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 explainable 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 de 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* tienden un 5.4-47.6% de divergencia genética entre especies del mismo complejo.

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

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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 (Bruschetii et al. 2009, Pan and Marcovál 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 (Riboa 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 (Cinar 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 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, 1844) (Alcázar and San Martín 2016). Hydroides nigras 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’s 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    | *H. dianthus*                        | 0.5       |                               |
| PAR-02         | Puerto de Palma  | *F. enigmaticus*                     |           |                               |
|               |                  | *H. dianthus*                        |           |                               |
|               |                  | *H. elegans*                         |           |                               |
| PAR-06         | Port of Palma    | *H. elegans*                         | 0.5       |                               |
| PAR-12         | Puerto de Palma  | *F. enigmaticus*                     |           |                               |
|                |                  | *H. elegans*                         |           |                               |
|                |                  | *H. dirampha*                        |           |                               |
| POR-01         | Portitxol        | *H. dianthus*                        | 1         | Concrete dock                 |
| POR-02         | Portitxol        | *F. enigmaticus*                     | 0.5       | Concrete dock covered with cirripeds |
| POR-03         | Portitxol        | *F. enigmaticus*                     | 0.5       | Concrete dock covered with cirripeds |
| POR-04         | Portitxol        | *F. enigmaticus*                     | 0.3       | Concrete pier with brown algae and sponges |
| POR-12         | Portitxol        | *F. enigmaticus*                     | 0.5       | Concrete dock                 |
| POR-13         | Portitxol        | *F. enigmaticus*                     | 0.5       | Concrete dock covered with cirripeds |
| POR-14         | Portitxol        | *F. enigmaticus*                     |           |                               |
|                |                  | *H. dianthus*                        |           |                               |
|                |                  | *H. elegans*                         |           |                               |
|                |                  | *H. nigra*                           |           |                               |
| PAD-03         | Puerto de Palma  | *F. enigmaticus*                     | 2         | Muddy sediment                |
| PABA-14        | Puerto de Palma  | *F. enigmaticus*                     | 1         | Fouling plate                 |
| PALC-01        | Port of Alcudia  | *H. elegans*                         | 0-1       | Fouling plate                 |
| PALC-02        | Port of Alcudia  | *H. dianthus*                        | 0-1       | Fouling plate                 |
| PALC-03        | Port of Alcudia  | *H. dianthus*                        | 0-1       | Fouling plate                 |
| PALC-04        | Port of Alcudia  | *H. dianthus*                        | 0-1       | Fouling plate                 |
| PALC-05        | Port of Alcudia  | *H. dianthus*                        | 0-1       | Fouling plate                 |
| PALC-06        | Port of Alcudia  | *H. dianthus*                        | 0-1       | Fouling plate                 |
| PALC-07        | Port of Alcudia  | *H. dianthus*                        | 0-1       | Fouling plate                 |
| PSOL-01        | Port of Sóller   | *H. dianthus*                        | 0-1       | Fouling plate                 |
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 Portoixol. 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. 1. – Map of the localities sampled around the island of Majorca.

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 thick), and examined with a HITACHI S-3400N scanning electron microscope at the University of Madrid; Table 2).

### Molecular data

A small portion, 1 to 2 mm, of each specimen’s thorn 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 oxidase 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 MgCl₂, 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/jgHCO2198, Hydro-COIF/Hydro-COIR or Cytb424F/corb825, 7.5 μl of MyTag 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 ethidium 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 PAR-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°33'39.7"N 2°37'54.7"E 0.5 m |
| *Hydroides dianthus* | MNCN 16.01/18776 | HYD14 NA MT215010 PARBAL-15 26-Jul-17 39°50'21.2"N 3°08'01.7"E 0.5 m |
| *Hydroides elegans* | MNCN 16.01/18777 | HYD22 NA MT215012 PARBAL-15 26-Jul-17 39°33'15.5"N 2°37'33.7"E |
| *Hydroides elegans* | MNCN 16.01/18778 | HYD23 NA MT215008 PAR-02 26-Jul-17 39°33'55.7"N 2°37'54.7"E 1 m |
| *Hydroides elegans* | MNCN 16.01/18779 | 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-FASC2D 09-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°37'54.7"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 | POR02i10 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 | PAR02i06 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 ABI 3130 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 PartitionFinder 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 cyt b 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 5000000. Convergence of each run and parameter was checked using Tracer 1.7.1 (Rambaut and Bouckaert 2015) and 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 CyPress 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 (Dinn 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) | TITCIACIAAYCAYAARGAYTGG | 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) | TAICACACGRTCACACATGCA | 4 min 95°C; 35x 40 s 94°C, 40 s 50°C, 60 s 72°C; 6 min 72°C |
|       | Hydro-COIF (Sun et al. 2012) | TCWRTWRTKACGTCKATGCTA | 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) | CMRAGGWSAARAAACCTAGTA | 72°C; 6 min 72°C |
| Cytb   | Cyb 424F (Boore and Brown 2000) | GGWTAYGWTWCCWGTGGRGWCARAT | 4 min 95°C; 35x 40 s 94°C, 40 s 50°C, 60 s 72°C; 6 min 72°C |
|       | cobr825 (Bouret et al. 2005) | AARTAYCAYTGYGTTRATRG | 4 min 95°C; 35x 40 s 94°C, 40 s 50°C, 60 s 72°C; 6 min 72°C |
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 dirampha*, two species were recovered. For one species, *H. dianthus* 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 5. – 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       | Clade 1 | Clade 2 | Clade 3 | Clade 4 | Clade 5 |
|-----------|---------|---------|---------|---------|---------|
| Ficopomatus enigmaticus | 0.02 | 0.11 | 0.16 | 0.58 | 0.61 |
| Ficopomatus enigmaticus | 0.01 | 0.01 | 0.15 | 0.51 | 0.58 |
| Ficopomatus enigmaticus | 0.16 | 0.15 | 0.55 | 0.56 | 0.57 |
| Hydroides dirampha | 0.58 | 0.51 | 0.55 | 0.53 | 0.54 |
| Hydroides dirampha Clade A | 0.56 | 0.58 | 0.56 | 0.25 | 0.31 |
| Hydroides dirampha Clade B | 0.59 | 0.58 | 0.57 | 0.27 | 0.30 |
| Hydroides elegans Clade E1 | 0.56 | 0.51 | 0.53 | 0.31 | 0.29 |
| Hydroides elegans Clade E2 | 0.51 | 0.49 | 0.49 | 0.30 | 0.30 |
| Hydroides elegans / ezoensis | 0.58 | 0.53 | 0.55 | 0.24 | 0.26 |
| Hydroides nigra | 0.61 | 0.59 | 0.55 | 0.32 | 0.32 |
| Hydroides triversisculosa | 0.63 | 0.54 | 0.52 | 0.29 | 0.31 |
| Hydroides bapsissinosa / gradata | 0.56 | 0.56 | 0.50 | 0.31 | 0.29 |
| Hydroides inornata / operculata | 0.52 | 0.51 | 0.52 | 0.28 | 0.30 |
| Hydroides operculata | 0.57 | 0.55 | 0.51 | 0.29 | 0.30 |
| Hydroides pseudounicornata | 0.62 | 0.59 | 0.57 | 0.28 | 0.36 |
| Hydroides crucigerina | 0.63 | 0.51 | 0.50 | 0.24 | 0.24 |
| Hydroides brachycanthata | 0.60 | 0.55 | 0.57 | 0.22 | 0.19 |
| Hydroides rigidicostata / dolabrata | 0.60 | 0.58 | 0.55 | 0.32 | 0.30 |
| Hydroides panamensis | 0.59 | 0.54 | 0.57 | 0.23 | 0.20 |
| Hydroides sanctaecrucis | 0.58 | 0.56 | 0.57 | 0.15 | 0.14 |
| Hydroides fusicola | 0.59 | 0.50 | 0.48 | 0.25 | 0.30 |

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       | Clade 1 | Clade 2 | Clade 3 | Clade 4 | Clade 5 |
|-----------|---------|---------|---------|---------|---------|
| Ficopomatus enigmaticus | 0.03 | 0.25 | 0.61 | 0.67 | 0.61 |
| Ficopomatus enigmaticus | 0.25 | 0.00 | 0.19 | 0.72 | 0.67 |
| Ficopomatus enigmaticus | 0.21 | 0.19 | 0.05 | 0.70 | 0.66 |
| Hydroides elegans Clade E1 | 0.64 | 0.72 | 0.00 | 0.26 | 0.44 |
| Hydroides norvegica | 0.71 | 0.70 | 0.66 | 0.20 | 0.01 |
| Hydroides dirampha Clade B | 0.61 | 0.64 | 0.44 | 0.41 | 0.02 |
| Hydroides dirampha | 0.65 | 0.67 | 0.38 | 0.42 | 0.25 |
| Hydroides nigra | 0.63 | 0.66 | 0.40 | 0.45 | 0.26 |
| Hydroides nigra | 0.65 | 0.68 | 0.65 | 0.43 | 0.47 |
| Hydroides pseudounicornata | 0.72 | 0.77 | 0.42 | 0.43 | 0.31 |
| Hydroides tuberculata | 0.62 | 0.68 | 0.65 | 0.40 | 0.43 |
| Hydroides triversisculosa | 0.64 | 0.65 | 0.57 | 0.40 | 0.36 |
| Hydroides maxima | 0.65 | 0.66 | 0.37 | 0.38 | 0.28 |
| Hydroides nigrae | 0.63 | 0.68 | 0.65 | 0.45 | 0.44 |
| Hydroides nigra | 0.63 | 0.68 | 0.64 | 0.37 | 0.36 |
| Hydroides gracilis | 0.57 | 0.63 | 0.43 | 0.46 | 0.23 |
| Hydroides sanctaecrucis | 0.60 | 0.66 | 0.60 | 0.42 | 0.37 |
| Hydroides brachycanthata | 0.63 | 0.67 | 0.67 | 0.38 | 0.39 |
| Hydroides brachycanthata b | 0.66 | 0.67 | 0.65 | 0.36 | 0.36 |
| Hydroides maxima | 0.74 | 0.76 | 0.69 | 0.43 | 0.42 |
| Hydroides fusicola | 0.67 | 0.72 | 0.70 | 0.43 | 0.36 |
| Hydroides gracilis | 0.68 | 0.78 | 0.69 | 0.40 | 0.24 |
| Hydroides novaenommeriana | 0.72 | 0.70 | 0.69 | 0.46 | 0.43 |

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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 Portixol 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 dispersal.

**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. Žibrowski 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.

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

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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*                 | ZMAVps5240   | France      | KP178713      | EU190467      |
| *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  |               | KP88293       |
| *Hydroides sanctaeacruis*                  | AMW46569     | Australia   | KU051504      |               |
| *Hydroides sanctaeacruis*                  | AMW40549     | Mexico      | JQ885943      | KP178717      |
| *Hydroides sanctaeacruis*                  | AMW46568     | Australia   | KU051501      |               |
| *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 funicola*                       | AMW40545     | Japan       | JQ885950      | KP178723      |
| *Hydroides elegans*                        | WT2NR-3      | Australia   | MH39602       |               |
| *Hydroides elegans*                        | WT5NS        | Australia   | MH39603       |               |
| *Hydroides elegans*                        | AMW40541     | Australia   | JQ885939      |               |
| *Hydroides elegans*                        | SA5MB-2      | USA         | KY235613      |               |
| *Hydroides elegans*                        | AMW40540     | China       | JQ885938      |               |
| *Hydroides elegans*                        | WT1NS-4      | Australia   | MH39601       |               |
| *Hydroides elegans*                        | U1S          | USA         | KY235618      |               |
| *Hydroides elegans*                        | WT5NS-2      | Australia   | MH39600       |               |
| *Hydroides elegans*                        | WT2MS-1      | Australia   | MH39596       |               |
| *Hydroides elegans*                        | CN1RT        | USA         | KY235603      |               |
| *Hydroides elegans*                        | AC74MR       | USA         | KY235596      |               |
| *Hydroides elegans*                        | SA2R-1       | USA         | KY235607      |               |
| *Ficopomatus enigmaticus*                  | KU697663     | Spain       | KL697663      |               |
| *Ficopomatus enigmaticus*                  | KU697660     | Spain       | KL697660      |               |
| *Ficopomatus enigmaticus*                  | KU697661     | Spain       | KL697661      |               |
| *Ficopomatus enigmaticus*                  | KU697662     | Spain       | KL697662      |               |
| *Ficopomatus enigmaticus*                  | KU697664     | Spain       | KL697664      |               |
| *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   | Cyb    |
|---------------------------------|----------------------|----------------|-------|--------|
| Ficopomatus enigmaticus         | H13_It               | Italy          | MK934533 |       |
| Ficopomatus enigmaticus         | H12_It               | Italy          | MK934532 |       |
| Ficopomatus enigmaticus         | Sw2                  | Australia      | KPS63738 |       |
| Ficopomatus enigmaticus         | Sw1                  | Australia      | KPS63736 |       |
| Ficopomatus enigmaticus         | H9_It_Av             | Italy / Portugal| MK934529 |       |
| Ficopomatus enigmaticus         | LA_36                | USA            | MK334109 |       |
| Ficopomatus enigmaticus         | LA_32                | USA            | MK334105 |       |
| Ficopomatus enigmaticus         | Cu6                  | Australia      | KPS63767 |       |
| Hydroides norvegica             | AMW46897             | Norway         | KPS178721 |       |
| Hydroides tuberculata           | SAME3596             | Australia      | EU190473 |       |
| Hydroides tuberculata           | AMW45419             | Australia      | KR905099 |       |
| Hydroides minax                 | SAME3597             |                | EU190475 |       |
| Hydroides nikae                 | SAME3530             | Australia      | KR905078 |       |
| Hydroides brachyachanta         | AMW45577             | Mexico         | KR905053 |       |
| Hydroides brachyachanta         | AMW46899             | Mexico         | KR905068 |       |
| Hydroides brachyachanta         | AMW46900             | Mexico         | KR905069 |       |
| Hydroides brachyachanta         | AMW47016             | Mexico         | KR905089 |       |
| Hydroides brachyachanta         | AMW46903             | Mexico         | KR905070 |       |
| 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              | POR02j09 – 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 |