixed Yule Coalescent Approach: A revised method and evaluation on simulated data sets. Syst. Biol. 62: 707-724.
https://doi.org/10.1093/sysbio/syt033

Geller J.B., Darling J.A., Carlton J.T. 2010. Genetic perspectives on marine biological invasions. Ann. Rev. Mar. Sci. 2: 367-393.
https://doi.org/10.1146/annurev.marine.010908.163745

Geller J., Meyer C., Parker M., et al. 2013. Redesign of PCR primers for mitochondrial cytochrome c oxidase subunit I for marine invertebrates and application in all-taxa biotic surveys. Mol. Ecol. Resour. 13: 851-861.
https://doi.org/10.1111/1755-0998.12138

Gil J. 2011. The European fauna of Annelida Polychaeta. PhD thesis, Univ. Lisboa, Portugal, 1554 pp.

Grube A.E. 1868. Beschreibungen einiger von Georg Ritter von Frauenfeld gesammelter Anneliden und Gephyreen des rothen Meeres. Verh. Zool.-Bot. Ges. Wien 18: 629-650.

Grube A.E. 1878. Annulata Semperiana. Beiträge zur Kenntniss der Annelidenfauna der Philippinen nach den von Herrn Prof. Semper mitgebrachten Sammlungen. Mem. Acad. Sci. St. Petersb. 25: 1-300.
https://doi.org/10.5962/bhl.title.85345

Guindon S., Dufayard J.F., Lefort V., et al. 2010. New algorithms and methods to estimate maximum-likelihood phylogenies: assessing the performance of PhyML 3.0. Syst. Biol. 59: 307-321.
https://doi.org/10.1093/sysbio/syq010

Gunnerus J.E. 1768. Om nogle Norske coraller. Kongel. Norske Vidensk. Selsk. Skr. 4: 38-73.

Halt M.N., Kupriyanova E.K., Cooper S.J., et al. 2009. Naming species with no morphological indicators: species status of *Galeolaria caespitosa* (Annelida: Serpulidae) inferred from nuclear and mitochondrial gene sequences and morphology. Invertebr. Syst. 23: 205-222.
https://doi.org/10.1071/IS09003

Heiman K.W., Micheli F. 2010. Non-native ecosystem engineer alters estuarine communities. Integr. Comp. Biol. 50: 226-236.
https://doi.org/10.1093/icb/icq036

Hewitt C.L., Gollasch S., Minchin D. 2009. The vessel as a vector-biofouling, ballast water and sediments. In: Rilov G., Crooks J.A. (eds) Biological Invasions in Marine Ecosystems. Ecol. Stud. Springer, Berlin, Heidelberg, pp. 117-131.
https://doi.org/10.1007/978-3-540-79236-9_6

Haswell W.A. 1883. On some new Australian tubicolous annelids. Proc. Linn. Soc. N.S.W. 7: 633-638.

Invasive Species Specialist Group (ISSG). 2011. We need to strengthen, not weaken, the struggle against harmful invasive species.
http://www.issg.org/pdf/rebuttal.pdf

Iroso I. 1921. Revisione dei serpulidi e sabellidi del Golfo di Napoli. PSZNI Mar. Ecol. 3: 47-91.

Katsanevakis S., Wallentinus I., Zenetos A., et al. 2014. Impact of invasive alien marine species on ecosystem services and biodiversity: a pan-European review. Aquat. Invasions 9: 391-423.
https://doi.org/10.3391/ai.2014.9.4.01

Kapli P., Lutteropp S., Zhang J., et al. 2017. Multi-rate Poisson tree processes for single-locus species delimitation under maximum likelihood and Markov chain Monte Carlo. Bioinformatics 33: 1630-1638.
https://doi.org/10.1093/bioinformatics/btx025

Katoh K., Standley D.M. 2013. MAFFT multiple sequence alignment software version 7: improvements in performance and usability. Mol. Biol. Evol. 30: 772-780.
https://doi.org/10.1093/molbev/mst010

Kearse M., Moir R., Wilson A., et al. 2012. Geneious Basic: an integrated and extendable desktop software platform for the organization and analysis of sequence data. Bioinformatics 28: 1647-1649.
https://doi.org/10.1093/bioinformatics/bts199

Kumar S., Stecher G., Li M., et al. 2018. MEGA X: Molecular Evolutionary Genetics Analysis across computing platforms. Mol. Biol. Evol. 35: 1547-1549.
https://doi.org/10.1093/molbev/msy096

Kupriyanova E.K., Nishi E., ten Hove H.A., et al. 2001. A review of life history patterns in Serpulimorph polychaetes: ecological and evolutionary perspectives. Oceanogr. Mar. Biol. 39: 1-101.

Lanfear R., Frandsen P.B., Wright A.M., et al. 2016. PartitionFinder 2: New methods for selecting partitioned models of evolution for molecular and morphological phylogenetic analyses. Mol. Biol. Evol. 34: 772-773.
https://doi.org/10.1093/molbev/msw260

Larsson A. 2014. AliView: a fast and lightweight alignment viewer and editor for large datasets. Bioinformatics 30: 3276-3278.
https://doi.org/10.1093/bioinformatics/btu531

Martínez-Taberner A., Forteza V., Fornós J.J. 1993. Colonization, structure and growth of *Ficopomatus enigmaticus* cf. ten Hove and Weerdenburg (Polychaeta, Serpulidae) in the Albufera of Menorca, Balearic Islands. Verh. Internat. Verein. Limnol. 25: 1031-1034.
https://doi.org/10.1080/03680770.1992.11900316

Miller M.A., Pfeiffer W., Schwartz T. 2010. Creating the CIPRES Science Gateway for Inference of Large Phylogenetic Trees. In: Proceedings of the Gateway Computing Environments Workshop. New Orleans, pp. 1-8.
https://doi.org/10.1109/GCE.2010.5676129

Molnar J.L., Gamboa R.L., Revenga C., et al. 2008. Assessing the global threat of invasive species to marine biodiversity. Front. Ecol. Environ. 6: 485-492.
https://doi.org/10.1890/070064

Mörch O. A. L. 1863. Revisio critica Serpulidarum. Et Bidrag til Rørormenes Naturhistorie. Naturh. Tidsskr. København 3: 347-470.

Muñoz-Colmenero M., Ardura A., Clusa L., et al. 2018. New specific molecular marker detects *Ficopomatus enigmaticus* from water eDNA before positive results of conventional sampling. J. Nat. Conserv. 43: 173-178.
https://doi.org/10.1016/j.jnc.2017.12.004

Nygren A. 2014. Cryptic polychaete diversity: a review. Zool. Scr. 43: 172-183.
https://doi.org/10.1111/zsc.12044

Obenat S.M., Pezzani S.E. 1994. Life cycle and population structure of the polychaete *Ficopomatus enigmaticus* (Serpulidae) in Mar Chiquita coastal lagoon, Argentina. Estuaries 17: 263.
https://doi.org/10.2307/1352574

Oliva M., De Marchi L., Vieira Sanches M., et al. 2020. Atlantic and Mediterranean populations of the widespread serpulid *Ficopomatus enigmaticus*: Developmental responses to carbon nanotubes. Mar. Poll. Bull. 156: 111265.
https://doi.org/10.1016/j.marpolbul.2020.111265

Pan J., Marcoval M.A. 2013. Top-down effects of an exotic serpulid polychaete on natural plankton assemblage of estuarine and brackish systems in the SW Atlantic. J. Coast. Res. 30: 1226-1235.
https://doi.org/10.2112/JCOASTRES-D-12-00163.1

Paradis E., Schliep K. 2018. ape 5.0: an environment for modern phylogenetics and evolutionary analyses in R. Bioinformatics 35: 526-528.
https://doi.org/10.1093/bioinformatics/bty633

Pawlik J.R. 1992. Chemical ecology of the settlement of benthic marine invertebrates. Oceanogr. Mar. Biol. 30: 273-335.

Peria J., Pernet B. 2019. Tolerance to salinity and thermal stress by larvae and adults of the serpulid annelid *Ficopomatus enigmaticus*. Invertebr. Biol. 138: e12271.
https://doi.org/10.1111/ivb.12271

Pernet B., Barton M., Fitzhugh K., et al. 2016. Establishment of the reef-forming tubeworm *Ficopomatus enigmaticus* (Fauvel, 1923) (Annelida: Serpulidae) in southern California. Bioinvasions Rec. 5.
https://doi.org/10.3391/bir.2016.5.1.03

Philippi A. 1844. Einige Bemerkungen über die Gattung Serpula, nebst Aufzählung der von mir im Mittelmeer mit dem Thier beobachteten Arten. Archiv für Naturgeschichte Berlin. 10: 186-198.
https://doi.org/10.5962/bhl.part.29558

Pons J., Barraclough T.G., Gomez-Zurita J., et al. 2006. Sequence-based species delimitation for the DNA taxonomy of undescribed insects. Syst. Biol. 55: 595-609.
https://doi.org/10.1080/10635150600852011

R Core Team. 2015. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria.
https://www.R-project.org/

Rafinesque C.S. 1815. Analyse de la nature ou tableau de l'univers et des corps organisés. Palermo, 224 pp.
https://doi.org/10.5962/bhl.title.106607

Rambaut A. 2014. FigTree 1.4.2 Software. Institute of Evolutionary Biology, Univ. Edinburgh, Edinburgh.

Rambaut A., Drummond A.J., Xie D., et al. 2018. Posterior Summarization in Bayesian Phylogenetics Using Tracer 1.7. Syst. Biol. 67: 901-904.
https://doi.org/10.1093/sysbio/syy032

Read G.B., Gordon D.P. 1991. Adventive occurrence of the fouling serpulid Ficopomatus enigmaticus (Polychaeta) in New Zealand. N. Z. J. Mar. Freshw. Res. 25: 269-273.
https://doi.org/10.1080/00288330.1991.9516478

Rioja E. 1923. Estudio sistemático de las especies Ibéricas del suborden Sabelliformia. Trab. Mus. Natl. Cienc. Nat. Ser. Zool. 48: 1-144.

Rioja E. 1941. Estudios Anelidologicos. II. Observaciones acerca de varias especies del genero Hydroides Gunnerus (sensu Fauvel) de las costas Mexicanas del Pacífico. An. Inst. Biol. Mex. 12: 161-175.

Rodriguez L.F. 2006. Can invasive species facilitate native species? Evidence of how, when, and why these impacts occur. Biol. Invasions 8: 927-939.
https://doi.org/10.1007/s10530-005-5103-3

San Martín G., Viéitez J.M. 1984. Anélidos poliquetos de los rizomas de Posidonia oceanica, en las costas de Cabo de Palos (Murcia, España). In: Bodouresque et al. (eds), International Workshop Posidonia oceanica Beds, GIS Posidonie publ. pp. 149-157.

Schwindt E., Bortolus A., Iribarne O.O. 2001. Invasion of a reef-builder polychaete: direct and indirect impacts on the native benthic community structure. Biol. Invasions 3: 137-149.
https://doi.org/10.1023/A:1014571916818

Schwindt E., De Francesco C.G., Iribarne O.O. 2004. Individual and reef growth of the invasive reef-building polychaete Ficopomatus enigmaticus in a south-western Atlantic coastal lagoon. J. Mar. Biol. Assoc. UK. 84: 987-993.
https://doi.org/10.1017/S0025315404010288h

Shumka S., Kashta L., Cake A. 2014. Occurrence of the non-indigenous tubeworm Ficopomatus enigmaticus (Fauvel, 1923) (Polychaeta: Serpulidae) on the Albanian coast of the Adriatic Sea. Turk. J. Zool. 38: 519-521.
https://doi.org/10.3906/zoo-1303-14

Smith A. M., Henderson Z. E., Kennedy M., et al. 2012. Reef formation versus solitariness in two New Zealand serpulids does not involve cryptic species. Aquat. Biol. 16: 97-103.
https://doi.org/10.3354/ab00444

Styan C.A., McCluskey C.F., Sun Y., et al. 2017. Cryptic sympatric species across the Australian range of the global estuarine invader Ficopomatus enigmaticus (Fauvel, 1923) (Serpulidae, Annelida). Aquat. Invasions 12: 53-65.
https://doi.org/10.3391/ai.2017.12.1.06

Sun Y., Kupriyanova E.K., Qiu J.W. 2012. COI barcoding of Hydroides: a road from impossible to difficult. Invertebr. Syst. 26: 539-547.
https://doi.org/10.1071/IS12024

Sun Y., Wong E., Tovar-Hernández M.A., et al. 2016. Is Hydroides brachyacantha (Serpulidae: Annelida) a widespread species? Invertebr. Syst. 30: 41-59.
https://doi.org/10.1071/IS15015

Sun Y., Wong E., Keppel E., et al. 2017a. A global invader or a complex of regionally distributed species? Clarifying the status of an invasive calcareous tubeworm Hydroides dianthus (Verrill 1873) (Polychaeta: Serpulidae) using DNA barcoding. Mar. Biol. 164: 28.
https://doi.org/10.1007/s00227-016-3058-9

Sun Y., Al-Kandari M., Kubal P., et al. 2017b. Cutting a gordian knot of tubeworms with DNA data: the story of the Hydroides operculata-complex (Annelida, Serpulidae). Zootaxa 4323: 39-48.
https://doi.org/10.11646/zootaxa.4323.1.3

Sun Y., Wong E., Ayhong S.T., et al. 2018 Barcoding and multi-locus phylogeography of the globally distributed calcareous tubeworm Hydroides Gunnerus, 1768 (Annelida, Polychaeta, Serpulidae). Mol. Phy. Evol. 127: 732-745.
https://doi.org/10.1016/j.ympev.2018.06.021

Ten Hove H.A., Kupriyanova E.K. 2009. Taxonomy of Serpulidae (Annelida, Polychaeta): The state of affairs. Zootaxa 2036: 1-126.
https://doi.org/10.11646/zootaxa.2036.1.1

Toonen R.J., Pawlik J.R. 2001. Foundations of gregariousness: A dispersal polymorphism among the planktonic larvae of a marine invertebrate. Evolution 55: 2439-2454.
https://doi.org/10.1111/j.0014-3820.2001.tb00759.x

Treadwell A.L. 1929. New species of polychaetous annelids in the collections of the American Museum of Natural History from Porto Rico, Florida, Lower California, and British Somaliland. Am. Mus. Novit. 392: 1-13.
https://doi.org/10.5479/si.00963801.75-2797.1

Ulman A., Ferrario J., Occhipinti-Ambrogi A., et al. 2017. A massive update of non-indigenous species records in Mediterranean marinas. PeerJ. 5: e3954.
https://doi.org/10.7717/peerj.3954

Ulman A., Ferrario J., Forcada A., et al. 2019. Alien species spreading via biofouling on recreational vessels in the Mediterranean Sea. J. Appl. Ecol. 56: 2620-2629.
https://doi.org/10.1111/1365-2664.13502

Venables W.N., Ripley B.D. 2002. Modern applied statistics with S. Fourth Edition, Springer, New York.
https://doi.org/10.1007/978-0-387-21706-2

Verrill A.E. 1873. XVIII. Report upon the invertebrate animals of Vineyard Sound and the adjacent waters, with an account of the physical characters of the region. Report on the condition of the sea fisheries of the south coast of New England in 1871 and 1872. Washington Gov. Print. Off. pp. 295-778.
https://doi.org/10.5962/bhl.title.57652

Vuillemin S. 1958. Fixation obtenue au laboratoire des larves de quelques Serpuliens (Annélides Polychètes) du lac de Tunis. C. R. Acad. Hebd. Seances Acad. Sci. D. 247: 2038-2040.

Willette D.A., Iñiguez A.R., Kupriyanova E.K., et al. 2015. Christmas tree worms of Indo-Pacific coral reefs: untangling the Spirobranchus corniculatus (Grube, 1862) complex. Coral Reefs 34: 899-904.
https://doi.org/10.1007/s00338-015-1294-y

Wright J.T., Gribben P.E. 2017. Disturbance-mediated facilitation by an intertidal ecosystem engineer. Ecology 98: 2425-2436.
https://doi.org/10.1002/ecy.1932

Yee A., Mackie J., Pernet B. 2019. The distribution and unexpected genetic diversity of the non-indigenous annelid Ficopomatus enigmaticus in California. Aquat. Invasions 14: 250-266.
https://doi.org/10.3391/ai.2019.14.2.06

Zenetos A., Çinar M.E., Pancucci-Papadopoulou M.A., et al. 2005. Annotated list of marine alien species in the Mediterranean with records of the worst invasive species. Mediterr. Mar. Sci. 6: 63-118.
https://doi.org/10.12681/mms.186

Zibrowius H. 1968. Étude morphologique, systématique et écologique des Serpulidae (Annelida Polychaeta) de la région de Marseille. Rec. Trav. Station mar. Endoume 43: 81-252.

Zibrowius H. 1971. Les espèces Méditerrannéennes du genre Hydroides (Polychaeta Serpulidae). Remarques sur le prétendu polymorphisme de Hydroides uncinata. Téthys 2: 691-746.

Zibrowius H. 1991. Ongoing modification of the Mediterranean marine fauna and flora by the establishment of exotic species. Mésogée 51: 83-107.

Zibrowius H. 2002. Assessing scale and impact of ship-transported alien fauna in the Mediterranean? CIESM Workshop Monographs 20: 62-68.

SUPPLEMENTARY MATERIAL

The following supplementary material is available through the online version of this article and at the following link: http://scimar.icm.csic.es/scimar/supplm/sm05062esm.pdf

Table S1. – Collection information on the specimens used for the phylogenetic analyses, vouchers, and GenBank accession numbers.

The elephant in the room: first record of invasive gregarious species of serpulids (calcareous tube annelids) in Majorca (western Mediterranean)

Maël Grosse, Roberto Pérez, Mateo Juan-Amengual, Joan Pons, María Capa

Supplementary material

Table S1. – Collection information on the specimens used for the phylogenetic analyses, vouchers, and GenBank accession numbers.

Species	Voucher / ID	Locality	COI	Cytb
Hydroides triversiculosus	AMW40553 / SAME3601	Australia	JQ885940	EU190476
Hydroides basispinosa	AMW48317	Australia	MF405948	
Hydroides gradata	AMW48313	Australia	MF405959	
Hydroides operculata	AMW40552	China	JQ885948	
Hydroides operculata	AMW40550	China		KP178722
Hydroides inornata	AMW48848	India	MF405941	
Hydroides operculata	AMW46604	Kuwait	MF405954	
Hydroides nigra	AMW42073	Spain	MF405952	
Hydroides pseudouncinata	AMW48679	Spain	MF405946	
Hydroides pseudouncinata	ZMAVPol5240	France	KP178713	EU190467
Hydroides pseudouncinata	AMW47500	Greece	MF405945	
Hydroides diramphus	AMW40539	China		KP178718
Hydroides crucigera	AMW40538	Mexico	JQ885947	KP178715
Hydroides branchyacanthus	AMW40536	Mexico	JQ885941	
Hydroides recurvispina	AMW40547	Mexico	JQ885945	
Hydroides dolabrus	AMW46906	Mexico	KP178712	KP178719
Hydroides panamensis	AMW47428	Costa Rica		KP988293
Hydroides sanctaecrucis	AMW46569	Australia	KU051504	
Hydroides sanctaecrucis	AMW40549	Mexico	JQ885943	KP178717
Hydroides sanctaecrucis		Australia	KU051501	
Hydroides sanctaecrucis	AMW46568	Australia	KU051503	
Hydroides dianthus	AMW48799	Turkey	KY123243	
Hydroides dianthus	AMW48789	Turkey	KY123249	
Hydroides dianthus	AMW48688	Italy	KY386653	
Hydroides dianthus	AMW48664	Italy	KY386640	
Hydroides dianthus	AMW48629	Italy	KY386635	
Hydroides dianthus	302666	USA	KU051463	
Hydroides dianthus	AMW48170	Ukraine	KU051522	
Hydroides dianthus	AMW47779	USA	KU051477	
Hydroides dianthus	AMW48167	Ukraine	KU051521	
Hydroides dianthus	AMW48156	USA	KU051512	
Hydroides dianthus	AMW47774	USA	KU051472	
Hydroides dianthus	AMW48152	USA	KU051508	
Hydroides dianthus	AMW48158	USA	KU051514	
Hydroides dianthus	AMW47780	USA	KU051478	
Hydroides fusicola	AMW40545	Japan	JQ885950	KP178723
Hydroides elegans	WT2NR-3	Australia	MH339602	
Hydroides ezoensis	AMW40544	Russia	JQ885951	KP178716
Hydroides elegans	WT5NS	Australia	MH339603	
Hydroides elegans	AMW40541	Australia	JQ885939	
Hydroides elegans	SA5MB-2	USA	KY235613	
Hydroides elegans	AMW40540	China	JQ885938	
Hydroides elegans	WT1NS-4	Australia	MH339601	
Hydroides elegans	UI5S	USA	KY235618	
Hydroides elegans	WT5NS-2	Australia	MH339600	
Hydroides elegans	WT2MS-1	Australia	MH339596	
Hydroides elegans	CN1RT	USA	KY235603	
Hydroides elegans	AC74MR	USA	KY235596	
Hydroides elegans	SA2R-1	USA	KY235607	
Ficopomatus enigmaticus	KU697663	Spain	KU697663	
Ficopomatus enigmaticus	KU697660	Spain	KU697660	
Ficopomatus enigmaticus	KU697661	Spain	KU697661	
Ficopomatus enigmaticus	KU697662	Spain	KU697662	
Ficopomatus enigmaticus	KU697664	Spain	KU697664	
Ficopomatus enigmaticus	KX840012	Spain	KX840012	
Ficopomatus enigmaticus	KX840013	Spain	KX840013	
Ficopomatus enigmaticus	Cu3	Australia		KP863760
Ficopomatus enigmaticus	Wi2	Australia		KP863759
Ficopomatus enigmaticus	Ho4	Australia		KP863758
Ficopomatus enigmaticus	H10_It	Italy		MK934530
Ficopomatus enigmaticus	H14_It	Italy		MK934534

Species	Voucher / ID	Locality	COI	Cytb
Ficopomatus enigmaticus	H13_It	Italy		MK934533
Ficopomatus enigmaticus	H12_It	Italy		MK934532
Ficopomatus enigmaticus	Sw2	Australia		KP863738
Ficopomatus enigmaticus	Sw1	Australia		KP863736
Ficopomatus enigmaticus	H9_It_Av	Italy / Portugal		MK934529
Ficopomatus enigmaticus	LA_36	USA		MK334109
Ficopomatus enigmaticus	LA_32	USA		MK334105
Ficopomatus enigmaticus	Cu6	Australia		KP863767
Ficopomatus enigmaticus	H11_It	Italy		MK934531
Hydroides norvegica	AMW46897	Norway		KP178721
Hydroides tuberculata	SAME3596			EU190473
Hydroides tuberculata	AMW45419	Australia		KR095099
Hydroides minax	SAME3597			EU190475
Hydroides nikae	SAME3530			EU190466
Hydroides nikae	AMW46923	Australia		KR095078
Hydroides brachyachanta	AMW45577	Mexico		KR095053
Hydroides brachyachanta	AMW46899	Mexico		KR095068
Hydroides brachyachanta	AMW46900	Mexico		KR095069
Hydroides brachyachanta	AMW47016	Mexico		KR095089
Hydroides brachyachanta	AMW46903	Mexico		KR095070
Hydroides novaepommeraniae	SAME3599			EU190478
Ficopomatus enigmaticus	H1_AV_IT	Italy / Portugal		MH271215
Ficopomatus enigmaticus	H6_IT	Italy		MH271220
Hydroides nigra	AM W48678	Spain		MF406020
Hydroides elegans	AMW48216	USA		MG892710
Hydroides elegans	AM W46406			MG892708
Hydroides dirampha	AM W48205	Panama	MG892525	
Hydroides dirampha	AM W42402	Australia	MG892524	
Ficopomatus enigmaticus	FIC01 – MNCN 16.01/18765	Majorca	MT044486	MT215015
Ficopomatus enigmaticus	FIC02 – MNCN 16.01/18766	Majorca	MT044489	MT215014
Ficopomatus enigmaticus	FIC04 – MNCN 16.01/18767	Majorca	MT044492	NA
Ficopomatus enigmaticus	FIC05 – MNCN 16.01/18768	Majorca	MT044491	NA
Ficopomatus enigmaticus	FIC12 – MNCN 16.01/18769	Majorca	MT044494	NA
Ficopomatus enigmaticus	FIC15 – MNCN 16.01/18770	Majorca	MT044488	NA
Ficopomatus enigmaticus	FIC16 – MNCN 16.01/18771	Majorca	MT044495	NA
Ficopomatus enigmaticus	FIC17 – MNCN 16.01/18772	Majorca	MT044490	NA
Ficopomatus enigmaticus	FIC20 – MNCN 16.01/18773	Majorca	MT044487	NA
Ficopomatus enigmaticus	FIC21 – MNCN 16.01/18774	Majorca	MT044493	NA
Hydroides nigra	HYD11 – MNCN 16.01/18775	Majorca	NA	MT215009
Hydroides dianthus	HYD14 – MNCN 16.01/18776	Majorca	NA	MT215010
Hydroides elegans	HYD22	Majorca	NA	MT215012
Hydroides elegans	HYD23 – MNCN 16.01/18777	Majorca	NA	MT215008
Hydroides dianthus	HYD26 – MNCN 16.01/18778	Majorca	NA	MT215011
Hydroides norvegica	HYD30	Norway	NA	MT215013
Hydroides dirampha	PAR06i05 – MNCN 16.01/18779	Majorca	MT044496	NA
Hydroides dianthus	POR02i09 – MNCN 16.01/18780	Majorca	MT044497	NA
Hydroides elegans	PAR02i05 – MNCN 16.01/18781	Majorca	MT044498	NA
Hydroides elegans	PAR02i04 – MNCN 16.01/18782	Majorca	MT044499	NA
Hydroides elegans	PAD01i03 – 16.01/18783	Majorca	MT044500	NA
Hydroides elegans	PAR02i08 – MNCN 16.01/18784	Majorca	MT044501	NA