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Molecular and morphological systematics of neustonic nudibranchs (Mollusca : Gastropoda : Glaucidae : *Glaucus*), with descriptions of three new cryptic species

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Abstract. A recent molecular phylogenetic study on *Glaucus*, a genus of neustonic aeolid nudibranchs, revealed undescribed cryptic diversity. *Glaucus atlanticus* is sister to the traditional species *Glaucus marginatus*, which is a complex of four genetically distinct cryptic species (Informal clade 'Marginatus'). The present paper revises the systematics of *Glaucus* and provides formal descriptions for three new species in the informal clade 'Marginatus' substantiated by species delimitation analyses. Molecular and morphological evidence confirms that the type species of *Glaucus*, *Glaucus atlanticus*, has a cosmopolitan subtropical distribution and is characterised by having a uniseriate ceratal arrangement, a penial spine and a longitudinal, medial silver stripe on the sole of the foot. Examination of type material indicates that the name *G. marginatus* should be retained for the most widespread of these species, found in the Indian and Pacific Oceans. This species is characterised by molecular diagnostic characters as well as the presence of a bursa copulatrix. *Glaucus marginatus* is sister to the undescribed species *Glaucus bennettae*, sp. nov., which is found in the South Pacific Ocean and lacks a bursa copulatrix. The other two undescribed species, *Glaucus thompsoni*, sp. nov. and *Glaucus mcfarlanei*, sp. nov. are only known from the North Pacific Ocean, and are characterised by molecular diagnostic characters as well as possessing and lacking a bursa copulatrix, respectively. Because sister species of *Glaucus* differ in their reproductive anatomy, we hypothesise that mating behaviour has played a role in cladogenesis in this group.

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Introduction

Glaucinin nudibranchs are the only lineage of truly pelagic sea slugs (Valdés and Angulo Campillo 2004). Floating upside-down at the surface of the planet's tropical and subtropical oceans, glaucinins are not strong swimmers, and thus are transported primarily by wind-driven surface currents (Thompson 1976; Lalli and Gilmer 1989). They maintain buoyancy by holding swallowed air in their chitinised gastric cavities (Valdés and Angulo Campillo 2004; Thompson 1976; Thompson and McFarlane 1967). Like bubble-rafting janthinid snails, glaucinins are neustonic predators, and primarily consume the drifting hydrozoan cnidarians *Porpita porpita* (Linnaeus, 1758), *Velella velella* (Linnaeus, 1758) and *Physalia physalis* (Linnaeus, 1758) (Lalli and Gilmer 1989). Although glaucinins display several unique adaptations to a neustonic ecology, including countershading, an expanded, flattened body shape and a muscular posterior sphincter in the gastric cavity for air bubble retention, many aspects of glaucinin morphology have not changed appreciably from their benthic counterparts (Thompson 1976). The presence of cnidosacs, muscular repositories of undischarged cnidarian nematocysts, in the tips of the cerata, characterises glaucinins as members of the Aeolidacea (Thompson and McFarlane 1967; Martin *et al*. 2009).

Since the first published record of a glaucinin in 1705 with the vernacular name 'Hirudinis marina' Breyn, 1705 (see Thompson and McFarlane 1967), there have been many published and unpublished descriptions of these unique sea slugs. Based on both anatomical and external differences, two monotypic genera have been traditionally recognised: *Glaucus* Forster, 1777 and *Glaucilla* Bergh, 1860 (Macnae 1954; Thompson and Bennett 1970). However, based on morphological examination of material from the Pacific Ocean, Valdés and Angulo Campillo (2004) concluded that *Glaucilla* should be synonymised with *Glaucus*, which contains two valid species *Glaucus atlanticus*

Forster, 1777 and *Glaucus marginatus* (Bergh, 1860) [sic.] (Fig. 1). Notwithstanding its derived ecology, *Glaucus* is the type genus (and *G. atlanticus* the type species) of the large family Glaucidae Gray, 1827. Synapomorphies for the family Glaucidae include simple oral glands, and the distinctive morphologies of the feeding structures (jaws and sharp radular teeth) and reproductive systems (Miller 1974), but family-level aeolid relationships remain unresolved (Valdés and Angulo Campillo 2004).

In a recent multigene molecular phylogeny of glaucinins and benthic aeolids (Fig. 2), Churchill *et al*. (2013) found that the Indo-Pacific species *G. marginatus* constitutes a cryptic complex of four distinct lineages. In the present study, we propose a new taxonomy for *Glaucus* based on published molecular data and newly conducted analyses, as well as an examination of the glaucinin reproductive system based on anatomy and histology. We also examine the level of genetic differentiation within *Glaucus* based on sequence data from two mitochondrial genes and specimens covering the entire range of this genus. These data indicate that all four *G. marginatus* cryptic lineages have speciated; thus, we redescribe *G. atlanticus*, *G. marginatus*, and three new species within the *G. marginatus* complex.

Materials and methods

Source of specimens

In total, 143 glaucinin specimens are included in this study. They were obtained from various sources: a global sampling of neustonic invertebrates collected over six years (2006–2012) (Churchill *et al*. 2013), donations by colleagues, or museum collections. Table 1 shows their respective locality data, museum catalogue numbers and GenBank accession numbers for sequences generated in this study. Table 2 shows, for every ocean gyre, the species present and indicates which species were collected in the same neuston tow (sympatric). Specimens were initially fixed and preserved in 95% EtOH. All the specimens are deposited at one of the following institutions: University of Michigan Museum of Zoology (UMMZ); Australian Museum Sydney (AMS); the Natural History Museum of Los Angeles County (LACM); the Department of Invertebrate Zoology and Geology, California Academy of Sciences, San Francisco (CASIZ); the Western Australia Museum (WAM); and the Natal Museum (NM). Additionally, type material from the Zoological Museum of the University of Copenhagen, Denmark (ZMUC) was examined (Fig. 3).

Morphological examination and histology

In total, 45 specimens, representing *Glaucus atlanticus* ($n = 10$) and all four cryptic lineages of *G. marginatus* ($n = 35$), were examined by either dissection or histology (Table 1). Reproductive systems were dissected as in Churchill *et al*. (2013). A representative sampling of glaucinin radulae, jaws and penial spines (*G. atlanticus* only) were dissected for examination with a scanning electron microscope (SEM) Hitachi S-3000N at the Natural History Museum of Los Angeles County. For the feeding structures, the buccal bulb was dissected by making a dorsal incision above the anterior gastric cavity; the penis was dissected by making a lateral incision on the right side of the body, posterior to the anterior peduncle. To isolate hard parts, tissue was digested in a 10% household bleach solution or sodium hydroxide for ~30 min until only chitinous structures remained. Jaws, radulae and penial spines were then rinsed with water, dried, mounted and sputter coated for examination with an SEM. For each of the five *Glaucus* lineages, two ethanol-preserved, reproductively mature specimens were prepared for transverse and sagittal plane sections. All histology was performed at the University of Michigan Medical School Microscopy and Image Analysis Laboratory (MIL) Biomedical Research Core Facility. Specimens were processed using a Leica ASP 300 paraffin tissue processor and embedded in paraffin using

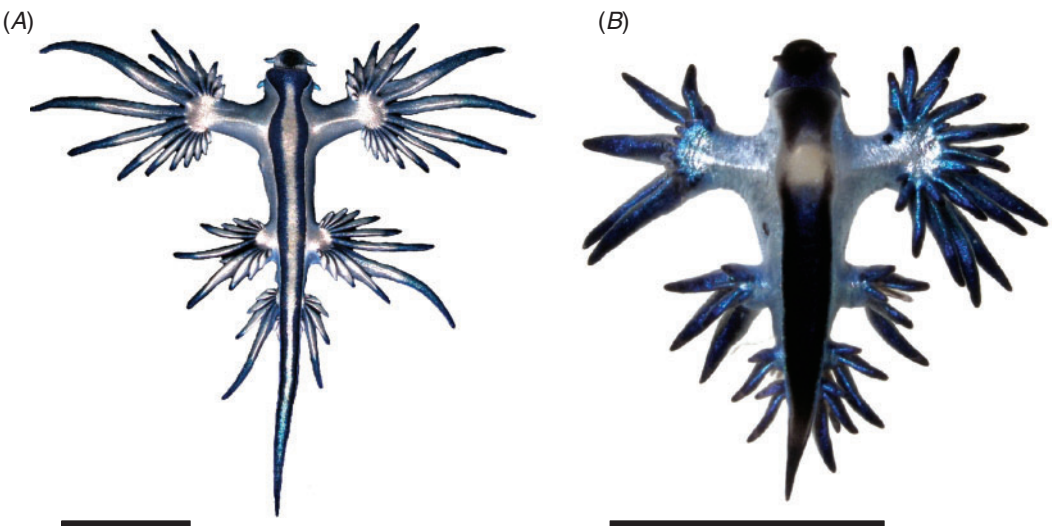

Fig. 1. Live photos of two glaucinin morphospecies. (*A*) *Glaucus atlanticus*; cosmopolitan range. (*B*) *Glaucus marginatus* (unknown S. Pacific lineage); Indo-Pacific range. Scale bars = 10 mm. Photos by Denis Riek.

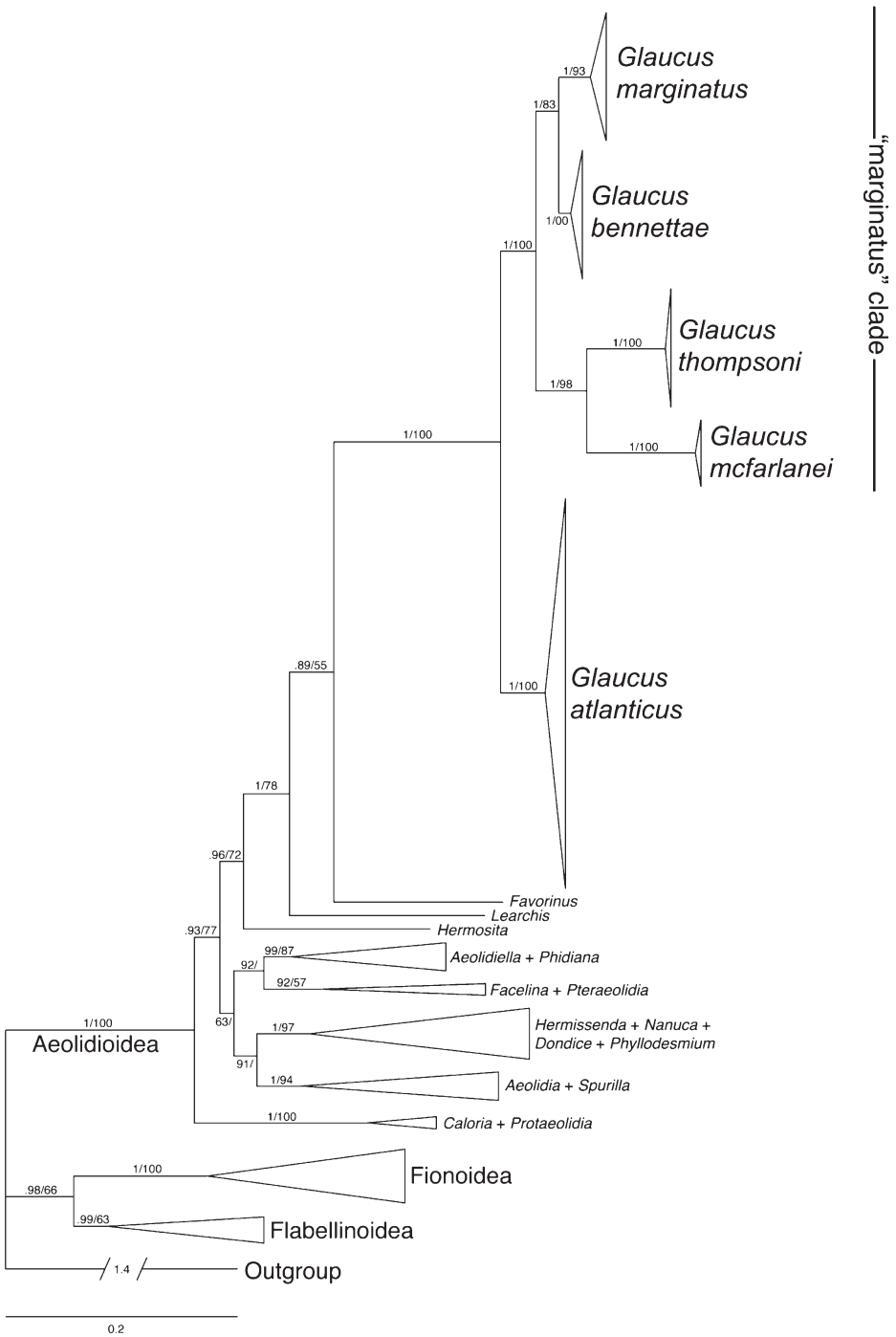

Fig. 2. Schematic representation of the Bayesian consensus phylogenetic tree of glaucinin nudibranchs based on four molecular markers (after Churchill *et al*. (2013): supplemental fig. 3, modified and republished with permission). Statistical support percentages are shown on internal branches. Bayesian posterior probabilities precede maximum-likelihood bootstrap values. The names for the new species here described are assigned to the appropriate clades.

a Leica Tissue-Tek paraffin embedding station. Serial sections were cut at 5–7 μm using a Leica 2155 rotary paraffin microtome. Sections were mounted and stained with haematoxylin and eosin. Slides were viewed with an Olympus BX-51 upright light microscope and photographed with an Olympus DP-70 high-resolution digital camera.

Table 1. Material examined in the molecular analyses
Specimens marked with an asterisk were examined morphologically

Species	Isolate	Voucher	Gyre	Decimal coordinates	COI	16S
G. atlanticus	01*	UMMZ 302975	North Atlantic	27.4965, –84.9965	JQ699574	JQ699488
G. atlanticus	02*	UMMZ 302975	North Atlantic	27.4965, –84.9965	JQ699575	JQ699489
G. atlanticus	03*	no voucher	North Atlantic	27.4965, –84.9965	JQ699576	JQ699490
G. atlanticus	04*	UMMZ 302975	North Atlantic	27.4965, –84.9965	JQ699577	JQ699491
G. atlanticus	05*	UMMZ 302975	North Atlantic	27.4965, –84.9965	JQ699578	JQ699492
G. atlanticus	06	no voucher	North Atlantic	28.6627, –85.488	JQ699579	JQ699493
G. atlanticus	07*	UMMZ 302976	North Atlantic	27.4997, –86.0057	JQ699580	JQ699494
G. atlanticus	08	UMMZ 302977	North Atlantic	28.0004, –87.4846	JQ699581	JQ699495
G. atlanticus	09*	UMMZ 302978	North Atlantic	24.4932, 83.5035	JQ699582	JQ699496
G. atlanticus	10*	UMMZ 302979	North Atlantic	26.0047, 86.0083	JQ699583	JQ699497
G. atlanticus	11*	UMMZ 302980	South Pacific	–4.265, –141.715	JQ699584	JQ699498
G. atlanticus	12*	UMMZ 302981	North Pacific	19.355, –156.7217	JQ699585	JQ699499
G. atlanticus	13*	UMMZ 302982	North Pacific	19.5212, –156.222	JQ699586	JQ699500
G. atlanticus	14	AMS C.462956	South Pacific	–33.738, 151.31	JQ699587	JQ699501
G. atlanticus	15*	UMMZ 302983	North Pacific	19.355, –156.7217	JQ699588	JQ699502
G. atlanticus	16*	UMMZ 302983	North Pacific	19.355, –156.7217	JQ699589	JQ699503
G. atlanticus	17	AMS C.462956	South Pacific	–33.738, 151.31	JQ699590	JQ699504
G. atlanticus	18*	UMMZ 302983	North Pacific	19.355, –156.7217	JQ699591	JQ699505
G. atlanticus	19	AMS C.462956	South Pacific	–33.738, 151.31	JQ699592	JQ699506
G. atlanticus	20	AMS C.462956	South Pacific	–33.738, 151.31	JQ699593	JQ699507
G. atlanticus	21	WAM S59354	Indian	–32.05, 115.7333	JQ699594	JQ699508
G. atlanticus	22	WAM S59354	Indian	–32.05, 115.7333	JQ699595	JQ699509
G. atlanticus	23	WAM S59354	Indian	–32.05, 115.7333	JQ699596	JQ699510
G. atlanticus	24	WAM S59354	Indian	–32.05, 115.7333	JQ699597	JQ699511
G. atlanticus	25	WAM S59354	Indian	–32.05, 115.7333	JQ699598	JQ699512
G. atlanticus	26	WAM S59354	Indian	–32.05, 115.7333	JQ699599	JQ699513
G. atlanticus	27	NM W7469	Indian	–30.095, 30.862	JQ699600	JQ699514
G. atlanticus	28	NM W7469	Indian	–30.095, 30.862	JQ699601	JQ699515
G. atlanticus	29	NM W7469	Indian	–30.095, 30.862	JQ699602	JQ699516
G. atlanticus	30	NM W7469	Indian	–30.095, 30.862	JQ699603	JQ699517
G. marginatus	A1*	CASIZ 176985	Indian	–21.8, 35.59	JQ699604	JQ699518
G. marginatus	A2	CASIZ 176985	Indian	–21.8, 35.59	JQ699605	JQ699519
G. marginatus	A3	CASIZ 176985	Indian	–21.8, 35.59	JQ699606	JQ699520
G. marginatus	A4	CASIZ 176985	Indian	–21.8, 35.59	JQ699607	JQ699521
G. marginatus	A5*	UMMZ 302984	North Pacific	19.5212, –156.222	JQ699608	JQ699522
G. marginatus	A6*	UMMZ 302985	North Pacific	19.355, –156.7217	JQ699609	JQ699523
G. marginatus	A7*	UMMZ 302985	North Pacific	19.355, –156.7217	JQ699610	JQ699524
G. marginatus	A8*	UMMZ 302986	North Pacific	25.082, –114.481	JQ699611	JQ699525
G. marginatus	A9	UMMZ 302986	North Pacific	25.082, –114.481	JQ699612	JQ699526
G. marginatus	A10	UMMZ 302987	South Pacific	–4.265, –141.715	JQ699613	JQ699527
G. bennettae	B1*	AMS C.462957	South Pacific	–33.738, 151.31	–	JQ699528
G. bennettae	B2*	AMS C.462957	South Pacific	–33.738, 151.31	–	JQ699529
G. bennettae	B3*	AMS C.462957	South Pacific	–33.738, 151.31	–	JQ699530
G. bennettae	B4*	AMS C.462957	South Pacific	–33.738, 151.31	–	JQ699531
G. bennettae	B5*	AMS C.462957	South Pacific	–33.738, 151.31	–	JQ699532
G. bennettae	B6*	AMS C.462957	South Pacific	–33.738, 151.31	–	JQ699533
G. bennettae	B7	AMS C.462957	South Pacific	–33.738, 151.31	–	JQ699534
G. bennettae	B8	AMS C.462957	South Pacific	–33.738, 151.31	–	JQ699535
G. bennettae	B9	AMS C.462957	South Pacific	–33.738, 151.31	–	JQ699536
G. bennettae	B10	AMS C.462957	South Pacific	–33.738, 151.31	–	JQ699537
G. thompsoni	C1*	UMMZ 302988	North Pacific	25.082, –114.481	JQ699614	JQ699538
G. thompsoni	C2*	UMMZ 302988	North Pacific	25.082, –114.481	JQ699615	JQ699539
G. thompsoni	C3*	UMMZ 302988	North Pacific	25.082, –114.481	JQ699616	JQ699540
G. thompsoni	C4	UMMZ 302988	North Pacific	25.082, –114.481	JQ699617	JQ699541
G. thompsoni	C5	UMMZ 302988	North Pacific	25.082, –114.481	JQ699618	JQ699542
G. thompsoni	C6*	UMMZ 302988	North Pacific	25.082, –114.481	JQ699619	JQ699543
G. thompsoni	C7*	UMMZ 302988	North Pacific	25.082, –114.481	JQ699620	JQ699544
G. thompsoni	C8	UMMZ 302988	North Pacific	25.082, –114.481	JQ699621	JQ699545

(*continued next page*)

Table 1. (*continued*)

Species	Isolate	Voucher	Gyre	Decimal coordinates	COI	16S
G. thompsoni	C9*	UMMZ 302988	North Pacific	25.082, –114.481	JQ699622	JQ699546
G. thompsoni	C10	UMMZ 302988	North Pacific	25.082, –114.481	JQ699623	JQ699547
G. mcfarlanei	D1*	UMMZ 302989	North Pacific	19.48, –156.2883	JQ699624	JQ699548
G. mcfarlanei	D2*	UMMZ 302989	North Pacific	19.48, –156.2883	JQ699625	JQ699549
G. mcfarlanei	D3*	UMMZ 302989	North Pacific	19.48, –156.2883	JQ699626	JQ699550
G. mcfarlanei	D4	UMMZ 302989	North Pacific	19.48, –156.2883	JQ699627	JQ699551
G. mcfarlanei	D5*	UMMZ 302990	North Pacific	19.355, –156.7212	JQ699628	JQ699552
G. mcfarlanei	D6	no voucher	North Pacific	19.48, –156.2883	JQ699629	JQ699553
H. hakunamatata	–	LACM 175023	–	–	JQ699631	JQ699555
L. poica	–	CPIC 00137	–	–	JQ699632	JQ699556
F. elenalexarium	–	CASIZ178875	–	–	HM16275	HM16267

Table 2. Glaucinin species present in each subtropical oceanic gyre
Species collected in the same neuston tow are grouped in parentheses

Gyre	*Glaucus* species present
North Atlantic	*G. atlanticus*
South Atlantic	*G. atlanticus*
North Pacific	(*G. atlanticus*, *G. marginatus*, *G. mcfarlanei*); (*G. marginatus*, *G. thompsoni*)
South Pacific	(*G. atlanticus*, *G. bennettae*)
Indian	*G. atlanticus*; *G. Marginatus*

DNA extraction, PCR amplification, sequencing and phylogenetic analysis

DNA extraction was performed as in Churchill *et al*. (2013) using the E.Z.N.A. Mollusc DNA Kit (Omega-Biotek). Up to 50 mg of pedal or ceratal tissue was cut into fine pieces before tissue digestion; thereafter the manufacturer's protocol was followed. Possible contamination with cnidarian DNA in ceratal tissue extractions was ruled out by checking all amplified sequences against the NCBI GenBank database to confirm aeolid taxonomic affinities. DNA was successfully amplified for all specimens, except for one population of *Glacus marginatus* (South Pacific), for which no COI sequences were obtained. PCR reactions were prepared as in Churchill *et al*. (2013) for two mitochondrial molecular markers, 16S rRNA and cytochrome oxidase I (COI). Sequences were assembled, edited and aligned with the same parameters as in Churchill *et al*. (2013).

Phylogenetic analyses

The four-gene analysis (nDNA 28S rRNA and H3; mtDNA 16S rRNA and COI; total of 2921 aligned nucleotides) conducted by Churchill *et al*. (2013) and reproduced in Fig. 2 is used as the phylogenetic framework for this study. Separate analyses were conducted for COI and 16S to test the congruency between gene trees and the concatenated analysis. The two nuclear genes (28S and H3) also included in the analysis by Churchill *et al*. (2013) did not provide enough resolution to delineate cryptic species within *Glaucus marginatus* and therefore were not included in this study. Based on the topology of the phylogeny produced by Churchill *et al*. (2013), *Hermosita hakunamatata* (Ortea, Caballer & Espinosa, 2003), which is closely related to *Glaucus*, was selected as the outgroup. Also, the species *Learchis poica* Marcus & Marcus, 1960 and *Favourinus elenalexarium* García & Troncoso, 2001 were included in both analyses. The Akaike information criterion (Akaike 1974) was executed in MrModeltest (Nylander 2004) to determine the best-fit model of evolution for each gene (GTR+I+Γ). The Bayesian analyses were executed in MrBayes 3.2.1 (Huelsenbeck and Ronquist 2001), partitioned by gene (unlinked). The Markov chain Monte Carlo analysis was run with two runs of six chains for fifty million generations, with sampling every 100 generations. The default 25% burn-in was applied before constructing majority-rule consensus tree(s). Convergence of runs was diagnosed using the program Tracer 1.3 (Rambaut and Drummond 2007). The maximum likelihood analyses were implemented in raxmlGUI 1.0 (Silvestro and Michalak 2012) using the bootstrap (thorough) + consensus option (10 000 replicates) and the GTR +I+Γ model.

Molecular diagnostic characters and species delimitation analyses

Molecular diagnostic characters for all clades recovered in the COI and 16S alignments were detected using the Characteristic Attribute Organization System (CAOS-Analyzer) (Sarkar *et al*. 2008) implemented in the online version of the software package (http://bol.uvm.edu/caos-workbench/caos_analyzer.php). CAOS-Analyzer can extract diagnostic character states from molecular sequences that have been hierarchically organised into phylogenetic trees. Only diagnostics with values of 1.00 (pure diagnostics) were considered, as in Jörger and Schrödl (2013).

Three different methods were used to provide support for species delimitations and substantiate the description of the new taxa. First, an F_{ST} analysis was implemented in Arlequin 3.5.12 (Excoffier and Lischer 2010) for 16S (no COI sequences are unavailable for one of the putative species) to determine the proportion of the total genetic variance contained in the candidate species relative to the total genetic variance. F_{ST} values were calculated as a measure of pairwise differences between *G. atlanticus* and all populations in the *G. marginatus* species complex. The significance of the pairwise F_{ST} value was estimated by performing 16 000 permutations. Second, the Species Delimitation plugin (Masters *et al*. 2011) for Geneious Pro 4.7.4 (Drummond *et al*. 2009) was used to provide a statistical framework to help determine whether clades obtained in the

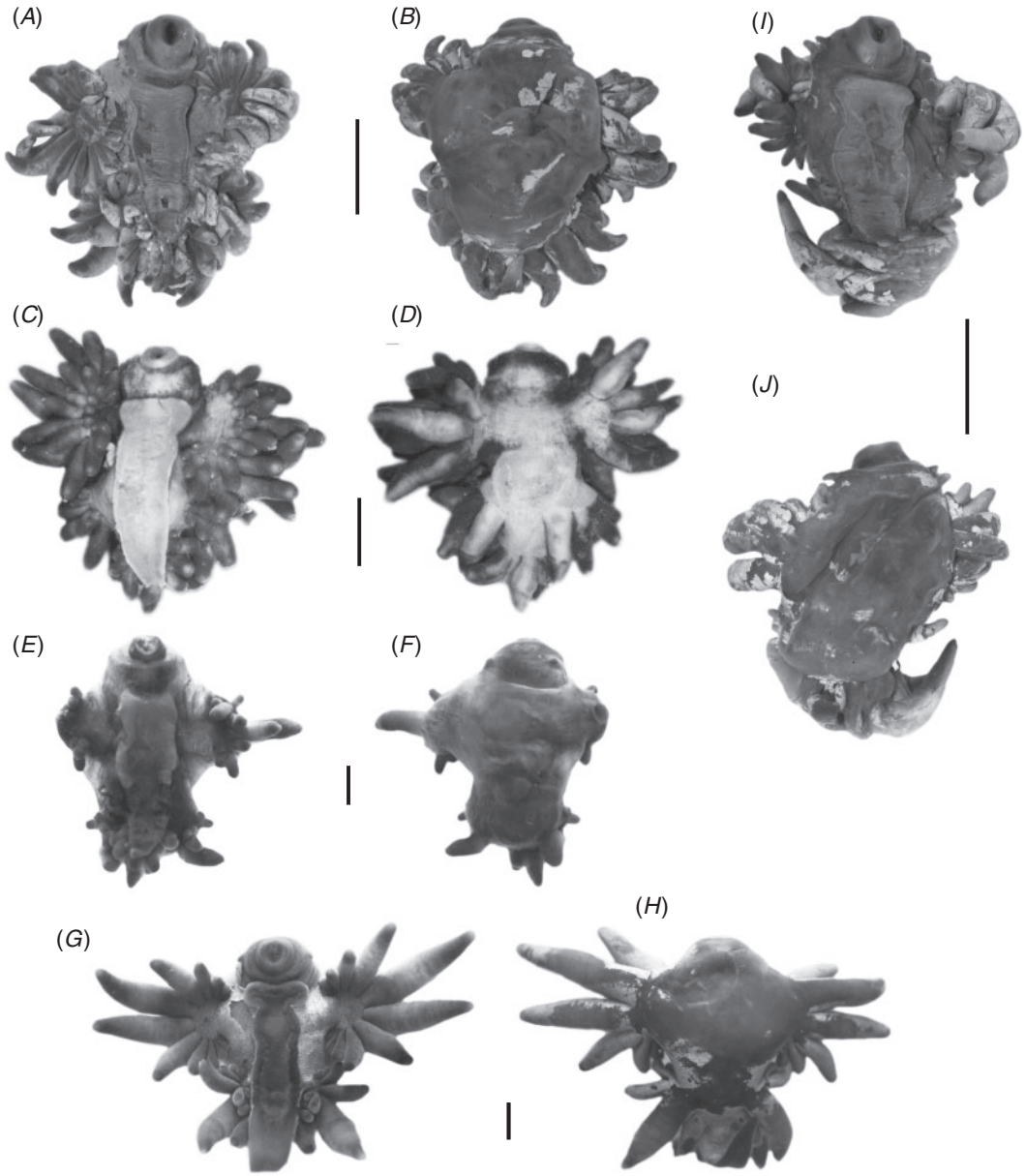

Fig. 3. Photographs of preserved types housed at the Zoological Museum of the University of Copenhagen (ZMUC) gastropod collection. (*A–B*) Syntype of *Glaucus lineatus* (ZMUC GAS-2157), scale bar = 5 mm. (*C–D*) Holotype of *Glaucilla briareus* (ZMUC GAS-2159), scale bar = 2 mm. (*E–F*) Syntype of *Glaucilla marginata* (ZMUC GAS-2158), scale bar = 1 mm. (*G–H*) Syntype of *Glaucus gracilis* (ZMUC GAS-2155), scale bar = 1 mm. (*I–J*) Syntype of *Glaucus longicirrus* (ZMUC GAS-2156), scale bar = 5 mm.

concatenated phylogenetic analyses have identity as distinct species. The statistics implemented were the ratio between the mean distance within the members of the clade and the mean distance of those individuals to the nearest clade (the larger the value of this ratio is, the more distinct is the identity of the group) and the p ID, which represents the mean probability, 95% confidence interval, for a member of the putative species to fit inside (strict p ID), or at least to be the sister group (liberal p ID) of the clade made up by the other individuals belonging to this species. Third, the Automatic Barcode Gap Discovery (ABGD) method (Puillandre *et al*. 2012) was implemented to compute the theoretical maximal limit of the intraspecific diversity (using a coalescent model). ABGD identifies in the entire distribution of pairwise distances whose gap (superior to the maximal limit of the intraspecific diversity) potentially corresponds to the so-called 'barcoding gap', a hypothetical limit between intra- and interspecific diversity (Puillandre *et al*. 2012). Inference of the limit and gap detection are then recursively applied to previously obtained groups to get finer partitions until there is no further partitioning. The online version of the software (http://wwwabi.snv.jussieu.fr/public/abgd/) was used to analyse the COI and 16S datasets. MEGA 4.0 (Tamura *et al*. 2007) was used to build the

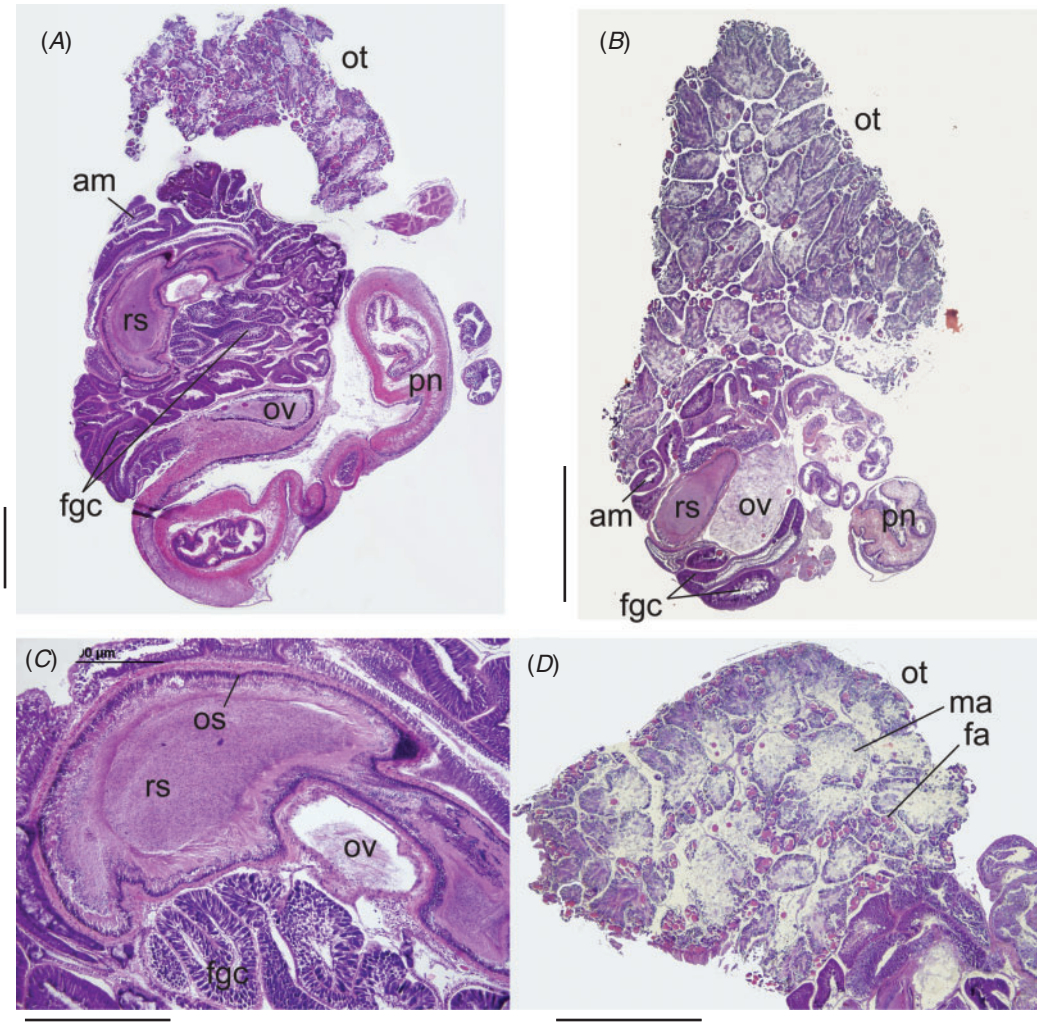

Fig. 4. Histology of the glaucinin reproductive system emphasising undescribed or under-described features. (*A*) *Glaucus atlanticus* (AMS C.462956), transverse section. View of the entire reproductive system from composite slide photographs. The proximal ovotestis (ot) is composed of evenly dispersed male and female acini. The receptaculum seminis is visible as a large organ distal to the ampulla. (*B*) *Glaucus marginatus* (AMS C.462957), transverse section. View of the entire reproductive system from composite slide photographs. Ovotestis resembles *G. atlanticus* in acinar distrubution. C. *Glaucus atlanticus* (AMS C.462956), transverse section. Detail of receptaculum seminis containing oriented spermatozoa. (*D*) *Glaucus marginatus* (AMS C.462957), sagittal section. Detail of ovotestis (ot) showing interspersed male (ma) and female (fa) acini. Abbreviations: am, ampulla; fa, female acinus; fgc, female gland complex; ma, male acinus; os; oriented spermatozoa; ot, ovotestis; ov, oviduct; pn, penis; rs, receptaculum seminis. Scale bars: A, B, D = 1.0 mm; C = 500 µm.

distance matrices for COI and 16S using a Tamura Nei model. The data were analysed using the two available models: Jukes–Cantor (JC69) and Kimura (K80). The program requires two user-specified values: P (prior limit to intraspecific diversity) and X (proxy for minimum gap width). To evaluate the effect on the datasets, X values from 0.1 to 5 were tested and the maximum Pmax value was extended from 0.1 to 0.2 (as in Jörger *et al.* 2012).

Results

Redescription of glaucinin reproductive system

Proximal reproductive system

Glaucinins are simultaneous hermaphrodites. The gonad is located on the right side of the body, immediately posterior to the gastric cavity. Unlike Thompson and McFarlane (1967), we did not observe a mediolateral gradient of male to female acini. In all specimens examined here, smaller female acini are interspersed between larger male acini. The former contain few numbers of oocytes at both previtellogenic and vitellogenic developmental stages, while the latter contain bunches of spermatozoa, most of which are oriented with their heads towards the acinar walls (Fig. 4). Ductules within the gonad transport female and male gametes to the hermaphroditic duct, which extends to the distal reproductive system.

Distal reproductive system

Glaucinins have diaulic distal reproductive systems. The hermaphroditic duct connects to the ampulla, a long muscular

tube that appears very convoluted when empty. The ampulla branches into the male and female reproductive tracts. On the male side, a long, thin, convoluted prostate connects to the protrusible penis. On the female side, a series of blind reproductive organs connect with the oviduct. The most proximal is a receptaculum seminis, originally observed by Thompson and McFarlane (1967) in *Glaucus atlanticus*, which is often impossible to view in dissections of smaller specimens. It is shown here in histological sections only (Fig. 4). The receptaculum seminis is oblong, fluid-filled, and contains oriented spermatozoa. In some specimens it appears dark purple or black. Fertilisation occurs in the oviduct around and distal to the receptaculum seminis. Zygotes continue distally into the female gland complex, which consists of membrane, mucus, and capsule glands as described in other aeolids (Klussmann-Kolb 2001). Finally, the oviduct terminates at the vaginal opening, which is located posterior to the penial opening, inside the gonopore. In *G. atlanticus* and two *G. marginatus* morphospecies lineages (Indo-Pacific and Eastern North Pacific), a blind bursa copulatrix branches off the oviduct immediately proximal to the vaginal opening; no bursa copulatrix is present in the other two *G. marginatus* morphospecies (South Pacific and Kona) (Fig. 4). The bursa copulatrix is normally a thin walled structure that receives sperm after copulation (Valdés *et al*. 2010).

Phylogenetic analyses

Both Bayesian gene trees for the mitochondrial COI and 16S genes (Fig. 5) are similar to that obtained by Churchill *et al*. (2013). Species of *Glaucus* form a well supported clade (pp = 1) and *G. atlanticus* is sister to the rest of the species ('Marginatus' clade). The 'Marginatus' clade is well supported in the 16S analysis (pp = 0.95) and composed of two sister clades. The first of those clades (pp = 1) includes the Indo-Pacific and South Pacific populations (both monophyletic and well supported, pp = 1). The second clade (pp = 1) includes the North Pacific and Kona populations (both monophyletic and well supported, pp = 1). The topology and support values of the COI tree are the same, with the exception that no samples from the South Pacific population were included.

Molecular diagnostic characters and species delimitation analyses

Molecular diagnostic characters for all clades recovered in the COI and 16S alignments are summarised in Table 3 and shown in Appendix 1. Several unique substitutions in both genes for each clade provide strong support to separate them into different species. Additional support for the classification of the clades recovered in the phylogenetic analysis into different species is provided by the results of the F_{ST} analysis (Table 4), which indicates that all four clades of *G. marginatus* are genetically distinct and also distinct from *G. atlanticus*. This conclusion is substantiated with results from the species delimitation analysis (Table 5). From these data, it was concluded that all the groups included in the phylogenetic analysis are monophyletic, except for the outgroups, for which monophyly was not tested. The values of the ratio between the average distance between the samples of one group (Intra Dist) and the average distance between those samples and the closest clade (Inter Dist) are small in all cases, below 0.25, meaning that genetic differences within all clades are small relative to the differences between clades, so there is a well defined separation of clades (see López-López *et al*. 2012). Additionally, the probability (p ID) of a new sequence fitting inside (strict) or at least as sister group (liberal) of its clade is high (above 0.8) in all cases. These data confirm the genetic distinctiveness of the clades recovered in the phylogenetic analysis by Churchill *et al*. (2013) (Fig. 2) as well as the single gene analyses (Fig. 5) and, together with their consistent differences in genital anatomies, they are therefore treated as distinct species and described as such below. Finally, the ABGD analysis with the standard settings recovered 4 and 5 groups for COI and 16S respectively. These groups correspond to the clades recovered in the phylogenetic analyses, providing further support for the taxonomic decisions. ABGD grouping results were independent from the chosen model (Jukes–Cantor and Kimura) and unaffected by changes of prior limit of intraspecific variation or X-value.

Systematics

Family **GLAUCIDAE** Gray, 1827

Genus ***Glaucus*** Forster, 1777

Type species *Glaucus atlanticus* Forster, 1777 (by monotypy)

Glaucus atlanticus Forster, 1777

(Figs 1*A*, 6)

For a full list of synonyms see Thompson and McFarlane (1967).

Material examined

Glaucus lineatus: 2 syntypes from the South Pacific Subtropical Gyre System, ~710 nautical miles south-east of the Pitcairn Islands (36°18′S, 125°13′W), 8.i.1847, leg. Reinhardt (ZMUC GAS-2157).

Glaucus longicirrus: 4 syntypes from the North Pacific Subtropical Gyre System, ~670 nautical miles north-east of the Hawaiian Islands (30°9′–31°30′N, 149°27′–148°48′W), 27–29.ix.1846, leg. Reinhardt (ZMUC GAS-2156).

Glaucus gracilis: 3 syntypes from the North Atlantic Subtropical Gyre System, ~300 nautical miles south-west of the Canary Islands (25°N, 23°W), 5.xi.1857, leg. Capt. Hygom (ZMUC GAS-2155); 4 specimens from the Indian Ocean (NM W7469); 6 specimens from the Indian Ocean (WAM S59354); 4 specimens from the South Pacific (AMS C.462956); 4 specimens from the North Pacific (LACM 111410); 8 specimens from the Gulf of Mexico (UMMZ 302975–302789); 1 specimen from the South Pacific Subtropical Gyre system (UMMZ 302980); 5 specimens from the North Pacific Subtropical Gyre system (UMMZ 302981–302983) (see Table 1).

Diagnosis

This species can be distinguished by 70 molecular diagnostic characters in COI and 39 in 16S (Appendix 1). Morphologically it is characterised by having a uniseriate ceratal arrangement, presence of a penial spine, and by the presence of a longitudinal, medial silver stripe on the sole of the foot (Fig. 1*A*).

External anatomy (Fig. 1*A*)

Body slender and elongate. Head short and not distinctly separated from body. Cephalic tentacles and rhinophores short

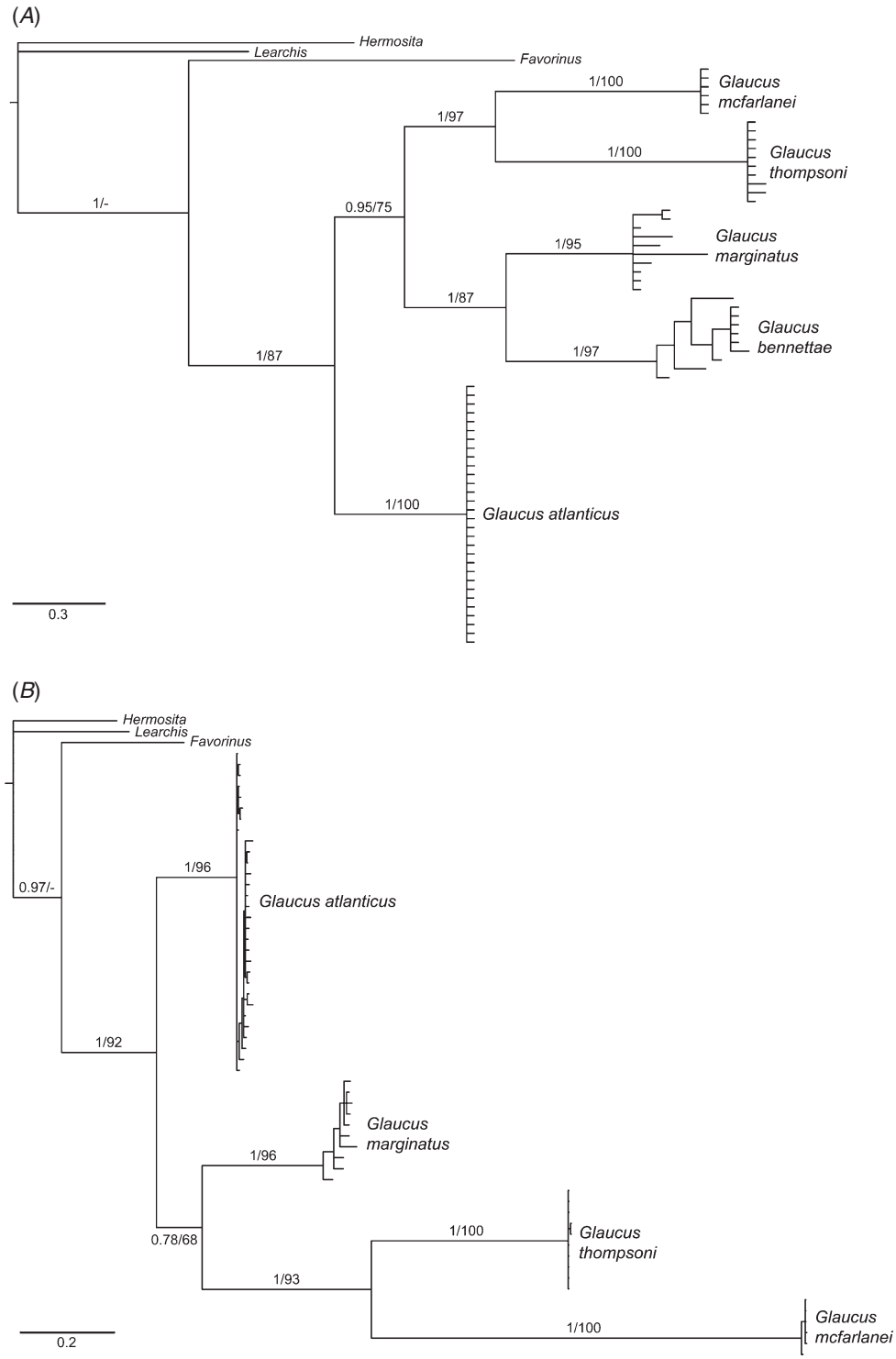

Fig. 5. Bayesian analysis consensus trees for the single mt gene analyses. Statistical support percentages are shown on internal branches. Bayesian posterior probabilities precede maximum-likelihood bootstrap values. The names for the new species here described are assigned to the appropriate clades. A. Large ribosomal (16S rDNA) gene; B. Cytochrome oxidase subunit one (COI) gene. Note that Churchill *et al*. (2013) were unsuccessful in obtaining mtCOI genotypes for *Glaucus bennettae*, sp. nov.

Table 3. Number of molecular diagnostic traits for *Glaucus atlanticus* and *Glaucus marginatus* morphospecies in mitochondrial 16S rRNA and COI obtained with CAOS-analyzer

For detailed information on the position in the alignments and the base letter see Appendix 1. Note that Churchill *et al*. (2013) were unsuccessful in obtaining mtCOI genotypes for *Glaucus bennettae*, sp. nov.

	G. atlanticus	*G. marginatus*	*G. bennettae*	*G. thompsoni*	*G. mcfarlanei*
COI	70	70	–	219	217
16S	39	17	19	34	36

Table 4. Matrix of the population group comparisons results for 16S, with F_{ST} values (lower triangular) and associated *P*-values (upper triangular)

After Bonferroni correction (10 comparisons) significant values are $P < 0.005$

	G. atlanticus	*G. marginatus* (Indo-Pacific)	*G. bennettae* (South Pacific)	*G. thompsoni* (North Pacific)	*G. mcfarlanei* (Kona)
G. atlanticus	–	0.00000	0.00000	0.00000	0.00000
G. marginatus (Indo-Pacific)	0.92666	–	0.00000	0.00000	0.00010
G. bennettae (South Pacific)	0.98969	0.97983	–	0.00000	0.00020
G. thompsoni (North Pacific)	0.96937	0.93854	0.99727	–	0.00010
G. mcfarlanei (Kona)	0.96867	0.92958	0.99645	0.99339	–

Table 5. Species delimitation results from the Bayesian consensus tree

Species	Closest species	Monophyly	Intra dist	Inter dist closest	Intra/Inter	p ID (strict)	p ID (liberal)
G. atlanticus	*G. bennettae*	yes	0.092	1.879	0.05	0.98	1.00
G. marginatus (Indo-Pacific)	*G. bennettae*	yes	0.202	1.170	0.17	0.88	0.96
G. bennettae (South Pacific)	*G. marginatus*	yes	0.147	1.170	0.13	0.91	0.97
G. thompsoni (North Pacific)	*G. bennettae*	yes	0.019	2.975	0.01	0.98	1.00
G. mcfarlanei (Kona)	*G. thompsoni*	yes	0.025	3.231	0.01	0.93	0.98

and smooth. Dorsal coloration white with silver reflective pigment. Ventral coloration blue and brownish on the lateral foot sole, with a medial longitudinal silver stripe. Lateral sides of the body coloured silver. Large, broad foot with rounded anterior edges. Four clusters of cerata on each side of the body. Three anterior ceratal groups pedunculate, posterior group sessile. Cerata conical, multiseriate, bluish silver at base, dark blue at tips. Cleioproct anus, situated between tops of second and third ceratal groups. Renal pore at the same level, in front of second ceratal group.

Internal anatomy

Diaulic reproductive system as described above. Because it was illustrated by Valdés and Angulo Campillo 2004) it is not figured here. Penis is muscular and possesses a spine, a single strong curved and hollow hook (Fig. 6*D–E*). The vagina connects to a small bursa copulatrix.

Geographic range

This species is widespread in the tropical and subtropical Atlantic, Indian and Pacific Oceans.

Informal clade 'Marginatus'

Remarks

We suggest the informal clade name, 'Marginatus,' for the cryptic species complex of the *Glaucus marginatus* morphospecies. *Glaucus marginatus* is both the oldest name for this morphospecies, and the cryptic lineage with the widest range (Indo-Pacific).

Glaucus marginatus (Reinhardt *in* Bergh, 1864)

(Figs 7 and 8)

Glaucilla marginata Reinhardt *in* Bergh, 1864: 296–300, fig. 9A.

Material examined

Glaucilla marginata: 3 syntypes from the North Pacific Subtropical Gyre System, ~640 nautical miles north-east of the Hawaiian Islands (29°37–52′N, 149°37′–150°12′W), 28–29.ix.1846, leg. Reinhardt (ZMUC GAS-2158); 6 specimens from the North Pacific Subtropical Gyre System (UMMZ 302984–302987) and 4 specimens from the Indian Ocean (CASIZ 176985) (see Table 1).

Diagnosis

This species can be distinguished geographically (if collected in the Indian Ocean) and by 70 molecular diagnostic characters in COI and 17 in 16S (Appendix 1). It differs morphologically from its sister species, *Glaucus bennettae*, in having a bursa copulatrix.

External anatomy

Body elongate but relatively wider than *G. atlanticus*, widest behind the head. Cephalic tentacles and rhinophores short and smooth. Dorsal coloration white with silver reflective pigment.

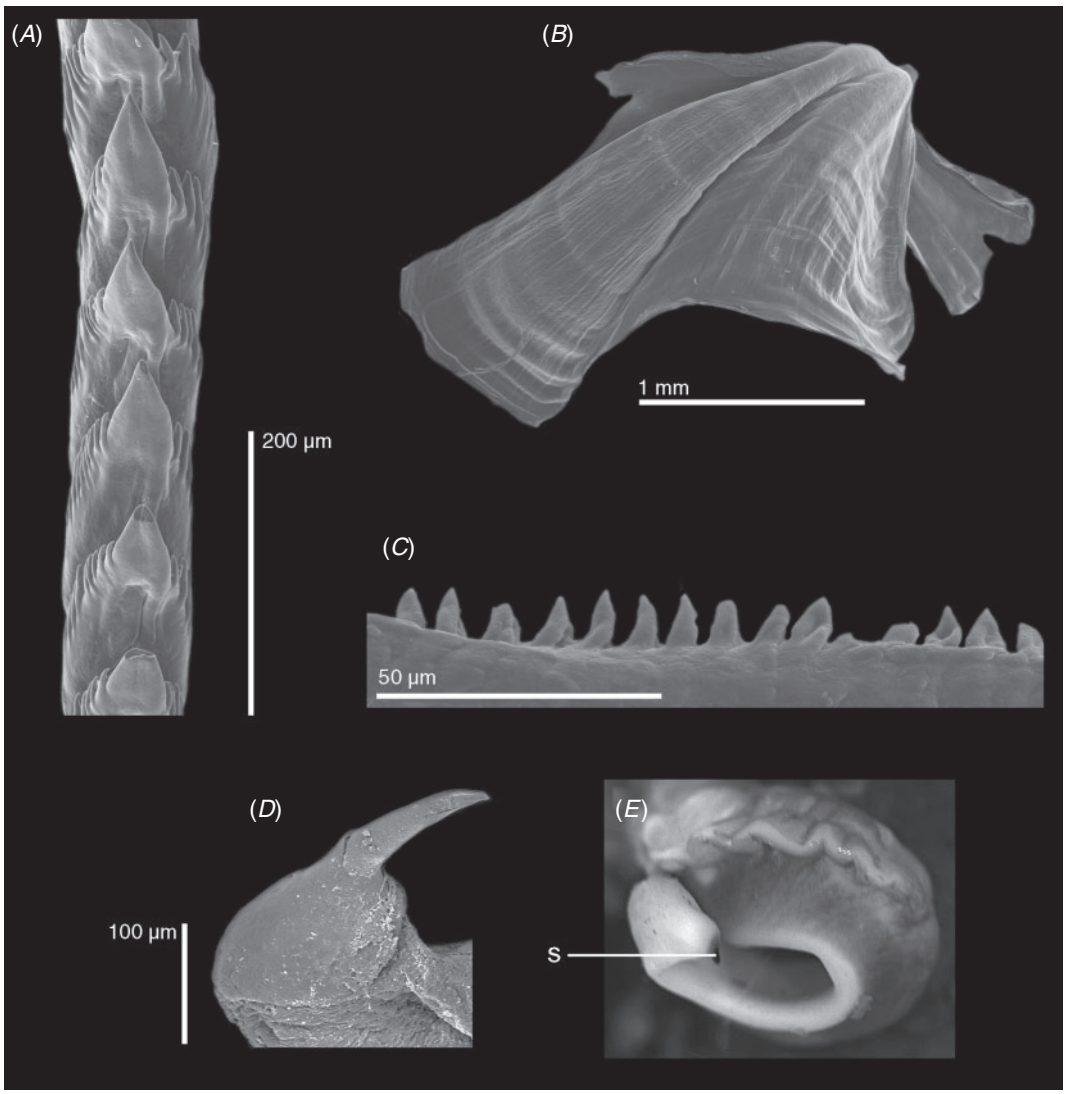

Fig. 6. Scanning electron micrographs and photographs of feeding and reproductive structures of *Glaucus atlanticus*. (*A*) Radular teeth of specimen AMS C.462956. (*B*) Jaw of specimen AMS C.462956. (*C*) Detail of the masticatory border of the jaw of specimen AMS C.462956. (Penial spine of specimen LACM 111410. (*E*) Photograph of the everted penis in preserved specimen AMS C.462956 showing the penial spine (s) on the tip.

Ventral coloration blue and brownish on the foot sole, white with silver reflective pigment around foot. Large, broad foot with rounded anterior edges. Four clusters of cerata on each side of the body. Anterior ceratal group angled towards head, three posterior groups perpendicular to midline of the body. Two anterior ceratal groups pedunculate, two posterior groups sessile and merged. Cerata conical, multiseriate, blue at base and white or transparent at tips. Cleioproct anus, situated between tops of second and third ceratal groups. Renal pore at the same level, in front of second ceratal group.

Internal anatomy

Diaulic reproductive system as described above (Fig. 7). Penis is muscular, lobed, and devoid of a spine. The vagina connects to a small bursa copulatrix. Radular formula 15 × 0.1.0 in a 6 mm long specimen and in a 4 mm long specimen (UMMZ 302985; respective sample codes 08GmNPKo and 22GmNPKo). Radular teeth elongate with a robust, conical central cusp, flanked by 3–9 denticles on each side (Fig. 7*A*). Denticles elongate and slightly curved with a sharp tip. Some specimens with small denticles on one of the sides of the central cusp. Masticatory border of the jaw with a single row of denticles (Fig. 7*B–C*). Denticles simple, conical with one or two small tubercles on some of them.

Geographic range

This species is widespread in the tropical and subtropical Indo-Pacific region.

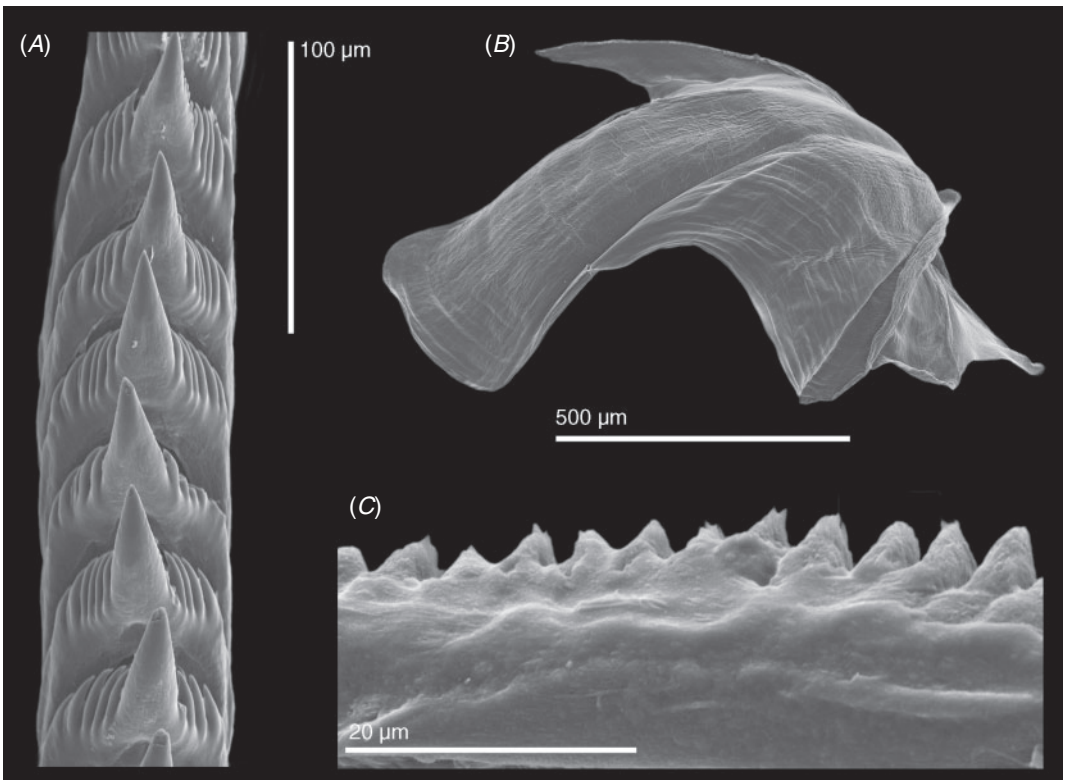

Fig. 7. Scanning electron micrographs of feeding structures of *Glaucus marginatus*. (*A*) Radula of specimen UMMZ 302985. (*B*) Jaw of specimen UMMZ 302985. (*C*) Masticatory border of the jaw of specimen UMMZ 302985.

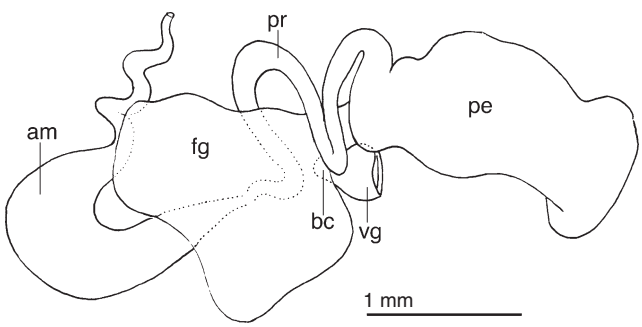

Fig. 8. Anatomical drawing of the distal reproductive system of *Glaucus marginatus* (UMMZ 302985). Abbreviations: am, ampulla; ag, accessory gland; fg, female gland complex; pe, penis; pr, prostate.

Glaucus bennettae, sp. nov.

(Figs 9*A*, 10, 11)

ZooBank LSID
http://zoobank.org/NomenclaturalActs/385821D1-51B9-496C-9B1E-DB23DF9DB9E8

Material examined

Holotype. Collaroy, Sydney, Australia (33.738S, 151.31E), 17.i.2009, leg. Phil Colman (UMMZ 400001).
Paratypes. Same data as for holotype, 9 specimens (AMS C.462957).

Diagnosis

This species can be distinguished by 19 molecular diagnostic traits in 16S (Appendix 1) and differs morphologically from its sister species, *Glaucus marginatus*, by lacking a bursa copulatrix.

External anatomy

External anatomy identical to *G. marginatus* (Fig. 9*A*).

Internal anatomy

Diaulic reproductive system as described above (Fig. 11). Penis is muscular, lobed, and devoid of a spine. No bursa copulatrix present; the vagina connects proximally to the oviduct. Radular formula 19 × 0.1.0 in a 14 mm long specimen (AMS C.462957). Radular teeth elongate with a conical, relatively short and robust central cusp, flanked by 4–9 denticles on each side (Fig. 10*A*, *B*). Denticles elongate and slightly curved. Masticatory border of the jaw with a single row of denticles (Fig. 10*C*–*E*). Denticles short, and widen covered by several small tubercles.

Geographic range

This species has only been found in the South Pacific Subtropical Gyre System.

Etymology

Named after the marine biologist and Australian Academy of Science member Isobel Bennett (1909–2008), who wrote the first

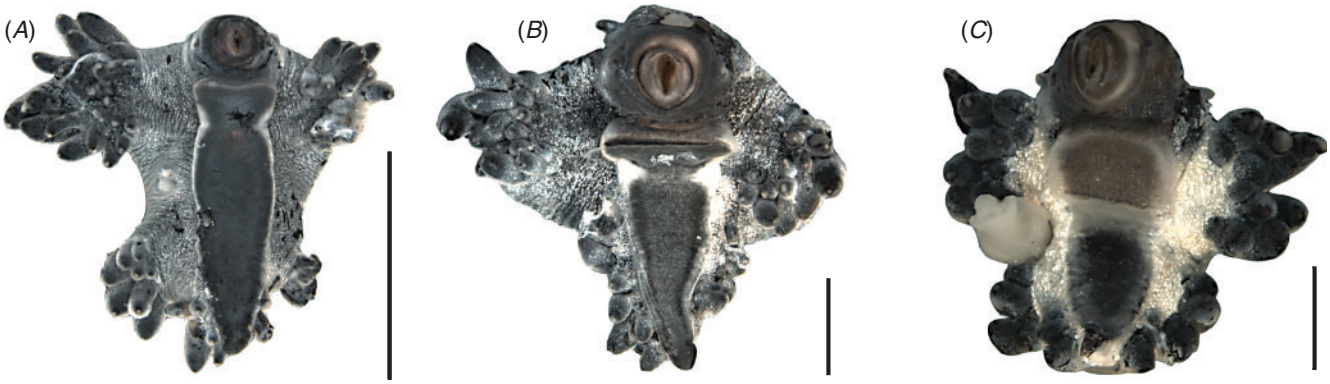

Fig. 9. Photographs of the preserved holotypes of three new species of glaucinin nudibranchs (ventral views). (*A*) *Glaucus bennettae*, sp. nov. (UMMZ 400001), scale bar = 5 mm. (*B*) *Glaucus thompsoni*, sp. nov. (UMMZ 400004), scale bar = 1 mm. (*C*) *Glaucus mcfarlanei*, sp. nov. (UMMZ 400006), scale bar = 1 mm.

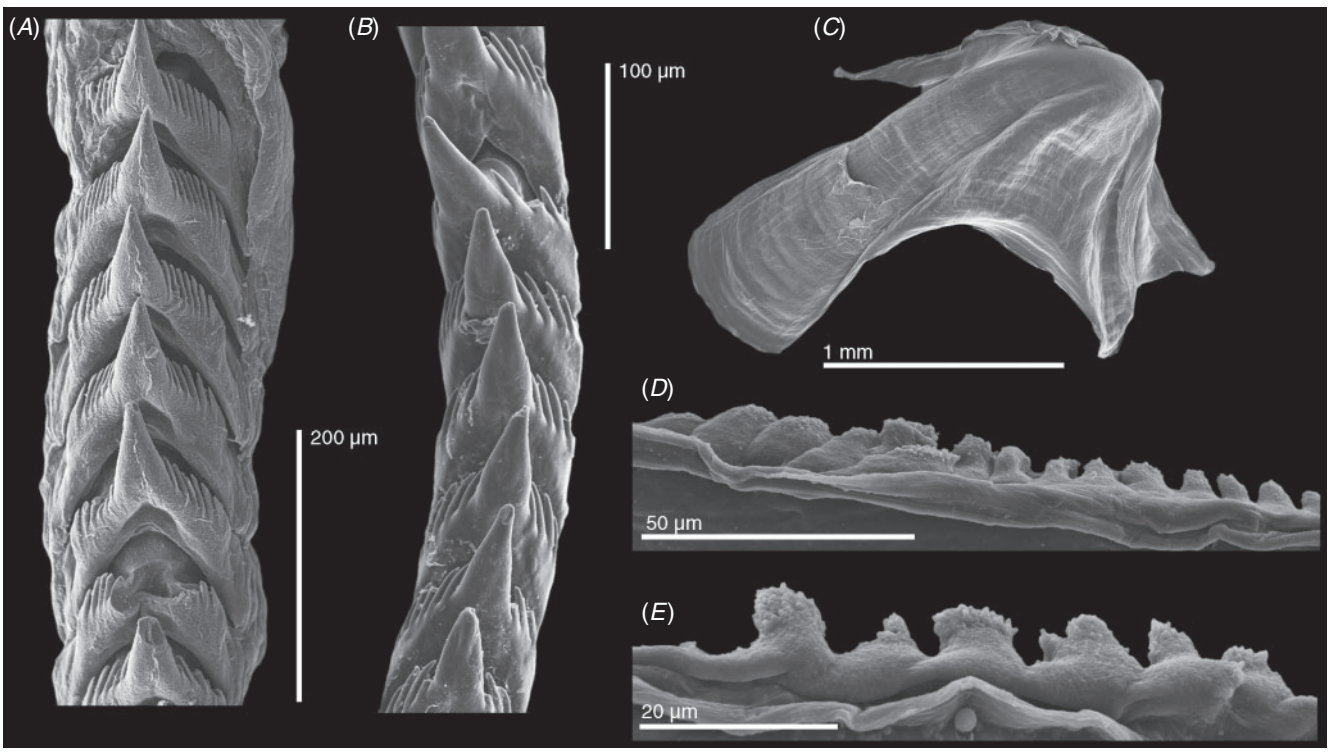

Fig. 10. Scanning electron micrographs of feeding structures of *Glaucus bennettae*, sp. nov. (*A*) Radula of specimen AMS C.462957. (*B*) Radula of specimen AMS C.462957. (*C*) Jaw of specimen AMS C.462957. (*D*) Masticatory border of the jaw of specimen AMS C.462957. (*E*) Masticatory border of the jaw of specimen AMS C.462957.

monograph on Australian plankton, and observed Australian glaucinins (Thompson and Bennett 1970).

Glaucus thompsoni, sp. nov.

(Figs 9*B*, 12, 13)

ZooBank LSID
http://zoobank.org/NomenclaturalActs/DFD1E8CF-3945-440B-AC74-DF0314919C17

Material examined

Holotype. North Pacific Subtropical Gyre System, ~120 nautical miles west of Cabo San Lazaro, Baja California Sur, Mexico (25.082N, 114.481W), 14.x.2011, leg. students and crew of the *SSV Robert C. Seamans* (SEA Semester Program) (UMMZ 400003).

Paratypes. Same data as for holotype, 9 specimens (UMMZ 400004).

Diagnosis

This species can be distinguished by 219 molecular diagnostic characters in COI and 34 in 16S (Appendix 1) and differs morphologically from its sister species, *Glaucus mcfarlanei*, sp. nov., by the presence of a bursa copulatrix.

External anatomy

External anatomy identical to *G. marginatus*.

Internal anatomy

Diaulic reproductive system as described above (Fig. 13). Penis is muscular, lobed, and devoid of a spine. The vagina connects to a small bursa copulatrix.

Radular formula $13 \times 0.1.0$ in a 4 mm long specimen (UMMZ 302988). Radular teeth elongate with a conical, very long, delicate central cusp, flanked by 4–6 denticles on each side (Fig. 12*A*). Denticles elongate and slightly curved, with a sharp apex. Masticatory border of the jaw with a single row of denticles (Fig. 12*B*, *C*). Denticles conical, elongate, some of them bearing small tubercles.

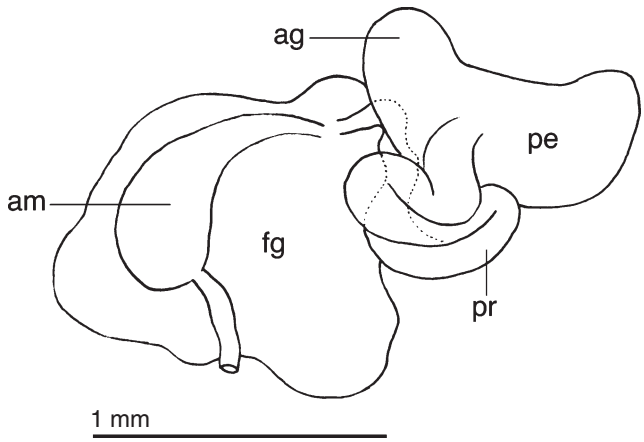

Fig. 11. Anatomical drawing of the distal reproductive system of *Glaucus bennettae*, sp. nov. (AMS C.462957). Abbreviations: am, ampulla; ag, accessory gland; fg, female gland complex; pe, penis; pr, prostate.

Geographic range

This species has only been found in the North Pacific subtropical gyre system.

Etymology

Named after Thomas E. Thompson, the prolific nudibranch biologist who coauthored several papers on glaucinins (e.g. Thompson and Bennett 1970; Thompson and McFarlane 1967).

Glaucus mcfarlanei, sp. nov.

(Figs 9*C*, 14, 15)

ZooBank LSID
http://zoobank.org/NomenclaturalActs/66DC5980-5D32-4FDF-A5D4-9E34F0C5E551

Material examined

Holotype. Approximately 20 nautical miles west of Kona, Hawaii (19.48N; 156.2883W), 16.ix.2006, leg. Robert Humphreys (NOAA) (UMMZ 400005).

Paratypes. Same data as for holotype, 5 specimens (UMMZ 302990).

Diagnosis

This species can be distinguished phylogenetically by 217 molecular diagnostic characters in COI and 36 in 16S (Appendix 1) and differs morphologically from its sister

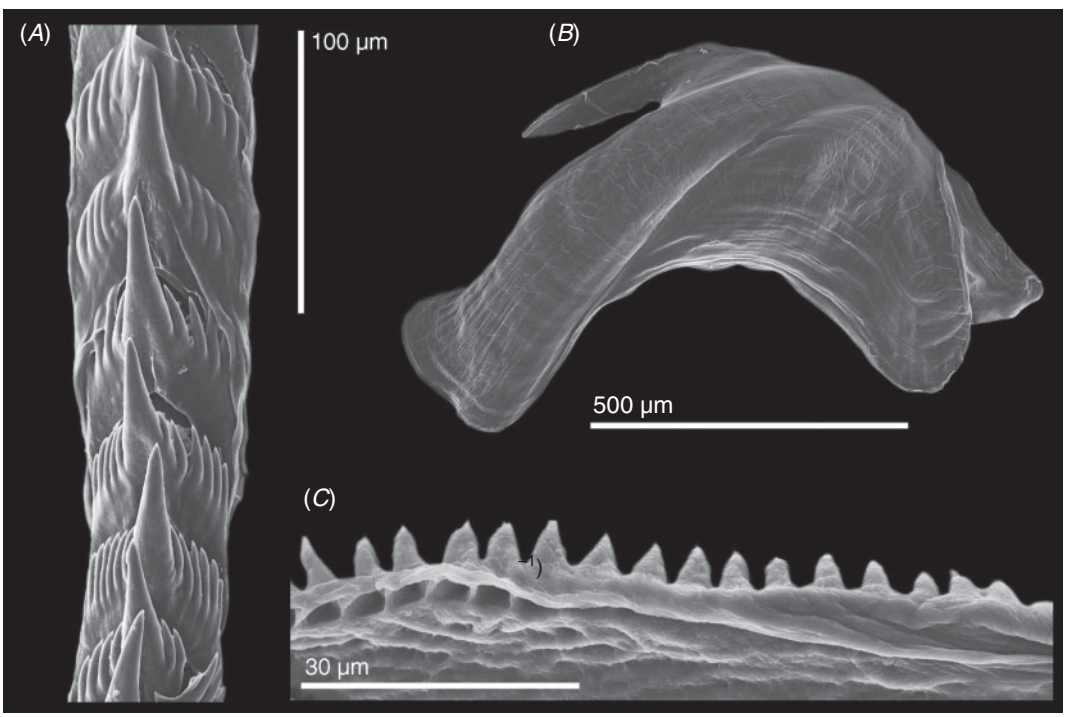

Fig. 12. Scanning electron micrographs of the chitinous feeding structures of *Glaucus thompsoni*, sp. nov. (*A*) Radula of specimen UMMZ 302988. (*B*) Jaw of specimen UMMZ 302988. (*C*) Masticatory border of jaw of specimen UMMZ 302988.

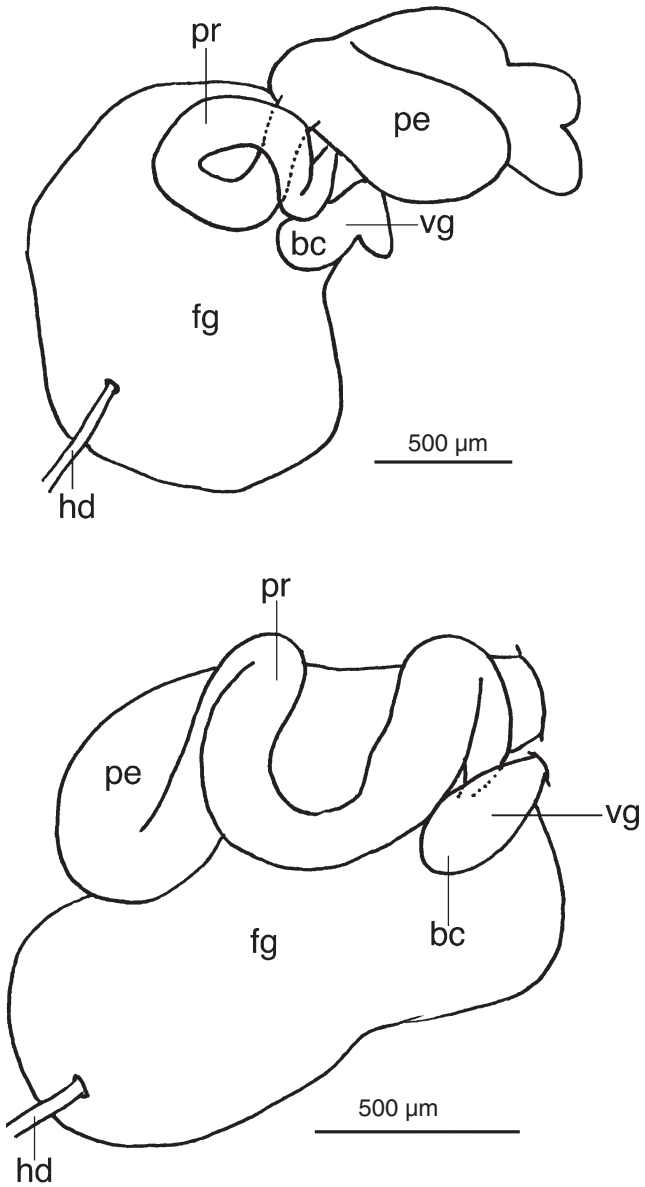

Fig. 13. Anatomical drawings of the distal reproductive system of two specimens of *Glaucus thompsoni*, sp. nov. (UMMZ 302988). Abbreviations: fg, female gland complex; hd, hermaphroditic duct; pe, penis; pr, prostate; vg, vagina.

species, *Glaucus thompsoni*, sp. nov., by the absence of a bursa copulatrix.

External anatomy

External anatomy identical to *G. marginatus* (Fig. 9*C*).

Internal anatomy

Diaulic reproductive system as described above (Fig. 15). Penis is muscular, lobed, and devoid of a spine. No bursa copulatrix present; the vagina connects proximally to the oviduct. Radular formula 16 × 0.1.0 in a 3 mm long specimen (UMMZ 302990). Radular teeth elongate with a conical, very long, delicate central cusp, flanked by 5–7 denticles on each side (Fig. 14*A*). Denticles elongate and slightly curved, with a sharp apex. Masticatory border of the jaw with a single row of denticles (Fig. 14*B*, *C*). Denticles short, rounded to conical, lacking small tubercles.

Geographic range

This species has only been found in the North Pacific subtropical gyre system.

Etymology

Named after Ian McFarlane, who co-authored the first histological study of a glaucinin (Thompson and McFarlane 1967).

Discussion

Nomenclatural history and species authorships

The first descriptions of glaucinin nudibranchs are pre-Linnean, non-binominal works, and therefore names introduced in those publications are not available. Bergh (1864) and Thompson and McFarlane (1967) provided an account and discussion of those early descriptions. Forster (1777) introduced the first binominal description of a glaucinin nudibranch, *Glaucus atlanticus*. Russell (1971) and McDonald (2006) considered Forster's description to be a *nomen dubium* and assigned the authorship of *Glaucus* to Gmelin (1791), who based the genus name on the species *Doris radiata* d'Orbigny, 1839. Forster (1777) described *Glaucus atlanticus* as follows: 'We had also at various intervals, found the sea [North Atlantic] covered with animals belonging to the class of *mollusca*, one of which, of a blue colour, in shape like a snail, with four arms, divided into many branches, was named *glaucus atlanticus*; . . .' This description unequivocally refers to the species recognised in this paper as *Glaucus atlanticus*, as all other recognised species are found in the Pacific and Indian Oceans. Therefore we agree with the current usage of the names *Glaucus* and *Glaucus atlanticus* in recent publications (Valdés and Angulo Campillo 2004; Churchill *et al*. 2013).

The original publication and authorship of *Glaucilla marginata* and *G. briareus* has also been the subject of confusion. Bergh (1860) introduced these two names for the first time (under Reinhardt's authorship), but did not provide a description of the animals; instead he provided descriptions of the ingested, exogenous nematocysts. In a footnote in the same paper, Bergh (1860) introduced the genus name *Glaucilla* and provided a description and a comparison with *Glaucus*. Jensen (personal communication) argued that Bergh's (1860) description of *G. marginata* and *G. briareus* are *nomina nuda* because the paper contains no descriptions of the species. We agree with Jensen that Bergh's (1860) species descriptions do not meet the criteria for a valid species description under Article 12 of the Code of Zoological Nomenclature (ICZN 1999), as no descriptions or illustrations of the animals, or indications are provided. In a subsequent paper (Bergh 1864) provided descriptions and illustrations of both species (based on manuscript notes from Reinhardt). We consider Bergh (1864) to be the original publication of the two species, while the original publication of the genus name *Glaucilla* remains Bergh (1860). The correct authorship of the two species names should then be *Glaucilla marginata* Reinhardt *in* Bergh, 1864 and *G. briareus*

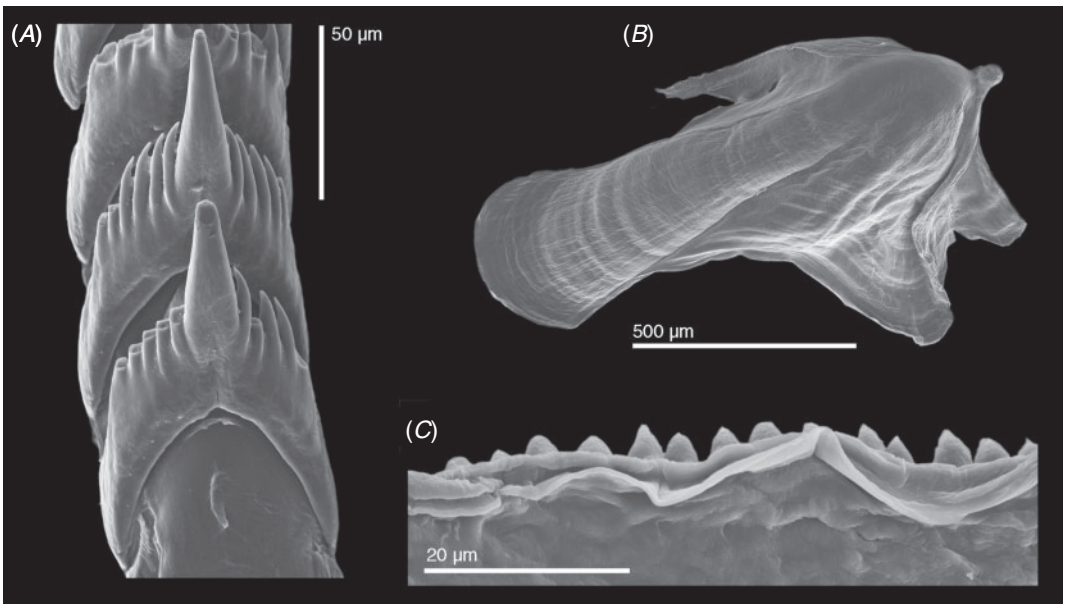

Fig. 14. Scanning electron micrographs of feeding structures of *Glaucus mcfarlanei*, sp. nov. (*A*) Radula of specimen UMMZ 302990. (*B*) Jaw of specimen UMMZ 302990. (*C*) Masticatory border of the jaw of specimen UMMZ 302990.

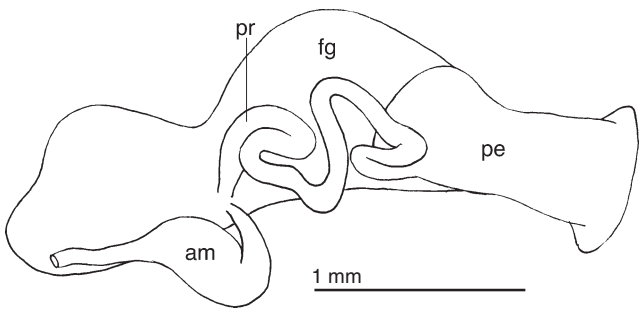

Fig. 15. Anatomical drawing of the distal reproductive system *Glaucus mcfarlanei*, sp. nov. (UMMZ 302990). Abbreviations: am, ampulla; fg, female gland complex; pe, penis; pr, prostate.

Reinhardt *in* Bergh, 1864. Bergh's (1864) paper is often erroneously cited as published in 1868 (Thompson and McFarlane 1967; Valdés and Angulo Campillo 2004).

Validity of previously described species

The genus name *Glaucilla* has been used for the two traditionally Pacific species with multiseriate cerata, *G. marginata* and *G. briareus* (Thompson and McFarlane 1967). Bergh (1864) proposed that *G. marginata* was a north Pacific species, whereas *G. briareus* was found in the south Pacific. Thompson and Bennett (1970) synonymised *G. briareus* with *G. marginata* and Valdés and Angulo Campillo (2004) synonymised *Glaucilla* with *Glaucus*. The most recent published opinion on the taxonomy of this group (Valdés and Angulo Campillo 2004) is that there is only one valid genus of glaucinin nudibranchs with two species, the cosmopolitan *Glaucus atlanticus* and the Pacific *Glaucus marginatus*. Examination of the type specimens of *Glaucus lineatus* Reinhardt *in* Bergh, 1864 (ZMUC GAS-2157), *Glaucus gracilis* Reinhardt *in* Bergh, 1864 (ZMUC GAS-2155) and *Glaucus longicirrus* Reinhardt *in* Bergh, 1864 (ZMUC GAS-2156) confirmed that these names are synonyms of *Glaucus atlanticus*. However, the conclusion of the present study is that four cryptic species with external morphological characteristics of *Glaucus marginatus* exist in the Indo-Pacific. Since there are two available names for these four Indo-Pacific species, it is important to determine whether these names can be assigned with confidence to any of the species recovered in the phylogenetic and species delimitation analyses.

Reinhardt *in* Bergh (1864) described *Glaucus marginatus* in great detail and included descriptions and illustrations of the reproductive anatomy and the radula. We had access to the type material and dissected one of the syntypes. The dissected syntype has a bursa copulatrix and the rest of the reproductive anatomy is consistent with that of the most widespread species in the '*marginatus*' clade. Additionally Bergh's (1864) illustrations of the radula (fig. 18) and reproductive system (fig. 24) are consistent with those of that species. Therefore, we can confidently assign the name *G. marginatus* to this species.

In the case of *Glaucilla briareus*, it is impossible to determine the identity of this species. Although the holotype of this species still exists (ZMUC GAS-2159) (Fig. 3*C*, *D*) we could not obtain permission to dissect it. Also Reinhardt *in* Bergh (1864) did not provide descriptions of the internal anatomy of the species and the external morphology is not distinctive enough to recognise this species. The type locality of *G. briareus* is north of Juan Fernandez, an area in which two of the cryptic species described in the present paper could potentially be found. Therefore we consider *G. briareus* to be a *nomen dubium*.

Implications for future aeolid systematics

Aeolidacea has been an extremely difficult group to resolve systematically (Moore and Gosliner 2011), and a full review is

out of the scope of this study; however, one critical hindrance has been the lack of readily-amplifiable nuclear molecular markers that are phylogenetically informative at the family level. Recent attempts to reconstruct the phylogeny (Pola and Gosliner 2010) of large groups of aeolids, such as the Aeolidiidae (Carmona *et al*. 2013) using COI, 16S and H3, resulted in well resolved trees at the family and genus level, but failed to provide resolution at the basal nodes. We suggest Churchill *et al*.'s (2013) protocol for 28S rRNA as potentially useful in resolving within-aeolid relationships. However, in that study, the molecular phylogeny does not support the monophyly of the aeolid families Glaucidae and Aeolidiidae; members of both families are interspersed throughout a well supported clade (Churchill *et al*. 2013).

Finally, considering the repeated cryptic speciation within the *Glaucus marginatus* complex versus the widespread *G. atlanticus*, an obvious question is: Why hasn't speciation occurred within *G. atlanticus*? Although higher resolution markers and/or population genetics methods may reveal population structure in *Glaucus atlanticus*, the species delimitation techniques used in the present paper suggest that Indo-Pacific and Atlantic populations of *G. atlanticus* constitute the same species. If speciation in the *G. marginatus* complex is related to reproductive morphology (Churchill *et al*. 2013), then any functional differences between the reproductive systems of *G. atlanticus* and the *G. marginatus* species complex could help explain their disparate rates of speciation. The major reproductive difference between the two glaucinin morphospecies is the presence of a penial spine in *G. atlanticus* (Fig. 6*D*, *E*; Valdés and Angulo Campillo 2004); in the *G. marginatus* species complex the penis is unarmed (Valdés and Angulo Campillo 2004). Miller (1974) considered the penial spine in *G. atlanticus* an adaptation for literally hooking individuals together in an unstable environment (the neuston) and between individuals with lateral, pedunculate cerata (a unique condition among aeolids). A single study of mating behaviour in *G. atlanticus* versus *G. marginatus* morphospecies individuals (unidentified South Pacific lineage) showed significantly longer copulation times in *G. atlanticus* (43–59 min *v.* 50–70 s; Ross and Quetin 1990). We hypothesise that the penial spine, when present, primarily governs the mechanics of penial interaction during mating. Aeolids have a variety of different forms of penial spines (Miller 1974, described nine types in facelinids), and their presence or absence varies within genera. Comparing species diversity levels between taxa with or without penial spines would be one potential way to test our hypothesis of the effect of the spine on nudibranch mating. In the *G. marginatus* morphospecies, the penis is unarmed, and thus the presence or absence of a bursa copulatrix immediately proximal to the gonopore might have a much greater effect on mating behaviour and penial interaction. The function of the bursa copulatrix as a sperm storage and digestion organ plays a fundamental role in mating interactions in hermaphroditic sea slugs, leading to sperm trading schemes (Anthes and Michiels 2005). The loss of the bursa copulatrix in *G. bennettae* and *G. mcfarlanei* might have resulted in a reduction in the male mating effort and selection for faster copulation, which would be advantageous for mating on the ocean surface. For example, Anthes *et al*. (2008) found positive evolutionary correlations between bursa copulatrix volume and both prostate and penis volume in aglajid sea slugs. Further research on mating behaviour in both morphospecies and the two cryptic species pairs is necessary to better understand the role sexual selection and mating behaviour may have played in the speciation and cladogenesis of glaucinin sea slugs.

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Appendix 1. Molecular diagnostic traits for *Glaucus atlanticus* and *Glaucus marginatus* morphospecies in mitochondrial 16S rRNA and COI obtained with CAOS-analyzer

Both the position in the alignments and the base letter are included. Only diagnostics with values of 1.00 are included (pure diagnostics). Note that Churchill *et al*. (2013) were unsuccessful in obtaining mtCOI genotypes for *Glaucus bennettae*, sp. nov.

G. atlanticus				*G. marginatus*				*G. bennettae*				*G. thompsoni*				*G. mcfarlanei*			
COI		16S		COI		16S		COI		16S		COI		16S		COI		16S	
0	T	5	G	3	C	8	G	–	–	8	A	2	C	4	T	2	T	4	C
1	A	27	A	15	C	39	C	–	–	39	G	4	T	8	G	4	C	8	A
2	C	31	A	48	A	136	G	–	–	136	A	6	A	12	C	6	T	12	A
3	T	32	G	69	C	180	T	–	–	142	T	9	C	13	A	9	T	13	T
15	T	33	T	72	A	181	T	–	–	143	G	10	T	14	C	10	A	14	A
39	A	34	G	81	T	260	A	–	–	181	A	13	C	28	T	13	A	28	G
48	G	35	A	91	T	269	C	–	–	228	A	15	A	36	A	15	C	35	A
69	T	85	G	99	T	270	G	–	–	233	T	18	C	55	T	18	C	36	C
72	G	101	A	120	A	271	T	–	–	260	G	21	T	136	A	21	A	55	C
75	A	126	A	156	T	283	T	–	–	269	T	22	A	138	A	22	T	136	G
81	C	135	G	162	A	301	A	–	–	270	A	24	G	142	C	24	G	138	G
91	C	137	A	165	A	302	A	–	–	271	C	25	T	144	C	25	G	142	T
99	A	139	T	183	A	304	T	–	–	283	C	27	G	145	A	27	C	144	A
120	T	140	C	192	T	310	C	–	–	301	T	33	G	178	T	33	T	145	T
156	C	145	T	198	A	315	A	–	–	302	T	34	A	180	G	34	T	178	G
162	G	146	C	201	A	326	G	–	–	304	A	36	G	182	A	36	A	180	A
165	G	177	T	205	C	351	A	–	–	310	T	37	G	185	A	37	C	182	G
183	T	179	T	207	T	–	–	–	–	315	G	38	T	224	A	38	A	185	T
189	T	180	A	228	G	–	–	–	–	326	A	39	T	225	A	43	G	224	T
192	C	214	A	237	T	–	–	–	–	–	–	43	A	227	G	45	C	225	T
198	G	217	C	240	G	–	–	–	–	–	–	45	C	234	C	57	C	227	A
201	C	225	C	243	A	–	–	–	–	–	–	57	G	239	G	60	G	234	T
205	T	226	A	264	G	–	–	–	–	–	–	60	A	259	G	63	C	239	C
207	A	227	C	267	T	–	–	–	–	–	–	63	C	270	G	66	T	259	A
216	A	229	A	270	A	–	–	–	–	–	–	66	G	271	T	69	G	268	A
228	A	230	T	283	C	–	–	–	–	–	–	69	C	284	C	79	C	270	A
231	A	231	T	286	C	–	–	–	–	–	–	79	A	288	G	80	A	271	G
237	G	232	T	288	T	–	–	–	–	–	–	80	C	289	A	82	G	284	T
240	A	233	G	291	A	–	–	–	–	–	–	82	A	310	C	84	G	288	T
243	G	239	A	297	C	–	–	–	–	–	–	84	A	346	A	87	C	289	C
264	A	269	T	303	T	–	–	–	–	–	–	87	A	351	C	88	T	310	T
267	A	270	T	306	A	–	–	–	–	–	–	88	T	381	A	90	C	346	T
283	T	272	A	312	A	–	–	–	–	–	–	90	T	405	A	93	C	351	T
286	T	285	A	321	G	–	–	–	–	–	–	93	T	427	T	94	C	381	G
288	A	286	G	342	A	–	–	–	–	–	–	94	T	–	–	96	A	405	G
291	G	288	A	345	T	–	–	–	–	–	–	96	T	–	–	99	C	427	A
306	G	290	G	346	C	–	–	–	–	–	–	99	A	–	–	102	T	–	–
315	G	301	A	348	T	–	–	–	–	–	–	102	C	–	–	103	A	–	–
346	T	405	T	372	G	–	–	–	–	–	–	103	G	–	–	108	C	–	–
348	A	–	–	385	T	–	–	–	–	–	–	108	T	–	–	109	A	–	–
385	C	–	–	411	G	–	–	–	–	–	–	109	T	–	–	117	C	–	–
411	A	–	–	427	C	–	–	–	–	–	–	117	C	–	–	120	G	–	–
423	A	–	–	429	T	–	–	–	–	–	–	120	A	–	–	126	T	–	–
427	T	–	–	474	G	–	–	–	–	–	–	126	C	–	–	132	C	–	–
429	A	–	–	477	A	–	–	–	–	–	–	129	G	–	–	138	A	–	–
474	C	–	–	483	T	–	–	–	–	–	–	132	G	–	–	141	C	–	–
477	G	–	–	486	C	–	–	–	–	–	–	138	T	–	–	144	C	–	–
483	G	–	–	489	C	–	–	–	–	–	–	141	T	–	–	147	A	–	–
486	T	–	–	498	T	–	–	–	–	–	–	144	G	–	–	157	A	–	–
489	T	–	–	499	T	–	–	–	–	–	–	147	G	–	–	158	C	–	–
499	C	–	–	501	A	–	–	–	–	–	–	157	G	–	–	159	T	–	–
501	T	–	–	507	T	–	–	–	–	–	–	158	T	–	–	162	C	–	–
507	A	–	–	510	A	–	–	–	–	–	–	159	G	–	–	168	T	–	–
510	G	–	–	513	T	–	–	–	–	–	–	162	A	–	–	169	T	–	–
513	G	–	–	516	A	–	–	–	–	–	–	168	C	–	–	171	A	–	–

(*continued next page*)

Appendix 1. (*continued*)

G. atlanticus				*G. marginatus*				*G. bennettae*				*G. thompsoni*				*G. mcfarlanei*			
COI		16S		COI		16S		COI		16S		COI		16S		COI		16S	
516	G	–	–	517	T	–	–	–	–	–	–	169	C	–	–	172	A	–	–
517	C	–	–	519	A	–	–	–	–	–	–	171	T	–	–	174	A	–	–
525	A	–	–	525	T	–	–	–	–	–	–	172	C	–	–	180	A	–	–
528	A	–	–	528	T	–	–	–	–	–	–	174	G	–	–	183	G	–	–
549	T	–	–	549	G	–	–	–	–	–	–	180	G	–	–	186	T	–	–
594	T	–	–	552	G	–	–	–	–	–	–	183	C	–	–	194	A	–	–
606	A	–	–	594	C	–	–	–	–	–	–	186	T	–	–	195	T	–	–
624	G	–	–	606	G	–	–	–	–	–	–	194	G	–	–	196	T	–	–
627	A	–	–	624	A	–	–	–	–	–	–	195	A	–	–	198	C	–	–
633	T	–	–	633	C	–	–	–	–	–	–	196	A	–	–	201	G	–	–
639	C	–	–	639	T	–	–	–	–	–	–	198	G	–	–	210	C	–	–
648	G	–	–	648	A	–	–	–	–	–	–	201	T	–	–	216	C	–	–
652	C	–	–	652	T	–	–	–	–	–	–	210	G	–	–	222	G	–	–
654	G	–	–	654	A	–	–	–	–	–	–	216	G	–	–	231	T	–	–
657	C	–	–	657	T	–	–	–	–	–	–	222	T	–	–	234	T	–	–
–	–	–	–	–	–	–	–	–	–	–	–	231	A	–	–	237	A	–	–
–	–	–	–	–	–	–	–	–	–	–	–	234	C	–	–	240	T	–	–
–	–	–	–	–	–	–	–	–	–	–	–	237	A	–	–	255	G	–	–
–	–	–	–	–	–	–	–	–	–	–	–	240	T	–	–	258	T	–	–
–	–	–	–	–	–	–	–	–	–	–	–	255	G	–	–	261	G	–	–
–	–	–	–	–	–	–	–	–	–	–	–	258	C	–	–	264	G	–	–
–	–	–	–	–	–	–	–	–	–	–	–	261	A	–	–	265	T	–	–
–	–	–	–	–	–	–	–	–	–	–	–	264	A	–	–	270	G	–	–
–	–	–	–	–	–	–	–	–	–	–	–	265	T	–	–	273	T	–	–
–	–	–	–	–	–	–	–	–	–	–	–	270	C	–	–	274	G	–	–
–	–	–	–	–	–	–	–	–	–	–	–	273	A	–	–	276	T	–	–
–	–	–	–	–	–	–	–	–	–	–	–	274	G	–	–	278	A	–	–
–	–	–	–	–	–	–	–	–	–	–	–	276	C	–	–	279	T	–	–
–	–	–	–	–	–	–	–	–	–	–	–	278	T	–	–	280	C	–	–
–	–	–	–	–	–	–	–	–	–	–	–	279	C	–	–	281	T	–	–
–	–	–	–	–	–	–	–	–	–	–	–	280	G	–	–	285	G	–	–
–	–	–	–	–	–	–	–	–	–	–	–	281	G	–	–	286	T	–	–
–	–	–	–	–	–	–	–	–	–	–	–	285	A	–	–	288	G	–	–
–	–	–	–	–	–	–	–	–	–	–	–	286	A	–	–	292	C	–	–
–	–	–	–	–	–	–	–	–	–	–	–	288	A	–	–	293	T	–	–
–	–	–	–	–	–	–	–	–	–	–	–	292	T	–	–	294	T	–	–
–	–	–	–	–	–	–	–	–	–	–	–	293	C	–	–	297	T	–	–
–	–	–	–	–	–	–	–	–	–	–	–	294	A	–	–	298	A	–	–
–	–	–	–	–	–	–	–	–	–	–	–	297	C	–	–	300	T	–	–
–	–	–	–	–	–	–	–	–	–	–	–	298	G	–	–	301	A	–	–
–	–	–	–	–	–	–	–	–	–	–	–	300	C	–	–	303	C	–	–
–	–	–	–	–	–	–	–	–	–	–	–	301	T	–	–	304	G	–	–
–	–	–	–	–	–	–	–	–	–	–	–	303	A	–	–	306	C	–	–
–	–	–	–	–	–	–	–	–	–	–	–	304	G	–	–	310	T	–	–
–	–	–	–	–	–	–	–	–	–	–	–	306	G	–	–	312	T	–	–
–	–	–	–	–	–	–	–	–	–	–	–	310	G	–	–	315	A	–	–
–	–	–	–	–	–	–	–	–	–	–	–	312	G	–	–	318	G	–	–
–	–	–	–	–	–	–	–	–	–	–	–	315	A	–	–	321	A	–	–
–	–	–	–	–	–	–	–	–	–	–	–	318	C	–	–	324	A	–	–
–	–	–	–	–	–	–	–	–	–	–	–	321	A	–	–	327	A	–	–
–	–	–	–	–	–	–	–	–	–	–	–	324	T	–	–	330	G	–	–
–	–	–	–	–	–	–	–	–	–	–	–	327	G	–	–	333	C	–	–
–	–	–	–	–	–	–	–	–	–	–	–	330	G	–	–	336	A	–	–
–	–	–	–	–	–	–	–	–	–	–	–	333	T	–	–	339	C	–	–
–	–	–	–	–	–	–	–	–	–	–	–	336	T	–	–	342	C	–	–
–	–	–	–	–	–	–	–	–	–	–	–	339	T	–	–	345	C	–	–
–	–	–	–	–	–	–	–	–	–	–	–	342	A	–	–	346	C	–	–
–	–	–	–	–	–	–	–	–	–	–	–	345	A	–	–	348	G	–	–

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Appendix 1. (*continued*)

G. atlanticus				*G. marginatus*				*G. bennettae*				*G. thompsoni*				*G. mcfarlanei*			
COI				16S				COI				16S				COI			
–	–	–	–	–	–	–	–	–	–	–	–	346	T	–	–	351	A	–	–
–	–	–	–	–	–	–	–	–	–	–	–	348	A	–	–	354	T	–	–
–	–	–	–	–	–	–	–	–	–	–	–	351	G	–	–	358	A	–	–
–	–	–	–	–	–	–	–	–	–	–	–	354	G	–	–	360	C	–	–
–	–	–	–	–	–	–	–	–	–	–	–	355	C	–	–	362	C	–	–
–	–	–	–	–	–	–	–	–	–	–	–	358	C	–	–	363	G	–	–
–	–	–	–	–	–	–	–	–	–	–	–	360	T	–	–	367	T	–	–
–	–	–	–	–	–	–	–	–	–	–	–	362	G	–	–	368	C	–	–
–	–	–	–	–	–	–	–	–	–	–	–	363	A	–	–	369	A	–	–
–	–	–	–	–	–	–	–	–	–	–	–	367	G	–	–	370	A	–	–
–	–	–	–	–	–	–	–	–	–	–	–	368	C	–	–	373	G	–	–
–	–	–	–	–	–	–	–	–	–	–	–	369	G	–	–	374	C	–	–
–	–	–	–	–	–	–	–	–	–	–	–	370	G	–	–	375	G	–	–
–	–	–	–	–	–	–	–	–	–	–	–	373	G	–	–	376	G	–	–
–	–	–	–	–	–	–	–	–	–	–	–	374	C	–	–	378	C	–	–
–	–	–	–	–	–	–	–	–	–	–	–	375	T	–	–	381	A	–	–
–	–	–	–	–	–	–	–	–	–	–	–	376	G	–	–	384	T	–	–
–	–	–	–	–	–	–	–	–	–	–	–	378	C	–	–	385	A	–	–
–	–	–	–	–	–	–	–	–	–	–	–	381	G	–	–	387	A	–	–
–	–	–	–	–	–	–	–	–	–	–	–	384	C	–	–	389	T	–	–
–	–	–	–	–	–	–	–	–	–	–	–	385	C	–	–	390	T	–	–
–	–	–	–	–	–	–	–	–	–	–	–	387	G	–	–	393	C	–	–
–	–	–	–	–	–	–	–	–	–	–	–	389	C	–	–	399	C	–	–
–	–	–	–	–	–	–	–	–	–	–	–	390	C	–	–	400	T	–	–
–	–	–	–	–	–	–	–	–	–	–	–	393	T	–	–	402	A	–	–
–	–	–	–	–	–	–	–	–	–	–	–	399	T	–	–	408	T	–	–
–	–	–	–	–	–	–	–	–	–	–	–	400	C	–	–	411	A	–	–
–	–	–	–	–	–	–	–	–	–	–	–	402	T	–	–	414	T	–	–
–	–	–	–	–	–	–	–	–	–	–	–	408	A	–	–	415	C	–	–
–	–	–	–	–	–	–	–	–	–	–	–	411	G	–	–	417	C	–	–
–	–	–	–	–	–	–	–	–	–	–	–	414	G	–	–	423	T	–	–
–	–	–	–	–	–	–	–	–	–	–	–	415	G	–	–	424	A	–	–
–	–	–	–	–	–	–	–	–	–	–	–	417	A	–	–	426	T	–	–
–	–	–	–	–	–	–	–	–	–	–	–	423	C	–	–	427	T	–	–
–	–	–	–	–	–	–	–	–	–	–	–	424	T	–	–	429	A	–	–
–	–	–	–	–	–	–	–	–	–	–	–	426	G	–	–	432	G	–	–
–	–	–	–	–	–	–	–	–	–	–	–	427	C	–	–	435	T	–	–
–	–	–	–	–	–	–	–	–	–	–	–	429	G	–	–	441	C	–	–
–	–	–	–	–	–	–	–	–	–	–	–	432	A	–	–	447	C	–	–
–	–	–	–	–	–	–	–	–	–	–	–	435	C	–	–	450	G	–	–
–	–	–	–	–	–	–	–	–	–	–	–	441	C	–	–	454	G	–	–
–	–	–	–	–	–	–	–	–	–	–	–	447	T	–	–	456	G	–	–
–	–	–	–	–	–	–	–	–	–	–	–	450	A	–	–	458	T	–	–
–	–	–	–	–	–	–	–	–	–	–	–	454	A	–	–	459	A	–	–
–	–	–	–	–	–	–	–	–	–	–	–	456	T	–	–	468	A	–	–
–	–	–	–	–	–	–	–	–	–	–	–	458	T	–	–	469	G	–	–
–	–	–	–	–	–	–	–	–	–	–	–	459	A	–	–	474	C	–	–
–	–	–	–	–	–	–	–	–	–	–	–	468	G	–	–	476	G	–	–
–	–	–	–	–	–	–	–	–	–	–	–	469	T	–	–	477	G	–	–
–	–	–	–	–	–	–	–	–	–	–	–	474	A	–	–	478	C	–	–
–	–	–	–	–	–	–	–	–	–	–	–	476	G	–	–	482	A	–	–
–	–	–	–	–	–	–	–	–	–	–	–	477	T	–	–	484	A	–	–
–	–	–	–	–	–	–	–	–	–	–	–	478	A	–	–	486	G	–	–
–	–	–	–	–	–	–	–	–	–	–	–	482	A	–	–	489	G	–	–
–	–	–	–	–	–	–	–	–	–	–	–	484	A	–	–	493	G	–	–
–	–	–	–	–	–	–	–	–	–	–	–	486	G	–	–	495	G	–	–
–	–	–	–	–	–	–	–	–	–	–	–	489	A	–	–	496	A	–	–
–	–	–	–	–	–	–	–	–	–	–	–	493	G	–	–	497	A	–	–

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Appendix 1. (*continued*)

G. atlanticus				*G. marginatus*				*G. bennettae*				*G. thompsoni*				*G. mcfarlanei*			
COI			16S	COI			16S	COI			16S	COI			16S	COI			16S
–	–	–	–	–	–	–	–	–	–	–	–	495	G	–	–	499	C	–	–
–	–	–	–	–	–	–	–	–	–	–	–	496	C	–	–	501	G	–	–
–	–	–	–	–	–	–	–	–	–	–	–	497	C	–	–	504	T	–	–
–	–	–	–	–	–	–	–	–	–	–	–	499	T	–	–	507	C	–	–
–	–	–	–	–	–	–	–	–	–	–	–	501	G	–	–	510	A	–	–
–	–	–	–	–	–	–	–	–	–	–	–	504	C	–	–	513	C	–	–
–	–	–	–	–	–	–	–	–	–	–	–	507	G	–	–	516	T	–	–
–	–	–	–	–	–	–	–	–	–	–	–	510	G	–	–	517	T	–	–
–	–	–	–	–	–	–	–	–	–	–	–	513	A	–	–	519	A	–	–
–	–	–	–	–	–	–	–	–	–	–	–	516	A	–	–	522	A	–	–
–	–	–	–	–	–	–	–	–	–	–	–	517	C	–	–	525	G	–	–
–	–	–	–	–	–	–	–	–	–	–	–	519	G	–	–	529	G	–	–
–	–	–	–	–	–	–	–	–	–	–	–	522	G	–	–	531	G	–	–
–	–	–	–	–	–	–	–	–	–	–	–	525	G	–	–	535	C	–	–
–	–	–	–	–	–	–	–	–	–	–	–	529	G	–	–	537	T	–	–
–	–	–	–	–	–	–	–	–	–	–	–	535	T	–	–	538	T	–	–
–	–	–	–	–	–	–	–	–	–	–	–	537	G	–	–	540	A	–	–
–	–	–	–	–	–	–	–	–	–	–	–	538	C	–	–	541	T	–	–
–	–	–	–	–	–	–	–	–	–	–	–	540	C	–	–	543	A	–	–
–	–	–	–	–	–	–	–	–	–	–	–	541	C	–	–	546	A	–	–
–	–	–	–	–	–	–	–	–	–	–	–	543	G	–	–	549	C	–	–
–	–	–	–	–	–	–	–	–	–	–	–	546	A	–	–	552	T	–	–
–	–	–	–	–	–	–	–	–	–	–	–	549	C	–	–	558	A	–	–
–	–	–	–	–	–	–	–	–	–	–	–	552	A	–	–	564	A	–	–
–	–	–	–	–	–	–	–	–	–	–	–	558	G	–	–	567	G	–	–
–	–	–	–	–	–	–	–	–	–	–	–	564	G	–	–	573	G	–	–
–	–	–	–	–	–	–	–	–	–	–	–	567	A	–	–	576	G	–	–
–	–	–	–	–	–	–	–	–	–	–	–	573	C	–	–	579	G	–	–
–	–	–	–	–	–	–	–	–	–	–	–	576	A	–	–	582	T	–	–
–	–	–	–	–	–	–	–	–	–	–	–	579	A	–	–	585	T	–	–
–	–	–	–	–	–	–	–	–	–	–	–	582	T	–	–	588	T	–	–
–	–	–	–	–	–	–	–	–	–	–	–	585	G	–	–	591	G	–	–
–	–	–	–	–	–	–	–	–	–	–	–	588	T	–	–	597	T	–	–
–	–	–	–	–	–	–	–	–	–	–	–	591	T	–	–	600	C	–	–
–	–	–	–	–	–	–	–	–	–	–	–	597	C	–	–	603	A	–	–
–	–	–	–	–	–	–	–	–	–	–	–	600	C	–	–	612	C	–	–
–	–	–	–	–	–	–	–	–	–	–	–	603	A	–	–	615	T	–	–
–	–	–	–	–	–	–	–	–	–	–	–	612	T	–	–	618	A	–	–
–	–	–	–	–	–	–	–	–	–	–	–	615	C	–	–	621	C	–	–
–	–	–	–	–	–	–	–	–	–	–	–	618	C	–	–	627	G	–	–
–	–	–	–	–	–	–	–	–	–	–	–	621	A	–	–	630	A	–	–
–	–	–	–	–	–	–	–	–	–	–	–	627	G	–	–	633	C	–	–
–	–	–	–	–	–	–	–	–	–	–	–	630	A	–	–	640	C	–	–
–	–	–	–	–	–	–	–	–	–	–	–	633	T	–	–	645	T	–	–
–	–	–	–	–	–	–	–	–	–	–	–	640	T	–	–	652	C	–	–
–	–	–	–	–	–	–	–	–	–	–	–	645	C	–	–	654	G	–	–
–	–	–	–	–	–	–	–	–	–	–	–	652	T	–	–	–	–	–	–
–	–	–	–	–	–	–	–	–	–	–	–	654	A	–	–	–	–	–	–