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ORIGINAL ARTICLE

Molecular phylogeny of the marine snail genus *Haminoea* (Gastropoda, Cephalaspidea): A framework to study marine diversity and speciation

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Abstract

Haminoea are herbivorous, coastal snails occurring in temperate and tropical waters of the Atlantic and Eastern Pacific oceans, with one species present in temperate South Africa (Indian Ocean). The genus is taxonomically difficult as several available nominal species were introduced based on shell descriptions alone, or described based on subtle differences in morpho-anatomical features, without a phylogenetic molecular framework. Fifteen species are currently accepted as valid in recent scientific literature and field guides (eight Eastern Atlantic, one temperate Indian Ocean, four Western Atlantic and three Eastern Pacific). Here we generate the first complete phylogeny (Bayesian and Maximum Likelihood) of this genus based on multilocus molecular data (COI, 12S rRNA, 16S rRNA, 28S rRNA) using a taxon set accumulated over a period of 15 years, coupled with species delimitation analyses methods (ABGD, ASAP, bPTP) and morpho-anatomical studies. The goal of this study is to provide insights into the taxonomy, phylogenetic relationships and geographical distributions of species while generating a framework for future systematic reviews of the genus, as well as to study speciation and historical biogeography. Our results rendered four possible hypotheses of species diversity: with 14, 15, 19 and 20 candidate species and point to the fact that several taxa presently regarded as valid might be conspecific (e.g. *H. orteai–H. templadoi–H. exigua*; and *H. alfredensis–H. antillarum–H. orbignyana*), while highlighting the existence of a complex of four or five species often identified as *H. elegans*. Pervasive nomenclatural problems in the genus, including with the type species *H. hydatis*, are highlighted and discussed.

KEYWORDS

Atlantic, biodiversity, Eastern Pacific, Haminoeidae, Mollusca, systematics

1 | INTRODUCTION

The marine gastropods of the genus *Haminoea* Turton & Kingston in Carrington, 1830 have been long considered to have a worldwide distribution, inhabiting temperate and tropical shorelines (Burn & Thompson, 1998; Malaquias & Cervera, 2006; Rudman, 1971). Yet, Oskars et al. (2019) and Oskars and Malaquias (2019) found *Haminoea* to be a paraphyletic assemblage of five distinct evolutionary lineages; three mostly tropical and restricted to the Indo-West Pacific (*Haloa* Pilsbry, 1921, *Lamprohaminoea* Habe, 1952 and *Bakawan* Oskars & Malaquias, 2020a), one confined to Australasian (*Papawera* Oskars & Malaquias, 2020b) and *Haminoea* proper, geographically restricted to the Atlantic Ocean (including the Mediterranean Sea), the Eastern Pacific Ocean and with a single lineage represented in temperate stretches of the Indian Ocean coastline of South Africa.

The small, globose, semi-translucent and thin shells of these five genera are similar in shape, colour and size, which coupled with a lack of broad comparative morphological studies and phylogenetic frameworks, led the majority of authors to accept *Haminoea* as the only valid genus across the world (e.g. Burn & Thompson, 1998; Cervera et al., 2004; Gosliner et al., 2008; Malaquias & Cervera, 2006; Thompson, 1981; Valdés et al., 2006). The modern concept of *Haminoea* was proposed by Oskars et al. (2019) and Oskars and Malaquias (2019) who identified several diagnostic features to separate it from their Indo-West Pacific closely related genera, namely the higher number of lateral radular teeth, the presence of a muscular penis and a Hancock organ with a perfoliate structure.

Species of *Haminoea* are herbivorous and live predominantly in estuaries and coastal lagoons, where they are often found on seagrass, algae or sandy-muddy bottoms, but can also occur on rocky shores in tidepools or shallow depths always among algal mats (Boulch-Bleas, 1983; Malaquias et al., 2002, 2004, 2009; Rudman, 1971).

At present, eight species of *Haminoea* are recognized as valid in the Eastern Atlantic (EA) between the southern shores of the British Isles and Angola in West Africa, namely *H. hydatis* (Linnaeus, 1758; type locality Mediterranean Sea), *H. navicula* (Da Costa, 1778; type locality Weymouth, Dorset, England), *H. orbignyana* (Férussac, 1822; type locality near La Rochelle, Bay of Biscay, France), *H. elegans* (Gray, 1825; type locality south of British Isles and Mediterranean Sea), *H. orteai* Talavera, Murillo & Templado, 1987 (type locality Salinas del Rasall, Murcia, Spain), *H. templadoi* García, Pérez-Hurtado & García-Gómez, 1991 (type locality Huelva, Spain), *H. exigua* (Schaefer, 1992; type locality Adriatic Sea, Italy/Croatia) and *H. fusari* (Álvarez, García & Villani, 1993; type locality Lake Fusaro, Italy) (Malaquias & Cervera, 2006; Martínez & Ortea, 1997; Rolán & Ryall, 1999). In the Western Atlantic (WA) four species are often accepted as valid in current literature occurring between Florida, USA and Rio Grande do Sul in Brazil, namely *H. elegans*, *H. antillarum* (d'Orbigny, 1841; type locality Saint Thomas, U.S. Virgin Islands), *H. petitii* (d'Orbigny, 1841; type locality Cuba) and *H. succinea* (Conrad, 1846; type locality Tampa Bay, Florida, USA) (Caballer et al., 2015; García et al., 2008; Rios, 2009; Valdés et al., 2006). In the Eastern Pacific (EP) three species are commonly recognized as valid between Alaska and Panama, namely *H. ovalis* Pease, 1868 (type locality Tahiti, French Polynesia), *H. virescens* (Sowerby, 1833; type locality Pitcarin Island or California, USA; see Valdés, 2019) and *H. vesicula* (Gould, 1855; type locality San Diego, California, USA) (Behrens & Hermosillo, 2005; Hermosillo et al., 2006; Valdés & Camacho-Garcia, 2004). In addition, one species of *Haminoea* occurs in temperate stretches of the Indian Ocean coastline of South Africa (tWIO), namely *H. alfredensis* Bartsch, 1915 (type locality Port Alfred, South Africa), distributed on both sides of the Cape Peninsula eastwards up to East London (Gosliner, 1987).

In total, 15 species of *Haminoea* are currently accepted as valid in current scientific literature and field guides. However, in a literature search we were able to identify 48 nominal species, most of them of uncertain taxonomic status because of short and ambiguous species descriptions based only on shells, which are similar in shape, colour and dimensions (e.g. Leach, 1852 for *H. dilatata*; A. Adams, 1850 for *H. glabra*; Baker & Hanna, 1927 for *H. angelensis*; Petuch, 1987 for *H. taylorae*).

Furthermore, even among the 'well-established' species, there are questions about the taxonomic status of several of them. For example, the definition of the type species of the genus—*H. hydatis*—is problematic. This species was described by Linnaeus (1758) based on shells (unclear if only one or several) from the Mediterranean Sea but later assumed by various authors to be conspecific with specimens occurring between the British Isles and the Adriatic Sea, and characterized by having a smooth shell, a bilobed prostate separated by a constricted region and a radula with the first lateral tooth denticulated (Pruvot-Fol, 1954; Tchang, 1931; Thompson, 1981; Thompson & Brown, 1976; Vayssière, 1885). Another case is the species name *H. elegans* introduced by Gray (1825) based on shells from the British Isles and the Mediterranean Sea, yet, the name is commonly attributed to one of the tropical western Atlantic species (e.g. Caballer et al., 2015; Malaquias, 2014; Marcus, 1976; Marcus & Marcus, 1967; Redfern, 2001; Valdés et al., 2006) and also to spiralled shells occurring in tropical West Africa (Gabon, Republic of the Congo, São Tomé and

Príncipe, Angola; Bernard, 1984; Martínez & Ortea, 1997; Rolán & Ryall, 1999). Likewise, the name *H. ovalis* is commonly employed to designate animals with tiny orange or yellow dots on the body occurring in the Eastern Pacific, between Mexico and Peru (Behrens & Hermosillo, 2005; Hermosillo et al., 2006; Oskars & Malaquias, 2019; Valdés & Camacho-Garcia, 2004); nonetheless, *H. ovalis* was described by Pease (1868) from Tahiti and was recently reassigned to the genus *Lamprohaminoea* by Oskars and Malaquias (2020c), who confirmed the species to be widespread in the Indo-West Pacific and absent from the Eastern Pacific. In fact, in a previous study, Oskars & Malaquias (2019, as *Haminoea* sp.1 475) showed that dotted orange haminoeids from Peru were phylogenetically related to all other Atlantic and Eastern Pacific *Haminoea* species.

In the present study, we generate the first complete phylogeny of the genus *Haminoea* based on multilocus molecular characters using a taxon set accumulated over a period of 15 years, which we believe to likely cover the entire diversity of the genus and include a comprehensive geographical coverage of the distribution of species. The main goals of this paper are to define the number of species in *Haminoea* and provide insights on their taxonomy, phylogenetic relationships and geographical distributions while establishing a framework for future detailed systematic reviews and studies on speciation and historical biogeography of this genus.

2 | MATERIALS AND METHODS

2.1 | Sampling of taxa

Specimens of *Haminoea* were obtained during fieldwork in Bermuda (2009), Venezuela (2010), Brazil (2012), Bahamas (2013), Portugal (2014) and Florida Keys, USA (2015), from donations from colleagues, and loans of museum collections; *e. g.*, University Museum of Bergen, Norway (ZMBN), The Natural History Museum, London, UK (NHMUK), The Natural History Museum of Florida, USA (UF), Museu Municipal do Funchal (História Natural) (MMF(HN)), California Academy of Sciences (CAS), Museo de Ciencias Naturales de Madrid (MCNM), Bavarian State Collections of Zoology (ZSM) and Cal Poly Pomona Invertebrate Collection, USA (CPIC).

For the majority of the 15 recognized valid species (see Introduction) our dataset includes specimens from the type localities or nearby places (50–100 km). The exceptions are *H. orbignyana*, *H. orteai*, *H. antillarum* and *H. succinea*, but in these last four cases, specimens were still assembled from the same biogeographical areas of the type localities (see Introduction and Table 1).

Outgroup taxa consisted of species from two additional genera, namely *Haloa* (represented by four species) and *Lamprohaminoea* (represented by one species). The trees were rooted with *Smaragdinella*, a genus closely related to *Haminoea* (Oskars et al., 2019). In total, this study includes 206 specimens (94 EA *Haminoea*, 69 WA *Haminoea*, 18 EP *Haminoea*, 5 tWIO *Haminoea* and 16 outgroup taxa) and a total of 608 sequences, of which 426 were newly generated for this study (Table 1).

2.2 | DNA extraction, amplification and sequencing

DNA was extracted from tissue obtained from the foot or parapodial lobes using the Qiagen DNeasy Blood and Tissue Kit (catalogue no. 69504) following the protocol recommended by the manufacturer. For small specimens with shell height between 2 and 3 mm, the whole specimen was digested and hard parts such as the shell, radula and gizzard plates were collected for morphological examination.

Partial sequences of the mitochondrial genes cytochrome *c* oxidase subunit I (COI; primers: LCO1490 (F) GGTCA ACAAATCATAAAGATATTGG and HCO2198 (R) TAAAC TTCAGGGTGACCAAAAATCA by Folmer et al., 1994; C_ GasF1_t1 (F) TGTAAAACGACGGCCAGTTTTCAACAAA CCATAARGATATTGG and GasR1_t1 (R) CAGGAAACAG CTATGACACTTCWGGRTGHCCRAARAATCARAA by Steinke et al., 2016), 16S rRNA (16S; primers: 16Sar-L (F) CGCCTGTTTATCAAAAACAT and 16Sbr-H (R) CCGGT CTGAACTCAGATCACGT by Palumbi et al., 1991), and 12S rRNA (12S; primers: 12SA-L (F) AAACTGGGATTAGA TACCCCACTAT and 12SB-H (R) GAGGGTGACGGGCG GTGTGT by Palumbi, 1996), as well as the nuclear gene 28S rRNA (28S; LSU5-F TAGGTCGACCCGCTGAAYTTAAGCA by Littlewood et al., 2000; 900-F CCGTCTTGAAACACGGA CCAAG by Olson et al., 2003; LSU1600-R AGCGCCATCCA TTTTCAGG by Williams et al., 2003; ECD2S-R CTTGGTCC GTGTTTCAAGACGG modified from Littlewood et al., 2000 by Williams et al., 2003) were amplified and sequenced. Polymerase chain reactions (PCR) were performed in 25 µL volume and for the COI and 28S genes followed the protocols described by Malaquias et al. (2009), whereas for the 16S the protocol described by Oskars et al. (2015) was used, and for the 12S gene we applied the protocol described by Oskars and Malaquias (2019). Annealing temperatures were 45°C for the COI gene, 51.5°C for 16S, 49.4°C for 12S and 52°C for the 28S gene.

For samples that did not amplify with Qiagen Taq, additional 25 µL reactions were set with TaKaRa Ex Taq Polymerase HS (250 U) (Cat. number: RR006A), following the protocol described by Oskars et al. (2015). For some

TABLE 1 List of specimens of *Haminoea* and outgroups used for phylogenetic analyses, with sampling localities, voucher numbers and GenBank accession numbers (numbers with prefix 'OR' are novel sequences generated for this study).

Taxon	DNA extraction code	Group No	Locality	Voucher No	COI	12S rRNA	16S rRNA	28S rRNA
H. navicula	C51	1	Hampshire, South England, UK	BMNH 20060324	DQ974676	OR343604	OR351715	DQ927233
H. navicula	129	1	Aveiro, Portugal	BMNH 20070018	KF615838	MK474364	MK474221	KF615804
H. navicula	130	1	Aveiro, Portugal	BMNH 20070020	KF615837	OR343606	OR351711	KF615803
H. navicula	131	1	Aveiro, Portugal	BMNH 20070020	KF615839	MK474365	MH933144	KF615805
H. navicula	147	1	Hampshire, South England, UK	BMNH 20070021	KF615836	MK474366	MH933145	KF615806
H. navicula		1	Aveiro, Portugal	BMNH 20070018.2	EU314804	–	–	–
H. navicula	398	1	France, Mediterranean	ZMBN 81646	OR352608	OR343608	OR351713	OR346082
H. navicula	294	1	France, Mediterranean	ZMBN 81647.1	OR352609	OR343607	OR351712	OR346083
H. navicula	295	1	France, Mediterranean	ZMBN 81647.2	OR352610	OR343605	OR351714	OR346084
Haminoea sp.	526	2	Žirje I., Croatia	ZMBN 121357	OR352611	–	–	–
Haminoea sp.	543	3	Roses, Catalonia, Spain, Mediterranean	ZMBN 112904	OR352612	OR343603	OR351650	OR346094
H. 'fusari'	535	4	Taranto, Italy	ZMBN 106885	OR352613	OR343609	OR351726	–
H. 'fusari'	167	4	Naples, Italy	BMNH 20070177	KF615840	MK474368	MH933152	KF615801
H. 'fusari'	511	4	Rovinj, Croatia	ZMBN 119729.1	OR352614	OR343610	OR351729	OR346085
H. 'fusari'	512	4	Rovinj, Croatia	ZMBN 119729.2	OR352621	OR343611	OR351728	OR346087
H. 'fusari'	522	4	Bene, Croatia	ZMBN 119735	OR352618	OR343612	OR351730	OR346086
H. 'fusari'	515	4	Žut I., Croatia	ZMBN 119731.1	OR352619	OR343613	OR351716	OR346097
H. 'fusari'	516	4	Žut I., Croatia	ZMBN 119731.2	OR352620	OR343614	OR351727	OR346098
H. 'fusari'	525	4	Žirje I., Croacia	ZMBN 121357	OR352615	–	–	–
H. 'fusari'	527	4	Žirje I., Croacia	ZMBN 121358.1	OR352616	–	–	–
H. 'fusari'	528	4	Žirje I., Croacia	ZMBN 121358.2	OR352617	–	–	–
Haminoea sp.	C50	5	Selvagem Grande I., Madeira Archipelago, Portugal	BMNH 20070024	OR352622	OR343615	OR351717	OR346081
Haminoea sp.	173	5	Selvagem Grande I., Madeira Archipelago, Portugal	BMNH 20070025	–	OR343616	–	–
H. 'hydatis'	524	6	Žut I., Croatia	ZMBN 121356	OR352623	–	–	–
H. 'hydatis'	530	6	Žut I., Croatia	ZMBN 121355	OR352625	–	–	–
H. 'hydatis'	513	6	Iž I., Croacia	ZMBN 119730.1	OR352626	–	–	–
H. 'hydatis'	514	6	Iž I., Croacia	ZMBN 119730.2	OR352624	–	–	–

(Continues)

TABLE 1 (Continued)

Taxon	DNA extraction code	Group No	Locality	Voucher No	COI	12S rRNA	16S rRNA	28S rRNA
H. 'hydatis'	529	6	Iž I., Croacia	ZMBN 121355	OR352627	–	–	–
H. 'hydatis'	540	6	Roses, Catalonia, Spain, Mediterranean	ZMBN 112900	OR352635	OR343617	OR351719	OR346092
H. 'hydatis'	541	6	Roses, Catalonia, Spain, Mediterranean	ZMBN 112901	OR352632	OR343624	OR351725	OR346093
H. 'hydatis'	542	6	Port – Leucate, France, Mediterranean	ZMBN 112902	OR352629	OR343622	OR351723	OR346089
H. 'hydatis'	545	6	Port – Leucate, France, Mediterranean	ZMBN 112906	OR352630	OR343623	OR351722	OR346090
H. 'hydatis'	546	6	Cerbére, France, Mediterranean	ZMBN 112907	OR352636	OR343619	OR351721	OR346095
H. 'hydatis'	166	6	Port Barcarès, France, Mediterranean	BMNH 20060326	KF615841	MK474367	KJ022796	KF615802
H. 'hydatis'	533	6	Mataró, Barcelona, Spain	ZMBN 106861	OR352634	OR343618	OR351710	–
H. 'hydatis'	561	6	Cadaqués, Caials, Catalonia, Spain	ZMBN 131054	OR352633	OR343625	OR351720	OR346096
H. 'hydatis'	C53	6	France, Mediterranean	BMNH 20060326	DQ974674	MK474369	MH933208	DQ927231
H. 'hydatis'	559	6	France Mediterranean	ZMBN 130226	OR352631	OR343621	OR351724	OR346091
H. 'hydatis'	259	6	Lazaro, Calabria, Italy	ZMBN 81714	OR352628	OR343620	OR351718	OR346088
H. 'elegans 4'	311	7	Guanacabibes, Cuba	MNCN Madrid	OR352637	OR343591	OR351697	OR346044
H. 'elegans 4'	461	7	Morrocoy, Venezuela	ZMBN 84942	–	–	OR351693	OR346045
H. 'elegans 4'	462	7	Morrocoy, Venezuela	ZMBN 84943	–	OR343592	OR351696	OR346046
H. 'elegans 4'	463	7	Morrocoy, Venezuela	ZMBN 84903.1	–	OR343593	OR351695	–
H. 'elegans 4'	464	7	Morrocoy, Venezuela	ZMBN 84903.2	–	OR343590	OR351694	–
H. 'elegans 4'	465	7	Morrocoy, Venezuela	ZMBN 84939.1	–	–	OR351692	OR346047
H. 'elegans 4'	466	7	Morrocoy, Venezuela	ZMBN 84939.2	–	OR343594	OR351698	–
H. vesicula	202	8	Bodega Harbor, Sonoma Co., California, USA	CAS97502	KF615843	MK474362	MH933161	KF615789
H. vesicula	574	8	Cat Harbor, Catalina I., California, USA	CPIC 01237	OR352641	OR343601	OR351690	OR346058
H. vesicula	575	8	Long Beach, California	CPIC 00606	OR352639	–	OR351691	OR346057
H. vesicula	577	8	San Pedro, California	CPIC 01030	OR352640	OR343599	OR351689	–
H. vesicula	571	8	San Diego, California	CPIC 00222	OR352638	OR343602	OR351688	–
H. vesicula		8	British Columbia, Indian Arm, Canada	BIOUG12670-G07	MG423188	–	–	–

TABLE 1 (Continued)

Taxon	DNA extraction code	Group No	Locality	Voucher No	COI	12S rRNA	16S rRNA	28S rRNA
H. vesicula		8	British Columbia, Indian Arm, Canada	10BCMOL-00307	KF643501	–	–	–
H. vesicula		8	Washington, USA		JQ693571	–	–	–
H. vesicula		8	False Bay, San Juan Island, Washington, USA	BMBM-0081	MH242779	–	–	–
H. vesicula		8	Haida Gwaii, British Columbia, Canada	10BCMOL-00152	KF643269	–	–	–
H. vesicula		8	Haida Gwaii, British Columbia, Canada	10BCMOL-00154	KF643444	–	–	–
H. vesicula		8	Haida Gwaii, British Columbia, Canada	10BCMOL-00348	KF643861	–	–	–
H. vesicula		8	Haida Gwaii, British Columbia, Canada	10BCMOL-00345	KF643877	–	–	–
H. vesicula		8	Haida Gwaii, British Columbia, Canada	10BCMOL-00346	KF643968	–	–	–
H. vesicula		8	Haida Gwaii, British Columbia, Canada	10BCMOL-00347	KF644011	–	–	–
H. vesicula	481	8	Long Beach, California	ZMBN 88214	–	–	–	MK474262
H. orteai		9	Greece	CPIC 01879	KX683878	–	–	–
H. orteai		9	Greece	CPIC 01880	KX683879	–	–	–
H. orteai		9	Greece	CPIC 01878	KX683877	–	–	–
H. orteai	197	9	Boca das Caldeirinhas, Faial I., Azores, Portugal	BMNH 20070458.1	KF615844	MK474363	MH933160	KF615791
H. orteai	198	9	Boca das Caldeirinhas, Faial I., Azores, Portugal	BMNH 20070458.2	KF615845	KF615790	MK474238	KF615790
H. orteai	508	9	Rovinj, Croatia	ZMBN 119726.1	OR352652	–	–	–
H. orteai	510	9	Rovinj, Croatia	ZMBN 119726.2	OR352653	–	–	–
H. orteai	517	9	Zadar, Croatia	ZMBN 119728.1	OR352654	–	–	–
H. orteai	518	9	Zadar, Croacia	ZMBN 119728.2	OR352655	–	–	–
H. orteai	519	9	Zadar, Croatia	ZMBN 119728.3	OR352656	–	–	–
H. orteai	520	9	Brac I., Maslinova Bay, Croatia	ZMBN 119734.1	OR352648	–	–	–
H. orteai	521	9	Brac I., Maslinova Bay, Croatia	ZMBN 119734.2	OR352658	–	–	–
H. orteai	523	9	Lubinski, Croatia	ZMBN 119736	OR352647	–	–	–

(Continues)

TABLE 1 (Continued)

Taxon	DNA extraction code	Group No	Locality	Voucher No	COI	12S rRNA	16S rRNA	28S rRNA
H. orteai	507	9	Rovinj, Croatia	ZMBN 119726	OR352643	–	–	–
H. orteai	281	9	Tenerife, Canary Is, Spain	BMNH 20030802	OR352659	–	–	–
H. orteai	258	9	Naples, Italy	ZMBN 81761	OR352657	–	OR351699	OR346048
H. orteai	282	9	Tenerife, Canary Is, Spain	BMNH 20030802	OR352642	OR343596	OR351706	–
H. orteai	369	9	Naples, Italy	ZMBN 83030	OR352644	–	OR351702	OR346054
H. orteai	292	9	Zoomarine, Algarve, Portugal	ZMBN 81696	OR352650	–	OR351701	OR346050
H. orteai	293	9	Zoomarine, Algarve, Portugal	ZMBN 81696	OR352645	–	OR351709	OR346051
H. orteai	C54	9	Cape Ferret, France, Atlantic	BMNH 20070030	OR352651	–	–	–
H. orteai	370	9	Naples, Italy	ZMBN 83030	OR352646	–	OR351703	OR346053
H. orteai	254	9	Naples, Italy	ZMBN 81701.2	KX383914	–	OR351708	OR346056
H. orteai	255	9	Naples, Italy	ZMBN 81701.3	KX383913	–	OR351704	OR346049
H. orteai	48	9	Tenerife, Canary Is, Spain	BMNH 20030836	KF615846	–	MK474239	KF615792
H. orteai	151	9	Sal I., Cape Verde Archipelago	BMNH 20070023	KX383915	–	OR351707	OR346052
H. orteai	283	9	Porto Velho do Varadouro, Faial I., Azores, Portugal	BMNH 20070459	KC404963	OR343598	KC404960	KC404962
H. orteai	536	9	Cap Ferret, France, Atlantic	ZMBN 112891	OR352649	OR343597	OR351700	OR346055
H. orteai	253	9	Naples, Italy	ZMBN 81701.1	KX383912	–	MH933172	MH933367
H. orteai	144	9	Porto Moniz, Madeira I., Portugal	MMF(HN) 36,229	–	–	OR351705	–
H. 'ovalis'	578	10	Baya de Banderas, Mexico	CPIC 00177	OR352660	–	OR351605	–
H. 'ovalis'	475	10	Máncora, Piura, Peru	ZSM Mol-20,100,737	–	OR343519	OR351606	–
H. virescens	489	11	Long Beach, California USA	ZMBN 88213	–	–	OR351609	MK474255
H. virescens	MT 572	11	Palos Verdes, California, USA	CPIC 01055	OR352662	OR343540	OR351607	OR346018
H. virescens	MT 573	11	Long Beach, California, USA	CPIC 00186	OR352661	OR343539	OR351610	OR346017
H. virescens	MT 580	11	Catalina I., California, USA	CPIC 01238	OR352663	OR343541	OR351608	OR346016
H. virescens	HVCal	11	Venice, California, USA		AF156142	AF156110	AF156126	–
Haminoea sp.	307	12	Rio de Janeiro, Brazil	ZMBN 81796.1	OR352665	OR343536	OR351601	OR346019
Haminoea sp.	308	12	Rio de Janeiro, Brazil	ZMBN 81796.2	OR352666	OR343538	OR351604	OR346020
Haminoea sp.	479	12	São Sebastião, São Paulo State, Brazil	ZMBN 88219.1	OR352664	OR343537	OR351602	–
Haminoea sp.	480	12	São Sebastião, São Paulo State, Brazil	ZMBN 88219.2	–	–	OR351603	–

TABLE 1 (Continued)

Taxon	DNA extraction code	Group No	Locality	Voucher No	COI	12S rRNA	16S rRNA	28S rRNA
H. alfredensis	174	13	Kariega river estuary, Kenton, South Africa	BMNH 20070314	KF615816	MK474355	MH933154	KF615774
H. alfredensis	182	13	Kenton on Sea, Kariega river estuary, South Africa	BMNH 20070315.1	KF615815	MK474353	OR351612	KF615775
H. alfredensis	183	13	Kenton on Sea, Kariega river estuary, South Africa	BMNH 20070315.2	KF615814	MK474354	MH933155	KF615773
H. alfredensis	459	13	Knysna lagoon, South Africa	ZMBN 86406.1	MK473513	MK474343	MK474188	MK474257
H. alfredensis	460	13	Knysna lagoon, South Africa	ZMBN 86406.2	MK473514	–	MK474184	MK474256
H. orbignyana	278	14	Niger Delta, Nigeria	ZMBN 81652.1	OR352681	OR343569	OR351629	OR346036
H. orbignyana	279	14	Niger Delta, Nigeria	ZMBN 81652.2	OR352682	OR343571	OR351624	OR346031
H. orbignyana	531	14	Praia Grande do Sul, Principe Is., São Tomé and Principe	ZMBN 106875	OR352680	OR343570	OR351625	OR346035
H. orbignyana	548	14	Ayamonte, Huelva, Spain	ZMBN 130159	OR352677	OR343556	OR351611	OR346021
H. orbignyana	391	14	Gran Canaria I., Canary Is., Spain	ZMBN 86408	OR352669	–	OR351640	–
H. orbignyana	396	14	Rabat, Morocco	ZMBN 81793	OR352671	OR343555	OR351619	–
H. orbignyana	397	14	Aghroud, Morocco	ZMBN 81797	–	OR343547	–	–
H. orbignyana	399	14	Djerba, Tunisia	ZMBN 86407	OR352678	OR343559	OR351614	OR346038
H. orbignyana	275	14	Rabat, Morocco	ZMBN 81791	MH933103	MK474352	MH933174	MH933369
H. orbignyana	277	14	Agadir, Morocco	ZMBN 81799	OR352672	OR343552	OR351623	OR346037
H. orbignyana	296	14	Aveiro, Portugal	ZMBN 81674.1	OR352674	OR343551	OR351622	OR346022
H. orbignyana	297	14	Aveiro, Portugal	ZMBN 81674.2	OR352668	OR343545	OR351639	OR346039
H. orbignyana	256	14	Naples, Italy	ZMBN 81714.1	KC404964.1	OR343548	OR351616	KC404961.1
H. orbignyana	257	14	Naples, Italy	ZMBN 81714.2	OR352670	OR343550	OR351616	OR346033
H. orbignyana	498	14	Lake Qarun, Egypt	ZMBN 99936.2	KT339766	–	–	–
H. orbignyana	499	14	Lake Qarun, Egypt	ZMBN 99936.1	KT339765	–	–	–
H. orbignyana	549	14	Isla Cristina, Huelva, Spain	ZMBN 130161	OR352679	OR343544	OR351618	OR346034
H. orbignyana	001	14	Faro, Ria Formosa, Portugal	BMNH 20030296	KF615813	MK474360	KJ022794	KF615776
H. orbignyana	557	14	Cartaya, Huelva, Spain	ZMBN 130169	OR352667	OR343557	OR351641	OR346027
H. orbignyana	558	14	Huelva, Spain	ZMBN 130197	OR352673	OR343558	OR351615	OR346028
H. orbignyana	249	14	Djerba, Golfo de Gabes, Tunisia	ZMBN 81710	OR352675	OR343546	OR351620	OR346040
H. orbignyana	250	14	Djerba, Golfo de Gabes, Tunisia	ZMBN 81710	OR352676	OR343549	OR351621	OR346041
H. orbignyana	148	14	Ria Formosa, Portugal	BMNH 20030296	KF615812	MK474359	OR351613	KF615777

(Continues)

TABLE 1 (Continued)

Taxon	DNA extraction code	Group No	Locality	Voucher No	COI	12S rRNA	16S rRNA	28S rRNA
H. orbignyana	367	14	Agadir, Morocco	ZMBN 83029.1	–	OR343553	–	–
H. orbignyana	368	14	Agadir, Morocco	ZMBN 83029.2	–	OR343554	–	–
H. orbignyana	390	14	Gran Canaria I., Canary Is., Spain	ZMBN 86408	–	–	OR351638	–
H. 'antillarum 2'	157	15	Caburná, Yucatan, Mexico	BMNH 20070091	KF615819	OR343542	OR351626	KF615782
H. 'antillarum 2'	158	15	Bocana, Sisal, Yucatan, Mexico	BMNH 20070094	KF615811	MK474356	MK474186	KF615779
H. 'antillarum 2'	159	15	Caburná, Yucatan, Mexico	BMNH 20070092	KF615818	OR343543	OR351627	KF615780
H. 'antillarum 2'	160	15	Bocana, Sisal, Yucatan, Mexico	BMNH 20070093	KF615820	MK474357	MH933150	KF615781
H. 'antillarum 1'		16	Indian River Lagoon, Florida, USA	FTP_0224	KP255198	–	–	–
H. 'antillarum 1'	176	16	Jupiter inlet, Palm Beach, Florida, USA	BMNH 20070316	KF615817	MK474358	MH933151	KF615778
H. 'antillarum 1'	210	16	Fort Pierce, Florida, USA	UF 369433	OR352683	OR343564	OR351628	OR346026
H. 'antillarum 1'	553	16	Key Largo, Florida Keys, USA	ZMBN 99926	OR352689	–	–	–
H. 'antillarum 1'	555	16	Key Largo, Florida Keys, USA	ZMBN 99908	OR352690	OR343568	OR351630	–
H. 'antillarum 1'	310	16	Florida Keys USA	ZMBN 81766.1	OR352685	OR343560	OR351632	OR346024
H. 'antillarum 1'	271	16	Long Key, Florida, USA	ZMBN 81769	OR352687	OR343565	OR351636	OR346032
H. 'antillarum 1'	309	16	Florida Keys USA	ZMBN 81766.2	OR352686	OR343561	OR351631	–
H. 'antillarum 1'	269	16	Long Key, Florida, USA	ZMBN 81767	OR352688	OR343567	OR351634	OR346025
H. 'antillarum 1'	364	16	Tobacco Bay, Bermuda	ZMBN 82989	OR352684	OR343562	OR351637	OR346029
H. 'antillarum 1'	270	16	Long Key, Florida, USA	ZMBN 81768	–	OR343566	OR351635	OR346030
H. 'antillarum 1'	303	16	Florida Keys USA	ZMBN 81751	–	OR343563	OR351633	OR346023
H. 'elegans 3'	47	17	Bimini Is, Bahamas	BMNH 20060100	–	OR343528	OR351658	OR346059
H. 'elegans 3'	265	17	Abaco Is, Bahamas	ZMBN 81771	–	OR343533	OR351652	OR346061
H. 'elegans 3'	266	17	Abaco Is, Bahamas	ZMBN 81765	OR352692	OR343535	OR351657	OR346066
H. 'elegans 3'	267	17	Abaco Is, Bahamas	ZMBN 81770	–	OR343532	OR351653	OR346062
H. 'elegans 3'	268	17	Abaco Is, Bahamas	ZMBN 81773	–	OR343531	OR351651	-
H. 'elegans 3'	304	17	Abaco Is, Bahamas	ZMBN 81713	–	OR343534	OR351655	OR346063
H. 'elegans 3'	305	17	Abaco Is, Bahamas	ZMBN 81764	–	OR343530	OR351654	OR346064
H. 'elegans 3'	306	17	Abaco Is, Bahamas	ZMBN 81763	–	–	OR351656	OR346065
H. 'elegans 3'	568	17	Great Oyster Pond, Eleuthera I., Bahamas	ZMBN 91089	OR352691	OR343529	OR351659	OR346060
H. 'elegans 1'	168	18	Sal-Rei, Boavista I., Cape Verde	BMNH 20020716	OR352693	–	–	–

TABLE 1 (Continued)

Taxon	DNA extraction code	Group No	Locality	Voucher No	COI	12S rRNA	16S rRNA	28S rRNA
H. 'elegans 1'	175	18	Kiwanis Park, Florida, USA	BMNH 20070318	KF615827	OR343573	OR351663	KF615800
H. 'elegans 1'	188	18	Banana River, Florida, USA	BMNH 20070448.1	KF615828	MK474349	MK474213	KF615798
H. 'elegans 1'	189	18	Banana River, Florida, USA	BMNH 20070448.2	KF615831	OR343574	OR351661	KF615799
H. 'elegans 1'	190	18	Pine Channel, Florida, USA	BMNH 20070603.1	KF615832	MK474350	MK474214	KF615795
H. 'elegans 1'	152	18	Banana River, Florida, USA	BMNH 20070180	KF615829	MK474348	MK474212	KF615797
H. 'elegans 1'	552	18	Summerland Key, Florida, USA	ZMBN 99935	OR352695	–	OR351665	OR346067
H. 'elegans 1'	554	18	Key Largo, Florida, USA	ZMBN 99925	OR352694	OR343572	OR351666	OR346068
H. 'elegans 1'	191	18	Pine Channel, Florida, USA	BMNH 20070603.2	KF615830	OR343575	OR351660	KF615796
H. 'elegans 1'	482	18	Banana River Lagoon, Florida, USA	ZMBN 88212	OR352696	–	OR351664	–
H. 'elegans 1'	162	18	Yucatan, Mexico	BMNH 20070089	–	–	OR351662	–
H. 'elegans 2'	154	19	Veracruz, Mexico	BMNH 20070175	KF615834	OR343584	OR351682	KF615794
H. 'elegans 2'	161	19	Bocana, Sisal, Yucatan, Mexico	BMNH 20070090	KF615833	MK474347	–	KF615793
H. 'elegans 2'	312	19	Guanacabibes, Cuba	MNCN Madrid	OR352700	OR343588	OR351669	OR346073
H. 'elegans 2'	313	19	Guanacabibes, Cuba	MNCN Madrid	OR352701	OR343582	OR351667	OR346074
H. 'elegans 2'	314	19	Guanacabibes, Cuba	MNCN Madrid	OR352702	OR343583	OR351668	OR346075
H. 'elegans 2'	550	19	Great Oyster Pond, Eleuthera I. Bahamas	ZMBN 91086	OR352698	OR343576	OR351672	OR346069
H. 'elegans 2'	566	19	El Ocho's lagoon, Morrocoy National Park, Venezuela	ZMBN 84926	OR352710	OR343589	OR351670	OR346071
H. 'elegans 2'	569	19	Sweeting Pond, Eleuthera I., Bahamas	ZMBN 91093	OR352699	OR343577	OR351683	–
H. 'elegans 2'	570	19	Turtle Pond, Eleuthera I., Bahamas	ZMBN 91103	OR352711	–	OR351673	–
H. 'elegans 2'	298	19	Abaco Is, Bahamas	ZMBN 81759	OR352712	OR343587	OR351674	OR346070
H. 'elegans 2'	365	19	Tom Moore's pond, Bermuda	ZMBN 82999.1	OR352707	OR343585	OR351684	OR346079
H. 'elegans 2'	366	19	Tom Moore's pond, Bermuda	ZMBN 82999.2	OR352706	OR343586	OR351685	OR346080
H. 'elegans 2'	371	19	Tom Moore's pond, Bermuda	ZMBN 83024.1	OR352705	OR343578	OR351675	OR346077
H. 'elegans 2'	372	19	Tom Moore's pond, Bermuda	ZMBN 83024.2	OR352709	OR343579	OR351676	OR346076
H. 'elegans 2'	373	19	Tom Moore's pond, Bermuda	ZMBN 82983.1	OR352703	OR343580	OR351671	OR346078
H. 'elegans 2'	374	19	Tom Moore's pond, Bermuda	ZMBN 82983.2	OR352704	OR343581	OR351677	OR346072
H. 'elegans 2'	484	19	São Sebastião, São Paulo, Brazil	ZMBN 88217.1	OR352697	OR343595	OR351680	–
H. 'elegans 2'	485	19	São Sebastião, São Paulo, Brazil	ZMBN 88217.2	–	–	OR351681	–
H. 'elegans 2'	486	19	Veracruz, Mexico	ZMBN 88204	OR352713	–	OR351679	–

(Continues)

TABLE 1 (Continued)

Taxon	DNA extraction code	Group No	Locality	Voucher No	COI	12S rRNA	16S rRNA	28S rRNA
H. 'elegans 2'	492	19	Ilha de Santo Amaro, Guarujá, São Paulo, Brazil	ZMBN 88218.1	–	–	OR351687	–
H. 'elegans 2'	493	19	Ilha de Santo Amaro, Guarujá, São Paulo, Brazil	ZMBN 88218.2	OR352708	–	OR351686	–
H. 'elegans 2'	477	19	Quintana Roo, Mexico	ZMBN 88203	–	–	OR351678	–
Haloa japonica	c52		Barcarès, France, Mediterranean	BMNH 20070029	KF615824	OR343525	OR351645	KF615786
Haloa japonica	149		Barcarès, France, Mediterranean	BMNH 20070065	KF615823	OR343523	OR351646	KF615787
Haloa japonica	164		Barcarès, France, Mediterranean	BMNH 20070028	KF615822	OR343526	OR351647	KF615785
Haloa japonica	251		Bahia de Ogrove, Galicia, Spain	ZMBN 81700.1	OR352606	OR343524	OR351648	OR346042
Haloa japonica	252		Bahia de Ogrove, Galicia, Spain	ZMBN 81700.2	OR352607	OR343527	OR351649	OR346043
Haloa japonica			San Juan Islands, WA		JN830723	–	–	–
Haloa japonica			Hokinawa, Japon	ZMBN 91233	MK473509	–	MK474248	MK474264
Haloa crocata			Beach House, Kauai, Hawaii	ZMBN 97228	MK473470	MK474393	MK474222	MK474278
Haloa pemphis			Hamamatsu, Japan	NHMUK 20070446	MH933106	MK474379	MH933156	MH933343
Haloa musetta			Heshikiya, Okinawa	ZMBN 112928	MK473467	MK474400	MK474232	MK474290
Lamprohaminoea ovalis			Manza Beach, Okinawa	ZMBN 88230	MK473488	MK474428	MK474194	MK474303
Lamprohaminoea ovalis			Airport Beach, Maui, Hawaii	NHMUK 20070031	MK473489	MK474427	MK474173	MK474250
Lamprohaminoea ovalis	262		Calabria, Italy	ZMBN 81762.1	–	OR343520	OR351644	OR346101
Lamprohaminoea ovalis	263		Calabria, Italy	ZMBN 81762.2	OR352604	OR343521	OR351642	OR346099
Lamprohaminoea ovalis	276		Malta	ZMBN 81803	OR352605	OR343522	OR351643	OR346100
Smaragdinella sp.			Mozambique	ZMBN 125447	MK473459	MK474345	MK474249	MK474253

samples, the amount of $MgCl_2$ and DNA was increased, and the volume of water adjusted accordingly in the PCR cocktail. In addition, 10x dilutions of DNA extractions were attempted for samples that did not yield results with all previous approaches.

The quality and quantity of PCR products were assessed by gel electrophoresis following standard methods (see Eilertsen & Malaquias, 2013). Successful PCR products were purified according to the EXO-SAP method described by Eilertsen and Malaquias (2013). Sequence reactions were run on an ABI 3730XL DNA Analyser (Applied Biosystems).

2.3 | Phylogenetic analyses

Geneious (v. R11, Kearse et al., 2012) was used to inspect, edit, and assemble the chromatograms of the forward and reverse DNA strands. All sequences were blasted in GenBank to check for contamination. Single gene sequences were aligned with Muscle (Edgar, 2004) implemented in Geneious. Alignments were trimmed to a position where at least 50% of the sequences had nucleotides and missing positions at the ends were coded as missing data (?). All sequences were deposited in GenBank (Table 1).

Blocks of ambiguous data in the single gene alignments of the ribosomal genes were identified and excluded using Gblocks with stringent and relaxed settings (Talavera & Castresana, 2007) (Appendix S1). The JModeltest software (Darriba et al., 2012) was used to find the best-fit model of evolution for each single gene dataset under the Akaike information criterion (Akaike, 1974). Seven individual gene analyses were initially performed: COI (Appendix S2; 681 bp; GTR + G), 12S Gblocks-relaxed (Appendix S3; 355 bp; TVM + G), 12S Gblocks-stringent (Appendix S4; 267 bp; TrN + I + G), 16S Gblocks-relaxed (Appendix S5; 420 bp; GTR + G + I), 16S Gblocks-stringent (Appendix S6; 393 bp; TVM + I + G), 28S Gblocks-relaxed (Appendix S7; 1047 bp; TrN + I + G) and 28S Gblocks-stringent (Appendix S8; 1036 bp; GTR + I + G).

Bayesian inference analyses (BI) using MrBayes (Huelsenbeck & Ronquist, 2001) were run through the portal CIPRES Science gateway V.3.3 (https://www.phylo.org) on the initial single gene datasets (Appendix S2–S8) and all-genes concatenated dataset (Figure 1, Appendices S9 and S10; 2492 bp). For the ribosomal genes, the datasets selected for concatenation were those that yielded the best-resolved trees with higher node support. All samples with sequences available for two or more genes were used in the concatenation analysis. In addition, samples with a single gene from unique geographical localities or with a unique phylogenetic position in the single gene trees were also included in the concatenated dataset. The analyses used three parallel runs of 5 million generations for the single gene analyses and 15 million generations for the concatenated dataset, with sampling every 100 generations. The concatenated dataset was partitioned by gene and each partition was run under the best-fit model of evolution. Convergence of runs was inspected in Tracer v1.7 (Rambaut et al., 2018) with a burn-in set to 25% by comparing the likelihood of trees drawn by the independent runs. Posterior probabilities (PP) higher than 0.95 were considered statistically significant (Alfaro et al., 2003; Huelsenbeck et al., 2001). A Maximum Likelihood analysis (ML) of the concatenated dataset was run with the RAxML (v.8.2; Stamatakis, 2014) plug-in implemented in Geneious. The analysis was partitioned by gene and run under the 'rapid bootstrapping and search for best scoring ML tree' algorithm, using a random starting tree and the model GTR + G + I with 1000 bootstrap (BS) replicates. Bootstrap values higher than 75% were considered significantly supported (Felsenstein, 1985). Consensus phylograms were converted to graphics in FigTree v1.3.1 (Rambaut & Drummond, 2009).

COI uncorrected *p*-distances were calculated in MEGA (ver. 7, Kumar et al., 2016) (Table 2) within and between candidate species, by plotting pairwise uncorrected *p*-distances against total distances (transversions + transitions). This is a common approach to calculate genetic distances in Heterobranchia taxonomic research (e.g. Austin et al., 2018; Carmona et al., 2011; Jörger et al., 2012; Kienberger et al., 2016).

2.4 | Molecular species delimitation analyses

We used the DNA sequences of the COI gene to evaluate candidate species by using the Automatic Barcode Gap Discovery delimitation method (ABGD) (Puillandre et al., 2012) and the Assembling Species by Automatic Partitioning (ASAP) (Puillandre et al., 2021) under default settings and three different models of molecular evolution (Jukes–Cantor (JC69), Kimura TS/TV = 2.0 (K80), Simple Distance). In addition, we used the bPTP method (Poison tree processes) on the same COI dataset. This method is intended to delimiting species that are consistent with the phylogenetic species concept and model speciation in terms of the number of substitutions (Zhang et al., 2013).

2.5 | Haplotype network analyses

Haplotype networks were generated based on the COI DNA sequences for the groups recognized by the phylogenetic analyses as putative candidate species, but which were

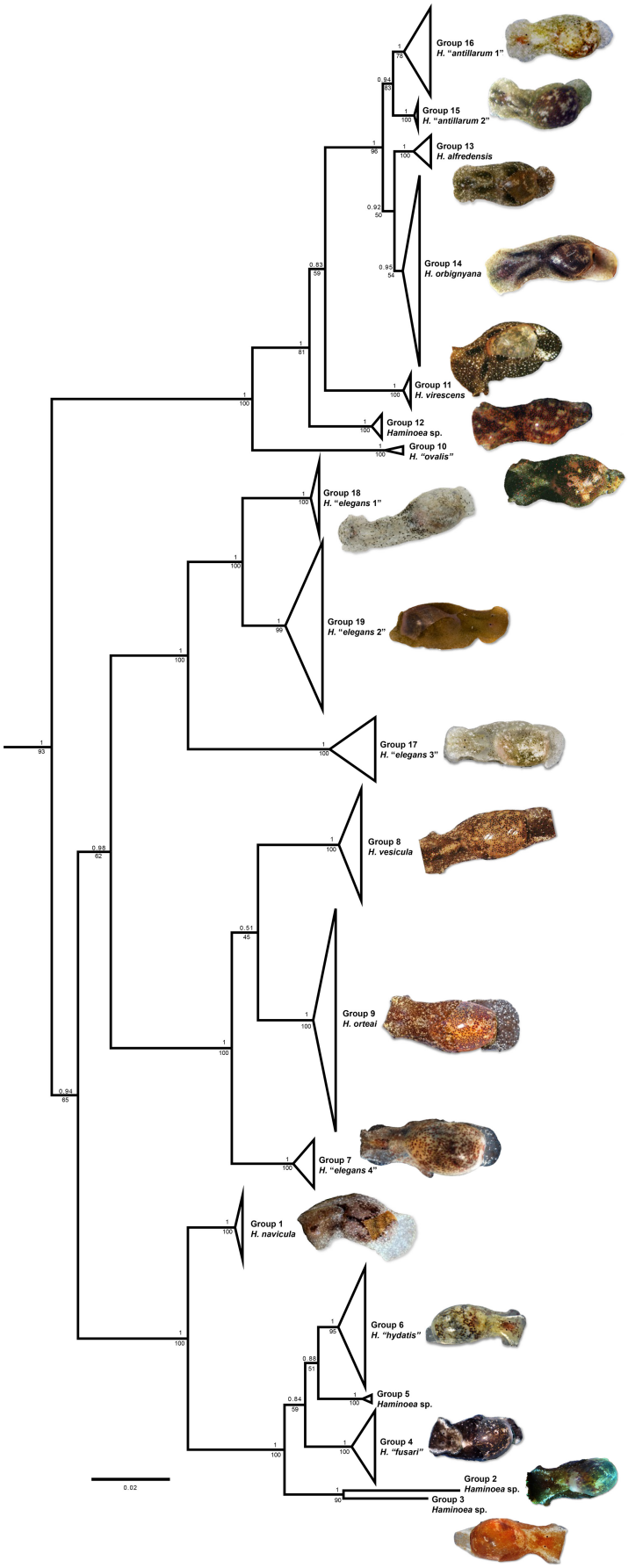

FIGURE 1 Cartoon based on the Bayesian phylogeny of *Haminoea* species depicted in Appendix S9 and resulting from the combined analysis of the mitochondrial COI, 12S rRNA and 16S rRNA and nuclear 28S rRNA gene markers. Figures above branches are Bayesian posterior probabilities and those below branches are bootstrap values derived by maximum likelihood. The trees were rooted with *Smaragdinella* sp. and representatives of the genera *Haloa* and *Lamprohaminoea* were included as outgroups. Both rooting and outgroups were removed for clarity (see Appendices S9 and S10 for complete trees). Images groups 1, 7, 14, 19 by Manuel Malaquias; images groups 8, 10, 11 by Ángel Valdés; images groups 2, 4, 6, 9 courtesy of Jakov Prkic; image group 3 courtesy of Marina Poddubetskaia; image group 12 courtesy of Joana Bahia; image group 13 courtesy of George Branch; image group 15 courtesy of Jazmin Ortigosa; images groups 16, 17 courtesy of Colin Redfern; image group 18 courtesy of Marlo Krisberg. Image not available for group 15.

TABLE 2 COI uncorrected genetic *p*-distances between and within groups of *Haminoea*. Figures are depicted as percentages (%). Distances within groups showed in bold font.

	Gp_1	Gp_2	Gp_3	Gp_4	Gp_5	Gp_6	Gp_7	Gp_8	Gp_9	Gp_10	Gp_11	Gp_12	Gp_13	Gp_14	Gp_15	Gp_16	Gp_17	Gp_18	Gp_19
Gp_1	**0.18**																		
Gp_2	14.3	**n/c**																	
Gp_3	13.2	11.3	**n/c**																
Gp_4	13.1	13.7	11.1	**0.5**															
Gp_5	12.7	13.0	10.0	7.4	**n/c**														
Gp_6	12.8	13.7	11.1	6.2	5.1	**1.2**													
Gp_7	16.1	19.8	19.4	18.4	17.6	17.6	**n/c**												
Gp_8	16.7	17.8	17.7	17.6	17.0	16.8	11.3	**0.6**											
Gp_9	16.0	18.5	17.2	17.3	16.1	17.0	10.4	11.0	**0.6**										
Gp_10	16.4	18.5	16.0	18.8	17.0	18.0	17.0	16.4	16.1	**n/c**									
Gp_11	15.8	17.1	17.3	17.3	17.1	18.1	16.3	17.6	16.2	10.7	**0.7**								
Gp_12	18.7	19.3	18.8	18.2	17.6	19.3	16.1	16.1	17.2	11.3	10.0	**0.3**							
Gp_13	16.4	18.1	16.4	18.5	17.7	18.6	16.7	17.3	17.0	12.8	10.7	11.1	**0.2**						
Gp_14	15.8	17.7	16.6	18.2	17.7	18.7	16.0	17.0	16.9	12.8	10.0	10.4	2.4	**0.5**					
Gp_15	15.6	16.5	15.7	17.1	17.0	18.0	16.7	16.5	15.9	12.7	10.4	10.1	3.8	4.7	**0.1**				
Gp_16	15.2	16.1	15.3	16.8	15.9	17.1	15.7	16.2	15.8	11.8	10.2	9.9	3.9	4.5	3.1	**0.9**			
Gp_17	15.5	15.5	16.2	16.1	15.4	16.5	16.3	15.2	14.7	15.8	14.4	14.2	15.8	16.1	15.2	14.5	**2.4**		
Gp_18	15.1	17.6	18.4	17.4	16.6	18.1	15.4	16.4	15.8	15.4	16.0	16.9	18.0	17.2	16.7	16.6	12.3	**0.3**	
Gp_19	15.9	18.4	18.8	18.4	17.2	18.1	16.1	16.1	15.9	15.6	15.9	16.2	19.4	18.5	18.2	18.1	12.1	7.5	**1.0**

clustered together by all or some of the ABGD and ASAP species delimitation analyses. This was the case for groups 4 + 5 + 6; groups 13 + 14 + 15 + 16; and groups 18 + 19. The COI alignment derived for phylogenetic inference was edited to remove all sequences from non-target groups using the text editor programme Notepad++ v.8.3.3. Empty positions at both ends of the alignments were treated as missing data, yielding final alignments of 677 bp. The programme DnaSP v.5 (Librado & Rozas, 2009) was used to identify the number and sequences of the different haplotypes. Notepad++ was additionally used to generate trait files with geographic area codes based on a binary coding where 0 stands for sample absent and 1 for sample present and to edit the file into nexus format (nex). Alignments and trait files were finally run in PopArt v. 1.7 (Population Analysis with Reticulate Trees; Leigh & Bryant, 2015) to create a standard tight compact spanning (TCS; Clement et al., 2002) network analysis to visualize the relationships and distances between the individual haplotypes from different groups and geographical areas. The TCS haplotype networks were edited in PopArt for more satisfying visualization.

2.6 | Morpho-anatomical analysis

The shell and anatomical features of selected specimens were studied to aid in interpreting the taxonomic status of some problematic lineages or to address complex taxonomic cases resulting from the molecular phylogenetic analyses (see Section 4 for details).

The shells were gently separated from the animals with the aid of forceps. The male reproductive system, gizzard and buccal bulb were dissected by a dorsal incision through the cephalic shield. Shells were photographed with a digital DSLR camera equipped with a macro lens and strobe lights. Shell height (*H*) was measured with a digital Vernier calliper. The reproductive system was drawn using a stereo microscope fitted with a drawing tube and the penial sheath was removed to expose the penial papilla. The gizzard and buccal bulb were placed in a solution containing 180 µL buffer ATL with 20 µL of proteinase K solution (both from the Qiagen DNeasy® Blood and Tissue Kit) and incubated at 56°C at night in order to clean the gizzard plates, jaws and radulae. The penial papillae, gizzard plates, jaws and radulae were mounted on metallic stubs using carbon sticky tabs and then sputter-coated with gold–palladium for scanning electron microscopy (SEM). Prior to sputter coating and SEM, the gizzard plates and penial papillae were dehydrated with Hexamethyldisilazane (HMDS) by covering each sample inside small square watch glasses and left to dry between 30 min and 1 h inside a fume hood. All samples were scanned and imaged with a Fei Quanta 450 scanning electron microscope.

3 | RESULTS

3.1 | Phylogenetic analyses

The ribosomal gene datasets selected for concatenation were the 12S-relaxed, 16S-relaxed and the 28S-stringent. Though, it must be stressed that differences between relaxed and stringent datasets were minor in all cases (Appendix S5–S8). Thus, the all-genes concatenated dataset was based on the COI (178 sequences), 12S-relaxed (136 sequences), 16S-relaxed (162 sequences) and 28S-stringent (134 sequences).

The COI gene analyses rendered 19 groups putatively compatible with candidate species of *Haminoea* (Appendix S2). All groups but one (*H. orbignyana*; PP = 0.88), received maximum or nearly maximum support. The 12S rendered 18 groups, but clade support was comparatively lower and often below statistical thresholds; the group missing is a singleton only represented in the COI dataset (*Haminoea* sp. 256 Croatia) (Appendix S2). The 16S tree rendered 15 groups and clustered together with no support (PP = 0.72) four groups recognized in the COI analyses (groups 13 + 14 + 15 + 16). None of these four groups formed supported sub-clades. Only group 13 (*H. alfredensis*) was nearly supported (PP = 0.91) but one representative branched apart (Appendix S5). The 28S gene tree was the less resolved with several of the groups recognized by the mitochondrial gene analyses, rendered non-monophyletic (Appendix S8). On the contrary, the concatenated analyses rendered the same 19 groups as the COI analysis, with two groups represented by singletons (*Haminoea* sp. 256 [Croatia; group 2] and *Haminoea* sp. 543 [Spanish Mediterranean; group 3]), one group (group 14) with moderate support (PP = 0.95) and all remaining 16 groups with maximum support (PP = 1) (Figure 1, Appendices S9 and S10).

3.2 | Molecular species delimitation analyses and genetic distances

The ABGD analyses using the simple distance (SD) and Jukes-Cantor (JC69) models suggested the presence of 13 candidate species of *Haminoea*, clustering together groups 4 + 5 + 6; groups 13 + 14 + 15 + 16; and groups 18 + 19. With the K2P model, the ABGD hypothesized the presence of 14 species by considering groups 15 and 16 distinct lineages (Appendix S11).

The ASAP results based on the three best ASAP-scores and for the same three evolutionary models used in ABGD rendered 11 candidate species of *Haminoea* (SD ASAP-score = 4; JC69 ASAP-score = 3.5; K2P ASAP-score = 3; SD genetic threshold = 9.3%), 14 species (SD ASAP-score = 5; JC69 ASAP-score = 5; K2P ASAP-score = 1.5; SD genetic threshold = 5.9%) and 17 species (SD ASAP-score = 4.5; JC69 ASAP-score = 4.5; K2P ASAP-score = 6; SD genetic threshold = 3.1%) (Appendix S12). The best ASAP score was 1.5 retrieved with the K2P model, suggesting 14 candidate species. This hypothesizes the same scenario as with the ABGD method under the same model, rendering 14 species and clustering groups 4 + 5 + 6 and groups 13 + 14 + 15 + 16, but separating groups 18 and 19 as distinct lineages.

The bPTP analysis suggested 20 candidate species, corresponding to the same 19 groups rendered by the COI and concatenated analyses, yet group 17 (*H.* '*elegans 3*') represented by two samples was inferred to correspond to two putative species (sample 266 from Abaco, Bahamas and sample 568 from Eleuthera, Bahamas; Table 1, Appendices S2 and S13).

The estimated uncorrected *p*-distances between the 19 groups depicted in Figure 1 varied between a maximum of 19.8% (*Haminoea 'elegans* 4'; Western Atlantic [group 7] and *Haminoea* sp. Mediterranean Sea [group 2]) and a minimum of 2.4% (*Haminoea alfredensis* [group 13] and *Haminoea orbignyana* [group 14]). Genetic distances between several sister groups, namely those considered by the species delimitation methods to be conspecific were comparatively low; for example, between groups 13, 14, 15, 16 (2.4%–4.7%), between groups 4, 5, 6 (5.1%–7.4%) and between groups 18, 19 (7.5%). All other sister groups have genetic distances equal to or higher than 10% (Table 2). The genetic distance between the two samples of *H.* '*elegans* 3' recognized as distinct lineages by the bPTP analyses was 2.4%. This corresponds to the intraspecific genetic distance for this species depicted in Table 2.

3.3 | Haplotype network analyses

The haplotype network of groups 4 + 5 + 6 formed by samples from the eastern and central Mediterranean Sea was well structured with 14 haplotypes and three recognizable haplogroups separated by 28 substitutions (between groups 4 and 6) and 21 substitutions (between groups 5 and 6). Only one case of shared haplotypes was detected in group 6 between samples from Spain and France (Figure 2). The haplotype network of groups 13 + 14 + 15 + 16 with samples from Europe, West Africa, temperate South Africa and Caribbean Sea, includes 19 haplotypes and four recognizable haplogroups connected through hypothetical haplotypes (black circles; Figure 3). The highest number of substitutions among haplogroups was 23 between group 13 (*H. alfredensis*) and 15 (*H.* '*antillarum* 2'). The haplotype network of groups 18 + 19 formed by samples from Brazil, Caribbean Sea and Cape Verde Islands includes 21 distinct haplotypes and two recognizable haplogroups separated by 36 substitutions (Figure 4).

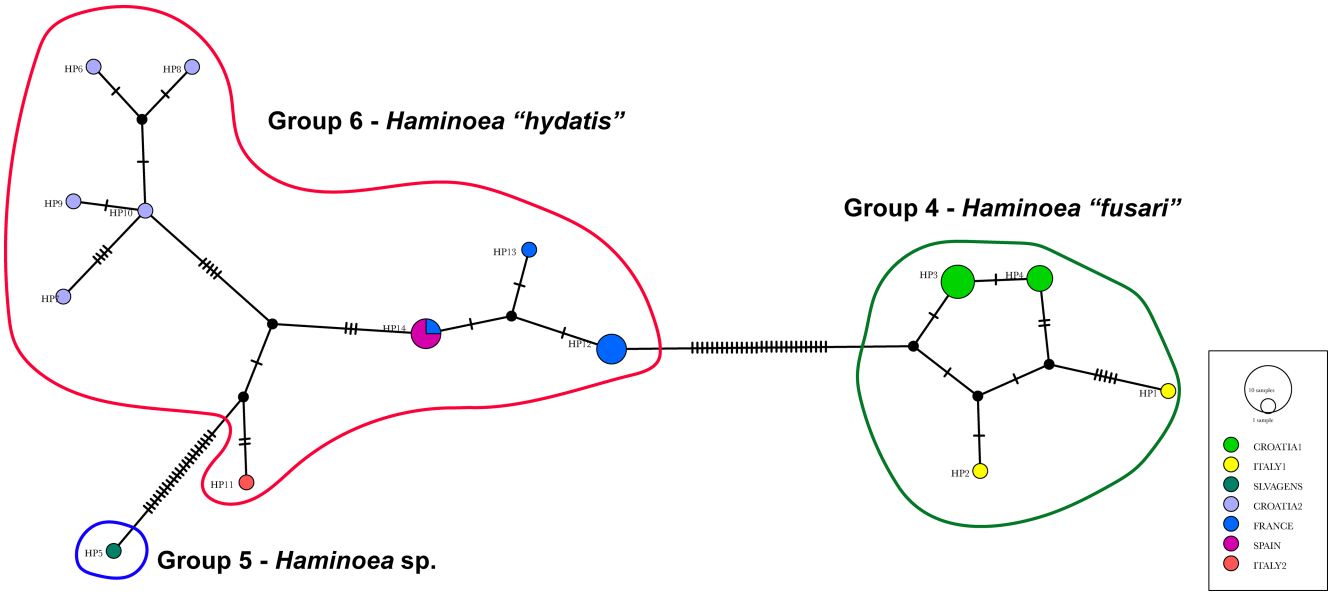

FIGURE 2 COI haplotype network produced with the TCS method in PopART for groups 4, 5 and 6. Colours of circles refer to the geographic origin of each haplotype. The relative size of circles is proportional to the number of sequences of that same haplotype. Black circles refer to hypothetical haplotypes and black bars to mutations.

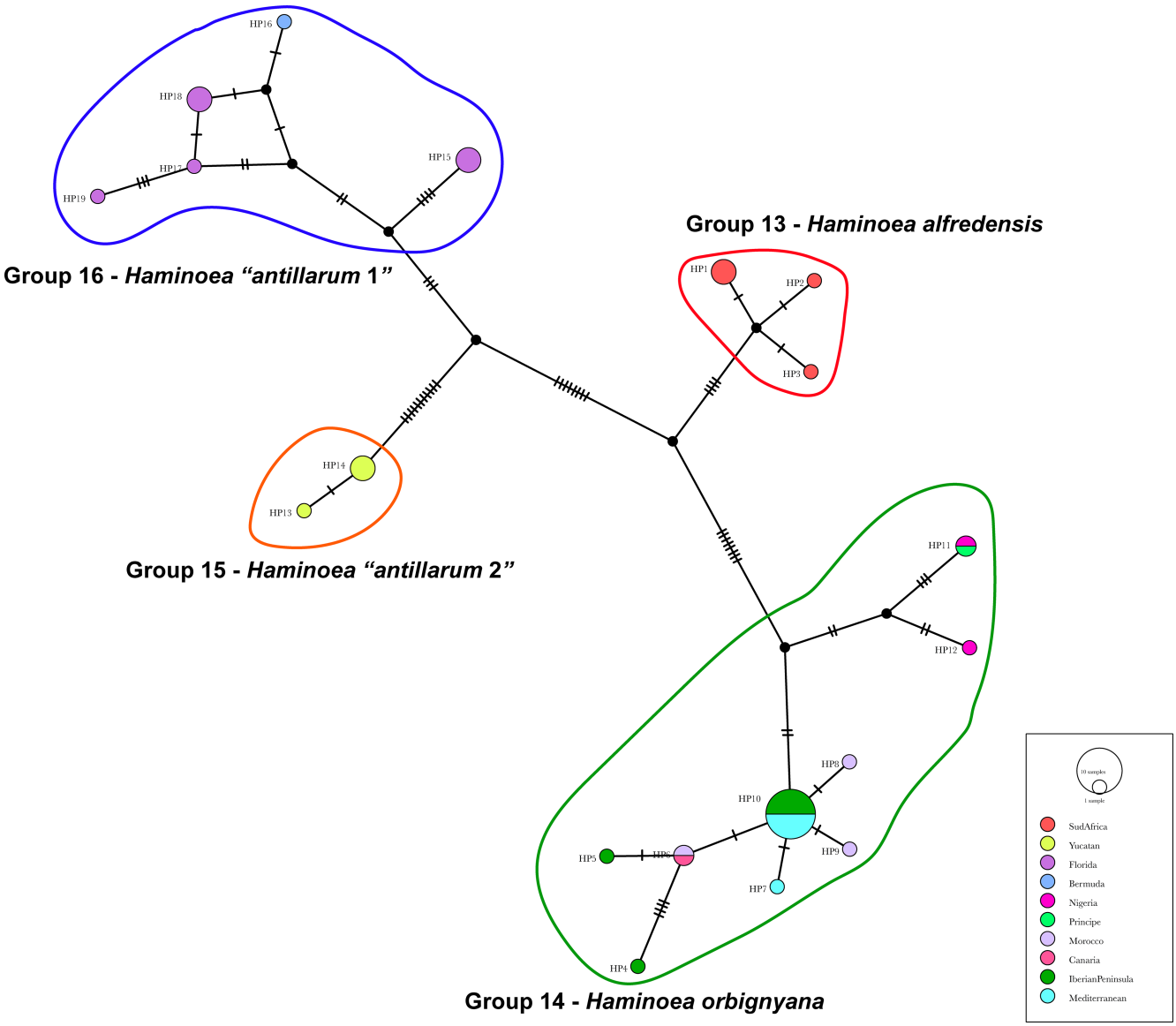

FIGURE 3 COI haplotype network produced with the TCS method in PopART for groups 13, 14, 15 and 16. Colours of circles refer to the geographic origin of each haplotype. The relative size of circles is proportional to the number of sequences of that same haplotype. Black circles refer to hypothetical haplotypes and black bars to mutations.

4 | DISCUSSION

Our results rendered four possible hypotheses of species diversity for the genus *Haminoea*: 19 candidate species consistent with the number of supported clades in the phylogenetic analyses (Figure 1); 14 species, where groups 4 (*H.* '*fusari*' Mediterranean) + 5 (*Haminoea* sp. Selvagens Islands) + 6 (*Haminoea* '*hydatis*' Mediterranean) and groups 13 (*H. alfredensis*) + 14 (*H. orbignyana*) + 15 (*H.* '*antillarum* 2' Gulf of Mexico) + 16 (*H.* '*antillarum* 1' Caribbean) are suggested to be single species; 13 species – a hypothesis similar to the latter, but where groups 18 (*H.* '*elegans* 1' EA + WA) + 19 (*H.* '*elegans* 2' WA) are hypothesized to be one candidate species; and 20 species—similar to the first hypothesis (19 species) but where group 17 (*H.* '*elegans* 3') splits in two candidate species.

4.1 | The *Haminoea orbignyana–alfredensis–antillarum* complex

Probably, the most surprising result of this study is the hypothetical conspecificity of the Eastern Atlantic and Mediterranean Sea species *H. orbignyana*, with *H. alfredensis* from the temperate Indian Ocean shores of South Africa, and the Western Atlantic lineages of *H. antillarum* (groups 15 and 16). Despite the fact that our phylogenetic analyses (Figure 1) rendered these four lineages

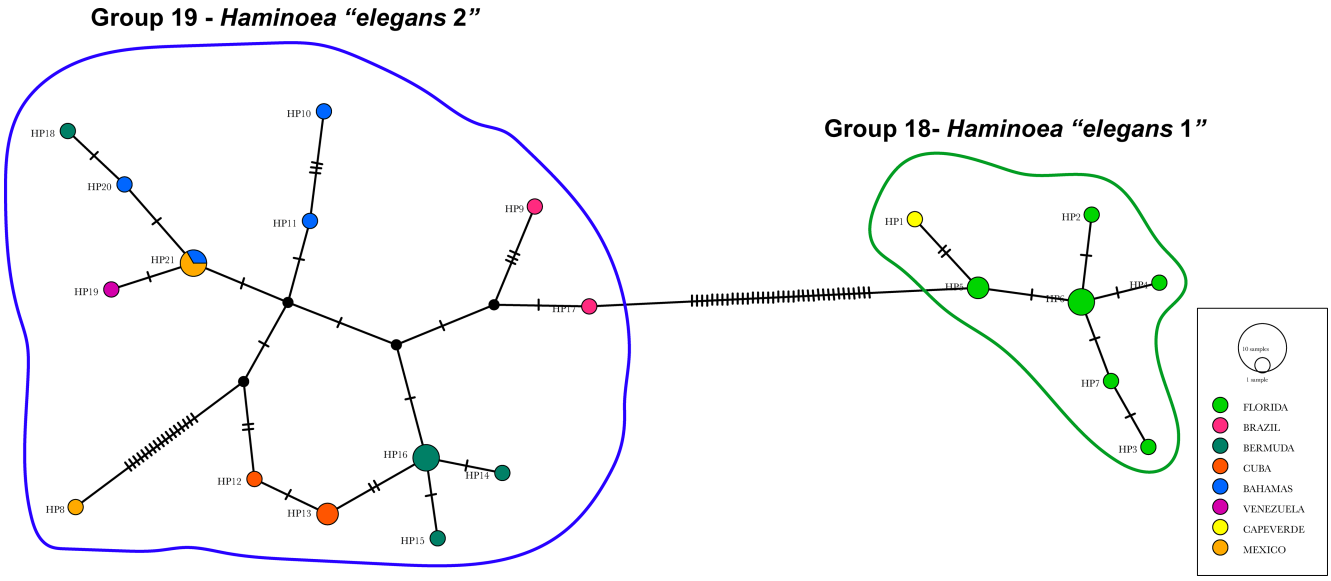

FIGURE 4 COI haplotype network produced with the TCS method in PopART for groups 18 and 19. Colours of circles refer to the geographic origin of each haplotype. The relative size of circles is proportional to the number of sequences of that same haplotype. Black circles refer to hypothetical haplotypes and black bars to mutations.

monophyletic, the genetic distances among them are comparatively lower varying between a minimum of 2.4% (*H. alfredensis* and *H.orbignyana*) and a maximum of 4.5% (*H. antillarum* group 16 and *H. orbignyana*), with the genetic distance between the two lineages of *H. antillarum* estimated at 3.9%. These four lineages are only supported by the COI gene, whereas the 12S and 28S genes rendered support for the clade containing the specimens of *H. antillarum* from Yucatan (group 15), and the latter gene also provided support for the clade with specimens of *H. alfredensis* (Figure 1, Appendices S2, S3, S8, S10, S11 and S11). None of the four lineages was supported by the 16S gene analysis, which nevertheless clustered all representatives together but with low node support (PP = 0.72).

The species *H. orbignyana*, *H. antillarum* and *H. alfredensis* are well established in the literature, yet they were never studied in a comparative framework. A closer look at the literature together with our own preliminary data on the morphology of specimens reveals that all these nominal species share a pear-shaped smooth shell and a body colouration characterized by dense black pigmentation along the edges of the cephalic shield and parapodial lobes. In contrast, *H. antillarum* has mildly denticulated inner lateral radular teeth, whereas in *H. alfredensis* and *H. orbignyana* these teeth are smooth. Likewise, whereas in the latter two species, the proximal lobe of the prostate is wider, conferring the prostate an acorn-like shape, in *H. antillarum* seems to be the opposite with the distal lobe wider compared to the proximal one (Gosliner, 1987; Macnae, 1962; Malaquias & Cervera, 2006; Marcus & Marcus, 1967; Thompson, 1981; Valdés et al., 2006; personal observations), but this requires further anatomical investigations in order to be confirmed.

Even if our molecular results based on the species delimitation analyses and genetic distances suggest the occurrence of a single ubiquitous species with amphi-Atlantic distribution encompassing the Iberian Peninsula, the Mediterranean Sea, West Africa including the Canary Islands, the temperate shores of South Africa in the Indian Ocean and the western Atlantic along the Yucatan Peninsula, Florida and Bermuda, this warrants caution and further corroboration by conchological and morpho-anatomical data. As highlighted above, *H. antillarum* seems to be characterized by relevant anatomical differences from the digestive and reproductive systems, and even if genetic distances are comparatively low, this could be due to different evolutionary rates between species of *Haminoea*.

On the contrary, and even in the absence of sound data on the duration of the pelagic larval stage of *Haminoea* (Schaefer, 1996), the confirmed occurrence of specimens attributed to *H. elegans* (group 18) on both sides of the Atlantic (Figure 1; only 0.3% different in the COI gene; Table 2) supports a high dispersal capability, at least in some species of the genus (Martínez & Ortea, 1997; current study as *H.* '*elegans* 1' [group 18]; Figure 1). Thus, we cannot discard that representatives of the *orbignyana-alfredensis-antillarum* complex may have larvae with high dispersal capability favouring gene flow between distant populations. However, we must admit that the genetic distance between the two putative lineages of *H. antillarum* from nearby locations, namely the Yucatan side of the Gulf of Mexico (group 15) and the Florida Keys/Florida

Peninsula–Bermuda (group 16), estimated at 3.9% and 14 substitutions between these two haplogroups (Figure 3) challenges this view. Even if the prevalent ocean current system in the area suggests putative connectivity between Yucatan and the Florida Peninsula through the Loop Current (Gyory et al., 2011), faunal breaks between tropical Florida and the more temperate/sub-tropical Gulf of Mexico have been documented for several groups of molluscs and fish (Briggs, 1974; Lee & Ó Foighil, 2004; Mikkelsen & Bieler, 2000; Reeb & Avise, 1990), likely reflecting seasonal changes in the current systems and water temperatures oscillations. These factors may hinder gene flow between the Yucatan and the Florida–Bermuda populations, creating periods of temporary isolation that could explain the observed genetic discontinuity.

Another interesting aspect is the sister relationship between the lineages *H. orbignyana* (Eastern Atlantic) and *H. alfredensis* (temperate South Africa). The cold water of the Benguela current established at the end of the Miocene (Siesser, 1980) is regarded as a strong barrier for temperate and tropical marine coastal species isolating the faunas of the Atlantic and Indian Oceans, while at the same time, paleontological and morphological evidence suggests that this barrier was sporadically bridged by several coastal invertebrate organisms (Briggs, 1995; Vermeij & Rosenberg, 1993). Few molecular evidence for dispersal from the Atlantic into the Indian Ocean is still available. This is the case for reef fish (Floeter et al., 2008; Rocha et al., 2005) and sea slugs (Churchill et al., 2014; Golestani et al., 2019), which seem to have taken advantage of the disruption of the Benguela and Agulhas currents during warmer interglacial periods of the Pleistocene. Because *Haminoea* is a genus of Atlantic and Eastern Pacific affinity, the sister relationship between *H. orbignyana* and *H. alfredensis* is more parsimoniously explained as being the result of dispersal of larvae or *H. orbignyana* into the Indian Ocean during these warmer periods, with the establishment of viable populations followed by isolation after the reestablishment of the current system.

4.2 | The *Haminoea elegans* complex

Haminoea elegans is characterized by having whitish translucent spiralled shells and it has been regarded as widely distributed in the Western Atlantic throughout the Gulf of Mexico and the Caribbean Sea southwards to Brazil (Valdés et al., 2006) with records in West Africa between the Gulf of Guinea and Angola (Bernard, 1984; Martínez & Ortea, 1997; Rolán & Ryall, 1999). However, the attribution of the name '*elegans*' to this tropical amphi-Atlantic species stems certainly from a misidentification perpetuated in the literature over time. The name *H. elegans* was introduced by Gray (1825) based on spiralled shells from the British Isles and the Mediterranean Sea, and the name is most certainly a junior synonym of *Haminoea navicula*, the only European species with a deeply spiralled shell (Malaquias & Cervera, 2006).

Our results showed the existence of cryptic diversity in this 'species' with specimens provisionally ascribed by us to *H. elegans* splitting in four (or five) clades of possible species status (groups 7, 17–19; Figure 1, Appendices S2, S9, S10 and S13). Representatives of groups 17, 18 and 19 clustered together with maximum support, whereas group 7 branched off elsewhere in the tree (Figure 1, Appendix S2, S9 and S10).

If, in contrast, our results unequivocally support group 7 as a good species, they are not conclusive about the eventual status of group 17, with one of the species delimitation analysis (bPTP), suggesting the possible occurrence of two lineages in this group. However, none of the single gene and combined analyses retrieved reciprocal monophyly between sub-clades within group 17. When present, the sub-clades are not statistically supported (Appendices S3–S10).

The results are also not entirely conclusive about the conspecificity of groups 18 and 19 (see Section 3 – theme 3.2). Groups 18 and 19 are the only two in the complex with a genetic distance between themselves below 10%, but still moderately high (=7.5%). Moreover, in the haplotype network analysis, they were separated by 36 substitutions, the largest number of substitutions between putative conspecific groups among all our haplotype network analyses (Figure 4).

There are several names available in the literature that could be regarded as previous attempts to describe the shells variability in the *H. elegans* complex (e.g. *H. guildingii* (Swainson, 1840) [shells globose with visible spiral striae], *H. petitii* (d'Orbigny, 1841) [shells lacking or with inconspicuous spiral striae], *H. succinea* (Conrad, 1846) [shells cylindrical with tightly arranged spiral striae], *H. taylorae* (Petuch, 1987) [shells globose with numerous fine spiral striae]). These names have been in part considered synonyms of *H. elegans* (MolluscaBase, 2022; Valdés et al., 2006) or hardly used in scientific literature, but our results show the need to carefully re-evaluate the status of these names since some of them may apply to lineages revealed by our analyses.

The only study that provided a comparative analysis of the various types of shells of '*H. elegans*' in the Western Atlantic was by Redfern (2013: 266–268). This author recognized five different types of whitish shells that could be associated with *H. elegans*; four with spiral striae and one apparently smooth. One of these forms was named by Redfern (2013) *Haminoea elegans* proper and the other four *Haminoea* A, B, C and D. According to the author,

Haminoea elegans and *Haminoea* sp. A are characterized by globose-quadrate opaque shells with wavy-spiral striae and a partially concealed involute spire; *Haminoea* sp. B by a more oval translucent shell, with numerous tightly arranged spiral striae and a spire concealed by a callus; *Haminoea* sp. C by a globose opaque smooth shell and a spire concealed by a callus; *Haminoea* sp. D by cylindrical translucent shells, with lightly impressed spiral striae and a spire concealed by a callus.

Here we provide for the first time a phylogenetic framework to properly explore the diversity of the *Haminoea* '*elegans*' species-complex. Yet confirming whether our four (or five) candidate species correspond to the shell types identified by Redfern (2013) and are compatible with the names available in the literature, requires additional taxonomic work based on detailed analyses of conchological and morpho-anatomical characters.

4.3 | The *Haminoea hydatis–fusari* complex

Another difficult case consists of groups 4, 5 and 6 in our phylogeny (Figure 1), which were rendered a single candidate species by the ABGD and ASAP molecular species delimitation methods. These three clades received maximum or nearly maximum support in both BI and ML analyses, but interestingly if considered together as a single clade the support lowers to 0.84 (PP) and 59% (BS), although this seems to be mostly influenced by the 16S gene data (Appendix S5). On the contrary, the haplotype network analysis (Figure 2) recovered the three groups as distinct, separated by 28 substitutions (between groups 4 and 6) and 21 substitutions (between groups 5 and 6) and showed a lack of shared haplotypes. Genetic distances were moderately high, ranging between 5.1% (between groups 5 and 6), 6.2% (between groups 4 and 6) and 7.4% (between groups 4 and 5).

This larger clade, including the three groups (4 + 5 + 6), contains only specimens from the Mediterranean Sea and one from the Eastern Atlantic island of Selvagem Grande (Madeira Archipelago). They are all characterized by a distinct anatomical feature among *Haminoea*, namely a prostate with a constricted zone between the proximal and distal lobes (see Thompson, 1988). This feature has been described for the type species of the genus *H. hydatis* (Talavera et al., 1987; Tchang, 1931; Thompson, 1976, 1981, 1988) and *H. fusari* (Álvarez et al., 1993). According to the literature these two species are basically distinguished by the presence of denticulated inner lateral teeth in *H. hydatis* (Talavera et al., 1987; Tchang, 1931; Vayssière, 1885) while they are smooth in *H. fusari* (Álvarez et al., 1993).

Haminoea hydatis is the type species of the genus described by Linnaeus (1758) based on shells from the Mediterranean Sea. The type specimen illustrated in the webpage of the Linnean Collections, London (https://linnean-online.org/16897/#?s=0&cv=0&z=0.0365%2C0.0109%2C1.232%2C1.503), is a shell about 9 mm in height with a smooth surface. Vayssière (1885) studied specimens from the Gulf of Marseille on the Mediterranean French coast with smooth shells and a radula with inner lateral teeth denticulated, which he identified as *H. hydatis*. Later, Tchang (1931) described the male reproductive system of specimens from the same region as having a prostate with the proximal and distal lobes separated by a narrow tubular region, and Talavera et al. (1987) mentioned a smooth, cylindrical pointed penis. Thus, progressively it became established in the scientific literature the idea that *H. hydays* (originally only known from shells) was characterized by having smooth shells, radulae with denticulated inner lateral teeth and a prostate with a narrow region separating the two lobes. This view was reinforced by the fact that up until the end of the first half of the 20th century, the European fauna of *Haminoea* was basically restricted to two accepted species; either *H. hydatis* with its small smooth shells or *H. navicula* with larger and deeply spiralled shells.

However, several species with smooth shells accepted as valid (see Introduction) were described during the second half of the last century, one of them (*H. fusari*) also with a prostate with two lobes separated by a narrow region, but with smooth radular inner lateral teeth (Álvarez et al., 1993). But the study of the holotype of *H. fusari* (MNCN 15.05/5356) revealed in fact the presence of mostly smooth inner lateral teeth, but interestingly some of them had the lower half denticulated. Intraspecific radular variability was described by Malaquias and Cervera (2006) for *H. navicula* and might occur also in specimens identified as *H. fusari*. This would basically make the two species anatomically undistinguishable and thus likely conspecific, rendering the name *H. fusari* a junior synonym of *H. hydatis*. In addition, the colour patterns of specimens in groups 4 and 5 are alike (data not available for group 3), with large unpigmented peri-ocular areas, dark upper sides of the parapodial lobes and fine bright-white dots along the edge of the cephalic shield, which further supports their conspecificity (see Figure 1).

4.4 | The eastern Pacific species

In the Eastern Pacific coastlines of North and Central America, there are three species of *Haminoea* commonly recognized as valid between Alaska and Panama, namely *H. ovalis*, *H. virescens* and *H. vesicula* (Behrens

& Hermosillo, 2005; Hermosillo et al., 2006; Valdés & Camacho-Garcia, 2004). For this region, our analyses recognized lineages compatible with these three species. The species *H. virescens* (group 11) with pear-shaped shells and monolobated prostates (Gibson & Chia, 1989; Valdés, 2019; personal observations) split off as sister to the eastern Atlantic/Indian Ocean complex *H. alfredensis–H. orbignyana–H. antillarum*, although with no support (PP = 0.83, BS = 59%; Figure 1). A similar pattern was found for the species *H. vesicula* (group 8) characterized by a globose-quadrate shell and bilobed prostate (Gibson & Chia, 1989; Valdés, 2019; personal observations), which was rendered sister to the eastern European *H. orteai*, but again with no support (PP = 0.51, BS = 45%; Figure 1). This phylogenetic pattern with putative speciation across these two oceanic realms could be explained by processes related to the uplift of the Isthmus of Panama, which separated the Atlantic from the eastern Pacific around 3 Mya (Coates & Obando, 1996), however, the low support values hamper any sound explanation.

Finally, we retrieved a lineage morphologically compatible with what has been named in recent literature as *H. ovalis* (Behrens & Hermosillo, 2005; Hermosillo et al., 2006; Valdés & Camacho-Garcia, 2004). However, as explained in the Introduction this name applies to an Indo-West Pacific species in the genus *Lamprohaminoea* (Oskars & Malaquias, 2020c; Pease, 1868). This Eastern Pacific species has globose smooth shells and a body doted by abundant tiny orange dots. We could not find any available name fitting the features known for this lineage, which thus may represent an undescribed species.

4.5 | Notes on other Atlantic species

An additional five species were recovered by the phylogenetic and species delimitation analyses (*H. navicula* [group 1], *Haminoea* sp. [group 2], *Haminoea* sp. [group 3], *H. orteai* [group 9], *Haminoea* sp. [group 12]). The species *H. navicula* is well established, characterized by large shells, with conspicuous spiral striae and an armed penis (for details see Lobo-da-Cunha et al., 2018; Malaquias & Cervera, 2006). The species *H. orteai* was the first described European species characterized by a penis with an apical crest with 10 lamellae and the peculiarity of lacking an unpigmented periocular area in the cephalic shield (Talavera et al., 1987). However, our observations revealed that the description of this latter feature is not entirely accurate; in fact, like all other species of the genus, *H. orteai* has an unpigmented periocular area yet rounded and of a much smaller diameter. This can even be seen in the original description of the species (Talavera et al., 1987: 66, figure 15). Later, two additional species also with penises with apical crests were described for European waters, namely *H. templadoi* (García et al., 1991) and *H. exigua* (Schaefer, 1992). These two species are morpho-anatomically very similar to *H. orteai* when it comes to the radula, the male reproductive system, the shell and the reduced diameter of the periocular area. *Haminoea templadoi* was described as having a shell with transverse folds interconnecting longitudinal growth lines and a radula with the first two inner lateral teeth denticulated (García et al., 1991: 396, figures 2, 3), but the study of the holotype (MNCN 15.05/854) showed a radula with only the inner lateral teeth denticulated. Moreover, according to our interpretation, the distinct shell structure likely relates to the comparatively larger size of the shell ($H = 21.6$ mm). A thorough systematic review of species combining detailed morphological work and the current phylogenetic framework is necessary to address the putative conspecificity of the species *H. orteai*, *H. templadoi* and *H. exigua*.

Three additional and unidentified species were rendered by our analyses; *Haminoea* sp. [group 12] from Brazil, and two species represented by single individuals, namely *Haminoea* sp. [group 2] from Croatia and *Haminoea* sp. [group 3] from Roses, Girona, Spain (Mediterranean Sea). The specimens from Brazil externally resemble *H.* '*ovalis*' from the eastern Pacific and are interestingly part of a fully supported clade which includes the later species (Figure 1). The remaining two Mediterranean species might represent undescribed taxa, but this requires additional work to be confirmed. The colourations of these species are quite unique among European species with more or less uniform orangish and brownish background colour patterns (Figure 1).

4.6 | Concluding remarks

The genus *Haminoea* is a difficult taxonomic group with many available names introduced based on shell descriptions alone or described based on their morpho-anatomical features outside a phylogenetic molecular framework, several of them grounded on subtle differences.

The current literature consensus accepts eight species as valid in the Eastern Atlantic (including the amphi-Atlantic *H.* '*elegans*'), four in the Western Atlantic (including *H.* '*elegans*'), three in the Eastern Pacific and one in temperate South Africa. As explained in the Discussion, we open the possibility that several of the Eastern Atlantic taxa, presently regarded as valid species, might be conspecific (e.g. *H. orteai*, *H. templadoi*, *H. exigua*). A re-evaluation of the literature and type material, preliminary anatomical work, combined with our molecular phylogenetic framework seem to indicate that several of the subtle differences used in the past to

introduce new species might not be sound enough. On the contrary, there were several cases where our results were not sufficient to reach definitive conclusions about the taxonomic status of certain species (e.g. *H. alfredensis*, *H. antillarum*, *H. elegans*, *H. orbignyana*) and further morphological work is necessary to understand their diversity and draw a robust taxonomic hypothesis. A preliminary combination of conchological, morphological and phylogenetic data demonstrated that putative cases of cryptic diversity may, in fact, reflect previously detected differences in shell characters that led to the description of species currently considered invalid (e.g. the cryptic species complex *H.* '*elegans*').

There are several paradigmatic examples of pervasive nomenclature confusion that need to be evaluated, such as the status of the type species *H. hydatis*, *H. elegans*—a name introduced for a European species but largely in use for western Atlantic animals, and *H. ovalis*—an IWP species in the genus *Lamprohaminoea*, but a name commonly used to also refer to an Eastern Pacific lineage.

With this work, progress was made to underhand the diversity of *Haminoea* snails and the relationships between species, and for the first time a phylogeny of the genus is presented. This new framework combined with a detailed study of shells, morpho-anatomy of wet specimens, revision of original descriptions and type material, can help solve the many issues remaining with the taxonomy of *Haminoea* snails in the Atlantic and Eastern Pacific Oceans.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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