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**Research Article**

# Confirmation of the exotic status of *Marphysa victori* Lavesque, Daffe, Bonifácio & Hutchings, 2017 (Annelida) in French waters and synonymy of *Marphysa bulla* Liu, Hutchings & Kupriyanova, 2018

Nicolas Lavesque[1,2,*], Pat Hutchings[3,4], Hirokazu Abe[5], Guillemine Daffe[6], Laetitia M. Gunton[3] and Christopher J. Glasby[7]
[1]*Univ. Bordeaux, EPOC, UMR 5805, Station Marine d'Arcachon, 33120 Arcachon, France*
[2]*CNRS, EPOC, UMR 5805, Station Marine d'Arcachon, 33120 Arcachon, France*
[3]*Australian Museum Research Institute, Australian Museum, Sydney, Australia*
[4]*Department of Biological Sciences, Macquarie University, North Ryde 2109, Australia*
[5]*Iwate Medical University, Iwate 028–3694, Japan*
[6]*CNRS, Université de Bordeaux, Observatoire Aquitain des Sciences de l'Univers, UMS 2567 POREA, 33615 Pessac, France*
[7]*Museum and Art Gallery Northern Territory, Darwin, Australia*
Author e-mails: *nicolas.lavesque@u-bordeaux.fr (NL), Pat.Hutchings@austmus.gov.au (PH), habe@iwate-med.ac.jp (HA), guillemine.daffe@u-bordeaux.fr (GD), laetitia.gunton@austmus.gov.au (LG), chris.glasby@nt.gov.au (CG)*
**Corresponding author*

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## Abstract

The bait worm *Marphysa victori* Lavesque, Daffe, Bonifácio & Hutchings, 2017 was originally described from Arcachon Bay, France. In the original description, the authors suggested that it may have been introduced, although definitive evidence was lacking at the time. In this paper, we confirm that *M. victori* is an exotic species originating from East Asia, probably a native of China or Japan. This species was most likely introduced into Arcachon Bay in the 1970s with non-native oysters *Crassostrea gigas* Thunberg, 1793. The Asiatic origin of this species is confirmed based on both morphological and molecular evidence. Comparison of the type specimens of *M. victori* with those of *Marphysa bulla* Liu, Hutchings & Kupriyanova, 2018 described from the Yellow Sea, China, showed no morphological or molecular differences and therefore the more recently named *M. bulla* is relegated to a junior synonym. This is the first example of a large-sized errant invertebrate species being introduced with oysters into Arcachon Bay.

**Key words:** Arcachon Bay, bloodworm, non-indigenous species, oyster translocations, polychaete, East Asia

## Introduction

Along the French coasts, Arcachon Bay is considered to be a hotspot for the presence of non-indigenous species (NIS) (e.g. Lavesque et al. 2013; Gouillieux et al. 2016a, b; Gouillieux and Massé 2019). More than 70 exotic species are reported in the lagoon, most of them originating from the Asiatic region (G. Bachelet 2007, *pers. comm.*). These species belong to diverse groups (Algae, Bryozoa, Cnidaria, Crustacea, Mollusca) but to date only two exotic species of polychaete worms have been reported in the bay: *Ficopomatus enigmaticus* (Fauvel, 1923) and *Boccardia semibranchiata*

**Figure 1.** Artistic representation of *Marphysa victori* Lavesque, Daffe, Bonifácio & Hutchings, 2017, Watercolour from Lisa Miroglio©.

(Guérin, 1990). However, *F. enigmaticus*, after being considered as an Australian species, is now thought to be native from France (Styan et al. 2017).

Recently, a new eunicid polychaete *Marphysa victori* Lavesque, Daffe, Bonifácio & Hutchings, 2017 was described from Arcachon Bay (Figure 1). This species has an important economic value as bait and is widely collected by recreational and professional fishermen. The number of worms collected in the lagoon by professionals (13 companies) could reach 1 million per year (Lavesque et al. 2017). When described, the authors put forward two hypotheses for its presence in Arcachon Bay. The first hypothesis is that this species has always been present in the bay but has possibly been confused with another very similar species such as *Marphysa sanguinea* (Montagu, 1813). This latter was considered for decades as a cosmopolitan species distributed worldwide (Hutchings and Kupriyanova 2018). However, recent studies have revealed and described a number of cryptic species in the "*sanguinea* complex" (see references in Lavesque et al. 2019). The second hypothesis is that this species is a non-indigenous species introduced in the bay, however without evidence of specimens resembling this species have not yet been recorded elsewhere in the world.

When *M. victori* was described the authors provided genetic data (COI and 16S genes) and deposited the sequences on Genbank (https://www.ncbi.nlm.nih.gov/genbank/). Recently these sequences matched with those of specimens of *Marphysa* sp. A, collected from Sendai and Ena bays, Japan (Abe et al. 2019). These authors suggested that *M. victori*, *M. bulla* Liu, Hutchings & Kupriyanova, 2018 (described from Rongcheng, Yellow Sea, China), and *Marphysa* sp. A, may be the same species since the COI sequences were almost identical. However, they also pointed out that the descriptions of *M. victori* and *M. bulla* indicate some morphological differences between these two species. In the present study, we have compared using morphological and molecular techniques specimens from France, China and Japan, confirming the exotic status of *M. victori*. We have also compared holotypes of *M. victori* and *M. bulla*, and found them to be identical, and relegated the latter to a junior synonym.

## Materials and methods

### *Material examined*

Holotypes of *M. victori* and *M. bulla* and specimens of *Marphysa* sp. A sensu Abe et al. (2019) from Japan were examined under Olympus SZX16 and Nikon SMZ25 stereomicroscopes and a Leica DLMB microscope. Scanning electron microscope images were obtained using a JEOL JSM 6480LA microscope and imaged with a secondary detector at Macquarie University, Sydney, Australia. The studied material is deposited at the Australian Museum, Sydney (AM), Muséum National d'Histoire Naturelle, Paris (MNHN) and additional material is lodged at Arcachon Marine Station (SMA).

– *Marphysa victori*: holotype, AM W.49047, paratypes MNHN-IA-TYPE 1083 and 1804, additional material SMA-Mar-02. All specimens collected in Arcachon Bay, Bay of Biscay, France (44°40′35″N; 1°6′58″W), intertidal, 20 September 2016.

– *Marphysa bulla*: holotype, AM W.49124 and paratype AM W.49125, Rongcheng coast, China (37°9′N; 122°24′W), intertidal, 2014.

*Marphysa* sp. A sensu Abe et al. (2019): AM W.52426, one specimen, Mangoku-ura inlet, Japan (38°25′06″N; 141°22′52″E), intertidal, coll. 20 April 2018. AM W.52430, one specimen, Ena Bay, Japan (35°08′40″N; 139°39′50″E), intertidal, 7 September 2018. SMA-Mar-20, one specimen, Matsushima Bay, Japan (38°21′08″N; 141°03′32″E), intertidal, coll. 1 July 2018.

### *Molecular analyses*

Sub-samples for DNA analysis were removed from holotype of *M. bulla* and from a small Japanese specimen. Extraction of DNA was done with ISOLATE II Genomic DNA kit (BIOLINE) following protocol supplied by the manufacturers. Approximately 600 bp of COI (cytochrome *c* oxidase subunit I) gene was amplified, using primers polyLCO and polyHCO COI (Carr et al. 2011). PCR (Polymerase Chain Reaction) occurred with Taq DNA Polymerase QIAGEN Kit in 20 µL mixtures containing: 2 µL of 10X

CoralLoad PCR Buffer (final concentration of 1X), 1.5 µL of $MgCl_2$ (25 Mm) solution, 1,5 µL of PCR nucleotide mix (final concentration of 0.2 mM each dNTP), 0.4 µl of each primer (final concentration of 0,2 µM), 0.1 µl of Taq DNA Polymerase (5U/µl), 1 µl template DNA and 13,1 µL of nuclease-free water. The temperature profile was as follows 94 °C / 60 s – (94 °C / 40 s–45 °C / 40 s–72 °C / 60 s)*5 cycles – (94 °C / 40 s–51 °C/ 40 s–72 °C / 60 s)* 35 cycles – 72 °C / 300 s – 4 °C. PCR success was verified by electrophoresis in a 1% p/v agarose gel stained with Gelred. Amplified products were sent to Macrogen Company to complete double strain sequencing, using same set of primers as used for PCR.

COI sequences of *M. victori* from Arcachon Bay (Lavesque et al. 2017) were compared with sequences of *Marphysa* sp. A, sensu Abe et al. (2019, and this study) from Japan and *Marphysa bulla* from China (this study) (Table 1). 16S genes were compared for specimens from France and Japan only (Table 1).

Forty-nine COI sequences were downloaded from GenBank or obtained during this study, forty-six COI sequences of *Marphysa* species and three outgroup species from closely related genera in the family Eunicidae (Table 1). Nine sequences of *M. victori* were included, these specimens were from France (4 sequences), China (1 sequence, *M. bulla*) and Japan (4 sequences, *Marphysa* sp. A) and obtained in previous studies and from this new one. Six 16S sequences were downloaded from GenBank (Table 1). 16S and COI sequences were aligned and Pairwise Kimura 2-parameter (K2P) genetic distances between these sequences was performed using MEGA version 7.0.26 (Kumar et al. 2016).

All COI sequences were aligned in Geneious Prime 2019.0.4 using the MUSCLE plugin and default settings. The AIC and BIC tests in jModeltest 2.2.10 (Darriba et al. 2012) were used to select the GTR + I + G model of molecular evolution as the best evolutionary model for the COI gene alignment. The phylogenetic analysis was performed in MrBayes v3.2.6 (Ronquist and Huelsenbeck 2003). The analysis was run for 10 million generations (sampled every 1000), 25% of the generations were discarded as burn-in and the standard deviation of split frequencies decreased below 0.01. FigTree v.1.4.4 (Rambaut 2007) was used to visualise the majority-rule consensus tree displaying all nodes with a posterior probability > 0.5.

## Results

### *Molecular data*

Molecular data using the COI gene confirmed that all specimens from France, Japan and China belong to a single species (Figure 2). Bayesian analysis of the COI dataset resulted in a phylogenetic tree in which all sequences of *M. victori* formed a well-supported monophyletic clade (100 % posterior probability, pp). For COI, intraspecific pairwise genetic distances

**Table 1.** Terminal taxa used in molecular part of the study (COI gene), with type localities, collection localities, GenBank accession numbers, genes analysed, voucher specimen catalog numbers and references.

| Species | Type locality | Collection locality | GenBank Accession Number | Gene | Voucher specimen | Reference |
|---|---|---|---|---|---|---|
| *Eunice* cf. *violaceomaculata* | Tortugas, Caribbean | Carrie Bow Cay, Belize | GQ497542 | COI | | Zanol et al. 2010 |
| *Palola viridis* | Samoa, Pacific Ocean | Kosrae, Micronesia | GQ497556 | COI | | Zanol et al. 2010 |
| *Leodice rubra* | Saint Thomas, Caribbean | Ceara, Brazil | GQ497528 | COI | | Zanol et al. 2010 |
| *M. aegypti* | Suez Canal, Egypt | Suez Canal, Egypt | MF196969 | COI | | Elgetany et al. 2018 |
| *M. aegypti* | Suez Canal, Egypt | Suez Canal, Egypt | MF196970 | COI | | Elgetany et al. 2018 |
| *M. bifurcata* | WA, Australia | Qld, Australia | KX172177 | COI | | Zanol et al. 2016 |
| *M. bifurcata* | WA, Australia | Qld, Australia | KX172178 | COI | | Zanol et al. 2016 |
| *M. brevitentaculata* | Tobago | Quintana Roo, Mexico | GQ497548 | COI | | Zanol et al. 2010 |
| *M. californica* | California, USA | California, USA | GQ497552 | COI | | Zanol et al. 2010 |
| *M. disjuncta* | California, USA | California, USA | GQ497549 | COI | | Zanol et al. 2010 |
| *M. fauchaldi* | NT, Australia | NT, Australia | KX172165 | COI | | Zanol et al. 2016 |
| *M. hongkonensa* | Hong Kong | Hong Kong | MH598525 | COI | | Wang et al. 2018 |
| *M. hongkonensa* | Hong Kong | Hong Kong | MH598526 | COI | | Wang et al. 2018 |
| *M. iloiloensis* | Iloilo, Philippines | Tigbauan, Philippines | MN106279 | COI | | Glasby et al. 2019 |
| *M. iloiloensis* | Iloilo, Philippines | Tigbauan, Philippines | MN106280 | COI | | Glasby et al. 2019 |
| *M. iloiloensis* | Iloilo, Philippines | Tigbauan, Philippines | MN106281 | COI | | Glasby et al. 2019 |
| *M. kristiani* | NSW, Australia | NSW, Australia | KX172160 | COI | | Zanol et al. 2016 |
| *M. kristiani* | NSW, Australia | NSW, Australia | KX172161 | COI | | Zanol et al. 2016 |
| *M. kristiani* | NSW, Australia | NSW, Australia | KX172162 | COI | | Zanol et al. 2016 |
| *M. kristiani* | NSW, Australia | NSW, Australia | KX172158 | COI | | Zanol et al. 2016 |
| *M. mossambica* | Mozambique | Iloilo, Philippines | KX172164 | COI | | Zanol et al. 2016 |
| *M. mullawa* | Qld, Australia | NSW, Australia | KX172166 | COI | | Zanol et al. 2016 |
| *M. mullawa* | Qld, Australia | NSW, Australia | KX172167 | COI | | Zanol et al. 2016 |
| *M. mullawa* | Qld, Australia | NSW, Australia | KX172168 | COI | | Zanol et al. 2016 |
| *M. mullawa* | Qld, Australia | NSW, Australia | KX172176 | COI | | Zanol et al. 2016 |
| *M. pseudosessiloa* | NSW, Australia | NSW, Australia | KY605405 | COI | | Zanol et al. 2010 |
| *M. pseudosessiloa* | NSW, Australia | NSW, Australia | KY605406 | COI | | Zanol et al. 2010 |
| *M. regalis* | Bermuda | Ceara, Brazil | GQ497562 | COI | | Zanol et al. 2010 |
| *M. sanguinea* | Devon, UK | Callot Island, France | GQ497547 | COI | | Zanol et al. 2010 |
| *M. sanguinea* | Devon, UK | Cornwall, UK | MK541904 | COI | | Lavesque et al. 2019 |
| *M. sanguinea* | Devon, UK | Arcachon Bay, France | MK950853 | COI | | Lavesque et al. 2019 |
| *M. sanguinea* | Devon, UK | Brest, France | MK967470 | COI | | Lavesque et al. 2019 |
| *M. tripectinata* | Beihai, China | Beihai, China | MN106271 | COI | | Glasby et al. 2019 |
| *M. tripectinata* | Beihai, China | Beihai, China | MN106272 | COI | | Glasby et al. 2019 |
| *M. tripectinata* | Beihai, China | Beihai, China | MN106273 | COI | | Glasby et al. 2019 |
| *M. tripectinata* | Beihai, China | Beihai, China | MN106274 | COI | | Glasby et al. 2019 |
| *M. tripectinata* | Beihai, China | Beihai, China | MN106275 | COI | | Glasby et al. 2019 |
| *M. tripectinata* | Beihai, China | Beihai, China | MN106276 | COI | | Glasby et al. 2019 |
| *M. tripectinata* | Beihai, China | Beihai, China | MN106277 | COI | | Glasby et al. 2019 |
| *M. tripectinata* | Beihai, China | Beihai, China | MN106278 | COI | | Glasby et al. 2019 |
| *M. victori* | Arcachon Bay, France | Arcachon Bay, France | MG384996 | COI | AM W.49048 | Lavesque et al. 2017 |
| *M. victori* | Arcachon Bay, France | Arcachon Bay, France | MG384997 | COI | MNHN-IA-TYPE 1803 | Lavesque et al. 2017 |
| *M. victori* | Arcachon Bay, France | Arcachon Bay, France | MG385000 | 16S | MNHN-IA-TYPE 1803 | Lavesque et al. 2017 |
| *M. victori* | Arcachon Bay, France | Arcachon Bay, France | MG384998 | COI | MNHN-IA-TYPE 1804 | Lavesque et al. 2017 |
| *M. victori* | Arcachon Bay, France | Arcachon Bay, France | MG385001 | 16S | MNHN-IA-TYPE 1804 | Lavesque et al. 2017 |
| *M. victori* | Arcachon Bay, France | Arcachon Bay, France | MG384999 | COI | MNHN-IA-TYPE 1806 | Lavesque et al. 2017 |
| *M. victori* | Arcachon Bay, France | Mangoku-ura Inlet, Japan | LC467767 | COI | AM W.52426 | Abe et al. 2019 |
| *M. victori* | Arcachon Bay, France | Mangoku-ura Inlet, Japan | LC467743 | 16S | AM W.52426 | Abe et al. 2019 |
| *M. victori* | Arcachon Bay, France | Sendai Bay, Japan | LC467769 | COI | AM W.52428 | Abe et al. 2019 |
| *M. victori* | Arcachon Bay, France | Sendai Bay, Japan | LC467745 | 16S | AM W.52428 | Abe et al. 2019 |
| *M. victori* | Arcachon Bay, France | Ena Bay, Japan | LC467772 | COI | AM W.52430 | Abe et al. 2019 |
| *M. victori* | Arcachon Bay, France | Ena Bay, Japan | LC467748 | 16S | AM W.52430 | Abe et al. 2019 |
| *M. victori* | Arcachon Bay, France | Matsushima Bay, Japan | LC530870 | COI | SMA-Mar-20 | This study |
| *M. victori* | Arcachon Bay, France | Matsushima Bay, Japan | LC530869 | 16S | SMA-Mar-20 | This study |
| *M. bulla* | Rongcheng coast, China | Rongcheng coast, China | MT012514 | COI | AM W.49124 | This study |
| *M. viridis* | Florida, USA | Ceara, Brazil | GQ497553 | COI | | Zanol et al. 2010 |

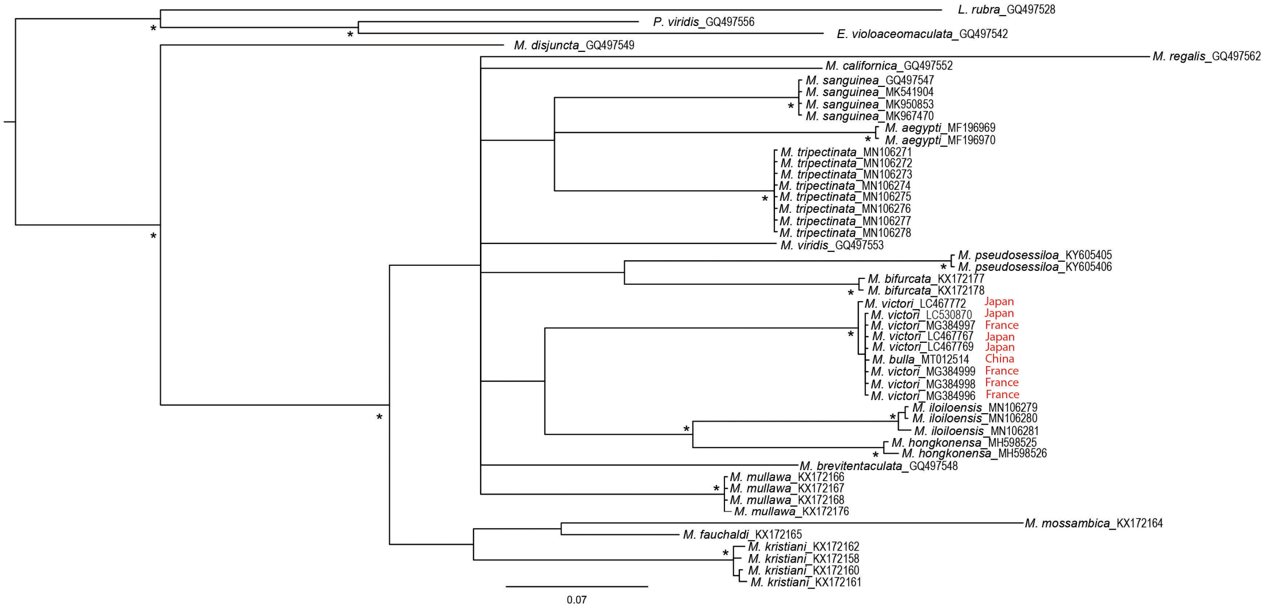

**Figure 2.** Majority-rule consensus tree of *Marphysa* spp. from Bayesian analysis using COI. Asterisk indicates posterior probability > 90%. Text in red indicates geographical localities of specimens used in this study.

were zero among all specimens, except for AM W.52429 (from Japan) which showed a difference of 0.4% with all other specimens. For 16S, intraspecific pairwise genetic distances were zero among all specimens. *Marphysa victori* formed a sister clade with *M. iloiloensis* Glasby, Mandario, Burghardt, Kupriyanova, Gunton & Hutchings, 2019 and *M. hongkongensa* Wang, Zhang & Qiu, 2018 with low support (63% pp).

Absence of variations in COI and 16S sequences confirm absence of genetic divergences between European and Asiatic populations.

### *Morphological characteristics*

Detailed morphological study confirmed that all specimens from France (n = 4), China (n = 2) and Japan (n = 3) belong to one and the same species: *M. victori* (Figure 3A). This species belongs to the "*sanguinea*" complex, characterised by the presence of compound spinigerous chaetae and absence of compound falcigerous chaetae, and branchiae present over most of the body. *Marphysa victori* is characterised by the presence of eyes (only visible on small specimens); the presence of pectinate branchiae from chaetigers 29 to 33 to almost the pygidium; the presence of only one pair of long (extending back over 15 chaetigers) pygidial cirri on the ventral margin (many other *sanguinea* group species have two pairs of pygidial cirri) (Figure 3B), the following maxillary formula: MF = 1+1, 4+4, 5(6)+0, 3(4)+6, 1+1 (see Figure 3E in Lavesque et al. 2017); the number of branchial filaments ranging from 3 in first chaetigers to a maximum 6 in the mid body (Figure 3C–D), and posterior chaetigers with 2 long filaments; the globular shape of parapodial ventral cirri of anterior chaetigers (Figure 3C–E); the presence of subacicular hooks on small specimens only (Figure 3E); the presence of three types of pectinate chaetae: isodont-narrow-slender (INS)

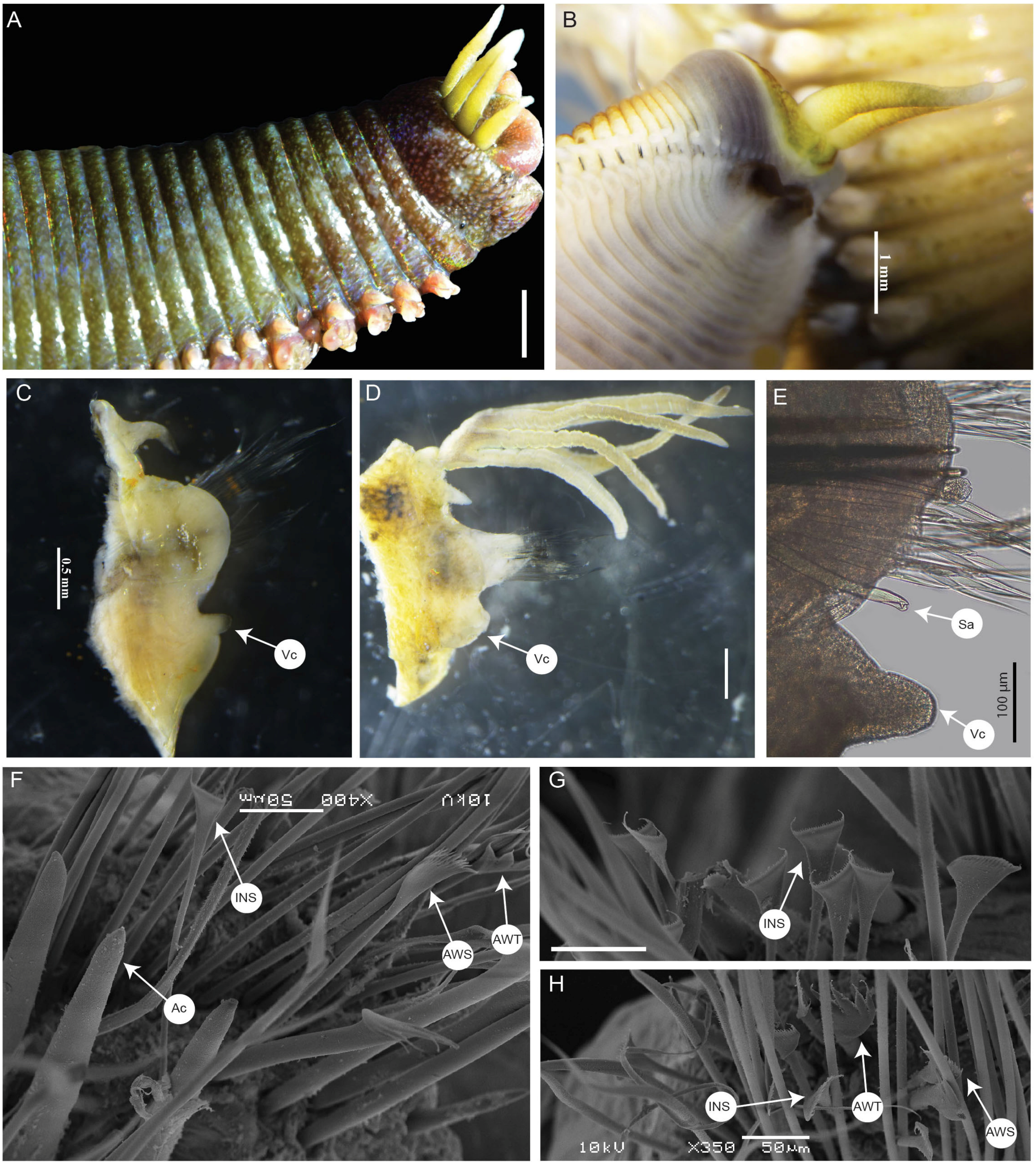

**Figure 3.** *Marphysa victori* Lavesque, Daffe, Bonifácio & Hutchings, 2017 (A) Anterior part, lateral view, (SMA-Mar-02, additional material); (B) Pygidium, ventral view (paratype MNHN-IA-TYPE 1804); (C–D) parapodium 31, 206 respectively, lateral view (paratype MNHN-IA-TYPE 1803); (E) parapodium 72, lateral view (SMA-Mar-20, additional material); (F–H) Different types of pectinate chaetae, from chaetiger 323 (F) and from chaetiger 430 (G–H), SEM images (holotype AM W.49047). Abbreviations: INS: isodont-narrow-slender; Ac: Acicula; AWS: anodont-wide-slender; AWT: anodont-wide-thick; Sa: subacicular hook; Vc: ventral cirri. Scale bars: (A): 2 mm; (D): 0.5 mm; (G): 50 µm. Photos by Lavesque N.

having 12–28 short teeth present from anterior to posterior chaetigers; anodont-wide-slender (AWS) having 9–14 short teeth and anodont-wide-thick (AWT) having 2–4 long teeth present from median to posterior chaetigers (Figure 3F–H).

## Discussion

### *Marphysa victori, a NIS for French waters*

This study confirmed both morphologically and molecularly that specimens from Arcachon Bay (France) and from Japan and China belong to one species: *Marphysa victori*. We confirm the exotic origin hypothesis for the Arcachon Bay population (Lavesque et al. 2017) and also the suspicions of Abe et al. (2019) that their *Marphysa* sp. A and *M. victori* were the same. In this case, the pathway of introduction is clearly linked to aquaculture oyster transfers between Japan and France. Arcachon Bay is one of the major French oyster farming sites with a production of 7000–8000 t per year of the exotic Pacific cupped Oyster *Crassostrea gigas* (Thunberg, 1793). Originally, oysters cultivated in the bay were the Portuguese cupped oysters *Crassostrea angulata* (Lamarck, 1819). However, in the early 1970s, a viral disease completely devastated the population and Pacific cupped oysters *C. gigas* were then introduced to maintain the local oyster industry (Grizel and Héral 1991). Between 1971 and 1975, about 1200 t of spat collected from Sendai Bay (Japan) were introduced into Arcachon Bay. Some of the Japanese specimens examined in this study were collected exactly in the same area; Mangoku-ura inlet (a branch of Sendai Bay). The habitat of *M. victori* in Arcachon Bay provides further support for the translocation of worms as this species is found only in, or close to, oyster reefs. Several other non-indigenous invertebrate species, introduced with oyster transfers, were recently found in Arcachon Bay (see references in introduction). However, these species are sessile invertebrates (like bryozoan or sponge) travelling from Japan attached to oysters or small-sized errant species (like amphipods or isopods) which have hitchhiked in or amongst oyster shells. This is the first example of a large-sized errant species, with adult specimens exceeding one meter, being introduced with oysters into the bay. Either worms were introduced as juveniles during transportation of live oysters from Japan, or perhaps *M. victori*, like other Asian species of *Marphysa*, can produce jelly cocoons, into which fertilized eggs are laid as recently shown (see Glasby et al. 2019, for review). When cocoons were transported attached to oysters, the eggs were consequently released into the bay.

As no importation of cupped oyster from Japan to Arcachon has been officially reported since the 1970s, *M. victori* has probably been present in Arcachon Bay since that time, remaining unnoticed or confused with the so-called cosmopolitan species *M. sanguinea*. This is not surprising as distinguishing between species belonging to this complex requires taxonomic expertise, molecular analysis and/or SEM photographs (Lavesque et al. 2017, 2019). However, a recent study showed that these two species occupy two different ecological niches in the bay, *M. sanguinea* being only present subtidally, inside peat slabs (produced from fossil pines forest) (Lavesque et al. 2019) and *M. victori* only under or close to oyster reefs on muddy flats.

To date, *M. victori* has not been found in other localities in France or in Europe. Nevertheless, its presence in other European shellfish farming areas is highly probable. Indeed, transfers of *C. gigas* among European regions occur daily and are responsible for introduction and spread of numerous alien species in northern Europe (Goulletquer et al. 2002). For example, the Asiatic amphipod *Grandidiriella japonica* Stephensen, 1938 has been recently recorded in Arcachon Bay (Lavesque et al. 2014), in Marennes-Oléron Bay (Jourde et al. 2013) and the Gulf of Morbihan (Droual et al. 2017), three major sites of oyster farming in France. In the same way, the Asiatic amphipod *Monocorophium uenoi* (Stephensen, 1932) has been sampled in intertidal Pacific oyster reefs in the Netherlands (Faasse 2014) and in Arcachon Bay (Gouillieux and Massé 2019). Furthermore, *M. victori* is widely collected in Arcachon Bay and worms are exported live and sold in western French Mediterranean fishing shops for recreational activities. Use of bait represents a potential source of secondary spread of an exotic species. First, live gravid females are used and could release their eggs in the water column. Second, a recent study highlighted that anglers threw their live baits away in the water after their fishing activities (Kilian et al. 2012). These two possibilities could lead to the establishment of new non-indigenous populations in Europe. Fortunately, because different *Marphysa* species seem to be habitat-dependent (Lavesque et al. 2019) the likelihood of successful introduction of *M. victori* into the Western Mediterranean, which lacks intertidal muddy oyster reefs, is considered low.

### Marphysa bulla *Liu, Hutchings & Kupriyanova, 2018: junior synonym of* M. victori

The second important result provided by this study is that there are no significant morphological and molecular differences between *M. victori* and *M. bulla*, and thus the names refer to a single species. Following the ICZN Principle of Priority, the valid name for the species is the oldest available name, in this case *M. victori*, as it was described in 2017 and *M. bulla* in 2018. *Marphysa bulla* is therefore synonymised with *M. victori*.

Detailed observations of the holotype of *M. bulla* and *M. victori* revealed some inaccuracies in the original descriptions. The first one concerns the subacicular hooks. Indeed, in the description of *M. bulla*, the authors stated the presence of “subacicular hooks transparent/pale yellow, unidentate, from mid-body parapodia (chaetiger 71) onwards, up to 3 per parapodium”. In contrast, *M. victori* was described with absence of subacicular hooks. Despite many new dissections of parapodia of the two holotypes of *M. victori* and *M. bulla*, and observations of previous SEM pictures, no acicular hooks were found. For *M. bulla*, these subacicular hooks were probably confused with emergent aciculae by the authors (Liu et al. 2018). Indeed, in this paper, Figure 3C clearly shows aciculae and not hooks. However, these two holotypes are very large (300 mm long for *M. victori*, about 230 mm for

*M. bulla*) and hooks could disappear on larger specimens, as previously reported for *Marphysa brevitentaculata* Treadwell, 1921 (Salazar-Vallejo and Carrera-Parra 1997). Indeed, observation of a small specimen (SMA-Mar-20, 30 mm long) of *M. victori* from Japan, with the same COI sequence, clearly shows the presence of bidentate subacicular hooks in posterior parapodia. Thus, this character should be used with caution. Secondly, the branchial filaments are more developed and very long in posterior chaetigers but it was not reported in the original description of *M. bulla*. The maxillary formula given by Liu et al. (2018) was correct and those given by Lavesque et al. (2017) was wrong (not given in the correct order). Thus the maxillary formula, with variations, is: MF = 1+1, 4+4, 5(6)+0, 3(4)+6, 1+1. Finally, observing and classifying the pectinate chaetae is very challenging and approximations occurred in both descriptions. The new classification of pectinate chaetae provided by Molina-Acevedo and Carrera-Parra (2017) permitted us to clarify the situation. *Marphysa victori* shows three different types of pectinate chaetae: isodont-narrow-slender (INS) having 12–28 short teeth present from anterior to posterior chaetigers; anodont-wide-slender (AWS) having 9–14 short teeth from median to posterior chaetigers; anodont-wide-thick (AWT) having 2–4 long teeth present from median to posterior chaetigers.

When they described *M. bulla*, the authors compared this new species with other *Marphysa* species occurring in China but not with *Marphysa* species outside the region. This is not surprising as species within this genus are known to live in restricted areas and habitats, and were never recorded as potential non-indigenous species. The single exception is *M. sanguinea*, whose type locality is SE England and introduced in the nearby Netherlands (Hutchings et al. 2011). Moreover, *Marphysa* is very speciose (72 valid species, Read and Fauchald 2020) and most of its species, described several decades ago, were documented very briefly, poorly illustrated and with type specimens often lost or damaged. Most authors describing new species of *Marphysa* have compared their materials with existing valid species known within a large biogeographical region (e.g. Lavesque et al. 2017; Liu et al. 2017; Wang et al. 2018; Glasby et al. 2019).

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## References

Abe H, Tanaka M, Taru M, Abe S, Nishigaki A (2019) Molecular evidence for the existence of five cryptic species within the Japanese species of *Marphysa* (Annelida: Eunicidae) known as “Iwa-mushi”. *Plankton and Benthos Research* 14: 1–12, https://doi.org/10.3800/pbr.14.303

Darriba D, Taboada GL, Doallo R, Posada D (2012) jModelTest 2: more models, new heuristics and parallel computing. *Nature Methods* 9: 772, https://doi.org/10.1038/nmeth.2109

Droual G, Le Garrec V, Cabelguen J, Gélinaud G, Grall J (2017) The spread goes on: the non-indigenous species *Grandidierella japonica* Stephensen 1938 (Amphipoda: Aoridae) has reached Brittany (Gulf of Morbihan). *An aod - les cahiers naturalistes de l’Observatoire marin* 5(1): 21–29

Elgetany AH, El-Ghobashy AE, Ghoneim AM, Struck TH (2018) Description of a new species of the genus *Marphysa* (Eunicidae), *Marphysa aegypti* sp.n., based on molecular and morphological evidence. *Invertebrate Zoology* 15: 71–84, https://doi.org/10.15298/invertzool.15.1.05

Faasse M (2014) The Pacific amphipod *Monocorophium uenoi* (Stephensen, 1932) introduced to The Netherlands (NE Atlantic). *BioInvasions Records* 3: 29–33, https://doi.org/10.3391/bir.2014.3.1.05

Fauvel P (1923) Un nouveau serpulien d’eau saumatre *Merceriella* n.g., *enigmatica* n.sp. *Bulletin de la Société Zoologique de France* 47: 424–430

Glasby CJ, Mandario MAE, Burghardt I, Kupriyanova E, Gunton LM, Hutchings PA (2019) A new species of the *sanguinea*-group Quatrefages, 1866 (Annelida: Eunicidae: *Marphysa*) from the Philippines. *Zootaxa* 4674: 264–282, https://doi.org/10.11646/zootaxa.4674.2.7

Gouillieux B, Massé C (2019) First record of *Monocorophium uenoi* (Stephenson, 1932) (Crustacea: Amphipoda: Corophiidae) in the Bay of Biscay, French Atlantic coast. *BioInvasions Records* 8: 87–95, https://doi.org/10.3391/bir.2019.8.1.09

Gouillieux B, Lavesque N, Leclerc JC, Le Garrec V, Viard F, Bachelet G (2016a) Three non-indigenous species of *Aoroides* (Crustacea: Amphipoda: Aoridae) from the French Atlantic coast. *Journal of the Marine Biological Association of the United Kingdom* 96: 1651–1659, https://doi.org/10.1017/S0025315415002027

Gouillieux B, Lavesque N, Blanchet H, Bachelet G (2016b) First record of the non-indigenous *Melita nitida* Smith, 1873 (Crustacea: Amphipoda: Melitidae) in the Bay of Biscay (NE Atlantic). *BioInvasions Records* 5: 85–92, https://doi.org/10.3391/bir.2016.5.2.05

Goulletquer P, Bachelet G, Sauriau PG, Noel P (2002) Open Atlantic coast of Europe - A century of introduced species into French waters. In: Leppäkoski E, Gollasch S, Olenin S (eds), Invasive Aquatic Species of Europe. Kluwer Academic Publishers, Netherlands, pp 246–290, https://doi.org/10.1007/978-94-015-9956-6_30

Grizel H, Héral M (1991) Introduction into France of the Japanese oyster (*Crassostrea gigas*). *Journal du Conseil International pour l’Exploration de la Mer* 47: 399–403, https://doi.org/10.1093/icesjms/47.3.399

Guérin JP (1990) Description d'une nouvelle espèce de spionidé (Annélides, Polychètes) *Boccardia semibranchiata*. *Annales de l’Institut Océanographique* 66(1–2): 37–45

Hutchings PA, Kupriyanova E (2018) Cosmopolitan polychaetes - fact or fiction? Personal and historical perspectives. *Invertebrate Systematics* 32: 1–9, https://doi.org/10.1071/IS17035

Hutchings PA, Glasby CJ, Wijnhoven S (2011) Note on additional diagnostic characters for *Marphysa sanguinea* (Montagu, 1813) (Annelida: Eunicida: Eunicidae), a recently introduced species in the Netherlands. *Aquatic Invasions* 7: 277–282, https://doi.org/10.3391/ai.2012.7.2.014

Jourde J, Sauriau P-G, Guenneteau S, Caillot E (2013) First record of *Grandidierella japonica* Stephensen, 1938 (Amphipoda: Aoridae) from mainland Europe. *BioInvasions Records* 2: 51–55, https://doi.org/10.3391/bir.2013.2.1.09

Kilian JV, Klauda RJ, Widman S, Kashiwagi M, Bourquin R, Weglein S, Schuster J (2012) An assessment of a bait industry and angler behavior as a vector of invasive species. *Biological Invasions* 14: 1469–1481, https://doi.org/10.1007/s10530-012-0173-5

Kumar S, Stecher G, Tamura K (2016) MEGA7: Molecular Evolutionary Genetics Analysis Version 7.0 for Bigger Datasets. *Molecular Biology and Evolution* 33: 1870–1874, https://doi.org/10.1093/molbev/msw054

Lamarck JBM (1819) Histoire naturelle des animaux sans vertèbres. Tome sixième, 1re partie. Paris, published by the Author, 343 pp

Lavesque N, Sorbe JC, Bachelet G, Gouillieux B, de Montaudouin X, Bonifácio P, Blanchet H, Dubois S (2013) Recent discovery of *Paranthura japonica* Richardson, 1909 (Crustacea: Isopod: Paranthuridae) in European marine waters (Arcachon Bay, Bay of Biscay). *BioInvasions Records* 2: 215–219, https://doi.org/10.3391/bir.2013.2.3.07

Lavesque N, Gouillieux B, de Montaudouin X, Bachelet G, Bonifácio P, Simonet E (2014) Premier signalement de l’espèce introduite *Grandidierella japonica* Stephensen, 1938 (Crustacea: Amphipoda: Aoridae) dans le bassin d’Arcachon. *An aod - les cahiers naturalistes de l’Observatoire marin* 3(1): 11–19

Lavesque N, Daffe G, Bonifácio P, Hutchings PA (2017) A new species of the *Marphysa sanguinea* complex from French waters (Bay of Biscay, NE Atlantic) (Annelida, Eunicidae). *ZooKeys* 716: 1–17, https://doi.org/10.3897/zookeys.716.14070

Lavesque N, Daffe G, Grall J, Zanol J, Gouillieux B, Hutchings P (2019) Guess who? On the importance of using appropriate name: case study of *Marphysa sanguinea* (Montagu, 1813). *ZooKeys* 859: 1–15, https://doi.org/10.3897/zookeys.859.34117

Liu Y, Hutchings PA, Sun S (2017) Three new species of *Marphysa* Quatrefages, 1865 (Polychaeta: Eunicida: Eunicidae) from the south coast of China and redescription of *Marphysa sinensis* Monro, 1934. *Zootaxa* 4263: 228–250, https://doi.org/10.11646/zootaxa.4377.2.3

Liu Y, Hutchings P, Kupriyanova E (2018) Two new species of *Marphysa* Quatrefages, 1865 (Polychaeta: Eunicida: Eunicidae) from northern coast of China and redescription for *Marphysa orientalis* Treadwell, 1936. *Zootaxa* 4377: 191–215, https://doi.org/10.11646/zootaxa.4377.2.3

Molina-Acevedo IC, Carrera-Parra LF (2017) Revision of *Marphysa* de Quatrefages, 1865 and some species of *Nicidion* Kinberg, 1865 with the erection of a new genus (Polychaeta: Eunicidae) from the Grand Caribbean. *Zootaxa* 4241: 1–62, https://doi.org/10.11646/zootaxa.4241.1.1

Montagu G (1813) Descriptions of several new or rare animals, principally marine, found on the south coast of Devonshire. *Transactions of the Linnean Society of London* 11: 18–21, https://doi.org/10.1111/j.1096-3642.1813.tb00035.x

Rambaut A (2007) FigTree. http://tree.bio.ed.ac.uk/software/figtree/

Read G, Fauchald K (eds) (2020) World Polychaeta database. *Marphysa* Quatrefages, 1865. http://www.marinespecies.org/aphia.php?p=taxdetails&id=129281 (accessed 2020-04-28)

Ronquist F, Huelsenbeck JP (2003) MRBAYES 3: Bayesian phylogenetic inference under mixed models. *Bioinformatics* 19: 1572–1574, https://doi.org/10.1093/bioinformatics/btg180

Salazar-Vallejo SI, Carrera-Parra LF (1997) Eunícidos (Polychaeta) del Caribe mexicano con claves para las especies del Gran Caribe: *Fauchaldius*, *Lysidice*, *Marphysa*, *Nematonereis* y *Palola*. *Revista biología tropical* 45(4): 1481–1498

Stephensen K (1932) Some new amphipods from Japan. *Annotationes Zoologicae Japonenses* 13(5): 487–501

Stephensen K (1938) *Grandidierella japonica* n. sp. a new amphipod with stridulating (?) organs from brackish water in Japan. *Annotationes Zoologicae Japonenses* 17(2): 179–184

Styan CA, McCluskey CF, Sun Y, Kupriyanova EK (2017) Cryptic sympatric species across the Australian range of the global estuarine invader *Ficopomatus enigmaticus* (Fauvel, 1923) (Serpulidae, Annelida). *Aquatic Invasions* 12: 53–65, https://doi.org/10.3391/ai.2017.12.1.06

Thunberg CP (1793) Tekning och Beskrifning på en stor Ostronsort ifrån Japan. *Kongliga Vetenskaps Academiens Nya Handlingar* 14(4–6): 140–142

Treadwell AL (1921) Leodicidae of the West Indian region. *Carnegie Institution of Washington Publication* 15: 1–131, https://doi.org/10.5962/bhl.part.20335

Wang Z, Zhang Y, Qiu JW (2018) A new species of the *Marphysa sanguinea* complex (Annelida, Eunicidae) from Hong Kong. *Zoological Studies* 57: 48, https://doi.org/10.6620/ZS.2018.57-48

Zanol J, Halanych KM, Struck TH, Fauchald K (2010) Phylogeny of the bristle worm family Eunicidae (Eunicida, Annelida) and the phylogenetic utility of noncongruent 16S, COI and 18S in combined analyses. *Molecular Phylogenetics and Evolution* 55: 660–676, https://doi.org/10.1016/j.ympev.2009.12.024

Zanol J, da Silva T dos SC, Hutchings P (2016) Integrative taxonomy of *Marphysa* (Eunicidae, Polychaeta, Annelida) species of the Sanguinea group from Australia. *Invertebrate Biology* 135: 328–344, https://doi.org/10.1111/ivb.12146