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Molecular and morphological systematics of *Elysia* Risso, 1818 (Heterobranchia: Sacoglossa) from the Caribbean region

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Abstract

The Caribbean is a biodiversity hotspot for photosynthetic sea slugs, with about 27 described species in the genus *Elysia* Risso, 1818. However, many species are poorly known or have complex taxonomic histories, complicating assessments of regional biodiversity and impeding studies of plastid symbiosis, speciation, and larval biology. Using an integrative approach, we address the taxonomy and systematics of Caribbean elysiids by performing robust tests of existing species hypotheses, and describe six new species. Species delimitation included DNA barcoding of up to 189 nominal conspecific specimens; nuclear gene sequences were then used to confirm that divergent lineages were genetically distinct candidate species. New synonymies and species descriptions are based on external anatomy, penial and radular morphology, developmental characters, and host ecology of all species described from the region, plus a critical review of the literature. We synonymized three species (*Elysia annedupontae* Ortea, Espinosa & Caballer *in* Ortea, Caballer, Moro & Espinosa, 2005, *Elysia clarki* Pierce *et al.* 2006, and *Elysia leeanneae* Caballer, Ortea & Espinosa *in* Ortea, Espinosa, Buske & Caballer, 2013), transfered one species from *Bosellia* (*Elysia marcusi*), and described six new species (*Elysia pawliki* **n. sp.**, *Elysia zemi* **n. sp.**, *Elysia christinae* **n. sp.**, *Elysia hamanni* **n. sp.**, *Elysia taino* **n. sp.**, and *Elysia buonoi* **n. sp.**). We resurrected the name *Elysia velutinus* Pruvot-Fol, 1947, a senior synonym of *Elysia tuca* Ev. Marcus & Er. Marcus, 1967. Based on a four-gene phylogeny of 76 *Elysia* spp., we identified shifts in host use and penial armature that may explain patterns of endemic diversification in *Elysia*, invoking both ecological and non-ecological mechanisms. Non-monophyly of stylet-bearing species rejects previous attempts to classify species based on presence of a stylet (i.e., the genus *Checholysia* Or-

tea, Caballer, Moro & Espinosa, 2005). Our findings show how integrative approaches can resolve the taxonomic status of problematic species (e.g., *Elysia papillosa* Verrill, 1901) for soft-bodied marine taxa.

Key words: development mode, ecological speciation, external morphology, host use, integrative taxonomy, reproductive anatomy, species delimitation

Introduction

Sacoglossa (Gastropoda: Heterobranchia) is a clade of sea slugs comprising the most specialized group of marine herbivores, and noted for the photosynthetic ability of some species that sequester functional chloroplasts from their algal hosts (Poore *et al.* 2008; Händeler *et al.* 2009; Christa *et al.* 2014b; Wägele *et al.* 2011). Sacoglossans have emerged as a model system for studies on herbivore-host coevolution and early-stage endosymbiosis (Jensen 1997a; Pierce & Curtis 2012), and also on the evolutionary ecology of larval development mode (Vendetti *et al.* 2012; Krug *et al.* 2015). As a basal lineage in Panpulmonata, Sacoglossa also occupies a pivotal position in the evolutionary radiation of gastropods (Kocot *et al.* 2013; Schrödl 2014; Zapata *et al.* 2014). However, the complex taxonomic history of many sacoglossan taxa and the prevalence of cryptic species have obscured our understanding of biogeography and diversity in this group (Jensen 1996, 2007; Krug *et al.* 2013, 2015).

The most species-rich sacoglossan genus, *Elysia*, contains 87 currently recognized species worldwide (Jensen 2007; Wägele *et al.* 2010; Ortea, Espinosa, Buske & Caballer 2013). Among sacoglossan genera, the widest range of food sources is consumed by species of *Elysia*, including diverse groups within Chlorophyta (e.g., *Caulerpa*, *Halimeda*, *Bryopsis*, *Penicillus*, *Udotea*, *Acetabularia*), Rhodophyta (e.g., *Griffithsia*, *Polysiphonia, Wrangelia*), Heterokontophyta (e.g., *Vaucheria*, *Biddulphia*), and angiosperm seagrasses (e.g., *Halophila*, *Halodule*, *Thalassia*) (Jensen 1997a; Händeler & Wägele 2007; Trowbridge *et al.* 2010; Christa *et al.* 2014b; 2015). A few *Elysia* spp. readily consume algae from up to five genera (Christa *et al.* 2014b; Middlebrooks *et al.* 2014; Christa *et al.* 2015), but most are host-specialized, associating with and primarily consuming algae from only one or two genera (Jensen 1993). As sister taxa often feed on different algae, host use can be a taxonomically informative character at the species level (Jensen 1980). Overall niche breadth (i.e., range of hosts used) may be an important driver of evolutionary success for a lineage such as *Elysia*, but tests of this hypothesis will require studies that document host ecology and also clarify species status for regional faunas. Species of *Elysia* are also important in drug discovery (Hamann & Scheuer 1993; Suárez *et al.* 2003), have been proposed as potential control agents of invasive marine algae (Thibaut *et al.* 2001), and are farmed commercially as model research organisms (Dionísio *et al.* 2013).

Although *Elysia* spp. are found in temperate and tropical regions worldwide, their biodiversity is concentrated in the tropical Indo-Pacific (Jensen 1992, 2007). The Caribbean region is also a diversity hotspot for *Elysia*, however, with substantially higher levels of diversity than other tropical regions outside of the Indo-Pacific. For example Valdés *et al.* (2006) reported 24 morphospecies from the Caribbean; in contrast, Camacho-García *et al.* (2005) reported only four for the entire tropical Eastern Pacific, and only seven *Elysia* spp. are known from the Mediterranean (Thompson & Jaklin 1988; Cervera *et al.* 2004). The taxonomy of Caribbean *Elysia* spp. therefore warrants special attention, to inform downstream studies of factors that influence the global distribution of marine species richness, and the differential evolutionary success of sacoglossan lineages.

The systematics of *Elysia* from the Caribbean region has been plagued by confusion, and the identity of some species vigorously debated. Taxonomic instability results partly from the lack of detail in some early descriptions, and absence of corresponding type material (e.g., Mörch 1863; Verrill 1901). Most of the taxonomic work on Caribbean *Elysia* was done by Eveline and Ernst Marcus, including numerous descriptions (Er. Marcus 1955, 1957; Ev. Marcus & Er. Marcus 1967; Ev. Marcus, 1972a, 1980) and re-descriptions, as well as interpretations of previously described species (e.g., Ev. Marcus & Er. Marcus 1960, 1963; Ev. Marcus & Hughes 1974; Ev. Marcus 1980). Although the Marcuses' work included new details on the anatomy of these species, such as the radula and reproductive system, they were often lacking in illustrations of live animals. More recently, papers by Ortea and collaborators have introduced a number of new elysiid taxa (Ortea & Espinosa 1996, 2002; Ortea *et al.* 2005; Ortea *et al.* 2011; Ortea *et al.* 2013) without consistently including important information such as photographs of live animals or data on the anatomy and feeding behavior of the new species. Recent field guides have provided illustrations of live animals (Redfern 2001, 2013; Valdés *et al.* 2006; García *et al.* 2008) but, lacking anatomical

data, these have not solved entrenched taxonomic problems. Jensen and Clark established baseline datasets on species distributions, reproductive and larval development, and host ecology for Caribbean elysiids, but problematic identifications and cryptic species compromise these efforts (Clark & Busacca 1978; Clark & Goetzfried 1978; Clark *et al.* 1979; Jensen 1980, 1981a; Clark & Jensen 1981; Jensen 1982; DeFreese & Clark 1983; Jensen 1983; Jensen & Clark 1983; Clark 1984; Jensen 1986; Clark & DeFreese 1987; Clark 1994).

Since Ev. Marcus (1980) last reviewed the group, there has been no comprehensive effort to clarify the systematics of *Elysia* in the Caribbean region (for reviews of narrower geographic and taxonomic scope see Thompson 1977; Jensen 1981a, 1982; Clark 1984; Jensen 1986). Here, we examine the systematics of *Elysia* in the Caribbean region using an integrative approach that includes molecular phylogenetics, illustrations of live animals and intraspecific variation, details of radular and penial morphology, ecological data on algal host use, and reproductive characters including mode of larval development, egg size, larval size and behavior, and pattern of extra-capsular yolk (ECY) deposition within egg masses. For the purposes of this paper, the Caribbean region is broadly defined as including Bermuda and the tropical northwestern Atlantic (from Florida and the Bahamas), the Caribbean Sea to northern South America, and the Gulf of Mexico. Our findings present a phylogenetic framework for future studies of elysiid systematics, and provide new data on ecology and development that contribute both to resolving species identities, and to broader efforts to study the importance of such characters in macroevolutionary processes.

Material and methods

Collection of specimens and ecological data. A total of 1,148 specimens were examined in this study. Species are discussed in chronological order according to description dates, and alphabetically if multiple species were described in the same year. Specimens were collected by the authors with permission of the state of Florida (Special Activity License #07SR-1034) or host country (Fig. 1, Table S1). Preserved specimens and any accompanying collection notes or photographs were also obtained from museum collections, or were donated by colleagues (Table 1, S1). Coenocytic green and red algae known to host sacoglossans were sampled by SCUBA or snorkeling from visited field sites, and small slugs removed in the laboratory; large slugs were collected in situ from rocky or sandy substrata. Host alga was recorded for all specimens obtained from an algal thallus. Live slugs and algae were transported to Los Angeles and maintained in aquaria for up to three months to observe feeding and reproduction. To confirm host use, slugs were observed feeding in aquaria or under a dissecting microscope, and ingestion of algal cytoplasm was visually verified. Limited host-choice experiments were performed in some cases by providing slugs with access to thalli of two different algae collected from the same field site, and determining the proportion of slugs physically found on a given algal thallus after a period of time.

Voucher specimens were deposited in the collections of the Natural History Museum of Los Angeles County (LACM), and the California State Polytechnic University Invertebrate Collection (CPIC). Types and voucher specimens and/or specimen data were obtained from other natural history collections, including: BMHN (The Natural History Museum, London), HMCZ (Harvard Museum of Comparative Zoology), LACM (Natural History Museum of Los Angeles County), MNHN (Museum National d'Histoire Naturelle, Paris), MSPC (Museu de Zoologia da Universidade de São Paulo, Brazil), MZUCR (Museo de Zoología, Universidad de Costa Rica), USNM (Smithsonian National Museum of Natural History, Washington, D.C.), YPMNH (Yale Peabody Museum of Natural History), ZMUC (Zoologisk Museum Københavns Universitet, Copenhagen). Type specimens from the following instututions were not examined: IESH (Instituto de Ecología y Sistemática, Havana, Cuba), IOH (Instituto de Oceanología, Havana, Cuba), MCNT (Museo de Ciencias Naturales de Tenerife, Spain). Specimens collected by PJK were given isolate codes using the following format: (1st letter of genus)(1st three letters of species name) (last two digits of year of collection)(location code)(specimen #). Isolates are included to indicate the specimens from which DNA was extracted and used in phylogenetic analyses. In addition to accession numbers for the LACM collection, isolate codes are referenced in NCBI accessions, and in some sections of this study under "material examined."

Reproductive data. Live specimens were isolated in small containers of sea water to observe mating behavior and obtain egg masses, from which the following reproductive characters were recorded: larval development mode (planktotrophic or lecithotrophic), pattern and color of extra-capsular yolk (ECY) deposits, diameter of uncleaved

ova, width of larval shell across the aperture at the time of hatching, and time to hatching of first larvae (Krug 2009; Krug *et al.* 2015). Lecithotrophy was confirmed by inducing metamorphosis in newly hatched larvae using the adult host alga and/or 20 mM excess K^+. Egg size and larval shell size were measured from calibrated digital images. Data are given as the mean size ± SD for one or more egg masses, or as a grand mean-of-means if mean values were obtained for replicate clutches. Sizes were typically measured for 25 ova or shells per clutch where possible, and up to 64 offspring per clutch; data were collected for one to eight replicate clutches, depending on the species.

Morphological examination. After reproducing, slugs were relaxed in $MgCl_2$ isotonic with seawater and photographed for external morphology. The number and pattern of raised vessels lining the inside of the parapodial flaps was noted, and specimens were scored for presence/absence of a pointed tail, color and shape of rhinophores (anterior sensory extensions), relative height of parapodial side-flaps, and color, shape and texture of parapodial sides and margins. Samples were preserved in 95–100% ethanol, or were fixed in 5% formalin and subsequently transferred to 70% ethanol. Morphological examinations of preserved specimens were made under magnification (Leica EZ4D) and drawn by eye or with a *camera lucida*.

Multiple specimens of each morphospecies were dissected if available. To isolate the radula, the slug's buccal mass was removed, dissolved in a 0.5 M solution of NaOH for 1-3 days, then rinsed in distilled water. Clean radulae were mounted on SEM stubs with fine forceps, then sputter coated with 60% gold, 40% palladium (@ 0.014 kÅ) using a Emitech K550x sputter coater, for examination under a Hitachi S-3000N scanning electron microscope (SEM). Radular features potentially described for a given species are diagrammed in Fig. 2.

Penises were removed for examination via an incision on the anterior-right of the body and drawn using a camera lucida. Penial tips were dehydrated in air, then mounted on a stub for SEM further examination, following the same methods described for radulae. Characteristics such as the presence of a chitinous or otherwise resistant penial apex, and the degree to which this tip extends and/or folds into a scoop or barb were noted. Tip morphology that we term a "stylet" follows Gascoigne (1974), who defined this structure as "a hollow, cuticular extension of the vas deferens." Thus, two categories of stylets are identified in Caribbean ellysiids herein described: (1) a cuticularized penial tip with little to no relief, but visibly distinct from surrounding tissue; and (2) a folded or scoop-shaped cuticle that extends beyond the fleshy tissue of the penial tip. SEM photomicrographs were captured using Hitachi imaging software and saved as digital image files. Image contrast and brightness were adjusted in Adobe Photoshop™ for clarity only. All photomicrographs were taken by the authors.

Species delimitation criteria. We used a four-step workflow (expanded below) to delimit all species among our available specimens. First, a conservative 8% COI distance threshold was used to identify mtDNA lineages so divergent that they likely represent distinct species, barring a lack of other diagnostic criteria. Second, a coalescent-based analysis of all available COI data (including as much intra-specific data as possible) was used to test whether less divergent but still supportable candidate species were present in four main subclades, which contained the bulk of Caribbean diversity within *Elysia*. Third, we confirmed that alleles at one nuclear locus differed among candidate species. Fourth, we used non-molecular characters to test the resulting species hypotheses, and to provide integrative species descriptions using a total evidence approach.

First, species delimitation began with a screen for divergent mtDNA lineages representing likely candidate species (Vieites *et al.* 2009). Genomic DNA was extracted with a QIAamp DNA Mini Kit (Qiagen; Valencia, CA) and stored in buffer at -20°C. Polymerase chain reactions (PCR) and custom versions of primers LCO1490 and HCO2198 (Folmer *et al.* 1994) were used to amplify the barcoding portion of the mitochondrial cytochrome *c* oxidase I (COI) gene. Prior work indicated that a conservative 8% COI distance distinguished species that were also diagnosable by morphological and/or reproductive characters—i.e., non-controversial candidate species (Krug *et al.* 2013; 2015). From two to 228 specimens of each nominal morphospecies were initially sequenced in unpublished phylogeographic studies (Rodriguez 2009; Trathen 2010; Rico 2012; Vo 2013); full details of molecular analyses will be published elsewhere. Lineages of COI haplotypes that were >8% divergent from their nearest relative were considered candidate species, and further tested under the following three criteria.

Second, for complexes of morphologically similar species, Automatic Barcode Gap Discovery (ABGD; Puillandre *et al.* 2012) tested whether closely related species were being lumped by our potentially conservative 8% threshold. This method estimates the genetic distance separating a coalescent (intra-specific) process of sequence evolution from a speciation process. We calculated a pairwise COI distance matrix (Tamura-Nei corrected) for three species complexes in MEGA 6.0 (Tamura *et al.* 2013), using pairwise deletion of missing data

for sequences shorter than 658 bp. Resulting mismatch distributions were plotted to visualize the barcoding gap. Each COI distance matrix was input into the ABGD web interface, with priors on intra- (pmin = 0.02) and inter-specific (pmax = 0.15) genetic distances based on previous results and default settings for other parameters (Puillandre *et al.* 2012, Krug *et al.* 2013).

We focused species delimitation analyses on three subclades recovered in multilocus phylogenetic analyses (see Results) that included complexes of co-occurring, morphologically similar Caribbean species. Subclades 1 (*E. papillosa* complex) and 4 (*E. tomentosa* complex) include all five of the new species described herein for which molecular data were available. Subclade 2 includes the taxonomically problematic lettuce sea slugs (*E. crispata*; "*E. clarki*"; *E. ellenae*; and *E. diomedea* from the eastern Pacific). Thus, ABGD analyses were performed on subclade 1 (six candidate spp. based on 8% threshold); subclade 2 (seven candidate spp. excluding *E. evelinae*, for which no COI sequence was obtained); and subclade 4 (11 candidate spp.). In addition to data generated for the present work, sequence data for subclade 1 were taken from Trathen (2010) and Rico (2012); data for subclade 2 were taken from Vo (2013); and data for subclade 4 were taken from Rodriguez (2009) and Krug *et al.* (2013). Theses are available from the California State University, Los Angeles library or from the first author's website, but taxon names have in some cases changed from those used in M.S. thesis work.

After delimiting provisional species based on mtDNA divergence, our third step was to examine the distribution of alleles at the nuclear H3 locus. Prior results (Krug *et al.* 2013; 2015) found almost no examples of H3 alleles shared between sister species. Therefore, if co-occurring individuals with divergent COI lineages were also fixed for different nuclear alleles, they were presumed to be reproductively isolated species, whereas a shared nuclear gene pool could indicate interbreeding and conspecificity. For most taxa included in intraspecific surveys of COI diversity, diversity at the nuclear histone III (H3) locus was thus also determined for multiple specimens using primers H3F and H3R (Colgan *et al.* 2000). Histone alleles were resolved using PHASE v. 2.1 for heterozygous individuals as needed (Stephens *et al.* 2001; Stephens & Scheet 2005; Vo 2013).

Finally, we tested species hypotheses based on analyses of molecular data by looking for consistent differences in (i) external anatomy; (ii) radular and/or penial morphology; (iii) larval development mode and/or extra-capsular yolk production; or (iv) algal host use. Unpaired, two-tailed *t* tests were used to determine if radular teeth differed significantly in length, width or angle if similar species had consistent but subtle differences in radular morphology. Presently, integrative methods of species delimitation that combine molecular and morphological data (e.g., iBPP; Solis-Lemus *et al.* 2014) cannot accommodate discrete characters; the dearth of continuous characters in deformable, soft-bodied taxa like sea slugs thus impedes fully integrated species delimitation. Instead, we used characters from morphology, ecology and reproduction to test species hypotheses based on analyses of molecular data, and then used fixed differences to inform integrative species descriptions of divergent and reproductively isolated lineages.

Molecular phylogenetic analyses. After initial species delimitation, our final molecular dataset comprised four genera: *Bosellia* (2 spp.), which was designated as the outgroup taxon; *Thuridilla* (13 spp.); *Plakobranchus* (10 candidate spp.; Krug *et al.* 2013); and *Elysia* (76 spp.), including 22 species examined in the present work. One exemplar per species was used in phylogenetic analyses except for two highly divergent representatives of *E. pusilla*. Portions of four gene regions were sequenced from each exemplar: (i) COI; (ii) the mitochondrial large ribosomal subunit rRNA (16S) gene, using primers 16Sa and 16Sb (Palumbi 1996); (iii) H3; and (iv) the nuclear large ribosomal subunit rRNA (28S) gene, amplified as three overlapping fragments using (i) primers 28SF3 and 28SR1, (ii) primers 28SF2 and 28SR3 (Morgan *et al.* 2002), and (iii) primers 28SC1 and 28SD2R (Vonneman *et al.* 2005). Reaction conditions were previously described (Krug *et al.* 2008; Händeler *et al.* 2009; Vendetti *et al.* 2012). PCR products were purified and amplified in both directions by Retrogen, Inc. (San Diego, CA). Chromatograms were edited and primer sequences removed using Geneious version 6.1.6 (http://www.geneious.com, Kearse *et al.* 2012).

Sequences from all available loci were initially aligned using MUSCLE with default settings in Geneious v6.1.6, refined by hand using secondary structure models for 16S and 28S, and sequence blocks masked by the least stringent criteria in Gblocks v.0.91b were removed (Castresana 2000); see Krug *et al.* (2015) for complete details. Ambiguous regions were removed accordingly, yielding aligned sequence partitions of 658 bp (COI), 429 bp (16S), 1392 bp (28S), and 328 bp (H3); NCBI accession numbers are given in Table 1.

TABLE 1. Specimens sequenced for this study and sequences obtained from GenBank, including species, locality, museum voucher number, isolate code, and GenBank accession numbers.

Species	Locality	Voucher number[1,2]	Isolate sequenced	GenBank Accession Numbers			
				COI	16S	H3	28S
E. abei	Kanagawa, Japan	-	Eabei_07Ja01	KM086374	JN819137	JN819171	KM230495
E. amakusana	Lizard Island, Australia	-	Eamakusana703	GQ996686	EU140851	-	GQ996621
E. asbecki	Vanuatu	-	Easb_06Van01	KM086360	KM204200	KM040808	KM230468
E. atroviridis	Choshi, Japan	-	Eatr_02Jap01	KC573760	KM204223	KC597184	KM230496
E. australis	Coolom, Queensland, Australia	-	Eaus_07Aus03	JN819109	JN819142	JN819176	KM230497
E. bangtawaensis	Bang Tawa, Thailand	-	Eban_09Tha01	KM086375	KM204224	KM040826	KM230498
E. bennettae	Upolu Island, Apia, Samoa	-	Ebennettae779	GQ996675	EU140868	-	GQ996637
***E. buonoi* n. sp.**	San Salvador, Bahamas	LACM 3316	Ebuo_04SSal02	JQ914619	JQ914621	JN819179	KM230540
E. canguzua	Dry Tortugas, Florida, USA	LACM 178644	Ecan_10Dry01	KM086376	KM204225	KM040827	KM230499
E. cf. *bennettae*	unknown	-	EBE2	DQ471216	DQ480183	DQ534778	-
E. cf. *marginata* sp.1	Pago Bay, Guam	-	E_cf_mar_sp1_09Gua01	JN819099	JN819128	JN819156	KM230469
E. cf. *marginata* sp.2	Pago Bay, Guam	-	E_cf_mar_sp2_09Gua02	KC573695	KM204201	KM040809	KM230470
E. cf. *marginata* sp.3	Sobe, Okinawa, Japan	-	E_cf_mar_sp3_08 Jap01	KC573752	KM204206	KM040810	KM230475
E. cf. *marginata* sp.4	Sobe, Okinawa, Japan	-	E_cf_mar_sp4_08Jap03	KC573754	-	KM230476	KC597177
E. cf. *tomentosa* sp.1	Sobe, Okinawa, Japan	-	E_cf_tom_sp1_05Jap01	KC573749	KM204204	KC597175	KM230473
E. cf. *tomentosa* sp.3	Toguchi, Okinawa, Japan	-	E_cf_tom_sp3_06Jap01	KC573752	KM204206	KM040810	KM230475
E. cf. *tomentosa* sp.4	Sobe, Okinawa, Japan	-	E_cf_tom_sp4_05Jap03	KC573754	-	KC597177	KM230476
E. cf. *tomentosa* sp.5	Krabi, Andaman Sea, Thailand	-	E_cf_tom_sp5_09Tha01	KC573755	KM204207	KC597178	KM230477
E. cf. *tomentosa* sp.6	Guam	-	E_cf_tom_sp6_09Gua01	KC573757	KM204208	KC597180	KM230478
E. chilkensis	Singapore	-	Echi_09Tha01	KM086361	KM204209	KM040811	KM230500
E. chlorotica	Martha's Vineyard, Mass., USA	LACM 178597	Echl_06Mas01	KM086377	KM204226	JN819183	GU191035
***E. christinae* n. sp.**	Bimini, Bahamas	LACM 3308	Echr_10Bim01	KM086366	KM204214	KM040817	KM230483
E. cornigera	Discovery Bay, Jamaica	*LACM 173227*	Ecor_06Jam01	JN819084	JN819125	JN819154	KM230501
E. crispata	Florida Keys, Florida, USA	*LACM 178636*	Ecri_06FL01	JN819090	JN819139	KM040828	KM230502
E. degeneri	Bile Bay, Guam	-	Edeg_09Gua01	KM086378	KM204227	KM040829	KM230503
E. diomedea	Los Rios, Bay of Panama	-	Edio_07BLR03	KM086379	KM204228	KM040830	KM230504
E. ellenae	New Providence, Bahamas	LACM 178663	Eell_10NPr01	JN819089	JN819141	JN819175	KM230484
E. evelinae	Brazil	-	Eeve_08Bra01	-	-	KM040831	KM230505

……*continued on the next page*

TABLE 1. (Continued)

Species	Locality	Voucher number[1,2]	Isolate sequenced	GenBank Accession Numbers			
				COI	16S	H3	28S
E. flava	Bocas del Toro, Panama	LACM 173252	Efla_06Pan1	-	KM204229	KM040832	KM230506
E. furvacauda	Lord Howe Is., Australia	*AM C.469614*	Efuv_11How02	KM086369	KM204218	KM040821	KM230488
E. hamanni	Banana River, Florida, USA	LACM 3310	JH S1167-69 c	-	-	-	-
E. hamatanii	MMBS, Kanagawa, Japan	-	Eham_02Jap01	JN819110	JN819143	JN819177	KM230507
E. hedgpethi	San Diego, California, USA	-	Ehedg_05SD01	KM086380	KM204230	KM040833	KM230508
E. leucolegnote	Bang Tawa, Thailand	-	Eleu_09Tha01	KM086381	KM204231	KM040834	KM230509
E. lobata	Maui, Hawaii, USA	-	Elob_11Maui01	KM086382	KM204232	KM040835	KM230510
E. macnaei	Bunaken Park, Sulawesi	-	Emacnaei726	GQ996689	EU140854	-	GQ996628
E. maoria	Auckland, New Zealand	-	Emao_06NZ01	KM086383	KM204233	KM040836	KM230511
E. marcusi	Discovery Bay, Jamaica	*LACM 17866*	Emar_06Jam01	KM086384	KM204234	KM040837	KM230512
E. mercieri	Bile Bay, Guam	-	Emer_09Gua01	KM086385	-	KM040838	KM230513
E. minima	Chinen, Okinawa, Japan	-	Emin_10Jap01	KM086386	KM204235	KM040839	KM230514
E. obtusa	Kominato, Japan	-	Eobt_09Jap01	KM086387	KM204236	KM040840	KM230515
E. orientalis	Florida, USA	CPIC 00842	Eori_13FL01	KP187842	KP187839	KP187833	KP187836
E. ornata	Discovery Bay, Jamaica	LACM 178581	Eorn_06Jam01	JN819093	JN819132	JN819157	KM230516
E. papillosa	Tampa, Florida, USA	*LACM 178600*	Epap_06Tar01	KP187843	KP187840	KP187834	KP187837
E. patina	Sweetings Cay, Bahamas	*LACM 178649*	Epat_04Swe04	JN819108	JN819145	KM040842	GU191033
***E. pawliki* n. sp.**	Sweetings Cay, Bahamas	LACM 3303	Epaw_03Swe01	KC573751	KM204205	KC597176	KM230474
E. pratensis	Plana Cays, Bahamas	-	Epra_07Pla04	JN819112	KM204237	JN819169	KM230518
E. pusilla (L)	Sobe, Okinawa, Japan	-	Epus_04Jap02	JQ914606	JN819152	KM040843	KM230519
E. pusilla (P)	Coolom, Queensland, Australia	-	Epus_07Aus01	JQ914593	JN819151	KM230520	KM040844
E. rufescens	Sobe, Okinawa, Japan	-	Eruf_08Jap01	KC573688	KM204247	KC597152	KM230530
E. serca	Lee Stocking Is., Bahamas	*LACM 172293*	Eser_06Lee03	DQ471244	KM204248	KM040846	KM230531
E. singaporensis	Western Johor Strait, Singapore	-	Esin_09Tha01	KM086398	KM204249	KM040847	KM230532
Elysia sp. 1	Namibia	-	E_sp1_Nam01	KM086367	KM204215	KM040818	KM230485
Elysia sp. 5	Maui, Hawaii, USA	CASIZ 166758	E_sp5_03Maui02	JN819113	JN819138	JN819172	KM230492
Elysia sp. 6	Playa Escondido, Mexico		E_sp6_08Mex01	KM086372	JN819134	KM230493	KM040824
Elysia sp. 9	Pago Bay, Guam	-	E_sp9_09Gua09	KM086373	KM204222	KM040825	KM230494
Elysia sp. 11	Azan Bay, Guam	-	E_sp11_09Gua16	KM086362	KM204210	KM040813	KM230479

... ...continued on the next page

TABLE 1. (Continued)

Species	Locality	Voucher number[1,2]	Isolate sequenced	GenBank Accession Numbers			
				COI	16S	H3	28S
Elysia sp. 12	Cocos reef, Guam	-	E_sp12_09Gua01	KM086363	KM204211	KM040814	KM230480
Elysia sp. 15	Cocos lagoon, Guam	-	E_sp15_09Gua01	KM086364	KM204212	KM040815	KM230481
Elysia sp. 16	Bile Bay, Guam	-	E_sp16_09Gua01	KM086365	KM204213	KM040816	KM230482
Elysia sp. 21	Lord Howe Is., Australia	*AM C.469612*	E_sp21_11How01	KM086368	KM204216	KM040819	KM230486
Elysia sp. 22	Lord Howe Is., Australia	AM C.469610	E_sp22_11How05	KC573759	KM204217	KM040820	KM230487
Elysia sp. 25	Lord Howe Is., Australia	AM C.469617	E_sp25_11How04	KM086370	KM204219	KM040822	KM230489
Elysia sp. 26	Palmyra Atoll, Line Islands	CASIZ 174215	E_sp26_06Pal01	KM086371	KM204220	KM230490	KM040823
Elysia sp. 30	Zanpa, Okinawa, Japan	-	E_sp30_08Jap02	-	KM204221	KM040845	KM230491
Elysia 'spec 5'	Lizard Island, Australia	-	Elysia5_806	GQ996691	EU140855	-	GQ996630
E. stylifera	Coolom, Queensland, Australia	-	Esty_07Aus01	GU191057	GU191046	KM040848	GU191032
E. subornata	Discovery Bay, Jamaica	*LACM 178630*	Esub_06Jam01	JN819111	JN819135	KM040849	KM230533
E. sugashimae	Sobe, Okinawa, Japan	-	Esug_05Jap01	KM086399	KM204251	KM040850	KM230534
***E. taino* n. sp.**	Discovery Bay, Jamaica	*LACM 178603*	Etai_06Jam06	JQ914617	JQ914620	KM040841	KM230517
E. thompsoni	Chinen, Okinawa, Japan	-	Ethom_10Jap01	JN819088	KM204252	KM040851	KM230535
E. timida	Banyuls-sur-Mer, France	-	Etim_07Fra01	KM086400	EU140857	JN819155	KM230536
E. transluscens	Banyuls-sur-Mer, France	-	Etran_845	HM187631	HM187602	-	-
E. trisinuata	Hayama, Japan	-	Etri_02Jap01	KM086401	KM204253	KM040852	KM230537
E. velutinus	Bocas del Toro, Panama	*LACM 178632*	Evel_04Boc01	KM086402	JN819133	KM040853	KM230538
E. viridis	Doolin Ferry, Galway, Ireland	-	Evir_05Ire01	KM086403	KM204254	KM040854	KM230539
***E. zemi* n. sp.**	Martinique	LACM 3305	Ezem_14Mar01	KP187844	KP187841	KP187835	KP187838
E. zuleicae	Sweetings Cay, Bahamas	*LACM 178657*	Ezul_07Swe01	JN819105	JN819146	JN819178	KM230541

[1]Italicized entries indicate the morphological voucher is a different specimen from the one used to obtain DNA sequences represented by NCBI accession numbers for that species.
[2]LACM = Los Angeles County Museum of Natural History malacology collection; AM = Australian Museum malacology collection; CPIC = Cal Poly Pomona Invertebrate Collection; CAS = California Academy of Sciences Invertebrate Zoology collection

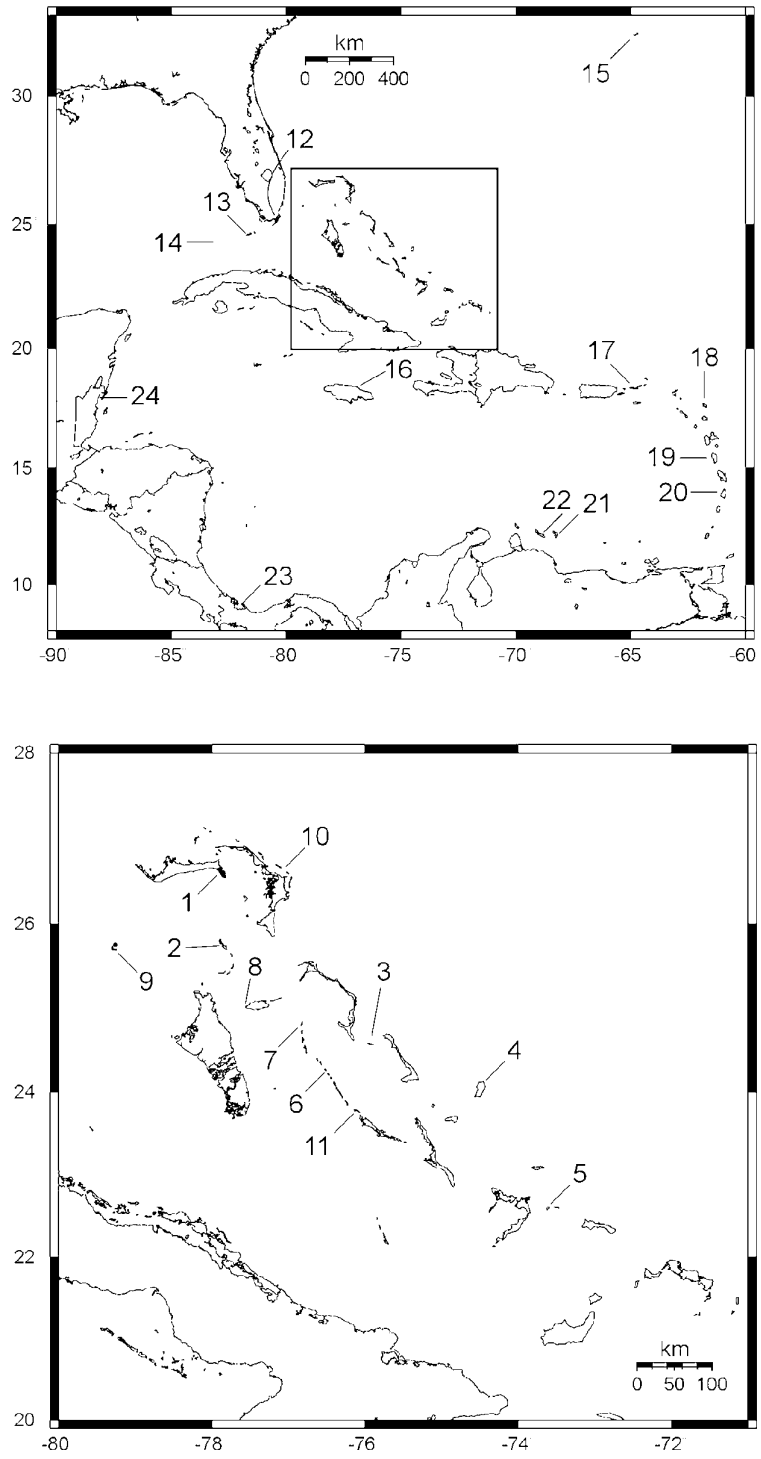

FIGURE 1. Primary sampling locations for specimens used in morphological and molecular analyses. Sites in the Bahamas included (1) Sweeting's Cay; (2) Great Stirrup Cay; (3) the lagoon in Little San Salvador island; (4) San Salvador island; (5) Plana Cays; (6) Compass Cay; (7) Northern Exumas; (8) New Providence; (9) Bimini; (10) Abaco; (11) Lee Stocking island. Major collecting sites in Florida sites were Lake Surprise, Key Largo (12); Geiger Beach, near Key West (13); and the Dry Tortugas (14). Other sites were Bermuda (15); Jamaica (16); the U.S. Virgin Islands (17); Antigua (18); Dominica (19); St. Lucia (20); Bonaire (21); Curacao (22); Bocas del Toro, Panama (23); and Belize (24). For each site, Table S1 gives the latitude and longitude; corresponding sample identifier abbreviation; sampling dates and collector(s); and more detailed information on collecting localities, as warranted.

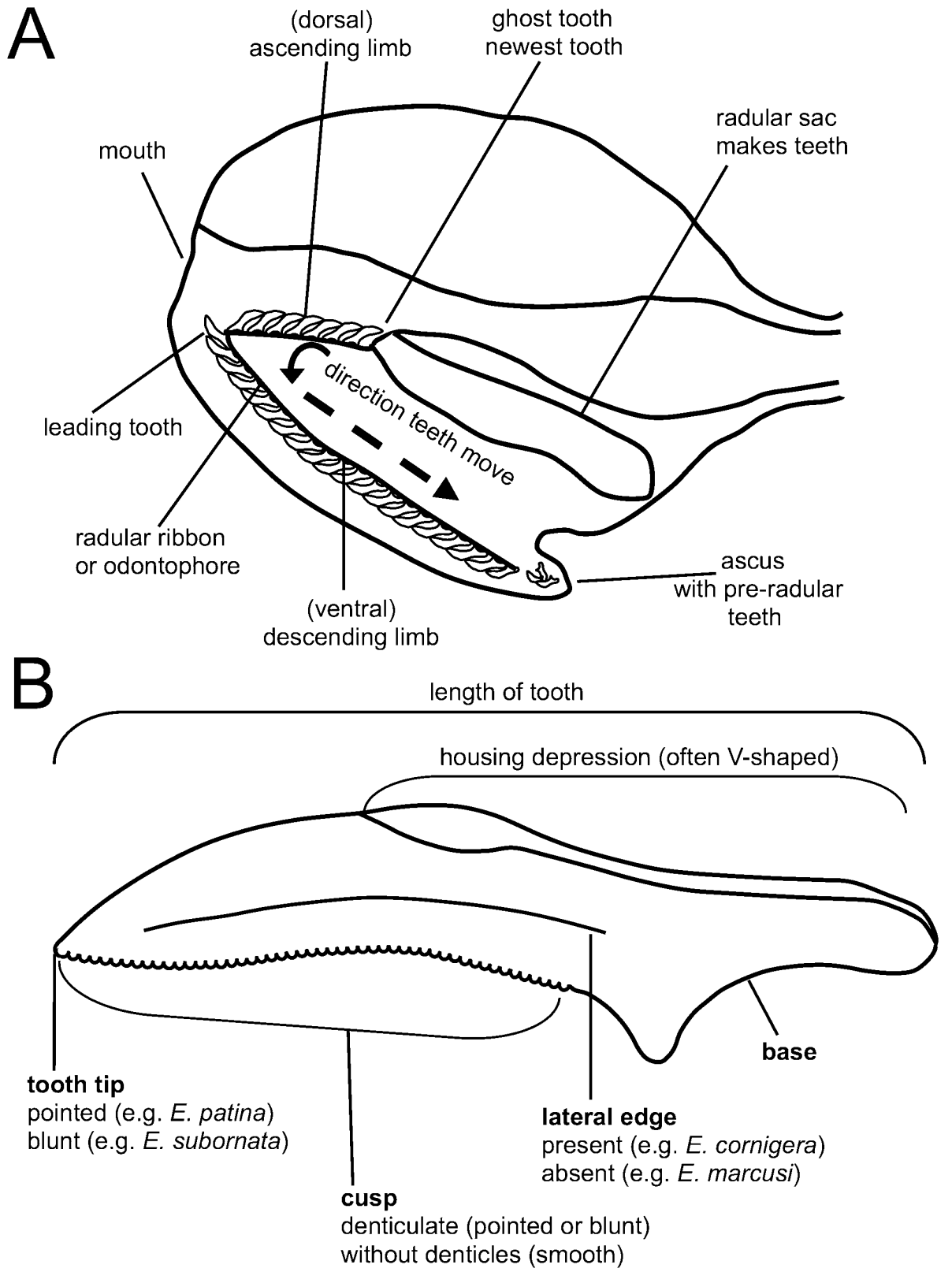

FIGURE 2. Diagram showing generic morphology of (a) a complete radula, and (b) a leading tooth, labeling features described or measured in the present study of *Elysia*.

Individual gene trees for all loci were initially inferred using Bayesian Inference (BI) and Maximum Likelihood (ML) methods as detailed below. Topologies were consistent among gene trees except in unresolved regions, supporting concatenation for final phylogenetic analyses. An alignment of all four loci was thus analyzed as described in Krug *et al.* (2015). Briefly, Markov-chain Monte Carlo (MCMC) methods were implemented with BayesPhylogenies software, using mixture models to capture among-site heterogeneity in substitution rates and base frequencies without partitioning (Pagel & Meade 2004). Four chains were run for 10^8 generations, each parameterizing three GTR + Γ models and assigning the best-fit model to each position in the alignment. Trees were saved every 5,000 generations, and L scores and parameter estimates inspected to confirm that runs reached stationarity. The final 400 trees from each run were pooled and a 50% consensus tree generated; nodal support was estimated as posterior probabilities (PP), with values ≥90% taken as significant (Huelsenbeck & Rannala 2004).

Maximum Likelihood analyses were run with RAxML v7.6.6 (Stamatakis 2006) through the CIPRES Science Gateway v3.3 (Miller *et al.* 2010), using a GTR + Γ model with 4 rate multipliers and no partitioning of the data (see Krug *et al.* 2015). Nodal support was assessed using 250 bootstrap pseudoreplicates, with values ≥70% taken as significant (Hillis & Bull 1993).

A separate analysis was performed to test the hypothesis that *Elysia crispata* and *Elysia clarki* were synonymous, by inferring evolutionary relationships among COI haplotypes from specimens provisionally classified by external morphology. Pierce *et al.* (2006) described *E. clarki* from the Florida Keys as distinct from *E. crispata* primarily using four characters: (i) COI distance of ~7% between specimens from Florida versus the U.S. Virgin Islands; (ii) in *E. crispata*, parapodia are highly ruffled with secondary and tertiary folding and largely white, whereas in *E. clarki* the parapodia are less ruffled with reduced surface area, and largely green; (iii) in adult *E. crispata*, the anterior edge of the parapodia fuse together behind the head, whereas in *E. clarki* (and juvenile *E. crispata*) the parapodia are unfused, leaving a notch or gap posterior to the head; (iv) in *E. crispata*, the foot is white (devoid of digestive diverticula) and blunt-ended, where in *E. clarki* the foot tapers to a point, and is green from diverticula with white spots, the same in appearance as the outer surface of the parapodia. A subset of COI haplotypes generated and analyzed by Vo (2013) were selected for 15 specimens that had been photographed and described while alive, representing (a) the traditional *E. crispata* morph, (b) the *clarki* morph described by Pierce *et al.* (2006), and (c) intermediate forms with combinations of features. A COI gene tree was inferred by ML analysis as described above, using *E. ellenae* as the outgroup based on our analysis of evolutionary relationships within *Elysia* (see Results).

Results

Species delimitation of Caribbean elysiids. The majority of Caribbean candidate species delimited by an 8% COI threshold (18 out of 22 spp.) fell within one of four major haplotype groups (subclades 1-4; see next section). Three of the remaining species were sister to a morphologically similar species from the Pacific (*E. velutinus*, *E. flava*) or Mediterranean (*E. cornigera*), while the fourth was morphologically derived and distinct from all congeners (*E. marcusi*); none of these cases warranted more focused delimitation analyses. One of the four major subclades (subclade 3, the *E. marginata* complex) was previously analyzed for species composition by ABGD, and no new molecular data were obtained for this clade in the present study (Krug *et al.* 2013). Each of the other three subclades was independently analyzed by ABGD using all available molecular data, to test whether closely related taxa were being lumped under an 8% divergence threshold.

Analyses of subclade 1 used 233 unique COI haplotypes sampled from 362 specimens, representing six putative candidate species; ABGD supported six species across all partitions (Fig. 3A). ABGD distinguished *E. papillosa* from its previously unrecognized sister species *E. taino* **n. sp.**, and *E. zuleicae* from its previously unrecognized sister species *E. buonoi* **n. sp.**, while also recovering *Elysia patina* and the undescribed *E. christinae* **n. sp.** as distinct. In all six species, specimens grouped as conspecific by ABGD also shared a distinctive pool of H3 alleles not sampled from other taxa, supporting the candidate species as non-interbreeding gene pools.

ABGD analysis of subclade 2 used 116 COI haplotypes sampled from 242 specimens, representing seven candidate species (excluding clade member *E. evelinae*). ABGD recovered seven species across all partitions below 6% intraspecific divergence (Fig. 3B). The bimodal distribution of COI distances below 8% was driven by a high degree of phylogeographic structure in *E. crispata*, which has relatively non-dispersive larvae. Notably, all *E. crispata* and nominal *E. clarki* morphotypes were recovered as a single species by ABGD. Moreover, H3 alleles were shared among nominal specimens of *E. crispata* and *E. clarki*.

Figure 3

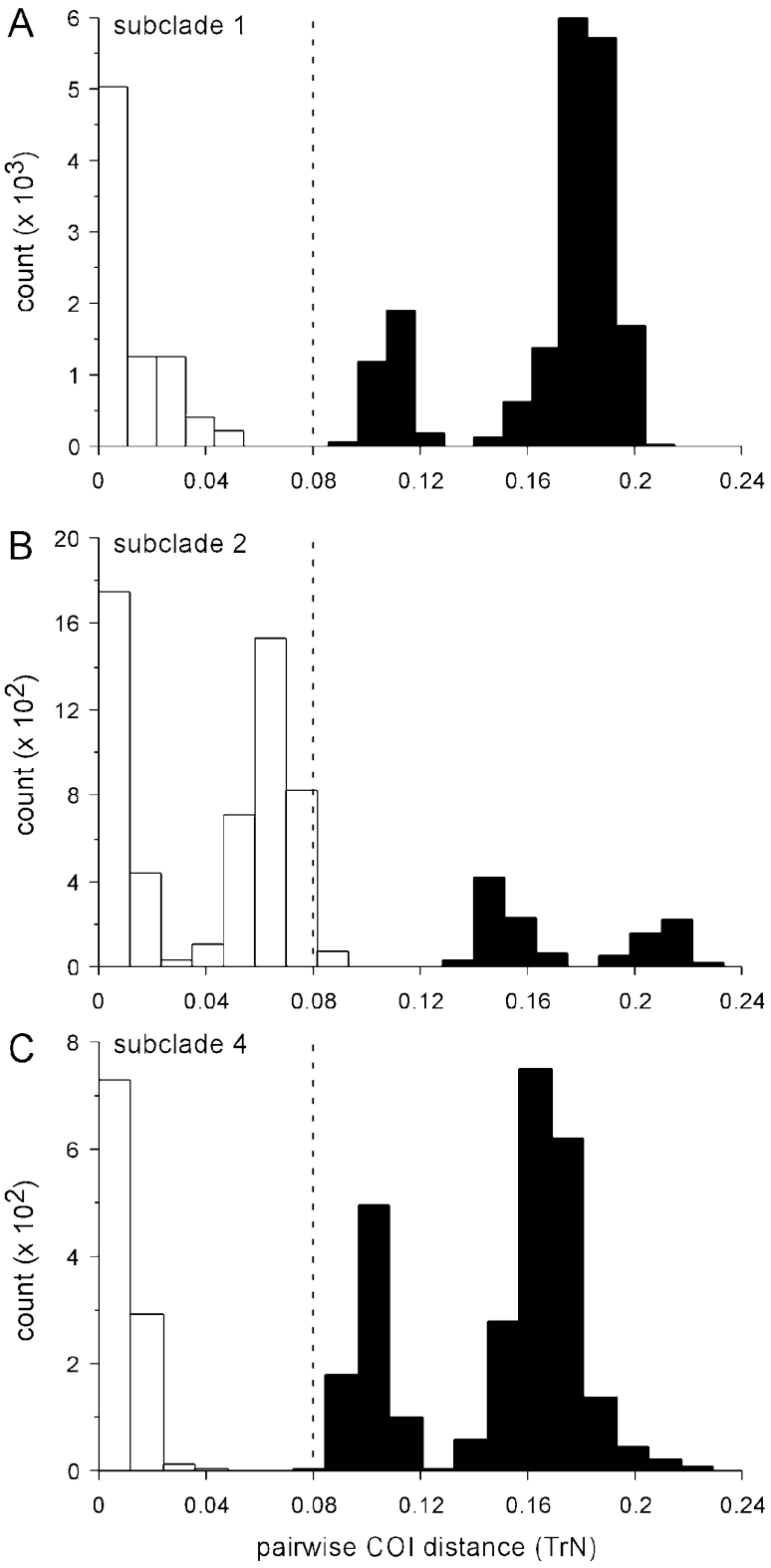

FIGURE 3. Mismatch distributions based on TrN-corrected pairwise distances at the COI locus for three subclades of *Elysia*. Based on ABGD analysis, genetic distances were classified as intra-specific (white bars) or inter-specific (black bars). Dashed horizontal line indicates the *a priori* threshold distance used provisionally to delimit candidate species.

Figure 4

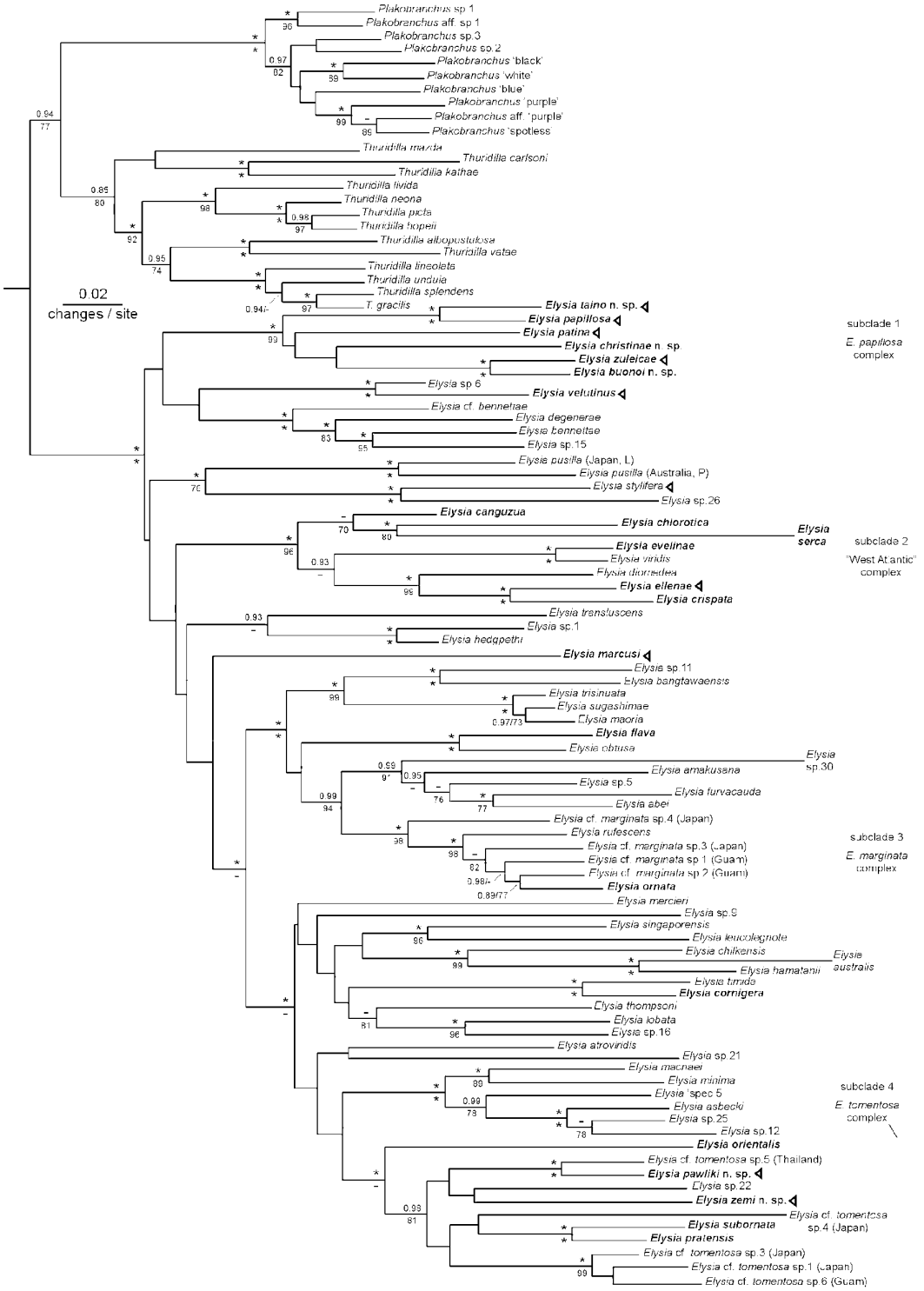

FIGURE 4. Phylogenetic hypothesis for family Plakobranchidae rooted on genus *Bosellia* (not shown) to illustrate relationships within the genera *Elysia*, *Plakobranchus* and *Thuridilla*. Topology and branch lengths are from Maximum Likelihood analysis of concatenated DNA sequences (2,807 bp total) representing portions of two mitochondrial (COI, 16S) and two nuclear (H3, 28S) genes. Significant support values are given as ML bootstrap percentages (below branch), or BI posterior probabilities (above branch); asterisk = 1.0 or 100% support. Species discussed in this study are bolded. Triangle denotes presence of a penial stylet.

Analysis of subclade 4 used 87 COI haplotypes sampled from 155 specimens, representing 11 candidate species (five Caribbean, six Indo-Pacific). As predicted, 11 species were supported by ABGD across all partitions, including two new species described herein (Fig. 3C). A subset of COI haplotypes in *E. pratensis* (restricted to some Bahamas islands) represented introgressed mtDNA from *E. subornata*, based on phylogenetic analysis; thus barcoding should be applied cautiously to this species pair (Rodriguez 2009). However, no H3 alleles were shared between Caribbean specimens grouped as different species by ABGD, again supporting each lineage as a distinct (non-interbreeding) nuclear gene pool.

Phylogenetic relationships within Elysia. Phylogenetic analyses supported two major clades within family Plakobranchidae: (1) a clade comprising outgroup genera *Thuridilla* and *Plakobranchus*, each supported as monophyletic and sister to each other; and (2) a fully supported clade comprising all 76 species of *Elysia* (Fig. 4). Sampled *Elysia* spp. included five new species described in this work, plus 24 candidate species (labeled sp. #, or "cf. sp." if anatomically similar to a named species) from other regions that are presently undescribed or unidentified. Within *Elysia*, sister-species relationships were recovered with significant support in most cases; however, higher-level relationships of lineages were not well resolved (Fig. 4), consistent with other recent studies that included fewer *Elysia* spp. (Christa *et al.* 2014, 40 spp.; Krug *et al.* 2015, 73 spp.).

Both species originally classified as *Elysiella* (*E. pusilla*, *E. stylifera*) formed a supported clade with candidate species *Elysia* sp. 26 from Palmyra Atoll (Fig. 4). Although no sister group was identified, this clade nested within *Elysia* in the ML and BI consensus trees, supporting prior work that synonymized *Elysiella* with *Elysia*. However, the uncertain placement of the lineage including "*Elysiella*" spp. leaves open the possibility that future work may recover this lineage sister to the rest of *Elysia*, in which case *Elysiella* could be resurrected.

Included in molecular analyses were 22 *Elysia* spp. from the Caribbean region (Fig. 4, bolded names). Most Caribbean taxa (n = 18) belonged to one of four supported subclades, which included all radiations occurring within the western Atlantic. Subclade 1 comprised six species feeding on udotacean green algae, and was termed the *E. papillosa* complex to reflect taxonomic confusion surrounding the identity of several included taxa. Three new species described herein belonged to this lineage. Subclade 2 included six species from the northern or tropical West Atlantic, including the taxonomically controversial lettuce slugs *E. crispata* and '*E. clarki*'. All members of subclade 2 lack ECY in their egg masses, a reproductive trait otherwise widespread in *Elysia* (Krug *et al.* 2015). Subclade 3 was the previously defined *E. marginata-ornata* complex (sensu Krug *et al.* 2013), including one Caribbean species. Subclade 4, the previously defined *E. tomentosa* complex, consisted primarily of *Caulerpa*-feeding taxa; this lineage comprised six Indo-Pacific species and five Caribbean taxa, including two new species described here. These major subclades within *Elysia* were also largely recovered in phylogenetic studies of Sacoglossa as a whole, including Christa *et al.* (2014) and Krug *et al.* (2015).

Species possessing a penial stylet, armature used to pierce the epidermis during hypodermic insemination, did not form a clade (Fig. 4). Stylets likely evolved six or more times within *Elysia*, and cannot be considered a genus-level synapomorphy of *Checholysia* as proposed by Ortea *et al.* (2005). Presence of a stylet may be plesiomorphic in *Elysia*; stylets are present in *Thuridilla* and *Plakobrachus*, and in some putatively basal *Elysia* spp. for which anatomical data are available, including most members of subclade 1, *E. stylifera*, and *E. velutinus*.

Systematics

Elysia Risso, 1818

Actaeon Oken 1815: 305 [non *Acteon* Montfort, 1810] (Type species *Aplysia viridis* Montagu, 1804 [=*Elysia viridis*]), rejected under plenary powers (ICZN 1956: Opinion 417).

Elysia Risso 1818: 375–376 (Type species: *Notarchus timidus* Risso, 1818 [=*Elysia timida*], by monotypy).

Aplysiopterus Delle Chiaje 1830: 31 (Type species: *Aplysiopterus neapolitanus* Delle Chiaje, 1830 [=*Elysia viridis*], by monotypy).

Rhyzobranchus Cantraine 1835: 384 (Type species: *Elysia viridis* (Montagu, 1804), by monotypy)

Rhycobranchus Herrmannsen 1846–47 [1846]: 17, error for *Rhyzobranchus*.

Thallepus Swainson 1840: 250, 359 (Type species: *Thallepus ornatu* Swainson, 1840 [=*Elysia ornata*], by monotypy).

Tridachia Deshayes 1857: 142 (Type species: *Elysia schrammi* Mörch, 1863 [=*Elysia crispata*], by subsequent monotypy).

Hydropsyche Kelaart 1858: 107 (Type species: *Elysia grandifolia* Kelaart, 1858, by monotypy).

Elysiella Verrill 1872: 283–284 (Type species: *Placobranchus catulus* Gould, 1870 [=*Elysia catula*], by monotypy).

Elysiella Bergh 1872: 201, pl. 9, fig. 3, pl. 24, figs. 20–25 [non *Elysiella* Verrill, 1872] (Type species: *Elysiella pusilla* Bergh, 1871 [=*Elysia pusilla*], by monotypy).

Pterogasteron Pease 1860: 35–36 (Type species: *Pterogasteron ornatum* Pease, 1860 [=*Elysia ornata*], here designated).

Thridachia P. Fischer 1880–87 [1883]: 545, unjustified emendation for *Tridachia*.

Elysiobranchus Pruvot-Fol, 1930: 230 (Type species: *Elysiobranchus mercieri* Pruvot-Fol, 1930 [=*Elysia mercieri*], by monotypy).

Tridachiella MacFarland 1924: 405 (Type species: *Tridachia diomedea* Bergh, 1894 [=*Elysia diomedea*], by original designation).

Elysiopterus Pruvot-Fol 1946: 39 (Type species: *Elysiopterus verrilli* Pruvot-Fol, 1946 [=*Elysia verrilli*], here designated).

Pattyclaya Ev. Marcus 1982: 17 (Type species: *Elysia arena* Carlson & Hoff, 1978, by original designation).

Checholysia Ortea, Caballer, Moro & Espinosa 2005: 512 (Type species: *Elysia patina* Ev. Marcus, 1980, by original designation) **n. syn.**

Diagnosis. Species of *Elysia* have a differentiated head bearing slender, dorsal rhinophores. Eyes located behind the rhinophores. Parapodia vary in size from narrow folds, barely covering the dorsal body surface, to wide extensions of the body. Body surface typically smooth, sometimes covered with papillae, which can be ramified. Dorsal vessels normally extensively branched, sometimes anastomosing distally. Body color usually green of different shades, but some species may be dark or light. In some species the parapodial margins may have brightly colored bands or spots, and spots of varying sizes and color may be distributed over the body. Pharynx lacking a pharyngeal pouch. Longitudinal ascus-muscle long and attached to the ventral surface of the pharynx throughout its length. Radular teeth blade-shaped, denticulate or smooth. Reproductive system triaulic, but a separate vaginal opening may be absent. There may be one, two or many ampullae. Penis is usually unarmed but in some species has a hollow apical stylet.

Remarks. The genus *Elysia* has a long and complex taxonomic and nomenclatural history. Several genus names are currently considered synonyms of *Elysia* for different reasons. Montagu (1804) described the species *Aplysia viridis* Montagu, 1804 (under the incorrect spelling "Laplysia") from Devonshire, England. Oken (1815) reexamined the original description of this species and considered it different from the true *Aplysia* Gmelin, 1791, thus erecting the new genus *Acteon* Oken, 1815 (under the incorrect spelling "Actæon"). However, Oken's name *Acteon* is preoccupied by *Acteon* Montfort, 1810, and subsequently Oken's (1815) publication was rejected for nomenclatural purposes by the ICZN (1956: Opinion 417). Risso (1818) named a new species from Nice, France as *Notarchus timidus* Risso, 1818, based on manuscript notes from 1812 in which he refers to the species as *Elysia timida*. Because Risso (1818) cites the species as the binomen *Elysia timida*, this work constitutes the original description of the genus *Elysia*. Another synonymous genus name based on Mediterranean-Atlantic species is *Aplysiopterus*, originally introduced by Delle Chiaje (1830) for the new species *Aplysiopterus neapolitanus* Delle Chiaje, 1830, which was later found to be a synonym of *Elysia viridis* (see Iredale & O'Donoghue 1923; Bouchet 1984). Five years later, Cantraine (1835) indicated that, in personal correspondence during 1827, he had created the name *Rhyzobranchus* for *Elysia viridis*, but now recognized that Risso's name, *Elysia,* had priority.

Two additional genus names were introduced for species with convoluted parapodial margins. Deshayes (1857) described the genus *Tridachia* based on a species to be named after Schramm, but did not name the species. Mörch (1863) introduced for the first time the binominal name *Tridachia schrammi* in reference to Deshayes' (1857) description, thus becoming the type species by subsequent monotypy. MacFarland (1924) described *Tridachiella* as different from *Tridachia* because the parapodia did not unite in front as in *Tridachia*. Because both *Tridachia* and *Tridachiella* are nested within *Elysia* in phylogenetic analyses based on both morphological (Gosliner 1995) and molecular (Händeler *et al.* 2009) data, these three names are considered synonyms.

Three additional genus names were introduced for members of the *Elysia ornata* species complex. Swainson (1840) described the genus *Thallepus* for *Thallepus ornatus* Swainson, 1840 but Verrill (1901) considered it to be a synonym of *Elysia*. Kelaart (1858) described the species *Elysia grandiflora* Kelaart, 1858 from Sri Lanka. At the end of the description he suggests to use the new genus name *Hydropsyche* for this species if it is later found that it does not belong to any know genus. Pease (1860) described the genus *Pterogasteron* for two species collected in the Hawaiian Islands, *Pterogasteron ornatum* Pease, 1860 and *Pterogasteron bellum* Pease, 1860. No type species was indicated. Pease's illustrations were published in Bergh (1881: pl. G, fig. 18–19), who transferred *Pterogasteron ornatum* Pease, 1860 to the genus *Elysia*, making it a homonym of *Elysia ornata* Swainson, 1840, of which it is also a synonym (Jensen 1992). Krug *et al.* (2013) showed that although there may be undescribed Indo-Pacific species, all members of the *Elysia ornata* species complex form a clade; thus, the genera *Thallepus*, *Hydropsyche*, and *Pterogasteron* are synonyms of *Elysia*.

In 1872 two authors independently introduced the same genus name for two different species of *Elysia*. Bergh (1872) described the genus *Elysiella* for *Elysiella pusilla* Bergh, 1871 and distinguished it from *Elysia* because of the short tentacles and the carinated side of the head. The name *Elysiella pusilla* was first introduced in the caption of plate 9 for Bergh's (1872) paper, which was published in 1871, a year before the actual text. Verrill (1872) introduced the genus *Elysiella* for *Placobranchus catulus* Gould, 1870 as different from *Elysia* and *Placobranchus* because the posterior end of the parapodia are fused together. According to Wheat (1918) *Elysiella* Verrill, 1872 was published earlier and therefore has priority, thus *Elysiella* Bergh, 1872 is unavailable. Jensen & Wells (1990) and Jensen (1992; 1997b) considered *Elysiella* a valid genus based on a broad, demarcated foot, short parapodia and rhinophores, an elongated renopericardial extension radiating dorsal vessels, and radular teeth with triangular, unidenticulate cusps. Jensen (1997b) described an additional species from Australia, *Elysiella stylifera* Jensen, 1997. However, *Elysia* is paraphyletic with respect to *Elysiella* in both morphological (Gosliner 1995; Jensen 1997a) and molecular (Händeler *et al.* 2009; Krug *et al.* 2015) phylogenetic analyses. Thus, *Elysiella* is considered a synonym of *Elysia*.

Similarly, the genus *Pattyclaya* Marcus, 1982 was erected for the species *P. arena* Carlson & Hoff, 1978, which has dorsal lamellae running perpendicular to the main body axis; a second species, *P. brycei* Jensen & Wells, 1990, was subsequently described in this genus. However, *Pattyclaya* nested within *Elysia* in all morphological phylogenetic analyses (Gosliner 1995; Jensen 1997a), and is considered a synonym of *Elysia* pending molecular confirmation.

Several additional genus-level names have been introduced more recently because of the presence of unique anatomical traits in some species. For example, Pruvot-Fol (1930) described the new genus *Elysiobranchus* for *Elysiobranchus mercieri* Pruvot-Fol, 1930, which has long and ramified tubercles. Later, Pruvot-Fol (1946) considered *Elysiobranchus* as a subgenus of *Elysia*, and Carlson & Hoff (1978) re-described *E. mercieri* treating *Elysiobranchus* as a synonym of *Elysia*. Pruvot-Fol (1946) introduced the subgenus *Elysiopterus* for Verrill's (1901) misidentification of *Elysia crispata* (as "*Elysia crispa*"), which she named *Elysiopterus verrilli* Pruvot-Fol, 1946. Also, Pruvot-Fol (1946) included *Placobranchus expansa* O'Donoghue, 1924 in this new subgenus. As discussed below, *Elysia velutinus* Pruvot-Fol, 1947 (=*Elysiopterus verrilli* Pruvot-Fol, 1946) is a senior synonym of *Elysia tuca* Ev. Marcus & Er. Marcus, 1967 which is nested within other Caribbean *Elysia*, and therefore there is no phylogenetic basis for the maintenance of *Elysiopterus*. Finally Ortea *et al.* (2005) introduced the genus *Checholysia* Ortea, Caballer Moro & Espinosa, 2005 for species with a penial stylet, with *Elysia patina* Marcus, 1980 as the type species. Because *Elysia velutinus* Pruvot-Fol, 1947 has a penial stylet, *Elysiopterus* is the oldest available genus-level name for such a group of species. However, according to the phylogenetic analysis here presented, the penial stylet in *Elysia* evolved multiple times, so there is no phylogenetic support for either *Elysiopterus* or *Checholysia* (Fig. 4).

Elysia ornata **(Swainson, 1840)**

(Figs. 4–5, 6A, 7–8)

Thallepus ornatus Swainson 1840: 250, 359 (Type locality: undetermined, probably St. Vincent, U.S. Virgin Islands).

Dolabrifera (?) *ornata* (Swainson, 1840)—Pilsbry 1895–96 [1896]: 126.

Elysia ornata (Swainson, 1840)—Verrill 1901: 28–29, pl. 4, fig. 5; Engel 1927: 113–115, figs. 29–30; Pruvot-Fol 1946: 33; Er. Marcus 1957: 414; Ev. Marcus 1976b: 128; Ev. Marcus & Er. Marcus 1963: 20–21, figs. 27–28, 64; Er. Marcus & Ev. Marcus 1970: 43–44, figs. 80–83; Marcus 1972a: 292, fig. 18; Bandel 1976: 95–96, fig. 8 (egg mass); Thompson 1977: 126–128, figs. 25d–e, 26a; Ev. Marcus & Hughes 1974: 507, fig. 17; Ev. Marcus 1980: 63–64, figs. 1–3, 38, 44; Jensen & Clark 1983: 5; Díaz Merlano & Puyana Hegedus 1994: 246; Clark 1994: 905; Espinosa & Ortea 2001: 44; García *et al.* 2002: 50, figs. 2E–F; Espinosa *et al.* 2005: 56; García *et al.* 2008: 69–70; Valdés *et al.* 2006: 62–63; Redfern 2013: 282–283, fig. 786; Krug *et al.* 2013: 1106–1108, fig. 1A–E, fig. 2A; Christa *et al.* 2014: fig. 3; Krug *et al.* 2015: 990–991, figs. 3B, 4.

Type material. *Thallepus ornatus*—type material untraceable.

Material examined. Discovery Bay, Jamaica, 1 March 2006, 2 specimens (LACM 178581–82); Playa Kanoa, Curaçao, 4 Jan 2009, 1 specimen (LACM 178579); Spanish Waters inlet, Curaçao, 9 Jan 2009, 1 specimen (LACM 178580).

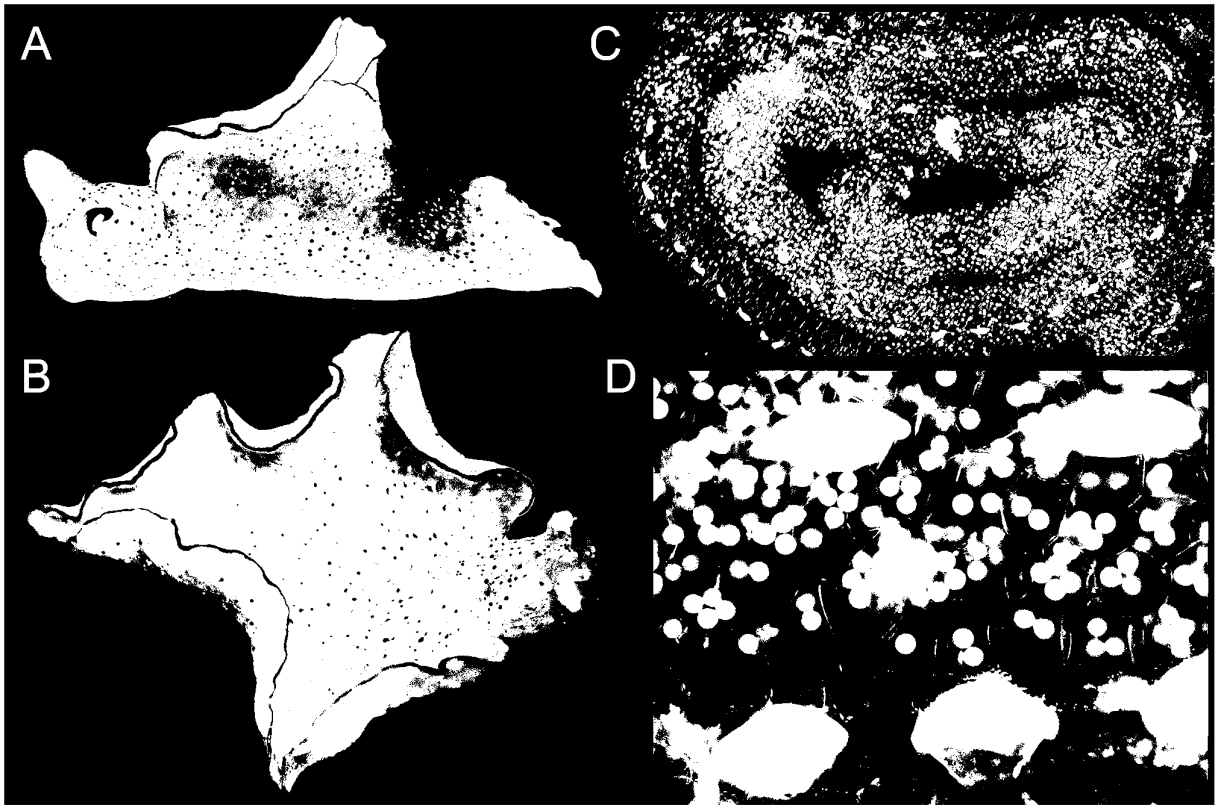

FIGURE 5. *Elysia ornata*, external anatomy and egg mass. **A,** Lateral view of a live animal, specimen from Jamaica (20 mm long). **B,** Dorsal view of same specimen. **C,** Egg mass of figured specimen from Jamaica (15 mm across). **D,** Detail of the eggs and ECY from a specimen from Curaçao, showing 1–3 uncleaved ova per capsule, and ECY inclusions imbedded in the upper face of the egg mass. Field = 1.48 mm across.

Additional material examined. Discovery Bay, Jamaica 1 March 2006, 1 specimen (isolate Eorn_06Jam03); Playa Kanoa, Curaçao, 4 Jan 2009, 1 specimen (isolate Eorn_09Cur01); 5 specimens, Bocas del Toro Panama, 30 July 2015.

Live animal. Brightly colored species, yet cryptic when buried among thalli of the alga *Bryopsis*; parapodial lines and mottled body coloration renders slugs difficult to see.

External anatomy. Overall color olive green, with black and smaller white spots scattered across head and parapodia; white dots sometimes forming medial line on head (Fig. 5A–B). Rhinophores short, tapering to a point at rolled tips; surface smooth, lacking dots otherwise scattered across head. Bright white streak extending from base halfway up rhinophores. Distal half of rhinophores orange, with black band at tips. Foot not clearly distinct from parapodia, same green color without spotting. Transverse groove separating underside of head from foot. Parapodia extending to posterior end of body, uniting to form pointed tail. High-arching parapodia forming three siphonal openings when covering dorsum, with middle opening forming a prominent raised chimney halfway along body (Fig. 5A). Parapodial margin somewhat undulating, with black marginal band slightly separated from orange submarginal band running along both outer and inner edge; some specimens with white dots running between marginal and submarginal band.

Pericardium small with short renopericardial extension, both white with scattered black spots, tiny red-orange dots and brown flecks. Large specimens with three dorsal vessels radiating from each side of renopericardial complex, branching irregularly and repeatedly; side branches anastomosing into complex network lining inner face of each parapodium (Fig. 7). Vessels often spaced at regular intervals, transparent but sometimes highlighted by large white spots or tiny red-orange dots. White reproductive glands visible within tissue of dorsum, divided by wide medial band of clear tissue running length of body to tail.

Internal anatomy. Radula with 9 teeth (LACM 178581), 6 teeth in ascending limb and 3 in descending limb (Fig. 8A). Leading tooth elongate, widest at mid-length, tapering and slightly curved towards tooth tip, with cusp

lacking denticles (Fig. 8B). Housing depression for interlocking teeth "V"-shaped and extending ½ of tooth length (Fig. 8B). Base of tooth approximately ⅓ of total tooth length. Ascus containing jumbled heap of discarded teeth (Fig. 8C).

Penis short and broad, almost as wide as long, devoid of armature (Fig. 6A). Deferent duct short and thin.

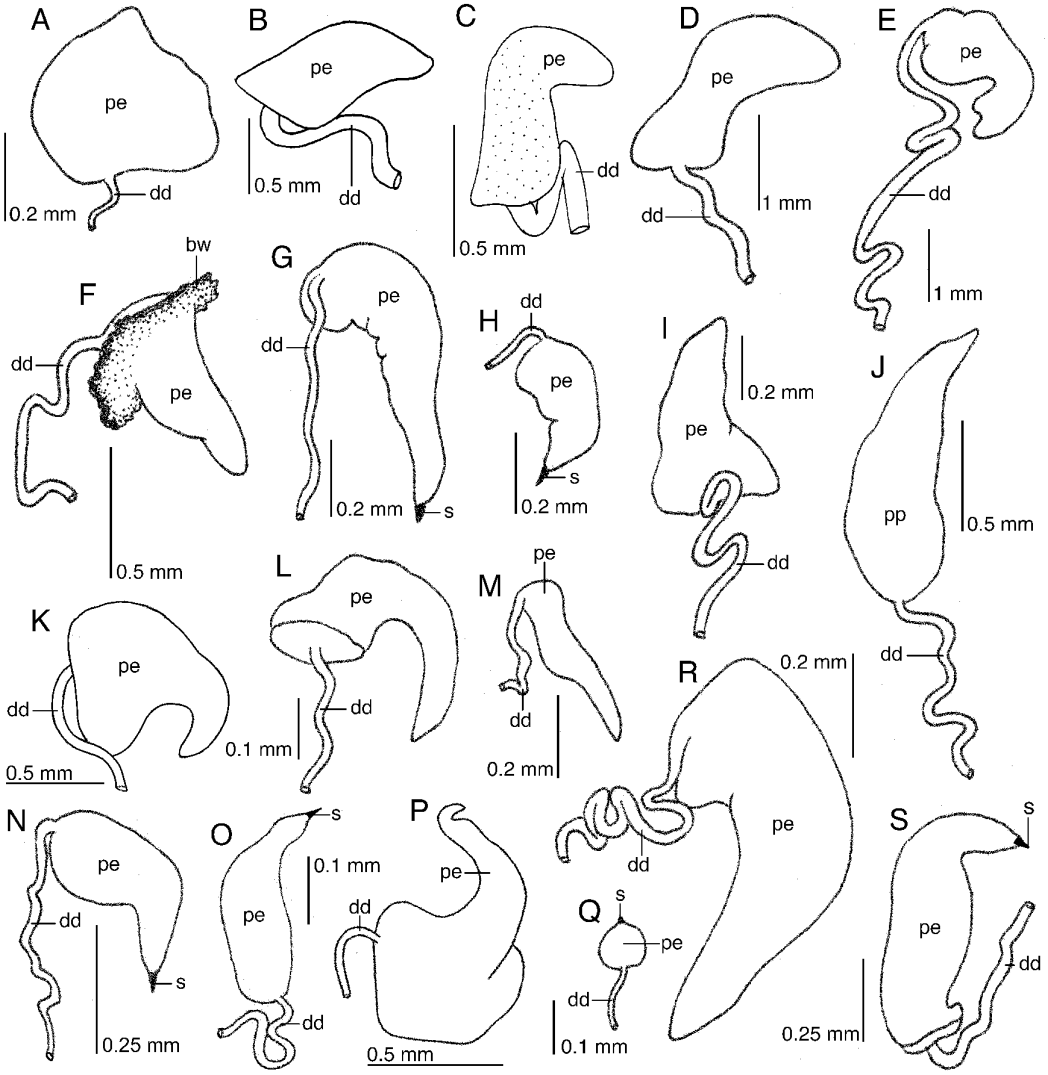

FIGURE 6. Penial morphology of some species examined. **A**, *Elysia ornata* (LACM 178583). **B–E**, *Elysia crispata* (LACM 178640) (B); (LACM 178641) (C); (LACM 2004.5.1) (D); (isolate Ecri_10LKS03) (E). **F**, *Elysia chlorotica* (LACM 178597). **G–H**, *Elysia patina* (LACM 178650) (G); (LACM 178651) (H). **I**, *Elysia flava* (LACM 178626). **J**, *Elysia subornata* (LACM 178629). **K**, *Elysia canguzua* (LACM 178644). **L**, *Elysia serca* (CPIC 00027). **M**, *Elysia evelinae* (MZUCR INB0003312779). **N**, *Elysia velutinus* (LACM 178642), **O**, *Elysia papillosa* (LACM 178607). **P**, *Elysia cornigera* (LACM 173227). **Q**, *Elysia marcusi* (LACM 178647), **R**, *Elysia pratensis* (CPIC 00068). **S**, *Elysia zuleicae* (LACM 178656). Abbreviations: bw, body wall; dd, deferent duct; pe, penis; s, stylet.

Reproduction and development. Clutches laid by *E. ornata* from Jamaica and Curaçao contained regularly spaced blobs of white ECY deposited along the inside of the upper face of the egg mass (Fig. 5C–D). The ECY was deposited as clumps of tiny granules within a thin casing; deterioration of the casing released the granules as larvae developed (Fig. 5D). In related candidate species from the Pacific, belonging to the "*E. marginata*" complex, ECY was deposited as a continuous black ribbon ("sp. 1"), or as regularly spaced blobs of bright yellow ("sp. 2" from Guam), darker gold ("sp. 3" from Japan), or orange ("sp. 4" from Japan) (Krug *et al.* 2013).

One clutch laid by a specimen from Curaçao had a mean egg diameter of 59.4 ± 2.6 μm (n = 14 ova), while a clutch deposited by a Jamaican specimen had a mean egg diameter of 55.8 ± 2.2 μm (n = 25 ova). Development is

planktotrophic. The encapsulated period was 6 d at room temperature (n = 2 clutches). At hatching, mean larval shell width was 111.1 ± 5.7 μm and 119.6 ± 8.3 μm for two clutches from Jamaica (n = 25 larvae per clutch). A characteristic of this species is the presence of multiple embryos developing within some capsules (Fig. 5D). Of three clutches laid by specimens from Jamaica, clutch #1 contained primarily 4–6 eggs per capsule; in clutch #2, most capsules contained 2–4 eggs; and in clutch #3 (the smallest), capsules held only one egg. All embryos completed development and hatched as veliger larvae.

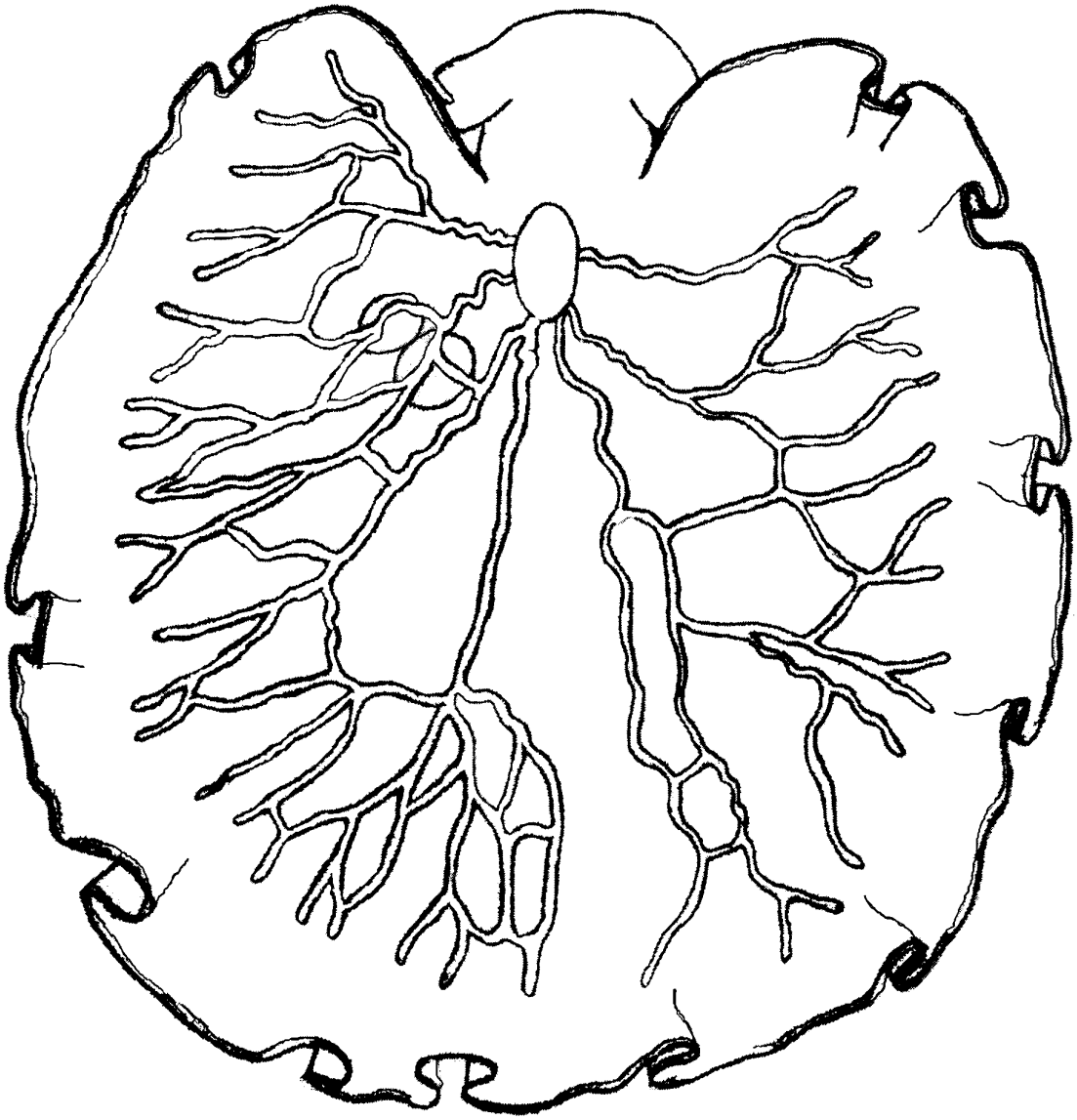

FIGURE 7. *Elysia ornata*, drawing of the renopericardium and dorsal vessel network of a preserved specimen (LACM 178580; 15 mm long × 11 mm wide).

Host ecology. The only host described in the literature for *E. ornata* and identified in the present study is *Bryopsis*, generally *B. plumosa*. Species of *Bryopsis* are also the host of the five Indo-Pacific species that form a clade with *E. ornata*, indicating speciation in this complex was not driven by host shifts.

Phylogenetic relationships. *Elysia ornata* is a derived member of subclade 3, which includes five tropical Indo-Pacific species: four candidate species in the *marginata-grandifolia* complex, plus *E. rufescens* (Fig. 4; Krug *et al.* 2013). Its sister species ("*E.* cf. *marginata* sp. 2") is morphologically similar but deposits yellow ECY in its egg masses, and is known from Japan, Guam and Vanuatu. The divergence between *E. ornata* and *E.* cf. *marginata* sp. 2 is ~8%, the minimum inter-specific distance proposed by Krug *et al.* (2013) for delimiting *Elysia* spp. using

COI barcodes. Subclade 3 likely diversified in the Indo-Pacific prior to colonization of the Caribbean by the ancestor of *E. ornata*, given its derived position.

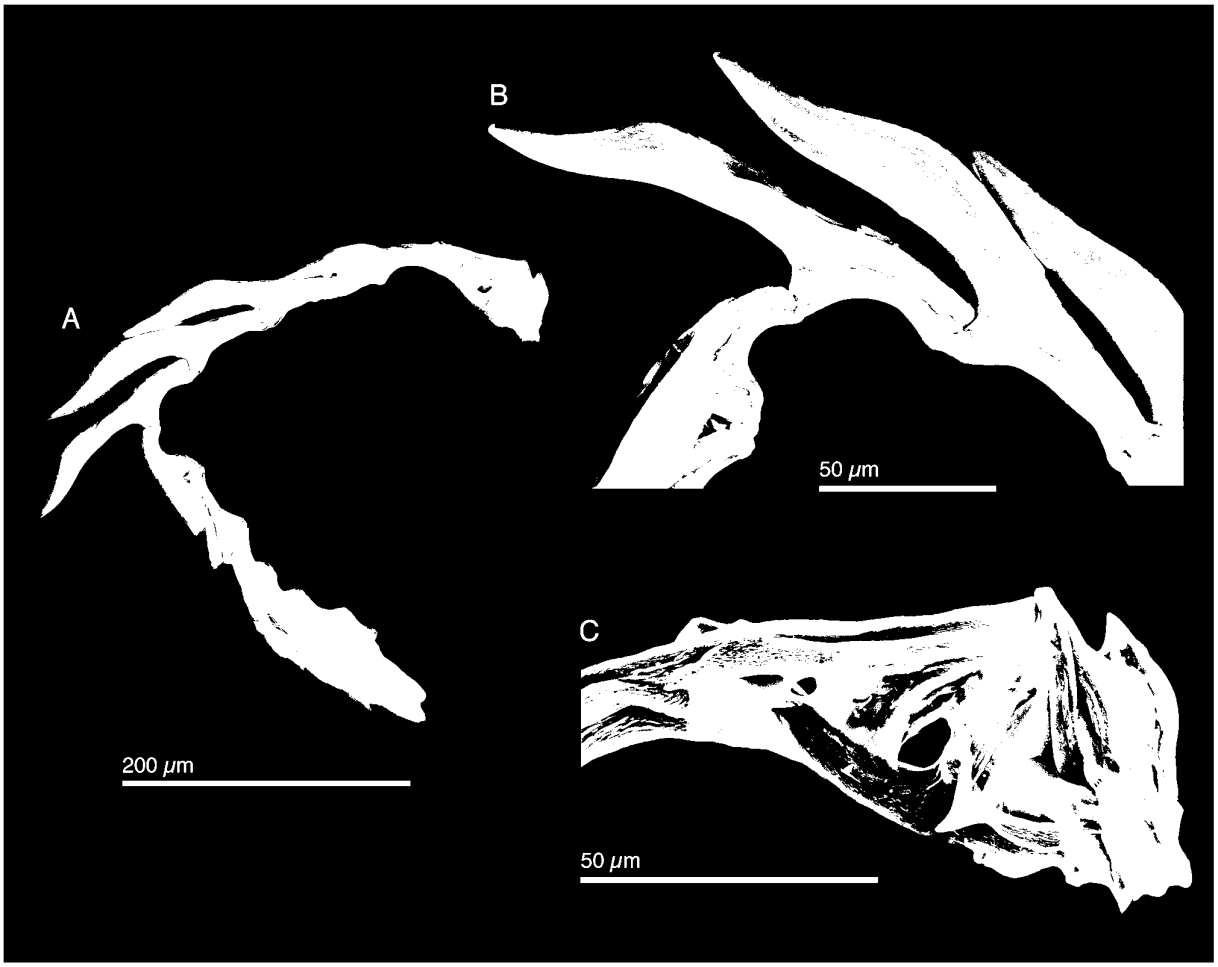

FIGURE 8. *Elysia ornata*, SEM of the radula (LACM 178581). **A,** Complete radula. **B,** Leading tooth. **C,** Ascus.

Range. Aruba (Valdés *et al.* 2006), Bahamas (Redfern 2013), Barbados (Ev. Marcus & Hughes 1974), Bermuda (Verrill 1901), Brazil (García *et al.* 2002, 2008), Colombia (Ev. Marcus 1976b), Costa Rica (Espinosa & Ortea 2001), Cuba (Espinosa *et al.* 2005), Curaçao (Engel 1927; Ev. Marcus & Er. Marcus 1963; Er. Marcus & Ev. Marcus 1970; present study), Florida (Ev. Marcus 1972a; Jensen & Clark 1983; Clark 1994), Guadeloupe (Valdés *et al.* 2006), Jamaica (Thompson 1977; present study), St. Vincent (Swainson 1840), Trinidad and Tobago (Valdés *et al.* 2006). Also recorded from the Eastern Atlantic in the Canary Islands and the Azores (Ortea *et al.* 2001; Malaquias *et al.* 2009).

Remarks. *Thallepus ornatus* was described by Swainson (1840: 250) based on an unpublished drawing by Reverend Lansdown Guilding. The animal was described as "sea green, covered with minute black and white dots; the edges or crests of the reflected mantle have a broad edging of the richest orange, bordered on their outer edge with a line of deep black; the tentacula are also orange, and formed like those of *Aplysia*." In another entry Swainson (1840: 359) added "Body more slender and fusiform [than *Aplysia*]; the lobes of the mantle short, and incapable of being used for swimming; tentacula two, large, ear-shaped; eyes not visible." This description matches the external morphology and coloration of the species commonly referred to as *Elysia ornata* in the Caribbean literature (see Ev. Marcus & Er. Marcus 1963; Er. Marcus & Ev. Marcus 1970; Ev. Marcus 1972a; Thompson 1977; Ev. Marcus & Hughes 1974; Espinosa & Ortea 2001; Valdés *et al.* 2006). Verrill (1901) transferred *T. ornatus* to *Elysia* and Ev. Marcus (1980) subsequently re-described as *E. ornata* based on Caribbean specimens.

However, other genetically distinct Indo-Pacific species have a similar external morphology and anatomy and

have been included in the same species (Jensen 1992), thus it is important to determine the type locality of all available names for this species complex. No type locality was specified in the original description of *Thallepus ornatus*, but because Reverend Guilding [1797–1831] lived in St. Vincent and worked exclusively on Caribbean natural history (Howard & Howard 1985), it is almost certain that the specimen used in the drawing was found in the Caribbean Sea. Two other large species of *Elysia* feeding on *Bryopsis* spp. were described from the tropical Pacific. Both have a black band along the parapodial edge and a submarginal orange band similar to those of *E. ornata*. The first species, *E. grandifolia* (Kelaart, 1858), was described from Sri Lanka as having black and gold marginal lines along parapodia that fused with the tail (Kelaart 1858). The second species, *E. marginata* (Pease, 1871) was originally described from the Hawaiian Islands and subsequently from Tahiti as having a white band between the orange and black marginal bands (Pease 1871). Authorities subsequently debated whether *E. grandifolia* had denticulate teeth (Eliot 1904, 1908; O'Donoghue 1932). Both *E. marginata* and *E. grandifolia* were synonymized with *E. ornata* based on morphological comparisons between Pacific and Caribbean material (Ev. Marcus 1980; Heller & Thompson 1983; Jensen 1992).

Recent integrative taxonomic work revealed that the *E. marginata-grandifolia* complex contained four candidate species in Pacific, all distinct from each other and from *E. ornata* by (1) molecular sequence analyses of two genetic loci; (2) external features including color of rhinophores and marginal bands, folding of parapodia into siphonal openings, tail shape, and pattern of dorsal vessels; and (3) color and pattern of ECY (Krug *et al.* 2013). *Elysia ornata* is therefore restricted to the Caribbean, and some related Pacific species await formal description.

Elysia crispata Mörch, 1863

(Figs. 4, 6B–E, 9–14)

Tridachia ornata [non *Elysia ornata* (Swainson, 1840)]—White 1952: 118–120, text figs. 19–20, pl. 6, fig. 6.

Elysia (Tridachia) crispata Mörch aff. Ørsted 1863: 40 (Type Locality: St. Croix).

Elysia crispata Mörch aff. Ørsted 1863—Clark 1994: 904; Redfern 2001: 162, figs. 670; Espinosa & Ortea 2001: 44; Collin *et al.* 2005: 690; Espinosa *et al.* 2005: 56, fig. 339; Valdés *et al.* 2006: 62–63; Redfern 2013: 282–283, figs. 784A–C; Zamora-Silva & Ortigosa 2012: 366, fig. 2H.; Händeler *et al.* 2009: figs. 6, 7; Krug 2009: 362–365, figs. 4D, 6; Christa *et al.* 2014: fig. 3; Krug *et al.* 2015: 990–991, figs. 3B, 4.

Tridachia crispata (Mörch aff. Ørsted, 1863)—Engel 1927: 115, fig. 32; Ev. Marcus & Er. Marcus 1960: 153–159, figs. 36–43; Ev. Marcus & Er. Marcus 1962: 461–463, figs. 7–8; Ev. Marcus & Er. Marcus 1967: 33–34, text fig. 38, pl. 1, figs. 7–8; Ev. Marcus & Er. Marcus 1963: 23–24; Er. Marcus & Ev. Marcus 1970: 50; Bandel 1976: 96–97, fig. 9 (egg mass); Ev. Marcus 1976b: 128–129; Ev. Marcus & Hughes 1974: 509, figs. 21–22; Thompson 1977: 131–132, figs. 22f, 28a–b; Ev. Marcus 1980: 75; Jensen & Clark 1983: 6, fig. 2A; Hess *et al.* 1994: 161–162, figs. 9.5–9.6.

Elysia (Tridachia) crispata var. *schiadura* Mörch 1863: 40–41 (Type locality: St. Croix).

Elysia schrammi Ørsted & Mörch *in* Mörch 1863: 41 (Type locality: Guadeloupe)—Er. Marcus 1957: 415–416.

Tridachia whiteae Er. Marcus 1957: 416 (Type locality: Dry Tortugas)—introduced for *Tridachia ornata* sensu White (1952) [non Swainson (1840)].

Elysia clarki Pierce *et al.* 2006: 26–36, figs. 1B, 1D, 2, 4A, 5A, 5C, 5E, 5G, 6A–B, 7 (Type locality: Eastern end of Vaca Key, Florida Keys, USA) **n. syn.**; Curtis *et al.* 2006: 340–343, figs. 3–6; Curtis *et al.* 2010: 299–302, figs. 1A, 1B, 2A, 3; Middlebrooks *et al.* 2011, 2014; Christa *et al.* 2014: fig. 1E; Curtis *et al.* 2015: 27, fig. 1

Type material. *Elysia crispata*—3 syntypes from St. Croix (ZMUC GAS-1584); *Elysia crispata* var. *schiadura*—1 syntype from St. Croix (ZMUC GAS-1572); *Tridachia schrammi*—4 syntypes from Guadeloupe (MHNH).

Material examined. A total of 189 specimens examined morphologically by PJK, 155 of which were also sequenced for the mitochondrial COI and nuclear H3 loci. Of these specimens, those with LACM specimen numbers range from LACM 178584–96.

Bocas del Toro, Panama, 19 February 2004, 1 specimen (LACM 2004-5.1); New Providence, Bahamas, July 2010, 3 specimens (LACM 178588–89, LACM 178641); Discovery Bay, Jamaica, 7 March 2006, 5 specimens (LACM 178591–92, LACM 178636–37, LACM 178640); Florida, USA: Geiger Beach, August 2007, 2 specimens (LACM 178590, LACM 178596), Dry Tortugas National Park, 2010, 2 specimens (LACM 178593–94), Mote Marine Laboratory and Aquarium, June 2007, 2 specimens (LACM 178587, LACM 178635), Lake Surprise Inlet, Key Largo, 26 October 2009, 2 specimens (LACM 178595, LACM 178638), November 2010, 4 specimens (LACM 178584–86, LACM 178639).

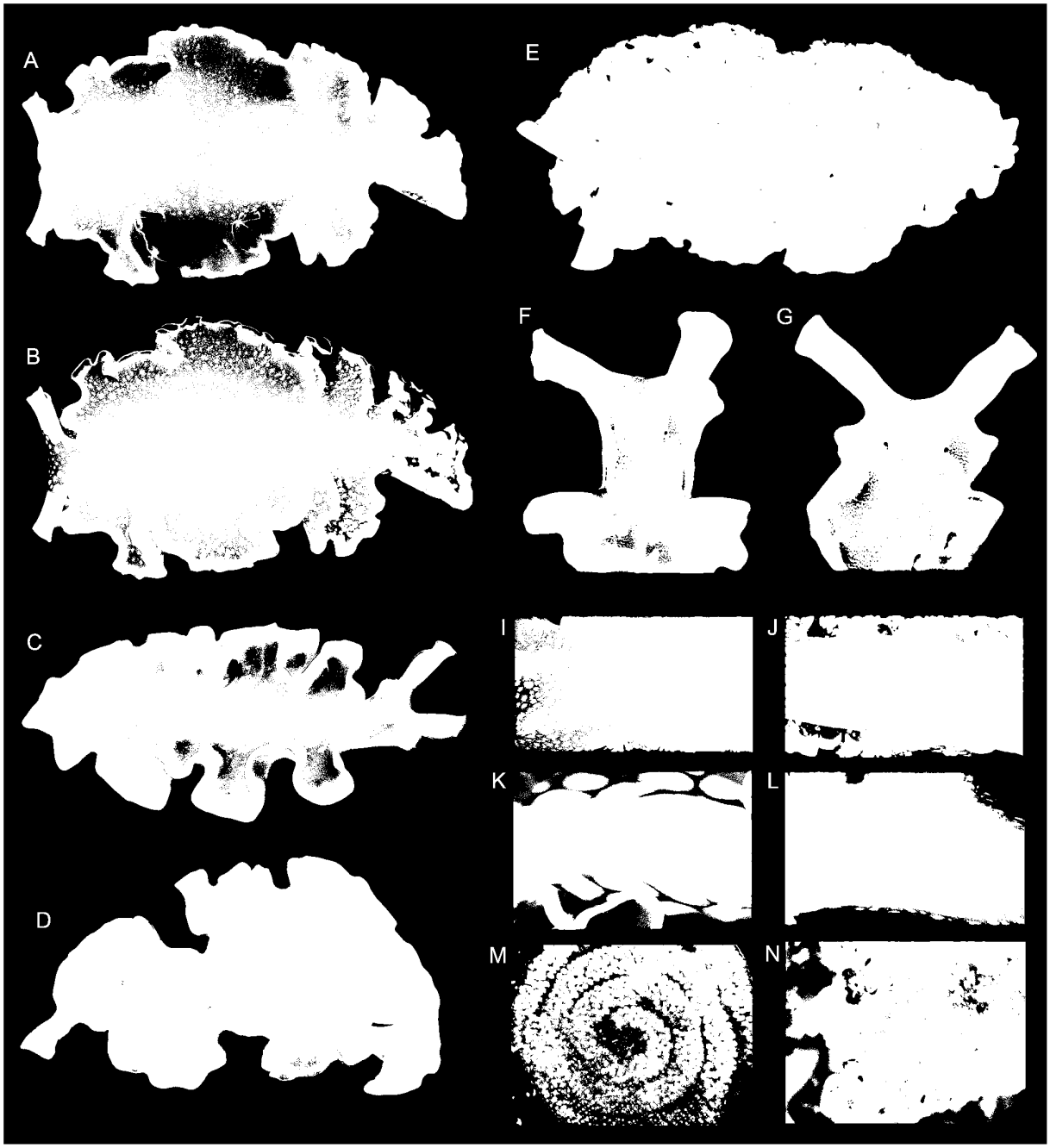

FIGURE 9. *Elysia crispata*, external anatomy and egg mass. **A,** Dorsal view of specimen from Florida Keys (LACM 178590; 14 mm). **B,** Dorsal view of specimen from boat channel at the Mote Tropical Research Laboratory, Florida Keys (LACM 178635; 23 mm). **C,** Dorsal view of specimen from Dry Tortugas (LACM 178593; 18 mm). **D,** Dorsal view of a '*clarki*' morph specimen from Lake Surprise, Florida (LACM 178595; 52 mm). **E,** Dorsal view of specimen from Bocas del Toro, Panama (35 mm). **F–G,** Variability in parapodial notch in two specimens from Yucatan, Mexico. **I–L,** Variability in foot sole pigmentation for specimens from the Florida Keys (LACM 178590) (**I**), (LACM 178635) (**J**), Dry Tortugas (LACM 178593) (**K**), and '*clarki*' morph from Lake Surprise, Florida (LACM 178595) (**L**). **M,** Egg mass of a specimen from Sweetings Cay, Bahamas. Field of view = 11.7 mm. **N,** Close-up of larvae from a clutch laid by a specimen from Lake Surprise, Florida; shelled larvae have lost their velar lobes and are metamorphosing on the egg mass at the time of hatching. Field of view = 1.8 mm.

Additional material examined. Bocas del Toro, Panama, December 2004, 17 specimens (Ecri_04Pan01-17); Bahamas: New Providence, July 2010, 17 specimens (isolate Ecri_10NPr01-07, isolate Ecri_10NPr10-20), Sweetings Cay, July 2007, 20 specimens (isolate Ecri_07Swe01-20), Little San Salvador, July 2007, 45 specimens

(isolate Ecri_07LSS01-45), San Salvador, July 2010, 6 specimens (isolate Ecri_10SSal01-06), Compass Cay, July 2010, 1 specimen (isolate Ecri_10Comp01), Northern Exumas, July 2010, 3 specimens (isolate Ecri_10NEx01-03), Bimini, July 2010, 14 specimens (isolate Ecri_10Bim01-14); Discovery Bay, Jamaica, 7 March 2006, 16 specimens (isolate Ecri_06Jam04, isolate Ecri_06Jam08-23); Florida, USA: Geiger Beach, August 2007, 9 specimens (isolate Ecri_07Gei03-11), Dry Tortugas National Park, 2010, 16 specimens (isolate Ecri_10Dry03-18), Mote Marine Laboratory and Aquarium, June 2007, 1 specimen (isolate Ecri_07Mote02), Lake Surprise Inlet, Key Largo, 26 October 2009, 8 specimens (isolate Ecri_09LKS04-11), November 2010, 1 specimen (isolate Ecri_10LKS03); English Harbor, Antigua, Antigua and Barbuda, 25 April 2008, 16 specimens (isolate Ecri_08Ant01-16); Dominica, 2007, 26 specimens (isolate Ecri_07Dom01-26); Son Friere Bay, St. Lucia, 27 March 2008, 12 specimens (isolate Ecri_08STL01-12); Spanish Waters inlet, Curaçao, 8 January 2009, 7 specimens (isolate Ecri_09Cur01-07); Bonaire, May 2012, 1 specimen (Ecri_012Bon01).

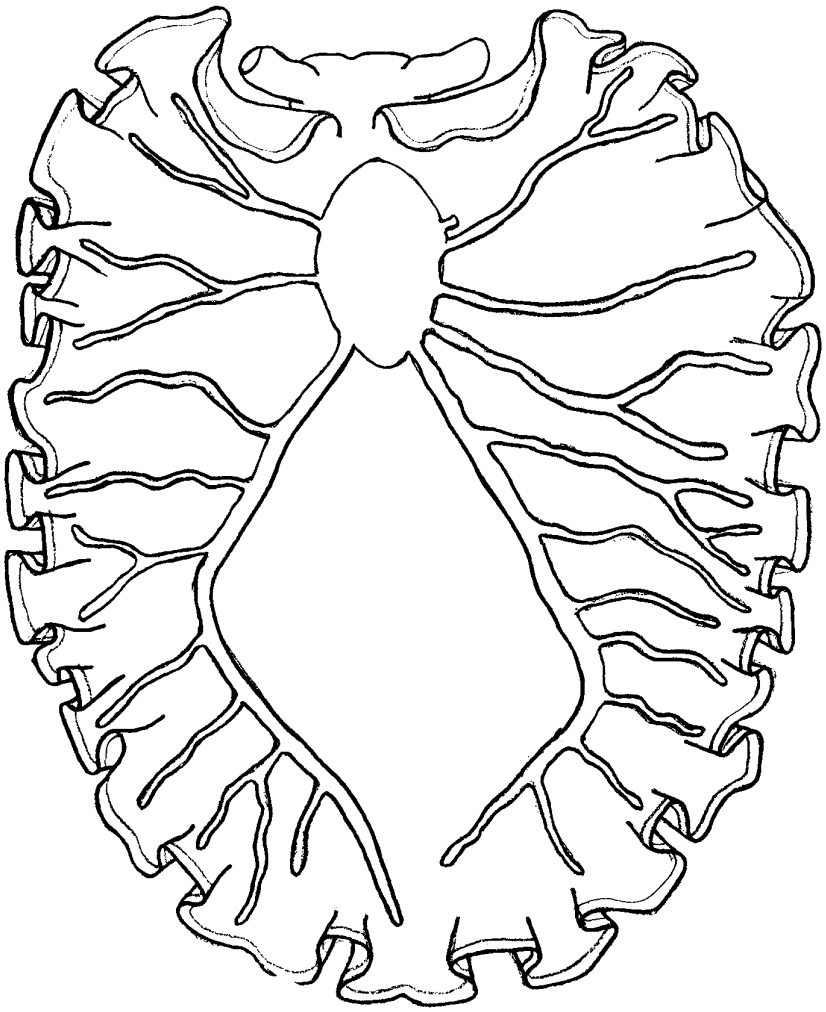

FIGURE 10. *Elysia crispata*, drawing of the pericardium and dorsal vessels of a preserved specimen (LACM 178589; 4.5 cm long × 3.3 cm wide).

Live animal. *Elysia crispata* is both the largest Caribbean elysiid, and the only species not commonly associated with a particular host alga. Most specimens in the present study were found resting or crawling on hard substrata, although some specimens were collected on *Halimeda incrasatta* (Key West, FL), *Penicillus capitatus* (Curaçao), and *Bryopsis plumosa* (Jamaica, Dry Tortugas). Lighter morphs with white foot are associated with areas of fast water flow and high light (e.g., tidal surge channels, patch reefs), whereas darker morphs are found in shaded areas of low light and reduced flow (e.g., rocky banks in mangrove lagoons, coastal borrow pits). The

frilled, undulating parapodia usually cover the dorsum. A few specimens were found ovipositing in the field on upright algal thalli (*Avrainvillea*, Key Largo, FL; *Penicillus*, Curaçao; *Udotea*, Sweeting's Cay, Bahamas).

FIGURE 11. *Elysia crispata*, SEM of the radulae of several specimens. **A**, Complete radula (LACM 178593). **B**, Leading tooth (LACM 178593). **C**, Close-up of leading tooth showing fine denticles (LACM 178593). **D**, Leading tooth (LACM 178636). **E**, Leading tooth (LACM 178594). **F**, Leading tooth (LACM 178637). **G**, Leading tooth (LACM 178591).

External anatomy. Large-bodied *Elysia* with highly variable external coloration, ranging from predominantly creamy white with green patches between large white spots (Fig. 9A–C), to dark green (Fig. 9D) or in the '*clarki*' morph purple with white spotting to entirely blue (Fig. 9E). Dorsal surface between parapodia also highly variable in color, generally green with pale cream (Fig. 9A–B) to white spots (Fig. 9C) varying in size and number, to uniformly green (Fig. 9D). Foot also highly variable in color, ranging from green with small pale spots (Fig. 9I, 9L) or large white spots (Fig. 9J) on specimens from lower light environments, to uniformly pale cream with no spots on specimens from high-light, high-flow habitats (Fig. 9K). Head relatively small for body size; ground color

green, with scattered large or small white spots, and/or iridescent blue pigment specks. Rhinophores short and wide, having same color pattern as rest of dorsum or lighter and lacking spots.

Parapodia undulating to varying degrees, often correlated with overall coloration and microhabitat. In typical specimens with lighter coloration from brightly lit, high-flow environments, undulations numerous and highly convoluted, resulting in parapodia with large surface area that cover entire dorsum (Fig. 9E). Undulations shallow and less numerous (*'clarki'* morph) on darker specimens from low-light, low-flow habitats, leaving most of dorsum uncovered (Figs. 9A–D). Anterior ends of parapodia either fused together (Fig. 9F) or separate (Fig. 9G) varying among specimens, but usually fused on adults with highly undulating parapodia, and unfused on *clarki* morphotypes and all juveniles. Parapodial margin thin, typically edged with thin white line followed by submarginal band of darker green or grey, and pale yellow to orange line. Submarginal band either continuous or interrupted; some specimens with neon blue pigment surrounding yellow-orange line. Specimens from Lake Surprise, Key Largo, Florida with distinctive orange marginal line.

Pericardium typically differing in color from rest of dorsal surface, being either darker or lighter. Renopericardial extension short. Two to three anterior dorsal vessels emerging from renopericardium on either side, asymmetrically placed, running perpendicular to body axis and branching near upper edge of parapodium (Fig. 10). Posterior paired vessels emerging before terminus of short renopericardial extension, running to posterior end of body and sending off numerous lateral vessels that fork once or not at all.

Internal anatomy. Radula with 12–18 teeth (LACM 178584, LACM 178586–89, LACM 178590–94, LACM 178636–38), 6–9 in ascending limb and 6–9 in descending limb (Fig. 11A). Leading tooth elongate and variable in shape from slender (Figs. 11E–F, 8C) to robust (Figs. 12A–B, D). Cusp bearing numerous very small denticles (e.g. Figs. 11C–D) not evident in all figured specimens (e.g. Fig. 12B). Housing depression for interlocking teeth "V"-shaped and extending ⅔ of tooth length (Figs. 11D, F, 12A–D). Some specimens with a sharp transition between denticulate edge and rest of tooth visible as a longitudinal line (labeled with white arrow in Fig. 12A; visible but unlabeled in Figs. 11B–F, 12E–F), but absent in other specimens (Fig. 12B–D). Base of tooth approximately ¼ total tooth length. Ascus containing jumbled heap of discarded teeth (not figured).

Penis highly variable (Figs. 6B–E), with no correlation between penial shape and position of an individual slug on a COI gene tree of all specimens. Penis typically curved slightly and devoid of armature. Deferent duct long, thin, and convoluted (e.g. Fig. 6E).

Reproduction and development. Development is lecithotrophic (Fig. 9M–N). Like all members of subclade 2, *E. crispata* does not produce ECY. From field observations, slugs preferentially oviposit on upright, flat surfaces such as the vertical thalli of *Udotea* or *Avrainvillea*, or the tops of *Penicillus capitatus*. Laboratory-held slugs that had not oviposited for several weeks rapidly laid eggs on plastic aquarium plants once introduced to the tank; the response to these structural mimics suggests that egg-laying behavior may be inhibited in the absence of preferred algal substrates.

Larvae lack any host-associated settlement cue and undergo spontaneous metamorphosis before hatching, or within a few days of hatching (Krug 2009). Earlier work described *E. crispata* as poecilogonous, with mangrove populations producing 'type 2' or swimming lecithotrophic larvae, and reef slugs producing 'type 3' larvae that underwent intracapsular metamorphosis and hatched as crawl-away juveniles (Clark & Jensen 1981; Defreese & Clark 1983; Clark 1994). These are not distinct types of larvae, however, but rather reflect inter-clutch variation in the timing of hatching or attainment of competence, common in many lecithotrophic species (Krug 2009).

Development of mangrove specimens from the Bahamas, and reef specimens from Curaçao, was described (Krug 2009). For two clutches, mean egg diameter was 113.8 ± 3.9 µm (Bahamas; n = 7) and 106.1 ± 2.2 µm (Curaçao; n = 67). Much larger mean egg sizes were previously reported: 205 mm by Clark & Jensen (1981), and 209 mm by Defreese & Clark (1983). No measures of variance were reported by Clark and coworkers, making it difficult to evaluate the reliability of these values. No sacoglossan has been reported to have eggs larger than ~130 µm in any recent study, and only three older studies reported egg diameters on the order of 200 µm (*Limapontia senestra*, Chia 1971; *Berthelinia* [= *Tamanovalva*] *limax*, Kawaguti & Yamasu 1960; *Thuridilla hopei*, Thompson & Salghetti-Drioli 1984). The older values for *E. crispata* and other taxa may indicate a recent decline in mean egg sizes in Sacoglossa.

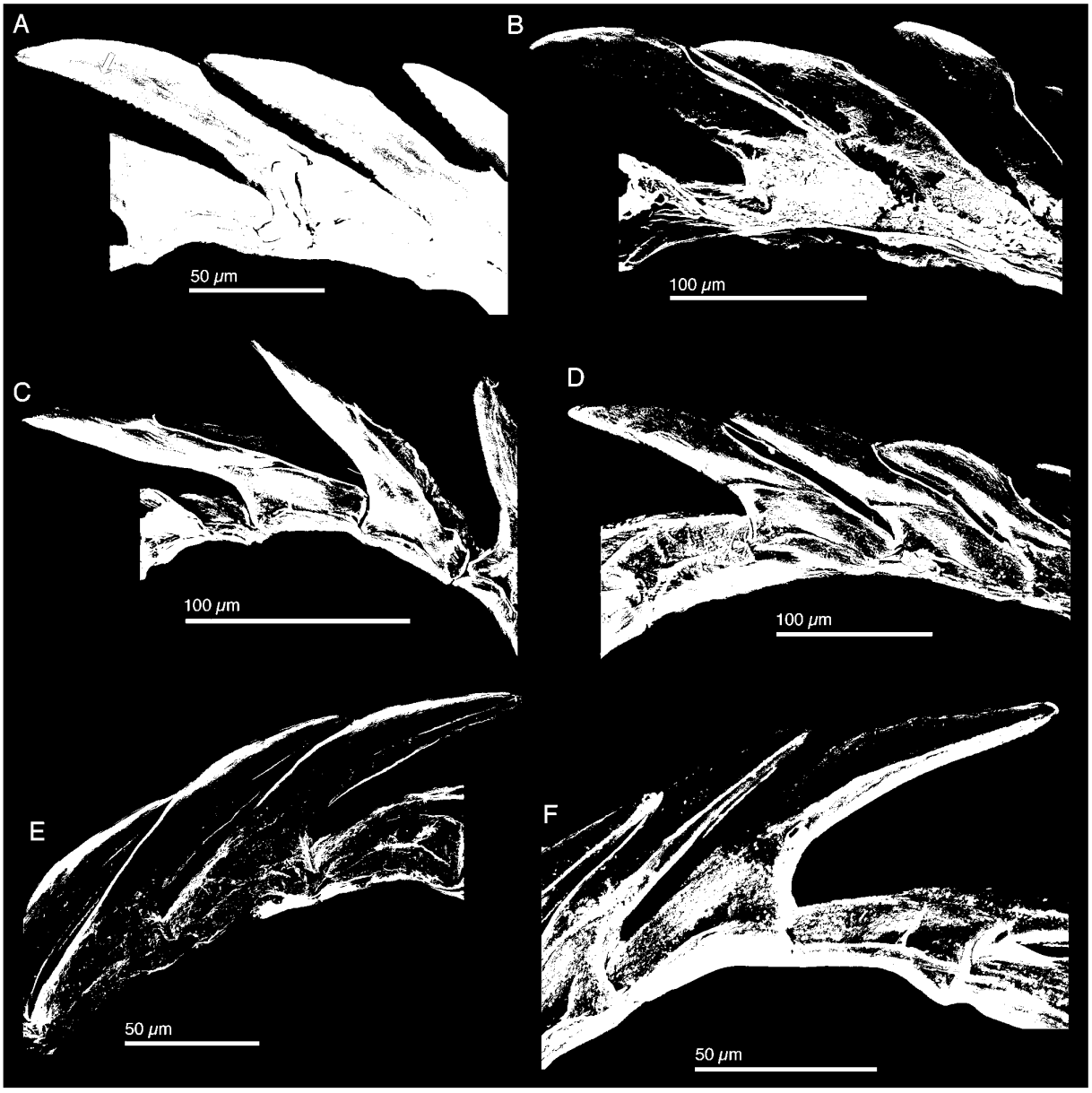

FIGURE 12. *Elysia crispata*, SEM of the radulae of additional specimens. **A**, Leading tooth (LACM 178592); white arrow indicates sharp transition between denticulate edge and rest of tooth. **B**, Leading tooth (LACM 178584). **C**, Leading tooth (LACM 178590). **D**, Leading tooth (LACM 178638). **E**, Leading tooth (LACM 178588). **F**, Leading tooth (LACM 178589).

Mean time to hatching was 14.9 ± 0.4 d at ~22°C (n = 9 clutches). In nine of 11 clutches laid by Bahamas slugs, all larvae metamorphosed prior to hatching, while in two clutches all larvae hatched but metamorphosed in filtered seawater within 2 d (Krug 2009). In egg masses laid by specimens of the *clarki* morph from Lake Surprise, Florida, larvae emerged in the early stages of metamorphosis, primarily crawling and in the process of velar resorption. Longer post-hatching larval periods of 4–5 d were reported by Pierce *et al.* (2003), and a longer encapsulated period for the *clarki* morph (28–35 d), for clutches incubated at 20°C. Differences in conditions during incubation of egg masses among studies may explain such variation in time to hatching and the proportion of encapsulated metamorphosis.

Significant among-clutch variation in larval shell size at hatching was reported, ranging from a mean shell width of 238.7 ± 5.93 μm (n = 40) for a clutch from Curaçao to a mean width of 299.2 ± 13.9 μm (n = 33) for a clutch from Sweetings Cay, Bahamas. Overall mean shell size per clutch was 283.0 ± 25.1 μm (n = 5 clutches).

Mean body length of post-metamorphic juvenile slugs was 517.1 ± 64.6 μm (n = 2 clutches) prior to feeding. Juveniles from egg masses laid by *clarki* morphs fed on *Bryopsis plumosa* or *Derbesia tenuissima*, but not *Caulerpa verticillata*; juveniles of typical *crispata* morphs did not feed on other *Bryopsis* spp., but *B. plumosa* was evidently not tested (Pierce *et al.* 2003).

Host ecology. *Elysia crispata* is one of the most polyphagous sacoglossans, but studies on its feeding ecology have a long and complicated history. Uncertainty has surrounded whether animals consume algae that they may be spatially associated with in the field. Confusion stems in part from the wide range of algae included in the diet of *E. crispata*; further, due to the highly photosynthetic nature of this species, specimens of *E. crispata* are not often observed feeding or physically resting upon a particular host. Feeding has been inferred from various lines of evidence including preferential association in field surveys or laboratory assays, visual observation, chlorophyll content of slug tissue, electron microscopy of retained chloroplasts, and DNA barcoding of chloroplasts retained in digestive gland cells of field-collected slugs. The latter two methods may best reflect algal consumption under ecologically relevant conditions; however, these methods may not fully capture all species eaten in the field if there is biased retention of (or PCR amplification from) plastids from a subset of consumed species.

It was long asserted that juveniles fed preferentially on *Caulerpa verticillata* (Jensen 1980; Jensen & Clark 1983; Clark 1994). The study cited to support this assertion contains no relevant data, however; Clark & Busacca (1978) tested adult feeding, and *C. verticillata* was not among the five *Caulerpa* spp. used in assays. Adding further confusion, Clark & Busacca (1978) state "… *Tridachia* used no species of *Caulerpa*" as a food source, yet listed "40% of *Caulerpa* spp." as "accepted" by *Tridachia* in their Table 2, and also reported that *Penicillus* spp. were consumed but *P. capitatus* was not.

The adult diet of *E. crispata* was reported to include *Bryopsis plumosa* and at least one species each of *Penicillus*, *Halimeda*, *Cymopolia*, and *Batophora* (Clark & Busacca 1978; Jensen 1980; Jensen & Clark 1983). In separate laboratory feeding assays, slugs were reported to feed on *Halimeda discoidea*, *Chaetomorpha* sp., and three *Caulerpa* spp. (*C. verticillata*, *C. racemosa* and *C. sertularioides*); however, slugs performed poorly on *C. verticillata* and died after a week on *C. sertularioides* (Thompson & Jarman 1989). The ecological relevance of captive feeding on toxic algae remains unclear, however.

Pierce and colleagues used a combination of microscopy, DNA barcoding, field surveys and lab feeding trials to establish the diet of Florida populations of the '*clarki*' morph of *E. crispata*. In the field and lab, slugs consumed at least six genera, including *Derbesia tenuissima*, seven *Bryopsis* spp. *(B. plumosa*, *B. pennata*, *B. pennatula*, four unidentified species), three *Penicillus* spp. (*P. capitatus*, *P. lamourouxii*, *P. pyroformus*), three *Halimeda* spp. (*H. incrassata*, *H. monile*, *H. opuntia*), *Acetabularia*, and an alga with genetic affinity to *Pseudochlorodesmis* (Pierce *et al.* 2003; Curtis *et al.* 2004, 2006; Middlebrooks *et al.* 2014). Plastids from *Caulerpa* were never detected in slugs preserved immediately after field collection (Middlebrooks *et al.* 2014); thus, *Caulerpa* is not consumed by *E. crispata* under field conditions. Consumed algae were not consistent among sites and did not always reflect algal abundance, indicating dietary preferences despite the breadth of suitable hosts. Using plastid barcoding, Christa *et al.* (2014) also documented the presence of retained chloroplasts from some of the above listed algal taxa, plus unidentified species in Pseudocodiaceae, Rhipiliaceae and Ulvophyceae. We have observed feeding *in situ* only on *Bryopsis*; however, we have occasionally observed a close association between slugs and either *H. incrassata* or *P. capitatus*, consistent with barcoding data showing preferential feeding on these algae.

Phylogenetic relationships. *Intra-specific relationships*. Within *E. crispata*, populations are highly genetically structured due to the limited dispersal ability of larvae. We compared the evolutionary relationships of mitochondrial lineages as inferred from ML analysis of COI haplotypes (Fig. 13) to the external morphology of 15 specimens (Fig. 14). Bolded isolate labels (Fig. 13) link a haplotype to the corresponding photographic vouchers (Fig. 14); plain text labels indicate other specimens that shared a given haplotype but that were not included in Fig. 14. The maximum distance between COI haplotypes, 7.8%, was greater than that noted between '*clarki*' from the FL Keys and *E. crispata* from the U.S. Virgin Islands (Pierce *et al.* 2006), yet was below our 8% cutoff for species-level distances in *Elysia*. Clades were not separated into different species by ABGD. On the ML gene tree, COI haplotypes fell into five divergent groups among which mean COI distances (TrN) ranged from 4.4 to 6.7%. Within each clade, mean distance between haplotypes was <1.5%. These results are consistent with a comprehensive phylogeographic analysis of *E. crispata* (216 specimens from 17 populations), which recovered eight COI clades ranging from 5–8% distant (Vo 2013). However, all populations shared at least one of three common H3 alleles, indicating populations likely represent one biological species.

Figure 13

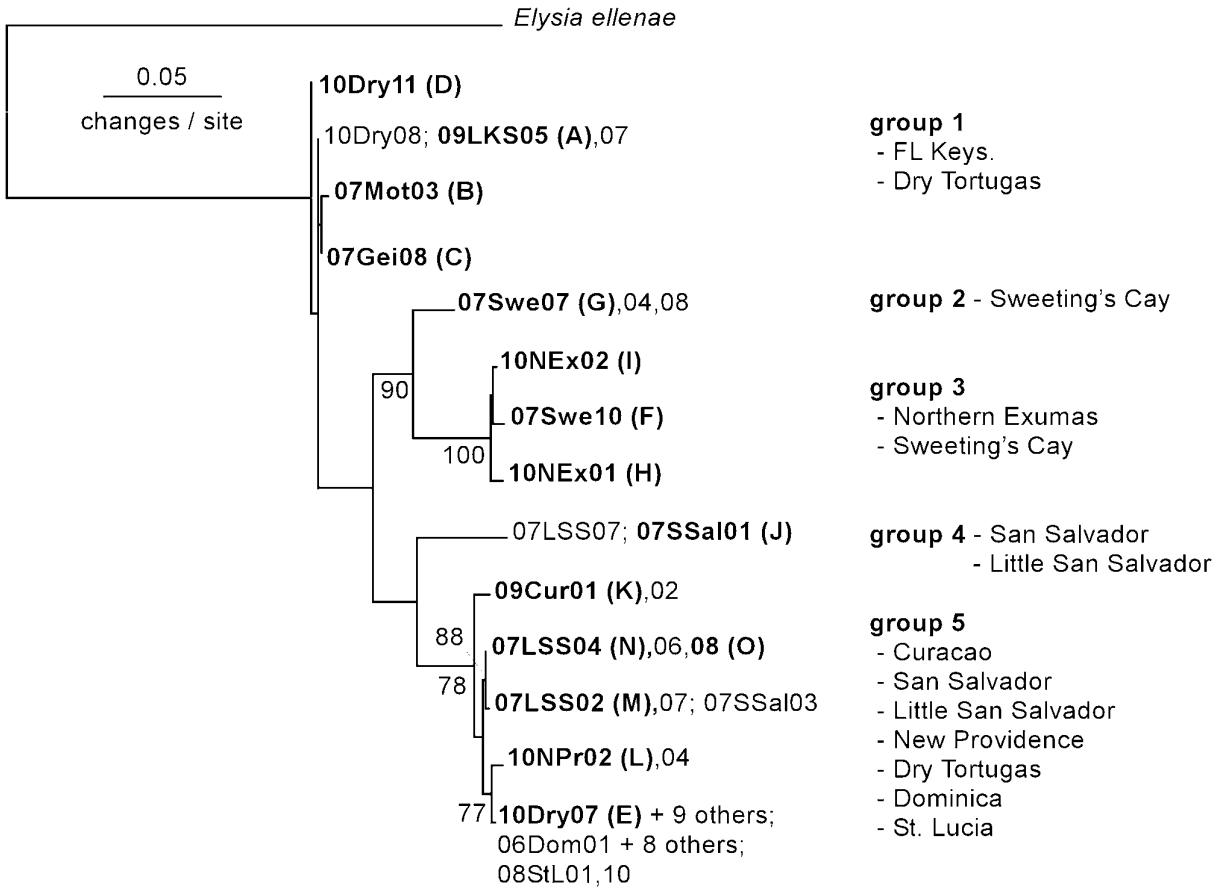

FIGURE 13. Evolutionary relationships among a subsample of COI haplotypes from specimens of *E. crispata*, inferred by Maximum Likelihood. Significant bootstrap values are given adjacent to supported nodes. External morphology of 15 specimens with bolded isolate codes is shown in Fig. 14, with the corresponding panel given in parentheses. Terminals with multiple isolates denote haplotypes sampled more than once; multiple specimens from the same site and year are indicated by two-digit numbers following the corresponding year-site combination, except the total number from two sites is given for the common haplotype sampled in the Dry Tortugas, Dominica and St. Lucia.

In our analysis, Group 1 comprised a grade of closely related COI haplotypes (maximum distance, 0.8%) from four Florida populations. Specimens from three Florida Keys sites (Lake Surprise, Mote Tropical Research Laboratory canal, Geiger Beach) had '*clarki*' features (unfused and unruffled parapodia, green foot; Fig. 14A-C), but grouped with two specimens sampled 120 km away in the Dry Tortugas that had typical *crispata* features (fused and ruffled parapodia, white foot; Fig. 14D). Notably, one *E. crispata* morph (10Dry08) shared a COI haplotype with a *clarki* morph from Lake Surprise, Florida (Fig. 14A, 09LKS05). The lack of genetic divergence at COI between morphologically distinctive specimens sampled from the area surrounding the Florida Keys supports the synonymy of *E. clarki* with *E. crispata*.

The suite of morphological characters that supposedly distinguish *E. clarki* from *E. crispata* also failed to covary among specimens sampled from shaded microhabitats across the Bahamas. For instance, Group 2 comprised one haplotype shared by three specimens from Sweeting's Cay, Bahamas, of intermediate morphology; specimen 07Swe07 (Fig. 14G) had features of both *clarki* (unruffled parapodia, green foot continuous with outer parapodia) and *crispata* (fused anterior parapodia). Group 3 comprised a specimen from Sweeting's Cay with a dark *clarki* morphology but fused parapodia (Fig. 14F), plus two specimens from the Northern Exumas.

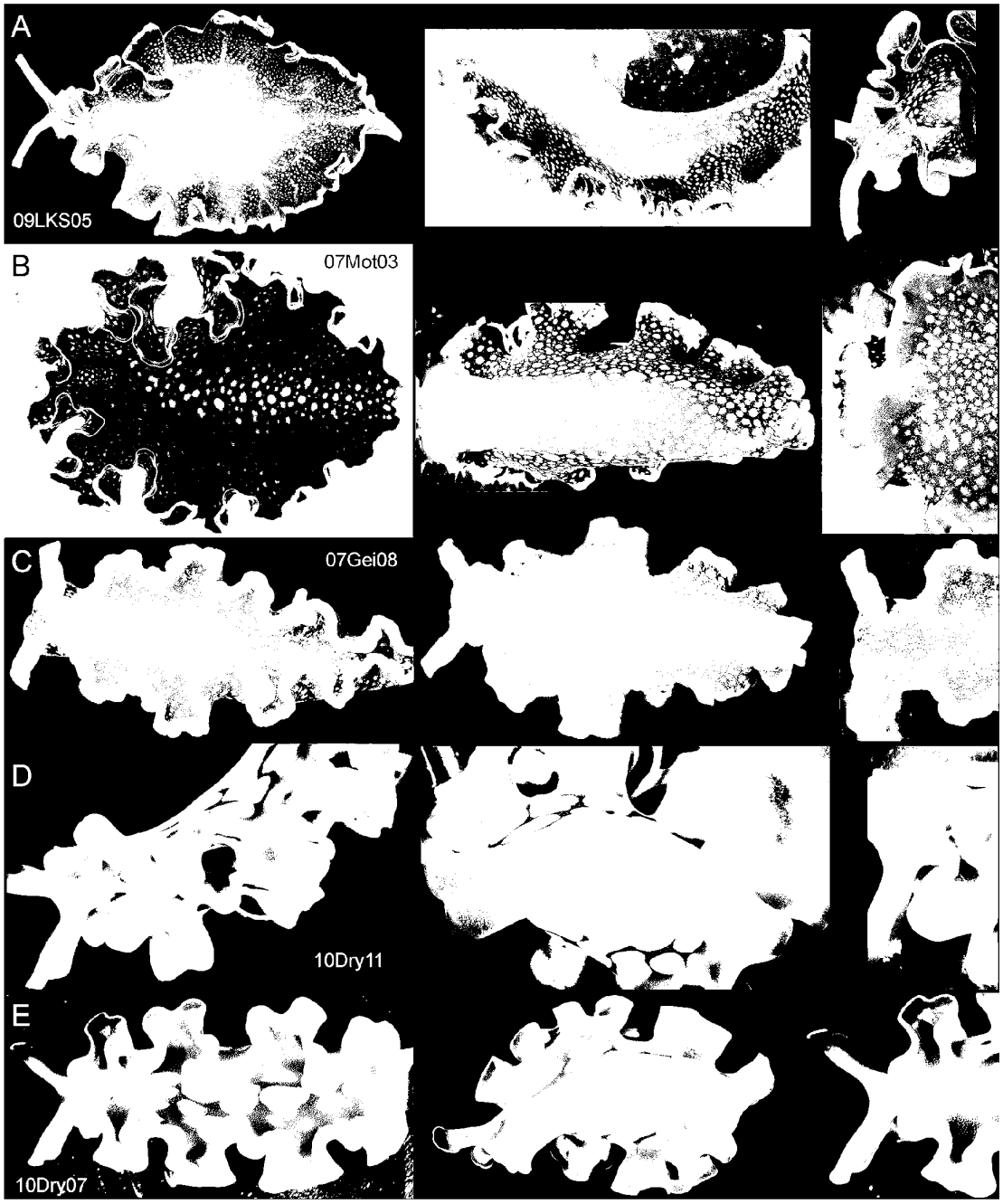

FIGURE 14. External morphology of specimens of *Elysia crispata* from which the COI haplotypes analyzed in Fig. 13 were obtained, showing parapodial ruffling and coloration (left panel), foot (center), and close-up view of the anterior parapodial margin (right) from each live specimen. **A,** Green *clarki* morph from Lake Surprise, FL (isolate Ecri_09LKS05; 60 mm). **B,** Dark *clarki* morph from Mote Tropical Research Laboratory, Florida Keys (Ecri_07Mot03; 20 mm). **C,** Intermediate *clarki* morph from Geiger Beach, Key West, FL (Ecri_07Gei08; 8 mm). **D,** Typical high-flow *crispata* morph from Dry Tortugas, FL (Ecri_10Dry11; 12 mm). Isolate Ecri_10Dry08 had external features nearly identical to this specimen. **E,** High-flow morph from Dry Tortugas, FL (Ecri_10Dry07; 13 mm). **F–G,** Intermediate morphs with mixed features from Sweeting's Cay, Bahamas (F, Ecri_07Swe10, 25 mm; G, Ecri_07Swe07, 25 mm). **H–I,** Intermediate morphs with mixed features from the Northern Exumas, Bahamas (H, Ecri_10NEx01, 15 mm; I, Ecri_10NEx02, 18 mm). **J,** Intermediate morph from San Salvador Island, Bahamas (Ecri_07SSal01, 15 mm). **K–L,** Typical *crispata* morphs from Curaçao (K, Ecri_ 09Cur01, 52 mm) and New Providence, Bahamas (L, Ecri_10NPr02, 30 mm). **M,** Green *clarki* morph from Little San Salvador, Bahamas (Ecri_07LSS02, 32 mm). **N,** Intermediate morph from Little San Salvador, Bahamas (Ecri_07LSS04, 25 mm). **O,** Dark *clarki* morph from Little San Salvador, Bahamas (Ecri_07LSS08, 28 mm).

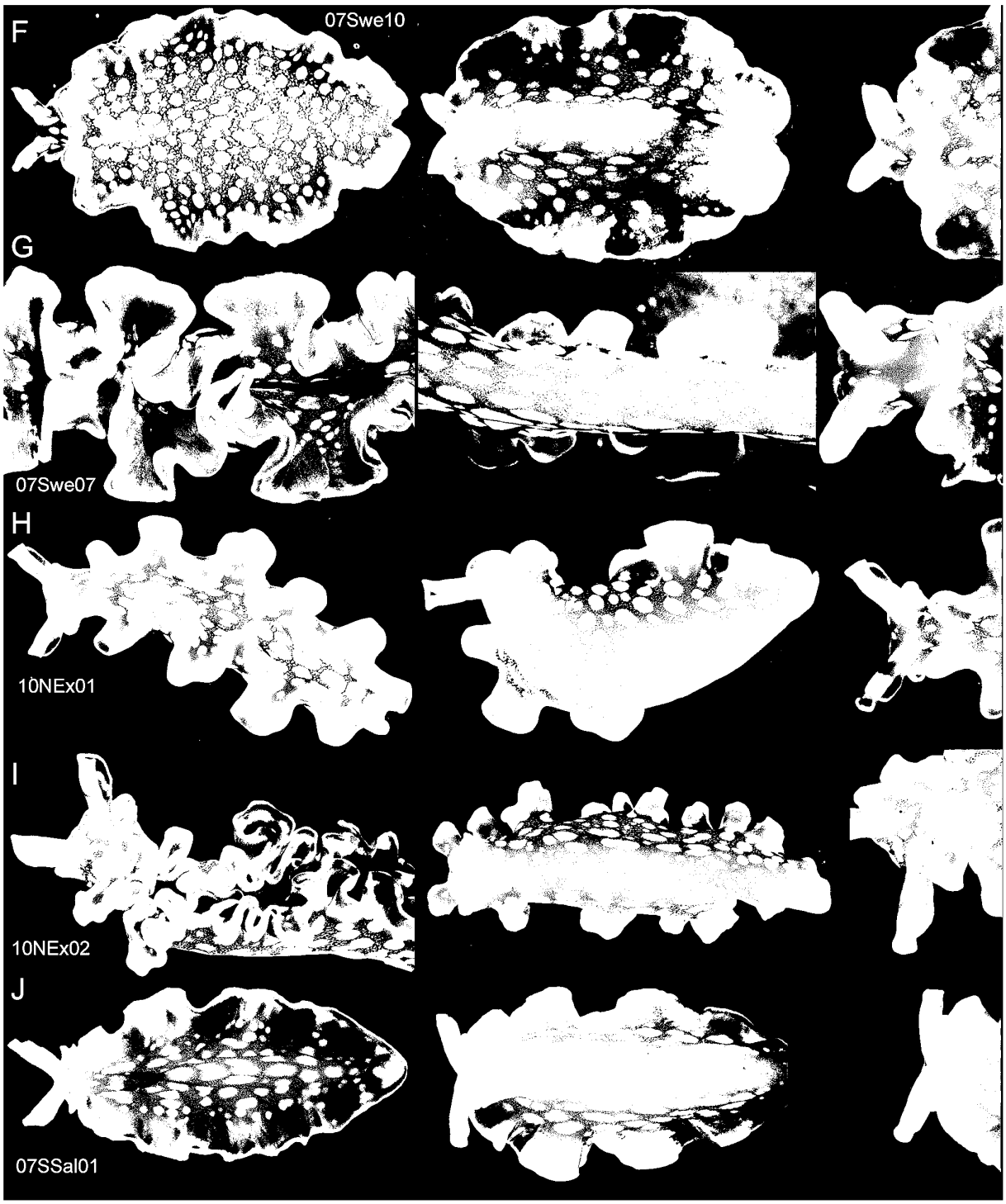

FIGURE 14. (Continued)

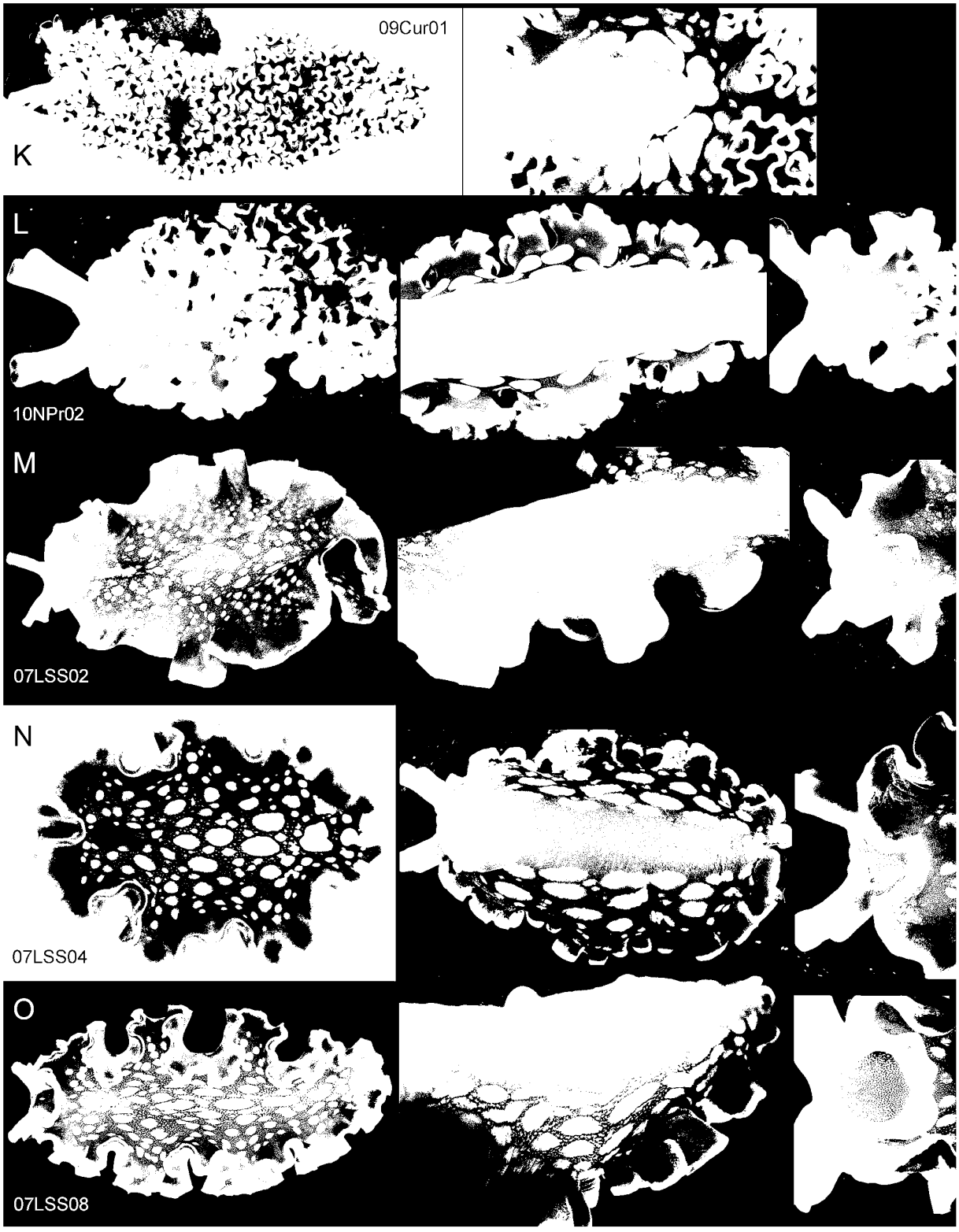

FIGURE 14. (Continued)

Both Exumas specimens had symmetrically ruffled parapodia (*crispata*-type) and a green foot (*clarki*-type); one had a parapodial notch (Fig. 14H), but the other did not (Fig. 14I). Group 4 included specimens from Sal Salvador and Little San Salvador with intermediate features such as a mostly white foot but pronounced parapodial notch (Fig. 14J). Thus, morphology could not be used to classify most specimens as either nominal species, based on the features used in the description of *E. clarki*.

Group 5 comprised typical *E. crispata* specimens collected from high-flow sites in Curaçao (Fig. 14K) and New Providence, Bahamas (Fig. 14L), together with 16 out of 18 Dry Tortugas specimens. Most Dry Tortugas slugs had a typical *crispata* morphology, but one specimen (Fig. 14E, 10Dry07) had an anterior parapodial notch ('*clarki*') together with ruffled parapodia and a white foot (*crispata*); this intermediate morph shared a haplotype with nine typical *crispata* morphs from Dry Tortugas, nine from Dominica, and two from St. Lucia. Moreover, most specimens from the sheltered lagoon at Little San Salvador also belonged to Group 5, yet presented a range of intermediate morphologies; all had reduced parapodial ruffling and dark coloration, but some had *clarki* features (green foot, parapodial notch; Fig. 14M, O) while genetically indistinguishable specimens had typically *crispata* features (white foot, no notch; Fig. 14N). Overall, the *clarki* morphotype was not monophyletic at the fast-sorting COI locus (Fig. 13) or the slow-sorting H3 locus (Vo 2013), and no suite of characters consistently covary and distinguish '*clarki*' morphs from co-occurring *E. crispata*. Thus, *E. clarki* cannot be considered a distinct species.

Inter-specific relationships. *Elysia crispata* is a member of subclade 2 (Fig. 4), which is largely restricted to the north and western Atlantic. The recently described *E. ellenae* (see Ortea *et al.* 2013) was recovered as sister to *E. crispata*, together forming a clade sister to the east Pacific *E. diomedea* (Fig. 4). This clade of three species occupies a derived position within subclade 2, recovered as sister to a clade comprising *E. viridis* (cold-temperate) and *E. evelinae* (tropical) with weak support. Both *E. crispata* and *E. ellenae* share a laterally undulating parapodial edge, but the parapodia of *E. ellenae* are greatly thickened. Speciation may have occurred within the Caribbean after formation of the Panamanian Isthmus isolated the ancestor of *E. diomedea* from Caribbean populations, but ecological and life-history data on *E. ellenae* are needed to formulate hypotheses regarding its divergence from *E. crispata*.

Range. Antigua (present study), Aruba (Ev. Marcus & Er. Marcus 1963; Valdés *et al.* 2006), Bahamas (Redfern 2001, 2013; Valdés *et al.* 2006), Barbados (Ev. Marcus & Hughes 1974), Belize (Valdés *et al.* 2006), Bonaire (Ev. Marcus & Er. Marcus 1963; Er. Marcus & Ev. Marcus 1970), Cayman Islands (Hess *et al.* 1994), Colombia (Ev. Marcus 1976b), Costa Rica (Espinosa & Ortea 2001), Cuba (Espinosa *et al.* 2005), Curaçao (Ev. Marcus & Er. Marcus 1963; Er. Marcus & Ev. Marcus 1970), Dominica (present study), Dry Tortugas (White 1952), Florida, USA (Ev. Marcus & Er. Marcus 1962; Jensen & Clark 1983; Clark 1994; Valdés *et al.* 2006), Guadeloupe (Valdés *et al.* 2006), Haiti (Valdés *et al.* 2006), Honduras (Ev. Marcus & Er. Marcus 1962; Valdés *et al.* 2006), Jamaica (Thompson 1977; Valdés *et al.* 2006), Mexico (Zamora-Silva & Ortigosa 2012), Panama (Collin *et al.* 2005), St. Lucia (present study), St. Martin/St. Maarten (Ev. Marcus & Er. Marcus 1963), Trinidad & Tobago (Valdés *et al.* 2006), Venezuela (Valdés *et al.* 2006), Virgin Islands (Mörch 1863; Ev. Marcus & Er. Marcus 1962; Valdés *et al.* 2006).

Remarks. Ørsted & Mörch *in* Mörch (1863) described *Elysia* (*Tridachia*) *crispata* in a brief Latin description based on unpublished drawings by Anders Sandøe Ørsted, later published by Bergh (1871: pl. 9, figs. 4–5). The main diagnostic characteristics included in the original description are the slug's curled parapodial edges (each side having 6–7 strong folds) that are fused together, as well as green body color with large, white, regularly arranged spots on the sides of the body. These external characteristics are only found in the species known as *Elysia crispata* in the Caribbean literature. Some years earlier, Deshayes (1857) described the genus *Tridachia* based on a species to be named after Schramm, but did not name the species. Mörch (1863) introduced for the first time the binominal name *Tridachia schrammi* in reference to Deshayes' (1857) description, which he considered to be a different species but the same genus as *Elysia crispata*. Since then, most authors have included *E. crispata* as the only member of *Tridachia*. Gosliner (1995) used a morphological phylogenetic analysis to show that *E. crispata* nests with other members of *Elysia*, thus rejecting the validity of *Tridachia*. Our molecular phylogenetic analysis confirms that *Tridachia* is a synonym of *Elysia*, as have all prior molecular analyses of Plakobranchidae or Sacoglossa (Bass & Karl 2006; Händeler *et al.* 2009; Wägele *et al.* 2010, 2011; Christa *et al.* 2014; Krug *et al.* 2015).

Despite the abundance of *Elysia crispata* in the field and its extreme variation in external morphology, few synonyms exist. Morphological examination of the type specimens of *Elysia crispata* (ZMUC GAS-1584), *Elysia*

crispata var. *schiadura* (ZMUC GAS-1572) and *Tridachia schrammi* (MNHN) confirmed that they all conform to the current use of the species name *E. crispata* and are therefore synonyms. White (1952) described a specimen from Dry Tortugas under the name *Tridachia ornata* (Swainson, 1840). However, the illustration of the external morphology and the radula of this animal match the description of *Elysia crispata*. Er. Marcus (1957) recognized that White's (1952) specimen was different from the original description of *Elysia ornata* but also from that of *Tridachia schrammi* (=*Elysia crispata*) and therefore he named it *Tridachia whiteae* Marcus, 1957. We here regard *Tridachia whiteae* as a synonym of *E. crispata*, as all 18 specimens examined from the Dry Tortugas were morphologically and genetically confirmed to be *E. crispata*.

Pierce *et al.* (2006) described *Elysia clarki* from mangrove swamps and canals in the Florida Keys. These authors compared specimens from low-flow Florida habitats with specimens identified as *E. crispata* from the Virgin Islands, and reported morphological differences including a nearly uniform green color with small white spots across parapodia and an almost transparent foot, non-fused anterior parapodial edges, and asymmetrical non-fixed folds in the parapodia of *E. clarki*. Pierce *et al.* (2006) also reported that radular teeth of *E. clarki* were ~10% longer (129 µm ± 4.1) than in *E. crispata* (114 µm ± 4.2), and also had a deeper and broader groove, and more prominent and widely spaced basal articulations. Finally, Pierce *et al.* (2006) reported a COI distance of ~7% between Florida specimens and specimens from the U.S. Virgin Islands, which was interpreted as a species-level genetic distance.

In this study, we examined over 200 specimens of *E. crispata* from across its range and found substantial variation in coloration and internal and external morphology, including all of the distinctive traits used to separate *E. clarki*. However, all specimens were less than 8% divergent at COI, consistently supported here and in prior work (Krug *et al.* 2013) as a threshold inter-specific COI distance for *Elysia*. The phylogenetic position of COI haplotypes (e.g., Fig. 13) was not related to morphology, but rather to location, with a high degree of differentiation among most populations. The 7% distance originally noted between Florida '*clarki*' and U.S. Virgin Islands '*crispata*' haplotypes is typical for among-population differentiation in this low dispersal species: Vo (2013) recovered seven COI clades that were 5–8% divergent in *E. crispata*, five of which are presented in Fig. 13. The high apparent COI distance between Florida '*clarki*' and U.S. Virgin Islands *E. crispata* thus reflected population differentiation, not genetic differences among morphotypes; U. S. Virgin Island specimens cluster with our group 5 samples (Vo 2013). The Florida group (including both '*clarki*' and *E. crispata* morphs) was no more divergent than any other COI clade of *E. crispata* from around the Caribbean.

More importantly, the broader sample size examined in the present study and in Vo (2013) revealed that specimens of *E. clarki* did not form a clade excluding *E. crispata*, based on molecular phylogenetic analysis of COI haplotypes. Most specimens from the Florida Keys were collected in low-flow, low-light habitats and had '*clarki*' morphology, but grouped genetically with some typical *E. crispata* from the nearby Dry Tortugas, and not with '*clarki*' morphs from low-flow habitats in the Bahamas, which grouped with typical *E. crispata* from the same location. Moreover, the three H3 alleles sampled in specimens from the Florida Keys and Dry Tortugas were common throughout the range of *E. crispata* (Vo 2013); thus, both mtDNA and the nuclear H3 gene fail to distinguish *clarki* morphs from *E. crispata*. Combined, all available morphological and molecular data indicate *E. clarki* cannot be considered a distinct species, as it is a polyphyletic ecotype.

Finally, the features proposed to distinguish *E. clarki* do not consistently co-occur in specimens from low-flow environments, but are frequently intermingled with *crispata*-type characters. For example, paired specimens of *E. crispata* of equivalent size collected side by side often had fused and unfused parapodia, respectively, including pairs from Yucatan, Mexico (Fig. 9F–G), Dry Tortugas (Fig. 14D–E), Northern Exumas (Fig. 14H–I), and Little San Salvador (Fig. 14N–O). The degree of undulation of the parapodia is extremely variable and the folds are not consistently fixed in all specimens assigned to *E. crispata*. The radular morphology of this species is also extremely variable, and we found no consistent differences between specimens of *E. crispata* from mangrove areas in the Florida Keys and the rest of the range, nor did size delineate *clarki* morphs from typical *E. crispata* (Figs. 11–12). Overall, neither morphological nor molecular analyses found consistent differences distinguishing *E. clarki* from *E. crispata*, and for all these reasons, *E. clarki* is here considered a synonym of *E. crispata*.

We do consider '*clarki*' to be an ecotype of *E. crispata* characterized by darker coloration, green diverticula in the foot, and reduced parapodial undulation. We have sampled this ecotype from low-light and low-flow habitats including mangrove lagoons, borrow pits, and shaded rock overhangs in shallow, protected areas throughout the Caribbean. In contrast, the light-colored, highly ruffled *crispata* ecotype predominates in high-flow, high-light

habitats such as drainage channels and subtidal reefs. The recurring association of *clarki* features with a particular habitat is consistent with either phenotypic plasticity during development, or local selection favoring the *clarki* ecotype. The lighter coloration and higher degree of parapodial ruffling may protect chloroplasts in diverticula lining the dorsum of the typical *crispata* morph from excess light, prolonging plastid function. The onshore Florida populations of *E. crispata* show coloration consistent with low-light adaptation (overall green to purple body coloration, including in the foot), possibly due to the greater turbidity of coastal waters compared to the rest of the Caribbean. Further study is needed to determine whether phenotypic plasticity or local selection produces the observed differences in external morphology between '*clarki*' ecotypes and typical *E. crispata* specimens. Indeed, given the ability to study diet via DNA barcoding of plastid DNA (Middlebrooks *et al.* 2014), *E. crispata* may provide a valuable system with which to investigate local adaptation, which is rare among marine herbivores (Sotka 2005).

Elysia chlorotica Gould, 1870

(Figs. 6F, 15–17)

Elysia chlorotica Gould 1870: 255–256, pl. 17, figs. 251–255 (Type locality: Cambridge Marshes, Massachusetts)—Verrill 1874: 363, pl. 25, fig. 172; Russell 1946: 96; Pfitzenmeyer 1960: 114; Russell 1964: 37–38; Bailey & Bleakney 1967: 353; Franz 1970: 7; Ev. Marcus 1972b: 308–310, fig. 5; Clark 1975: 42; Eyster 1980: 581–583; Boone 1982: 29–37, figs. 1–3; Jensen & Clark 1983: 4; Christa *et al.* 2014, fig. 3; Krug *et al.* 2015: 990–991, figs. 3B, 4.

Type material. *Elysia chlorotica*—unknown location (Johnson 1964).

Material examined. Martha's Vineyard, Massachusetts, USA, 2006, 3 specimens (LACM 178597–99).

Additional material examined. Martha's Vineyard, Massachusetts, USA, 2006, 8 specimens (isolate Echl_06Mas04-11).

Live animal. Resting slugs often hold parapodia wide open and flattened against the substratum. Slugs have an exceptional ability to osmoconform and hence great tolerance for low salinity, presumably adaptive in their estuarine habitat where salinities can drop rapidly due to runoff from storms (Pierce *et al.* 1983, 1984). This is the only species of *Elysia* that remains green across the entire body surface throughout extended periods of starvation (>1 month), due to prolonged survival of diet-derived chloroplasts.

External anatomy. Overall color deep emerald green; external surfaces dotted with minute speckles of white, light blue and red (Fig. 15). White speckles concentrated into larger white patches spaced at roughly regular intervals across sides of parapodia, and intermediate-sized patches on head (Fig. 15A–C). Body surface everywhere smooth with no papillae. Eyespots small, with no other distinguishing markings. Distinct and elongated neck flowing posteriorly into pericardium. Sides of head extend laterally into pointed oral tentacles, whitish at tips (Fig. 15B). Rhinophores elongated and tapering to a point, color fading to whitish at tips. Foot not clearly distinct from parapodia, with same overall coloration, narrowing posteriorly.

Wide parapodia covering dorsum when folded up, but often held flat and open by live animal (Fig. 15D). Parapodial margin smooth, pale yellow in color from absence of digestive diverticula. Inner parapodial surface emerald green with scattered white and red speckles concentrated along anterior end and margins, and scattered medially along dorsum. Parapodia forming ovoid side flaps, anterior margin with rounded corners; remainder of parapodia equal in width along most of body, gradually tapering to form short triangular tail.

Pericardium round or ovoid, green with dense speckling of white and red dots overlying wider, white renopericardium (Fig. 15A–D). Renopericardial extension only slightly posterior of the pericardium, short. Wide, clear dorsal vessels emerging asymmetrically from renopericardial extension, usually three on the right side and three to five on the left side (Fig. 16). Except for posterior pair, vessels fork or send off lateral branches at irregular intervals, side branches sometimes anastomosing; most branches forking immediately before reaching parapodial margin (Fig. 15B, D, Fig. 16). Posterior vessel on either side immediately forking, then one long branch running whole body length to end of parapodial flaps, and sending off six to nine lateral branches. Vessels transparent, with occasional white or red speckles. Reproductive ducts visible as faint, thin network within dorsal tissue underlying vessel network.

Internal anatomy. Radula with 25 teeth, 13 teeth in ascending limb and 12 in descending limb (Fig. 17A). Leading tooth elongate, narrowing to a point, with cusp bearing numerous very small denticles (Figs. 17B-C).

Housing depression for interlocking teeth extending approximately $^{4}/_{5}$ total tooth length (Fig. 17B). Base of tooth approximately ¼ total tooth length. Ascus containing jumbled heap of discarded teeth (not figured).

Penis robust, elongate, and nearly conical with attachment to the body wall, devoid of armature. Deferent duct long, thin, and convoluted (Fig. 6F).

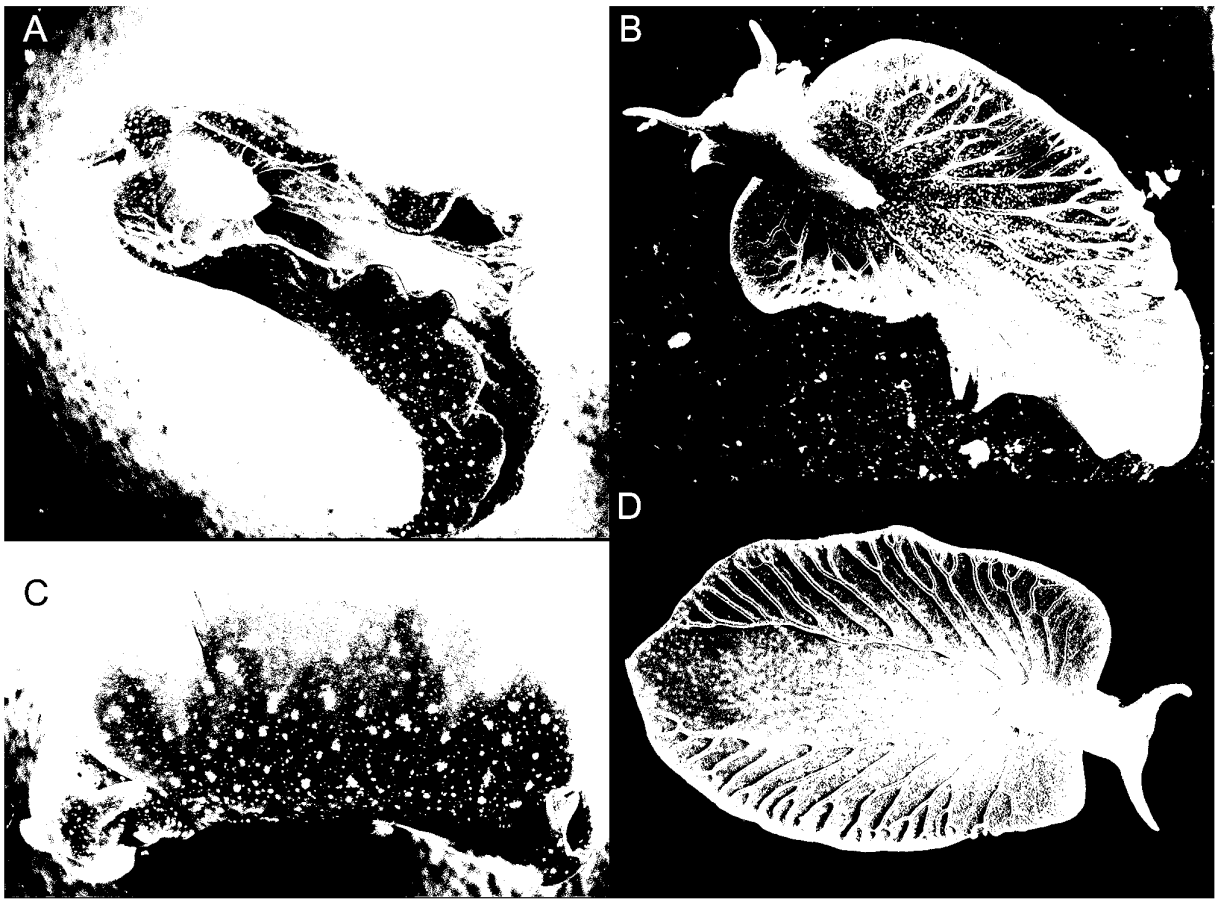

FIGURE 15. *Elysia chlorotica*, external anatomy of specimens from Martha's Vineyard, MA, U.S.A. **A–B,** Dorsal view with the parapodia partially folded. **C,** Lateral view. **D,** Dorsal view of specimen flattened against the substratum. Approximate size of live specimens was 2 cm resting.

Reproduction and development. Like other members of subclade 2, *E. chlorotica* does not produce ECY. Development is poecilogonous, with alternative larval modes fixed in different populations in Massachusetts, U.S.; all data are taken from West *et al.* (1984) and errors are SD. Planktotrophic larvae were produced by slugs from Martha's Vineyard, whereas lecithotrophic larvae with encapsulated metamorphosis were produced by a mainland population at Ipswitch. Planktotrophic clutches contained ~8900 eggs, with a mean diameter of 79 ± 3 μm SD; larvae hatched after 6–7 d at an unspecified temperature, with a mean shell length of 146 ± 11 μm. Larvae cultured on phytoplankton metamorphosed after 2 weeks when presented with either *Vaucheria compacta* or *V. litorea* (~50%), suitable adult hosts, with little spontaneous metamorphosis (~1%) occurring on non-host algae (*Enteromorpha* or *Bryopsis*).

Lecithotrophic clutches contained ~175 eggs on average, with a mean diameter of 96 ± 8 mm. Larvae metamorphosed before hatching after 14 ± 1 d (full salinity, 33‰) or 9 ± 1 d (17‰ salinity); faster development at low salinity suggests adaptation to the euryhaline conditions typical of estuaries inhabited by *E. chlorotica*. Lecithotrophic larval shells were 217 ± 8 μm. Inter-population crosses were successful through two generations, indicating conspecificity. Egg masses of F1 offspring had characteristics similar to their maternal population, while F2 hybrids produced offspring with intermediate characteristics. Despite the rarity of poecilogony, no follow-up studies were published on the lecithotrophic population of *E. chlorotica*.

Host ecology. *Elysia chlorotica* feeds on at least two species of the heterokont alga *Vaucheria* (*V. compacta*

and *V. litorea*), and may be cultured in the laboratory on the latter (West *et al.* 1984; Pelletreau *et al.* 2012). Although also reported to feed on *Cladophora* (Clark 1975), no supporting data were published. At least three independent radiations onto *Vaucheria* have thus occurred in Sacoglossa: the genus *Alderia*, one lineage of *Costasiella* (2 spp.; Jensen *et al.* 2014), and *E. chlorotica*. Most *Vaucheria* spp. grow in the intertidal zone of estuaries, a stressful environment with low salinities that may impede adaptive shifts onto *Vaucheria*.

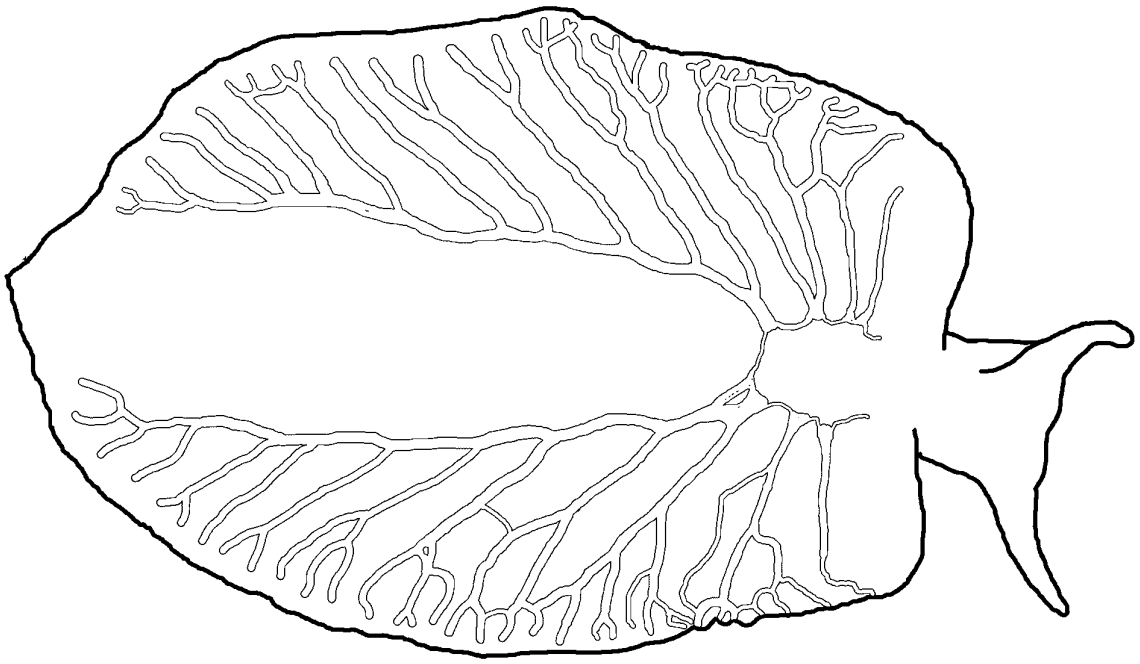

FIGURE 16. *Elysia chlorotica*, drawing of the pericardium and dorsal vessel network traced from a photograph of a live animal from Martha's Vineyard, MA.

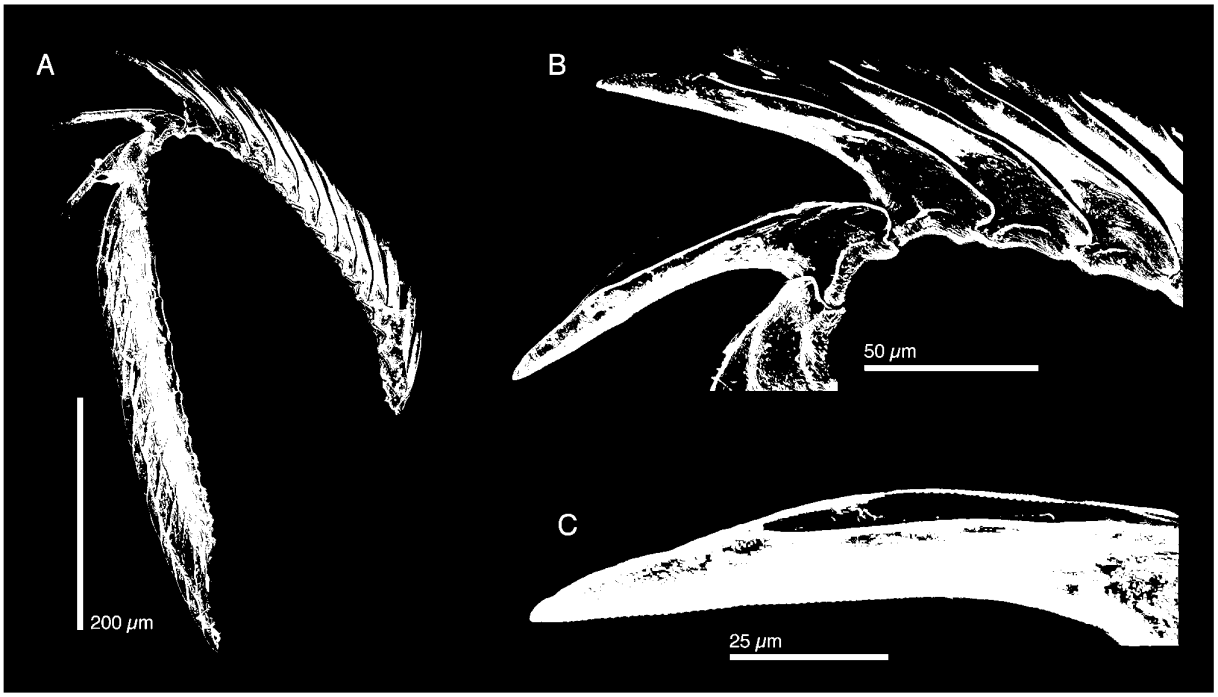

FIGURE 17. *Elysia chlorotica*, SEM of the radula (LACM 178597). **A,** Radula, without ascus. **B,** Leading tooth. **C**, Close-up of cusp showing denticles.

Phylogenetic relationships. Known from the temperate to subtropical northwestern Atlantic, *E. chlorotica* grouped within subclade 2 as sister to the seagrass-feeding *E. serca* with strong support. However, the North Atlantic seagrass-feeding *E. catulus* was not available for phylogenetic analysis, and may be more closely related to *E. serca* than *E. chlorotica*, requiring future study.

Range. Nova Scotia (Bailey & Bleakney 1967; Raymond & Bleakney 1987), Massachusetts (Gould 1870; Verrill 1874; Russell 1946, 1964), New Jersey (Franz 1970), Connecticut (Clark 1975), Maryland (Pfitzenmeyer 1960), North Carolina (Abbott 1974), South Carolina (Eyster 1980), Florida (Jensen & Clark 1983), Texas (Boone 1982).

Remarks. Sacoglossans in the clade Plakobranchoidea have long been studied for kleptoplasty, the maintenance of diet-derived plastids in their body tissues for several weeks. A few species exhibit "long-term retention" of plastids for over a month (*Elysia crispata*, *E. timida*, *Plakobranchus* spp.; Händeler *et al.* 2009). Exceptionally, *E. chlorotica* may retain functional chloroplasts for up to nine months, and is the only photosynthetic species with an overall green color due to ramifying digestive diverticula and associated cells harboring plastids (Pierce & Curtis 2012 and references therein). Other long-term retention species may shelter plastids from high sunlight, covering the digestive gland with white or colored parapodia to prevent burn-out of nuclear-encoded proteins from the light-harvesting complex; alternatively, long-term retainers may specialize on algae that still have key genes encoded by the plastid genome (deVries *et al.* 2013). Regardless, the ability of *E. chlorotica* to remain green over its entire surface for most of its life is unique even among sacoglossans, prompting special focus on this taxon.

Recent transcriptomic analysis suggested at least 52 genes from the *Vaucheria* nuclear genome were horizontally transferred into the genome of *E. chlorotica*, and expressed in adult cells harboring plastids (Pierce *et al.* 2009, 2012; Schwartz *et al.* 2014). Another study failed to detect algal genes in genomic sequences derived from slug eggs, but confirmed that *Vaucheria* genes were expressed in adult slugs, suggesting algal genes persist as non-integrated or extra-chromosomal DNA after initial feeding (Bhattacharya *et al.* 2013). Current evidence is thus consistent with facilitation of long-term retention by expression of algal nuclear genes in *E. chlorotica*, but the mechanism remains unclear. Recent work showed that after juvenile *E. chlorotica* were allowed to establish plastid symbiosis for 4 weeks, those held under partial or constant light survived and delayed the loss of body mass for two months without subsequent feeding, whereas slugs held in the dark shrank linearly for a month and then began dying (Pelletreau *et al.* 2014). Transfer of lipids biosynthesized by photosynthetically active plastids to the slug was key to the establishment and maintenance of kleptoplasty, and to long-term slug survival without food, highlighting a mechanism by which this symbiosis may function.

Elysia papillosa Verrill, 1901

(Figs. 6O, 18–20)

Elysia papillosa Verrill 1901: 31, pl. 4, fig. 3 (Type locality: Hungry Bay, Bermuda)—Pruvot-Fol 1946: 36; Er. Marcus 1957: 410, 415; Ev. Marcus & Er. Marcus 1967: 27–28, figs. 22–25; ?Er. Marcus & Ev. Marcus 1970: 45; Ev. Marcus 1980: figs. 9, 48; Jensen 1980: fig. 1C, tables 2,5; Clark 1984 figs. 15, 17; ?Hess *et al.* 1994: 161–163; Redfern 2013: 284, figs. 787A–B.

Elysia patina [non *Elysia patina* Ev. Marcus, 1980]—Ortea, Caballer, Moro & Espinosa 2005: 497–498, 505–512, fig. 5, pl. 1C; Händeler *et al.* 2009: figs. 6, 7; Curtis, Schwartz & Pierce 2010: 299–302, figs. 1C, 5; Christa *et al.* 2014: figs. 1C, 3; Curtis *et al.* 2015: 27, fig. 2

Checholysia patina [non *Elysia patina* Ev. Marcus, 1980]—Espinosa, Ortea, Caballer & Moro 2005: 56; Ortea *et al.* 2005: 512.

Elysia annedupontae Ortea, Espinosa & Caballer *in* Ortea, Caballer, Moro & Espinosa 2005: 502–505, fig. 3, pl. 1B (Type locality: Ensenada de Bolondrón, Guanahacabibes, Cuba) **n. syn.**

Checholysia annedupontae (Ortea, Espinosa & Caballer *in* Ortea, Caballer, Moro & Espinosa 2005)—Espinosa *et al.* 2005: 56; Ortea *et al.* 2005: 512 **n. syn.**

Elysia sp. 1—Valdés *et al.* 2006: 72–73.

Type material. *Elysia papillosa*—untraceable, not found at the (YPMNH); *Elysia annedupontae*—holotype at IESH (no registration number given); paratype at MCNT (no registration number given).

Material examined. Bocas del Toro, Panama, 19 February 2004, 4 specimens (LACM 178616–19); Discovery Bay, Jamaica, 7 March 2006, 1 specimen (LACM 178620); Bermuda, 2006, 4 specimens (LACM 178600–02,

LACM 178621); Florida, USA: 2009, 1 specimen (LACM 178624), Geiger Beach, 6 October 2006, 4 specimens (LACM 178612–13, LACM 178622–23); Great Stirrup Cay, Bahamas, July 2007, 2 specimens (LACM 178608, LACM 178611); Sweetings Cay, Bahamas, July 2010, 2 specimens (LACM 178614–15); Stocking Island, Exumas, Bahamas, 29 January 2009, 2 specimens (CPIC 00070, CPIC 00073).

Additional material examined. Bocas del Toro, Panama, 19 February 2004, 4 specimens (isolate Epap_04Pan01, isolate Epap_ 04Pan04, isolate Epap_04Pan07, isolate Epap_04Pan08); Discovery Bay, Jamaica, 7 March 2006, 1 specimen (isolate Epap_06Jam09); Bermuda, 2006, 3 specimens, (isolate Epap_06Ber05-07); Geiger Beach, Florida, USA, 6 October 2006, 1 specimen (isolate Epap_06Gei24); Bahamas, July 2010, 1 specimen (isolate Epap_10LSS01).

Live animal. Specimens swim readily when disturbed by undulating their parapodial margin.

External anatomy. External coloration highly variable. Overall body color generally light green, but ranging from white or tan to olive green. Parapodial margin tan to dark brown (Fig. 18C–D). Medial band running longitudinally along head between eyes, from front of face to pericardium, color ranging from cream to tan to brown; some specimens with darker brown to black streaks along sides of this band, running through eyes (Fig. 18E). Sides of head lighter green to white (Fig. 18D, F). Most specimens with one or two large white papillae between eyes, with scattered, smaller white papillae across head on some. Rhinophores elongated, rolled, with white to tan ground color; rounded white papillae of varying sizes dot surface. Rhinophores blunt-ended, sometimes with gently curving edge. Distinctive wide, dark band appearing about ⅓ of the way up rhinophore, not perpendicular to rhinophoral axis but rather at an angle such that band forms a rhomboid shape when viewed from above (Fig. 18A–E). Second, fainter band or streak present ⅔ of way up rhinophore on some specimens.

Parapodia relatively low, not covering pericardial complex. Outer parapodial surface bearing rows of white papillae, varying in size (Fig. 18C–F). Parapodia dotted with black spots same size as eyes, and with scatted brown specks. Lower portion of parapodia green to tan, grading to tan-brown along distal portion. Margin thickened into row of white-tipped, rounded protrusions (Fig. 18F–H). Color of margin either not distinct from upper parapodial surface, or more pronouncedly brown. Parapodial margin with scalloped edge, laterally undulating with three-pronged, pointed side flaps regularly spaced along entire length, forming series of siphonal openings (Fig. 18C–E).

Inner surface of parapodia and dorsum varying greatly in color among specimens. Ground color generally mottled light to dark green, usually pigmented by patches of white and minute flecks of iridescent blue, green or orange. Inner parapodial surface and dorsum lightly to heavily speckled with brown or black spots, and with scattered white, rounded papillae (Fig. 18G–H). Posterior end of body narrowing to form short, triangular, pointed tail, much elongated on some specimens (Fig. 18C–F).

Pericardium round, with brown streaks and spots and low white papillae (Fig. 18G–H). Renopericardial extension emerges from pericardium as narrower tube, clear with orange-brown spots and faint speckling of white to light blue. Renopericardial extension running less than halfway down body, relatively straight, terminating in pair of wide posterior vessels. Dorsal vessels sometimes clear, otherwise lined with patches of white or light blue speckles. Two to three vessels typically emerging on either side of the pericardium, and a further four to five vessels on each side of the renopericardial extension (Fig. 19). Vessels relatively symmetric in placement, but usually one extra vessel emerging on right side. Vessels wider than in most elysiids, initially straight and unforked, angling back towards posterior end of body. Vessels with extending thin side branches appearing at irregular intervals near parapodial margin, with terminal branches anastomosing on some specimens. Elongated pair of posterior vessels emerging from terminus of renopericardium, and running almost to tail with numerous lateral branches, some anastomosing with side branches of more anterior vessels.

One pair of large sperm-storage vesicles, typically visible on large adult specimens, and not juveniles, as irregularly shaped, greyish protrusions (Figs. 18G–H, 19 lower panel). Sperm-storage vesicles shall herein be referred to as "gametic" vesicles, *sensu* Clark (1984) [not "gametolytic" vesicles *sensu* Marcus (1983)] to denote that vesicles are filled with allosperm after mating without implying that sperm are lysed or digested there. Gametic vesicles usually between 5th and 6th, or 6th and 7th dorsal vessels, just anterior to end of renopericardial extension.

Internal anatomy. Radula with 10–13 teeth (CPIC 00070, CPIC 00073, LACM 178608, LACM 178613–18, LACM 178620, LACM 178622–23, isolate Epap_06Ber07, isolate Epap_10LSS01), 4–6 in ascending limb and 6–9 in descending limb (Fig. 20A). Leading tooth elongate and slender, with cusp bearing 18–30 denticles (Fig. 20B). Tooth length, width in lateral profile, and degree of mid-tooth angle variable among specimens. Variability in length of leading tooth from 60–200 μm, tooth width in lateral profile from 5–28 μm, with width to length ratios

from 8.3–15.5, and mid-tooth angle from 11° to 33° (n=18). Housing depression for interlocking teeth "V"-shaped and extending ¾ total tooth length (Fig. 20C). Base of tooth ½ to ⅓ total tooth length. Ascus containing jumbled heap of discarded teeth (not figured).

Penis thin and elongate, with rigid musculature that did not deform after drying (Fig. 6O, Fig. 20D), bearing a spoon-shaped stylet (LACM 178600, LACM 178616, LACM 178618–21) opposite a medial flange (Fig. 20D–E). Curved hook on stylet tip considered anomalous as observed in only one specimen (Fig. 20E). Deferent duct long, thin, and convoluted (Fig. 6O).

Reproduction and development. Development was planktotrophic for specimens from Panama, Curaçao, and the Bahamas. Newly hatched larvae lacked eyespots, fed readily on cultured phytoplankton, and did not metamorphose. Egg masses contained an irregular ribbon of white ECY which contacted most, but not all, egg capsules (Fig. 18I). Mean egg diameter was 67.2 µm ± 2.4 SD (n = 17 ova) for one clutch from Curaçao. Mean shell length at hatching was not determined for specimens that could be confirmed as *E. papillosa* by DNA sequencing or internal morphology.

Development was described as lecithotrophic for "*E. papillosa*" by Clark & Goetzfried (1978), with eggs measuring 91.9 µm in diameter and having a flat watch-spring spiral of ECY. Unfortunately, ECY color was not described. Either *E. papillosa* is poecilogonous and some specimens from Florida produce lecithotrophic larvae, or Clark & Goetzfried (1978) and Clark & Jensen (1981) misidentified *E. patina* (which is lecithotrophic) as *E. papillosa*. Krug (2009) described development for *E. patina* under the name "*E. papillosa*", following Ortea *et al.* (2005).

Host ecology. Field surveys and laboratory observations confirm that *E. papillosa* specializes on the algal genus *Penicillus*, as described by Jensen (1980). In field surveys, over 300 specimens were collected from *Penicillus* spp. at 12 sites over a decade of sampling. In the laboratory, some starved specimens fed on *Rhipocephalus brevicaulus*, but only a single large specimen was found on *R. brevicaulus* in the field. In an unreplicated choice experiment, 15 specimens of *E. papillosa* from Panama were placed in a dish with 200 mL seawater and stipes of two different algae, and left for 24 hr. Choices were: (1) *P. capitatus* vs. *Udotea flabellum*; (2) *P. capitatus* vs. *Halimeda incrassata*; and (3) *U. flabellum* vs. *H. incrassata*. After a day, 10 of 15 specimens were physically associated with *P. capitatus* in dish 1, and 12 of 15 with *P. capitatus* in dish 2; none were associated with the non-host alga, and the remainder were crawling on the glass or undersurface of the water. In dish 3, two slugs chose *U. flabellum*, one chose *H. incrassata*, and 12 of 15 chose neither alga. All available data thus indicate that the only ecologically relevant host for *E. papillosa* is the algal genus *Penicillus*.

Numerically, *E. papillosa* is one of the most abundant species of *Elysia* in the Caribbean; a collection of *P. capitatus* in Panama yielded 181 specimens from 364 individual algal stipes, or about one slug per two stipes. A discussion of maintenance of host chloroplasts in *E. papillosa* is provided by Curtis *et al.* (2010) under the name *E. patina* (due to misidentifications based on Ortea *et al.* (2005); see below). Like most *Elysia* spp., *E. papillosa* has short-term retention of functional chloroplasts from *Penicillus*, with degradation delayed for up to a week after phagocytosis by cells lining the digestive tubules (Curtis *et al.* 2010).

Based on the COI barcode used to identify slug species, Christa *et al.* (2014) also report the diet-derived plastids of *E. papillosa* under the name "*E. patina*", and vice-versa report the diet of *E. patina* as "*E. papillosa*", reflecting the prior reliance of most authors on the misidentification in Ortea *et al.* (2005) which swapped the names of these two species. Christa *et al.* (2014) reported plastids from *Udotea* and an unidentified alga in Udoteacea as the diet of *E. papillosa* (their "*E. patina*") based on barcoding of plastid DNA. However, inspection of the tree used to match plastid sequences to algal reference sequences (Figure S1 in Christa *et al.* 2014) reveals that plastid sequences from the true *E. papillosa*, and also *Cyerce antillensis,* match perfectly or closely the reference sequence for *Penicillus capitatus*, which is the primary food of both slug species. *Udotea* spp. are not monophyletic on the reference tree used to identify food sources by Christa *et al.* (2014), and plastid sequences could equally well correspond to *Penicillus*, *Rhipocephalus*, *Udotea*, or *Chlorodesmis* on this tree; why *Udotea* was reported as the diet source of "*E. patina*" is thus unclear. This inability to match unambiguously plastid barcodes to reference sequences highlights a serious concern with inferring diet via tree-based methods when algal relationships are not resolved by the available sequence data. The species reported as "*E. papillosa*" by Christa *et al.* (2014) is actually *E patina*, for which the ecologically relevant host genus *Halimeda* was indeed recovered.

Phylogenetic relationships. *Elysia papillosa* belongs to subclade 1, a putatively basal lineage of Caribbean elysiids, and was recovered as sister to *E. taino* **n. sp.** (described subsequently) (Fig. 4).

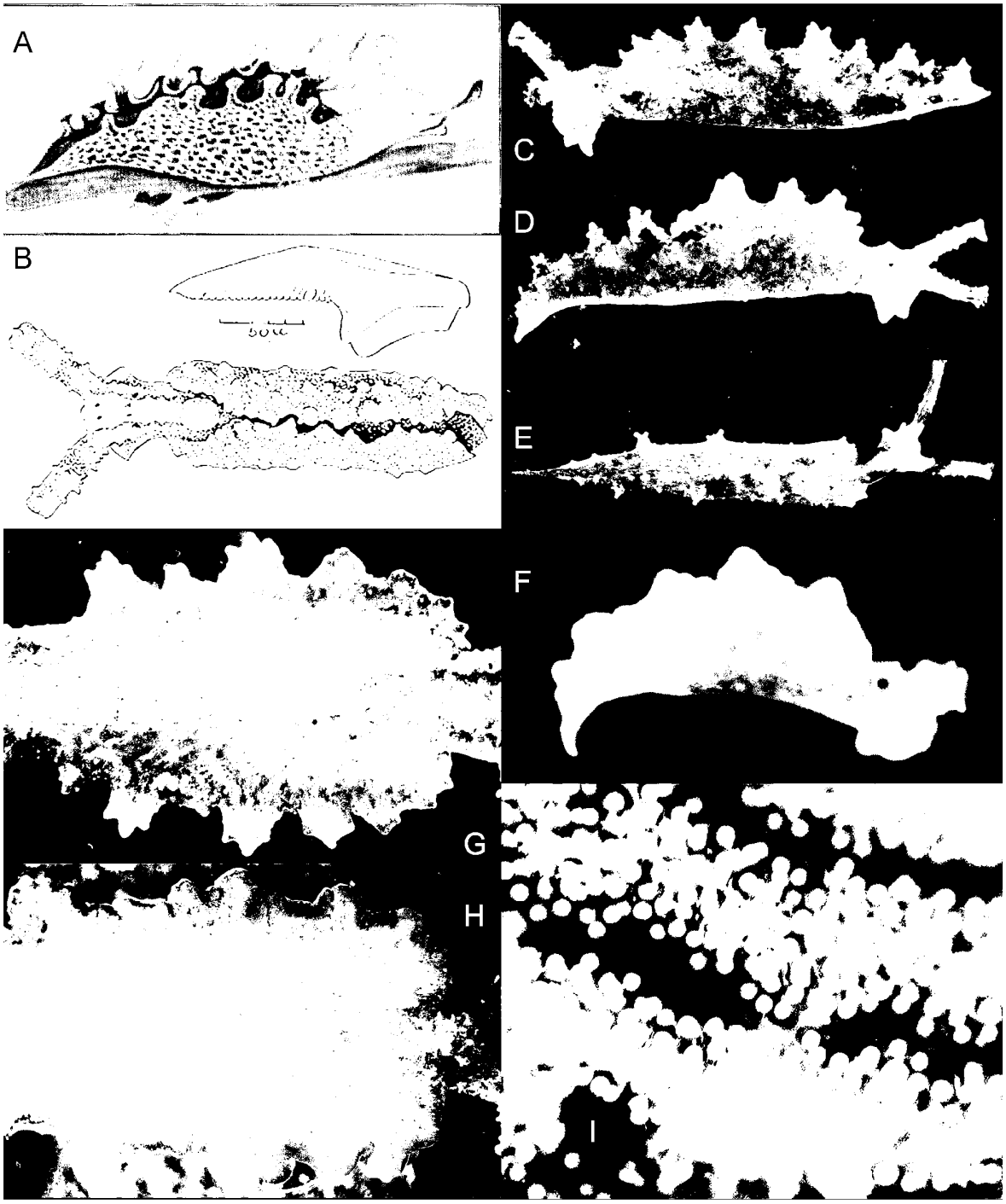

FIGURE 18. *Elysia papillosa*, external morphology and egg mass. Live specimens were photographed upon field collection from Bocas del Toro, Panama (**C–E**, **G–H**) or Discovery Bay, Jamaica (**F**). **A,** Illustration accompanying original description (Verrill 1901: fig. 2), showing lateral undulations of the parapodial margin but no branching papillae; specimen is shown resting on stipe of *H. incrassata*. **B,** Illustration accompanying re-description of *E. papillosa* (Ev. Marcus & Er. Marcus 1967: figs. 22 and 23). **C,** Side view of isolate Epap_04Pan01 (8 mm length) showing brown parapodial margin with crown-like lateral undulations. **D,** Side view of isolate Epap_04Pan04 (5 mm). **E,** Top view of isolate Epap_04Pan08 (6 mm), showing elongated tail. **F,** Side view of juvenile LACM 178620 (3 mm). **G–H,** Dorsal surface of relaxed isolates Epap_04Pan07 (**G**) and Epap_04Pan01 (**H**), showing renopericardial complex, vessel network and greyish-white gametic vesicles. **I** (bottom right panel), Egg mass from Curaçao specimen showing irregular ribbon of white ECY twisting around uncleaved ova; field of view = 1.75 mm.

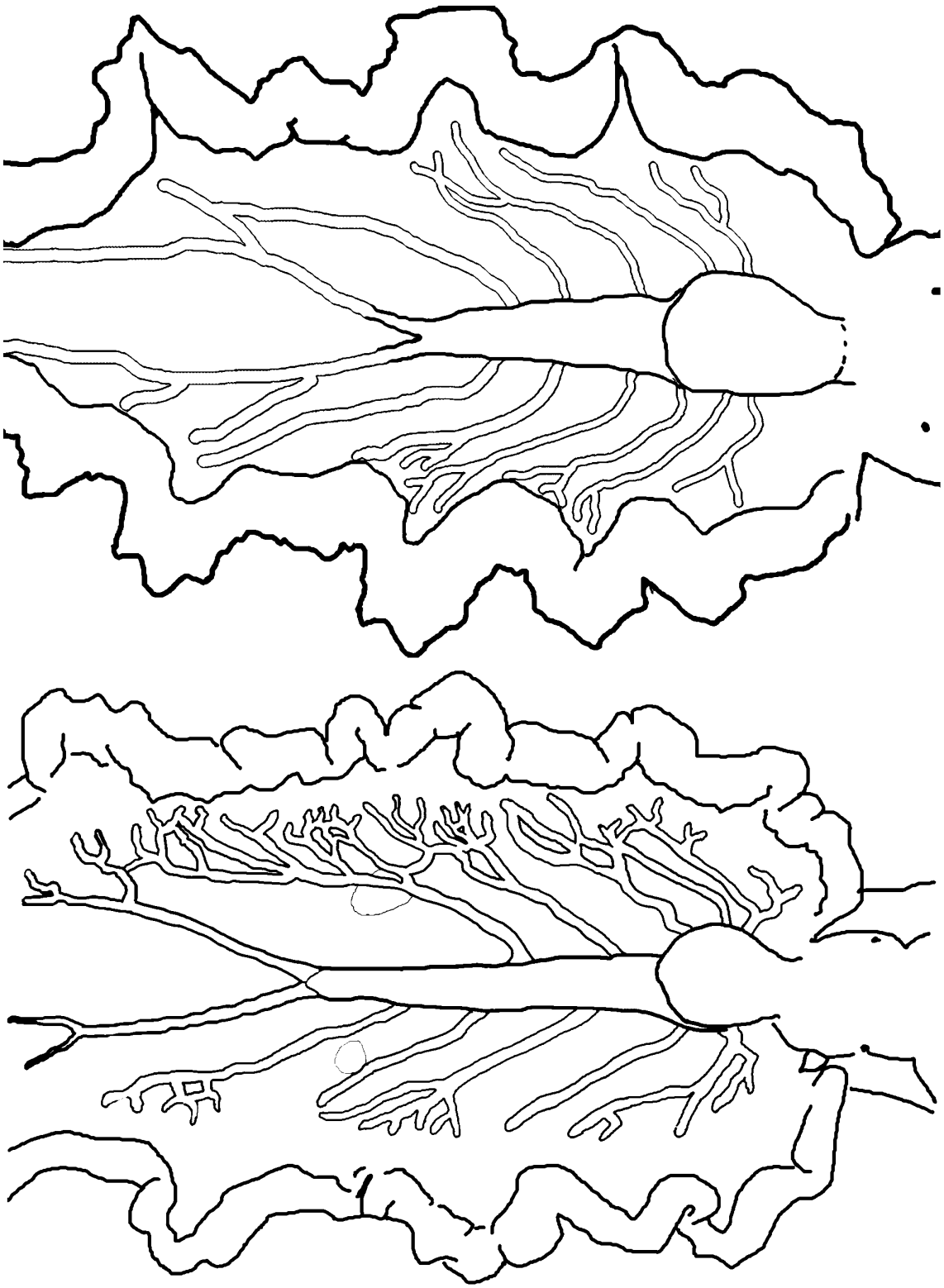

FIGURE 19. *Elysia papillosa*, renopericardial complex and dorsal vessel network traced from digital photograph of live specimens from Panama. Top, isolate Epap_04Pan01, length = 12 mm. Bottom, Epap_04Pan07, length = 10 mm. Grey areas represent sperm-storage vesicles.

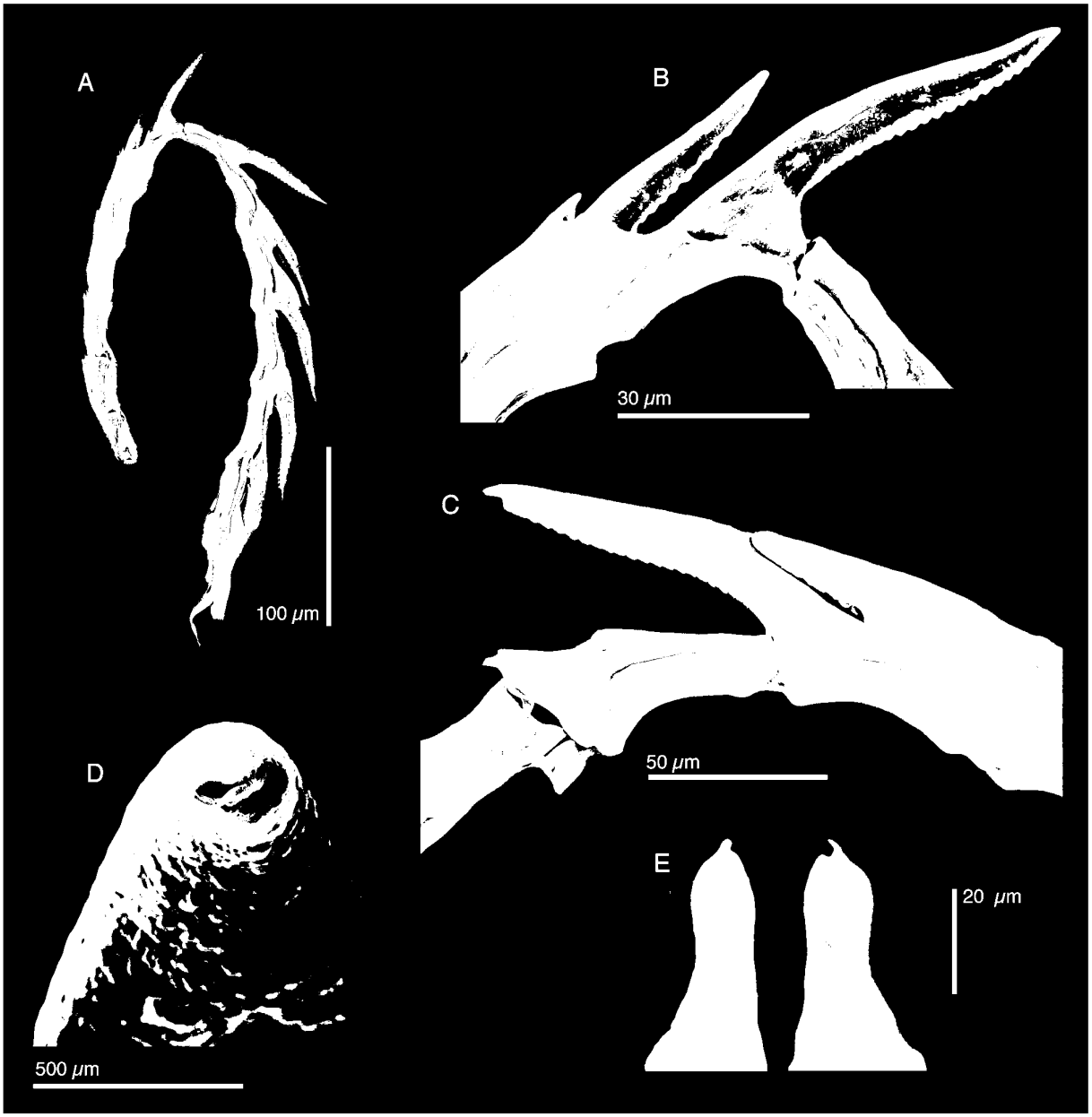

FIGURE 20. *Elysia papillosa*, SEM of the radula and penis. **A**, Radula minus a jumbled ascus (LACM 178613). **B**, Leading tooth (LACM 178613). **C**, Leading tooth, (LACM 178600). **D**, Penis, with stylet (LACM 178600). **E**, Penial stylet, dorsal and ventral views (LACM 178618).

Range. Range data for *E. papillosa* are compromised by frequent misidentifications in the literature. We confirm the species is present in Bermuda (the type locality), Panama, Jamaica, Florida, USA, Bahamas, U.S. Virgin Islands, Antigua, and Curaçao. Records from Mexico and Cuba (Ortea *et al.* 2005) and Florida (Curtis *et al.* 2010) as *E. patina* and *E. annedupontae* also refer to *E. papillosa*.

Remarks. Perhaps no Caribbean elysiid has had as complex a taxonomic history as *E. papillosa*. Confusion stemmed in part from the absence of type material and lack of relevant anatomical data in the initial description, and was compounded by the presence of a cryptic sister species in the central Caribbean. We distinguished *E. papillosa* from its cryptic sister species based on molecular phylogenetic analyses combined with subtle differences in radular morphology. In a population genetic survey, the barcoding COI gene and the nuclear H3 gene were sequenced from 174 specimens that superficially resembled *E. papillosa* (Trathen 2010 as "*E. patina*" sensu

Ortea *et al.* 2005; authors' unpublished data). In phylogenetic analyses, COI haplotypes formed two clades between which TrN distance ranged from 8.8 to 12.8% (mean distance = 10.9%), above the 8% threshold for species-level divergence in *Elysia* (Krug *et al.* 2013). In contrast, maximum pairwise COI divergence was 5.6% within *E. papillosa*, consistent with moderate phylogeographic structure in each species (as in other *Elysia* spp.; Krug 2011; Krug *et al.* 2013). Different alleles at the H3 locus were fixed in the two divergent COI clades, even where both clades were sympatric, indicating the two distinctive COI lineages do not interbreed. When these data were re-analyzed in the present study by ABGD, two distinct *E. papillosa*-like species were recovered across a wide range of priors on allowable intraspecific divergence (Fig. 3A). Branch lengths on the ML tree based on a concatenated four-gene alignment also show a comparable level of genetic divergence between these two taxa as exists for many other pairs of well-recognized sister species of *Elysia* (Fig. 4). Thus, all available molecular data support the distinction of *E. papillosa* from *E. taino* **n. sp.**

All *E. papillosa*-like specimens from Bermuda, the type locality of *E. papillosa*, grouped genetically with all specimens from Florida, Curaçao, and Panama, and with most specimens from three Bahamas islands (Sweetings Cay, Bimini, and Little San Salvador); these specimens formed 'clade 1' in Trathen (2010). Conversely, all specimens from Dominica grouped with most specimens from Jamaica and the remaining Bahamas islands (Stirrup Cay, San Salvador, Plana Cays, Compass Cay, Northern Exumas), forming a divergent lineage ('clade 2' of Trathen 2010). This lineage is subsequently described as *E. taino* **n. sp.** based on genetic divergence and differences in radular morphology from *E. papillosa*. The two species also had minimally overlapping geographical distributions, with only 1-3 specimens of *E. papillosa* sampled from each site where *E. taino* **n. sp.** was the predominant *Elysia* feeding on *Penicillus*.

Despite the existence of the morphologically similar sister species *E. taino* **n. sp.**, much of the confusion in the literature over the identify of *E. papillosa* involved even more distantly related species, including *E. patina*, *E. zuleicae*, and *E. pawliki* **n. sp.** The original description of *E. papillosa* by Verrill (1901) was brief, but included important details:

"A small, grayish, distinctly papillose species. Body rather elongated in extension; head large; neck long; rhinophores large; strongly folded and wide at the tips. Side-flaps large, thin, usually with the edges deeply undulated. Whole surface of body, head, and outside of flaps thickly covered with small conical papillae. Color of head, neck, and outside of flaps grayish blue, paler anteriorly, and spotted with darker gray on the outside of the flaps, and speckled with flake-white over the whole surface. Inside of flaps darker ash-gray; the edges bordered with white. Rhinophores are like the head, but with two indistinct transverse bands of orange-brown on the posterior side. Length, about 12 mm in extension. Hungry Bay, under stones, at a very low-tide, April 5, 1901. (A.H.V.) Rare. This species can swim freely by means of its ample lateral flaps."

The transverse brownish bands on the rhinophores and papillose body surface match the first authoritative re-description of *E. papillosa* by Ev. Marcus & Er. Marcus (1967), who noted that the specimen they examined conformed to the limited details in the original description. The teeth were described as ~200 μm long and serrulated with coarse denticles; the penis as muscular, 700 × 300 μm, with a triangular stylet 60 μm long (Ev. Marcus & Er. Marcus 1967). The species we recognize as *E. papillosa* is the only Caribbean species that matches both Verrill (1901) and Ev. Marcus & Er. Marcus (1967) in having the following distinguishing characteristics: (i) common in Bermuda; (ii) external morphology of some specimens fits the original description; (iii) swims readily when disturbed; (iv) has a penial stylet; (v) has a coarsely serrated, straight-edged radula. The morphologically similar species *Elysia taino* **n. sp.** was not sampled in Bermuda, and has shorter, wider radular teeth (see remarks of *E. taino* for more details). Radular characters readily distinguish *E. papillosa* from all other related species that swim, allowing us to match our material to *E. papillosa* by Ev. Marcus & Er. Marcus (1967), and the description of radular anatomy and feeding ecology by Jensen (1980). Related swimming species (*E. zuleicae*, *E. buonoi* **n. sp.**, *E. patina*) have curved, narrow, pointed radular teeth, which was termed the "*Halimeda* spur" (Clark & DeFreese, 1987). The pointed tip of such curved teeth is used to pierce the narrow utricles accessible on the surface of the heavily calcified, inter-utricular matrix of udotacean algae such as *Halimeda* and *Udotea*. The serrated, straight, blade-shaped tooth of *E. papillosa* and *E. taino* **n. sp.** is used to feed on the long, wide filaments of *Penicillus*, and has diverged rapidly from the tooth shape of all closely related species. Host ecology similarly helps distinguish *E. papillosa* from most related species, but not from its sister taxon.

Ev. Marcus (1980) described the dorsal vessels of *E. papillosa* as having only one pair of vessels with a posterior orientation and many lateral side branches (pg. 57: fig. 9); she may have illustrated a specimen with

multiple side branches emerging from the elongated posterior vessels, and failed to note smaller, transparent vessels anterior to the last pair (Fig. 19). Otherwise, the dorsal vessels figured by Ev. Marcus (1980) for *E. papillosa* do not match the pattern on any specimens we have seen, nor do they match any other Caribbean elysiid. Vessels on *E. papillosa* appear to be slightly wider on average than the vessels of *E. taino* **n. sp.**, and sperm-storage vesicles form closer to the posterior end of the renopericardium on *E. papillosa*.

Some similarities exist between *E. papillosa* and *E. zuleicae*; *E. zuleicae* can swim, occurs in Bermuda, and the planktotrophic egg masses of *E. zuleicae* are similar to those of *E. papillosa*. However, the coloration of typical specimens, rhinophores, and extended tail of *E. zuleicae* are not consistent with the description of *E. papillosa*. Clark (1984) lumped specimens of *E. zuleicae* (pg. 89: figs. 16, 18–20) in with *E. papillosa* (pg. 89: figs. 15, 17); his posthumously obtained notes and drawings indicate he recognized that his 1984 material included species other than *E. papillosa*, and Clark (1994) acknowledged that his *E. papillosa* was a species complex.

Another species often confused with *E. papillosa* is *E. patina*. The coloration described by Verrill also describes well typical specimens of *E. patina*, which also swims, and the dorsal vessels of *E. papillosa* and *E. patina* are very similar. Indeed, the specimen of *E. papillosa* illustrated by Ev. Marcus & Er. Marcus (1967) (reproduced here as Fig. 18B) resembles specimens of both *E. papillosa* (e.g., Fig. 18F) and *E. patina* (e.g., Fig. 42A–B). Small specimens of *E. patina* and *E. papillosa* can be very difficult to distinguish externally, but the species can clearly be distinguished by a number of criteria. Their host algae and radulae are entirely different: *E. patina* feeds on *H. opuntia* and its radular teeth have a curved "*Halimeda* spur". Egg mass characters clearly discriminate between the two: *E. patina* has lecithotrophic development and orange ECY in a flat ribbon (Fig. 42G), whereas *E. papillosa* is planktotrophic in all surveyed populations, with white ECY (Fig. 18I). The sperm-storage vesicles form posterior to the renopericardium in *E. patina*, but are more anterior in *E. papillosa*. Molecular data also clearly differentiate the taxa (Figs. 3–4).

Despite these differences, Ortea *et al.* (2005) identified specimens of *E. papillosa* as *E. patina* based on their dorsal vessel pattern. Ortea *et al.* (2005) confusingly assert that Ev. Marcus mixed two species in her original description of *E. patina*, presumably including one with a blade-shaped, serrated tooth, despite the absence of any supporting evidence. While Ev. Marcus (1980) suggested her paratype specimen from the Bahamas was likely a different species from *E. patina*, inspection of the holotype material of *E. patina* confirms that the type specimen had the curved, pointed radula illustrated in the description, and not the blade-shaped, heavily serrated tooth of *E. papillosa*. The species Ortea *et al.* (2005) called *E. patina* thus cannot be *E. patina* Ev. Marcus 1980. Based on the radular teeth drawn in Ortea *et al.* (2005: fig. 4D), their *E. patina* is most likely *E. papillosa*, but could potentially be *E. taino* **n. sp.** Also, Ortea *et al.* (1998) reported *E. papillosa* from the Canary Islands, but this is another misidentification.

Verrill (1901) drew the parapodial margin of *E. papillosa* undulating in a series of scalloped segments (reproduced here as Fig. 18A); the margin on some specimens bears unbranched conical papillae that create the appearance of points on a crown (e.g., Fig. 18C–D), whereas on other specimens, the margin has only low papillae and appears relatively smooth (Fig. 18E–F). The lateral undulations allow the parapodia of *E. papillosa* to interlock and cover the dorsum, as in the drawing by Ev. Marcus & Er. Marcus (1967) (Fig. 18B). Verrill (1901) drew *E. papillosa* resting on a stipe of *Halimeda incrassata* (Fig. 18A); the alga was misinterpreted by Ortea *et al.* (2005) as a row of digitiform, branching papillae along the parapodial margin of the animal. The species called *E. papillosa* by Thompson (1977) and Ortea *et al.* (2005) does not swim, is unknown from Bermuda, and has other features that are incompatible with the details provided by Verrill (1901) for *E. papillosa*, and must therefore be a different species (which we describe subsequently as either *E. pawliki* **n. sp.** or *E. zemi* **n. sp.**)

Based on external, radular and penial morphology, *E. annedupontae* (Ortea, Espinosa & Caballer *in* Ortea, Caballer, Moro & Espinosa, 2005) is a junior synonym of *E. papillosa*. Ortea *et al.* (2005) noted that key anatomical features of *E. papillosa* given by Ev. Marcus & Er. Marcus (1967) were all present in *E. annedupontae*, including radular characters, penial morphology, and shape and banding pattern of the rhinophores. Ortea *et al.* (2005) claimed *E. annedupontae* was a distinct species because they erroneously interpreted Verrill's drawing as indicating long, branching papillae along the parapodial rim of *E. papillosa*; as noted above, no such papillae are indicated on the drawing, nor are the papillae of *E. papillosa* described as branching by Verrill (1901). By all criteria, *E. annedupontae* is therefore synonymous with *E. papillosa*.

***Elysia flava* Verrill, 1901**
(Figs. 6I, 21–23)

Elysia flava Verrill 1901: 30, pl. 4, fig. 1 (Type Locality: Castle Harbor at Waterloo, Bermuda)—Pruvot-Fol 1946: 35; Er. Marcus 1957: 414; Thompson 1977: 124–125, figs. 25a, 26e; Ev. Marcus 1980: 66; Clark 1984: 90–91, figs. 22–24; Espinosa & Ortea 2001: 44; García *et al.* 2002: 50, fig. 2G; García *et al.* 2008: 71; Collin *et al.* 2005: 690; Espinosa *et al.* 2005: 56; Valdés *et al.* 2006: 68–69; Krug *et al.* 2015: 990–991, figs. 3B, 4.

Type material. *Elysia flava*—untraceable, not at YPMNH.

Material examined. Isla Chimana Grande, Venezuela, July 1989, 3 specimens (LACM 178625, LACM 178625 [2 in lot]); Cayo Solarte, Bocas del Toro, Panama, 21 February 2004, 1 specimen (LACM 2004-9.1); Piscadera Bay, Curaçao, July 2010, 1 specimen (LACM 173252).

Live animal. Animals are typically found under rocks during the day and not associated with any specific alga.

External anatomy. Color yellowish-orange, with some conical opaque white papillae on sides and edges of parapodia (Fig. 21). Edges of parapodia with line of opaque white pigment. Some scattered white spots also present on head and rhinophores. In most specimens digestive gland visible through skin as blotches of dark grey or black pigment. Body relatively short, wide and tall. Rhinophores relatively large, rolled, thick, with rounded blunt tips. Parapodia tall, thick, undulated edges when animal is resting and straight when animal is moving. Eyes conspicuously visible.

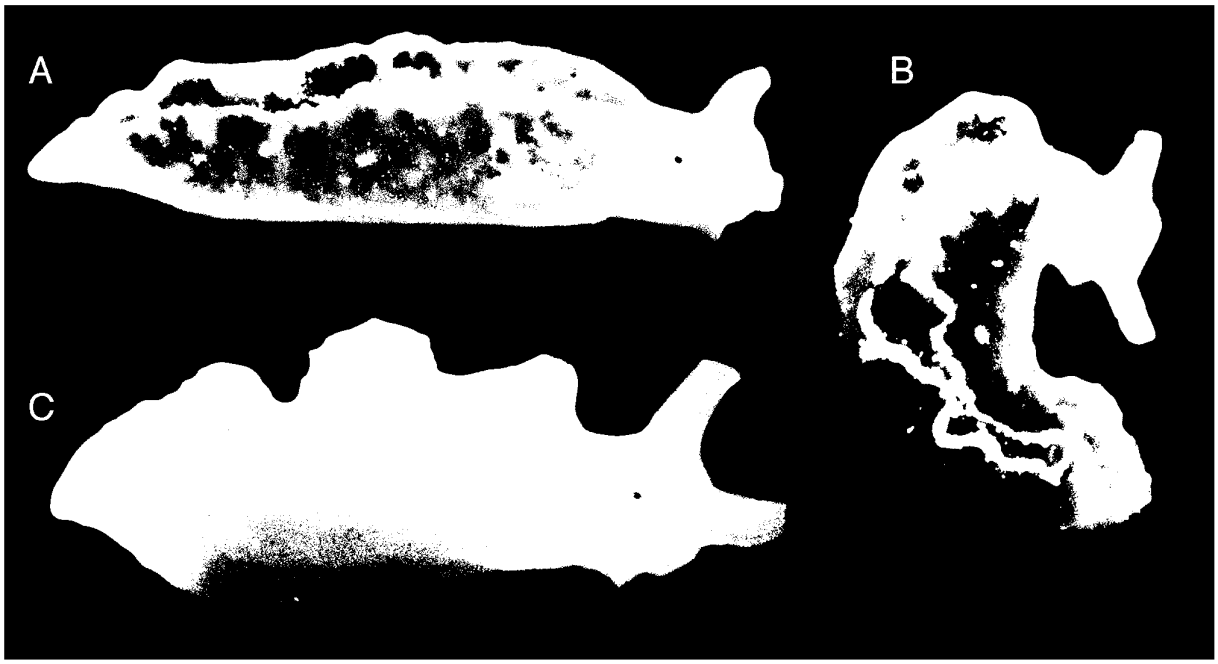

FIGURE 21. *Elysia flava*, external morphology. **A,** Dark specimen in motion from Young Island, St. Vincent and the Grenadines. **B,** Dark specimen stationary from Bocas del Toro, Panama (LACM 2004-9.1). **C,** Light specimen stationary from Freeport Harbor, Grand Bahama, Bahamas.

Renopericardium indistinct from pericardium on preserved specimens. One long pair of posterior dorsal vessels running length of body (Fig. 22). Numerous lateral vessels emerging from each main vessel; side vessels running up side of parapodium, most unbranched but a few forking once or twice.

Internal anatomy. Radula with 18 teeth (LACM 178626), 6 teeth in the ascending limb and 11–12 in the descending limb (Fig. 23A). Leading tooth elongate, with a slightly curving cusp tip, bearing a short denticulate keel and at least one smooth lateral edge (Fig. 23B). Housing depression for interlocking teeth extending ½ total tooth length (Fig. 23A). Base of the tooth approximately ½ total tooth length. Ascus containing jumbled heap of discarded teeth (not figured).

Penis robust and cone-shaped, devoid of armature. Deferent duct long, thin, and convoluted (Fig. 6I).

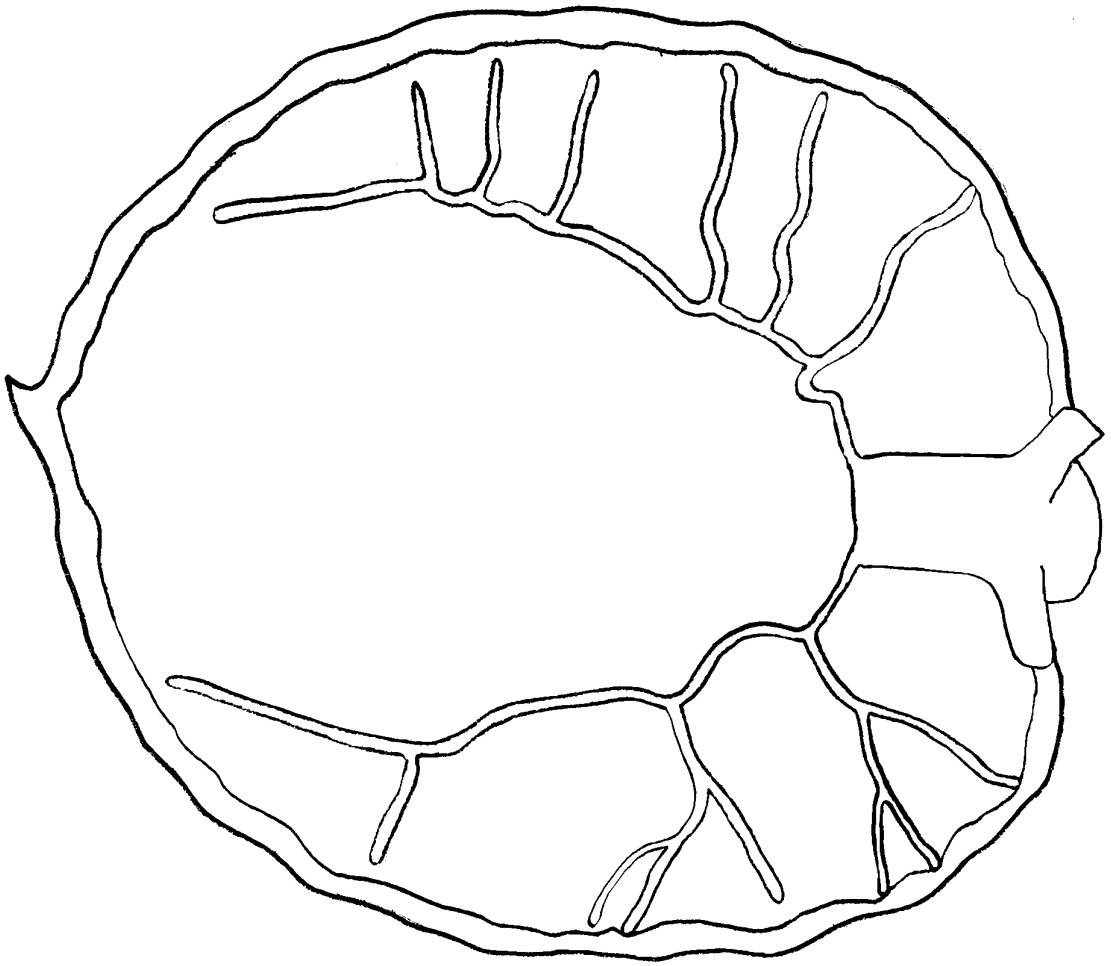

FIGURE 22. *Elysia flava*, dorsal vessels drawn of a preserved specimen from Venezuala (LACM 178625; 5.5 mm long × 5 mm wide).

Reproduction and development. No data available.

Host ecology. Little is known about this enigmatic species, with no published data on larval development mode or host alga. In the eastern Atlantic Marín & Ross (1988) found intact chloroplasts in the digestive system possibly of *Cladophora* sp., suggesting this alga could constitute the diet of *E. flava*. The sister species *E. obtusa* (see below) feeds on *Bryopsis*, which is another potential host alga for *E. flava*.

Phylogenetic relationships. The sister species of *E. flava* is the morphologically similar *Elysia obtusa* Baba, 1938 from the Pacific; these two species form a clade sister to a diverse clade including many *Bryopsis*-feeding taxa (Fig. 4).

Range. Belize (Clark & DeFreese 1987), Bermuda (Verrill 1901; Clark 1984), Brazil (García *et al.* 2002, 2008), Costa Rica (Espinosa & Ortea 2001; Valdés *et al.* 2006), Cuba (Espinosa *et al.* 2005), Curaçao (present study), Jamaica (Thompson 1977), Panama (Collin *et al.* 2005), Puerto Rico (Ev. Marcus 1980), Venezuela (Valdés *et al.* 2006), and Mediterranean Sea (Thompson & Jaklin 1988).

Remarks. *Elysia flava* is easily recognizable alive by its translucent yellowish color with the dark green branching of the parapodial digestive gland visible though the skin, and the presence of opaque white rounded papillae along the edge of the parapodia.

This species was first described by Verrill (1901) from Bermuda, in the western Atlantic, followed by several records from the Caribbean (see range section). The first eastern Atlantic record was by Thompson & Jaklin (1988) from the eastern Mediterranean. The Pacific species *Elysia obtusa* Baba, 1938 is very similar externally and has

been considered a synonym (Gosliner *et al.* 2008). However, Trowbridge *et al.* (2011) questioned this synonymy based on the geographic range separation between *E. obtusa* and *E. flava*. Our molecular phylogenetic analyses confirm that Indo-Pacific specimens of *E. obtusa* are genetically distinct from *E. flava* (Fig. 4), and indeed represent a valid species.

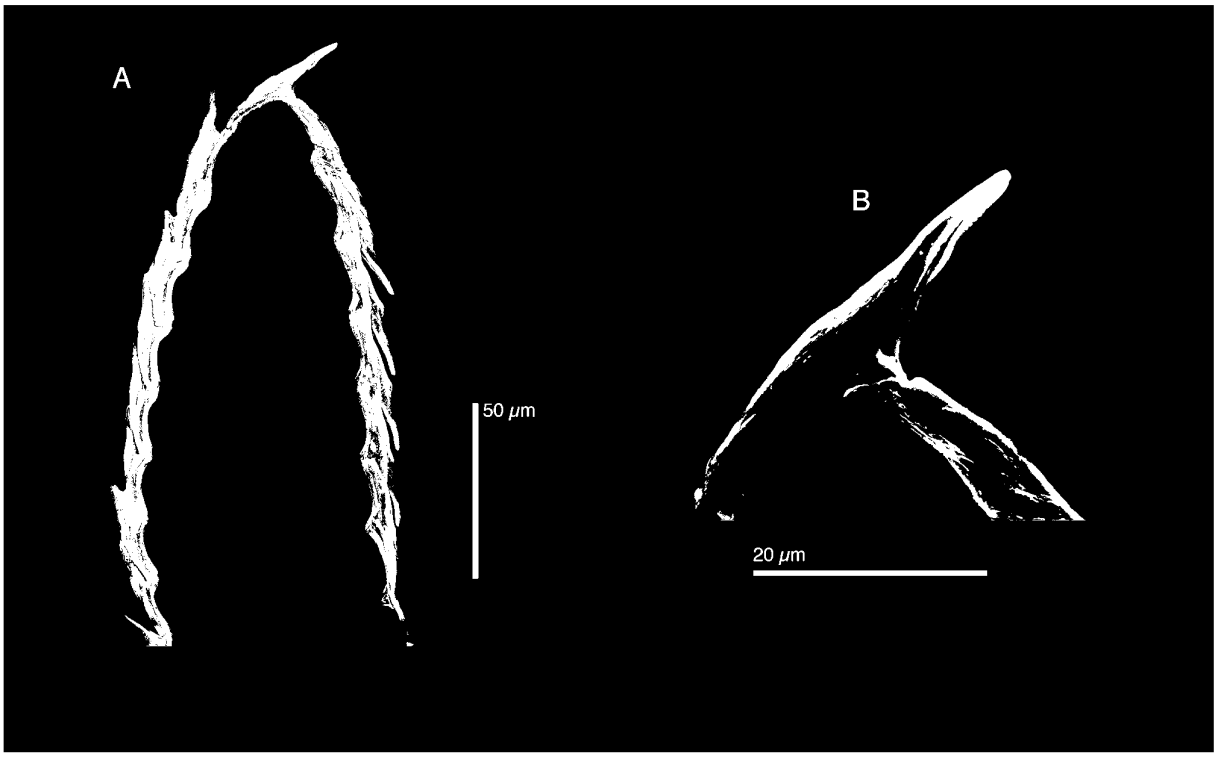

FIGURE 23. *Elysia flava,* SEM of the radula (LACM 178626). **A,** Radula. **B,** Leading tooth.

Elysia subornata Verrill, 1901
(Figs. 6J, 24–26)

Elysia subornata Verrill 1901: 29–30, pl. 4, fig. 4 (Type Locality: Castle Harbor, Bermuda)—Pruvot-Fol 1946: 33; Er. Marcus 1957: 414; Ev. Marcus 1980: 66; Clark 1984: 88–89, figs. 10–14; Hess *et al.* 1994: 163; Clark 1994: 905; Redfern 2001: 162, figs. 673A–B; Valdés 2006: 66–67; Krug 2009: 362–365, figs. 4, 6; Händeler *et al.* 2009: figs. 6–7; Redfern 2013: 285, figs. 790A–C; Zamora-Silva & Ortigosa 2012: 366; Krug *et al.* 2013: 1109–1113, figs. 2C, 4; Ortigosa *et al.* 2013: 65; Christa *et al.* 2014: fig. 3; Krug *et al.* 2015: 990–991, figs. 3B, 4.

Elysia cause Er. Marcus 1957: 405–410, figs. 35–44 (Type Locality: São Sebastião Island and near Ubatuba, São Paulo, Brazil)—Ev. Marcus & Er. Marcus 1960: 153, fig. 34; Ev. Marcus & Er. Marcus 1963: 22–23; Er. Marcus & Ev. Marcus 1970: 44–45, fig. 81; Ev. Marcus & Hughes 1974: 505–507, figs. 13–14; Thompson 1977: 124, figs. 25h–j, 26f; Clark, Busacca & Stirts 1979: 14–19, figs.1–7; Jensen & Clark 1983: 4.

Type material. *Elysia subornata*—untraceable, not at YPMNH; *Elysia cauze*—possible type specimen, ex. Marcus collection (HMCZ 288301).

Material examined. Anse du Bourg, Terre-de-Haut island, Guadeloupe, January 1986, 1 specimen (LACM 178629); Carriacou island, St. Vincent and the Grenadines (present study), Panama (present study). July 1987, 1 specimen (LACM 178628); Bahamas: Great Exuma, 29 Jan 2009, 1 specimen (LACM 172297), Stocking Island, 29 Jan 2009, 1 specimen (CPIC 00076), 16 Feb 2009, 1 specimen (CPIC 00077); Martinique, June 1986, 1 specimen (LACM 178631), July 1987, 1 specimen (LACM 178627), October 2013, 1 specimen (LACM 178630); Water Bay, St. Thomas, U.S. Virgin Islands, 11 April 2006, 1 specimen (CPIC 00142).

Live animal. Usually found in association with *Caulerpa* spp. Animal does not swim when disturbed. Parapodia may be held partly open when resting.

FIGURE 24. *Elysia subornata*, external morphology and egg masses. Live specimens photographed upon collection from the Bahamas (**A**), or near the type locality in Bermuda (**B–F**). **A,** Specimen from Sweetings Cay (length = 11 mm) resting on a stipe of *Caulerpa racemosa*, on which the specimen was collected. **B–C,** Dorsal view of specimens from Bermuda collected on *C. racemosa*, showing dark marginal line; length = 2.7 mm (B), 3 mm (C). **D,** Dorsal view of highly papillose specimen from Bermuda collected on *C. cupressoides*; length = 6 mm. **E–F** Side view (E) and dorsal surface (F) of specimen from Bermuda (length = 4.5 mm) showing elongate renopericardial complex and dorsal vessel network lining inner parapodial margin. **G,** Egg mass from Bahamas specimen showing thick ribbon of orange ECY embedded around egg capsules containing uncleaved ova; field of view = 1.73 mm. **H,** Subsequent development of egg mass from (G), showing newly metamorphosed juveniles and cast-off larval shells inside egg mass.

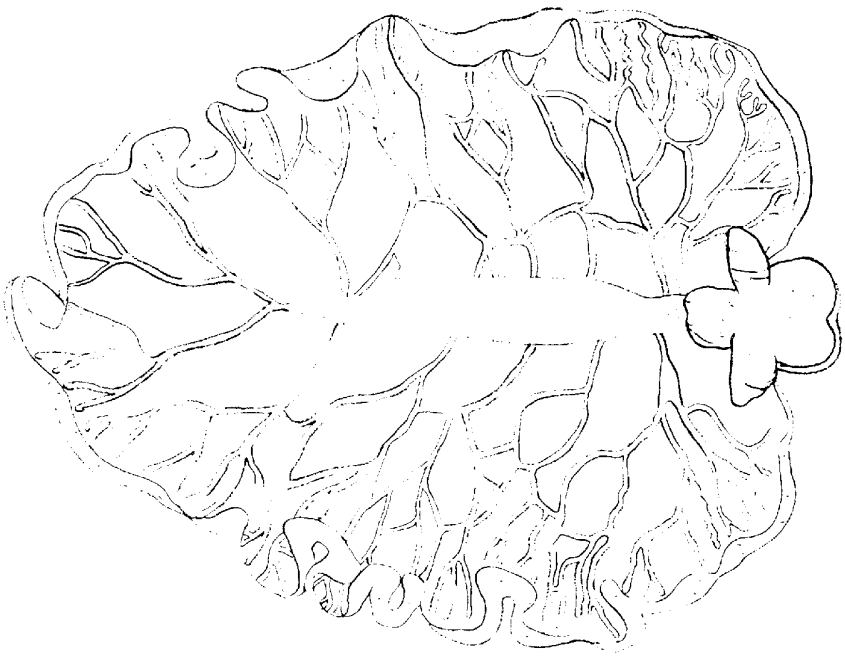

FIGURE 25. *Elysia subornata*, drawing of renopericardial complex and dorsal vessel network of preserved specimen collected in Martinique, 2013 (LACM 178630; 15 mm long × 9 mm wide).

External anatomy. Base color ranging from yellow to olive green to dark green. Sides of parapodia pigmented by white to varying degrees; white pigment often arranged in star-shaped clusters around base of white papillae. Scattered tiny black or brown dots all over head and body. Rows of white conical papillae scattered across parapodia and head to varying degrees (Fig. 24A–E); some specimens with few papillae (Fig. 24B), others densely covered in elongated papillae and associated patches of white pigment (Fig. 24D). White, grey or tan patch on top of head starting between rhinophores, bounded by eyes laterally and pericardium posteriorly. White streaks typically extending posteriorly from base of rhinophores over eyes. Anterior end of head trimmed with tan band and dark brown line along the edge. Rhinophores short relative to body length; color tan to lavender to dark brown with rows of white papillae, and white patches concentrated at tips. Parapodia high, covering most of renopericardial complex unless held partly open. Parapodial margin tan to dark brown, sometimes with white speckling. Distinctive fine, black marginal line running along edge of parapodia (Fig. 24A–F). Inner surface of parapodia and dorsum green with white speckling (Fig. 24B–C, E–F). Posterior end of body narrowing to short, triangular tail, not elongated.

Pericardium small, round, white to brown. Renopericardium light green or whitish, a straight tube running almost entire body length (Fig. 24B–C, F). Dorsal vessels clear to whitish, 10 or more emerging on either side of the renopericardium in large specimens (Figs. 24F, 25). Vessels relatively symmetric in placement on either side of renopericardium, but branch irregularly and repeatedly; side branches anastomosing into dense network lining upper half of inner parapodial surface (Fig. 25). Posterior vessels not longer than any other vessel pair due to length of renopericardial extension.

Internal anatomy. Radula with 28–29 teeth (LACM 172297, CPIC 00076, CPIC 00142), 6 teeth in ascending limb and 21–22 in descending limb (Fig. 26A, E). Leading tooth elongate with cusp bearing very fine, blunt denticles. Housing depression for interlocking teeth "V"-shaped and extending ⅔ total tooth length (Fig. 26B, F). Base of the tooth approximately ⅓ total tooth length. Ascus with 10 teeth arranged in a short row with some disorganized teeth at the end (Fig. 26C).

Penis elongate, often curved (CPIC 00076–77, CPIC 00142) and devoid of armature (Fig. 6J, 26D). Deferent duct long, thin, and convoluted.

Reproduction and development. Development is lecithotrophic with 100% encapsulated metamorphosis (Fig. 24G–H). Early reports by Clark and colleagues held that *E. subornata* (as *E. cauze*) was poecilogonous, seasonally progressing from planktotrophy ('type 1' development) to swimming lecithotrophic development ('type

2') to encapsulated metamorphosis ('type 3') (Clark & Goetzfried 1978; Clark *et al.* 1979). Jensen & Clark (1983) acknowledged that the type 1 and possibly the type 2 egg masses initially attributed to *E. subornata* were in fact laid by different species, and that *E. subornata* had encapsulated metamorphosis, the only mode of development we have personally observed.

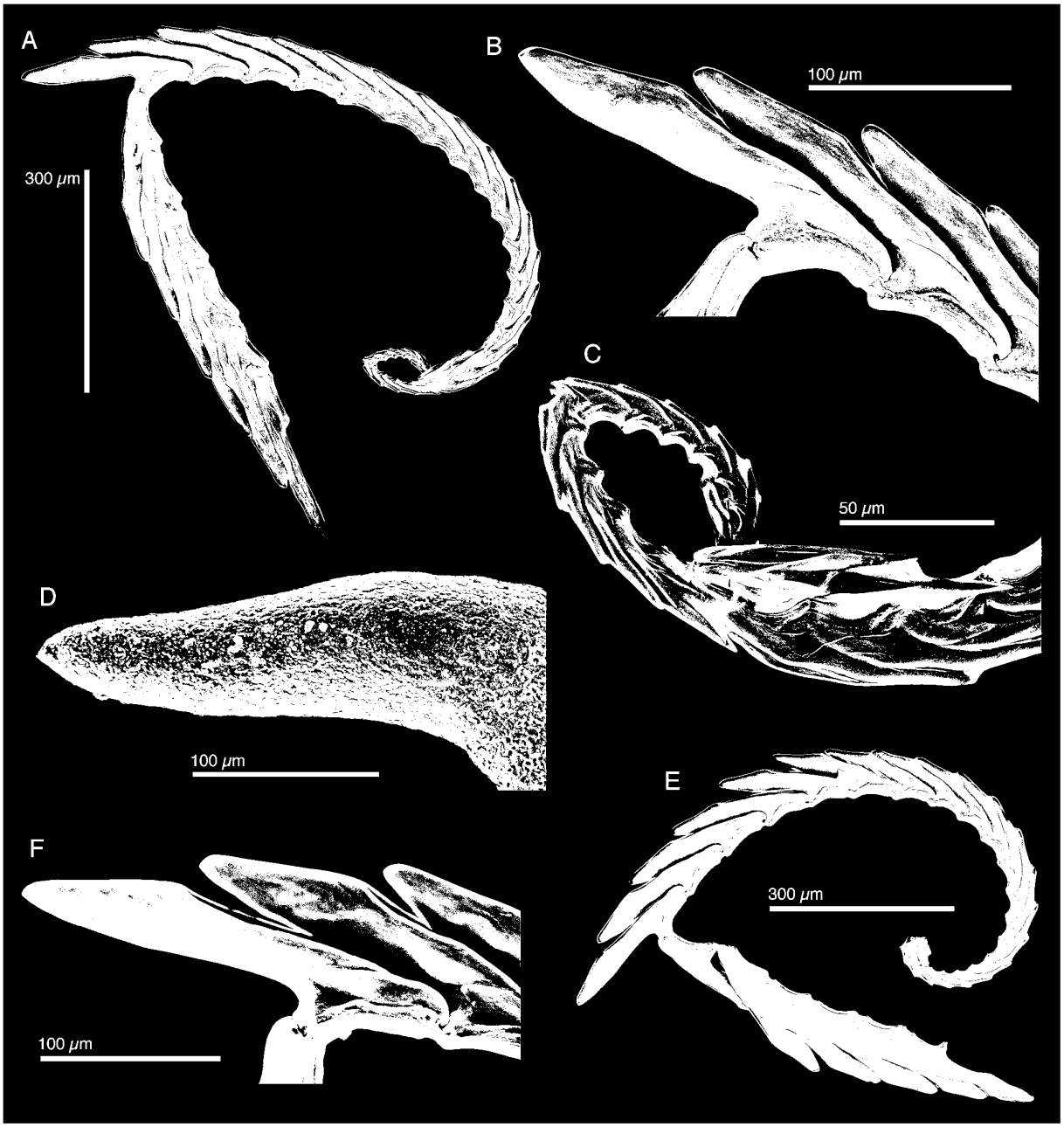

FIGURE 26. *Elysia subornata*, SEM of the radula and penis. **A,** Complete radula (CPIC 00077). **B,** Leading tooth (CPIC 00077). **C,** Ascus (CPIC 00077). **D,** Penis (CPIC 00076). **E,** Complete radula (LACM 172297). **F,** Leading tooth (LACM 172297).

E. subornata produces a continuous string (120–450 μm wide) of bright orange ECY that winds throughout the center of the egg mass, contacting each capsule embedded in the jelly matrix filling the egg spiral (Fig. 24G) (Clark & Goetzfried 1978; Clark *et al.* 1979; Krug 2009). Embryos develop through a veliger stage with a partially reduced velum, and metamorphose prior to hatching (Fig. 24H); a detailed description is provided by Clark *et al.* (1979). The ECY granules may be absorbed or ingested by larvae during encapsulated development, but can also

be consumed directly by juveniles that do not exit the egg mass after metamorphosis; juveniles that remain inside the egg mass and feed on ECY emerge at a significantly larger size than juveniles exiting immediately post-metamorphosis (Krug 2009).

Mean egg diameter for one clutch from the Florida Keys was 119.2 µm (± 2.0 SD; n = 21 ova), matching the 120 µm diameter reported previously (Clark & Goetzfried 1978; Clark *et al.* 1979; Clark & Jensen 1981). Mean larval shell length at metamorphosis for two clutches from Florida of 302.0 µm (± 9.5 SD; n = 26) and 311.1 µm (± 12.8 SD; n = 29), again closely matching prior reports of ~300 µm (Clark *et al.* 1979). Clark *et al.* (1979) reported a maximum fecundity of ~1200 eggs per clutch, and time to hatching of 14 d at 23–25°C; Krug (2009) reported a comparable development time of 14.8 d (± 0.9 SD; n = 4 clutches) at 22°C.

Host ecology. Like most members of subclade 4, *E. subornata* feeds on *Caulerpa* spp. While other sacoglossans in this group may specialize to some degree on particular species of *Caulerpa* (Baumgartner *et al.* 2009), *E. subornata* feeds on at least eight species: *C. racemosa* (Jensen 1981b), *C. sertularoides* (Jensen 1981b), *C. paspaloides* (Jensen 1981b), *C. cupressoides* (Jensen 1981b), *C. taxifolia* (Coquillard *et al.* 2000), *C. mexicana* (Jensen 1981b) and *C. ashmeadii* (Clark & Busacca 1978; Clark *et al.* 1979), and *C. verticillata* (Jensen 1981b; Jensen & Clark 1983).

Phylogenetic relationships. In our analyses the sister taxon of *E. subornata* was *E. pratensis* (Fig. 4), together forming a clade nested within subclade 4 (*E. tomentosa* complex). Species in subclade 4 share an elongated renopericardial complex, which runs almost the full length of the dorsum in both *E. subornata* and *E. pratensis*. Molecular data were not available from *E. hamanni* **n. sp.**, which likely belongs to subclade 4 given its elongated renopericardium and specialization on *Caulerpa*; future analyses including this species may alter our understanding of the evolutionary relationships of *E. subornata*.

Range. Aruba (Ev. Marcus & Er. Marcus 1963; Valdés *et al.* 2006), Bahamas (Redfern 2013), Barbados (Ev. Marcus & Hughes 1974), Belize (Clark & DeFreese 1987; Valdés *et al.* 2006), Bermuda (Verrill 1901; Clark 1984), Brazil (Er. Marcus 1957), Cayman Islands (Hess *et al.* 1994), Bonaire (Ev. Marcus & Er. Marcus 1963); Curaçao (Ev. Marcus & Er. Marcus 1963; Er. Marcus & Ev. Marcus 1970), Florida (Ev. Marcus & Er. Marcus 1960; Er. Marcus & Ev. Marcus 1970; Jensen & Clark 1983; Clark 1994), Guadeloupe (present study), Jamaica (Thompson 1977), Martinique (Thibaut *et al.* 2001), Mexico (Valdés *et al.* 2006; Zamora-Silva & Ortigosa 2012; Ortigosa *et al.* 2013), Puerto Rico (Er. Marcus & Ev. Marcus 1970), St. Martin/St. Maarten (Ev. Marcus & Er. Marcus 1963; Er. Marcus & Ev. Marcus 1970), St. Thomas, U.S. Virgin Islands (present study), St. Vincent and the Grenadines (present study), Virgin Islands (Valdés *et al.* 2006).

Remarks. Clark (1984) synonymized *Elysia cauze* with *Elysia subornata*. Clark indicated that different species with planktotrophic and pelagic lecithotrophic development were initially misidentified as *E. subornata*, leading to the erroneous reports of seasonally varying development mode in this species; however, the identities of those other two species were never published. Clark's notes indicate he took *E. pratensis* to be an ecotype of *E. subornata*, but as *E. pratensis* also has lecithotrophic development with encapsulated metamorphosis, it cannot be one of the misidentified species with swimming larvae. The elongated renopericardium distinguishes *E. subornata* from all other Caribbean species except *E. pratensis*. Radular characters and host use distinguish *E. subornata* from *E. pratensis*: *E. subornata* has nearly smooth teeth and feeds on *Caulerpa* spp., whereas *E. pratensis* has coarsely serrated teeth and feeds on *Rhipocephalus phoenix*.

***Elysia velutinus* Pruvot-Fol, 1947**

(Figs. 6N, 27–29)

"*Elysia crispa*" [not available] Verrill 1900: 547, pl. 66, fig. 4 (error for *Elysia crispata*).

Elysia verrilli Pruvot-Fol 1946: 39 [non Thiele, 1931] (Type locality: Bailey Bay, Bermuda)—new name for "*Elysia crispa*" sensu Verrill (1900).

Elysia velutinus Pruvot-Fol 1947: 115 (Type locality: Bailey Bay, Bermuda)—replacement name for *Elysia verrilli* Pruvot-Fol, 1946 [non Thiele, 1931].

Elysia pruvotfolae Er. Marcus 1957: 415 (Type locality: Bailey Bay, Bermuda)—replacement name for *Elysia verrilli* Pruvot-Fol, 1946 [non Thiele, 1931].

Elysia papillosa [non Verrill, 1901]—Ev. Marcus & Er. Marcus 1963: 21–22, fig. 29.

Elysia tuca Ev. Marcus & Er. Marcus, 1967: 29–31, figs. 28–32 (Type locality: Soldier Key, Biscayne Bay, Florida)—Er. Marcus & Ev. Marcus 1970: 46–47, figs. 81, 84–85; Ev. Marcus & Hughes 1974: 507, figs. 15–16; Thompson 1977: 128–

129, figs. 26c, 27a–b; Clark & Goetzfried 1978: 285, fig. 1; Ev. Marcus 1980: 70–72, figs. 16–17, 55; Clark 1984: 90, fig. 21; Jensen & Clark 1983: 5–6; Hess *et al.* 1994: 163; Clark 1994: 904; Espinosa & Ortea 2001: 44; Redfern 2001: 162–163, figs. 674A–B; Collin *et al.* 2005: 690; Espinosa *et al.* 2005: 56; Valdés *et al.* 2006: 66–67; Krug 2009: 360–365, figs 1, 2A, 3A, 4, 6; Redfern 2013: 285, figs. 791A–B; Ortigosa *et al.* 2013: 65–66 **n. syn.**; Christa *et al.* 2014: figs. 1F, 3; Krug *et al.* 2015: 990–991, figs. 3B, 4.

Type material. *Elysia velutinus*—untraceable, not at YPMNH; *Elysia tuca*—Syntype (USNM 576286).

Material examined. Punta Uva, Gandoca-Manzanillo, Costa Rica, 20 September 1999, 1 specimen (CPIC 00148); Stocking Island, Bahamas, 23 January 2008, 1 specimen (CPIC 00012), 15 Dec 2007, 2 specimens (CPIC 00013–14); Geiger Beach, Florida, USA, 2006, 1 specimen (LACM 178632); Southwest Flamingo Bay, Water Island, St. Thomas, U.S. Virgin Islands, March 1985, 1 specimen (LACM 178633); Union Island, St. Vincent and the Grenadines, 1987, 1 specimen (LACM 178634); Prince Rupert Bay, Dominica, 19 April 2008, 1 specimen (LACM 178642).

Live animal. Parapodia held together when resting. Slugs do not swim when disturbed. Although normally living in association with *Halimeda* spp., large specimens were occasionally found crawling on other algae in dense beds of mixed algae.

External anatomy. Overall coloration light to dark green, with spots or large patches of pigment ranging from white to tan (Fig. 27). Head with large "Y"-shaped pigment patch (usually white, sometimes tan) behind head, starting anterior of pericardium and running up to base of each rhinophore, usually extending laterally to just above small eyespots (Fig. 27B–D, F). Front of head rounded and smooth, no oral lobes. Rhinophores green at base but increasingly white or tan towards tip; uniform in width along entire length, sometimes dotted with small papillae. Foot pale yellow-green. Specimens from Panama (Fig. 27A–C) generally lacking papillae or white patches on parapodia; Bahamas specimens often with scattered white papillae across body, head and rhinophores, and larger patches of white concentrated along parapodial margins.

Parapodia high and thick, usually held closed to cover dorsal surface and pericardium. Edges of parapodia bow out to form one small siphonal opening about halfway along body (Fig. 27B–D). Outer parapodial surface ranging from smooth to dotted with white papillae, small and rounded. Parapodia dull to dark green, with regularly spaced patches or speckles of white to tan pigment. Larger patches of white sometimes concentrated at intervals along parapodial margin (Fig. 27D–F). Margin with smooth, even edge, sometimes lined along inner and/or outer edge with white to tan pigment. Inner surface of parapodia, dorsum and pericardium speckled (sometimes densely) with iridescent blue-green dots (Fig. 27F–G). Posterior end of body sometimes blunt-ended, or else narrowing to form short, triangular tail. Dorsal surface sometimes pierced by egg masses of parasitic copepod, colored light blue-green, not observed on other species (Fig. 27C).

Pericardium large and rounded, pale green to white in ground color, with scattered iridescent blue-green dots (Fig. 27F–G). Renopericardium short and not distinct from pericardium, giving rise to one pair of posterior dorsal vessels often densely coated with iridescent blue-green dots (Fig. 27G). Each vessel forks into two main branches, one curving towards anterior end and the other running towards posterior end of body. Each branch sending off 8–15 short lateral side branches each forking 0–3 times, and sometimes anastomosing. All branches extending only three-quarters of the way up inner parapodial surface before terminating at a distance from parapodial margin. Branching network of white reproductive ducts visible through dorsum around renopericardial complex.

Internal anatomy. Radula with 17 teeth (CPIC 000148), 9 teeth in ascending limb and 8 in descending limb (Fig. 28A–B). Leading tooth elongate and narrow, with slightly curved cusp, bearing numerous minute denticles (Fig. 28A). Housing depression for interlocking teeth "V"-shaped and extending ½ total tooth length (Fig. 28A). Base of the tooth approximately ⅓ of total tooth length.

Penis wide and elongate with rigid musculature resistant to desiccation (Fig. 6N) and tapering distally into a conical apex bearing a unique "ridged" stylet (Fig. 28C). Deferent duct narrow and convoluted.

Reproduction and development. Larval biology described by Krug (2009) is summarized below. Development is lecithotrophic, with a ribbon of bright orange ECY zigzagging through egg mass, contacting every egg capsule (Fig. 27H). Veligers ingest granules of ECY that enter their capsule, or absorb yolk material during development, acquiring an orange hue. Mean number of eggs per clutch for Bahamas specimens was 113.7 ± 20.2 SE (n = 7; range = 32–194), and for Florida specimens was 177.3 ± 32.7 SE (n = 19; range = 6–580). Mean egg diameter for one clutch from Bahamas was 104.8 μm (± 0.5 SE; n = 32).

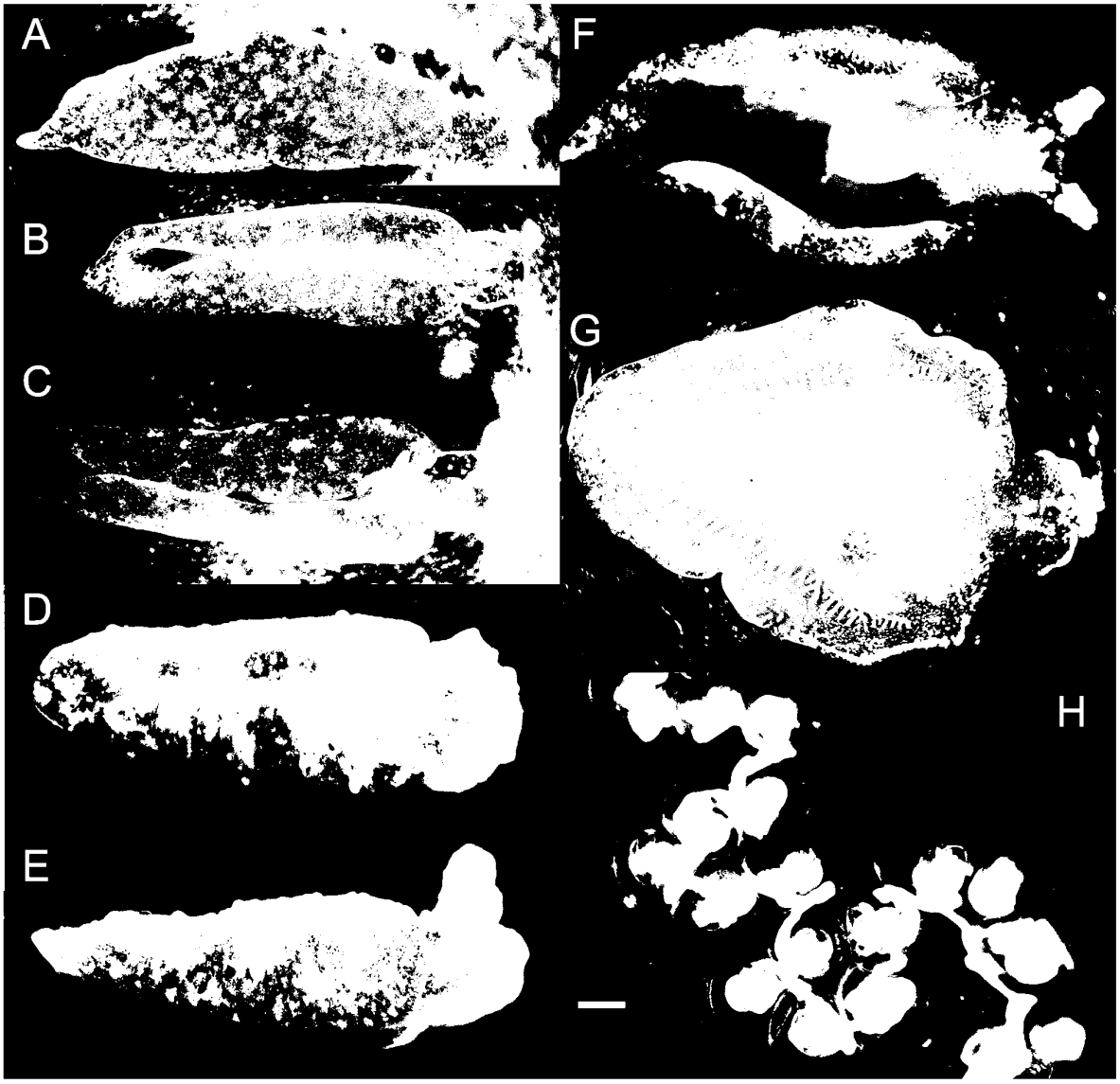

FIGURE 27. *Elysia velutinus*, external morphology and egg mass. Specimens photographed following field collection from Bocas del Toro, Panama (A–C, G; Dec 2004); Sweetings Cay, Bahamas (D–E; Jul 2007); or San Salvador, Bahamas (F; Jul 2007). All measurements give body length of specimens. **A–B,** Specimen (11 mm) with light green ground color and scattered tan spots. **C,** Darker green specimen, top view only (14 mm). **D–E,** Specimen (7 mm) showing characteristic white patch on head, and large white patches across parapodia. **F,** Specimen (9 mm) with sparse white patches along parapodial margin. **G,** Specimen (10 mm) with parapodia open, showing renopericardial complex and dorsal vessel network. **H,** Close-up of egg mass laid by specimen from San Salvador, Bahamas, showing orange ECY ribbon folding back and forth between capsules, each containing one lecithotrophic veliger larva. Scale bar = 200 μm.

Mean time to commencement of hatching was a comparable 17.7 d (± 0.3 SE; n = 3) for egg masses from Bahamas slugs, and 18.1 d (± 0.7 SE; n = 19 clutches) for Florida egg masses. Krug (2009) described extensive intra-clutch variation in time to hatching of siblings, with larvae in outermost whorl of egg mass hatching about a week earlier than siblings from innermost whorl; mean time from initial to final hatching was 8.6 d (± 3.9 SD; n = 19 clutches; range = 2–16 d). Time necessary to complete hatching scaled linearly with clutch size. Egg masses often deposited on seagrass *Thalassia testudinum* (this study; Jensen & Clark 1983) as well as on *H. incrasatta.*

Mean larval shell length at hatching varied substantially among five clutches, ranging from 261.9 μm to 284.1 μm (grand mean length = 275.8 μm ± 3.9 SE; n = 5). No intracapsular metamorphosis occurred, and less than 0.5% of larvae metamorphosed in absence of an algal substrate over a week in filtered sea water (FSW). About half of

larvae were induced to metamorphose by exposure to one of three species of adult host genus *Halimeda* (*H. incrassata*, *H. monile*, *H. opuntia*), whereas negligible settlement occurred in response to three non-host algae (*Udotea flabellum*, *Caulerpa verticillata*, *Batophora oerstedii*). Larvae settled directly onto blades of *Halimeda* and metamorphosed over 2–3 d. Some larvae successfully metamorphosed after 12 d with no planktonic food. Newly metamorphosed juveniles measured 358.2 µm in length (± 30.5 SE; n = 2 clutches) when crawling.

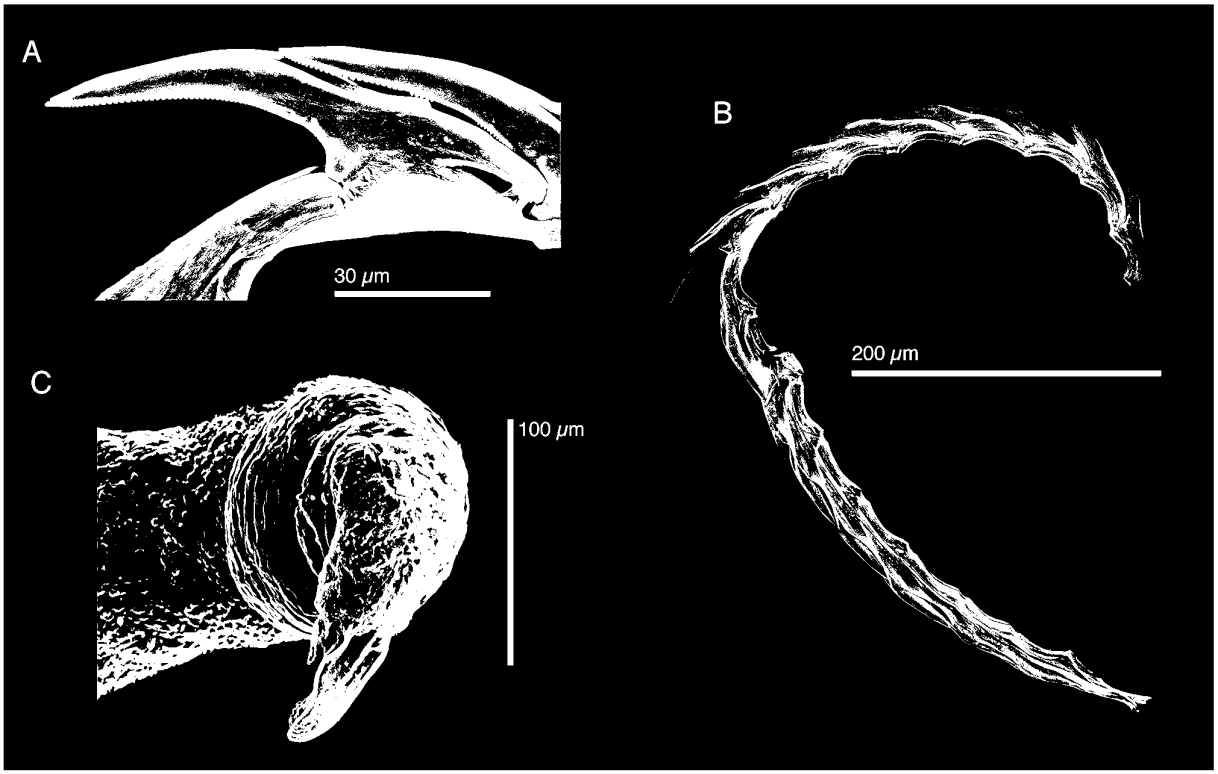

FIGURE 28. *Elysia velutinus*, SEM of the radula and penis. **A**, Leading tooth (CPIC 00148). **B**, Radula (CPIC 00148). **C**, Penis, (LACM 178634).

Host ecology. *Elysia velutinus* feeds on various species in the genus *Halimeda*, and is most commonly associated with the upright branching species *H. incrassata* and *H. monile* (this study) and *H. discoidea* (Jensen 1983; Jensen & Clark 1983). Being large, mobile and abundant, *E. velutinus* is also commonly found crawling on non-host algae in the field. Clark & Busacca (1978) reported that in the laboratory, starved specimens of *E. velutinus* consumed *Avrainvillea nigricans*, *Udotea* sp., three species of *Caulerpa* (*C. racemosa*, *C. mexicana*, *C. sertularoides*), and possibly *Batophora* or *Rhipocephalus*; however, the metric used to assess feeding was unclear, and may have been observed ingestion, growth, or maintenance of chlorophyll levels relative to starved slugs. These results do not reflect the typical host association of field-surveyed animals and are considered unreliable without further confirmation. Further, we have observed starved slugs of several species feeding on algae with which they are not associated in the field; starved animals are thus capable of feeding on non-host algae, but will not normally do so if preferred (host) species are present, and typically cannot sustain growth or long-term survival on non-host algae.

Phylogenetic relationships. *Elysia velutinus* was recovered as sister to an undescribed but morphologically similar species (*Elysia* sp. 6) from the eastern Pacific coast of Central America with complete support (Fig. 4). No other closely related species were identified. Bayesian analyses indicated the clade (*E. velutinus* + *E.* sp. 6) was sister to a clade of Pacific *Elysia* spp. that feed on other udotacean algae (*Chlorodesmis*, *Udotea*), including *Elysia bennettae* Thompson, 1973, *Elysia degeneri* Ostergaard, 1955, and two undescribed species (*E.* cf. *bennettae*, *Elysia* sp. 15).

Range. Bahamas (Redfern 2013), Barbados (Ev. Marcus & Hughes 1974), Belize (Clark & DeFreese 1987); Bermuda (Verrill 1900; Ev. Marcus & Er. Marcus 1963; Clark 1984), Brazil (Ev. Marcus 1980), Cayman Islands

(Hess *et al.* 1994), Costa Rica (Espinosa & Ortea 2001), Cuba (Espinosa *et al.* 2005), Curaçao (Ev. Marcus & Er. Marcus 1963; Er. Marcus & Ev. Marcus 1970; Valdés *et al.* 2006), Dominica (present study), Florida, USA (Er. Marcus & Ev. Marcus 1970; Jensen & Clark 1983; Waugh & Clark 1986; Clark 1994), Jamaica (Thompson 1977), Mexico (Valdés *et al.* 2006; Ortigosa *et al.* 2013); Panama (Collin *et al.* 2005), St. Thomas, U.S. Virgin Islands (present study). Union Island, St. Vincent and the Grenadines (present study).

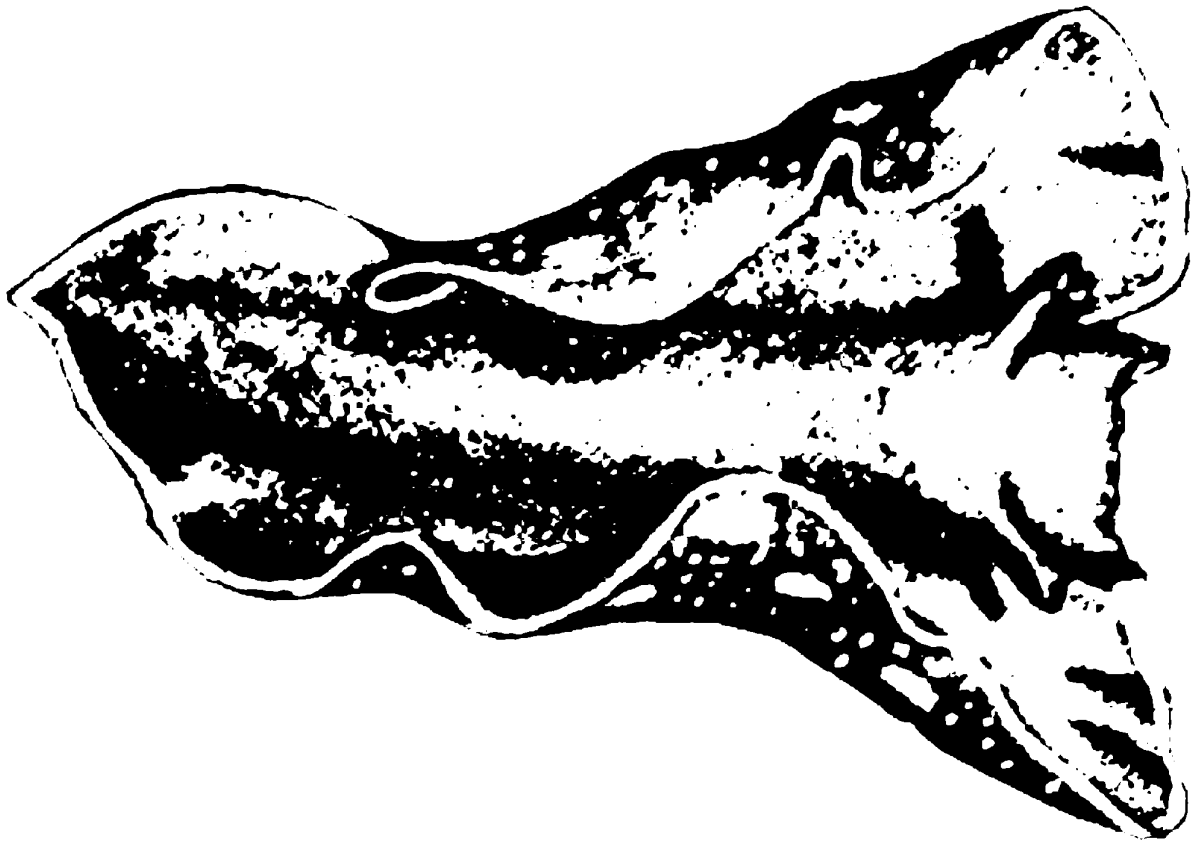

FIGURE 29. Original drawing of "*Elysia crispa*" by Verrill (1900) from Bermuda showing the characteristic white patch behind the rhinophores, and white patches with smaller white dots across the parapodia, characteristic of *E. velutinus*.

Remarks. Pruvot-Fol (1946) introduced the name *Elysia* (*Elysiopterus*) *verrilli* Pruvot-Fol, 1946 for the specimens identified as "*Tridachia crispa* Mörch" by Verrill (1900), which she argued were different from the true *Tridachia crispata*. In a later note, Pruvot-Fol (1947) mentioned that *Elysia verrilli* was preoccupied by *Elysia* (*Elysiella*) *verrilli* Thiele, 1931 and therefore introduced the replacement name *Elysia* (*Elysiopterus*) *velutinus* Pruvot-Fol, 1947. Unaware of Pruvot-Fol's (1947) paper, Er. Marcus (1957) introduced the replacement name *Elysia* (*Elysiopterus*) *pruvotfolae* Er. Marcus, 1957 for *Elysia* (*Elysiopterus*) *verrilli* Pruvot-Fol, 1946, realizing that it was preoccupied by Thiele's name.

The characteristics of the animals described and illustrated by Verrill (1900) from Bermuda include the presence of a white spot on the head and relatively smooth parapodia (Fig. 29). All described features are consistent with the species commonly known in the Caribbean literature as *Elysia tuca* Ev. Marcus & Er. Marcus, 1967, which is common in Bermuda. Clark (1984) noted for the first time that the specimens described from Bermuda by Verrill (1901) as "*E. crispa*" were indeed *E. tuca*. Because the name *E. tuca* is widely used in Caribbean literature it is desirable to maintain the usage of the name. However, under the provisions of the Code of Zoological Nomenclature (ICZN 1999: Article 23.9), a senior synonym can only be replaced automatically by a commonly used junior synonym if the former has not been used as a valid name after 1899. Because *Elysia velutinus* was introduced in 1947, following the Principle of Priority and the available evidence we propose to reinstate the name *Elysia velutinus* for this species.

The penial stylet in this species is only clearly visible under SEM (LACM 178634, CPIC 00013) as a "cuticular tube with several folds" (Ev. Marcus 1980). Such morphology is consistent with the "three spines" morphology of the penis mentioned by Ortea *et al.* (2005).

Elysia canguzua Er. Marcus, 1955

(Figs. 6K, 30–32)

Elysia canguzua Er. Marcus 1955: 111–113, figs. 45–48, 60–65 (Type locality: São Sebastião Island, Brazil)—Er. Marcus 1957: 415, fig. 47; Ev. Marcus 1980: 67–68, figs. 10, 49; Jensen & Clark 1983: 4; Valdés *et al.* 2006: 64–65; Ortigosa *et al.* 2013: 65; Christa *et al.* 2014: fig. 3; Krug *et al.* 2015: 990, fig. 3B.

Elysia eugeniae Ortea & Espinosa 2002: 130–133, figs. 1–2; pl. 1, fig. A (Type locality: Manzanillo, Limón, Costa Rica) **n. syn.**

Elysia purchoni Thompson 1977: 129–130, figs. 25f–g, 26h (Type locality: Lazaretto Cairn, approaches to Kingston Harbour, Jamaica) **n. syn.**

Type material. *Elysia canguzua*—untraceable, not at MZSP (Siqueira Dornellas & Simone 2011); *Elysia eugeniae*—Holotype (MZUCR INB0001497478); *Elysia purchoni*—Holotype (BMNH 19775.W)

Material examined. Dry Tortugas National Park, Florida, USA, 2010, 1 specimen (LACM 178644); Martinique, 14 July 2013, 1 specimen (LACM 178643); Puerto Vargas, Cahuita, Costa Rica, 7 January 2006, 1 specimen (LACM 178645); Manzanillo, Limón, Costa Rica, 1 specimen (MZUCR INB0001497478).

Additional material examined. Martinique, 14 July 2013, 2 specimens (isolate Ecang_13Mar02, isolate Ecang_13Mar03; Carriacou island, St. Vincent and the Grenadines, July 1987, 1 specimen (LACM 178645); Bocas del Toro, Panama, 30 July 2015, 10 specimens.

Live animal. The Dry Tortugas specimen, a juvenile, emerged from a collection of *Bryopsis plumosa* in 2 m depth from a seawall. Specimen fed on alga in the lab for 4.5 months, reaching 12 mm in length. While feeding, a strand of white exudate was frequently released from the anus. Parapodia were typically held open when resting. Specimen did not swim when disturbed. Specimens from Panama (n=10) were also obtained from *B. plumosa* growing in a sheltered cove, and conformed in all other respects to the previous specimen. Copulation was observed frequently.

External anatomy. Overall color dark to olive green on head and outer surface of parapodia, due to ramifying digestive diverticula. Exterior body surface generally smooth with sparse, low papillae; covered with dense, uniformly scattered tiny orange or red spots, and smaller iridescent blue specks. White spots scattered in uneven rows across sides of parapodia and head. White patches visible through epidermis from underlying white glands. Body shape dominated by a large siphonal opening just posterior to pericardium, with two smaller openings at the middle and end of body (Fig. 30A–B).

Raised bump at center top of head, between large eye spots. Upper lip with moustache of tiny black spots; lower lip lined in white. Digestive diverticula extend into both top and bottom lips. Rhinophores short relative to body length (1.5 mm on relaxed 12 mm animal), as long as distance from bump on head to anal papilla. Blunt tipped, with terminal white patch. Surface of rhinophores with same texture and color as rest of head, penetrated by green digestive diverticula throughout. Foot distinct from parapodia, set off by longitudinal groove on either side of body, but coloration same as parapodial surface (Fig. 30C). Transverse groove separating underside of head from the rest of the body. End of foot tapering to an elongated, pointed tail.

Large, anterior siphonal opening formed by laterally extended side-flaps of parapodia, which fold away from body; parapodia are one half body-length in width at widest point (Fig. 30C). Parapodia narrow about halfway down body, then widening into second, smaller siphonal opening. From anterior end to widest part of first siphon, parapodial margin green, crossed by regular white bars. Thereafter, thick white band running along margin, bordered by diverticula and white spots, with intermittent yellow-green splotches along margin; band extending to posterior end of second siphonal opening, thereafter margin green with regularly spaced white spots. Interior of parapodia milky white, with scattered clumps of dark green diverticula, and patches of white or light blue dots.

Large, raised anal papilla on dorsal surface, anterior and right of renopericardium inside large siphonal opening (Fig. 30A, D). Anus opening from center of papilla, ringed in white. Rounded pericardium covered by dense cap of green diverticula; short, tapering renopericardium, yellow-white with orange dots (Fig. 30D). One pair of dorsal

vessels emerging from posterior end of pericardial complex, clear except for a few scattered white spots (Fig. 31). Vessels thick, ~100 μm diameter, bifurcating. Posterior to first large siphon opening, dorsal vessels and accompanying clear swellings fusing one or both interior edges of parapodia to dorsal body surface, making parapodia thickened. At posterior end of body, parapodia form a third, smaller siphonal opening. Interior of parapodia and dorsal surface dominated by irregularly sized and shaped swollen white pustules, around which vessels may branch or wind; may be clear or filled with milky white fluid.

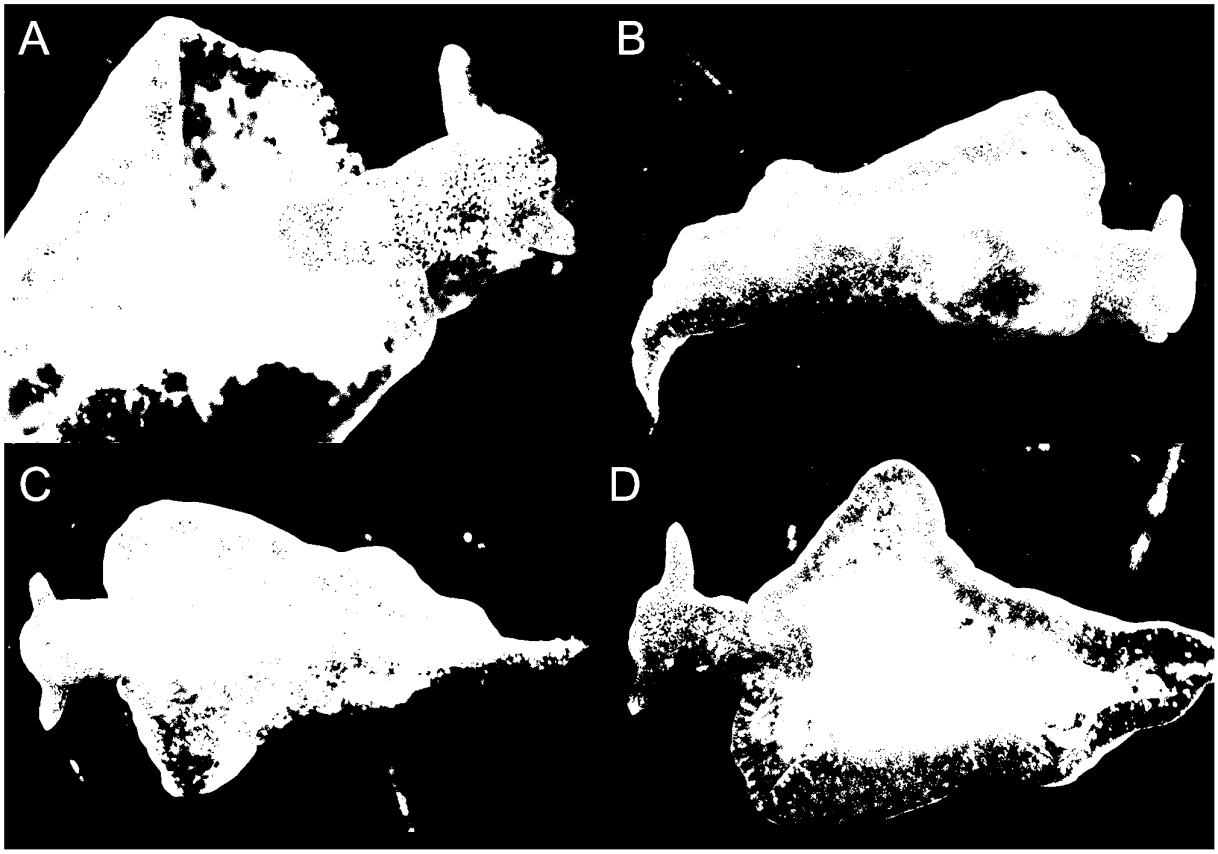

FIGURE 30. *Elysia canguzua*, external morphology of live specimen from Dry Tortugas, FL, USA (length of slug = 12 mm). **A,** Dorsal view of head, showing raised anal papilla and renopericardium. **B,** Side view of same specimen. Swollen white pustular glands visible inside parapodia. **C,** Ventral surface of foot, showing transverse groove separating the head. **D,** Interior of parapodia, showing renopericardial complex and dorsal vessels.

Internal anatomy. Radula with 14–16 teeth (LACM 178643–44, LACM 178646), 8–9 teeth in ascending limb and 6–7 in descending limb (Fig. 32A,C). Leading tooth elongate and robust with a subtle and smooth lateral edge on each side and cusp bearing approximately 67 very small, rounded denticles (Fig. 32B, D–F). Housing depression for interlocking teeth "V"-shaped and extending ¾ total tooth length (Fig. 32B). Tooth cusps, in lateral view, with two sections divided longitudinally, a thin and sharp basal half and a wider, thicker upper half. Base of tooth about ¼ total tooth length. Ascus containing jumbled heap of discarded teeth (not figured).

Penis large and elongate with rigid musculature resistant to desiccation (LACM 178644, LACM 178646), and tapering into a rounded tip devoid of armature (Fig. 6K). Deferent duct narrow and simple.

Reproduction and development. Development was reported as planktotrophic by Jensen & Clark (1983). Ortea & Espinosa (2002) reported an egg cordon of irregular shape, containing white eggs 85–103 μm, irregularly arranged in the jelly matrix. No ECY was present.

Host ecology. The Dry Tortugas specimen was collected from *Bryopsis plumosa*, on which it fed readily. Er. Marcus (1955) described specimens from Brazil as feeding on *Codium*, while Jensen & Clark (1983) reported both *B. plumosa* and *Codium* sp. as preferred hosts.

Phylogenetic relationships. *Elysia canguzua* was recovered as a member of subclade 2, sister to a clade

comprising *E. chlorotica* + *E. serca* (Fig. 4). The host algae of *E. canguzua* are also consumed by some other members of subclade 2, *E. viridis* (*Codium* and *Bryopsis*) and *E. crispata* (*Bryopsis*).

Range. Brazil (Er. Marcus 1955; Ev. Marcus 1980), Costa Rica (Ortea & Espinosa 2002; Valdés *et al.* 2006), Florida, USA (Jensen & Clark 1983), Jamaica (Thompson 1977), Martinique (present study), Mexico (Ortigosa *et al.* 2013), Carriacou island, St. Vincent and the Grenadines (present study), Panama (present study).

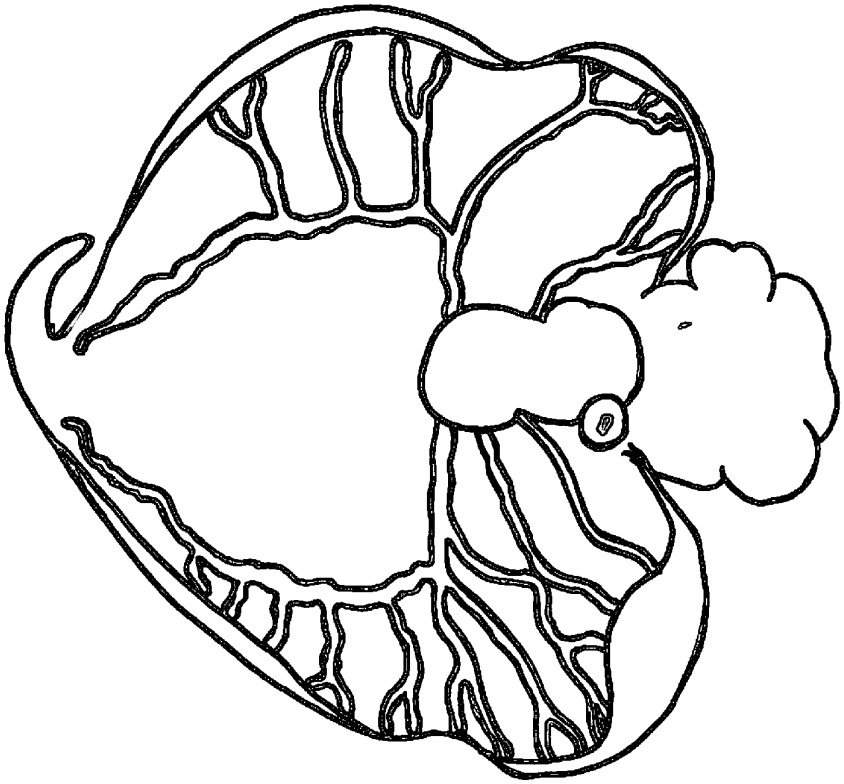

FIGURE 31. *Elysia canguzua*, drawings of renopericardium and dorsal vessel network from a preserved specimen (LACM 178643; 5.5 mm long × 5 mm wide).

Remarks. Thompson (1977) described *E. purchoni* from a single specimen, 5 mm long, based on purportedly distinguishing characteristics: a row of black spots on the oral lobes; "numerous orange specks on all exposed surfaces;" a lateral ridge on the radular tooth; and the large anterior siphonal opening. His description of external morphology and radular teeth entirely conform to the description of *E. canguzua*. No mention of *E. canguzua* was made in Thompson's (1977) remarks on *E. purchoni*, suggesting he was unaware of Er. Marcus' (1955) description of *E. canguzua*. Er. Marcus (1955) referred to the moustache of black spots on the upper "lip", or front of the oral lobes, as a "transverse arc on each side of the head," and depicted the row of black spots in his illustration of the feeding animal (Er. Marcus 1955: figs. 61–63). Although a similar moustache of black spots is present on *E. cornigera*, the extended lateral wing-flaps of *E. cornigera* do not form a rounded siphonal opening as depicted for *E. purchoni* (Thompson 1977: fig 25f) whereas those of *E. canguzua* do form such an opening. Thus, *E. purchoni* is a junior synonym of *E. canguzua*.

Ortea & Espinosa (2002) described *E. eugeniae* (12 mm in body length) from Costa Rica, found on *Bryopsis muscosa*. Their description conforms in all respects to that of *E. canguzua*, without the crucial penial anatomy that is distinctive to this species. They noted that *E. canguzua* was similar in its dorsal vessels, color pattern and radular teeth, but identified their material as distinct from *E. canguzua* based on (1) the very prominent anal papilla, (2) absence of black pigment inside the rhinophores, and (3) longer radular teeth. To the first point, although an anal papilla was not explicitly mentioned in Er. Marcus's (1955) description, the position of the anus is well described ("anus lies dorso-laterally in the shoulder fold and beneath it the single female opening"), and a raised papilla is clearly present on the close-up figure of the head (Er. Marcus 1955: fig. 64). In terms of pigmentation, the type material was described as having black pigment lining the inside of the rhinophores, and forming a transverse arc

on each side of the head, a patch on the shoulder fold, and a line along the parapodial margin in some specimens (Er. Marcus 1955). Our specimens lacked any notable black pigment, but were otherwise identical to the description of Er. Marcus (1955); we therefore consider the presence or absence of black pigment to reflect intra-specific polymorphism. Fittingly, Er. Marcus (1955) noted in his remarks on *E. canguzua* that "the separation of the species of *Elysia* cannot continue on the basis of colour, thickness and extension of the parapodia, form of the head, and radula," placing emphasis on the reproductive anatomy including the vaginal and penial morphology.

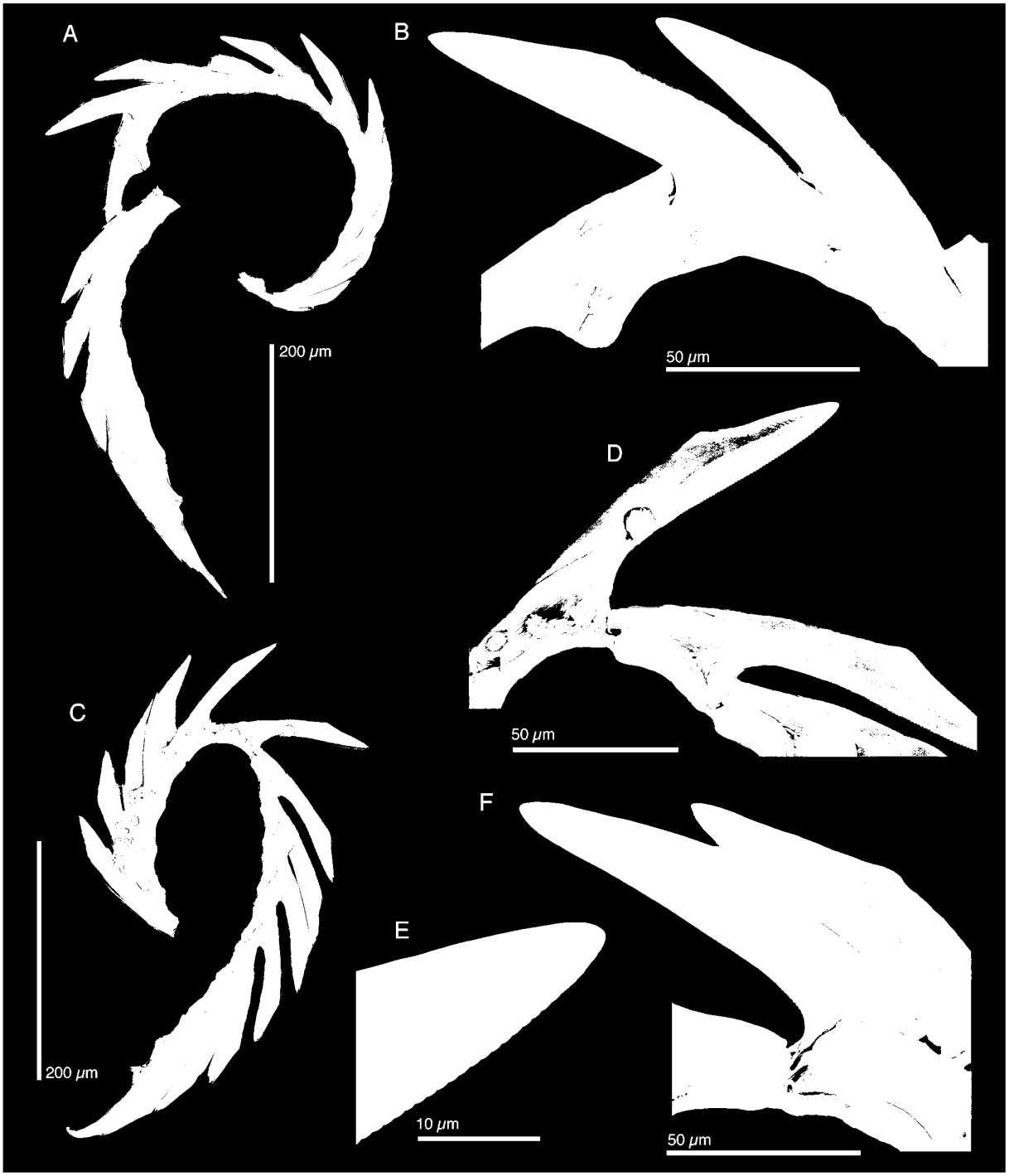

FIGURE 32. *Elysia canguzua*, SEM of radulae. **A,** Radula, without ascus (LACM 178643). **B,** Leading tooth (LACM 178643). **C,** Radula, without ascus (LACM 178644). **D,** Leading tooth, showing smooth lateral edge (LACM 178644). **E,** Close-up of cusp showing denticles. **F,** Leading tooth (LACM 178645).

In terms of radulae, Er. Marcus (1955) described the tooth of *E. canguzua* as 80 µm long from a 9 mm long slug, having "a more or less pointed cusp, slightly marked lateral crests and a very finely serrulate medial crest." Thompson (1977) reported a similar shape for the tooth of *E. purchoni*, with maximum length of 99 µm and minimum length of 36 µm, on a 5 mm long slug. Ortea & Espinosa (2002) reported a 125-µm-long tooth for *E. eugeniae* specimens measuring 12 mm in body length, with a lateral ridge and serrated medial cutting edge consistent with the descriptions of Er. Marcus (1955) for *E. canguzua*, and Thompson (1977) for *E. purchoni*. Given that Ortea & Espinosa (2002) studied larger slugs than Er. Marcus (1955) or Thomson (1977), it is not surprising that they measured longer teeth, and tooth length alone is not considered a species-diagnostic character in Sacoglossa. In the absence of any diagnostic difference, *E. eugeniae* is therefore a junior synonym of *E. canguzua*.

More recently, Ortea *et al.* (2011) proposed that *E. cornigera* was a junior synonym of *E. purchoni*, which they then "redescribed" from Caribbean material. The basis for their synonymy was that both species had some jumbled teeth in the ascus, red spots, a moustache of black dots on the oral lobes, and radulae with a lateral crest as well as a serrated cutting edge. Confusing matters further, the authors make no mention of *E. eugeniae*, despite the fact that it shares this suite of characters. Aside from the radular character noted, none of the other traits are unique to one species; for instance, numerous elysiids have an arc of black dots on the oral lobes, or tiny red spots on the body. These characters are therefore inappropriate for the basis of a synonymy in the absence of other data. In terms of the radula, Thompson (1977) referred to a lateral ridge on the tooth of *E. purchoni*, similar in drawings and descriptions to what Er. Marcus (1955) called a "slightly marked lateral crest" on the tooth of *E. canguzua*. The lateral ridge on the tooth of *E. canguzua* (= *purchoni*) is not described as serrated by Thompson or Marcus, nor is it drawn with serrations in their respective figures. The tooth of *E. canguzua* is therefore distinctly different from the double, serrated cutting edge of the radular tooth in *E. cornigera*. Moreover, the siphonal opening depicted by Thompson (1977) for *E. purchoni* is consistent with the opening formed by the curved parapodia of *E. canguzua*, but not the extended lateral parapodial wing-flaps of *E. cornigera*. Thus, *E. cornigera* is not a synonymn of *E. purchoni*.

Developmentally, *E. canguzua* lacks ECY, a derived character state shared by all members of subclade 2. Given the biogeographic distribution of members of subclade 2, this lineage may have evolved in the warm-temperate West Atlantic; secondary loss of ECY may be favored in more productive temperate waters, while ECY could be selectively maintained in oligotrophic tropical waters to buffer larval offspring against starvation (Krug *et al.* 2015).

Elysia chitwa Er. Marcus, 1955

(Not figured)

Elysia chitwa Er. Marcus 1955: 115–117, figs. 53–56, 58 (Type locality: São Sebastião Island, Brazil), ***nomen dubium***—Er. Marcus 1957: 415, fig. 46; Ev. Marcus 1980: 68 figs. 11, 37, 39, 51.

Type material. *Elysia chitwa*—Untraceable, not at MZSP (Siqueira Dornellas & Simone 2011).

Material examined. No specimens available.

Live animal. No live specimens were observed.

External anatomy. Er. Marcus (1955) briefly described this species as follows. "The green diverticles of the liver and red dots produce a general aspect similar to *E. canguzua*. Black pigment occurs in form of coarse granules in the shoulder furrow, on the outer border of the rhinophores, and between mouth and rhinophores. Small melanophores are scattered over the dorsal surface and the parapodia." An illustration of the preserved specimen (Er. Marcus 1955: fig. 53) shows a small *Elysia* with indistinct parapodia and lacking any visible dorsal vessels.

Internal anatomy. Er. Marcus (1955: fig. 58) illustrated the penis of this species as elongate and thin with no penial stylet.

Radula with an undetermined number of teeth. Teeth short and robust with cusp lacking denticles. Housing depression for interlocking teeth "V"-shaped (Er. Marcus 1955: fig. 56).

Reproduction and development. No data available.

Host ecology. No data available.

Phylogenetic relationships. No specimens available.

Range. Brazil (Er. Marcus 1955).

Remarks. This species has not been collected since its original description. Although it is possibly a valid and distinct species, its taxonomic status remains unknown.

Elysia serca Er. Marcus, 1955

(Figs. 6L, 33–35)

Elysia catulus [non Gould, 1870]—Espinosa *et al.* 2005: 56.

Elysia serca Er. Marcus 1955: 113–115, figs. 49–52, 59 (Type locality: Island of São Sebastião and Ubatuba, State of São Paulo)—Er. Marcus 1957: 415, fig. 45; Ev. Marcus 1970: 209–210, fig. 8, non fig. 7 (see Ev. Marcus 1976a); Ev. Marcus 1976a: 6; Ev. Marcus 1980: 68, figs. 12, 28, 40, 50; Hosoe, 1956: 1–6. pl. 1; Jensen 1982: 87–93, figs. 2–5; Jensen & Clark 1983: 5; Clark 1994: 905; Valdés *et al.* 2006: 68–69; Espinosa *et al.* 2007: 83; Händeler *et al.* 2009: fig. 7; Christa *et al.* 2014: fig. 3; Krug *et al.* 2015: 990–991, figs. 3B, 4.

Elysia clena Er. Marcus & Ev. Marcus, 1970: 49, figs. 81, 89–90 (Type locality: Piscadera Bay, Curaçao and Barbados)—Ev. Marcus 1972a: 292–293; Ev. Marcus & Hughes 1974: 507–509, fig. 18–20; Ev. Marcus 1980: 72, figs. 18, 56.

Type material. *Elysia serca*—untraceable, not at MZSP (Siqueira Dornellas & Simone 2011); *Elysia clena*—untraceable, not at USNM or MZSP (Siqueira Dornellas & Simone 2011).

Material examined. Bahamas: Exuma, 15 December 2007, 1 specimen (CPIC 00050), 9 March 2008, 1 specimen (CPIC 00027), Stocking Island, 14 January 2005, 1 specimen (LACM 172293), Feb 2009, 1 specimen (CPIC 00075).

Live animal. Er. Marcus (1955) described the live animals as "nearly cylindrical."

External anatomy. Body coloration green-brown to dark brown, with white patches scattered along parapodial flanks and margin. White patches concentrated on top and front of head, with head entirely white on some specimens (Fig. 32); on others, sides of head including area around eyespots having background green-brown color. Parapodia reduced, not covering rounded pericardium. Parapodial margin thickened. Rhinophores short, white; tips rounded or slightly pointed at one end (Fig. 32A–B). Foot with same color as parapodial flanks, not clearly demarcated from sides of parapodia; clear medial line running down foot (Fig. 32C). End of body forms elongated tail, narrowing to rounded tip.

Pericardium raised, rounded; color ranging from green-brown with white speckling, to all white (Fig. 32B). Renopericardium elongated, gradually narrowing; running up to half of body length on larger specimens. Six to seven dorsal vessels emerging irregularly on either side of renopericardium, some branching once near margin of parapodium (Fig. 33).

Internal anatomy. Radula with 31–35 teeth (CPIC 00027, LACM 173228), 12–13 teeth in ascending limb and 19–22 in descending limb (Fig. 35A) in a closely packed arrangement (Fig. 35A–B). Leading tooth elongate, slightly curved, robust, and lacking denticles, with a subtle bend at distal ¼ of tooth. Tooth base tall, nearly cuboid (Fig. 35B). Housing depression for interlocking teeth "V"-shaped and extending ¾ of tooth length. Base of tooth narrow, about ¼ or less total tooth length.

Penis narrow with a long, curved tip tapering from a wide base (CPIC 00027, CPIC 00050, CPIC 00075) and devoid of armature (Fig. 6L). Deferent duct long, narrow, and highly convoluted.

Reproduction and development. Larval development is planktotrophic, with a mean egg diameter of 61 µm and no ECY (Clark & Jensen 1981).

Host ecology. Er. Marcus (1955) described *E. serca* from Brazil, collected from "Phaeophyceae" (*Sargassum* or *Padina*) and *Ulva*, which were later asserted to be the host algae (Er. Marcus 1957). Later work indicated this was a mistaken assumption that the algae on which animals were found also constituted their diet. Studies by Jensen (1982, 1983a) clearly demonstrated that *E. serca* specializes on seagrasses in at least three genera: *Halophila engelmanni*, *Halodule wrightii*, and *Thalassia testudinum*. Slugs prefer young, fully developed leaves free from epiphytes (Jensen 1982). Epithelial cells are pierced by the radula to allow suctorial feeding on larger mesophyll cells. Slugs grew fastest on, and preferentially associated with, *Halophila engelmanni*, which has large but thin epidermal cells easily pierced by the radula overlying large mesophyll cells (Jensen 1983a). In *H. wrightii*, epidermal cells are comparably thin, but small relative to the underlying mesophyll cells, necessitating a zigzag feeding behavior to avoid repeatedly penetrating the epidermis above an already-emptied mesophyll cell. In *T. testudinum*, the epidermal cells are thicker than the length of the radular tooth, slowing feeding on this least preferred host.

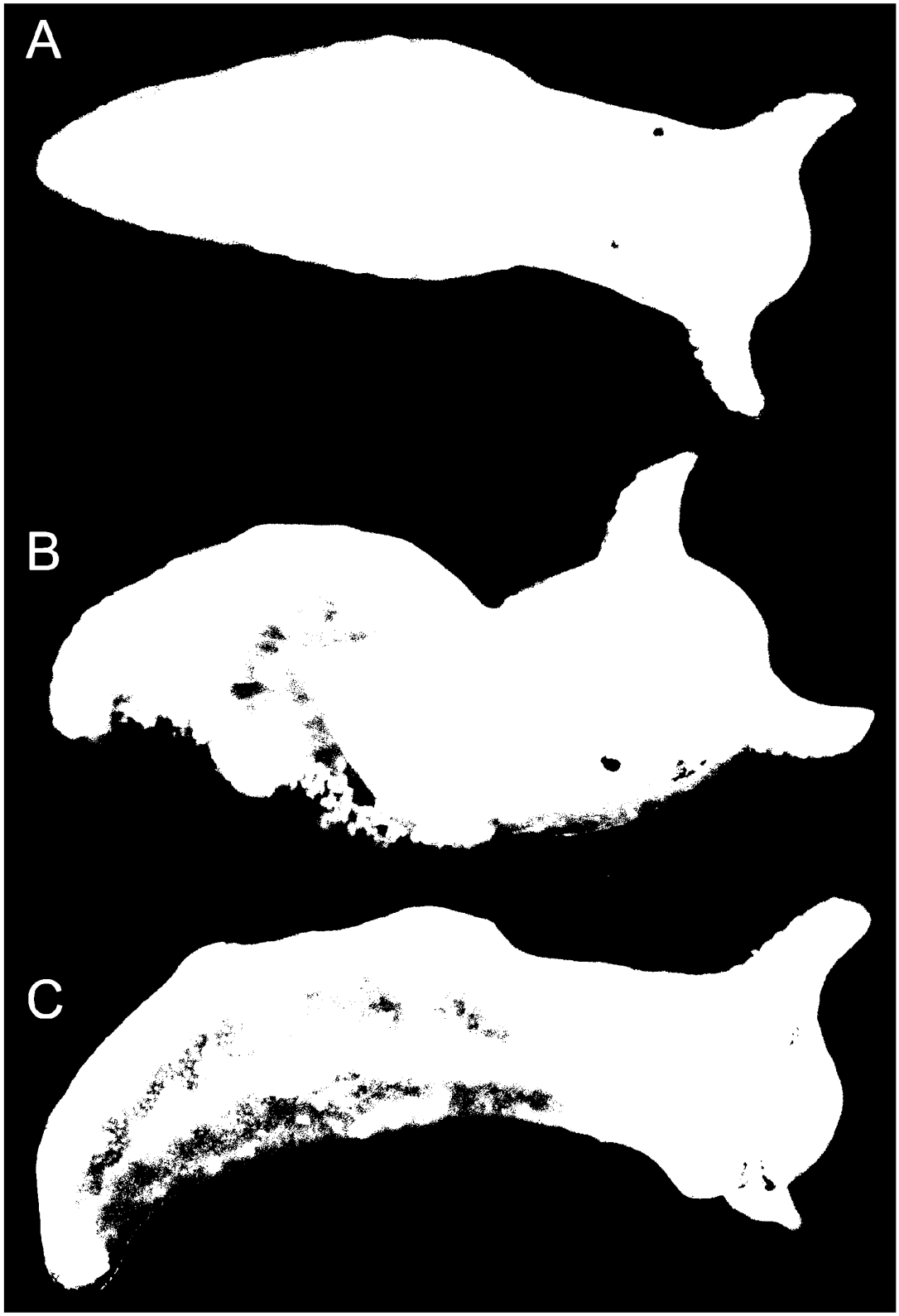

FIGURE 33. *Elysia serca*, external morphology of specimen from Geiger Beach, Florida Keys (4 mm long). **A**, Dorsal view of live specimen, showing pointed rhinophores and reduced parapodia. B, Dorsal view showing white, rounded pericardium and vessels. **C**, Ventral view of foot.

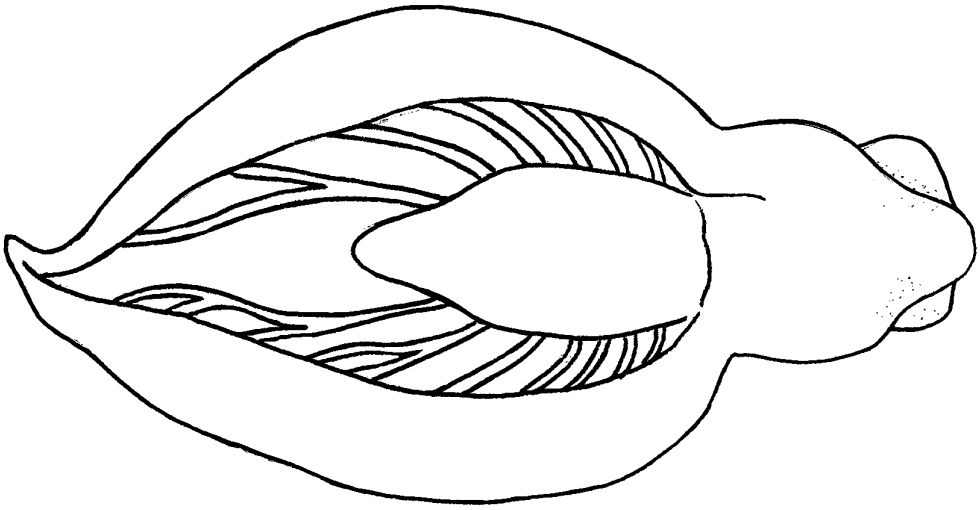

FIGURE 34. *Elysia serca*, dorsal vessels drawn of a preserved specimen collected on Lee Stocking Island, Bahamas (LACM 172293; 2.5 mm long × 1.2 mm wide).

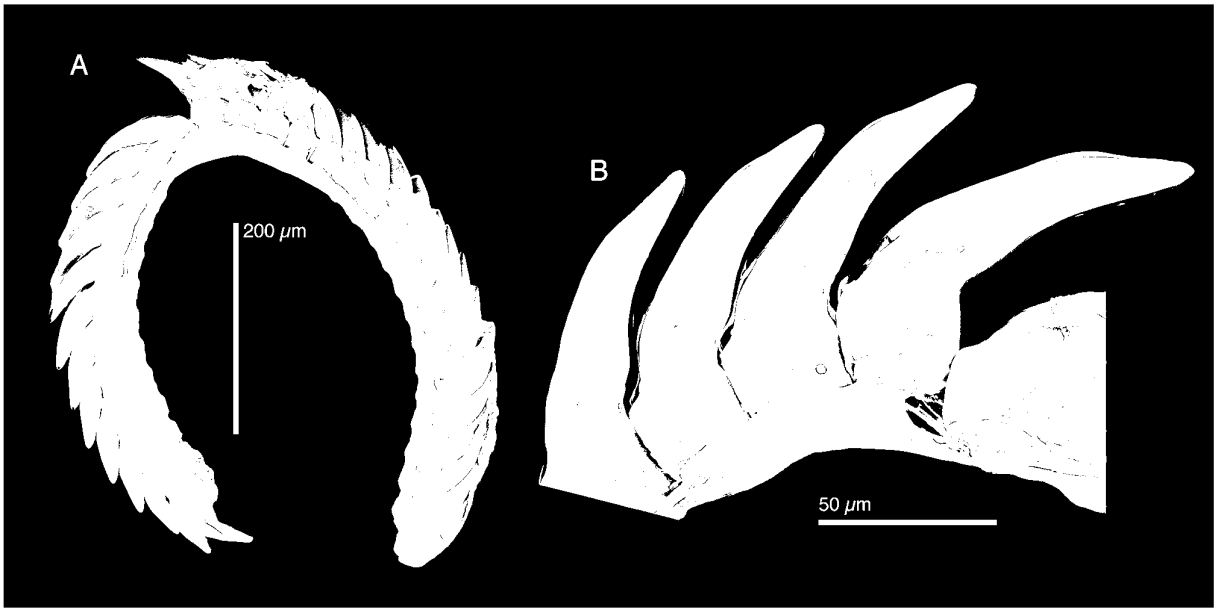

FIGURE 35. *Elysia serca*, SEM of the radula. **A**, Radula (LACM 173228). **B,** Leading tooth (CPIC 00027).

Phylogenetic relationships. A member of subclade 2, *Elysia serca* was recovered as sister to *E. chlorotica* (Fig. 4); however, the seagrass-feeding species *E. catulus* was not available for inclusion in our phylogeny. Based on their derived seagrass diet, we predict that *E. catulus* is the true sister species of *E. serca*.

Range. Bahamas (Valdés *et al.* 2006), Barbados (Er. Marcus & Ev. Marcus 1970; Ev. Marcus & Hughes 1974), Belize (Clark and DeFreese 1987), Brazil (Er. Marcus 1955, 1957; Ev. Marcus 1970), Curaçao (Er. Marcus & Ev. Marcus 1970), Cuba (Espinosa *et al.* 2005; Espinosa *et al.* 2007), Florida (Jensen 1982; Jensen & Clark 1983; Clark 1994; Valdés *et al.* 2006), U.S. Virgin Islands (Jensen 1983b).

Remarks. The pharynx of *E. serca* is exceptionally large for the body size, relative to other small *Elysia* spp. (Er. Marcus 1957). Maximum body length reported for the collection of slugs that yielded the type material for *E. serca* was 8 mm alive and 3.5 mm preserved. In the original description, freshly collected specimens of *E. serca* were noted to differ in external coloration depending on the substrate. The darker morph from brown algae was described as "brownish with a reddish violet area between the parapodia behind the region of the heart ... There are

three large white spots, one in front of the heart and two in the middle of the free border of the parapodia." A lighter morph from *Ulva* was "light green with darker green alimentary organs. They have the same three large white spots and white stipples as the brownish slugs and a black line along the margin of the parapodia that may also occur in the brownish animals."

Both forms from Brazil had a roughly serrated cutting edge on the radular tooth, which was figured as having the tooth cusp bent at a right angle to the base (Er. Marcus 1955: fig. 52). Er. Marcus & Ev. Marcus (1970) later described *E. clena* as having similarly shaped, but smooth, radular teeth, and a slightly different pattern of dorsal vessel venation. Jensen (1982, 1983b) synonymized *E. clena* Er. Marcus & Ev. Marcus 1970 with *E. serca*, based on population-level variation in radular denticulation and ontogenetic changes in dorsal venation pattern. Four of five populations from Florida had smooth radular teeth (*clena*-type), but in a fifth population, teeth were coarsely denticulate (*serca*-type). In specimens from St. Thomas, teeth were predominantly smooth but some were faintly denticulate (Jensen 1983b). There was also inter-population variation in the number of teeth, and in the ratio of outer-to-inner cusp length for teeth. Jensen hypothesized tooth morphology varied depending on the species of seagrass included in the diet of an individual. Further, juvenile slugs had the pattern of dorsal vessels described for *E. clena*, but upon maturation in the lab, developed the pattern described for *E. serca* (Jensen 1982).

Jensen (1982, 1983b) further hypothesized that *E. serca* was itself a junior synonym of *E. catulus* (Gould, 1870). The poorly studied *E. catulus* was the most common sacoglossan in Connecticut, U.S. (Clark 1975), but is restricted to colder waters, ranging from northern New England to North Carolina, U.S. (Ev. Marcus 1980). Both *E. serca* and *E. catulus* share an ascus displaced to the right side of the pharynx, weakly developed parapodia, and bent radular teeth, but *E. catulus* is typically black whereas dark specimens are rare in *E. serca* (Ev. Marcus 1972b, Jensen 1982). Diet distinguishes the species, but is a covariate of range: *E. catulus* feeds on the seagrass *Zostera*, restricted to temperate waters in the western Atlantic, whereas *E. serca* feeds on tropical seagrasses not found in the range of *E. catulus*. We do not consider the synonymy of *E. serca* and *E. catulus* here, as typical *E. catulus* are not found in the Caribbean, but we consider it unlikely that one sacoglossan species could span such different biogeographical provinces as the tropical Caribbean and northeastern U.S.

Elysia evelinae Er. Marcus, 1957

(Figs. 6M, 36–38)

Elysia evelinae Er. Marcus 1957: 410–416, figs. 48–57 (Type locality: Ilhabela, island of São Sebastião, and Enseada de Guarujá, island of Santa Amaro, state of São Paulo, Brazil)—Ev. Marcus & Er. Marcus 1960: 153, fig. 35; Ev. Marcus & Er. Marcus 1967: 27, fig. 21; Jensen & Clark 1983: 4–5; Clark 1994: 904–905; Valdés *et al.* 2006: 64–65; García *et al.* 2008: 72; Krug *et al.* 2015: 990–991, figs. 3B, 4.

Type material. *Elysia evelinae*—possible type specimen, ex. Marcus collection (HMCZ 288304).

Material examined. Manzanillo, Limón, Costa Rica, 13 March 2001, 1 specimen (MZUCR INB0003312779).

Live animal. There is little information on the behavior of this species.

External anatomy. Animal small, short and wide. Color variable, from pale cream to brownish gray, with numerous red and opaque white spots covering head or entire body. Conspicuous black circle surrounding anal papilla, a distinctive characteristic of this species (Fig. 36, black arrows). Anal papilla situated on right side of body, at base of head. Rhinophores short, wide, pale cream in color. Posterior end of head pigmented with dark brown or black. Tail short, conical. Eyes conspicuous, black, each crossed by a longitudinal dark line running from the base of the rhinophores to the anterior end of the parapodia.

Parapodia short, thick. Pericardium large, rounded, not covered by parapodia. Paired posterior dorsal vessels emerging from end of short renopericardium and running to end of body, with short lateral side branches shooting off at intervals (Fig. 37). One or two short anterior vessels emerging from renopericardial sac, branching not evident on preserved specimen.

Internal anatomy. Radula with 11 teeth (MZUCR INB0003312779), 5 teeth in ascending limb and 6 in descending limb (Fig. 38A). Leading tooth elongate and straight, with characteristic blunt cusp lacking denticles (Fig. 38B). See Jensen (1997) for additional views of the radular teeth. Base of tooth elongate.

FIGURE 36. *Elysia evelinae*, external morphology of two specimens; actual sizes: 5 mm (A), 4 mm (B).

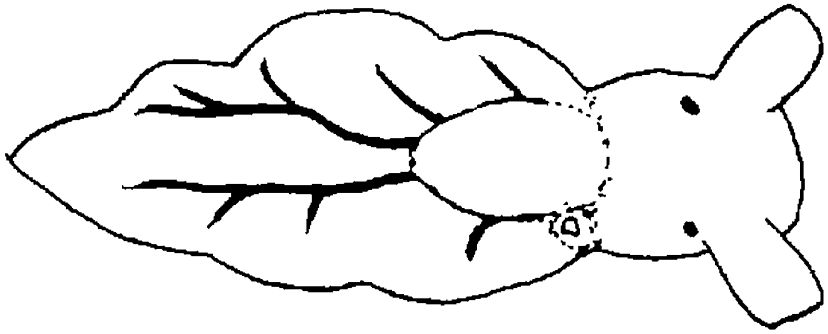

FIGURE 37. *Elysia evelinae*, dorsal vessel diagram reproduced from Ev. Marcus (1980).

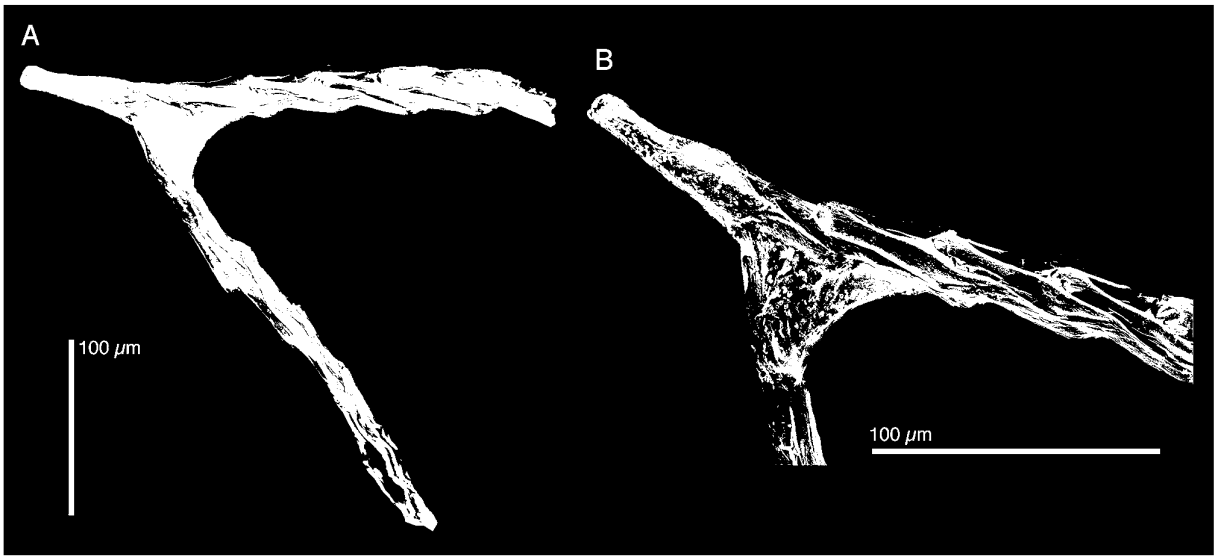

FIGURE 38. *Elysia evelinae*, SEM of the radula (MZUCR INB0003312779). **A**, Radula. **B**, Leading tooth.

Penis narrow and elongate, tapering into a conical apex devoid of armature. Deferent duct narrow and convoluted (Fig. 6M).

Reproduction and development. Larval development is lecithotrophic. Most veligers hatched prior to metamorphosis ('type 2' development) but some larvae in many clutches underwent encapsulated metamorphosis ('type 3') (Clark & Jensen 1981; Jensen & Clark 1983). Egg diameter was given as 104 μm; there is no ECY, but cloudy albumen inside the egg capsules vanished once cleavage began (Clark & Jensen 1981). Larvae hatched after 12 d at 20°C, at a shell length of ~210 μm. Larvae had eyespots, swam little and actively explored available

substrata. Starved slugs produced clutches with reduced proportions of encapsulated metamorphosis, as noted for the lecithotrophic morph of *Alderia willowi* Krug, Ellingson, Burton & Valdés, 2007 (see Krug 2001).

Host ecology. The host was erroneously reported in the original description (Er. Marcus 1957). *Elysia evelinae* primarily feeds on chain-forming benthic diatoms in the genus *Biddulphia*, but also on epiphytic diatoms fouling algae such as *Bryopsis* and *Caulerpa* in shaded areas (Jensen & Clark 1983). Jensen (1981a) described feeding; the pharyngeal bulb is extruded from the mouth, engulfing a diatom cell which is then pierced by the tip of the leading tooth; slugs emptied 5–10 cells per minute, selecting *Biddulphia* over other diatoms but not exhibiting preference for any particular cell size. Slugs preferred to feed on diatoms intermingled with other algal substrata. No other *Elysia* is known to feed suctorially on diatoms.

Phylogenetic relationships. *Elysia evelinae* was recovered within subclade 2, sister to the North Atlantic species *E. viridis* with 100% support in all phylogenetic analyses, despite their lack of apparent similarities in morphology or ecology (Fig. 4).

Range. Brazil (Er. Marcus 1957; García *et al.* 2008), Costa Rica (Espinosa & Ortea 2001), Florida (Ev. Marcus & Er. Marcus 1960, 1967; Jensen & Clark 1983; Clark 1994).

Remarks. Externally, the most distinctive feature of *E. evelinae* is the anus, which opens slightly anterior to, and to the right of, the pericardial bulge. The anus opens at the center of a large dark spot that is visible even on preserved specimens. Among Atlantic elysiids, only *E. canguzua* has a similarly placed and prominent anus, but lacks the dark circum-anal band of *E. evelinae*.

No pharyngeal pouches (used to pump algal cytoplasm in other species) are present in *E. evelinae*, being replaced by a pair of what Er. Marcus (1957: fig. 66) termed "muscular pharyngeal diverticula … on both sides of the radular pouch." The intestine is elongated in *E. evelinae* relative to body length, compared with other *Elysia* spp. (Er. Marcus 1957).

Elysia scops Ev. Marcus & Er. Marcus, 1967

(Not figured)

Elysia cauze scops Ev. Marcus & Er. Marcus 1967: 28–29, figs. 26–27 (Type locality: Biscayne Bay, Florida), ***nomen dubium***.
Elysia scops Ev. Marcus & Er. Marcus, 1967—Valdés *et al.* 2006: 72–73.

Type material. *Elysia cauze scops*—2 syntypes (USNM 576283, USNM 576273).

Material examined. No specimens available.

Live animal. No specimens available.

External anatomy. Ev. Marcus & Er. Marcus (1967) described *Elysia cauze scops* as a subspecies of *E. cauze* with a similar coloration to *E. cauze cauze*: "in part brownish, in part greenish grey." A description from the collector included few additional details: "color dark greenish; papillate surface." The border of the parapodia are variable in color, with one specimens having black only on the anterior end, another having the entire border black, and two more with no black pigment on the border of the parapodia. Examination of two syntypes revealed an elongated renopericardial complex running almost to the tail, and inner parapodial surfaces lined with a network of branching dorsal vessels. Neither specimen could be distinguished externally from *E. subornata*.

Internal anatomy. Ev. Marcus & Er. Marcus (1967: fig. 27) illustrated a single radular tooth of this species, with a elongate, thick cusp bearing numerous minute denticles, "V"-shaped housing depression, and short tooth base.

Reproduction and development. No data available.

Host ecology. No data available.

Phylogenetic relationships. No specimens available.

Range. Florida (Ev. Marcus & Er. Marcus 1967; Valdés *et al.* 2006).

Remarks. Ev. Marcus & Er. Marcus (1967) described *E. scops* from Florida as a subspecies of *E. cauze* because of some differences in the radular morphology, namely the size of the denticles and the shape of the ascus. However, a few years later Er. Marcus & Ev. Marcus (1970) changed their mind and no longer considered two subspecies in *E. cauze*. Valdés *et al.* (2006) illustrated a specimen that they identified as *E. scops* based on external similarities to the original description. The specimen illustrated in Valdés *et al.* (2006) is lost and we do not have access to additional specimens resembling the original description of *E. scops*.

The status of *E. scops* remains uncertain. External features suggest it could be a synonym of *E. subornata*, but the radular teeth appear to be shorter. It also resembles *E. hamanni* **n. sp.**, described below, but the coloration, part brownish part green described by Ev. Marcus & Er. Marcus (1967) is different from the pale green with a pinkish tinge of *E. hamanni* **n. sp.** It is also possible that *E. scops* is a distinct, uncommon species.

Elysia marcusi (Ev. Marcus, 1972)

(Figs. 6Q, 39–41)

Bosellia marcusi Ev. Marcus 1972a: 293–295, figs. 19–22 (Type locality: Grassy Key and Bear Cut Rocks, Florida, USA)—Ev. Marcus 1973: 819–820, figs. 1, 7 (part), 11–12, 15; Ev. Marcus 1980: 76; Thompson 1977: 132–133, figs. 22e, 29a–b; Clark 1994: 905–906; Krug 2009: 361–365, figs. 1B, 4C, 5C, 6; Christa *et al.* 2014: fig. 1D

Elysia marcusi (Ev. Marcus, 1972)—Händeler *et al.* 2009: fig. 7; Redfern 2013: 282–283, fig. 785; Christa *et al.* 2014: fig. 3; Krug *et al.* 2015: 990–991, figs. 3B, 4.

Type material. *Bosellia marcusi*—Untraceable, not at USNM. A specimen lot labeled "type specimen" at the MZSP (76043) contains a piece of calcareous algae (Siqueira Dornellas & Simone 2011).

Material examined. San Salvador Island, Bahamas, 10 July 2010, 2 specimens (LACM 178647–48).

Additional material examined. Bahamas: San Salvador Island, Bahamas, 23 June 2007, 23 specimens (isolate Emar_07Ssal02-24), 9 July 2010, 2 specimens (isolate Emar_10Ssal02-03), Goulding Point, New Providence, 13 July 2010, 21 specimens (isolate Emar_10NPr01-21); Discovery Bay, Jamaica, March 2006, 48 specimens (isolate Emar_06Jam01-48); Geiger Beach, Key West, Florida, USA, 12 June 2007, 58 specimens (isolate Emar_07Gei01-58).

Live animal. Body highly plastic. Resting slugs contract into perfectly round circles, thick in the center like gumdrops; crawling specimens elongate into a long, thin, typical slug form. Specimens observed ranged from 2–5 mm in length (Fig. 39A–C)

External anatomy. Overall coloration uniformly light to dark green (Fig. 39A), overlaid by white pigment patches if present (Fig. 39B–C). Body edge may be rimmed by whitish-blue spots or short, lateral lines perpendicular to edge (Fig. 39C). White patches scattered across dorsal surface in some specimens, absent in others. White spots may be concentrated in patch behind head, where anterolateral groove terminates (Fig. 39C–D). Front of head smooth-edged, no oral lobes. Widely spaced eyes. Rhinophores solid white, simple, flattened (not rolled); fully retractable into head. Some specimens with white bar extending between parapodia, posterior to eyes, like a solid eyebrow. Most Florida specimens with iridescent deep blue spots scattered across body. Specimens from San Salvador Island uniformly green. Specimens from Jamaica with scattered white spots across dorsum, denser along side of foot, sometimes with scattered blue dots.

Parapodia superficially absent, having secondarily fused to body; fusion line forming mid-dorsal line and antero-lateral furrow on right side behind head (Fig. 39A). Body surface uniformly smooth. Curved, anterolateral furrow extending up right side of body, starting from side of foot about one-quarter of total body length from front of head. Furrow narrowing and straightening to form mid-dorsal longitudinal line, running straight down midline of dorsum; terminating just anterior to tail, where vaginal opening appears in a white patch (Fig. 39A). White pigment patches clustering along midline in some specimens.

Internal anatomy. Radula with 15 teeth (LACM 178647), 7 teeth in ascending limb and 8 in descending limb (Fig. 40A). Radula with 18 teeth according to Ev. Marcus (1973). Leading tooth robust and elongate with 7–8 triangular denticles on cusp from mid-tooth to sharp tooth tip (Fig. 40B). Housing depression for interlocking teeth "V"-shaped and extending ½ of tooth length (Fig. 40A). Base of tooth ½ total tooth length. Ascus containing jumbled heap of discarded large teeth.

According to Ev. Marcus (1972a; 1973), pharynx small (230 μm long × 150 μm high, on 3–4 mm long animals). Esophagus narrowing into small stomach. Anus opening into anterior transverse groove. Heart and kidney reduced, not visible externally.

Penis small and oval to spherical in shape (LACM 178647–48), with a pointed stylet (Fig. 6Q, Fig. 40C). Deferent duct narrow.

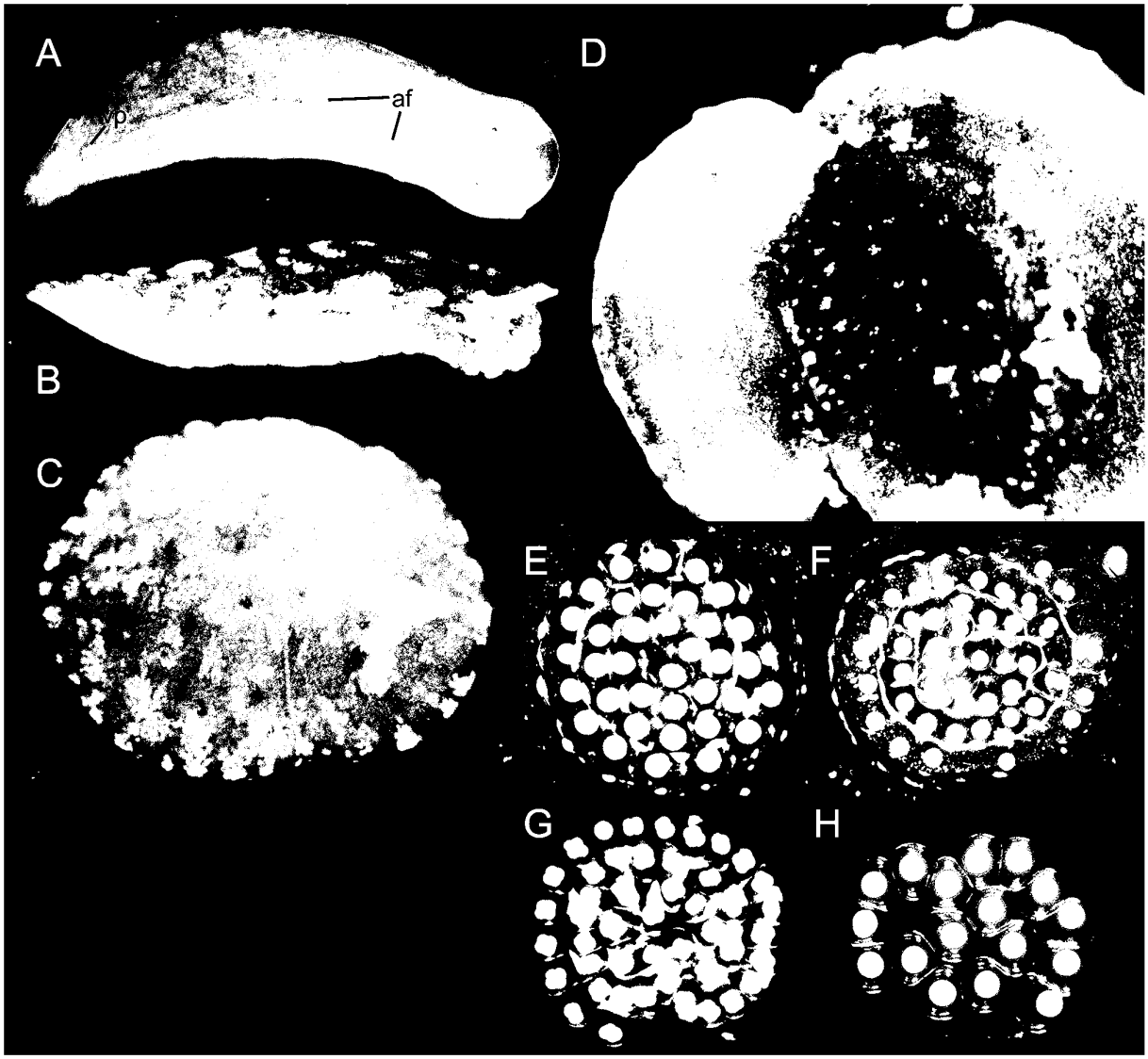

FIGURE 39. *Elysia marcusi*, external morphology and egg masses. **A,** Crawling specimen from Jamaica showing vaginal pore (vp) and antero-lateral furrow (af). **B,** Crawling specimen from Florida with white spots. **C,** Resting specimen from Jamaica, showing rounded "gumdrop" posture adopted by slugs on algae. Size = 4 mm (A), 5 mm (B), 4 mm (C). **D,** Copulating specimens from Jamaica. Translucent penis of slug (left) is visibly being inserted into vaginal pore of larger slug (right); note antero-lateral furrows on both slugs. Field of view = 5 mm. **E–H,** Egg masses showing variation in ECY. Clutches deposited by Jamaica specimens had either regularly spaced blobs of whitish ECY, one attached to the outside of each capsule (E; width of egg mass = 1.2 mm), or else a thread of yellow ECY running along the inside edge of the string of egg capsules (F; width of egg mass = 1.6 mm). An egg mass from San Salvador (G) containing both white and yellow ova at the four-cell stage had ECY deposited as one white blob or streak per capsule for the first turn of the egg spiral, but thereafter as one yellow blob or streak every third capsule. Width of egg mass = 1.5 mm. An egg mass from Florida lacked ECY (H; width of egg mass = 1.0 mm).

Reproduction and development. Ev. Marcus (1973) described the reproductive system as triaulic, with anterior male and female apertures and a posterior vaginal pore leading to bursa for receipt of allosperm. Follicles (n = 5–6) of ovotestes large (250 μm across). Penis transparent, saclike, round and flat at the end with a curved, pointed stylet. Female opening in dorsal furrow on right side, posterior to male aperture containing penial sheath. Bursa copulatrix inside posterior end of body, connected to outside by vaginal pore; duct running forward along midline of body to region where sperm duct and oviduct separate (Ev. Marcus 1973: fig. 15). The specimens here examined also had a round penis with a hollow apical stylet (Figs. 6Q, 40C), confirming Ev. Marcus' (1973) description.

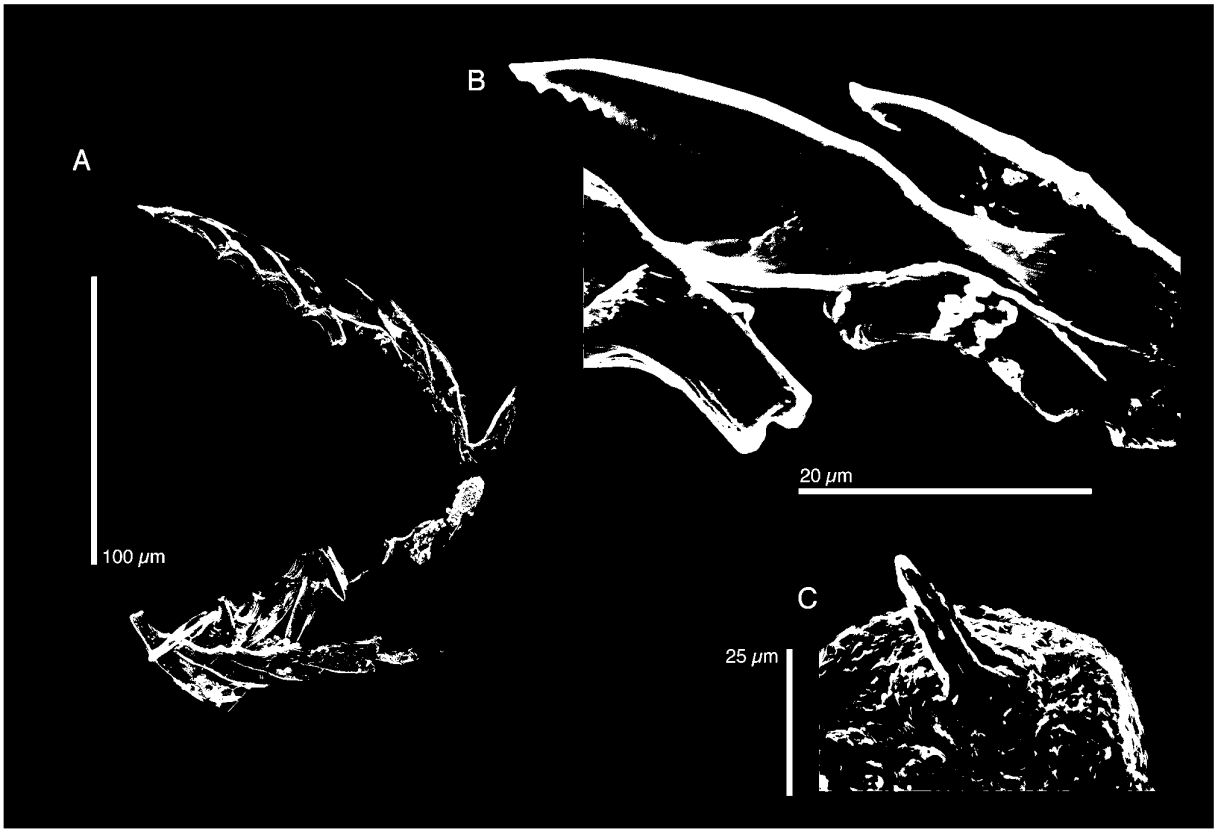

FIGURE 40. *Elysia marcusi*, SEM of the radula and penis (LACM 178648). **A,** Partly disarticulated but complete radula. **B,** Tooth showing denticles. **C,** Penial stylet.

During mating, some pairs lined up with the head of one aligned with the tail of the other, and reciprocally inseminated for periods of >1 hour. Occasional group mating was observed, with three slugs forming a circle, each inseminating the slug before it. Inseminations occurred either into the posterior furrow, at or near the location of the terminal vaginal pore leading to the internal bursa copulatrix (Fig. 39D), or into the antero-lateral furrow. Slugs settled into their round, flat resting morphology immediately after mating.

Larval development is described in Krug (2009) as "*Bosellia*" *marcusi*. Development is lecithotrophic. Mean clutch size was 26.0 ± 14.3 SD, including clutches from Florida (n = 4), Jamaica (n = 9) and San Salvador, Bahamas (n = 3). Grand mean diameter of uncleaved ova was 103.9 μm ± 2.8 SD (n = 8 clutches; range of mean egg diameters from different clutches was 99.5 ± 3.3 to 108.5 ± 4.4). White or yellow eggs are laid in a typical spiral, one egg per capsule, usually with ECY of the same color attached to the outside of the egg capsules (Fig. 39E–H). One clutch from San Salvador had both white and yellow ova, and white and yellow ECY (Fig. 39G). Of 13 clutches observed sufficiently early in development, ECY was present as one rounded blob per capsule (6 clutches; Fig. 39E); two small streaks per capsule (one clutch); continuous threads of almost translucent pale yellow (two clutches; Fig. 39F); one irregular blob or streak for every third capsule (one clutch; Fig. 39G); or was absent (all three clutches from Florida; Fig. 39H). Capsules were deposited with an opaque material, presumably albumen, surrounding the uncleaved ova; capsular fluid cleared within an hour.

Clutches held at ~25°C developed large eyespots after about 10 d, and in some clutches the encapsulated larvae developed a brick-red color shortly before hatching. Intra-capsular metamorphosis was only recorded in one of 11 clutches (Krug 2009). Larvae hatched after 14 d (± 0.7 SD, n = 2), releasing swimming veligers that swam actively for 4–7 d before either undergoing spontaneous metamorphosis or dying in the absence of an algal cue (Krug 2009). Mean larval shell length was 190.3 μm (± 13.9 SD, n = 5 clutches, grand mean; range = 175.5 ± 7.63 to 210.3 ± 13.3), the smallest lecithotrophic larvae reported for any elysiid, and the second-smallest out of 22 sacoglossans for which data exist; only the poecilogonous *Alderia willowi* has smaller lecithotrophic larvae (Krug 2007).

Host ecology. In Florida and Jamaica, specimens were collected from clumps of *Halimeda opuntia*, generally in shaded and protected habitats, in water as shallow as a few cm. Slugs maintained in aquaria fed readily on *H. opuntia* and rarely crawled off their host, on which they are highly cryptic. In Bahamas sites, specimens were collected from clumps of *H. goreaui* that grew hanging down in highly shaded pockets of dead coral (San Salvador) or under rock ledges (New Providence). Specimens were not collected from *H. incrassata*, *H. tuna* or *H. monile*.

Phylogenetic relationships. Our phylogenetic analyses indicated *E. marcusi* is a derived species of *Elysia* (Fig. 4) consistent with prior molecular analyses (Händeler *et al.* 2009; Christa *et al.* 2014; Krug *et al.* 2015). Its misclassification as a *Bosellia* resulted from the fusion of parapodia over the dorsum, and convergence on a flattened boselliid shape due to their shared niche (adhering tightly to flat pieces of *Halimeda*). *Elysia marcusi* was not closely related to any of the seven other sampled *Elysia* spp. that feed on *Halimeda*, and was one of the few species for which no sister taxon was identified with significant support (Fig. 4). The relatively long branch of *E. marcusi* could result from a failure to sample related species, and/or from the high degree of adaptive evolution that this taxon has undergone, reflected in both the highly derived morphology of this taxon and a large number of fixed mutations.

Range. Bahamas (Redfern 2013; present study), Costa Rica (Espinosa & Ortea 2001), Cuba (Espinosa *et al.* 2005), Florida, USA (Ev. Marcus 1972a; Clark 1994; present study), Jamaica (Thompson 1977; present study).

Remarks. Ev. Marcus (1972a, 1973) placed this species in *Bosellia* presumably due to the absence of apparent parapodia, despite the absence of dorsal vessels. Preceding molecular phylogenetic (Händeler *et al.* 2009; Christa *et al.* 2014; Krug *et al.* 2015) and developmental studies (Krug 2009) noted that *E. marcusi* was a derived *Elysia* species, but no formal taxonomic transfer has been published previously. Anatomical study indicates the parapodia secondarily fused to the body, forming the curved antero-lateral furrow and the mid-dorsal line. Thompson (1977) speculated about this, noting the "dorsal longitudinal line resembled the line of fusion of lateral parapodial lobes found in some aplysiomorphs". The fusion of parapodia would have covered the ancestral dorsal vessel network and renopericardial complex, becoming hidden from external observation. Ev. Marcus (1972a, 1973) did not question her assignment of this species to *Bosellia*, despite remarking on the numerous features that distinguished *E. marcusi* (slender, curved radular teeth; ascus storing discarded teeth; no evident dorsal vessels, pericardium or pharyngeal crop; triaulic reproductive system with few, large follicles in the ovotestes; penial stylet) from *B. mimetica*. Ev. Marcus (1973) described *B. corinnae* from Florida, which in most respects was more similar to *E. marcusi* than to *B. mimetica*, but had the exposed dorsal vessels of *Bosellia* yet highly distinct radular teeth. Given the absence of any subsequent studies, the identity and generic placement of *B. corinnae* remains unclear; it could be a derived *Elysia* that has lost parapodia, like *E. serca*, or a second Atlantic *Bosellia* sp.

Together with its small size, the secondary fusion of parapodia over the dorsum allows *E. marcusi* to flatten its body onto blades of *Halimeda opuntia* or *H. goreaui* in protected microhabitats. Several elysiids have a flattened body shape and reduced parapodia (*E. pusilla*, *E. stylifera*, *E. serca*) analogous to the fused parapodia in *E. marcusi*, likely reflecting convergent evolution. Selection may favor a flattened morphology for greater adherence to hosts presenting flat surfaces such as seagrass (*E. serca*) and some *Halimeda* spp. (*E. pusilla*, *E. stylifera*, *E. marcusi*, *Bosellia mimetica*). Homoplasy in body form resulted in the erroneous generic placement of *E. marcusi*, highlighting the need to consider ecological information when making taxonomic diagnoses.

Elysia nisbeti Thompson, 1977

(Fig. 41)

Elysia nisbeti Thompson 1977: 126, figs. 25b–c, 26d (Type locality: Port Royal mangroves of the Palisadoes, Jamaica)—Valdés *et al.* 2006: 70–71.

Type material. *Elysia nisbeti*—Holotype (BMNH 20070189)

Material examined. Holotype (BMNH 20070189), corresponding to specimen #2364 of T. E. Thompson collection, and BMNH 19774.W in Thompson (1977); collected October 30, 1974. One specimen represented as a headless body, missing pericardial region.

Live animal. No specimens available.

External morphology. Summarized from Thompson (1977): Three specimens ranged from 5.5–10 mm in

length. Body slender, rich green in color; foot pale yellow-green. Rhinophores dark olive-green, sometimes arched backwards over dorsum and held in this position. Dorsal surface of head with conspicuous pale brown blotches forming "Y"-shape extending up rhinophores. Oral lobes shelf-like with dark brown edges and white pustules. Parapodial margin pale brown, thickened, swollen at intervals with white vessicles. Parapodial sides with black specks, retained through preservation (Fig. 41A). Rows of low white papillae running along body and up rhinophores.

From our inspection of the holotype: Body lacking head, 5.5 mm wide by 10 mm in length (Fig. 41A). Renopericardium extending half of remaining body length as a wide tube. Eight paired dorsal vessels emerging on either side of renopericardium, plus one vessel emerging from the terminus of the renopericardial extension and running straight towards tail (Fig. 41B). Vessels bifurcate repeatedly, with side branches sometimes anastomosing. Posterior vessel initially straight, then branching irregularly, with side branches anastomosing with those of posterior-most paired vessels. Parapodial margin densely lined with straight ends of terminal branches.

Internal morphology. Radula (specimen 10 mm long) with 20 smooth teeth and a large number of discarded teeth in ascus, according to Thompson (1977). Tooth drawn with notch at base (Fig. 41C), not mentioned in the text or depicted in any other radular figure by Thompson (1977) . Penis morphology undescribed.

Reproduction and development. No specimens available.

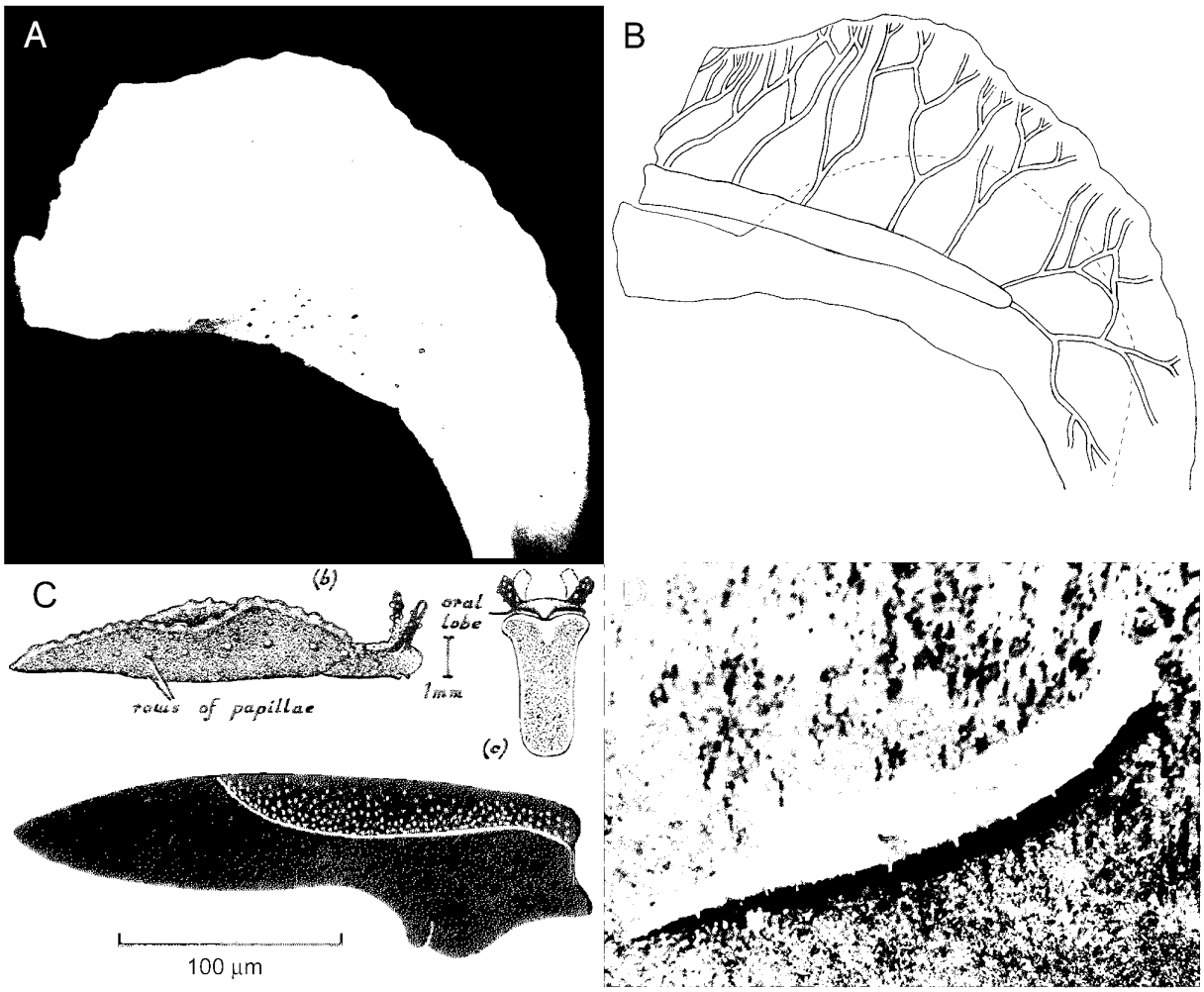

FIGURE 41. *Elysia nisbeti,* external morphology, dorsal vessels and radula. **A,** Photograph of holotype (BMNH 19774.W), showing dorsal vessels on right inner parapodial surface; holotype lacking head. **B,** Renopericardium and dorsal vessel network drawn from right side of holotype. **C,** Drawings of live animal (fig. 25b, c) and radula (fig. 26d) reproduced from Thompson (1977). **D,** Live specimen tentatively identified as *E. nisbeti* by external morphology, including the distinctive "Y"-shaped marking on head; photographed by J. Hamann in Bermuda.

Host ecology. Thompson (1977) reported collecting *E. nisbeti* from *Caulerpa verticillata* growing in drainage channels among mangroves, but no observations of feeding were recorded.

Phylogenetic relationships. No specimens available.

Range. Jamaica (Thompson 1977), Bermuda (Valdés *et al.* 2006; tentative assignment from photographic voucher of missing specimen)

Remarks. This enigmatic species has not been reported since it was described. Live animals did not swim. On the holotype, the renopericardial extension runs halfway along the body; the length of the renopericardium, and the pattern of the dorsal vessel network, are distinct from all known species that could loosely fit the description (e.g., *E. velutinus* or *E. subornata*). A specimen photographed in Bermuda fit well the original description in its external coloration, arrangement and appearance of papillae, and brown "Y"-shaped marking on head flowing into dark rhinophores (Fig. 41D); however, this specimen was not available for morphological study.

Elysia patina Ev. Marcus, 1980

(Figs. 6G–H, 42–44)

Elysia patina Ev. Marcus 1980: 72–73, figs. 23–24, 36, 41–43, 57 [non Ev. Marcus 1980: figs. 59–60] (Type locality: Florida Keys)—Jensen & Clark 1983: 5; Clark 1994: 905; Krug *et al.* 2015: 990-991, figs. 3B, 4.

Elysia papillosa [non Verrill 1901: 31, pl. 4, fig. 3]—Jensen & Clark 1983: 5; Clark 1984: 89, figs. 15, 17; Krug 2009: 361-365, figs. 2C, 5D, 6; Händeler *et al.* 2009: figs. 6, 7; Christa *et al.* 2014: figs. 1F, 3.

Type material. *Elysia patina*—10 syntypes (USNM 770515).

Material examined. Bahamas: Sweetings Cay, July 2007, 1 specimen (LACM 178650), July 2010, 3 specimens (LACM 178649, LACM 178651–52); Mia Reef Isla Mujeres, Mexico, August 1993, 1 specimen (LACM 178653).

Additional material examined. Mote Marine Laboratory and Aquarium, Florida, USA, June 2007, 4 specimens (isolate Epat_07Mote01-04); Bahamas: Sweetings Cay, July 2007, 11 specimens (isolate Epat_07Swe01, 02, 04-11), July 2010, 8 specimens (isolate Epat_10Swe01-05, 07-09), Stirrup Cay, 1 specimen (isolate Epat_07Stir01), Northern Exumas, 1 specimen (isolate Epat_07NEx01), Bimini, 7 specimens (isolate Epat_07Bim01-07).

Live animal. When resting, slugs hunker down with head tucked between parapodia. Disturbed slugs either cover their head with their parapodia in a similar manner, or swim by flapping (undulating) their parapodia.

External anatomy. Specimens from Sweetings Cay ranged from 1.5–8 mm in length, while Florida specimens were 6–8 mm in length. Overall color mottled white, grey or yellowish-brown, with scattered brown patches on sides of head and parapodia (Fig. 42A–D). Body elongate when crawling. Head predominantly white. Faint orange-brown spots, small, dotting front of face; no moustache of spots on oral lobes. Thin brown line running across side of head under large eyespots, up onto proximal portion of each rhinophore. Rhinophores elongate, rolled, thick, with rounded blunt tips. Outer surface of rhinophores covered with white rounded papillae, both large and small. Thick brown transverse band dividing proximal third of each rhinophore from the distal two-thirds (Fig. 42A–D).

Foot not clearly distinct from parapodia. Foot same yellowish-brown color as parapodial base, with scattered faint iridescent blue dots. Transverse groove separates underside of head from foot. End of body narrowing at ends of parapodia; foot then widening to form short, triangular tail with pointed end.

High-arching parapodia covering dorsum, often held over head of live animal. Upper half of parapodia predominantly white, grey or tan, with few scattered brown spots. Parapodial surface with streaks and patches of gold-yellow and white, with scattered dark brown spots. Parapodial surface everywhere dotted with white, rounded papillae varying in size. Parapodial margin even (not scalloped), with row of white papillae sometimes interspersed with light brown dots. Inner parapodial surface yellow or brown with white upper edge along margin; scattered blue iridescent dots speckle inner surface, sometimes highlighting dorsal vessels (Fig. 42E–F).

Renopericardium running over half the body length, sometimes bending about halfway along, and may undergo a constriction at posterior end. Coloration of renopericardium white, with sparse brown dots. Dorsal vessels emerging from renopericardial extension, initially wide and straight, bifurcating once about halfway along the distance to parapodial edge, then each branch forking again near parapodial margin (Fig. 43). Vessels

transparent, or pigmented by white or iridescent blue speckles (Fig. 42E–F). Four to five pairs of vessels on specimens 5–8 mm long, including elongated posterior pair, which sends a short branch over the gametic vesicle on each side of body. Vessels roughly symmetrical, do not anastomose. Prostate gland visible as network of white tubes underlying renopericardium and dorsal vessels.

One pair of large vesicles visible as rounded protrusions inside parapodia, either milky white or cloudy but partly clear (Figs. 42E–F, 43). Vesicles irregular in size, each contacted by terminus of one or more branches of dorsal vessels; often located between branches forking from 4th or 5th vessel on each side. Vesicles not apparent on juvenile specimens <3 mm in length; likely function as sperm-storage receptacles.

Internal anatomy. Radula with 14 teeth (LACM 178649, LACM 178652–53), 6 teeth in ascending limb and 8 in descending limb (Fig. 44A). Leading tooth thin and elongate with approximately 55 very small denticles (Fig. 44B–D). Radular teeth smooth according to Marcus (1980). Teeth without typical "V"-shaped depression of many other elysiids. Instead, teeth overlapping slightly with their tips resting in triangular-shaped depressions on side of adjacent teeth (Fig. 44F). Base of the tooth approximately ½ total tooth length. Ascus containing jumbled heap of discarded teeth (not figured).

Penis variable in length, with rigid musculature that did not deform after drying (LACM 178652–53) (Fig. 6G–H), often bent, bearing a folded or scoop-shaped stylet (Fig. 44E). Deferent duct long, thin, and loosely convoluted.

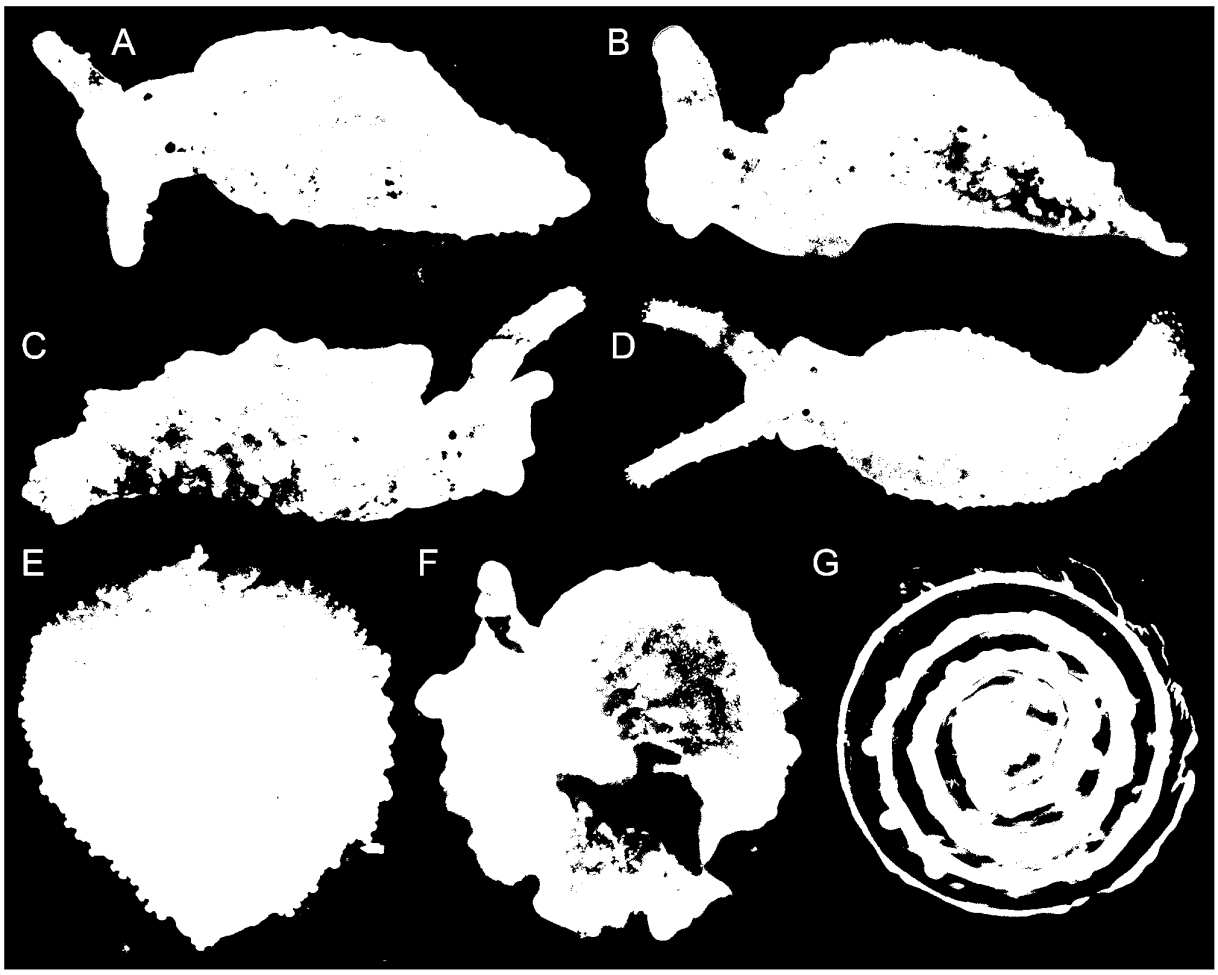

FIGURE 42. *Elysia patina*, external morphology and egg mass; all specimens 6–8 mm in length from Sweetings Cay, Grand Bahamas Island, Bahamas. **A–D**, Views of four different live specimens showing brown transverse bands on rhinophores, and color variation from white to tan to brown-green, with white papillae. **E,** Relaxed specimen (8 mm) showing elongated renopericardial complex, dorsal vessel network, and opaque white gametic vesicles. **F**, Live specimen (6 mm) with parapodia held open showing dorsal vessels lined in iridescent blue, and gametic vesicles in a semi-transparent state. **G**, Egg mass spiral showing flat orange ECY ribbon above uncleaved ova. Actual diameter = 2 mm.

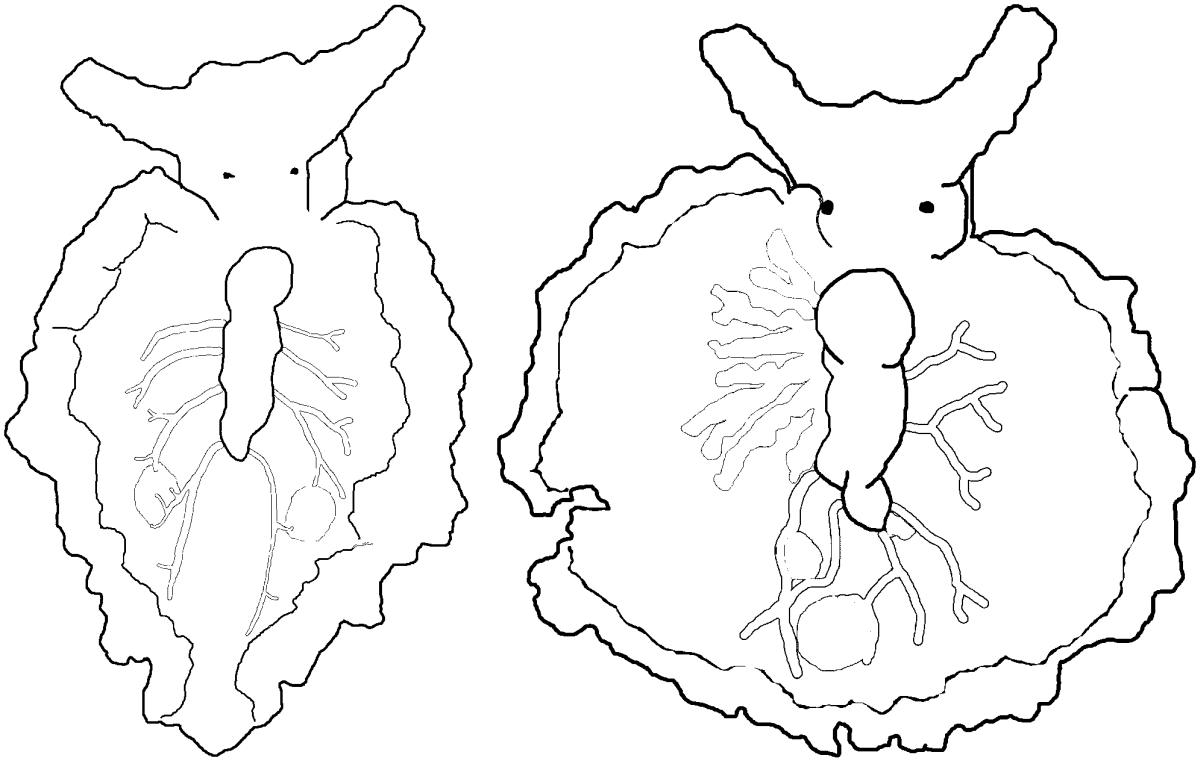

FIGURE 43. *Elysia patina*, drawing of renopericardial complex and dorsal vessel network traced from photographs of live specimens collected from Sweetings Cay, Bahamas in 2007: left, isolate Epat_07Swe01; right, isolate Epat_07Swe02. Grey areas represent sperm-storage vesicles.

Reproduction and development. Marcus noted as unusual placement of the male aperture in the groove separating the head from the right parapodium, which we could not corroborate. Krug (2009) presented data on larval development but reversed the names *E. patina* and *E. papillosa*, following Ortea *et al.* (2005). Development in *E. patina* is lecithotrophic. Eggs are laid in a typical elysiid spiral, one egg per capsule, with a flat ribbon of bright orange ECY on the upper surface of the egg strand, under the outer covering of the egg mass (Fig. 42G). Mean clutch size was 45.0 (± 28.3 SD, n = 2). Mean diameter of uncleaved eggs was 116.3 µm (± 3.4 SD; n = 15 ova). Clutches held at ~25°C hatched after 19.5 d (± 0.7 SD, n = 2), releasing swimming veliger larvae that swam actively for 4 d before undergoing metamorphosis in the absence of any inductive cue. Mean larval shell length was 337.3 µm (± 12.7 SD; n = 65 shells), the largest larval size out of 59 sacoglossan species for which data exist (Krug *et al.* 2015). Larvae released white mucus when disturbed. Juveniles fed immediately on *H. opuntia* when the alga was provided.

Host ecology. No prior report has documented the correct algal host for *E. patina*. In this study, all specimens of *E. patina* were collected from clumps of the alga *Halimeda opuntia*, generally growing in shaded and protected habitats, in water as shallow as a few cm. Slugs maintained in aquaria fed readily on *H. opuntia* for 6 weeks, but never consumed other udotacean algae (*Udotea*, *Penicillus*, *Caulerpa*). In the Florida Keys, *E. patina* and *E. marcusi* both feed on *H. opuntia* but have not been found co-occurring, even in similar habitats a few km apart; the species may be partitioned by competitive exclusion or distinct but as yet unidentified microhabitat preferences. Repeated assertions in the literature that *E. patina* feeds on *Udotea* (Jensen & Clark 1983; Clark & DeFreese 1987; Clark 1994) stemmed from misidentification of *E. zuleicae*, which does specialize on *Udotea*. Similarly, assertions that *E. papillosa* feeds on *Halimeda* likely reflect misidentifications of *E. patina* as *E. papillosa* (Clark 1984).

Christa *et al.* (2014) reported several *Halimeda* spp. as the food of "*E. papillosa*" but the specimens were actually *E. patina* based on DNA barcodes of the slugs (and vice-versa: their "*E. patina*" were actually *E. papillosa*). For unclear reasons, Christa *et al.* (2014) did not list *H. opuntia* as a source of plastids from *E. patina* even though some algal barcodes matched closely the lone included reference sequence for Caribbean *H. opuntia* (NCBI accession #AY942174, mis-entered as AY942147 on Fig. S1 of Christa *et al.* 2014). Their data suggest that

E. patina may occasionally consume a broader range of *Halimeda* spp. than is reflected by their tight ecological association with clumps of *H. opuntia*, the only microhabitat in which we have consistently (and indeed, exclusively) sampled this elysiid.

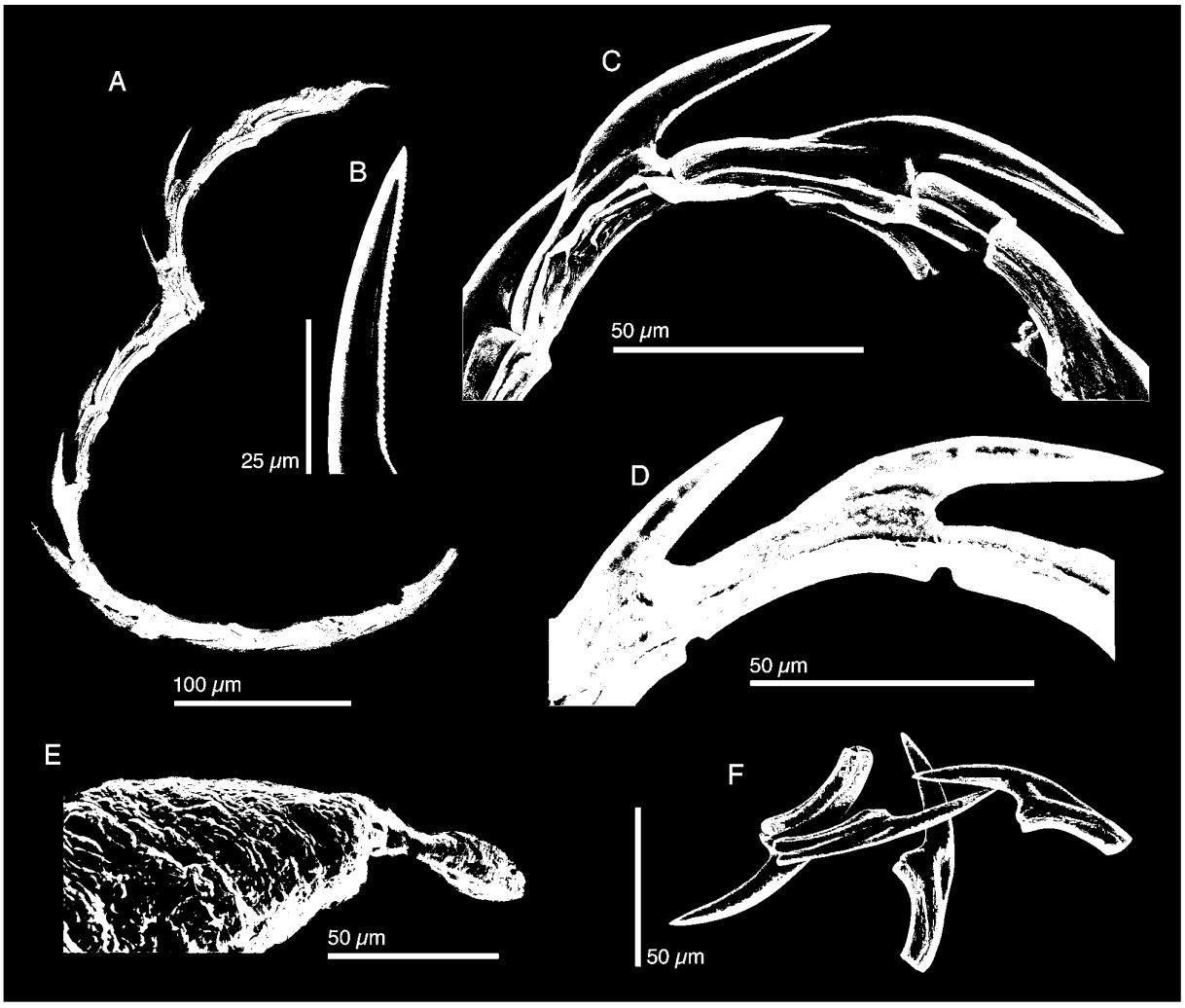

FIGURE 44. *Elysia patina*, SEM of the radula and penis. **A,** Complete radula (LACM 178652). **B,** Leading tooth (LACM 178649). **C,** Leading tooth (LACM 178652). **D**, Penis (LACM 178652), **E**, Loose teeth, showing triangular-shaped depressions.

Phylogenetic relationships. *Elysia patina* was recovered within subclade 1, but no sister species was identified with meaningful support (Fig. 4). Ortea *et al.* (2005) casually erected a new genus, *Checholysia*, with *E. patina* as the type species, as a "*precaución taxonomica*" or taxonomic precaution, with a penial stylet as the supposed distinguishing synapomorphy of the new genus. However, penial stylets are present in all three sacoglossan superfamilies, and are phylogenetically distributed throughout *Elysia* (Fig. 4). Therefore, our phylogenetic analysis reveals that *Checholysia* is polyphyletic and invalid, as stylets have evolved independently several times in *Elysia*. The proposal was not taxonomically "cautious," as erecting a new genus rendered *Elysia* paraphyletic with respect to *Checholysia*, a problem not discussed by Ortea *et al.* (2005).

Range. Florida, USA (Ev. Marcus 1980; present study), Bahamas: Sweetings Cay, Grand Bahamas Island; Stirrup Cay; Northern Exumas; Bimini (present study), Mia Reef Isla Mujeres, Mexico (present study).

Remarks. Marcus (1980) described *E. patina* from a preserved specimen and had not seen the species alive. Ortea *et al.* (2005) described *E. papillosa* as *E. patina*, inexplicably ignoring the completely different radular tooth morphology shown for *E. patina* in the original description, and without examining the type material. Ortea *et al.* (2005) asserted that Marcus (1980) had mixed two species in her type series for *E. patina*, but we examined the

holotype (a mounted series of thin sections) and found no evidence of multiple species; Marcus (1980) clearly states that her diagnosis of *E. patina* was based on a single specimen from Florida. A specimen from the Bahamas (fig. 59-60 in Marcus 1980) was noted in the text as a different species, distinct from *E. patina*, but was confusingly labeled as *E. patina* in the legend to figures 59 and 60 in Marcus (1980). This may have led Ortea *et al.* (2005) to conclude that two different species were mixed in the description of *E. patina*, but there is no evidence of that in the type series.

Specimens of *E. patina* can be superficially similar to whitish specimens of *E. papillosa* and *E. taino* **n. sp.**, both in external appearance and in their pattern of dorsal vessel venation, but a number of characters differentiate these species. First, *E. patina* has a curved finely denticulate radular tooth—the "*Halimeda* spur" of DeFreese & Clark (1987)—whereas *E. papillosa* and *E. taino* **n. sp.** both have a coarsely serrulated, blade-shaped tooth. Second, the gametic vesicles of *E. patina* are located at the posterior end of the renopericardium, often contacted by terminal branches of the vessels; in contrast, the gametic vesicles of *E. papillosa* and *E. taino* **n. sp.** are found midway along the renopericardium, lying between the primary vessels. Third, the coloration of *E. patina* consistently grades from dark brown to yellow to white moving from the foot up to the parapodial margin, in contrast to the more variable coloration and especially the irridescent speckling inside the parapodia of *E. papillosa*. *E. patina* also lacks red spots on, or red lines crossing, the parapodial margins, whereas red spots or bands commonly occur on specimens of *E. taino* **n. sp.**

Host use further distinguishes *E. patina* from all related species: among the udotacean specialists comprising subclade 1, only *E. patina* feeds on *H. opuntia*, while similar species feed on *Penicillus* (*E. papillosa* and *E. taino* **n. sp.**) or *Udotea* (*E. zuleicae*, *E. buonoi* **n. sp.**). Finally, developmental characters also distinguish *E. patina* from morphologically similar elysiids in subclade 1: only *E. patina* produces egg masses containing a flat ribbon of orange ECY, from which hatch swimming lecithotrophic larvae of exceptionally large size. Although *E. velutinus* sometimes feeds on *H. opuntia* and produces lecithotrophic clutches containing orange ECY, external and radular morphology clearly differentiate *E. velutinus* from *E. patina*, and the species are not closely related.

A similar curved radular tooth morphology is shared by *E. patina*, *E. zuleicae* and *E. buonoi* **n. sp.** However, the dorsal vessel pattern and tail of *E. zuleicae* differentiate this species from *E. patina*, as do host use and the production of white ECY, and the shape of the penial stylet. These species were confused in the literature by Jensen & Clark (1983) and Clark (1994), who reported details on *E. zuleicae* under the name *E. patina* before *E. zuleicae* was described.

Elysia cornigera Nuttall, 1989

(Figs. 6P, 45–47)

Elysia timida [non Risso, 1818]—Ortea, Moro, & Espinosa 1997: 143–145 (part), fig. 1C; Valdés *et al.* 2006: 68–69.

Elysia cornigera Nuttall 1989: 302–306, figs. 1–8 (Type locality: Spanish Harbor, West Summerland Key, Florida Keys)—Hess *et al.* 1994: 161; Clark 1994: 905; Händeler *et al.* 2009: figs. 6, 7; Krug, Händeler & Vendetti 2011: 481-484, fig. 1A–D, 2-3; Redfern 2013: 282, fig. 783A–B; Ortigosa *et al.* 2013: 65; Christa *et al.* 2014: figs. 1E, 3; Krug *et al.* 2015: 990-991, figs. 3B, 4.

Elysia purchoni [non Thompson, 1977]—Ortea *et al.* 2011: 200–203, pls. 1–2.

Type material. *Elysia cornigera*—holotype (USNM 859144) and 5 paratypes (USNM 859145).

Material examined. St. Anne's Bay, Jamaica, 25 May 2006, 1 specimen (LACM 173243); Bahamas: North Bimini, 21 March 2006, 1 specimen (CPIC 00127), Stocking Island, 15 December 2007, 1 specimen (CPIC 00015), 18 January 2009, 1 specimen (CPIC 00078), 29 January 2009, 2 specimens (CPIC 00080–81), Great Exuma, 29 December 2005, 1 specimen (LACM 173227), 18 February 2005, 1 specimen (LACM 172283).

Additional material examined. Geiger Beach, Florida, USA, August 2007, 1 specimen (isolate Ecor_07Gei01); Discovery Bay, Jamaica, 7 March 2006, 1 specimen (isolate Ecor_06Jam01); Little San Salvador, Bahamas, July 2010, 1 specimen (isolate Ecor_10LSS01).

Live animal. Specimens frequently cover their head with anterior parapodial flaps, as if shy. Slugs do not swim, and were not observed to perform the regular head bobbing movement described for *E. timida* (Thompson & Jaklin 1988; Wirtz & Anker 2009).

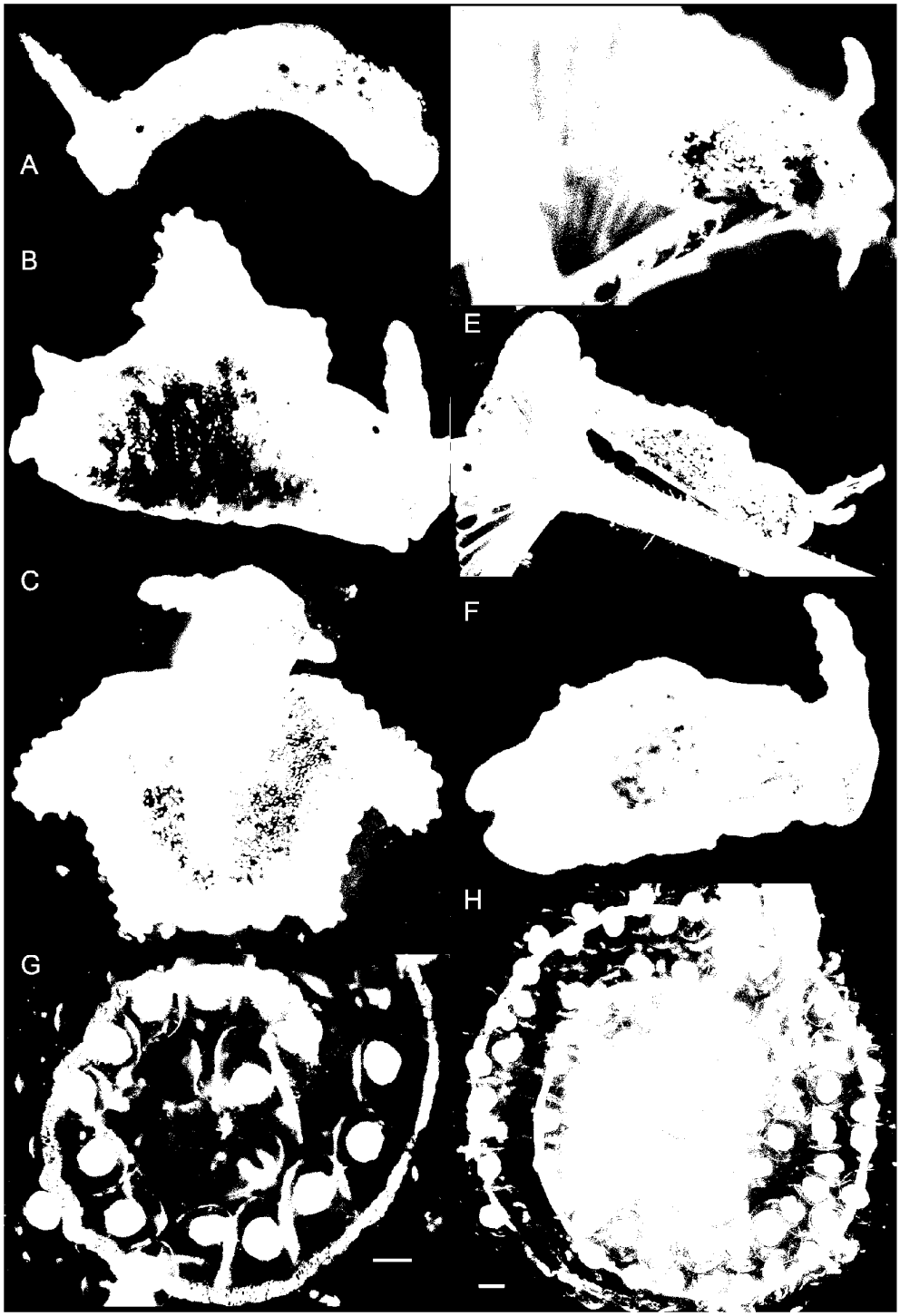

FIGURE 45. *Elysia cornigera*, external morphology and egg masses. **A**, Side view of live specimen from Geiger Beach, Key West, Florida (isolate Ecor_07Gei01; 4 mm long); note elongated, tapering rhinophores with knobby appearance, and orange vesicles in the epidermis. **B–C**, Specimen from Discovery Bay, Jamaica (isolate Ecor_06Jam01; 3 mm long). Side view showing larger anterior and smaller posterior wing-flap extensions to the parapodium, and large brown epithelial granules (B); dorsal view showing renopericardium (C). **D–F**, Specimen from Geiger Beach, Key West, Florida (isolate Ecor_07Gei01; 6 mm), showing red and orange spots on white parapodial flanks (D), larger brown granules embedded in anterior edge of parapodium (E), and, moustache of black spots on the oral lobes (F). **G–H,** Egg masses laid by two Florida specimens, showing semi-transparent (G) or pale yellow (H) ribbon of ECY and early-stage embryos; scale bars = 130 μm.

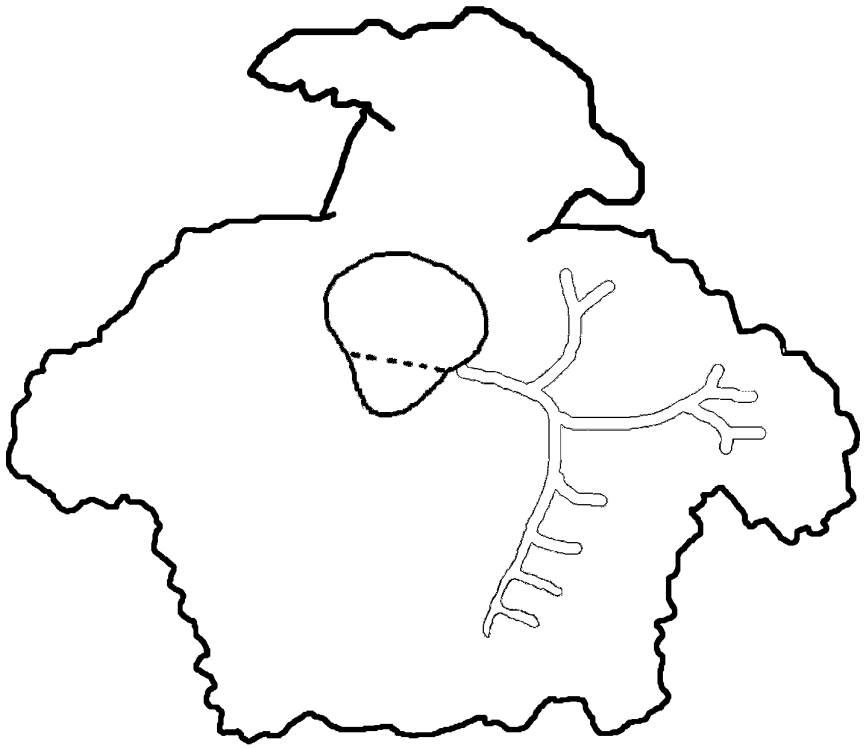

FIGURE 46. *Elysia cornigera*, drawing of renopericardial complex and dorsal vessel network from photograph of live specimen (isolate Ecor_06Jam01, 3 mm long).

External anatomy. Summarized from Krug *et al.* (2011): Body small (to 8 mm), color white to grey on parapodia and head, with numerous papillae giving warty appearance (Fig. 45A–B). Inside of parapodia densely penetrated by green digestive diverticula, conferring light green tinge to outside of fed specimens (Fig. 45C). Foot, sides of head, anterior juncture of parapodia all green (Fig. 45D–F). Red granules dot head and rhinophores, with smaller red spots scattered over parapodia (Fig. 45D). Rhinophores long, curled; widening medially then tapering to a point; edge of rhinophores uneven, with undulating appearance due to many papillae, hence the etymology behind "horned *Elysia*" (Fig. 45D–F). Outer surface of rhinophores white with red spots, inner surface yellow-green with branches of digestive diverticula. Upper edge of oral lobes lined with "moustache" of small black spots (Fig. 45B, F).

Parapodia rise into prominent anterior peaks halfway along body, with second posterior set of smaller peaks on large specimens. White papillae in rows along parapodia. Parapodial margin scalloped due to row of white papillae (Fig. 45C). Parapodia covered with tiny red sots and larger orange spots. Larger spherical granules of irregular size embedded haphazardly in parapodial surface, ranging from golden-brown to orange, appearing as small craters due to elevated surrounding tissue (Fig. 45A–B, D, F).

Pericardium rounded, white with red spots, with one pair of dorsal vessels emerging at posterior end (Fig. 45C, Fig. 46). Vessels opaque, covered in dense white spots, with lateral branches extending up towards parapodial margin. Short renopericardium followed by a clear furrow running down the midline of most of the remaining body length.

Internal anatomy. Radula with 25 teeth (CPIC 00078, CPIC 00081, LACM 173243), 4 teeth in ascending limb and 9 teeth in descending limb (Fig. 47A). Nuttall (1989) reported 2–5 teeth in the ascending limb and 6–7 teeth in the descending limb. Unlike other Caribbean elysiids, radula of *E. cornigera* with two cutting edges. Leading tooth elongate with cusp bearing a denticulate keel, one smooth lateral edge (Fig. 47B), and one denticulate edge (47C). Nuttall (1989) reported teeth of up to 216 μm in length. Base of teeth thickened and elongate, almost as long as tooth cusp. Ascus (CPIC 00080) containing jumbled heap of discarded teeth (not figured). Ascus with >12 teeth according to Nuttall (1989).

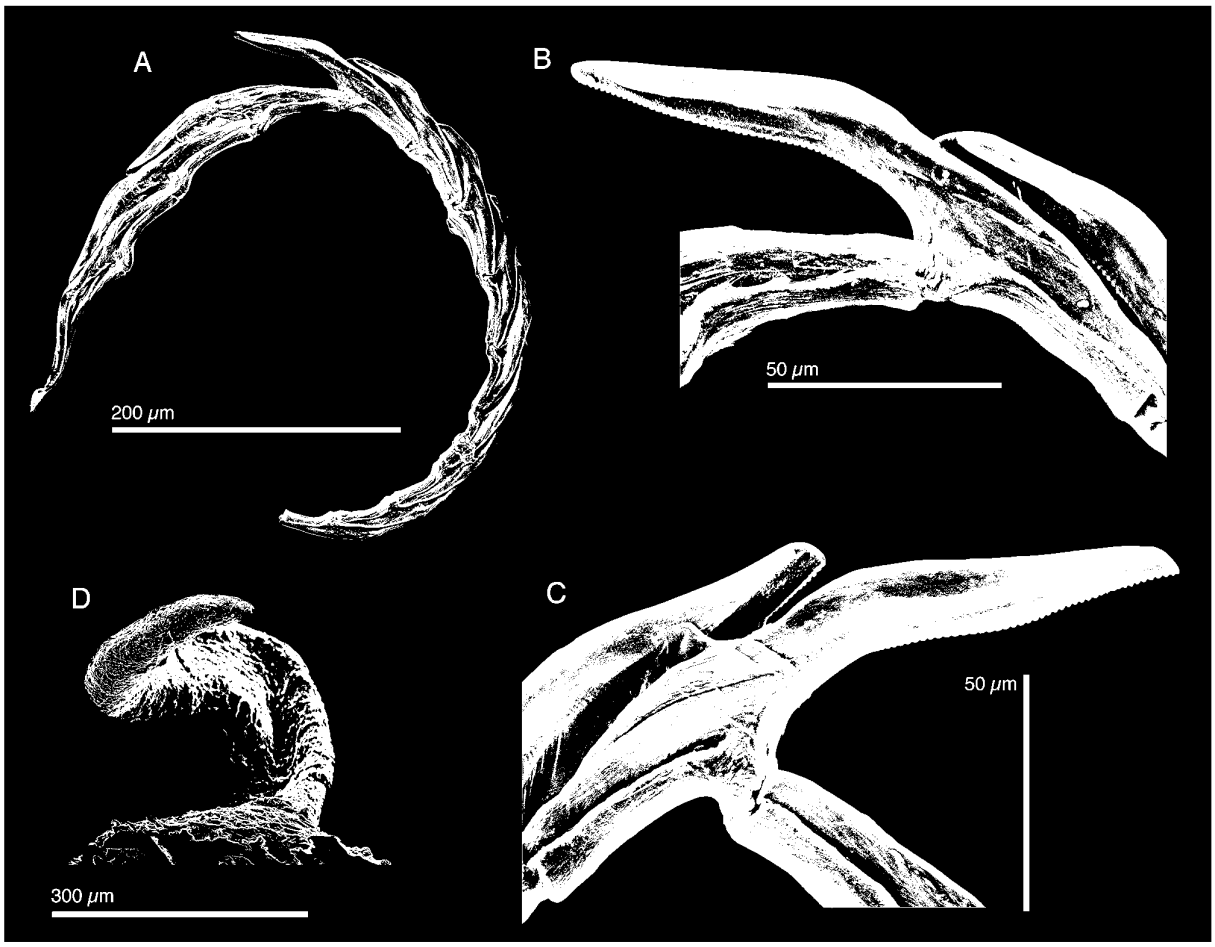

FIGURE 47. *Elysia cornigera*, SEM of the radula and penis. **A,** Radula, without ascus (CPIC 00078). **B,** Leading tooth, showing denticulate keel and smooth lateral edge (CPIC 00078). **C,** Leading tooth, showing serrated keel and denticulate lateral edge (CPIC 00081). **D,** Penis (LACM 173227).

Penis elongate and coiled with with rigid musculature that did not deform after drying, devoid of armature (Fig. 6P, 47D). Base of penis wide. Deferent duct short and thin.

Reproduction and development. Development is lecithotrophic. Clutches contain 20–137 ova ~105 μm in diameter (Nuttall 1989). A flat ribbon of pale yellow or semi-transparent ECY, granular in appearance, lines the upper surface of the egg mass, contacting each capsule (Fig. 45G–H). Larvae develop in 15–19 d at 22°C, with most undergoing encapsulated metamorphosis (95.8 ± 7.2%, n = 3 clutches) and emerging as crawl-away juveniles (Krug 2009). Mean larval shell length for two clutches from Florida Keys specimens was 243.6 μm (± 10.0 SD, n = 22 shells) and 253.1 μm (± 6.2, n = 19), small larvae for Caribbean sacoglossans with lecithotrophic development (Krug 2009). The shell aperture has a distinctive extension diagnostic of *E. cornigera* (Nuttall 1989).

Host ecology. The only known host is *Acetabularia crenulata*. Utricles atop the stipe are individually drained of cytoplasm. Whereas *E. timida* retains diet-derived chloroplasts for 3–4 weeks, *E. cornigera* quickly digests chloroplasts, showing a linear decline in photosynthetic activity over 11 d after feeding (Krug *et al.* 2011).

Phylogenetic relationships. *Elysia cornigera* was recovered as sister to the Mediterranean species *E. timida*, with which it was formerly synonymized (Fig. 4). The resurrection of *E. cornigera* (Krug *et al.* 2011, 2013) was based in part on the divergence between the two species at COI (11.2% TrN), an inter-specific distance for *Elysia* spp. No sister group was recovered with support for the clade of (*E. cornigera* + *E. timida*); the most closely related clade comprised three species, including *E. thompsoni*, which is similar in appearance and also feeds on *Acetabularia*, and *E. lobata*. Within *E. cornigera*, the Bahamas population was highly genetically differentiated from the Florida population, with the Florida clade of COI haplotypes nesting within a basal grade of Bahamas haplotypes; the lone Jamaica haplotype grouped with Florida (Krug *et al.* 2011).

Range. Bahamas (Valdés *et al.* 2006; Krug *et al.* 2011; Redfern 2013), Cayman Islands (Hess *et al.* 1994; Valdés *et al.* 2006), Florida, USA (Nuttall 1989; Krug *et al.* 2011), Jamaica (present study), Mexico (Ortigosa *et al.* 2013).

Remarks. Nuttall (1989) described *E. cornigera* from specimens collected about 5 km from our Florida collection sites, based on live specimens, radular SEM images, and larval development. Ortea *et al.* (1997) synonymized *E. cornigera* with *E. timida* based on material from Cuba (Ortea *et al.* 1997: fig. 1C, pl. 1B), but Ortea *et al.* (2011) rejected their own earlier synonymy and proposed that Caribbean material previously identified as *E. timida* could be *E. cornigera*. Ortea *et al.* (2011) also suggested the possibility of the existence of two cryptic species under the name *E. cornigera*. Genetic data in Carmona *et al.* (2011) indicate that a specimen of *E. timida* from the Mediterranean was sampled in Florida, whereas most Floridian samples were genetically distinct *E. cornigera*. It is thus possible that *E. timida* was introduced to Florida and/or Caribbean populations.

Elysia timida is larger (6–13 mm in body length), with outer parapodia, head and rhinophores colored pure white in contrast to the deep green diverticula visible inside the parapodia. In *E. cornigera* the head and parapodia are greyer and more papillose, the red spots proportionally smaller, and the distinctive large golden-brown spherical inclusions giving an overall brownish tint. Rhinophores of *E. cornigera* have an uneven edge, curve backward, and taper to a point, whereas those of *E. timida* are smooth-edged and terminate in a rounded tip. The rhythmic contraction of rhinophores and head-bobbing of *E. timida* have not been described from *E. cornigera*. Developmentally, the ECY of *E. cornigera* is distinctively semi-transparent, rather than bright yellow as in *E. timida*, and egg size appears to be smaller in *E. cornigera* (105 μm) than in *E. timida* (120 μm).

Ortea *et al.* (2011) described specimens of *E. cornigera* as *E. purchoni*, which we noted previously is a synonym of *E. canguzua*; Ortea *et al.* (2011) further suggest that *E. cornigera* can be a junior synonym of *E. purchoni*. There are many similarities shared by *E. canguzua* (= *E. purchoni*) and *E. cornigera*: both have scattered red spots across the head and body, a "moustache" of black spots on the upper oral lobes, and parapodial margins that widen considerably at the anterior edge. However, the parapodial extensions of *E. canguzua* (= *E. purchoni*) are rounded, and form a pronounced siphonal opening (Thompson 1977: fig. 25f); in contrast, the anterior parapodial edge of *E. cornigera* extends into angular, straight-edged side flaps. The shape of the rhinophores and the rounded siphonal opening drawn for *E. purchoni* are only consistent with *E. canguzua*, a species that Thompson was evidently unaware of, and not *E. cornigera*. Although the radular tooth of both species possesses lateral edges on the sides of the cusp, the tooth of *E. canguzua* (= *E. purchoni*) is only finely denticulate (and only evident by SEM), while that of *E. cornigera* has both a prominently denticulate keel and a serrated lateral edge (Marcus 1955; Thompson 1977; Nuttall 1989; present study).

Finally, Ortea *et al.* (2013) state that "Redfern ... illustrates *Elysia purchoni* and *Elysia timida* under the name *Elysia cornigera* Nuttall, 1989 (species 783A and 783B respectively)." We find these remarks incoherent, given that Ortea *et al.* (2011) proposed *E. cornigera* may be present in Cuba and was not in fact *E. timida*, and that *E. cornigera* could be synonymous with their *E. purchoni*. In fact, Redfern (2001) correctly identified his material as *E. cornigera*, as per Nuttall (1989) and Krug *et al.* (2011).

Elysia pratensis Ortea & Espinosa, 1996

(Figs. 6R, 48–50)

Elysia pratensis Ortea & Espinosa 1996: 116–119, figs. 1–3—Redfern 2001: 163, figs. 675; Espinosa *et al.* 2005: 56; Valdés *et al.* 2006: 70–71; Krug 2009: table 2, figs. 4G, 5E–F; Krug *et al.* 2013: 1109-1113, figs. 2C, 4; Redfern 2013: 285, fig. 789; Christa *et al.* 2014: figs. 1C, 3; Krug *et al.* 2015: 990-991, figs. 3B, 4.

Type material. *Elysia pratensis*—Holotype at MCNT (no registration number provided).

Material examined. Bahamas: San Salvador, July 2010, 2 specimens (LACM 178654–55), Stocking Island, 20 February 2009, 2 specimens (CPIC 00068–69).

Additional material examined. Florida, USA: Anne's Beach, Lower Matecumbe Key, June 2007, 1 specimen (isolate Eprat_07Ann01), Geiger Beach, 25 October 2009, 2 specimens (isolate Eprat_09Gei01, isolate Eprat_09Gei01); Discovery Bay, Jamaica, 07 March 2006, 1 specimen (isolate Eprat_06Jam01;

Bahamas: Sweetings Cay, July 2007, 26 specimens (isolate Eprat_07Swe01-26), Stirrup Cay, July 2007, 14 specimens (isolate Eprat_07Stir01-14), Little San Salvador, July 2007, 4 specimens (isolate Eprat_07LSS01-04),

San Salvador, July 2010, 13 specimens (isolate Eprat_10SSal02, Eprat_10SSal04-15), Bimini, July 2010, 10 specimens (isolate Eprat_10Bim01-10), Compass Cay, July 2010, 4 specimens (isolate Eprat_10Comp01-04), Plana Cays, July 2004, 8 specimens (isolate Eprat_04Pla01-08).

Live animal. Specimens moderately cryptic on host alga, with white longitudinal lines mimicking white borders of individual blades of *Rhipocephalus* on which slugs have fed, removing the green chloroplasts. Large slugs can be seen atop stipes of the alga in situ, however, due to their large body size and inability to hide within the algal thallus. Parapodia are usually held together to cover dorsum.

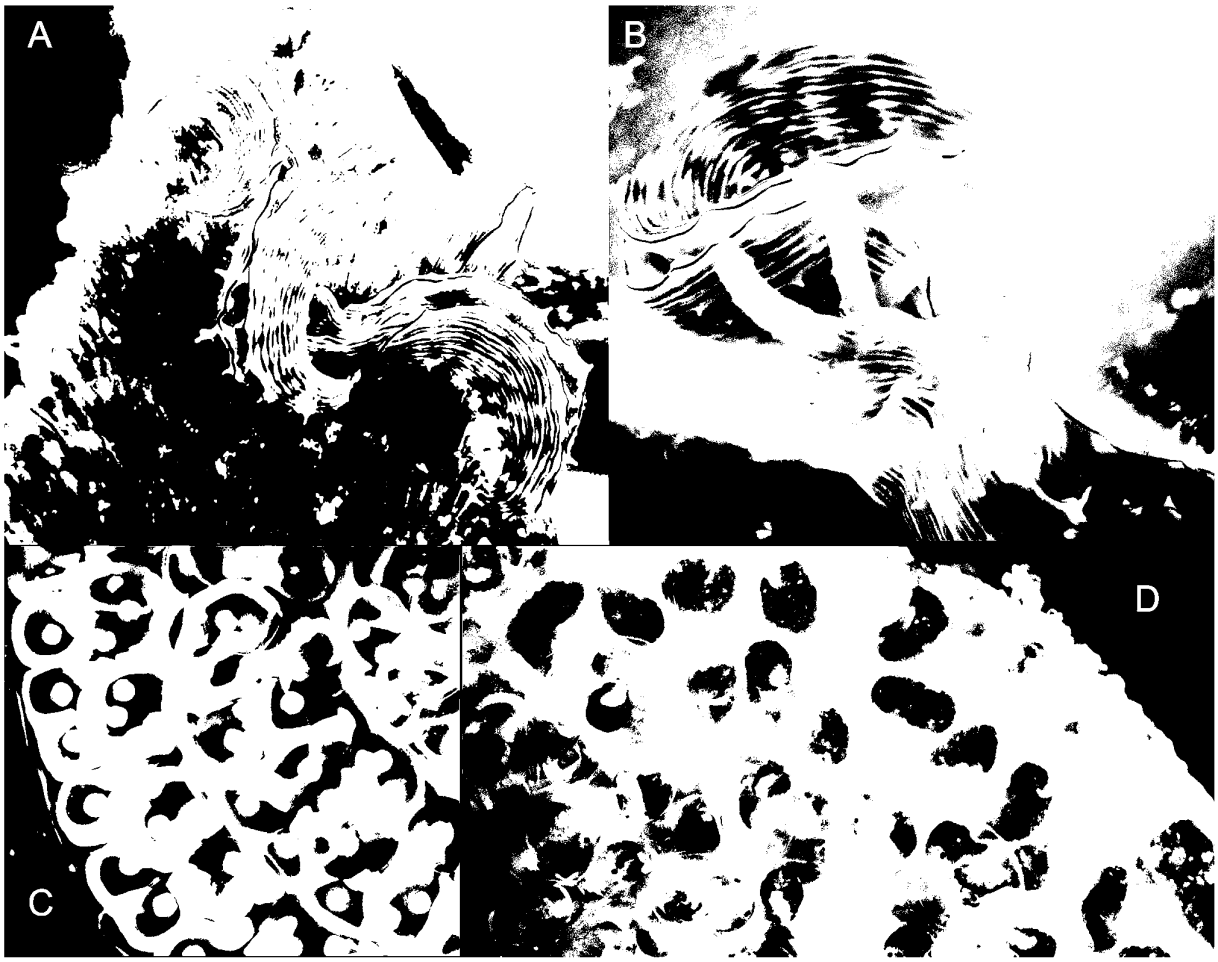

FIGURE 48. *Elysia pratensis*, external morphology and egg masses. **A**, Specimens from Bahamas in July 2004, feeding on *Rhipocephalus brevicaulus*. Note how longitudinal white stripes render slugs cryptic against green algal blades with white tips from emptied algal filaments. Field of view = 4 cm wide. **B**, Mating specimens showing reciprocal insemination. The long, flexible penis of each slug contacts the partner between the right anterior parapodial edge and the pericardium. Field of view = 15 mm. **C**, Egg mass spiral showing orange ECY ribbon winding around capsules containing early-stage embryos. Field of view = 2.5 mm. **D**, Egg mass with larvae undergoing encapsulated metamorphosis in the inner whorl (lower left), and darker, post-metamorphic juvenile siblings in the outer whorl (top and right). Note depletion of orange ECY, compared to earlier in development (**C**). Field of view = 4 mm.

External anatomy. Large specimens to 35 mm in body length. Overall coloration green, with longitudinal white stripes running the length of the body along parapodia, over head, and along rhinophores (Fig. 48A–B). Body elongated. Head roughly square, eyes small. Rhinophores mostly smooth with a few papillae near distal end. Tips of rhinophores yellow-orange.

Parapodia with black marginal line; on either side, white line with papillae running along edge. Rows of elongated white papillae spaced at regular intervals, emerging from white longitudinal stripes, running length of parapodia. No stripes inside parapodia.

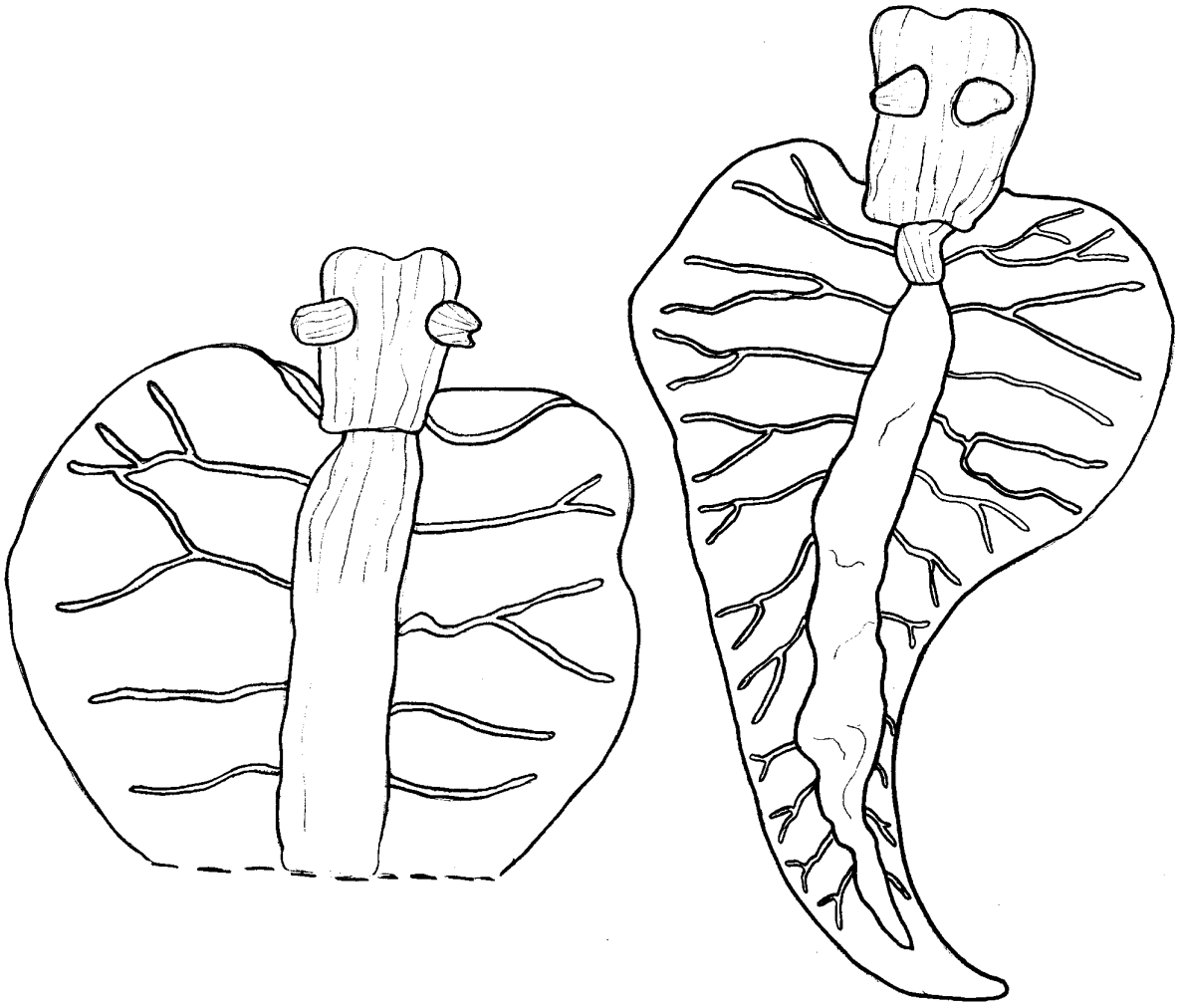

FIGURE 49. *Elysia pratensis*, drawing of renopericardial complex and dorsal vessel network from two preserved specimens (left: LACM 178654, 3 mm wide; right: LACM 178655, 6 mm long × 4 mm across at widest point).

Pericardium green, covered with white longitudinal stripes continuing from head. Renopericardial extension runs entire length of body, with eight or more lateral vessels emerging from along the entire length (Fig. 49). Vessels branch repeatedly and anastomose along inner parapodial margin.

Internal anatomy. Radula with 21 teeth (CPIC 00068), 8 teeth in ascending limb and 13 in descending limb (Fig. 50A). Leading tooth elongate and robust (CPIC 00068–69), with 16–22 small, irregular, and coarse denticles on cusp (Fig. 50B). Housing depression for interlocking teeth "V"-shaped and extending 80% of tooth length (Fig. 50B). Base of tooth approximately ¼ of total tooth length. Ascus containing jumbled heap of discarded teeth (Fig. 50C).

Penis elongate with rigid musculature resistant to desiccation (CPIC 00068–69), tapering to an apex devoid of armature. Deferent duct long and highly convoluted (Figs. 6R, 50D).

Reproduction and development. Mating was observed for two Florida specimens (Fig. 48B). Insemination was largely reciprocal, and similar in all respects to the behaviors described for *E. subornata* (see Jensen 1986). The penis of each slug contacted its partner between the right anterior parapodial surface and the pericardium for an extended period. Insemination appeared to be hypodermic, with sperm transferred into the epithelial tissue around the pericardium without a penial stylet or puncturing.

A wide flat ribbon of orange ECY weaves around each individual egg capsule (Fig. 48C–D). Grand mean clutch size was 146.6 eggs (± 106.4 SE; n = 11 clutches; range = 13 to 1,209 ova per clutch), but only 40.4 eggs ±

6.2 SE if the largest egg mass was excluded. Mean egg diameter ranged from 109.8 µm (± 0.6 SE) to 122.4 µm (± 0.7 SE) for five clutches, with a grand mean egg diameter of 117.9 µm (± 2.2 SE).

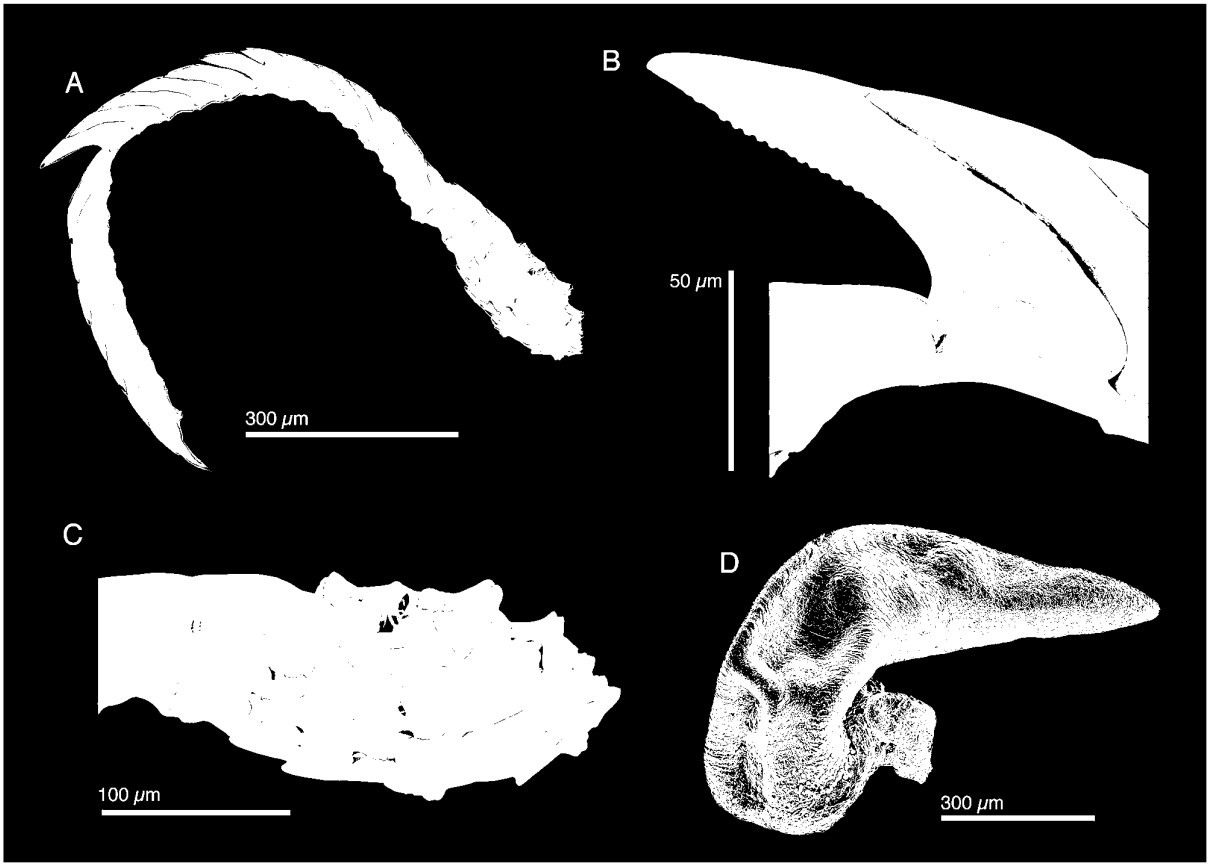

FIGURE 50. *Elysia pratensis,* SEM of the radula and penis (CPIC 00068). **A**, Complete radula. **B**, Leading tooth. **C**, Ascus. **D**, Penis.

Mean larval shell length at metamorphosis was determined for 12 clutches (two from Florida Keys, and five each from Sweetings Cay and Little San Salvador, Bahamas), and ranged from 301.2 µm (± 18.3 SD, n = 20) to 334.5 µm (± 18.2, n = 30); both size extremes were sampled from Little San Salvador parents. Overall, the grand mean shell length was 321.2 µm (± 9.9, n = 12 clutches), close to the median value of 323.4 µm; this is the second largest larval size recorded for a Caribbean sacoglossan, smaller only than *E. papillosa*. All larvae metamorphosed within the egg mass and emerged as crawl-away juveniles.

Larvae began metamorphosing after 18.4 d (± 0.7 SE, n = 13 clutches) at 22°C, but juveniles required an additional 4.8 d (± 0.7 SE, n = 6) to begin hatching, and about another 5 d for the last juvenile slug to leave the egg mass. Overall, mean developmental time until juveniles began hatching was 23.3 d (± 1.0 SE, n = 6), the longest encapsulated period for any Caribbean elysiid. During the extended post-metamorphic period, juveniles that remained in the egg mass fed readily on ECY, and grew to more than twice the size of siblings that emerged from the egg mass without consuming ECY post-metamorphosis (Krug 2009). Juveniles of *E. pratensis* were the largest measured for any Caribbean sacoglossan, with a grand mean juvenile length of 651.9 µm (± 42.0 SE, n = 5 clutches).

Host ecology. The host *of E. pratensis* has not been previously reported; the species takes its name from the seagrass "meadows" from which it was initially discovered by seine netting. Throughout the Caribbean, we have found *E. pratensis* associated exclusively with the alga *Rhipocephalus brevicaulis*. Notably, only one small clump of five stipes of *R. brevicaulis* was found in Discovery Bay, Jamaica, with one large specimen of *E. pratensis* feeding on the alga. We have observed feeding on *R. brevicaulis* in the lab; slugs drain cytoplasm from the tips of the filaments which, pressed together, form the scale-like blades. The longitudinal white stripes on *E. pratensis* render it cryptic against its host, especially when filament tips are white after being fed upon (Fig. 48A). Ortea &

Espinosa (1996) erroneously presumed the species fed on *Thalassia* and compared it to other seagrass-feeding species, even discussing the coloration as cryptic on *Thalassia*; large, mobile specimens have occasionally been found crawling on other substrates in the field, but feed exclusively upon *R. brevicaulis*.

Phylogenetic relationships. *Elysia pratensis* was sister to *E. subornata* in phylogenetic analyses (Fig. 4), although molecular data were not available for at least one related species (*Elysia hamanni* **n. sp.**); thus, the inclusion of other taxa in subsequent analyses may alter our understanding of relationships in this group. However, a phylogeographic study uncovered hybrid introgression of mitochondria from *E. subornata* into *E. pratensis*, resulting in local fixation of mitochondria from *E. subornata* in northern and central Bahamas populations of *E. pratensis* (Rodriguez 2009). Such cyto-nuclear discordance can lead to misidentification in DNA barcoding studies that employ only mitochondrial markers.

Notably, *E. pratensis* occupies a highly derived position within subclade 4, in which all other species feed on *Caulerpa* spp. (Krug *et al.* 2013). Several features are shared by *E. pratensis* and other members of the *E. tomentosa* clade, including a long renopericardium, complex anastomosing network of dorsal vessels, and meandering tubes of bright orange ECY. The derived diet of *E. pratensis* and its sympatric distribution with *E. subornata* are consistent with ecological speciation via host shift onto *Rhiphocephalus* by the ancestor of *E. pratensis*.

Range. Bahamas (Redfern 2013; present study), Belize (Valdés *et al.* 2006), Honduras (Valdés *et al.* 2006), Cuba (Espinosa *et al.* 2005), Florida, USA, Jamaica (present study), Mexico (Ortea & Espinosa 1996).

Remarks. The longitudinal white stripes distinguish *E. pratensis* from *E. subornata*, and the long renopericardial extension running almost the whole body length distinguishes *E. pratensis* and *E. subornata* from related elysiids in which the renopericardium runs only partway down the body. There has been little taxonomic confusion surrounding this species; Clark and coworkers appear to have regarded *E. pratensis* as a variant of *E. subornata* but undertook no taxonomic study, partly explaining why such a common species was undescribed until relatively recently.

Both diet and pointed, coarsely serrated teeth distinguish *E. pratensis* from all related and co-occurring species: *E. subornata*, *Elysia pawliki* **n. sp.**, and *Elysia zemi* **n. sp.** feed on *Caulerpa*, and have blunt-tipped, nearly smooth teeth. The radula of *E. pratensis* is presumably adapted to piercing the calcified filaments of *R. brevicaulis*; indeed, tooth morphology is strikingly convergent between *E. pratensis* and the distantly related *E. papillosa* and *E. taino* **n. sp.**, which feed on the related and morphologically similar alga *Penicillus*. In other Caribbean members of the *E. tomentosa* clade, the descending limb of the radula also forms a pronounced spiral absent from *E. pratensis*.

Elysia zuleicae Ortea & Espinosa, 2002

(Figs. 6S, 51–53)

Elysia zuleicae Ortea & Espinosa 2002: 133–139, figs. 3–7; pl. 1, fig. B (Type locality: Marina Hemingway, Cuba)—Valdés *et al.* 2006: 70–71; Händeler *et al.* 2009: figs. 6, 7; Krug 2009: 361-365, fig. 3B, 4B, 5A,B, 6; Redfern 2013: 286, figs. 792A–E; Ortigosa *et al.* 2013: 66; Christa *et al.* 2014: figs. 1, 3; Krug *et al.* 2015: 990-991, figs. 3B, 4.

Elysia papillosa [non Verrill 1901]—Clark 1984: 89–90 [part], figs. 16, 18–20 [non Clark 1984: figs. 15, 17]; Clark 1994: 905

Elysia patina [non Ev. Marcus 1980]—Jensen & Clark 1983; Clark 1994: 905; Jensen 1986: figs. 1,3–4.

Elysia leeanneae Caballer, Ortea & Espinosa *in* Ortea, Espinosa, Buske & Caballer 2013: 188–190, pl. 11, fig. F–H; pl. 13 (Type locality: Petit Cul-de-Sac Marin, Guadeloupe) **n. syn.**

Type material. *Elysia zuleicae*—holotype at IOH, paratypes at MCNT and MZUCR (no registration numbers given); *Elysia leeanneae*—holotype (MNHN 26978).

Material examined. Great Lameshur Bay, U.S. Virgin Islands, 2014, 1 specimen (LACM 178662); Union Island, Chatham Bay, St. Vincent and the Grenadines, 1987, 2 specimens (LACM 178660–61); Bahamas: Abaco Islands, 2003, 1 specimen (LACM 178656), Sweetings Cay, July 2007, 1 specimen (LACM 178657), San Salvador, July 2007, 1 specimen (LACM 178658), Plana Cays, July 2007, 1 specimen (LACM 178659), Stocking Island, Exumas, 16 February 2009, 1 specimen (CPIC 00089).

Additional material examined. Geiger Beach, Florida, USA, October 2006, 38 specimens (isolate Ezul_06Gei01-38); Bocas del Toro, Panama, December 2004, 51 specimens (isolate Ezul_04Pan01-51); Discovery Bay, Jamaica, 07 March 2006, 31 specimens (isolate Ezul_06Jam01-31); Bermuda, June 2006, 48

specimens (isolate Ezul_06Ber01-48); Piscadera Bay, Curaçao, 5 January 2009, 12 specimens (isolate Ezul_09Cur01-12), 7 January 2009, 13 specimens (isolate Ezul_09Cur13-25); Bahamas: Stirrup Cay, July 2007, 14 specimens (isolate Ezul_07Stir01-14), Sweetings Cay, July 2007, 34 specimens (isolate Ezul_07Swe01-34), July 2010, 25 specimens (isolate Ezul_10Swe01-25), Little San Salvador, July 2007, 22 specimens (isolate Ezul_07LSS01-22), July 2010, 18 specimens (isolate Ezul_10LSS01-18), San Salvador, July 2007, 27 specimens (isolate Ezul_07Ssal01-27), Plana Cays, July 2007, 28 specimens (isolate Ezul_07Plana01-28), Northern Exumas, July 2010, 21 specimens (isolate Ezul_10NEx01-21), New Providence, July 2010, 1 specimen (isolate E_cf_zul10NPr01), Bimini, July 2010, 14 specimens (isolate Ezul_10Bim01-14).

Live animal. Specimens swim readily by undulating their parapodia when disturbed. Slugs mate by hypodermic insemination (sometimes in groups), using a long, highly flexible penis that can extend out for half the body length. Slugs were observed to react violently to touch of a penis, and executed escape maneuvers including back-flips to avoid insemination during bouts of group mating. Juveniles hold parapodia flat against *Udotea*, and have notably darker coloration than adults.

External anatomy. Coloration variable but generally olive to dark green body, parapodia and head, with variously colored marginal bands along parapodia (Fig. 51A–C, E–H). Head light to dark green, sometimes with red, brown or rust-colored patches. Rhinophores long, rolled, colored white to brown-purple along whole length; with scattered, rounded white papillae. White patches of pigmentation concentrated at distal end of rhinophores, which are squared-off and uncurled at tip. Eyes large, usually not surrounded by pigment. White or brown patch sometimes bisecting head along midline between (and sometimes overlying) eyes (Fig. 51A, C, E–H). In many specimens, pronounced, narrow tail extending out a few mm beyond end of parapodia, which do not cleanly delimit posterior end of body. Some specimens without a tail at all. Color and pattern of tail usually matching that of parapodial margin. Foot continuous with parapodia, same in coloration, with thin transverse groove separating head (Fig. 51D). Light orange streaks on head and parapodia of some specimens.

Parapodia thin, sometimes with slightly undulating edge but not forming siphonal openings. Outer parapodial surface predominantly green with scattered white specks and low, small white papillae. Row of larger white papillae running along parapodial margin, papillae sometimes forming clusters of 3 or 5 with middle papilla being the tallest, creating alternating "crowns" rising and falling along margin (Fig. 51C, F–G). Color of margin highly variable, ranging from white to brown. Large adults sometimes with thick, light purple marginal band with submarginal bands of white to brown (Fig. 51E); some specimens (especially juveniles) with thin black marginal line surrounded by thicker white submarginal band (Fig. 51H). Inside parapodia, dorsum green with scattered brown flecks and iridescent blue to white speckles. Juveniles (<4 mm) tend to lay parapodia completely flat (Fig. 51G). Parapodial margin dominated by individual white papillae separated by black marginal line, creating appearance of alternating white peaks and black bars. Juveniles often with proximal pigment patches of black at base of rhinophores, with distal portion white.

Pericardium small and rounded, with color ranging from white to black but usually white with scattered tiny black or brown speckles. Renopericardial extension wider than pericardium but similar in color, extending about one quarter of body length (Fig. 51E–F). Dorsal vessels white, sometimes outlined in iridescent blue. All examined specimens had two paired vessels per side, one pair emerging from renopericardial extension just posterior to pericardial bulge, and a posterior pair extending from corners of squared-off end of renopericardial extension (Fig. 52). Anterior vessels branch immediately, then fork or send out lateral branches at irregular intervals, rarely anastomosing. Posterior vessels extend to about ¾ of body length, with roughly symmetrical pattern of lateral side branches, which sometimes fork 1–2 times while extending towards parapodial margin. Vessels variable in thickness among specimens.

One pair of large sperm-storage vesicles typical present as roughly spherical greyish protrusions (Fig. 51E, 52). On all specimens, vesicles positioned between posterior-most branch of 1st pair of vessels, and anterior-most branch of 2nd (posterior) pair of vessels. Vesicles sometimes bordered on one side, but not surrounded, by dorsal vessel branches.

Internal anatomy. Radula with 20–34 teeth (CPIC 00089, LACM 178659), 7–12 teeth in ascending limb and 13–22 in descending limb (Fig. 53A). Leading tooth elongate and narrow with a curved cusp bearing approximately 45–60 sharp and minute denticles (Fig. 53B). Teeth without typical "V"-shaped housing depression of many other elysiids. Instead, teeth overlapping with ½–¾ of tooth cusp resting on lateral edge of tooth base on adjacent tooth (Fig. 53A). Base of tooth tall and approximately ½ total tooth length. Ascus either an ordered series of small teeth in sequence (Fig. 53A) or a small jumbled heap of discarded teeth.

Penis wide and elongate tapering into a conical apex (CPIC 00089, LACM 178660, LACM 178662), bearing a pointed and scoop-shaped stylet (Fig. 6S, Fig. 53C–D). Deferent duct long and convoluted.

Reproduction and development. Mating behavior was observed for specimens from the Bahamas, and conformed to the description given by Jensen (1986) for specimens identified as *E. patina*. Three specimens performed a prolonged mating ritual that involved jostling for position and probing with the tip of the extended, highly flexible penis to act as a sperm donor, but avoiding contact from the penises of the other two specimens. Contact between a penis and the exterior of the parapodium or foot caused the intended sperm recipient to recoil violently; one specimen did a back-flip to avoid being hypodermically inseminated. Jensen (1986) reported that only contact near the sperm vesicles on the inner parapodial surface would be accepted without triggering a recoil response. The male opening is under the right rhinophore, and the female opening is near the anus in the groove separating the right anterior parapodial edge from the head.

Development is predominantly planktotrophic, but specimens from some Bahamas sites (Sweetings Cay, San Salvador Is., Plana Cays) produced lecithotrophic larvae (Krug 2009). DNA sequencing of the COI gene from lecithotrophic larvae confirmed conspecificity with planktotrophic *E. zuleicae* (Trathen 2010, and unpublished data). Both developmental morphs produced egg masses containing a ribbon of white ECY. In planktotrophic egg masses (Fig. 51I), the ECY ribbon is thin and meanders among the egg capsules, similar to the egg mass of *E. patina*. In lecithotrophic egg masses, the ECY ribbon is thicker and wider, and tends to cover the upper face of the egg mass, more similar to the ECY ribbon of *E. papillosa* but white (Fig. 51J–K). No other lecithotrophic elysiid from the Caribbean produces a white ECY ribbon.

Mean egg diameter was 67.6 µm (± 2.3 SD, n = 23 ova) for a planktotrophic clutch from Little San Salvador, Bahamas, and 64.5 µm (± 1.9 SD, n = 28 ova) for a clutch from Jamaica; grand mean diameter of planktotrophic eggs was thus 66.1 µm ± 2.3 SD. Planktotrophic larvae developed in 5.7 d (± 0.7 SE, n = 3 clutches), and mean shell length at hatching was 109.5 µm (± 5.1 SD, n = 80) for a clutch from Little San Salvador.

Lecithotrophic clutches contained 104.0 eggs (± 10.4 SE, n = 4 clutches). Lecithotrophic egg diameter was not determined, as clutches were collected after cleavage had begun. Grand mean shell length at hatching for lecithotrophs was 253.9 µm (± 8.9, n = 4 clutches), ranging from 244.6 µm (± 7.4, n = 21) for a clutch from Plana Cays, to 265.3 µm (± 8.9, n = 27) for a clutch from Sweetings Cay. Larval shell sizes previously reported for the lecithotrophic morph of *E. zuleicae* were plotted on the wrong Y-axis (Krug 2009: fig. 4B). Lecithotrophic larvae developed in 18.5 d (± 0.5, n = 2 clutches) and then hatched over an additional 4–5 d. No intracapsular metamorphosis occurred in four clutches. Significant metamorphosis was induced only by the adult host *Udotea flabellum*, which triggered about half of larvae to metamorphose; no larvae metamorphosed in the presence of *P. capitatus*, and fewer than 10% settled in response to *Caulerpa verticillata* or FSW only (Krug 2009).

Host ecology. Field surveys and laboratory observations indicate that *E. zuleicae* specializes on the green algal genus *Udotea*, particularly *U. flabellum*, the host identified in the species description (Ortea & Espinosa 2002). In field surveys by PJK of 15 sites over a decade, >400 specimens were collected from *Udotea*. Juvenile specimens (<5 mm) in particular have only been collected in association with *U. flabellum*, and both juvenile and adult slugs were observed to feed readily and exclusively on *U. flabellum* in the laboratory. *Elysia zuleicae* is generally less abundant than *E. papillosa*; a 500 g collection of *Udotea* (wet weight) yielded only 9 specimens in Jamaica. However, *E. zuleicae* is far more common and widespread than the related, morphologically similar species *E. buonoi* **n. sp.**, of which only four specimens were collected from *Udotea* at a single location (San Salvador Island, Bahamas), two in 2004 and two more in 2007.

Phylogenetic relationships. *Elysia zuleicae* and its sister species *E. buonoi* **n. sp.** were recovered within subclade 1, a lineage of Caribbean elysiids feeding on udotacean algae (Fig. 4). The morphologically similar *E. buonoi* **n. sp.** was genetically distinct at the COI locus (minimum distance: 10.2%) and fixed for different alleles at the nuclear H3 locus where the two species co-occurred on San Salvador Island, Bahamas (Trathen 2010; unpublished data); COI divergence, ABGD analysis, and fixed allelic differences at H3 all support treatment of *Elysia zuleicae* and *E. buonoi* **n. sp.** as distinct.

Range. Bahamas (Redfern 2013), Bermuda (present study), Costa Rica (Valdés *et al.* 2006), Cuba (Ortea & Espinosa 2002), Curaçao (present study), Florida, USA (present study), Jamaica (Valdés *et al.* 2006), Mexico (Ortigosa *et al.* 2013), Bocas del Toro, Panama (present study), U.S. Virgin Islands (present study), Union Island, St. Vincent and the Grenadines (present study).

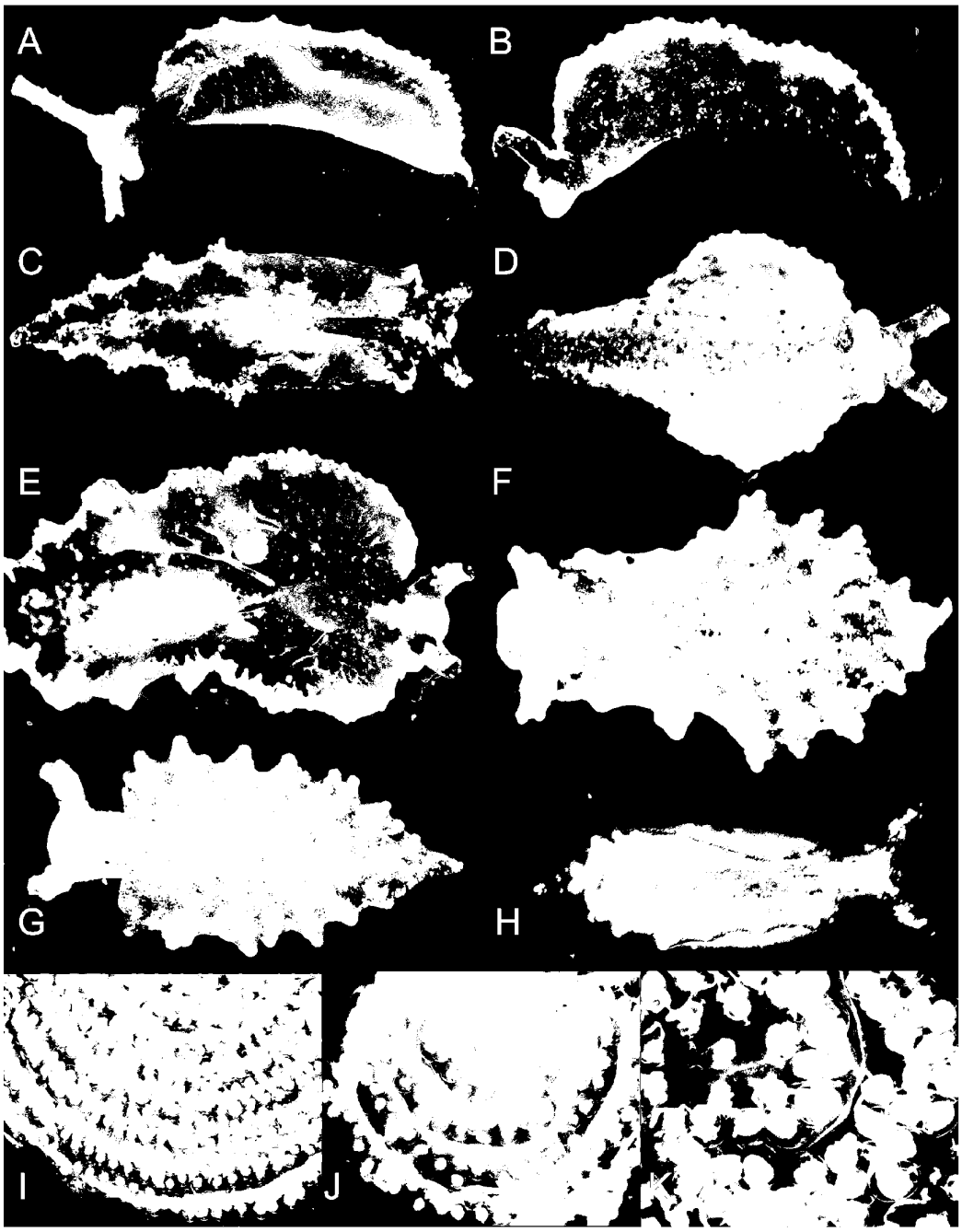

FIGURE 51. *Elysia zuleicae*, external morphology and egg masses from Discovery Bay, Jamaica (A; March 2006); Little San Salvador, Bahamas (B–D, F, H; July 2007); or Bocas del Toro, Panama (E, G; December 2004). All measurements give body length of specimens. **A,** Specimen (8 mm) with light-colored rhinophores, parapodial margin and tail. **B,** Side view of specimen (12 mm) with dark maroon rhinophores, marginal bands of purple and brown on dark green parapodia, and thin, dark-colored tail. **C,** Top view of same specimen, showing parapodial margin, short renopericardial extension, and head. **D,** Ventral view of foot. **E,** Dorsal view with parapodia open, showing renopericardial complex, dorsal vessel network, and white gametic vesicles. **F,** Light-colored specimen (8 mm) with black speckling inside parapodia and on dorsal vessels. **G,** Light-colored juvenile (3 mm) with parapodia held flat against the substrate. **H,** Dark-colored juvenile (3 mm) with black marginal line. **I,** Planktotrophic egg mass laid by specimen from Jamaica, showing white ECY ribbon intercalated among early-stage embryos. Field of view = 2.1 mm. **J,** Lecithotrophic egg mass laid by specimen from Plana Cays, Bahamas, showing thick white ECY ribbon on upper inside face of egg mass, and encapsulated veliger larvae. Field of view = 6.8 mm. **K,** Lecithotrophic egg mass laid by specimen from Sweetings Cay, Bahamas, showing remnants of white ECY ribbon intercalated among encapsulated late-stage veliger larvae, close to the point of hatching. Field of view = 2.1 mm.

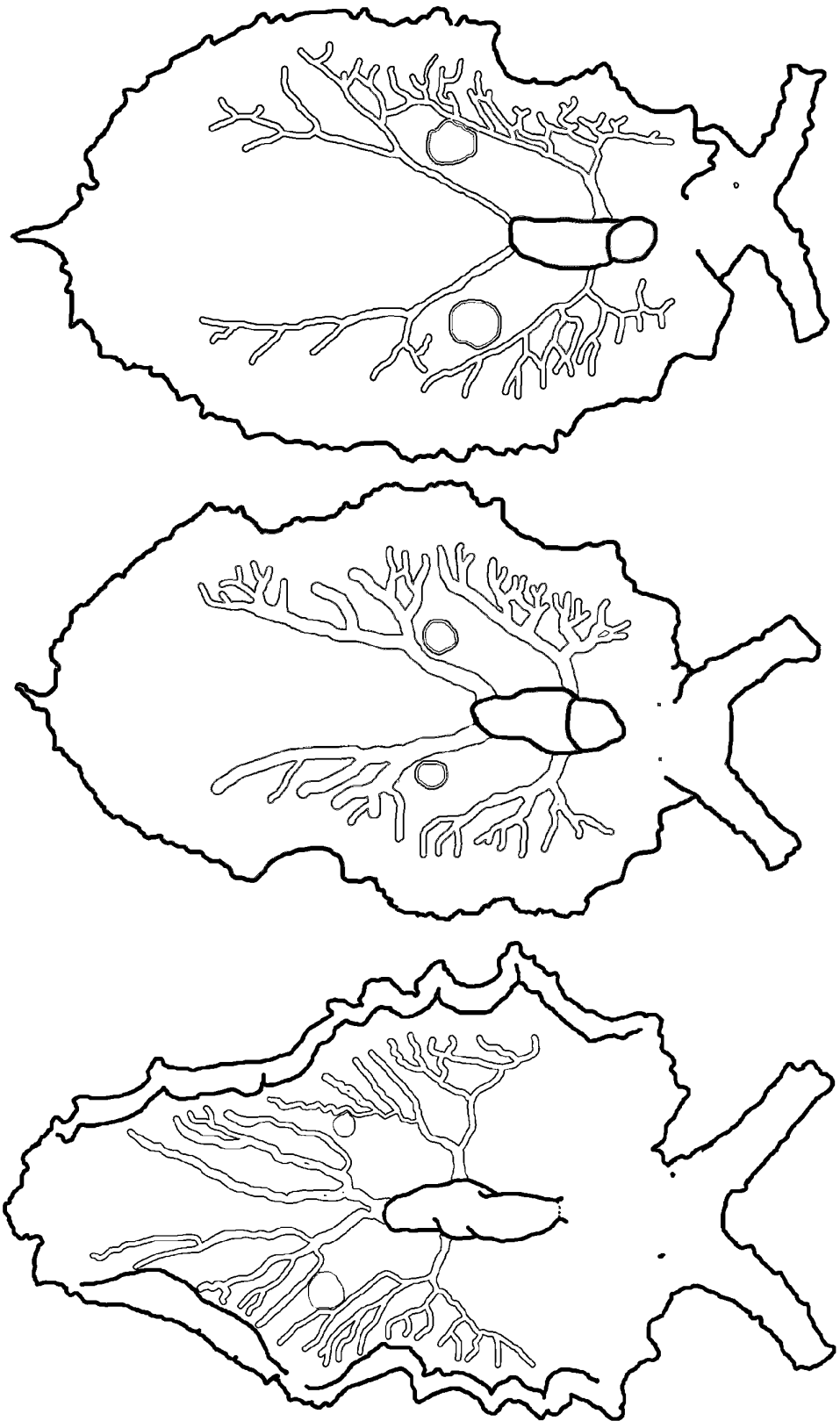

FIGURE 52. *Elysia zuleicae*, schematic drawing of renopericardial complex and dorsal vessel network traced from photographs of three live specimens from Jamaica. Gray areas represent sperm-storage vesicles.

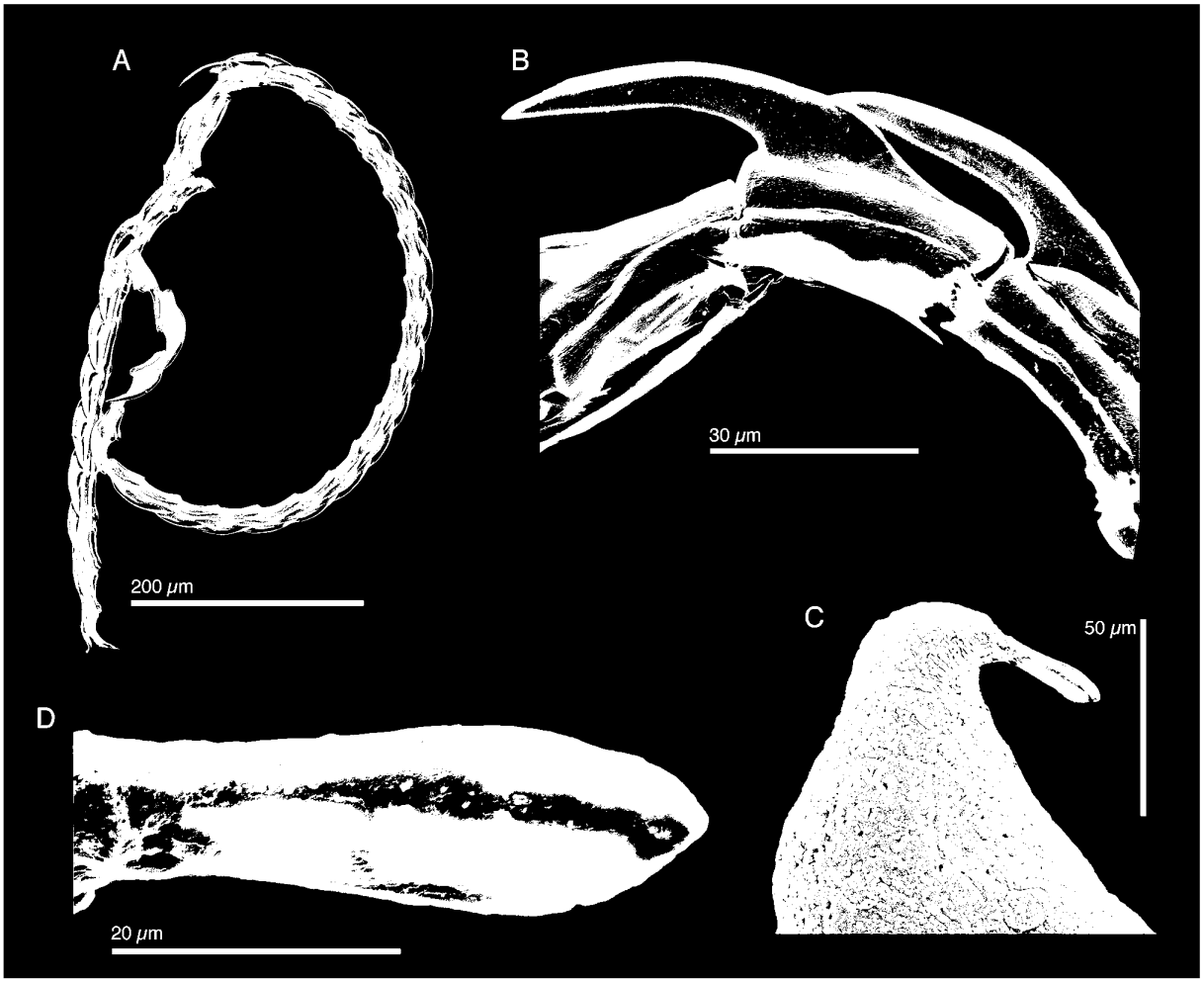

FIGURE 53. *Elysia zuleicae*, SEM of the radula and penis (CPIC 00089). **A**, Complete radula including ascus. **B,** Leading tooth. **C,** Penis. **D,** Close-up view of penial stylet.

Remarks. *Elysia zuleicae* was widely confused with *E. papillosa* and *E. patina* prior to its description by Ortea & Espinosa (2002). Clark (1984: figs. 16, 18–20) drew *E. zuleicae* as *E. papillosa* but noted that his *papillosa* probably represented a species complex. Contributing to the confusion, radular tooth morphology is highly similar among *E. zuleicae*, *E. buonoi* **n. sp.**, and *E. patina*, and *E. zuleicae* is common at the type locality of *E. patina*. Thus, Jensen & Clark (1983) and Clark (1994) identified the host of *E. patina* as *Udotea*, which is not correct; *E. patina* feeds on *Halimeda opuntia*, but both studies actually focused on *E. zuleicae* under the name *E. patina*. Similarly, Jensen (1986) described the mating behavior and reproductive anatomy of "*E. patina*" specimens that were *E. zuleicae*, having been collected from *Udotea* and matching *E. zuleicae* (undescribed at the time) in all respects. The discrepancy in the location of the male opening noted by Jensen (1986) further confirms that her "*E. patina*" was not *E. patina* Ev. Marcus 1980, but rather was *E. zuleicae*.

Ortea & Espinosa (2002) made no mention of penial morphology in the original description of *E. zuleicae*. Ortea *et al.* (2005) subsequently stated *E. zuleicae* lacked a penial stylet, and claimed that the presence of a stylet was a major character difference supporting their new species *E. deborahae*. However, a penial stylet was present on most specimens of *E. zuleicae* examined in the present study, as noted by Jensen (1986), and was even visible at low magnification on living specimens. Specimens of *E. zuleicae* flinch in response to contact by a penis, and execute escape behaviors consistent with traumatic mating by hypodermic insemination (Lange *et al.* 2013). One specimen of *E. zuleicae* (confirmed genetically by sequencing two genes) lacked a stylet, suggesting stylets may sometimes be torn out by the violent "escape" movements that occur during mating. Thus, Ortea *et al.* (2005) may have examined a specimen that lacked its stylet, but a stylet is unambiguously present in *E. zuleicae*.

The pointed, elongated, black tail of *E. zuleicae* is a variable character that, when present, distinguishes *E. zuleicae* from similar Caribbean species. However, the extent to which a tail is present varies within *E. zuleicae*, especially among juveniles; we misidentified some juvenile *E. zuleicae* as *E. papillosa* in three populations due to the lack of an apparent tail, only uncovering the correct identity of these specimens through DNA barcoding. Moreover, half the preserved specimens confirmed genetically as *E. zuleicae* from U.S. Virgin Islands lacked any tail, whereas half had the typical elongated tail.

Small specimens of *E. zuleicae*, especially if pale from lack of recent feeding activity, may be confused with small *E. papillosa*, *E. taino* **n. sp.**, or *E. patina*. However, *E. zuleicae* is distinct from all three species in its pattern of dorsal vessels, and the shape of its penial stylet. Adult specimens of *E. zuleicae* have only two pairs of dorsal vessels on each side of the renopericardium, in contrast to the greater number seen in equivalently sized specimens of *E. patina*, *E. papillosa* or *E. taino* **n. sp.** Radular tooth morphology distinguishes *E. zuleicae* from *E. papillosa* and *E. taino* **n. sp.**

Like the related species in this clade (*E. patina*, *E. papillosa*, *E. taino* **n. sp.**), *E. zuleicae* swims when disturbed, and has gametic vesicles that swell with sperm following insemination. The position of gametic vesicles is distinctive for *E. zuleicae*: vesicles are located between the primary forks of the posterior dorsal vessel on each side of the body. In contrast, sperm vesicles are located midway along the renopericardial extension in *E. papillosa*, while those of *E. patina* are found posterior to the end of the renopericardial extension, surrounded by the terminal branches of the posterior-most vessels. Ecologically, these three species also feed on different host algae.

The recently described *E. leeanneae* Caballer, Ortea & Espinosa *in* Ortea, Espinosa, Buske & Caballer, 2013 is highly similar to *E. zuleicae*, only differentiated by (a) overall white body coloration, (b) a jumbled ascus, and (c) fewer teeth: 10 in the ascending limb and 19 in the descending limb for *E. leeanneae*, versus a reported 24–25 teeth in the descending limb of *E. zuleicae*. However, specimens of *E. zuleicae* can be very white (e.g., Fig. 51F), and vary considerably in the degree to which parapodial margins are papillose or ruffled. Two of four specimens of *E. zuleicae* examined in the present study had a disorganized (or jumbled) ascus, while the teeth of ascus were organized in the other two specimens. One specimen had only 22 teeth (Fig. 53A). Thus, radular characters do not clearly differentiate *E. leeanneae* from *E. zuleicae*. Although the external appearance of the holotype of *E. leeanneae* looks different from most *E. zuleicae*, given the intra-specific variability of the latter species, we consider *E. leeanneae* a junior synonym of *E. zuleicae* due to their identical dorsal vessel patterns and radular teeth.

Elysia deborahae Ortea, Espinosa & Moro *in* Ortea, Caballer Moro & Espinosa, 2005

(Not figured)

Elysia patina [non Ev. Marcus 1980: 72–73, figs. 23–24, 36, 41–43, 57]—Ev. Marcus 1980: figs. 59, 60).
Elysia deborahae Ortea, Espinosa & Moro *in* Ortea, Caballer Moro & Espinosa 2005: 509–512, fig. 5, pl. 1D.
Checholysia deborahae (Ortea, Espinosa & Moro, 2005)—Ortea *et al.* 2005: 512; Espinosa *et al.* 2005: 56.

Type material. *Elysia deborahae*—holotype at IOH (no catalogue number provided).

Material examined. No specimens available.

Live animal. According to Ortea *et al.* (2005) the foot of the live animals secretes an adhesive mucus that allows the animals to attach themselves firmly to the substrate. When disturbed the animals can swim by slowly flapping the parapodia.

External anatomy. Summarized from Ortea *et al.* (2005). Body orange-brown with microscopic blue dots and small yellow tubercles. Parapodia with straight edge, pigmented with brown along its entire length or in segments. In live animal parapodia enter in contact with each other in center of body, becoming separate towards posterior and anterior ends. In area of contact there is an olive-oil elongate patch with a bright blue spot on its anterior half and snow white pigment on its posterior half. Sides of the body with 2–3 snow white conical papillae with blue pigment around their bases. Head white or orange with blue spots and two orange lines running from eyes to parapodia and several ramified green lines. Foot sole translucent orange with viscera visible through skin. Rhinophores elongate, about ¼ of total length of animal, reddish-brown with small white papillae. Pericardium globose anteriorly and elongate posteriorly extending backward beyond the center of body, pigmented with orange and white. Dorsal vessels orange, arranged in two main anterior branches that are ramified from their proximal end and two main posterior branches, which ramified closer to their distal end.

Internal anatomy. Summarized from Ortea *et al.* (2005). Radula with 15 teeth, 7 in ascending limb (plus one in development) and 7 in descending limb. Leading tooth elongate with a sharp cusp and lacking denticles. Base of tooth thickened, wide and elongate, about ½ total tooth length. Ascus with two teeth. Penis pyriform with a distal stylet, 55 μm long.

Reproduction and development. No data available.

Host ecology. Ortea *et al.* (2005) reported collecting live animals on *Halimeda* and *Lobophora* but there is no direct evidence for the actual diet of this species.

Phylogenetic relationships. No data available.

Range. Cuba (Espinosa *et al.* 2005)

Remarks. Confoundingly, Ortea, Caballer, Moro & Espinosa *in* Ortea *et al.* (2005) described *E. deborahae* but asserted that this species was synonymous with *E. patina*, referring to figures of both the type specimen (Ev. Marcus 1980: figs. 23–24) as well as a second specimen not included in the type series of *E. patina* (Ev. Marcus 1980: figs. 59–60). Ortea *et al.* (2005) inferred that Ev. Marcus (1980) had two species mixed together in her description of *E. patina*, but Ev. Marcus (1980) clearly differentiated the non-type specimen from the Bahamas (figs. 59–60) as likely representing a distinct species. If *E. deborahae* is synonymous with the type specimen of *E. patina* as figured by Ev. Marcus (1980) in figs. 23–24, then it is a junior synonym. If *E. deborahae* is instead only synonymous with the material figured by Marcus (1980) in figs. 59–60, then it could be a valid species, although the basis for this claim is uncertain as the dorsal vessel networks are not comparable.

Elysia deborahae closely resembles *E. zuleicae*, and while Ortea *et al.* (2005) presented a series of supposedly distinguishing features that separate *E. deborahae*, most do not appear to be diagnostic. A major supposed difference between *E. deborahae* and *E. zuleicae* was that a penial stylet was present in *E. deborahae* but reported to be absent from *E. zuleicae*; however, a stylet is normally present in *E. zuleicae*, so this is not a distinguishing feature. Ortea *et al.* (2005) described *E. deborahae* as having a lighter coloration, but specimens of *E. zuleicae* show tremendous intra-specific variation in external coloration (Fig. 51); some are very pale, making color a potentially unreliable character. Similarly, the parapodial margin was described as smooth in *E. deborahae* versus papillose in *E. zuleicae*, but parapodial morphology is highly variable in *E. zuleicae*.

The radular tooth of *E. deborahae* was described and figured as smooth by Ortea *et al.* (2005), and curved like that of *E. zuleicae* with a "*Halimeda* spur" morphology. Ortea *et al.* (2005) proposed two differences between the radulae of *E. zuleicae* and *E. deborahae*: (1) in *E. zuleicae*, the maximum height of the curved blade of radular teeth was attained behind the anterior edge of the base of the tooth, whereas in *E. deborahae*, the maximum height attained after the edge of the base; and (2) more teeth were present in the descending limb of the radula in *E. zuleicae*. However, the number of teeth in the descending limb depends on age, and the specimens of *E. deborahae* examined may have been juveniles (3–4 mm). Our SEMs reveal that the maximum tooth height in *E. zuleicae* is attained after the front of the base of the tooth, as described for *E. deborahae*, and not before the front edge as was reported for *E. zuleicae*; therefore, no radular character distinguishes *E. deborahae*.

An anatomical character that was also proposed to differentiate *E. deborahae* from *E. zuleicae* was the absence of an extended tail in *E. deborahae*. In *E. zuleicae*, a narrow, pointed tail usually extends beyond the point where the posterior edge of the parapodia fuse with the dorsal surface of the body. However, half the specimens of *E. zuleicae* we examined from the U.S. Virgin Islands lacked a tail, yet were genetically indistinguishable from the tailed specimens; indeed, as our figured specimens show (Fig. 51), there is great intra-specific variability in tail size, color and even presence in *E. zuleicae*. It is possible that there is a species similar to *E. zuleicae* with smooth, curved radular teeth and no tail, but we have sampled no such species. We describe a species (see below) that is sister to *E. zuleicae*, lacks a tail, but that has serrated, curved teeth, making it distinct from *E. deborahae*.

Elysia jibacoaensis Ortea, Caballer & Espinosa *in* Ortea, Moro, Caballer & Espinosa, 2011

(Not figured)

Elysia jibacoaensis Ortea, Caballer & Espinosa *in* Ortea, Moro, Caballer & Espinosa 2011: 203–205, pls. 3–4 (Type locality: Playa de Jibacoa, Provincia Mayabeque, Cuba)—Ortea *et al.* 2013: 184, pl. 14, figs. A–B.

Type material. *Elysia jibacoaensis*—holotype at IESH (no catalogue number provided).

Material examined. No specimens available.

Live animal. According to Ortea *et al.* (2011) the live animals are very active and move around continuously by expanding the anterior border of the foot beyond the head and then retracting it. This species is unable to swim, but can hang from the water surface by bending the posterior end of the foot forward forming a black "V"-shape. According to Ortea *et al.* (2013) when resting, the animal flattens its body "opening the parapodia and shrugging back the rhinophores, or it flattens and rolls on itself as a spiral turn" [sentence of unclear meaning].

External anatomy. Summarized from Ortea *et al.* (2011, 2013): Color pale olive green with red dots regularly arranged all over body and foot sole, except for rhinophores. Sides of body with white dots and small white papillae. Head with a triangular white patch and red dots. Rhinophores with a vertical black line, a few light green papillae and a large white spot with orange tinges in center. Parapodia forming a large siphonal opening in center of body just behind pericardium and a smaller one posteriorly. Siphonal openings whitish, with round white spots alternating with black areas, and brownish-orange pigment. Parapodial margin thin, with a black marginal line and a fragmented submarginal orange line. Edges of parapodia ornamented with a row of transparent papillae, each one having a white granule inside. Pericardium oval with white and red spots and patches. Inner side of parapodia light green, also with white and red spots. There are only two large dorsal vessels (only visible in the preserved animals) that merge together near posterior end of body and have faint branches towards center of body.

Internal anatomy. Summarized from Ortea *et al.* (2011). Radula with 11 teeth, 4 in ascending limb (plus one in development) and 7 in descending limb. Ortea *et al.* (2011: pl. 4, fig. D) illustrated teeth as having an elongate, sharp cusp, with no denticles and a "V"-shaped housing depression. Ascus with 2-5 teeth forming a spiral or whorl. Penis not described.

Reproduction and development. No data available.

Host ecology. *Elysia jibacoaensis* may be associated with the green alga *Penicillus* (Ortea *et al.* 2013).

Phylogenetic relationships. No data available.

Range. Cuba (Ortea *et al.* 2011); Guadeloupe (Ortea *et al.* 2013)

Remarks. The species description was based only on external and radular anatomy. Teeth were slightly curved, smooth and pointed, and were therefore distinct from other *Penicillus*-eaters (*E. papillosa*, *E. taino* **n. sp.**), which have coarsely serrated teeth. This appears to be a distinct species, but we could not obtain specimens to verify its taxonomic status and phylogenetic relationships.

Elysia orientalis Ortea, Moro & Espinosa *in* Ortea, Moro, Caballer & Espinosa, 2011
(Fig. 56B)

Elysia orientalis Ortea, Moro & Espinosa *in* Ortea, Moro, Caballer & Espinosa 2011: 205–206, pl. 5 (Type locality: Playita de 14-16, Miramar, Havana, Cuba).

Type material. *Elysia orientalis*—holotype at IESH (no catalogue number provided).

Material examined. Blue Heron Bridge/Phil Foster Park, Lake Worth Lagoon, Florida, USA, 8 March 2013, 1 specimen (CPIC 00842).

Live animal. The live animal moves actively while keeping the parapodia open.

External anatomy. Ortea, Moro & Espinosa *in* Ortea *et al.* (2011) described this species as snow white dorsally and pinkish laterally, with numerous red dots and scattered blue dots as well as some white bumps. Two longitudinal green striations posterior to the eyes and a transverse green band posterior to the parapodia. Rhinophores translucent with two white bands. Body rhomboidal with "flat" parapodia raised posteriorly. Video of the specimen we examined from Florida shows a small *Elysia* with a general opaque white color and darker pigment. The body is short, with a well-differentiated tail. Rhinophores translucent with two transversal opaque white bands. Parapodia forming ovoid side flaps.

Internal anatomy. Radula lost during preparation; not described by Ortea *et al.* (2011). Penis small and curved (CPIC 00842) with no apparent armature (Fig. 56B).

Reproduction and development. No data available.

Host ecology. No data available.

Phylogenetic relationships. A specimen conforming to the description of *E. orientalis* from Florida (CPIC 00842) was sequenced, and was genetically distinct from all other Caribbean species of *Elysia* studied in the

present work; however, further morphological analysis is needed. Our *E.* cf. *orientalis* was sister to the rest of subclade 4, the *E. tomentosa* complex, although without significant support in the ML analysis.

Range. Cuba (Ortea *et al.* 2011); Florida (present study)

Remarks. Our specimen from Florida matches the original description of the holotype from Cuba. However, further specimens are needed to better characterize this poorly known and recently described species, for which detailed data on development, ecology and internal anatomy are lacking.

Elysia sarasuae Ortea & Espinosa *in* Ortea, Moro, Caballer & Espinosa, 2011

(Not figured)

Elysia sarasuae Ortea & Espinosa *in* Ortea, Moro, Caballer & Espinosa 2011: 206, pl. 6 (Type locality: Playa de Rancho Luna, Cienfuegos, Cuba), ***nomen dubium***.

Type material. *Elysia sarasuae*—holotype at IESH (no registration number provided).

Material examined. No specimens available.

Live animal. No specimens available.

External anatomy. Summarized from Ortea *et al.* (2011). Head orange anteriorly and snow white with white papillae posteriorly with orange sides. External sides of the parapodia dark green; internal sides also green but paler, with bright blue lines or stripes. Rhinophores wide, brown externally with a few white papillae distally. Parapodial borders straight, dark brown, with a few white papillae. Pericardium horseshoe shaped, orange. Dorsal vessels not described.

Internal anatomy. Not described by Ortea *et al.* (2011).

Reproduction and development. No data available.

Host ecology. No data available.

Phylogenetic relationships. No data available.

Range. Cuba (Ortea *et al.* 2011)

Remarks. This species is impossible to recognize from the description, which included only external characters, a sketch with no anatomical detail, and a low-resolution photograph of a tiny specimen. Although *E. sarasuae* was not compared to any other species in its description, the external anatomy as drawn looks very similar to *E. zuleicae*.

Elysia ellenae Ortea, Espinosa & Caballer *in* Ortea, Espinosa, Buske & Caballer, 2013

(Figs. 54–55, 56C, 57)

Elysia ellenae Ortea, Espinosa & Caballer *in* Ortea, Espinosa, Buske & Caballer 2013: 185–188, pl. 11 (Type locality: South of Port Louis and west from Petit Canal, Guadeloupe); Krug *et al.* 2015: 990, fig. 3B.
Thuridilla sp.—Redfern 2013: 288, fig. 797.
Elysia sp. 4—Turner *et al.* 2012: 54.

Type material. *Elysia ellenae*—holotype (MNHN IM-26975), paratype (MNHN IM-26976).

Material examined. Bahamas: Stocking Island, Exumas, Bahamas, 15 January 2009, 1 specimen (CPIC 00071), Goulding Point, New Providence, 13 July 2010, 1 specimen (LACM 178663). Photographs of four additional specimens from Cayman Islands (courtesy of Everett Turner) examined.

Live animal. According to Ortea *et al.* (2013) "this species moves crawling, not jumping, not swimming. When at rest, it flattens and expands the parapodia, taking the appearance of a heart-shaped leaf."

External anatomy. Head white. Light blue patch or streak above each eye, like an eyebrow, extending part way up rhinophores on some specimens (Fig. 54A–C, E–F). Black row of dots forming "moustache" above upper lip of mouth (Fig. 54D). Rhinophores short relative to length of animal, and wider at tip than base. Rhinophores papillose; overall color white, with green digestive diverticula scattered across anterior surface. Green digestive diverticula scattered across anterior face of rolled rhinophores. Rose-red or brick-red spot at midpoint of rhinophores; red patch or curved line located at base of rhinophores, just anterior to each eye (Fig. 54C).

Foot white with sparse diverticula visible, and covered with scattered patches of light blue iridescence (Fig. 54D). Transverse groove extends halfway across left side of foot but does not fully delimit head from foot. No distinct tail; foot narrowing to a point, where ends of parapodia fuse.

Parapodia notably thick, separated at anterior edge by gap. Parapodia form three siphonal openings on living animal, with slightly undulating margins (Fig 54A, C, F–G). Overall color white. Outer face of each parapodium has two distinct halves: bottom half penetrated by green diverticula, and spotted with large white papillae, irregularly spaced; top half lacks visible diverticula, dotted with small white papillae except for smooth band just below parapodial margin. Entire outer parapodial face uniformly dotted with minute red spots. Parapodial margin with dark red band and row of regularly spaced yellow spots; marginal edge thickened, with regularly spaced white papillae. Faint submarginal band of iridescent blue may be present on outer parapodial surface (Fig. 54C, G). Inner face of parapodium dotted with white papillae and dark green diverticula. Inner submarginal band of iridescent light blue, widest inside siphonal openings.

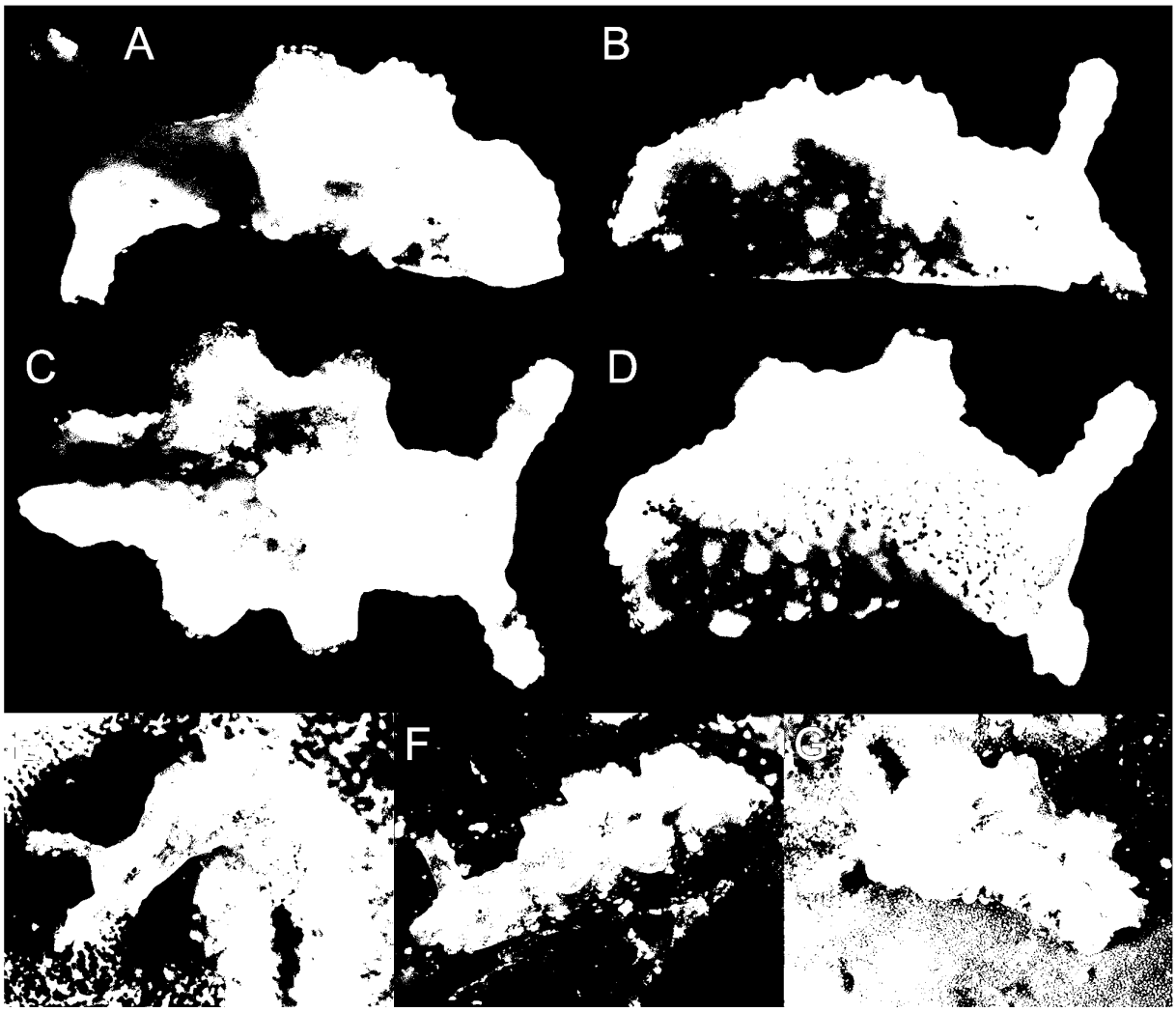

FIGURE 54. *Elysia ellenae*, external morphology of live specimen from New Providence, Bahamas (**A–D,** Eell_10NPr01) and additional specimens from the Cayman Islands (**E–G,** images courtesy of Everett Turner). **A,** Live specimen immediately following collection, showing characteristic red and blue patches above eye, three siphonal openings formed by the parapodia, and the red and blue bands along the parapodial margin. Length of slug = 5 mm. **B,** Side view showing dark green digestive diverticula penetrating the distal (bottom) half of the right parapodium. **C,** View of dorsum showing rounded pericardium, from which red-speckled dorsal vessels extend out to the parapodial margin. **D,** Pedal view, showing digestive and blue iridescent spots on the pointed foot. **E–G,** Three different specimens photographed underwater in the Cayman Islands, showing individual variation in the degree to which parapodia are papillate, pigmented, and penetrated by digestive diverticula.

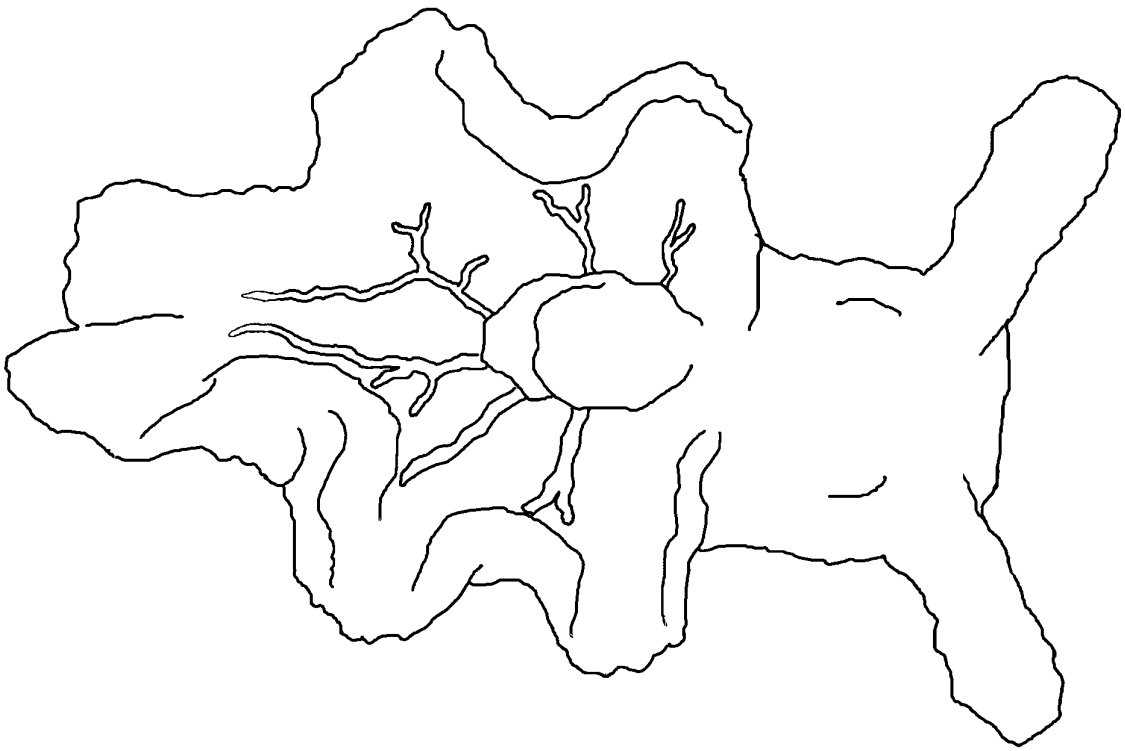

FIGURE 55. *Elysia ellenae*, drawing of the pericardium and dorsal vessels of a preserved specimen (LACM 178663; 3.5 mm long).

Pericardium large, white, round; completely filling anterior siphonal opening (Fig. 54C). Pericardium swelling to height of anterior parapodial edge, clearly visible as dorsal bulge on living animal. Renopericardial extension running about one-third of body length, more grey in color than pericardium. In a specimen used for DNA analysis, three dorsal vessels radiating from right side of renopericardial complex, and two vessels from left side, outlined in red spots (Figs. 54C, 55). Vessels unbranched or forking once terminally, except elongated posterior vessels having two lateral side branches reaching up to parapodial margin, respectively at midpoint and posterior end of second siphonal fold of parapodium.

Internal anatomy. Radula with 11–14 teeth (CPIC 00071, LACM 178663), 5–6 teeth in ascending limb and 6–8 in descending limb (Fig. 57A). Leading tooth elongate and robust with cusp bearing a finely denticulate keel and smooth lateral edges on each side (Fig. 57B). Housing depression for interlocking teeth "V"-shaped and extending ½ of tooth lengh. Base of tooth ½ to ⅓ total tooth length. Ascus containing three slightly smaller discarded teeth, according to Ortea *et al.* (2013)

Penis curved and elongate with rigid musculature that did not deform after drying (Fig. 54C), bearing a resistant, hollow tip (Fig. 57C) visible by SEM, but not light microscopy. Deferent duct long, thin, and convoluted.

Reproduction and development. No data available.

Host ecology. *Elysia ellenae* was not found associated with any algal host in either the Bahamas or Cayman Islands, and no food source has been reported.

Phylogenetic relationships. In phylogenetic analyses, *E. ellenae* was recovered as sister to *Elysia crispata*, while the eastern Pacific *E. diomedea* was sister to (*E. ellenae* + *E. crispata*) (Fig. 4). The morphological similarity of *E. ellenae* to *E. crispata* is consistent with their phylogenetic affinity. The clade comprising *E. diomedea*, *E. crispata* and *E. ellenae* is a derived member of subclade 2. The nested position of the clade containing *E. ellenae* suggests an ancestral lineage colonized the Caribbean from the northwestern Atlantic, after which closure of the Isthmus of Panama isolated the ancestor of *E. diomedea* in the eastern Pacific from the last common ancestor of *E. ellenae* and *E. crispata*, which subsequently diverged in the Caribbean.

Range. Bahamas: Abaco (Redfern 2013), New Providence (present study), Stocking Island (present study), Exumas (present study), Cayman Islands (present study); Guadeloupe (Ortea *et al.* 2013).

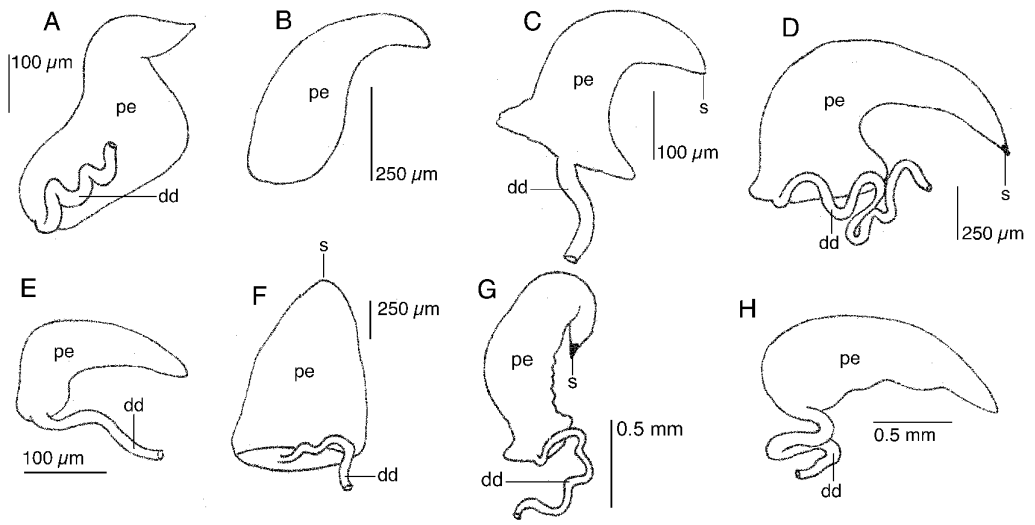

FIGURE 56. Penial morphology of some species examined. **A,** *Elysia buonoi* **n. sp.** (LACM 178675) **B,** *Elysia orientalis* (CPIC 00842). **C,** *Elysia ellenae* (LACM 178663). **D,** *Elysia pawliki* **n. sp.** (LACM 3303). **E,** *Elysia christinae* **n. sp.** (LACM 3309). **F,** *Elysia zemi* **n. sp.** (LACM 3307). **G,** *Elysia taino* **n. sp.** (LACM 178607). **H,** *Elysia hamanni* **n. sp.** (LACM 178667). Abbreviations: dd, deferent duct; pe, penis; s, stylet.

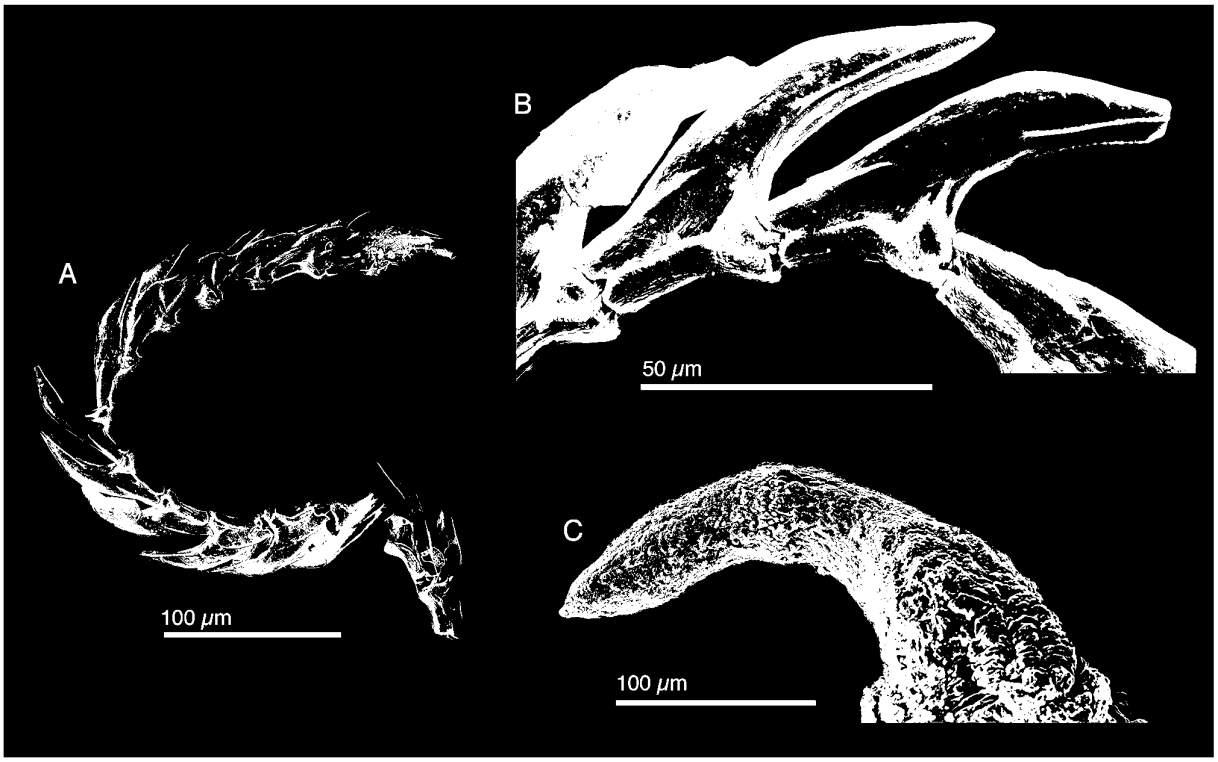

FIGURE 57. *Elysia ellenae* SEM of the radula and penis (LACM 178663). **A**, Complete radula. **B**, Leading tooth. **C**, Penis.

Remarks. Where visible, major features described from the Bahamas specimen were also present on photographs of additional specimens from the Cayman Islands and type material depicted from Guadeloupe, including the distinctive red and blue patches on the head, and bright iridescent blue marginal band. Morphologically, *E, ellenae* resembles its sister taxon *E. crispata* but has a much larger and more rounded pericardium and thicker parapodia. *Elysia crispata* has more undulating parapodia, and lacks the distinct siphonal openings of *E. ellenae*.

The probable presence of an apical penial stylet in *E. ellenae* but not in *E. crispata* suggests that divergence in reproductive armature may have been involved in the speciation process for these sister taxa, which have sympatric ranges. Confusion about this structure qualifying as a true stylet has to do with the penis apex bearing a resistant tip, but no obvious barb, spike, or scoop. However, as Gascoigne (1974) defined a penial stylet as simply "a hollow, cuticular extension of the vas deferens," we conclude that *E. ellenae* possesses a stylet.

Patchy coverage of the foot by thick digestive diverticula also distinguishes *E. ellenae* from both the typical morph of *E. crispata*, which lacks diverticula in the foot, and the '*clarki*' morph, in which the foot has an overall green appearance due to uniform coverage by small diverticula. In *E. crispata*, the foot is also more blunt-ended rather than tapering to a point as in *E. ellenae*.

Interestingly, although the algal host of *Elysia ellenae* is unknown, feeding in this species causes marked wear on its radular teeth (Fig. 57B). It is clear that such wear is the outcome of feeding because the leading tooth has a blunt tip, but teeth in the ascending radular row do not. Evidence of extreme tooth wear was not observed in any other Caribbean elysiid.

Elysia pawliki new species

(Figs. 56D, 58–60)

Elysia papillosa [non Verrill, 1901]—Thompson 1977: figs. 26, 27; Espinosa & Ortea 2001: 44; Espinosa *et al.* 2005: 56; Ortea *et al.* 2005: 498–502 (part), figs. 1–2, pl. 1, fig. A; Redfern 2001: 162, figs. 672A–F; Collin *et al.* 2005: 690; Valdés *et al.* 2006: 65.

Elysia sp. A—Redfern 2013: 286–287, figs. 793A–B.

Elysia cf. *tomentosa* sp.2—Krug *et al.* 2013: 1109-1113, figs. 2C, 4; Krug *et al.* 2015: 990, fig. 3B.

Type material. Sweetings Cay, Bahamas, 2003, (Holotype LACM 3303), collected by PJK; Abaco Islands, Bahamas, 2003, (Paratype LACM 3304), collected by Colin Redfern.

Type locality. Sweetings Cay, Bahamas

Material examined. Bahamas: Abaco Islands, 2003, 2 specimens (Paratype LACM 3304, LACM 178674); Sweetings Cay, 2003, 1 specimen, 35 mm long alive (isolate Epaw_03Swe01, Fig. 58A); July 2007, 1 specimen, 25 mm long alive (Holotype LACM 3303, isolate Epaw_07Swe01, Fig. 58B–E).

Live animal. Both Sweetings Cay specimens were collected four years apart in the same tidal channel, which opens to a mangrove lagoon. Slugs were collected off a large clump of *Caulerpa racemosa*. Resting slugs held their parapodia apart, forming a series of siphonal openings (Fig. 58A–B). One specimen was observed to associate, and potentially attempt to mate with, a large *E. subornata* when the two were held together in a container. Slugs often rested with their head tucked inside their expansive parapodial flaps. Both specimens from Sweetings Cay, Bahamas were observed in the laboratory for 3–4 weeks after collection; neither slug was ever observed to swim or flap its parapodia when disturbed. When stressed, slug released a cloud of iridescent blue-white mucus from the parapodial margin, and contorted its parapodia, but did not swim.

External anatomy. Overall coloration yellow-green with patches of brown. Body turning dark green after feeding, due to digestive diverticula ramifying throughout body, head and parapodia, visible through epidermis at low density. Overall body shape dominated by large parapodia with series of three laterally extended side-flaps folding away from body (Fig. 58A–B). When parapodia close over dorsum, flaps create three siphonal openings, the middle being the largest. Anterior-most opening small, representing little more than a fold over pericardium. When held open, middle parapodial flaps extending out roughly as wide, tip to tip, as body length from pericardium to tail. Elongated middle flaps forming largest opening when parapodia are closed, giving live animal a cruciform appearance. Third, posterior-most pair of parapodial flaps intermediate in size. Larger specimens with 2–3 additional siphonal openings present along posterior half of body, including a posterior pair of laterally extended parapodial flaps; examples include isolate Epaw_03Swe01 (Fig. 58A), and a specimen from Jamaica in the BMHN labeled "*E. papillosa*" by T. E. Thompson, 30 mm long. Exterior surface of parapodia covered by elongate, white papillae rising from tan-brown patches of pigment (Fig. 58C). Upper portion of parapodia grey-white inside and out, with occasional blotches of plum color; one large plum-color patch appearing on anterior-most parapodial flap near margin. Brown band running along inner and outer edge of parapodial margin. Row of

papillae extending straight up from edge, running to end of parapodia; marginal papillae grey-brown tipped with white. Interior of parapodia featuring white patches, pink at center, scattered about.

Outer surface of body heavily papillose. Irregular, white patches like lichen covering head and upper portion of parapodia; long, conical papillae rising out of these white patches, spotted with irregular small patches of pink. Glands appearing as scattered brown spots cover sides of parapodia, head and rhinophores; black-edged openings slightly elevated above surface of epidermis, with brown spherical inclusion (gland) lying beneath, discharging heavy mucus secretion when animal is alarmed.

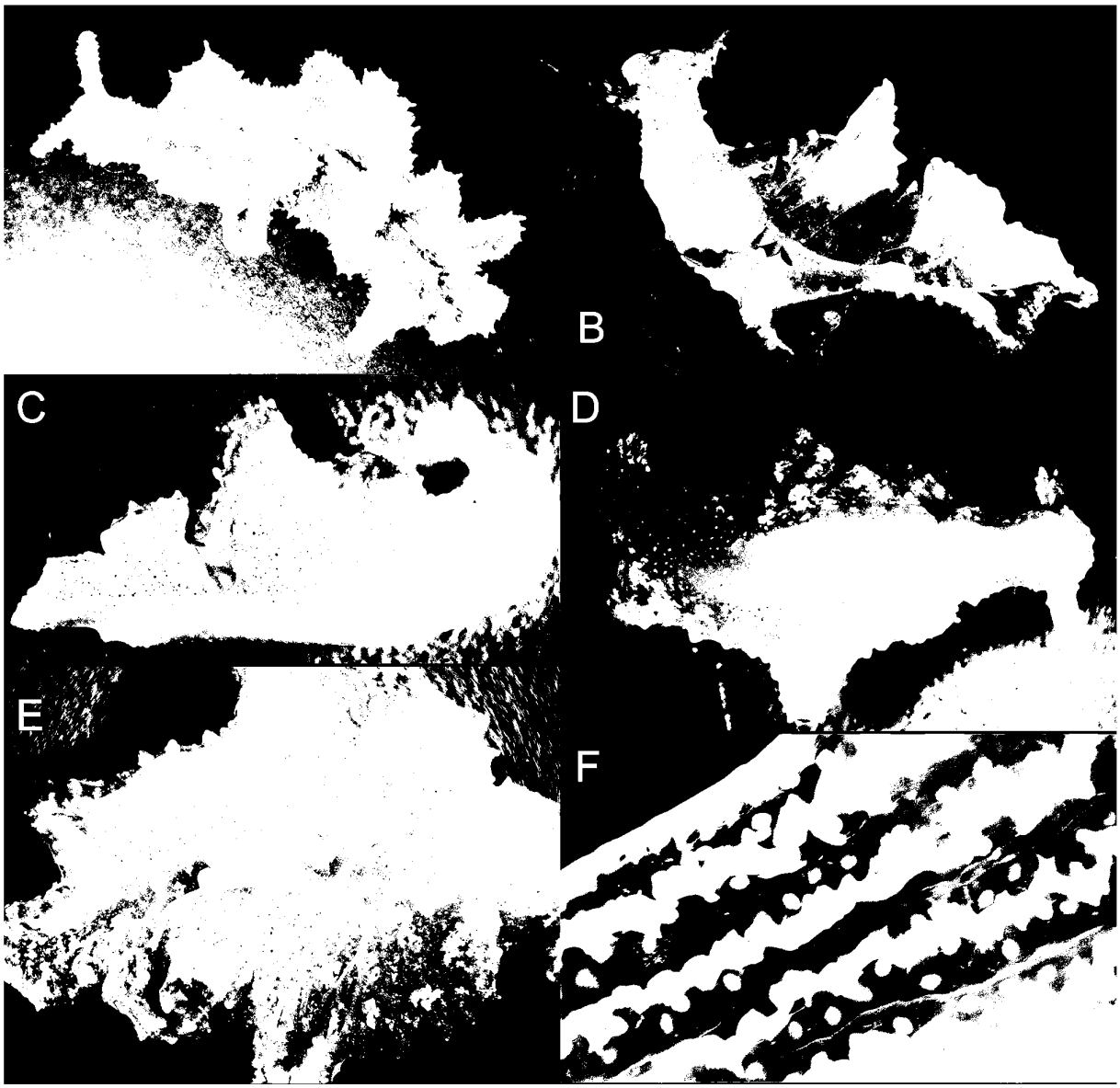

FIGURE 58. *Elysia pawliki* **n. sp.**, external morphology and egg mass of specimens from Sweetings Cay, Bahamas. **A,** Live specimen with six siphonal openings formed by parapodial flaps (LACM 3303; length = 35 mm). **B–E,** Live specimen viewed from above (B), right side (C), underneath (D), or with parapodia spread open (E) to show renopericardial complex and dorsal vessels (isolate Epaw_07Swe01; length = 25 mm). Purple egg masses of parasitic copepod visible. **F,** Egg mass (deposited by specimen LACM 3303), showing orange ECY ribbon and early-stage embryos. Field of view = 3 mm.

FIGURE 59. *Elysia pawliki* **n. sp.**, drawing of the renopericardial complex and dorsal vessel network traced from a photograph of a live animal (isolate Epaw_07Swe01; 2.5 cm long). Grey shapes are egg masses of parasitic copepod.

Front of head covered by large patches of tan, white and pink, over yellow-green background color. Eyes tiny, located in patch of background coloration posterior to base of rhinophores. Upper lip split into two curved sections; lower lip flattened, broader. Diverticula extend into both top and bottom lips. Upper lip with moustache of brown spots. Rhinophores short relative to body length (3 mm at maximum extension on 25 mm animal); rolled, blunt-ended, with long white papillae. Surface of rhinophores white-brown, dotted with dark brown spots (glands) and pink spots of equal size, and occasional irregular white patches. Faint, longitudinal white stripes run tip to base. At base, inner surface of rhinophores penetrated by green digestive diverticula, but outer surface white and devoid of diverticula.

Foot with same yellow-green or brown color as rest of body, with rows of minute white papillae but no brown glands or white patches (Fig. 58C–D). Transverse groove separating underside of head from foot, opening into wider genital groove on right side of head, with a white genital aperture at the top of the groove. End of foot wide, blunt, not narrowing to a tip; no extended tail.

Pericardium large, rounded, white-pink patch on top, dotted with brown glands. Thick renopericardial extension runs to halfway point of body, between second and third parapodial flaps (Fig. 58B, E). Dorsal vessels asymmetric; type specimen with four vessels emerging on left side, five on right side of pericardial complex (Fig. 59). Vessels branch and anastomose forming complex network running up to inner parapodial margin. Main branch of elongated posterior vessel running to tail on each side; posterior vessel otherwise notably asymmetric in placement and branching pattern. Vessels run under, or terminate in, papillae that dot inner parapodial surface.

Internal anatomy. Radula with ~24 teeth (LACM 3303, LACM 3304), 6 teeth in ascending limb and ~18 in descending limb (Fig. 60A). Leading tooth wide and flat, with fine, blunt denticles on cusp, with slightly rounded apex (Fig. 60B, E). Housing depression for interlocking teeth "V"-shaped and extending ⅔ of tooth length. Base of tooth approximately ¼ total tooth length. Ascus of small teeth in a single row with some jumbled teeth at the end (Fig. 60F).

Penis elongate and curved (Fig. 60C) with rigid musculature resistant to desiccation and tapering distally into a conical apex bearing a resistant, hollow tip (Fig. 60D). Penial stylet is not a scoop or barb; hardened penial tip visible by SEM, but not light microscopy. Deferent duct long, narrow, and highly convoluted.

Reproduction and development. One egg mass was produced by specimen 03Swe01 (Fig. 58F). The egg strand was wound in a typical elysiid spiral on the surface of the bubble-like "grapes" of the host alga *C. racemosa*.

Egg capsules alternated around a continuous, thick ribbon of bright orange ECY on the upper surface of the egg strand, under the outer covering of the egg mass. The ribbon was molded around each individual capsule. The clutch released swimming, lecithotrophic veliger larvae with eyespots; neither egg nor larval shell size was obtained, however.

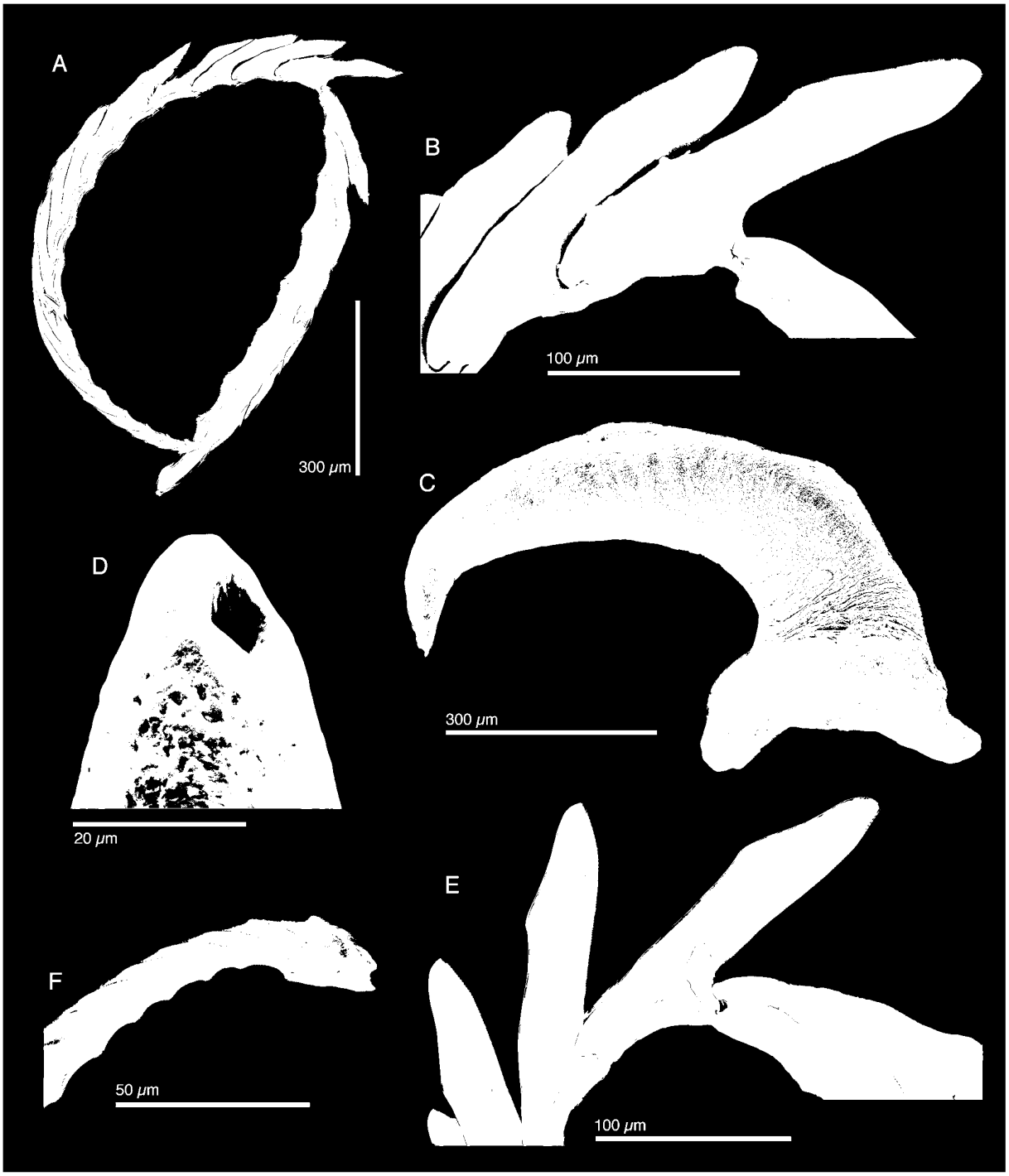

FIGURE 60. *Elysia pawliki* **n. sp.**, SEM of the radula and penis. **A**, Radula (isolate Epaw_07Swe01). **B,** Leading tooth (isolate Epaw_07Swe01). **C,** Penis (isolate Epaw_07Swe01). **D,** Detail of the penis tip (isolate Epaw_07Swe01). **E,** Leading tooth (LACM 3304). **F,** Ascus (LACM 3304).

Host ecology. Both live specimens of *E. pawliki* **n. sp.** were recovered from *Caulerpa racemosa*, on which both specimens fed readily in the laboratory. Slugs became green upon feeding, but reverted to a brown color after a few days without food. Specimens collected at different times from the Bahamas each had one pair of vivid purple egg masses of a parasitic copepod poking out of the dorsal surface near the pericardium; (Fig. 58B, E). Although copepods parasitize a wide range of opisthobranchs, the purple eggs of this unknown copepod species were only observed on the related *E. zemi* **n. sp.** and not on any other sacoglossan over the past 12 years, suggesting a specialized relationship.

Phylogenetic relationships. *Elysia pawliki* **n. sp.** belongs to the *E. tomentosa* species complex, together with at least five distinct species from the Indo-Pacific, and three sympatric Caribbean species: *E. subornata, E. pratensis* and *E. zemi* **n. sp.** (Fig. 4). All species in this clade feed on *Caulerpa* spp., except *E. pratensis*. We recovered as sister to *E. pawliki* **n. sp.** an undescribed, morphologically similar species collected from *Caulerpa cupressoides* in Australia and an unknown *Caulerpa* sp. in Thailand (*Elysia* cf. *tomentosa* sp. 5; Krug *et al.* 2013). Both species are brown, highly papillose, and share a characteristic cruciform body shape due to the wide lateral extension of the parapodia. Molecular data were not available for the morphologically similar species *E. manriquei* Ortea & Moro, 2009 from the Canary Islands.

Range. Bahamas (Redfern 2001, 2013; present study), Costa Rica (Espinosa & Ortea 2001), Venezuela (Valdés *et al.* 2006)

Etymology. Named in honor of colleague and "evil twin" Joseph R. Pawlik, in recognition of his landmark achievements studying Caribbean reef ecosystems, and the larval and chemical ecology of sea slugs. Without the opportunity to participate in four research cruises on which Dr. Pawlik was Chief Scientist, the present study would not have been possible, and the holotype specimen would not have been collected.

Remarks. Both Thompson (1977) and Ortea *et al.* (2005) described material most closely matching *E. pawliki* **n. sp.** (but potentially also *E. zemi* **n. sp.**) as *E. papillosa*. However, *E. pawliki* **n. sp.** has many features that are incompatible with the details provided by Verrill (1901) in his description of *E. papillosa*. The only similar feature shared by both species is a highly papillose body surface, but the papillae of *E. pawliki* **n. sp.** are notably different from the "small conical papillae" described and figured by Verrill (1901) for *E. papillosa*. Ortea *et al.* (2005) claim that Verrill's illustration shows branching, digitiform papillae, but they misinterpreted Verrill's drawing (Verrill, 1901: pl. 4, fig 3), which shows the specimen of *E. papillosa* sitting on a branching stipe of the alga *Halimeda incrassatta*; the alga was interpreted as papillae on the slug by Ortea *et al.* (2005). Further, although Verrill's type material from the Bermuda expedition was lost, we found among Verrill's surviving material a preserved specimen of *E. pawliki* **n. sp.** marked "*Elysia* sp."; thus, Verrill considered *E. pawliki* **n. sp.** to be distinct from his *E. papillosa*. Moreover, *E. pawliki* **n. sp.** has never been reported from the type locality of *E. papillosa* (Bermuda).

A major behavioral difference further confirms that *E. pawliki* **n. sp.** is distinct from *E. papillosa* Verrill 1901, which was described as swimming freely using its parapodia. Two live specimens of *E. pawliki* **n. sp.** were observed over a period of weeks, but never swam no matter the degree to which they were disturbed; thus, *E. pawliki* **n. sp.** cannot be *E. papillosa* Verrill 1901. At least five Caribbean species swim when disturbed by flattening and rapidly undulating their thin parapodia; all five belong to subclade 1, and are phylogenetically distinct from *E. pawliki* **n. sp.**

Morphologically, no named Caribbean species is similar in gross anatomy to *E. pawliki* **n. sp.** In *E. pawliki* **n. sp.**, as in most species belonging to subclade 4, the renopericardial extension runs about halfway down the body, whereas the renopericardial extension of related species *E. subornata* and *E. pratensis* runs the whole body length. The most similar Atlantic species is *E. manriquei* Ortea & Moro 2009 from the Canary Islands off West Africa. In addition to its east Atlantic type locality, *E. manriquei* differs from *E. pawliki* **n. sp.** in its external morphology in having symmetrical and non-anastomosing dorsal vessels, large black spots dotting the entire body and head, shorter rhinophores, and more vertically exaggerated siphonal openings. The radular teeth are also markedly different. The tooth of *E. manriquei* has a flat, serrated cutting edge, and a curving top edge; in *E. pawliki* **n. sp.**, both the cutting and non-cutting surfaces taper together towards a rounded tooth tip.

The radular teeth of *E. pawliki* **n. sp.** are most similar to those of *E. zemi* **n. sp.** in overall morphology, both bearing fine denticles and possessing a sharp tooth tip on a rounded apex, and downward-facing angle at ⅔ the length of the tooth. The teeth of *E. pawliki* **n. sp.** are distinct in having straighter tooth cusps and less club-like tooth apices. Like in *E. ellenae* and *E. zemi* **n. sp.** the resistant penial tip in *E. pawliki* **n. sp.** is not scoop or barb-like, but based on Gascoigne's (1974) description we refer to it as a stylet.

Developmentally, all members of the *E. tomentosa* clade studied to date have orange ECY ribbons. However, the lecithotrophic larvae of *E. pawliki* **n. sp.** swim upon hatching; thus, development mode (pelagic lecithotrophy) differentiates *E. pawliki* **n. sp.** from *E. subornata* and *E. pratensis* (non-pelagic lecithotrophy with encapsulated metamorphosis), and from its Pacific sister species *E.* cf. *tomentosa* sp. 5, which is planktotrophic (Krug *et al.* 2013). In terms of host ecology, *E. pawliki* **n. sp.** feeds on *Caulerpa racemosa*, which is also consumed by most related species. The co-occurring *E. subornata* feeds on several *Caulerpa* spp. including *C. racemosa*, but is much more common throughout the Caribbean.

Elysia zemi new species

(Figs. 56F, 61–63)

Type material. Martinique, 5 March 2014, (Holotype LACM 3305), collected by Yan Buske; Cul de sac du Marin, Martinique, 1987, (Paratype LACM 3306), collected by Jeff Hamann; Martinique, 14 March 2013, (Paratype LACM 3307), collected by Yan Buske.

Type locality. Martinique.

Material examined. Martinique, 5 March 2014, 1 specimen, 12 mm long × 10 mm wide (Holotype LACM 3305), 14 March 2013, 1 specimen, 16 mm long × 14 mm wide (Paratype LACM 3307), 2 February 2014, 1 specimen (isolate Ezem_14Mar02); Cul de sac du Marin, 1987, 1 specimen, 12 mm long (Paratype LACM 3306, LACM 178664–65); Petit Nevis Island, Saint Vincent and the Grenadines, January 1987, 1 specimen (LACM 178666). Underwater photographs of live specimens from the Cayman Islands were also examined, courtesy of Evertt Turner.

Live animal. Specimens were collected subtidally in association with the alga *Caulerpa racemosa*. Parapodia were often held open by the resting animal, exposing the dorsum (Fig. 61A–B).

External anatomy. Overall coloration mottled orange-brown overlaying dark green (Fig. 61A–C), ranging to dark red (Fig. 61D, F), with pinkish-rose or whitish patches on some specimens (Fig. 61E). Body shape dominated by large parapodia with two pairs of laterally extended side-flaps with rounded margins. When parapodia close over dorsum, flaps create three siphonal openings—a small anterior opening over the pericardium, and two prominent openings at the middle and posterior end of the body (Fig. 61A–B, D, F). These large siphonal openings may be same size, or posterior opening smaller. Elongated middle flaps giving cruciform appearance to live animal when held open. Outer surface of body, head and rhinophores heavily dotted with elongated, hair-like, branching papillae, grey-white in color, often splitting into 2–4 branches with the central branch the longest (Fig. 61A–B, D, F). Papillae imparting a hairy appearance to live animal. Posterior of body narrows to blunt end or slightly pointed tip, but no extended tail.

Head rounded in front. Top of head bearing species-diagnostic feature, a triangular or acorn-shaped patch of light grey-purple pigment, with anterior point placed medially along head and directly between eyes; posterior end narrowing slightly just before pericardium. Second patch of light grey-purple situated between rhinophores, sometimes extending anteriorly onto face, or posteriorly toward top of head. Paired white patches on upper sides of head, one above each eye, running alongside the region between the two purple patches (usually of background color) (Fig. 61B–C, E–F). Irregular pigment patches like lichen may occur on sides or top of head, ranging from white to pink to grey (Fig. 61E). Eyes tiny, located in patch of background coloration posterior to base of rhinophores, sometimes directly under a white pigment patch located on either side of the central purple patch atop head. Rhinophores short relative to body length, rolled, tips blunt-ended or rounded; may be held at 90° angle to head on crawling specimens, giving hammerhead shark-like appearance (Fig. 61A). Rhinophores matching background body color but with light purple or grey patches at tips, and bearing rows of long white papillae.

Exterior surface of parapodia uniform in color, but dotted with elongate, white papillae of varying lengths, mostly unbranched, densely covering sides of some specimens but sparse on others. Parapodial margin thin and tan, with submarginal band of brown or dark orange. Row of elongated, thin papillae running along margin, white to clear, sometimes with swollen white tips (Fig. 61A–B, D, F). Tiny, scattered, dark brown glandular inclusions dotting exterior and interior of parapodia. Interior of parapodia with scattered white flecks across whole surface.

Pericardium with prominent and distinctive white round patch on top (Fig. 61A–B, D, F). Thick renopericardial extension same color as dorsum, difficult to see, running halfway down body to start of second

parapodial side-flap. Irregular splotch of dark pigment (brown to black) forming band on dorsum immediately posterior to end of renopericardial extension, followed by larger irregular white patches of pigment.

Dorsal vessels asymmetric, with five to seven vessels per side emerging from renopericardial complex (Fig. 62). Vessels branch into anastomosing network covering most inner parapodial surface. Elongated posterior vessel running to tail and sending off numerous lateral side branches which run to parapodial margin. Vessels clear on some specimens (Fig. 61A), but notably lighter in color (Fig. 61B) or much darker than dorsal surface (Fig. 61D, F) on other specimens.

Internal anatomy. Radula with ~24 teeth (LACM 3307, LACM 178664, LACM 178666), 5–7 teeth in ascending limb and ~14–17 in descending limb (Fig. 63A, F). Leading tooth wide and flat, with fine, blunt denticles on slightly convex cusp, with pointed tip on club-shaped apex (Fig. 63B, G). Housing depression for interlocking teeth extending approximately ⅔ of the tooth length. Base of tooth approximately ⅓ total tooth length. Ascus of small teeth in a variable spiral or whorl (Fig. 63C, H).

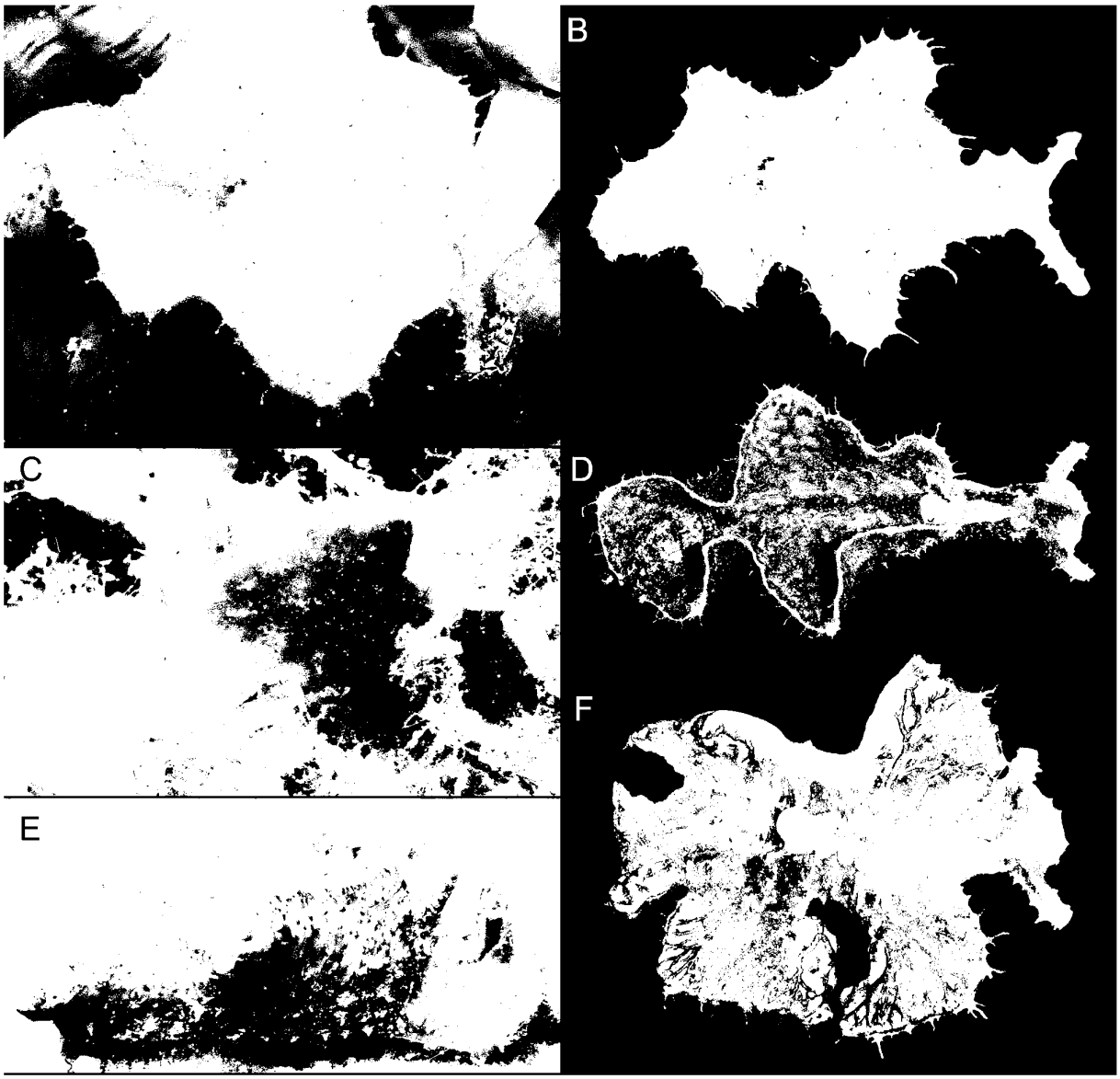

FIGURE 61. *Elysia zemi* **n. sp.**, external morphology of specimens from Martinique. **A–B,** Dorsal view of specimens with parapodia held open. **C**, Crawling specimen photographed underwater. **D**, Dorsal view of live specimen with parapodia partly closed, showing elongated marginal papillae. **E**, Side view of live specimen photographed underwater. **F**, Dorsal view of damaged specimen (LACM 3307) showing network of dark dorsal vessels. All photographs courtesy of Yan Buske.

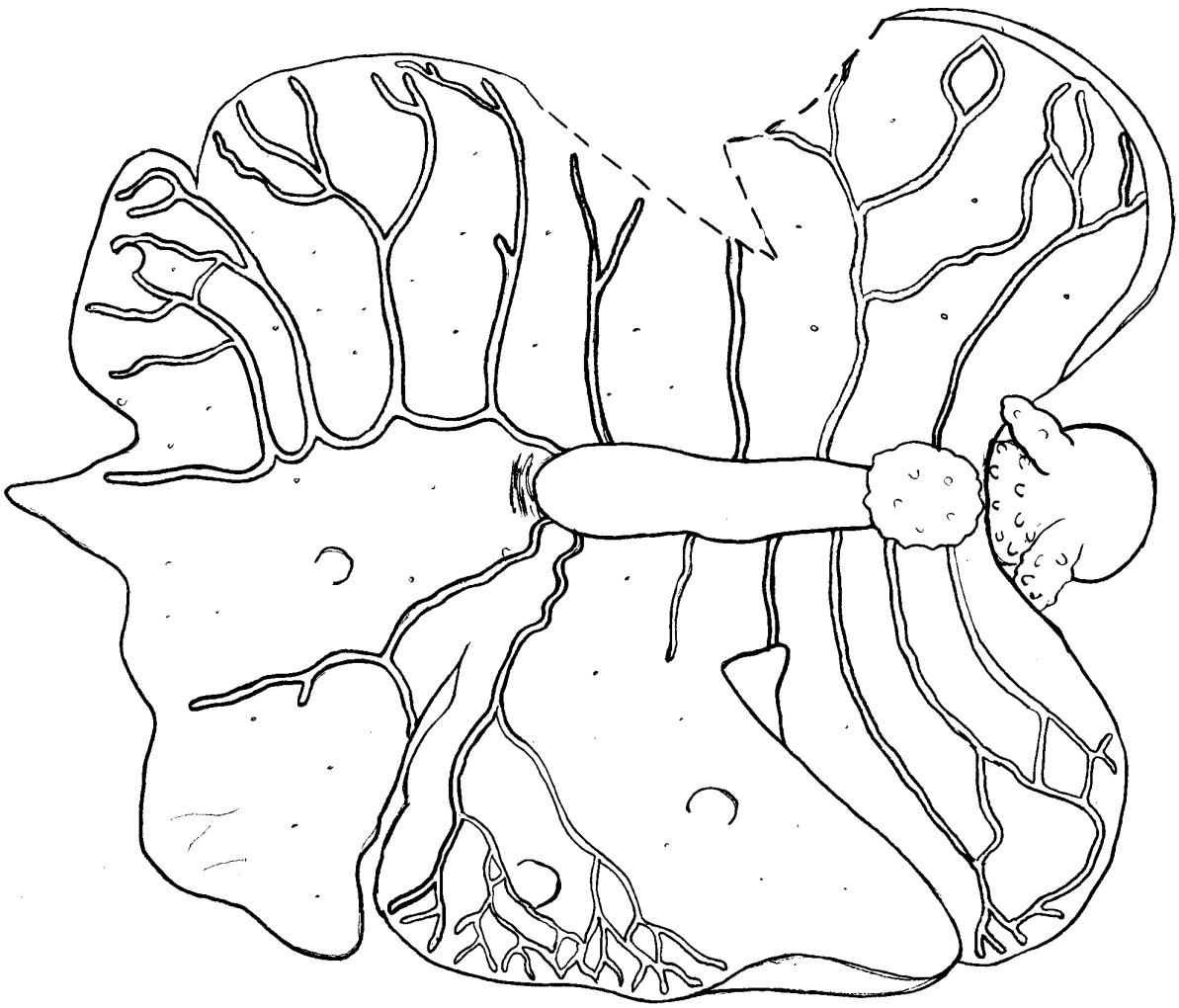

FIGURE 62. *Elysia zemi* **n. sp.**, dorsal vessel network drawn from preserved specimen (LACM 3307; dimensions 16 mm long × 14 mm wide). Shaded areas represent the external surface of the animal.

Penis elongate and curved (Fig. 56F) with rigid musculature that did not deform after drying, tapering into a conical apex bearing a resistant, hollow tip (Fig. 63D–E). Penial stylet is not a scoop or barb; hardened penial tip visible by SEM, but not light microscopy. Deferent duct long, thin, and highly convoluted.

Reproduction and development. No data available.

Host ecology. Specimens of *E. zemi* **n. sp.** were associated with the alga *Caulerpa racemosa*.

Phylogenetic relationships. *Elysia zemi* **n. sp.** belongs to subclade 4, the *E. tomentosa* complex, which includes related Caribbean species *E. subornata*, *E. pratensis* and *E. pawliki* **n. sp.** (Fig. 4). No sister species was recovered with significant support.

Range. Cayman Islands (present study), Martinique (present study), Saint Vincent and the Grenadines (present study), Venezuela (Valdés *et al.* 2006).

Etymology. Named after zemí, an embodiment of natural forces and ancestral spirits of the Taíno, an indigenous people of the Lesser Antilles. The distinctive triangular purple patch on the head of this species resembles the shape of the three-pointed sculptural zemí carved by the Taíno.

Remarks. A large elysiid, *E. zemi* **n. sp.** appears to be rare in most of the Caribbean, but locally common in Martinique and the Cayman Islands. Morphologically, the most similar Caribbean species is *E. pawliki* **n. sp.**, while the most similar species from the eastern Atlantic is *E. manriquei* Ortea & Moro 2009, from the Canary Islands off of West Africa. The purple patch on top of the head, white pericardium, brown marking just posterior to the renopericardial extension, and elongated hair-like papillae all differentiate *E. zemi* **n. sp.** from *E. pawliki* **n. sp.**

In addition to its east Atlantic type locality, *E. manriquei* differs in having symmetrical and non-anastomosing dorsal vessels, large black spots dotting the entire body and head, and more vertically exaggerated siphonal openings. Molecular data clearly differentiate *E. zemi* **n. sp.** from all other members of subclade 4.

FIGURE 63. *Elysia zemi* **n. sp.**, SEM of the radula and penis. **A**, Complete radula (LACM 178666). **B**, Leading tooth (LACM 178666). **C**, Ascus (LACM 178666). **D–E**, Penis and detail of penis tip showing stylet (LACM 178666). **F**, Radula (LACM 3306). **G**, Leading tooth (LACM 3306). **H**, Ascus (LACM 3306).

The radular teeth of *E. zemi* **n. sp.** are most similar to *E. pawliki* **n. sp.** in overall morphology, but can be clearly distinguished. Radulae of both species have minute denticles, possess a sharp tooth tip on a rounded apex, and a bend of the radula at ⅔ the length of the tooth. The teeth of *E. zemi* **n. sp.** are distinct in having convex tooth cusps, concave "V"-shaped depressions, and wider, more club-like tooth apices. The ascus of *E. zemi* **n. sp.** also forms a spiral that varies in size and number of teeth (Fig. 63C, H), not featured in *E. pawliki* **n. sp.** (Fig. 60F). The tooth of *E. manriquei* has a flat, serrated cutting edge, and smooth top edge that curves towards a pointed tip, like a disposable plastic knife.

Like in *E. pawliki* **n. sp.**, the resistant penial tip of *E. zemi* **n. sp.** is not scoop or barb-like. Instead, the stylet of *E. zemi* **n. sp.** appears as a curved cuticle with a "seam" observable under SEM (Fig. 63E).

Elysia christinae new species
(Figs. 56E, 64–66)

Elysia sp. 2—Valdés *et al.* 2006: 74–75.
Elysia sp. 18—Krug *et al.* 2015: 990-991, figs. 3B, 4

Type material. Bimini, Bahamas, July 2010, (Holotype LACM 3308, Paratype LACM 3309 [2 in lot]), collected by PJK.

Type locality. Bimimi, Bahamas

Material examined. Bimini, Bahamas, July 2010, 10 specimens, (Holotype LACM 3308, Paratype LACM 3309 [2 in lot], isolate Echr_10Bim04-10).

Live animal. Slugs resting on the algae held their parapodia open and flattened against the algal surface (Fig. 64A–B). When crawling, slugs typically elevated the parapodia along the anterior two-thirds of the body length, assuming a more typical slug-like shape, but the posterior third of the parapodia remained open and flattened, creating a widened, rounded end of the body (Fig. 64C–D). On crawling slugs, parapodial edges undulate. The head can be used to grip the substrate, and the front two-thirds of the body can rear up like a snake.

External anatomy. Four largest specimens ranged from 8–11 mm, remaining six slugs were 3–6 mm in length. Overall coloration dark green due to digestive diverticula, which ramify throughout body, head, and parapodia (Fig. 64A–D). Epidermis of head and body covered by network of brownish-rust colored lines and patches. Body elongate when parapodia contracted over dorsum. Head green and brown; prominent white patch just anterior to eyes and posterior to rhinophores on some specimens (Fig. 64B, F). Faint blue and white flecks dot sides of head. Underside of head green-brown with scattered blue dots, and brown line on lower "lip" of mouth (Fig. 64D). Rhinophores short, rolled, with flat tips. Proximal half of rhinophores green with brown specks, distal half mostly white due to dense covering of white speckles; white patches form transverse white line at beginning of distal half of each rhinophore. Penis extends from relaxed animals from under right rhinophore; white, tapering to blunt end.

Foot pale green. Transverse groove separates underside of head from foot, connecting to genital groove (Fig. 64D). Digestive diverticula present in foot but concentrated on either side, missing entirely from medial pale strip giving appearance of pale stripe down center of foot. Edges of foot not delineated from parapodia.

Body not wider than head, narrowing towards posterior end. Some specimens with parapodia fusing at posterior end with no visible tail (Fig. 64A); other specimens with short, pointed tail (Fig. 64D). Live animal sometimes having pear-shaped appearance due to widening parapodia at posterior end of body (Fig. 64A, D). Exterior of parapodia almost uniformly green with scattered white spots irregular in size and shape, more dense near outer parapodial margin, and fewer small blue spots. Interior of parapodia densely penetrated by digestive diverticula; pigmented by brown flecks (and larger black spots on some specimens) (Fig. 64B, E–F). Parapodial margin scalloped, with thin brown-black marginal line, but margin otherwise pale relative to rest of parapodia due to absence of digestive diverticula. Row of white spots runs along margin, with one spot under each pointed tip of scalloped margin (Fig. 64A–B). On largest specimen, front third of parapodia was twice as high as posterior portion of parapodia, forming wing-flaps on both sides just behind head. Anterior parapodial flaps held flat against substrate in resting animal; anterior region of parapodia sometimes folded over dorsum on living animal when crawling actively.

Pericardium small and rounded, pale or white in center, brown around periphery, dotted with a few white spots

(Fig. 64E–F). Anterior face of pericardium flattened where it merges with head, and penetrated by digestive diverticula. Anal papilla emerges from right side of pericardium, just posterior and distal to a dark circle, tapering to a rounded tip. Female genital aperture a white, eye-shaped opening at anterior edge of right parapodium near point of fusion with body; some specimens with small papilla directly above this opening.

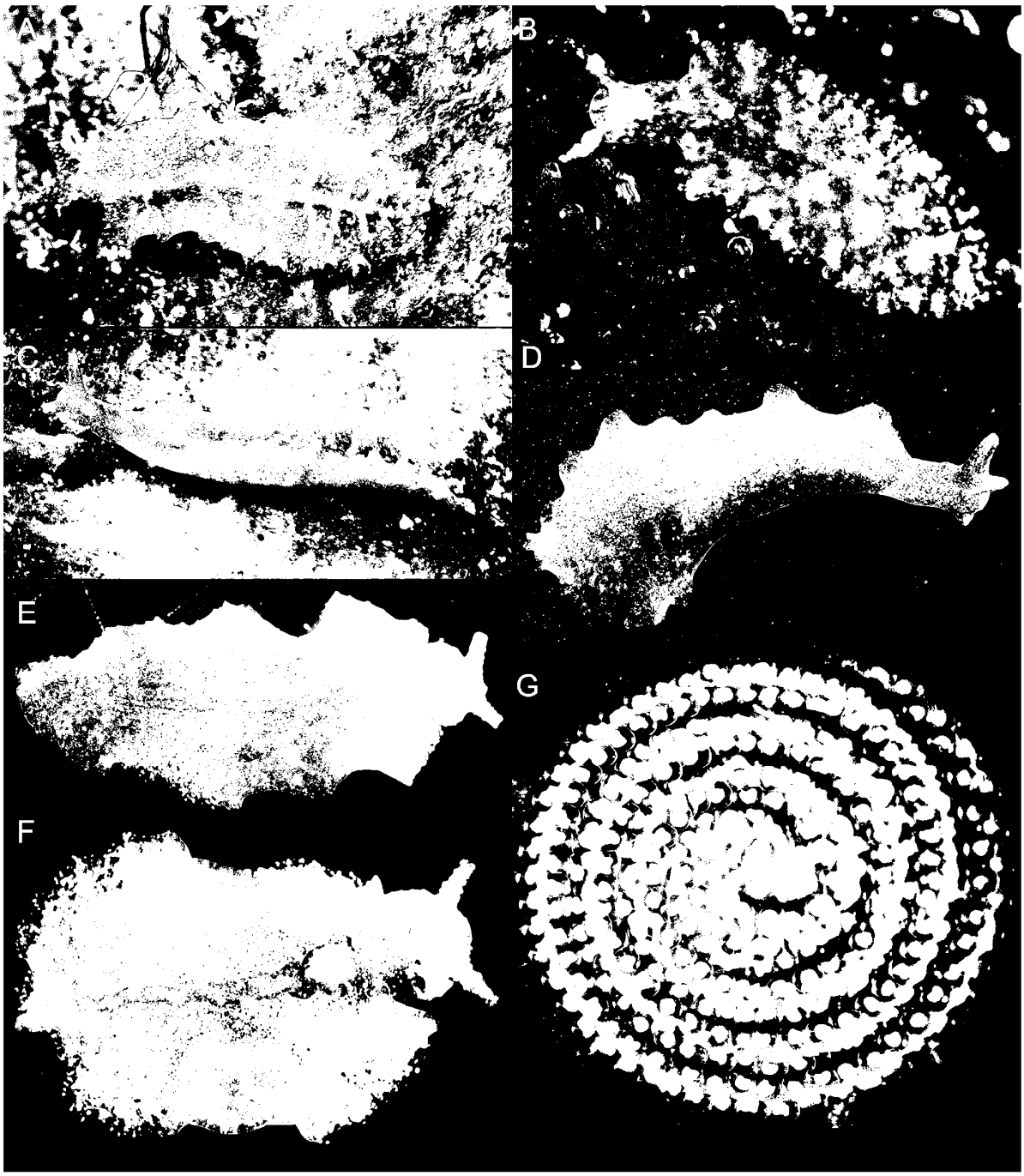

FIGURE 64. *Elysia christinae* **n. sp.**, external morphology and egg mass. **A–B,** Live specimens resting on host alga *Rhipilia tomentosa*, showing characteristic flattening of parapodia. Images taken within 2 weeks of field collection. Length of slugs = 11 mm (A), 8 mm (B). **C,** Crawling specimen showing tendency of posterior parapodia to bell out and remain flattened, giving the body a pear-shaped appearance. Length = 10 mm. **D,** Crawling specimen with body fully elongated and parapodia folded over dorsum; length = 9 mm. **E–F,** Relaxed specimens showing elongated renopericardial extension and dorsal vessel network. Images taken after specimens were held in aquaria for three months, at which point algal food was depleted and color faded to brownish-yellow. Length = 6 mm (E), 10 mm (F). **G,** Egg mass showing orange ECY ribbon and gastrula-stage embryos. Actual diameter = 2 mm.

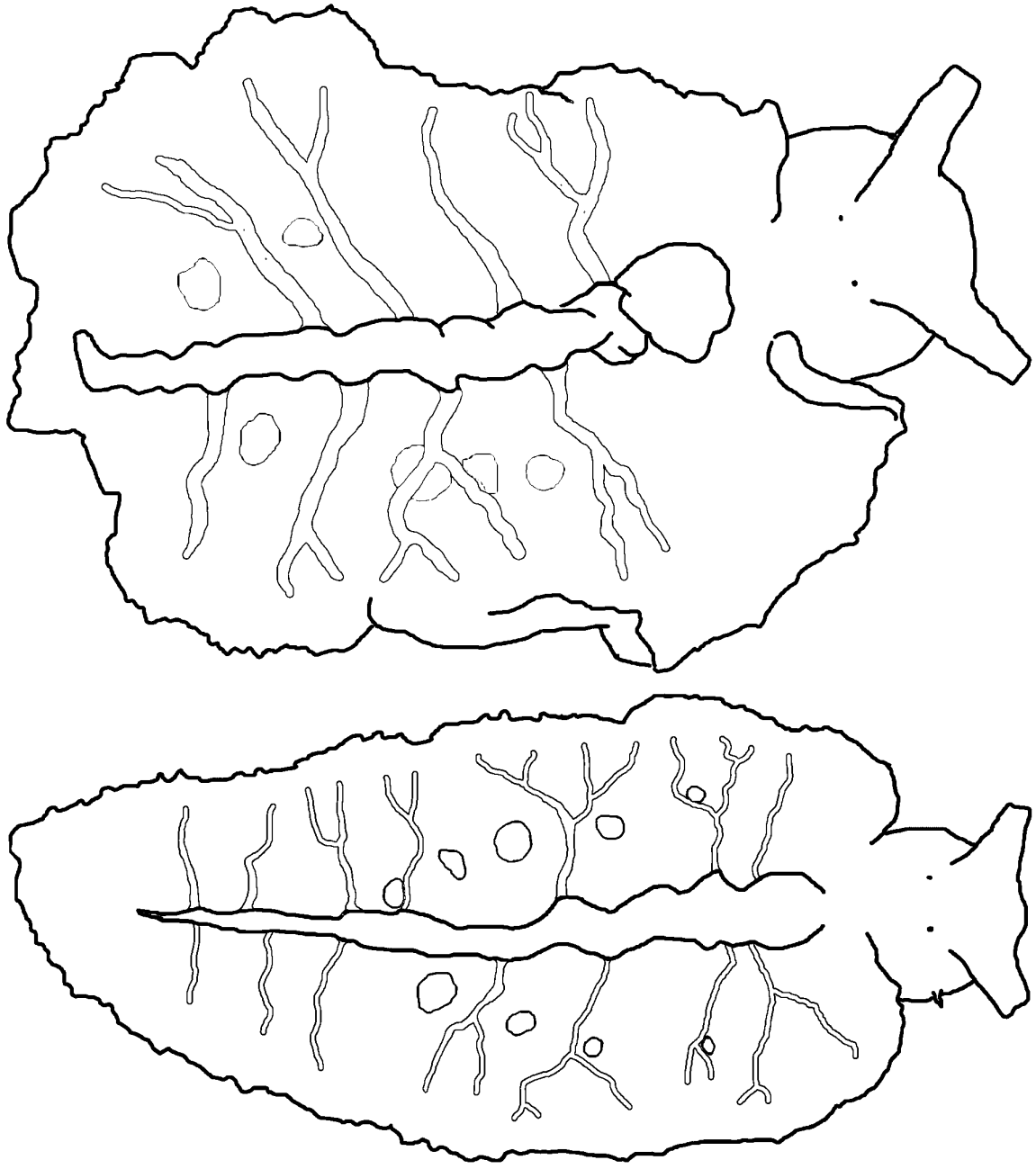

FIGURE 65. *Elysia christinae* **n. sp.**, drawing of renopericardial complex and dorsal vessel network traced from photographs of live animals from Bimini, Bahamas (A: 10 mm long; B: 6 mm long). Grey areas represent sperm-storage vesicles.

Renopericardium runs length of body, red-brown in outline and with red-brown dots across its length (Fig. 64F). Renal extension makes immediate S-turn upon exiting pericardium. Thick dorsal vessels (>500 μm wide) are either unbranched, or bifurcate about halfway to parapodial margin (Fig. 64E–F, 65). Some branches anastomose near point of bifurcation, about midway along parapodial surface; vessels do not anastomose at parapodial margin, terminating in blunt ends at point where digestive diverticula cease filling parapodium. Either one or no vessel emerges from pericardium on each side; remaining vessels emerge from renopericardial extension at irregular intervals along whole body length. Most vessels not paired with a vessel on opposing side of body. On slugs 8–10 mm in length, 6–8 vessels emerged from renopericardial extension on each side of body; largest specimen (11 mm long) had at least twelve vessels per side. Vessels densely spotted with white.

Sperm storage vesicles apparent on both sides of body as white rounded protrusions inside parapodia, 3–5 per

side on four largest specimens, dotting posterior ⅔ of body (Fig. 64E–F, 65). Vesicles irregular in size but typically about width of rhinophores; occur at irregular intervals along body, and not mirrored on opposite side. Anterior-most vesicle on each side closest to parapodial margin, with successive vesicles more proximal to midline, creating a "V"-shape towards posterior end of dorsum.

Internal anatomy. Radula with 10 teeth (LACM 3309), 5 teeth in ascending limb and 5 in descending limb (Fig. 66A). Leading tooth elongate with a serrated, curved cusp, bearing 20 sharp denticles (Fig. 66B). Housing depression for interlocking teeth "V"-shaped and extending approximately ½ of total tooth length. Base of tooth approximately ½ of total tooth length. Ascus lost during radular preparation.

Penis small, narrow, and elongate (Fig. 56E), with rigid musculature that did not deform after drying, tapering into a conical apex lacking armature (Fig. 66C). Deferent duct short, thin, and loosely convoluted.

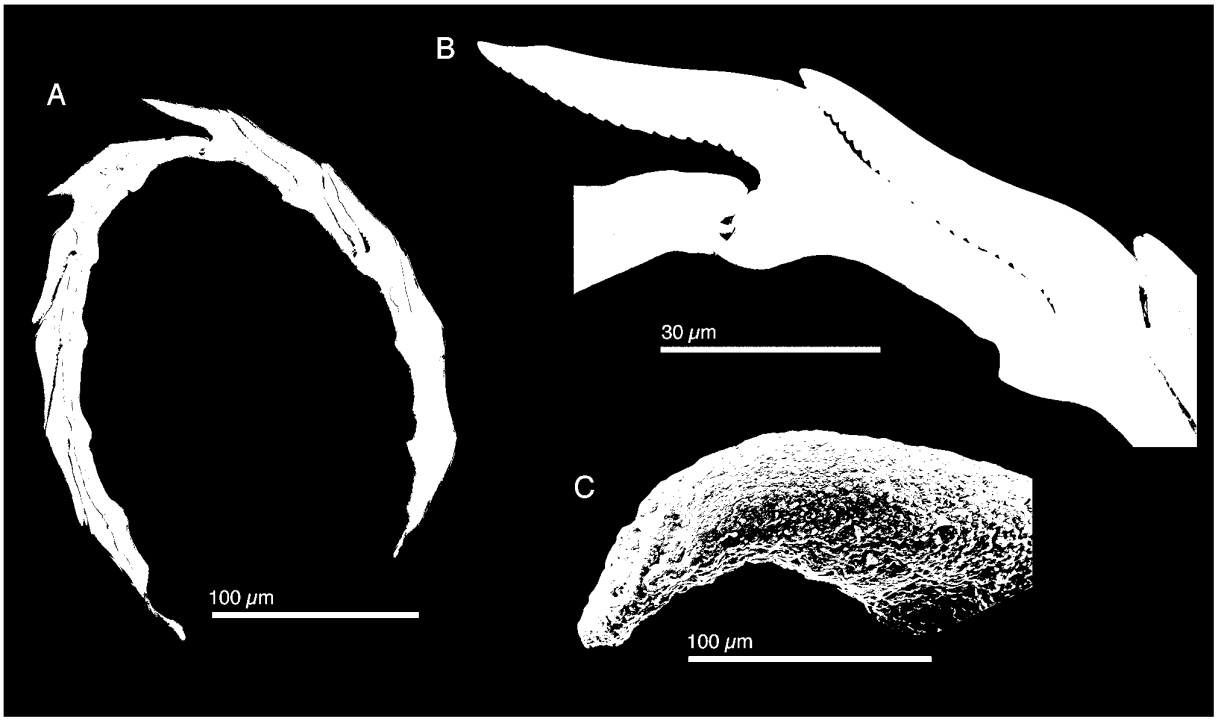

FIGURE 66. *Elysia christinae* **n. sp.**, SEM of the radula and penis (LACM 3309). **A,** Complete radula. **B,** Leading tooth. **C,** Penis.

Reproduction and development. Slugs held in aquaria from Jul–Oct 2010 produced six egg masses. The egg strand formed a tight spiral, with one egg per capsule. Within the strand, capsules alternated on either side of a continuous ribbon of bright orange ECY (Fig. 64G). The ECY ribbon twisted around on the upper surface of the egg strand, under the outer covering of the egg mass. The ribbon was not perfectly flat, but rather formed raised peaks or folds in between capsules.

Mean clutch size was 523.8 eggs (± 254.8 SD, n = 5; range: 239–820). Mean diameter of uncleaved ova was 61.2 μm (± 1.8 SD, n = 14) for one clutch and 58.7 μm (± 1.7, n = 25) for a second clutch; grand mean egg size was thus 60.0 μm ± 1.8 SD. For one egg mass, the long axis of egg capsules measured roughly 870 μm, while capsules in a second clutch were about 950 μm wide.

Clutches held at ~25°C hatched relatively synchronously after 12.3 d (± 1.0 SD, n = 4; range: 11–13), releasing veliger larvae that swam actively. Mean larval shell length per clutch ranged from 102.0 μm (± 3.8 SD, n = 25) to 115.3 μm (± 10.0 SD, n = 25); grand mean shell length for five clutches was 107.8 μm ± 5.0 SD. Larvae were not cultured to competence, but their small size, short encapsulated period, and absence of eyespots or a developed propodium all indicated larvae were planktotrophic.

Host ecology. About 15 specimens of *E. christinae* **n. sp.** were recovered from a sample of the udotacean alga *Rhipilia tomentosa*. The alga was growing at ~6 m depth, in a sandy patch within a large seagrass bed. Slugs were mostly juveniles <5 mm long. Slugs were maintained in aquaria on *R. tomentosa* and observed for three months.

All specimens preferentially associated with *R. tomentosa* and not other related algae growing in the aquaria (*Udotea*, *Caulerpa*, *Halimeda*). Slugs were observed feeding only on *R. tomentosa* in the laboratory. White spots on a green background color make live animals highly cryptic on their host alga, due to the numerous white calcareous structures made by fouling organisms on the surface of *Rhipilia* (Fig. 64A–B).

Phylogenetic relationships. *Elysia christinae* **n. sp.** belongs to subclade 1, a group of Caribbean species. Within this lineage, no sister species was recovered with significant support. Subclade 1 includes lineages that have radiated onto multiple genera of udotacean algae, and are physically associated with their hosts—*Penicillus* (*E. papillosa*, *E. taino* **n. sp.**), *Udotea* (*E. zuleicae*, *E. buonoi* **n. sp.**), *Rhipilia* (*E. christinae*), and *Halimeda* (*E. patina*). These lineages thus remain ecologically partitioned at a fine spatial grain due to host specificity, and being co-distributed but differentiated by host use, may represent an adaptive radiation driven by ecological speciation.

Range. Bimini, Bahamas (present study); Cozumel, Mexico (Valdés *et al.* 2006)

Etymology. Named in honor and fondest memory of Christine Marie Donnelly Lee and her daughter Christine Marie Lee, loving grandmother and aunt of PJK.

Remarks. No other Caribbean elysiid resembles *E. christinae* **n. sp.** in morphology, development or host use, and the species is genetically distinct from all sampled species. The characteristically flattened, open parapodia of *E. christinae* **n. sp.** are distinctive, although a similar flattening behavior is sometimes expressed by specimens of *E. zuleicae* resting on *Udotea*. Like other members of subclade 1, *E. christinae* **n. sp.** shares the feature of sperm storage vesicles that form after mating. However, only in *E. christinae* **n. sp.** do multiple vesicles form along both sides of the body, rather than one pair as in other subclade members. No specimens of *E. christinae* **n. sp.** were observed to swim, which is a characteristic of all related species.

Elysia christinae **n. sp.** is the only Caribbean elysiid currently known to have both planktotrophic development and orange ECY; other taxa with orange ECY (*E. velutinus*, *E. patina*, *E. subornata*, *E. pratensis*, *E. pawliki* **n. sp.**) have lecithotrophic development, although the larval type of *E. hamanni* (which also has orange ECY) remains undetermined. In terms of host ecology, *E. christinae* **n. sp.** is the only *Elysia* sp. known to feed on *Rhipilia*, a tropical genus with 11 species; at least one undescribed Indo-Pacific species (*Elysia* sp. 11 in Krug *et al.* 2015) feeds on the related alga *Tydemania* (Lam & Zeckman 2006). *Rhipilia* is typically a deep-water alga in the Caribbean, and is often misidentified as *Avrainvillea*, factors that may have previously impeded collection and obscured the host-association of *E. christinae*.

Elysia hamanni new species
(Figs. 56H, 67–69)

Elysia sp. 5—Valdés *et al.* 2006: 76–77.

Type material. Banana River, Florida, USA, May 1988, (Holotype LACM 3310) collected by Jeff Hamann; True Blue Point, True Blue Bay, Grenada, December, 1987, (Paratype LACM 3311 [8 in lot]), collected by Jeff Hamann.

Type locality. Banana River, Florida, USA

Material examined. Banana River, Florida, USA, May 1988, 5 specimens (Holotype LACM 3310, LACM 178668 [1 in lot], LACM 178669 [3 in lot]); Grenada: September, 1981, 3 specimens (LACM 178671), True Blue Point, True Blue Bay, December, 1987, 8 specimens (Paratype LACM 3311); Isla Mujeres, Mexico, December, 1993, 8 specimens (LACM 178670 [5 in lot], LACM 178673 [3 in lot]); Ranguana Caye, Belize, 31 March 1992, 1 specimen (LACM 178667); Port Antonio, Jamaica, August 1990, 5 specimens (LACM 178672).

Live animal. Resting slugs held their parapodia apart, forming a series of irregular openings (Fig. 67A–B). The animals leave characteristic circular feeding marks on their algal host, *Caulerpa prolifera*. When stressed the animals release a milky white substance.

External anatomy. Holotype (LACM 3310) measuring 21 mm long, 10 mm wide at widest point with parapodia flattened; paratype (LACM 3311) 17 mm long, 6 mm wide. Other examined specimens: LACM 178672, 19 mm long × 10 mm wide; LACM 178667, 18 mm long.

Background coloration pale green with a pinkish tinge on external side of parapodia. Epidermis of head and body covered by small dark purple to black spots. Body elongate when parapodia contracted over dorsum. Head pale green with pinkish pigment dorsally, and elongate lighter area on each side running from eyes into base of

rhinophores. Rhinophores elongate, rolled, with flat tips. Body covered with numerous conical papillae of various sizes, larger papillae occur mainly on external sides of parapodia. Parapodia margins lighter, with faint, thin, dark grey line, also covered with papillae.

Pericardium small and oval, pale green, dotted with few white spots. Renopericardial extension runs almost entire length of body, pale green. Extension narrows immediate upon exiting pericardium and expands again. Numerous very thin dorsal vessels, opaque white. One vessel extending out of pericardium and running along dorsal surface of renal extension (Fig. 68). Two dorsal vessels emerging from pericardium on each side, numerous remaining vessels emerging from renopericardial extension at regular intervals along whole body length. Vessels symmetrical, with most paired with a vessel on opposing side of body. Most vessels bifurcating about halfway up inner parapodial surface margin and then forking again near parapodial margin; vessels anastomosing into complex network (Fig. 68).

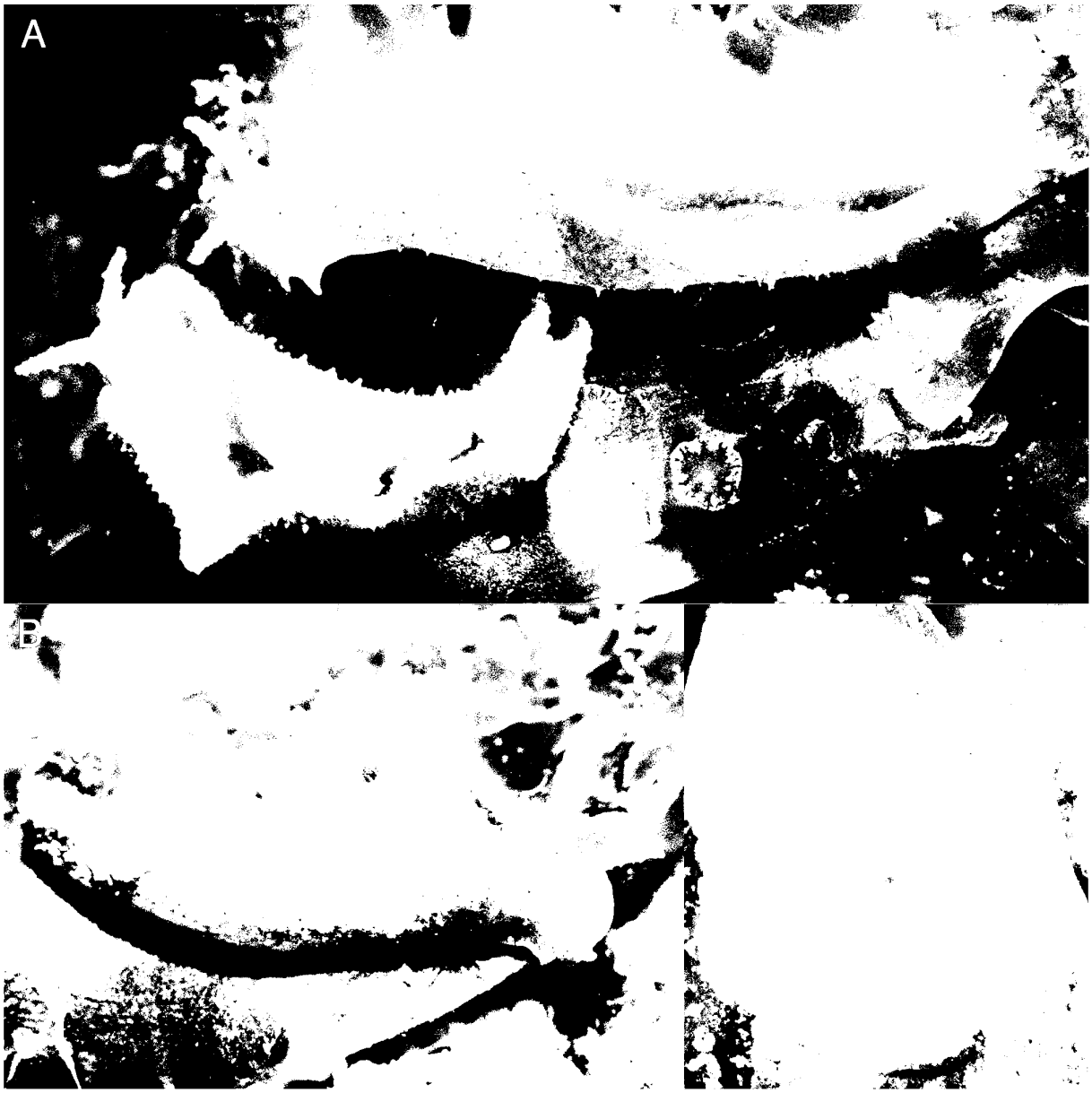

FIGURE 67. *Elysia hamanni* **n. sp.**, external morphology and egg mass. Characteristic round feeding scars on host alga *Caulerpa prolifera* visible in all panels. **A–B** Live specimens from Grenada. **C**, Egg mass showing orange ECY ribbon.

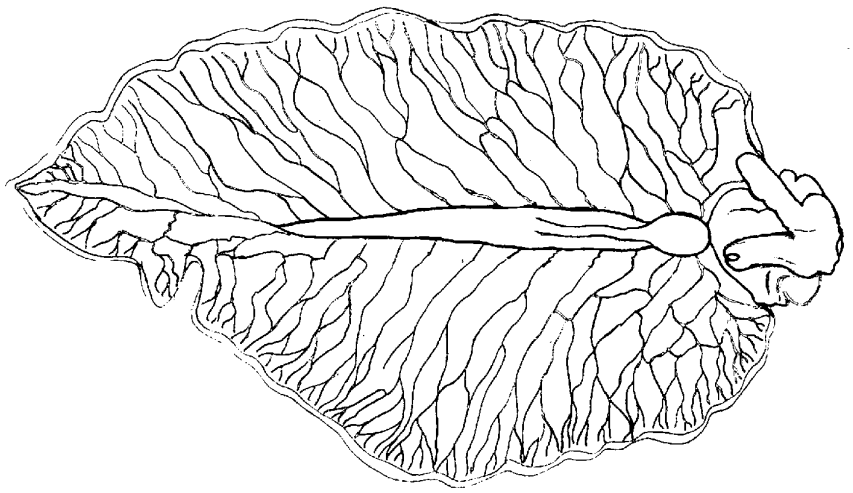

FIGURE 68. *Elysia hamanni* **n. sp.**, drawing of renopericardial complex and dorsal vessel network traced from preserved specimen from Florida (LACM 178668, 5 cm long × 3 cm width).

Internal anatomy. Radula with 28 teeth (LACM 3311), 6 teeth in ascending limb and 22 in descending limb (Fig. 69A). Leading tooth wide and robust with a parallelogram-like shape and lacking denticles. Housing depression for interlocking teeth extending ⅔ the tooth length (Fig. 69B). Base of tooth approximately ⅓ of total tooth length. Ascus with 16 teeth, becoming progressively smaller in size, in a spiral or whorl (Fig. 69C).

Penis broad, and relatively large with rigid musculature resistant to desiccation (Fig. 56H), tapering distally into a conical apex lacking armature (Fig. 69D–E). Deferent duct long, narrow, and highly convoluted.

Reproduction and development. Egg masses were photographed in the field along with the specimens. The egg strand formed a tight elysiid spiral, with one egg per capsule. Within the strand, capsules alternated on either side of a continuous ribbon of bright orange ECY (Fig. 67C). The egg ribbon was flat. Development mode could not be determined from the photographs.

Host ecology. All specimens were found on the alga *Caulerpa prolifera*, on which they leave a characteristic round feeding scar (Fig. 67A–B).

Phylogenetic relationships. No specimens of *E. hamanni* **n. sp.** were available for molecular work, so the phylogenetic relationships of this species are unknown. Based on its diet, radula, and elongated renopericardial complex, we hypothesize this species belongs to subclade 4, the *E. tomentosa* complex.

Range. Ranguana Caye, Belize (present study), Banana River, Florida, USA (present study), Grenada (Valdés *et al.* 2006), Jamaica (present study), Isla Mujeres, Mexico (present study).

Etymology. Named in honor of Jeff Hamann, who over several years compiled a comprehensive collection of sea slugs from the Caribbean, including numerous new species such as this one.

Remarks. *Elysia hamanni* **n. sp.** is clearly different from other species of *Elysia* described to date in the Caribbean region. Morphologically, the most similar species may be *E. subornata*, which also has a body covered with conical papillae, similar dorsal vessels, and wide parapodia with a marginal line. Although the dorsal vessel pattern is similar in these two species, *E. hamanni* **n. sp.** has comparatively more vessels. Both species also feed on *Caulerpa*. However, the radular morphology of these two species is somewhat different: *E. subornata* has narrower, more elongate radular teeth and the ascus is highly disorganized. The radular teeth of *Elysia hamanni* **n. sp.** are proportionally shorter and wider and the ascus forms a highly organized spiral or whorl. Radular morphology of *E. hamanni* **n. sp.** is also similar to *E. pawliki* **n. sp.** and *E. zemi* **n. sp.**, although teeth are more pointed and blade-like in *E. hamanni* **n. sp.** The tightly spiraled ascus is a notable similarity between *E. hamanni* **n. sp.** (Fig. 69C) and *E. zemi* **n. sp.** (Fig. 63H).

An unusual feature shared between *E. hamanni* **n. sp.** and the Indo-Pacific species *Elysia* (=*Pattyclaya*) *brycei* is a single dorsal vessel extending out from the pericardium and running along the dorsal surface of the elongated renal gland (fig. 20 in Jensen & Wells 1990). As both species feed on *Caulerpa*, it is possible this may be a synapomorphy, and that "*Pattyclaya*" spp. are derived members of subclade 4 with lateral lammellae. Molecular

data are needed to resolve the phylogenetic placement of both *E. hamanni* **n. sp.** and *Pattyclaya* spp. to assess homology versus convergence in characters such as the ascus and dorsal vessel patterns.

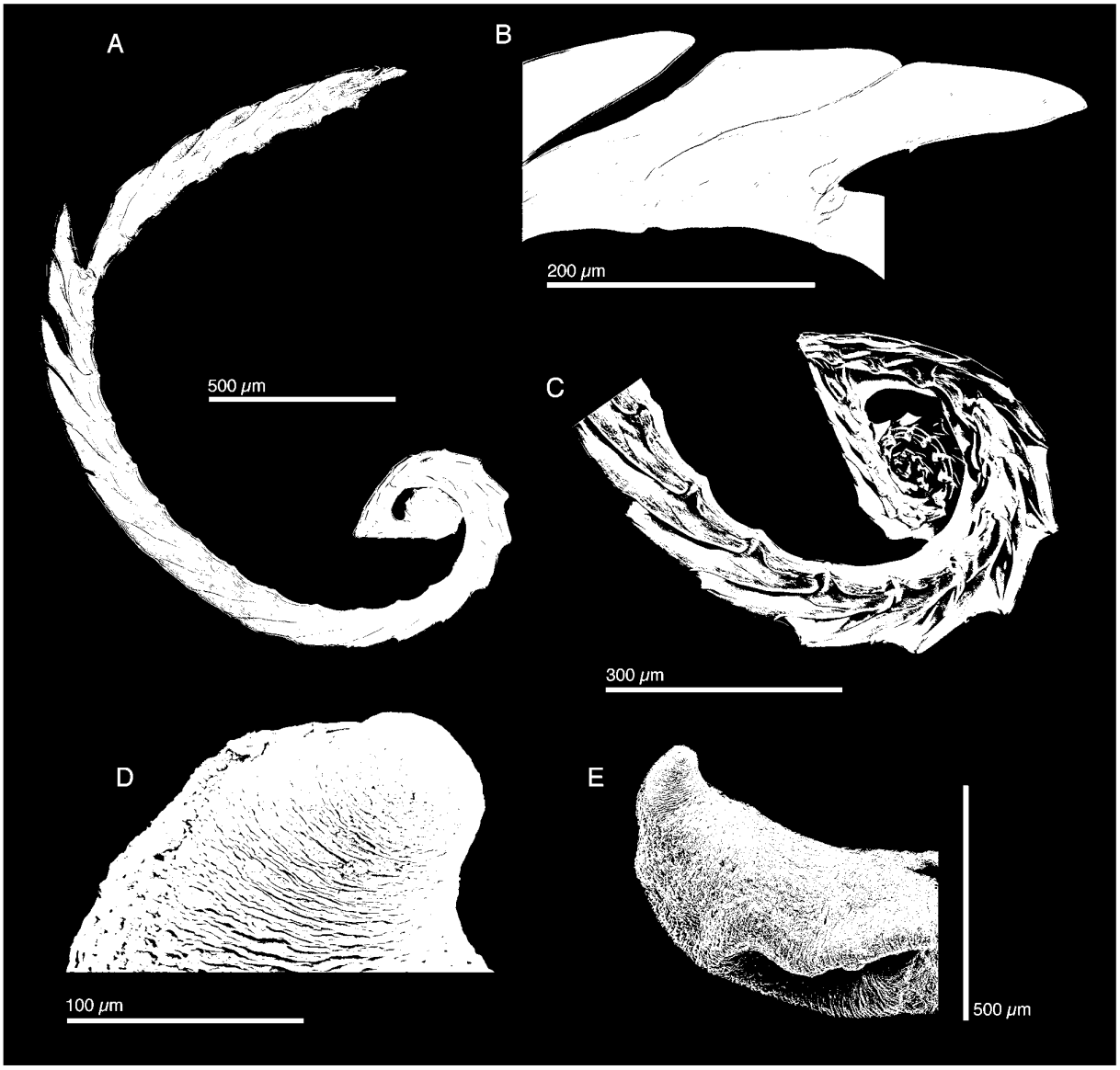

FIGURE 69. *Elysia hamanni* **n. sp.**, SEM of the radula and penis. B, Complete radula (LACM 3311). **B,** Leading tooth (LACM 3311). **C,** Ascus (LACM 3311), **D**, Penis (LACM 178668), **E**, Penis tip (LACM 178668).

Elysia taino new species

(Figs. 56G, 70–72)

Elysia papillosa [non Verrill 1901]—Krug *et al.* 2015: 990-991, figs. 3B, 4

Type material. Discovery Bay, Jamaica, March 2006, (Holotype LACM 3312, Paratype LACM 3313) collected by PJK; Stirrup Cay, Bahamas, July 2007 (Paratype LACM 3314), collected by PJK.

Type locality. Discovery Bay, Jamaica.

Material examined. Discovery Bay, Jamaica, March 2006, 5 specimens (Holotype LACM 3312, Paratype LACM 3313, LACM 178605–07); Stirrup Cay, Bahamas, July 2007, 2 specimens (Paratype LACM 3314, LACM 178609).

Additional material examined. Discovery Bay, Jamaica, March 2006, 8 specimens (isolate Etai_06Jam01,

isolate Etai_06Jam05-08, isolate Etai_06Jam11-12, isolate Etai_06Jam14); Bahamas: Stirrup Cay, July 2007, 3 specimens (isolate Etai_07Stir02, Etai_07Stir08, Etai_07Stir10), Little San Salvador, July 2007, 3 specimens (isolate Etai_07LSS09, Etai_07LSS17, Etai_07LSS19), San Salvador, July 2010, 6 specimens (isolate Etai_10Ssal01, isolate Etai_10Ssal03-07), Plana Cays, July 2007, 8 specimens (isolate Etai_07Pla01, isolate Etai_07Pla04-07, isolate Etai_07Pla10-12), Compass Cay, July 2010, 4 specimens (isolate Etai_10Comp01-04), Northern Exumas, July 2010, 2 specimens (isolate Etai_10NEx01-02), New Providence, July 2010, 5 specimens (isolate Etai_10NPr01-05), Bimini, July 2010, 1 specimen (isolate Etai_10Bim01); Dominica, 2007, 6 specimens (isolate Etai_07Dom01-06); US Virgin Islands, 2014, 2 specimens (isolate Etai_14USVI01-02).

Live animal. Specimens swim readily when disturbed by undulating their parapodial margin. Slugs perform hypodermic insemination (sometimes mating in groups) using a long, highly flexible penis that can extend out for half the body length, ~10 mm on a large adult specimen.

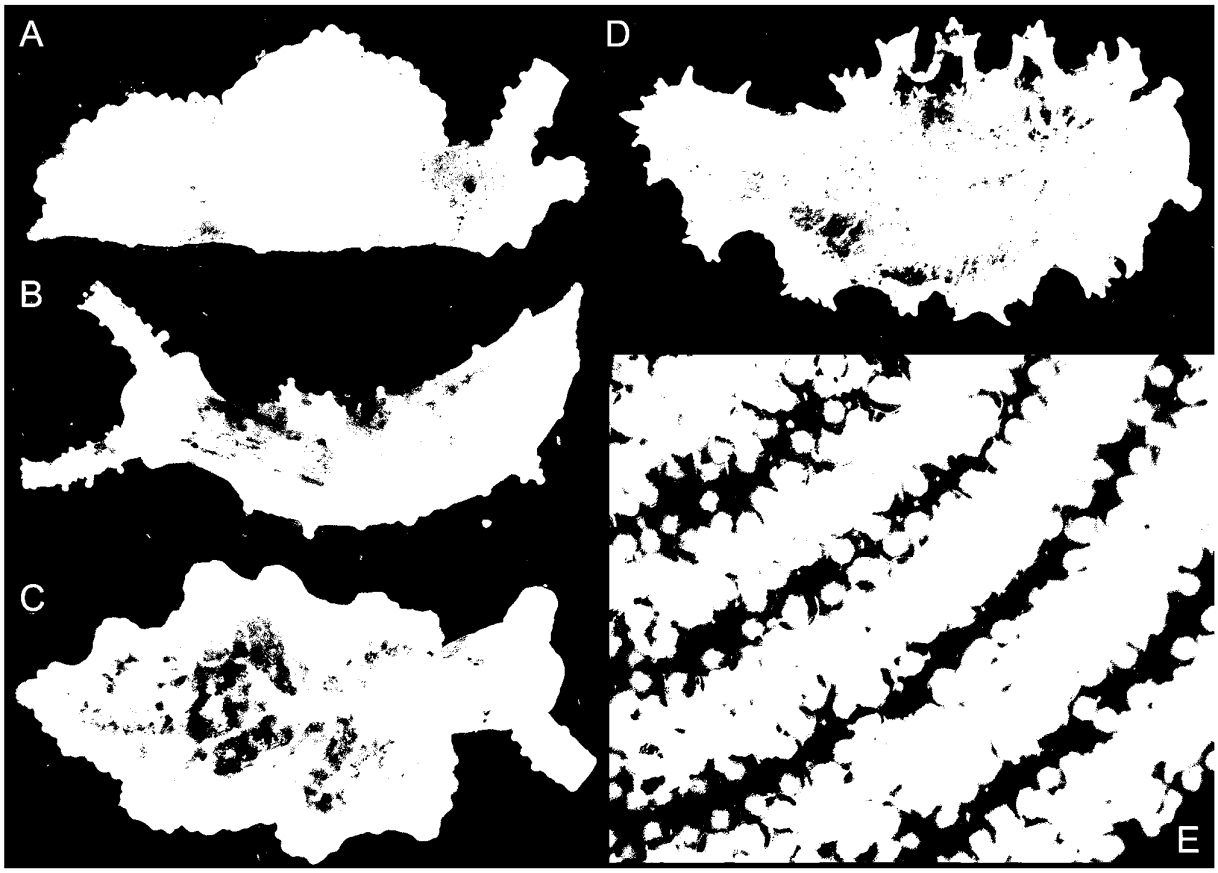

FIGURE 70. *Elysia taino* **n. sp.**, external morphology and egg mass. Live specimens were photographed immediately following field collection from Discovery Bay, Jamaica (March 2006). **A,** Specimen (length = 6 mm) typifying the all-white morph, with brown bands on rhinophores. **B,** View of specimen (10 mm) from above, showing thin red lines crisscrossing parapodial margin. **C** Relaxed specimen (10 mm) with parapodia open, showing elongated renopericardial complex, vessel network, and iridescent coloration of dorsum. **D,** Relaxed specimen (18 mm) with parapodia open; greyish-white gametic vesicles appear midway down and alongside renopericardial extension. **E,** Portion of egg mass spiral showing irregular ribbon of white ECY twisting around early-stage embryos. Actual field of view = 3 mm across.

External anatomy. Coloration and gross external morphology highly variable. Overall color ranging from entirely white (Fig. 70A) to mostly light green on the dorsum and base of parapodia, grading to white or brown on the upper parapodial face (Fig. 70B–D). Some specimens with longitudinal stripes (Fig. 70B) of darker color, and many with scattered tiny brown, red or orange dots. Head and body covered in low white conical papillae. Head generally white to light brown, sometimes with green band under large eyes if white pigment does not cover digestive diverticula. Most specimens with single, large white papilla between eyes; head bisected along midline by row of smaller white papillae in many specimens. Rhinophores relatively short, rolled, with white ground color

and rounded white papillae giving a knobby appearance. Dark band appearing about ⅓ of the way up rhinophores, from absence of white pigment and/or concentration of brown-orange dots. Scattered red-orange or brown spots dot head and rhinophores.

Parapodia usually held partially open, not fully covering pericardial complex. Edges of parapodia undulating laterally, producing series of loose siphonal openings along the body. Outer parapodial surface bearing rows of white papillae, varying in size. Lower portion of parapodia adjacent to foot variable in color, ranging from light or dark green to tan, brown or white; distal portion of parapodia typically white on smaller specimens, brown on larger specimens (Fig. 70A, C–D). Brick-red or orange flecks scattered over outer parapodial surface (Fig. 70A–B).

Parapodial margin with scalloped edge, giving a vertically undulating appearance, due to three-pointed extensions regularly spaced along entire length of margin. Rows of red dots forming thin lines or bars crisscrossing parapodial margin at regularly spaced intervals on most specimens (Fig. 70A, D). Ground color of inner parapodial surface and dorsum light to olive green, dotted with white papillae. Dorsum usually pigmented by iridescent blue, green or red-orange speckles, giving glittery appearance. Larger dark green, brown or black spots haphazardly scattered across dorsum. Posterior end of body varying among specimens, sometimes tapering to short, triangular tail, but on other specimens body ending bluntly with no extended tail.

Pericardium small, raised, rounded; white with orange-brown spots (Fig. 70C–D). Renopericardial extension distinct from pericardium, clear with orange-brown spots, running less than halfway down dorsal surface; undulating side to side (snake-like) on some larger specimens (Fig. 70D). Dorsal vessels often pigmented by iridescent speckles or colored spots creating marked contrast with ground color of dorsal surface (e.g., white vessels against green dorsum, or iridescent blue-green speckles against a white dorsum). Typically 5–6 dorsal vessels emerging on left side and 6–7 on right side of renopericardial complex, with two paired vessels emerging from either side of pericardium and the remaining vessels branching off renopericardial extension (Figs. 70C–D, 71). On most specimens, vessels loosely symmetrical in arrangement but with one extra vessel on right side; however vessels highly asymmetrical on other specimens. Vessels wider than in most elysiids, initially straight and unbranched until reaching upper region of inner parapodial surface; vessels then forking or extending thinner side branches at irregular intervals (Figs. 70C–D, 71). Terminal branches anastomosing on some specimens but remaining distinct on others. Elongated pair of posterior vessels emerges from terminus of renopericardium, running almost to tail, with numerous anastomosing lateral branches on larger specimens.

One pair of large sperm-storage vesicles typically visible as grey-white swellings of irregular shape (Figs. 70D, 71). Sperm vesicles positioned between 3rd and 4th dorsal vessels on either side, about halfway along renopericardial extension, often surrounded on all sides by vessel branches. Vesicles not apparent on juvenile specimens. Upon osmotic shock, swollen vesicles burst releasing sperm.

Size of three specimens: isolate Etain_06Jam17 (LACM 3313), 8.5 mm long × 4.5 mm wide at widest point with parapodia flattened; isolate Etai_06Jam33 (LACM 178605), 7.0 mm long × 4.5 mm wide; isolate Etain_06Jam34 (LACM 178606), 7.5 mm long × 5.0 mm wide.

Internal anatomy. Radula with 11–16 teeth (LACM 3312–3314, LACM 178605–06, LACM 178609), 5–7 in ascending limb and 6–9 in descending limb (Fig. 72A, showing only part of ascending limb). Leading tooth elongate and robust bearing 16–24 denticles on cusp (Fig. 72B), though one specimen observed with 39 irregular denticles on cusp (LACM 178606). Tooth length, width, and shape somewhat variable. Leading tooth measuring between 120–240 μm long, 20–40 μm wide, with width to length ratio 12.5–20.8 (n=6). Housing depression for interlocking teeth extending ½ the length of tooth (Fig. 72B). Base of tooth just less than ½ total tooth length. Ascus containing small, jumbled teeth; not figured (lost during radular preparation).

Penis large and elongate with rigid musculature that did not deform after drying (Fig. 56G, 72C), bearing a stylet with a scoop or spoon (LACM 3312–3314, LACM 178605-07) opposite to one medial and two distal flanges (Fig. 72D). Deferent duct long, thin, and convoluted.

Reproduction and development. Development was planktotrophic for specimens collected from Jamaica and San Salvador (Bahamas). Egg masses contain an irregular ribbon of white ECY which contacts most, but not all, egg capsules (Fig. 70E). Diameter of uncleaved ova was not determined. Mean larval shell length at hatching was 118.3 μm ± 7.4 (n = 20) for a clutch from Jamaica, and 124.9 μm ± 5.7 (n = 20) for a clutch from San Salvador.

Host ecology. In all field surveys, *E. taino* **n. sp.** was found on species of the green algal genus *Penicillus* (either *P. capitatus* or *P. dumetosus*), on which slugs were also observed to feed in the laboratory. Species-level host

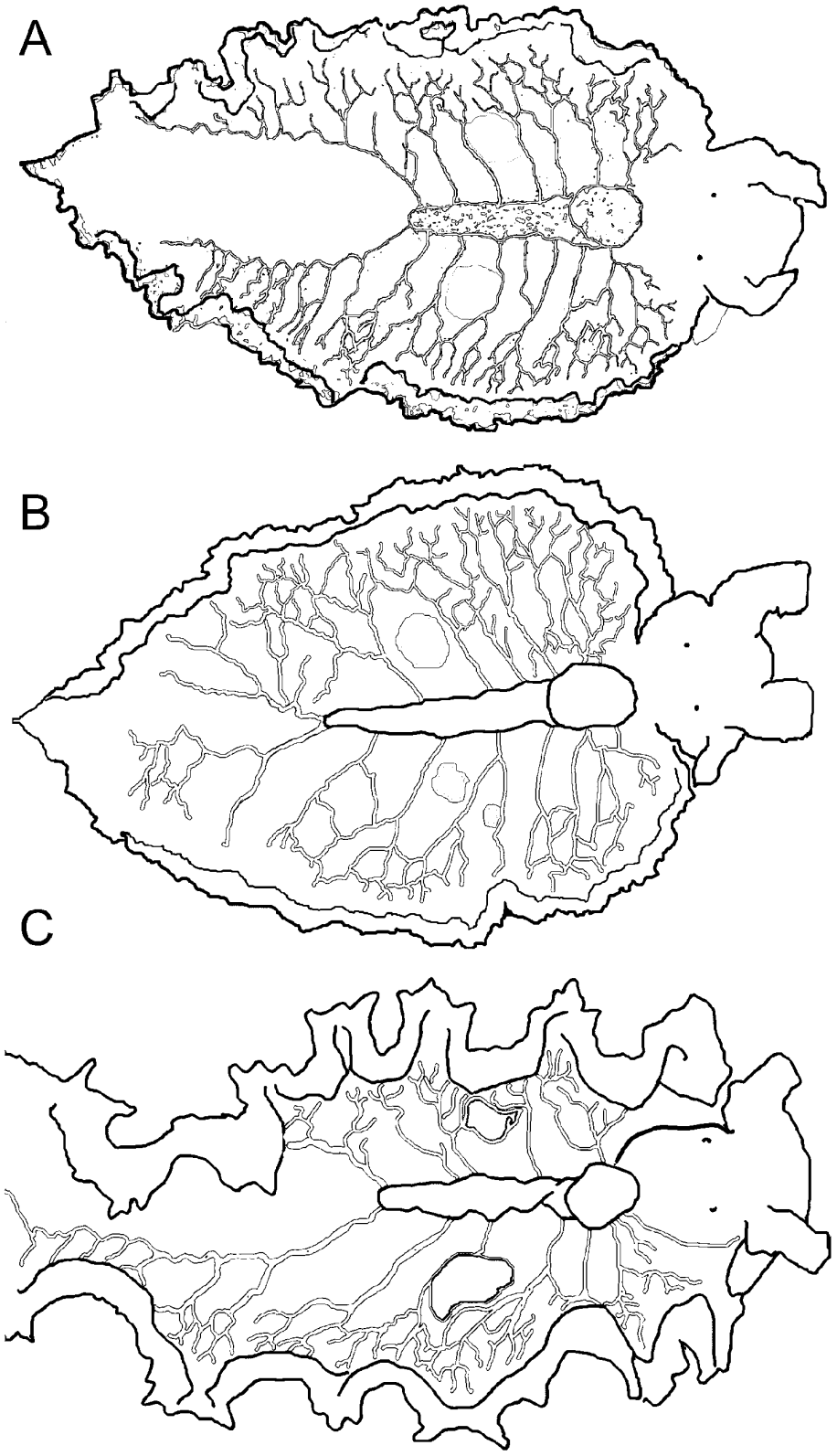

FIGURE 71. *Elysia taino* **n. sp.**, renopericardial complex and dorsal vessel networks traced from digital photographs of live specimens from Jamaica; actual lengths: 16 mm (**A–B**), 18 mm (**C**). Gray areas represent sperm-storage vesicles. Extended penis shown for all specimens.

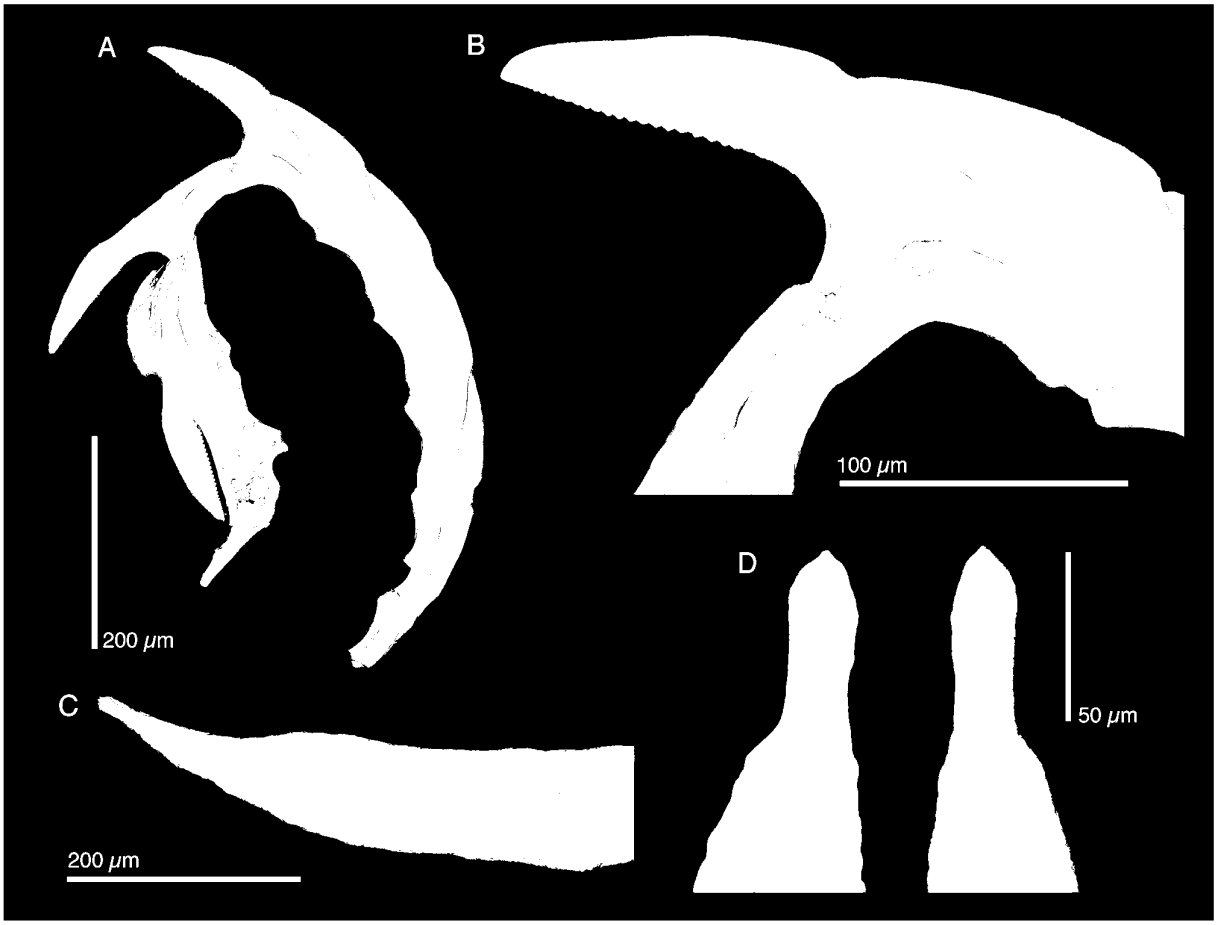

FIGURE 72. *Elysia taino* **n. sp.**, SEM of the radula and penis. A, Radula minus a jumbled ascus (LACM 3313). B, Leading tooth (LACM 3313). C, Penis (LACM 3312). D, Penial stylet, dorsal and ventral views (LACM 178605).

preferences are unclear, but in Jamaica, a collection of *P. dumetosus* (wet weight: 400 g) yielded 34 specimens, while a comparable amount of *P. capitatus* yielded only 7 specimens. Most specimens on field-collected algae were juveniles <5 mm in body length; in Jamaica, mean length of larger adults was 15.8 mm ± 3.4 SD (n = 9, range = 10–20 mm).

Phylogenetic relationships. *Elysia taino* **n. sp.** belongs to subclade 1, and was recovered as sister to the morphologically similar *E. papillosa*, which also consumes *Penicillus* (Fig. 4). While host partitioning of lineages onto different algae implicates ecological speciation early in the adaptive radiation of subclade 1, recent divergence between *E. taino* **n. sp.** and *E. papillosa* may have been allopatric as parts of their ranges are non-overlapping. Further study is needed to determine whether *E. taino* **n. sp.** and *E. papillosa* prefer different *Penicillus* spp. as their host (e.g., *P. capitatus* versus *P. dumetosus*), which could alternatively indicate a role for disruptive selection on host use during the speciation process.

Range. Specimens confirmed through molecular analyses in the present study were from Jamaica, U.S. Virgin Islands, Dominica, and the following Bahamas sites: Sweetings Cay, Stirrup Cay, Little San Salvador, San Salvador, Plana Cays, New Providence, Compass Cay, Northern Exumas, and Bimini.

Etymology. Named in honor of the Taíno, an indigenous people of the Greater Antilles who had a rich mythology of the ocean, and prior to arrival of European colonists were historically distributed throughout the central Caribbean locations where *E. taino* **n. sp.** is now common,.

Remarks. The long-unrecognized sister taxon of *E. papillosa*, *E. taino* **n. sp.** was uncovered in our preliminary screen for cryptic species using DNA barcoding. At the COI locus, all sampled *E. taino* **n. sp.** specimens (n = 54) formed a monophyletic lineage that was sister to a clade comprising all *E. papillosa* specimens (n = 120) (Fig. 3A; Trathen 2010; authors' unpublished data). These two clades were a minimum 8.8% divergent.

In comparison, maximum pairwise COI distance within *E. taino* **n. sp.** was 3.1%. Moreover, specimens of *E. taino* **n. sp.** shared a set of nuclear H3 alleles distinct from the alleles sampled from *E. papillosa*, even in Jamaica and Bahamas sites where both species co-occur; thus, the two divergent COI lineages are non-interbreeding species, one comprising *E. papillosa* and the other, *E. taino* **n. sp.** Branch lengths from phylogenetic analyses of our four-gene dataset shown in Fig. 4 reflect the species-level divergence between these two taxa.

Elysia taino **n. sp.** is difficult to distinguish from *E. papillosa* by morphology, ecology or development. Both species swim when disturbed, have transverse brownish bands on the rhinophores and papillose body surfaces, and possess serrated, straight blade-shaped radular teeth; both feed on *Penicillus*, have scoop or spoon-like penial stylets, and produce planktotrophic clutches with white ECY. However, molecular data differentiate the two species, and although *E. papillosa* and *E. taino* **n. sp.** co-occur in many localities, parts of their ranges are non-overlapping. All "*papillosa*-like" specimens sampled in Dominica (n=7) and two Bahamas sites (New Providence, Compass Cay) were *E. taino* **n. sp.**, as were the majority of such specimens sampled in Jamaica (86%) and four Bahamas islands: Stirrup Cay (70%), San Salvador (86%), Plana Cays (80%), and Northern Exumas (67%). In contrast, *E. taino* **n. sp.** was the rarer species in three Bahamas sites: Sweetings Cay (6%), Bimini (8%), Little San Salvador (15%). The geographic partitioning of the two species may reflect historical isolation during Pleistocene periods of low sea level, followed by gradual expansion during the Holocene. The lack of admixture at the nuclear H3 locus where the two species are sympatric indicates reproductive isolation has evolved, and thus that the two lineages are biologically good species.

Because no specimens of *E. taino* **n. sp.** were collected in Bermuda, this species cannot be the same as *E. papillosa* Verrill, 1901. Prior studies on *E. papillosa* from Curaçao (Ev. Marcus & Er. Marcus 1967) and Florida (Jensen 1980) concerned populations from which *E. taino* **n. sp.** has not been sampled, and thus likely comprise valid records of *E. papillosa*.

The main difference in internal anatomy that permits morphological differentiation is the shape of the radular tooth, which is narrower and more blade-like in *E. papillosa* versus thicker and more curved in *E. taino* **n. sp.** The leading tooth is significantly longer in *E. taino* **n. sp.** (188.5 µm ± 21.7 SE; n = 6 specimens) than in comparably sized specimens of *E. papillosa* (129.7µm ± 11.0 SE; n = 18 specimens) (results of an unpaired two-tailed *t* test: df = 22, $t = 2.58$, $P = 0.017$). Furthermore, the leading tooth is about twice as wide in *E. taino* **n. sp.** (30.0 µm ± 3.4 SE; n = 6 specimens) than in *E. papillosa* (15.6 µm ± 1.6 SE; n = 18 specimens), a highly significant difference (unpaired two-tailed *t* test: df = 22, $t = 4.23$, $P = 0.0003$). Overall, the ratio of tooth width to length is much greater for *E. taino* **n. sp.** (16.2 ± 1.1 SE) than in *E. papillosa* (11.8 ± 0.5 SE), permitting distinction based on radular anatomy that fully accorded with molecular phylogenetic analysis. The angle of curvature for the convex side of the leading tooth is also markedly different between the species. The smooth upper edge of the tooth in *E. taino* **n. sp.** angles more sharply from base to tip, making a mean angle of 31.0° ± 3.7 SE. In contrast, the upper surface of the tooth curves only 23.4° ± 1.3 SE in *E. papillosa*, making the tooth significantly straighter from base to tip (unpaired two-tailed *t* test: df = 22, $t = 2.42$, $P = 0.025$). The tooth of *E. papillosa* (Fig. 20C) also lacks the lateral keel of *E. taino* **n. sp.** (Fig. 72B).

The dorsal vessel pattern is similar between *E. taino* **n. sp.** and *E. papillosa*, but the vessels may be consistently narrower in *E. taino* **n. sp.**, and form a more densely anastomosing network along the inner parapodial margin on large specimens (Fig. 71). The sperm-storage vesicles typically form near the middle of the renopericardial extension in *E. taino* **n. sp.**, but occur near the posterior end of the renopericardial extension in *E. papillosa* (Fig. 19). Many specimens of *E. taino* **n. sp.** also have fine red lines crisscrossing the parapodial margin, a feature not observed in *E. papillosa*. The same features that differentiate *E. papillosa* from all other related Caribbean elysiids similarly distinguish *E. taino* **n. sp.**

***Elysia buonoi* new species**
(Figs. 56A, 73–74)

Elysia cf. *zuleicae*—Krug *et al.* 2015: 990, fig. 3B

Type material. San Salvador, Bahamas, July 2007, (Holotype LACM 3315, Paratype LACM 3316), collected by PJK.

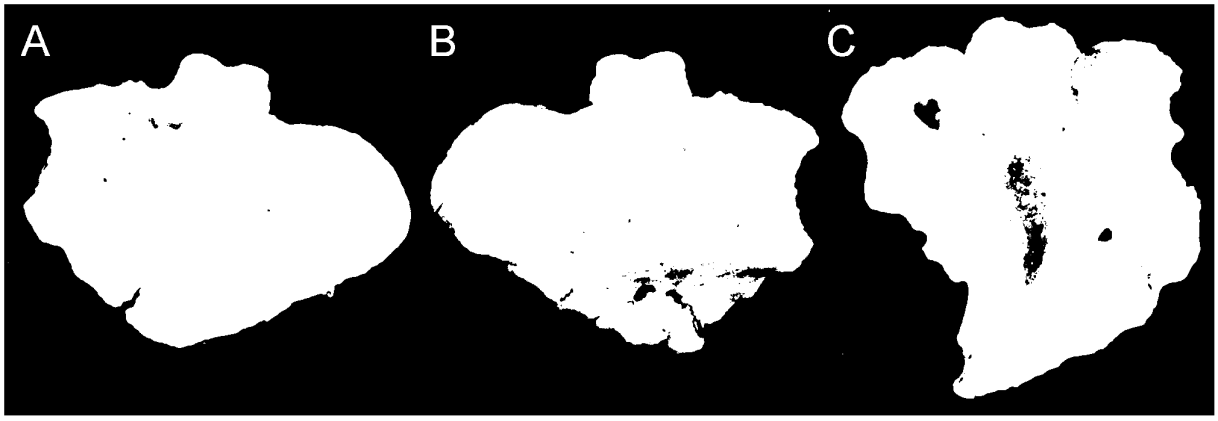

FIGURE 73. *Elysia buonoi* **n. sp.**, external morphology of preserved type specimens. **A,** Dorsal view of preserved holotype (LACM 3315); body 3.5 mm long × 3.8 mm wide at widest part of parapodia. **B**, Ventral view of preserved holotype. **C,** Dorsal view of preserved paratype (LACM 3316); body length = 3.7 mm.

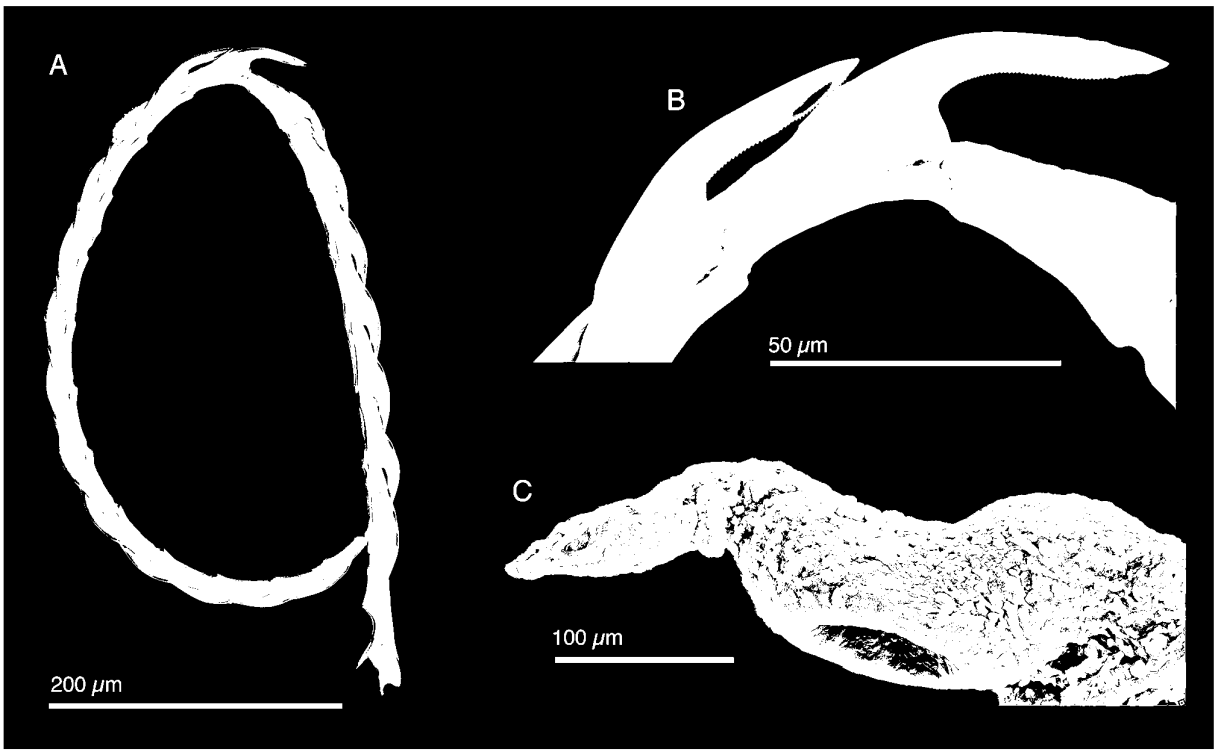

FIGURE 74. *Elysia buonoi* **n. sp.**, SEM of the radula and penis. **A**, Radula (LACM 3316). **B**, Leading tooth (LACM 3316). **C**, Penis (LACM 3315).

Type locality. San Salvador, Bahamas

Material examined. Bahamas: San Salvador, July 2004, 2 specimens (isolate Ebuo_04Ssal02, isolate Ebuo_04Ssal03), July 2007, 2 specimens (Holotype LACM 3315, Paratype LACM 3316).

Live animal. No data available; live specimens were among a bulk collection of *E. zuleicae* from *Udotea*, and were not initially recognized as distinct by external morphology.

External anatomy. Four preserved specimens examined, similar in general shape to *E. zuleicae*. Preserved holotype specimen ~3.5 mm long, and 3.8 mm across at widest point of parapodia when flattened; paratype ~3.7 mm in length (Fig. 73). Scattered brown spots along inner parapodial surface. Smooth parapodial margin. Large rounded pericardium; no dorsal vessels evident (Fig. 73A, C). No extended tail visible on any specimens.

Internal anatomy. Radula with 21 teeth (LACM 3316), 8 teeth in ascending limb and 13 in descending limb (Fig. 74A). Leading tooth narrow and elongate with approximately 50 small denticles on cusp (Fig. 74B). Teeth without the typical "V"-shaped depression of many other elysiids. Instead, teeth overlapping slightly with their tips resting on lateral side of adjacent teeth. Base of the tooth thickened, tall, and elongate, about ½ total tooth length.

Penis elongate with kink in distal end and tapering into a conical apex, devoid of armature (Fig. 56A, Fig. 74C). Deferent duct long, narrow, and highly convoluted.

Reproduction and development. No data available.

Host ecology. Found on *Udotea flabellum* together with specimens of the more common species, *E. zuleicae*.

Phylogenetic relationships. *Elysia buonoi* **n. sp.** was detected in a population-genetic study of *E. zuleicae*, its sister species; the two taxa were minimally 10.2% divergent at COI, above our initial threshold for species delimitation, and were recovered as a distinct species by ABGD analysis of COI data (Figs. 3A, 4). The four Bahamas specimens also shared distinctive alleles at the nuclear H3 locus that were not sampled in any population of *E. zuleicae*, and were distinguished from all *E. zuleicae* alleles by a fixed difference at position 34 of the 328-bp fragment amplified (G for *E. buonoi* **n. sp.**, A for *E. zuleicae*) (Trathen 2010; authors' unpublished data).

Range. Known only from San Salvador Island, Bahamas.

Etymology. Named in honor of Thomas Buono, for profoundly deepening PJK's appreciation of marine life at many unforgettable family dinners.

Remarks. Further work integrating molecular data with morphological and developmental characters is needed to more fully describe *E. buonoi* **n. sp.**, known only from a single Bahamas island. *Elysia buonoi* **n. sp.** closely resembles its sister species *E. zuleicae*, but may be distinguished by the absence of an extended tail and penial stylet in *E. buonoi*; moreover, the two species differ genetically at both mitochondrial and nuclear loci. The COI distance between *E. buonoi* **n. sp.** and *E. zuleicae* exceeds the threshold gap proposed for delimiting species of *Elysia* (Krug *et al.* 2013). Distinctive alleles and a fixed difference at the nuclear H3 locus distinguished sympatric specimens from San Salvador, indicating *E. buonoi* **n. sp.** does not interbreed with *E. zuleicae* when they co-occur. The combined divergence at COI and H3 support recognition of *E. buonoi* **n. sp.** as a distinct species. The radular morphology of *E. zuleicae* and *E. buonoi* **n. sp.** is virtually undistinguishable, both having radular teeth with small denticles, curved cusps and a thickened base. Both dissected specimens of *E. buonoi* **n. sp.** lacked a penial stylet, whereas most specimens of *E. zuleicae* possess a scoop-shaped stylet. Although additional specimens must be examined to confirm that a penial stylet is always absent in *E. buonoi* **n. sp.**, differences in penial armature could result in reproductive isolation, and thus explain how sympatric sister species avoid interbreeding when they co-occur.

Ortea, Caballer, Moro & Espinosa *in* Ortea *et al.* (2005) described *E. deborahae* as similar to *E. zuleicae* but lacking a tail, while having a lighter coloration, a penial stylet, and subtly different radular tooth morphology. In contrast to *E. deborahae*, our specimens of *E. buonoi* **n. sp.** lacked a penial stylet. Furthermore, radular teeth were described as smooth in *E. deborahae*, whereas the teeth of *E. buonoi* **n. sp.** are finely denticulate. The radula of *E. buonoi* **n. sp.** also does not match the description of the radula in *E. deborahae*, in which the maximum height was attained after the edge of the base, and fewer teeth were present in the descending limb. We could not determine the living color of *E. buonoi* **n. sp.**, as our specimens were preserved, but no specimens collected from San Salvador, Bahamas matched the description of external coloration given for *E. deborahae*. Thus, we conclude that *E. buonoi* **n. sp.** is not conspecific with *E. deborahae* Ortea, Espinosa & Moro, 2005.

Discussion

Prior to this work, *Elysia* contained 87 species generally considered valid, updating the list in Jensen (2007) to include eight more recently described species from the Atlantic (Ortea & Moro 2009; Ortea *et al.* 2005, 2011, 2013) and one from the Indo-Pacific (Wägele *et al.* 2010); the total was 89 when treating *Pattyclaya* as a junior synonym of *Elysia*. In this study, we described six new species and synonymized three (*E. annedupontae*, *E. clarki*, *E. leeanneae*) from the Caribbean. We also consider *E. chitwa*, *E. scops* and *E. sarasue* as nomina dubia, given the inadequate initial descriptions and lack of data associated with these names. The described species diversity in *Elysia* (+ *Pattyclaya*) thus stands at 90 identifiable species, pending further monographic treatment of other regions. However, true diversity in *Elysia* is likely much greater, with over 40 candidate species that have not yet

been matched to existing descriptions (Gosliner *et al.* 2015; Krug *et al.* 2015). Thus, further work is needed to characterize fully the species diversity of *Elysia*. Most undescribed diversity has been sampled in the Indo-Pacific, but the present study and recent studies by Ortea and colleagues show that considerable species richness remains undescribed even in well-studied areas like the Caribbean.

Elysia comprises about one-third of the species diversity in Sacoglossa. Ongoing studies will test whether trait-mediated diversification accounts for this disproportionate concentration of species richness, focusing on potential 'key characters' like kleptoplasty, as well as the state of frequently changing characters like larval type or host use. For instance, species with lecithotrophic larval development are concentrated in *Elysia*, but such lineages have reduced rates of diversification compared to planktotrophic lineages (Krug *et al.* 2015); thus, shifts in larval development do not explain why *Elysia* is so speciose. *Elysia* spp. are generally photosynthetic, either for short or long periods of time (Poore *et al.* 2008; Händeler *et al.* 2009; Christa *et al.* 2014b), and are often both host-specific and host-associated *in situ*. Moreover, as a group, *Elysia* spp. utilize a wider array of algal hosts than any other taxon within Sacoglossa. Host shifts, kleptoplasty, or their evolutionary synergy may explain the exceptional success of *Elysia* compared to other sacoglossan genera, which generally each have fewer than 20 species.

Phylogenetic hypotheses also provide a framework for identifying local radiations, and untangling the various evolutionary processes that gave rise to a regional fauna. Ocean basins may differ in opportunities for vicariance or transient allopatry to sunder ancestral taxa, which may explain why some basins are biodiversity hotspots while others remain relatively depauperate in species. Vicariance likely explains the origin of some geminate pairs separated by the Isthmus of Panama, particularly *E. vetulinus* from the Caribbean and *Elysia* sp. 6 from the eastern Pacific. Other candidate geminates include *E. ornata*, *E. pawliki* **n. sp.**, and *E. zemi* **n. sp.**, each of which has an Indo-Pacific sister taxon not yet known from the eastern Pacific. However, several within-Caribbean radiations challenge the classical paradigm of marine speciation by allopatric divergence among ocean basins (Mayr 1954, 1963). Speciation may occur via ecological divergence with gene flow, and our phylogeny suggests frequent host shifts may partly account for increased diversification in *Elysia*. We hypothesize that short-term kleptoplasty (retention of photosynthetically active chloroplasts from an algal diet) facilitates exploration of host space by depressing the rate at which slugs must feed on novel hosts during the early stages of a host switch. Specimens that feed on a new host will be initially maladapted to toxic secondary metabolites, the degree of calcification, structural polysaccharides in the algal cell wall, and filament dimensions or cell thickness (Jensen 1983; Cimino & Ghiselin 2009). By acquiring some fixed carbon through kleptoplasty, elysiids may not need to feed as often on a novel host to survive, facilitating the initial stages of a host switch. Adaptive evolution in radular morphology and detoxification systems would follow, as a host race evolved into an incipient species. Candidate examples include the recent sympatric shift from *Caulerpa* to *Rhipocephalus* in the ancestor of *E. pratensis*, and various shifts to heterokont algae and seagrasses by species in subclade 2.

Notably, subclade 1 comprised four Caribbean lineages specialized on different host genera: *Penicillus* (*E. papillosa* + *E. taino* **n. sp.**), *Udotea* (*E. zuleicae* + *E. buonoi* **n. sp.**), *Halimeda* (*E. patina*), and *Rhipilia* (*E. christinae* **n. sp.**). Host partitioning suggests divergent selection on resource use may have been an important factor driving the initial stages of this adaptive radiation. However, two pairs of sister taxa collected from the same host suggest more recent non-ecological speciation occurred within the Caribbean. Another example is *E. crispata* and *E. ellenae*, sister taxa that are not strongly host-associated in the field and overlap in range. Alternative mechanisms must be invoked to explain recent divergence within basins in these lineages. Allopatric divergence may have occurred during periods of low sea level in the Pleistocene, as suggested by the partially non-overlapping ranges of *E. papillosa* and *E. taino* **n. sp.** Evolutionary change in penial armature or female sites for sperm receipt and storage may also play an important role in the early stages of reproductive isolation. Notably, in two sympatric species pairs (*E. crispata-E. ellenae*, *E. zuleicae-E. buonoi* **n. sp.**), one member possesses a stylet and one does not. Such sexually selected traits can directly affect reproductive compatibility among subpopulations and may contribute to speciation in marine heterobranchs, which have highly complex reproductive systems (e.g., Churchill *et al.* 2013, 2014).

Jensen (1992) stated that penial stylets are rare in *Elysia*, but common in superfamily Limapontioidea. Recent phylogenetic hypotheses for Sacoglossa indicate that Limapontioidea is a paraphyletic grade basal to Plakobranchoidea (Christa *et al.* 2014, Krug *et al.* 2015); thus, the ancestor of *Elysia* may have possessed a stylet, but improved phylogenetic resolution within *Elysia* is needed to resolve this question. It is possible that a stylet independently evolved in one ore more putatively basal lineages (e.g., subclade 1, *E. velutinus*, *E. stylifera*) as well

as multiple times in derived *Elysia* spp. (e.g., *E. marcusi*, *E. cornigera*, *E. zemi* **n. sp.**). Ancestral state reconstructions may be premature, as the literature often fails to note accurately whether a stylet was present, and examination of multiple specimens by SEM is necessary to determine reliably this character state. We found a high frequency of stylets among taxa in the present study (10 out of 23 Caribbean species), some of which were erroneously reported to lack a stylet in their original description (e.g., *E. zuleicae*). Thus, stylets are either rare in the Indo-Pacific and Mediterranean, or have been systematically under-documented leading to the misapprehension that most *Elysia* spp. lack penial armature. Further study of species from other regions is needed to clarify if the Caribbean is unusual in the high incidence of stylets among its *Elysia* spp., or rather if stylets have been overlooked in Pacific taxa due to a lack of careful examination by SEM. Stylet-possessing taxa were clearly non-monophyletic, rejecting the hypothesized genus *Checholysia* (Ortea *et al.* 2005).

Our phylogeny, combined with morphological and developmental data, suggest that characters such as penial stylets, radular morphology, parapodial size and shape, and lecithotrophic development are all surprisingly plastic (Table 2). For any trait, a given state likely evolved multiple times, and/or was repeatedly lost. Adaptation to similar hosts may drive convergent evolution of coloration and shape, such as the parallel reduction in parapodia seen in *E. pusilla* and *E. marcusi*, species that are not closely related but both feed on *Halimeda*. Even radular tooth characters show evidence of homoplasy, such as the similar tooth shapes of *Halimeda*-feeders *E. patina* and *E. marcusi*, or the strikingly similar teeth of *E. taino* **n. sp.** and *E. pratensis*, distantly related taxa that feed respectively on the related and morphologically similar algae *Penicillus* and *Rhipocephalus*. Population-level changes in penial morphology (Churchill *et al.* 2013, 2014), larval development (Ellingson & Krug 2016) or host use may each contribute to the early stages of speciation, and thus evolve in a cladogenetic manner. For these reasons, we did not attempt to use morphological character data in a combined or independent phylogenetic analysis, as the available data suggest a high degree of homoplasy in most of the characters we scored. However, the combined evidence from all characters yields the most robust species descriptions (Table 2), and should facilitate downstream studies of trait evolution and state-dependent diversification.

Our results support the integrative approach to species discovery proposed by Krug *et al.* (2013, 2015), including DNA barcoding using both COI and H3, an initial screen for divergent mtDNA using an 8% COI distance threshold, and more detailed delimitation analyses to ensure that more closely related species were not overlooked. Unfortunately, current methods for integrative species delimitation that combine coalescent analysis of molecular data with modeled evolution of morphological traits can only accommodate continuous character data, rather than the discrete characters available for soft-bodied sea slugs. Key traits such as presence/absence of a stylet, tooth shape, diaulic vs triaulic reproductive systems, and color patterns cannot presently be included in delimitation steps, but fixed differences in morphological, ecological or reproductive traits can be used to test species hypotheses generated using molecular data (Vieites *et al.* 2009; Krug *et al.* 2013). Such traits then form the natural basis for integrative species descriptions, and reinforce conclusions drawn from molecular data. Notably, in the present study, the members of two previously unrecognized species pairs (*E. papillosa* + *E. taino* **n. sp.**, *E. zuleicae* + *E. buonoi* **n. sp.**) were first distinguished as genetically divergent COI lineages that also showed fixed differences at H3. Morphological differences in the radula or penis were then identified which supported the hypothesis that the divergent lineages were distinct species, consistent with molecular analyses. In contrast, clades within *E. crispata* were <8% divergent, were not separated by ABGD analysis, and did not differ consistently in external or internal morphology, supporting the synonymy of *E. clarki* and consideration of *E. crispata* as a highly polymorphic species with genetically subdivided populations.

Taxonomic problems arise often from over-reliance on one phrase picked from a description that may have been based on few specimens, or disproportionate focus on one aspect of a drawing, both of which can lead to false impressions regarding the identity of common species. For instance, Ortea *et al.* (2005) misinterpreted the piece of *Halimeda incrassata* on which Verrill (1901) drew *E. papillosa* resting, assuming the algae represented long branching papillae and disregarding Verrill's text which clearly stated "small conical papillae" on the animal. Similarly, Ortea *et al.* (2005) note Ev. Marcus' drawing of a second species in her description of *E. patina* (Marcus 1980: figs 59–60), but ignore that her drawings of radular teeth from the type specimen (figs. 23–24, 42) clearly differentiate *E. patina* from *E. papillosa* (the species Ortea *et al.* (2005) misidentified as *E. patina*). Lastly, Ortea *et al.* (2013) rejected the synonymy of *E. eugeniae* with *E. canguzua* proposed by Valdés *et al.* (2006), arguing that Er. Marcus (1955) described *E. canguzua* as having "rabbit's ear-like" rhinophores. Ortea *et al.* (2013) characterized the rhinophores of *E. eugeniae* as "tubular … rolled up like a parchment," yet the drawings of the

TABLE 2. Summary of characters for Caribbean elysiids including internal and external morphology, development, and algal host use.

		radular tooth					dorsal vessels					
***Elysia* species**[1]	**page #**	**cutting edge**[2]	**denticles**	**tip**	**penial stylet**[3]	**renoperi-cardium**	**# per side**	**branching**	**anasto-mosing**	**larval type**	**ECY**	**host alga**
ornata	18	curved	N	pointed	N	very short, rounded	3	complex	Y	P	white blobs	*Bryopsis*
crispata	23	straight, bent or curved; slight lateral edge	N or Y (fine)	pointed	N	very short, rounded	3–4	may fork once	N	L (EM or swim)	none	*Acetabularia* *Bryopsis* *Derbesia* *Halimeda* *Penicillus*
chlorotica	35	straight	Y (fine)	pointed	N	very short, rounded	3–4	repeatedly forking or branching	anterior vessels	P or L (EM)	none	*Vaucheria*
papillosa	40	straight or slightly bent	Y (coarse)	pointed	Y (sc)	½ body length	6–7	no to complex at tips	N or Y	P	white ribbon	*Penicillus*
flava	50	curved; lateral edge	Y, keel; N, lateral edge	pointed	N	very short, blunt	1	simple lateral branches	N	?	?	?
subornata	52	straight or angled to tip	Y (fine)	blunt	N	body length	6–8 or more	complex	Y (all)	L (EM)	orange ribbon	*Caulerpa* spp.
velutinus	57	curved (spur)	Y (fine)	pointed	Y (r)	short, rounded	1	may fork once	N	L (swim)	orange ribbon	*Halimeda* spp.
canguzua	62	straight; lateral edge	Y (fine)	pointed	N	short, rounded	2–3	may fork once	N	P	none	*Bryopsis*
chitwa (n.d.)	68	short, wide	N	pointed	N	?	?	?	?	?	?	?
serca	69	wide; doubly bent	N	pointed	N	½ body length	6–7	may fork once	N	P	none	seagrasses
evelinae	73	pipe-like, straight	N	blunt	N	½ body length, ovoid	2–3	few short lateral branches	N	L (EM or swim)	none	diatoms
scops (n.d.)	76	short, straight	Y (fine)	?	?	body length	6–8 or more	complexly branching	Y (all)	?	?	?
marcusi	78	straight	Y (coarse)	pointed	Y (p)	internal	0	n/a	n/a	L (swim)	white or yellow blobs	*H. opuntia*, *H. goreaui*
nisbeti	83	curved	N?	pointed	?	⅔ body length	>9	repeatedly	Y (all)	?	?	?
patina	85	straight (spur)	Y (fine)	pointed	Y (sc)	½ body length	4–5	1–2 forks	N or just posterior	L (swim)	flat orange ribbon	*H. opuntia*

……*continued on the next page*

TABLE 2. (Continued)

Elysia species[1]	page #	radular tooth: cutting edge[2]	radular tooth: denticles	radular tooth: tip	penial stylet[3]	renoperi-cardium	dorsal vessels: # per side	dorsal vessels: branching	dorsal vessels: anasto-mosing	larval type	ECY	host alga
cornigera	90	curved; 2 lateral edges	Y, keel + 1 of 2 edges	uneven, curved	Y	short, tapered	1	simple or forked side branches	N	L (EM or swim)	white or yellow blobs	*Acetabularia*
pratensis	95	short, straight	Y (coarse)	pointed	N	body length	>10	1 fork	N	L (EM)	orange ribbon	*Rhipocephalus*
zuleicae	99	curved (spur)	Y (fine)	pointed	Y (sc)	short	2	complex	Y	P or L (swim)	white ribbon	*Udotea*
deborahae (?)	107	curved (spur)	N	pointed	Y	½ body length	3–4?	complex	Y	?	?	*Halimeda?*
jibacoaensis (?)	110	angled or curved	N	pointed	?	short?	1?	lateral branches?	N?	?	?	*Penicillus?*
orientalis	112	?	?	?	N	?	?	?	?	?	?	?
sarasuae (n.d.)	114	?	?	?	?	short?	?	?	?	?	?	?
ellenae	115	short, wide; bent; 2 lateral edges	Y, keel; N, lateral edges	pointed prior to use	Y (ht)	prominent, round, short	3	1 fork	N	?	?	?
pawliki n. sp.	119	straight, but angled to tip	Y (fine)	blunt	Y (ht)	½ body length	4–5	complex	Y	L (swim)	orange ribbon	*C. racemosa*
zemi n. sp.	125	straight, but angled to tip	Y (fine)	blunt	Y (ht)	½ body length	5–7	simple to complex	N or Y	?	?	*C. racemosa*
christinae n. sp.	129	narrow, curved	Y (coarse)	pointed	Y	body length	4–7	1–2 forks	N	P	orange ribbon	*Rhipilia*
hamanni n. sp.	134	short, wide; straight	N	pointed	N	⅔ body length	13	complex	Y	?	orange ribbon	*C. prolifera*
taino n. sp.	137	straight	Y (coarse)	pointed or blunt	Y (sc)	½ body length	5–8	complex	Y	P	white ribbon	*Penicillus*
buonoi n. sp.	144	curved (spur)	Y (fine)	pointed	N	short, rounded	none visible	n/a	n/a	?	?	*Udotea*

[1](?) = species of uncertain status; (n.d.) = *nomen dubium*
[2]bent = cusp with two straight portions forming an angle at one `point (doubly = two points); curved = cusp makes a smooth arc towards tip; spur = narrow tooth curving to pointed tip *sensu* Clark & DeFreese (1987)
[3]Stylet type: ht = hardened, hollow tip; p = pointed; r = ridged; sc = scoop

species in their respective descriptions are essentially indistinguishable, and no other species has the prominent anal papilla unique to this (one) species. Good taxonomic practice cannot rely on subjective interpretations of what "rabbit's ear-like" means, while disregarding major features of the anus and reproductive system (*i.e.*, penial morphology) that distinguish *E. canguzua* (and other species).

Similarly, conspecifics can be falsely split into nominal 'species' when taxonomists do not examine multiple specimens or sample different populations, and fail to characterize intra-specific variation. Many characters can be taxonomically informative in some cases yet phenotypically plastic in others, reflecting individual diet or history, including presence of a penial stylet, radular morphology, shape or size of body and tail, and coloration. Radulae can change shape depending on diet (Jensen 1993), and the length and number of radular teeth varies with age and size of an individual; thus, the number and size of teeth is not a reasonable basis to treat otherwise indistinguishable specimens as different species. Penial stylets can evidently break off during traumatic mating, as we found variation in the presence of a stylet across specimens of *E. zuleicae*. Ortea *et al.* (2013) assert that sperm-storage vesicles ("parapodial sacs") were absent from *E. annedupontae*, but these structures transiently form upon receipt of allosperm and thus may not be apparent on all specimens, depending on recent mating history. Local, population-level adaptation can also account for morphological variation and moderate levels of sequence divergence, leading to erroneous classification of conspecifics as different species. Such differences among populations can be evolutionarily significant and should not be overlooked, but neither should they be considered grounds for erecting a new species without careful study of specimens sampled from across the range.

We urge taxonomists working on sacoglossans to make use of all available information present in original descriptions, and to examine type material whenever possible, to assess whether a specimen represents a described species. Minor differences in coloration or size of features (without normalizing to body size) are not appropriate for proposing new species in the absence of data on differences in reproductive anatomy, or genetic data showing divergence from described species consistent with species-level differences. Paratypes suitably preserved for molecular analysis should be archived if DNA barcodes cannot be generated and included in descriptions, to facilitate later identification as well as placement of new species in a phylogenetic framework. Such practices will achieve maximal taxonomic stability and allow accurate assessment of biodiversity in Sacoglossa, facilitating evolutionary studies on mechanisms of speciation and trait-based diversification in this group.

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TABLE S1. Caribbean sampling sites & coordinates.

Site #	**Site** (code)	Latitude, Longitude	Collection dates	Collector
	Bahamas			
1	Sweetings Cay (Swe)	26° 33' 43" N, 77° 51' 15" W	6/2004, 6/2007, 7/2010	PJK
2	Great Stirrup Cay (Stir)	25° 49' 12" N, 77° 53' 56" W	2007/6/1	PJK
3	Little San Salvador (LSS)	24° 34' 30" N, 75° 56' 30" W	6/2004, 6/2007	PJK
4	San Salvador (SSal)	24° 08' 30" N, 74° 28' 18" W	6/2004, 6/2007, 7/2010	PJK
5	Plana Cays (Pla)	22° 36' 40" N, 73° 33' 52" W	6/2004, 6/2007	PJK
6	Compass Cay (Comp)	24° 16' 29" N, 76° 30' 35" W	7/2010	PJK
7	Northern Exumas (NEx)	24° 46' 00" N, 76° 49' 01" W	7/2010	PJK

.....continued on the next page

TABLE S1. (Continued)

Site #	**Site** (code)	Latitude, Longitude	Collection dates	Collector
8	New Providence (NPr)	25° 00' 35" N, 77° 34' 00" W	7/2010	PJK
9	Bimini (Bim)	25° 44' 19" N, 79° 16' 20" W	7/2010	PJK
10	Abaco (Aba)	25° 30' N, 77° 05' W	2004-2006	AB
11	Lee Stocking Island (Lee)	23° 46' 18" N, 76° 06' 16" W	2006-2009	ADP
	Florida Keys, U.S. (FL)			
12	Lake Surprise, Key Largo (LKS)	25° 10' 55" N, 80° 23' 05" W	6/2007, 10/2009, 7/2010	PJK
	Anne's Beach	24° 50' 54" N, 80° 44' 27" W		
	Summerland Key (Sum)	24° 39' 47" N, 81° 27' 48" W	6/2007	YG
	Mote marine lab	24° 39' 42" N, 81° 27' 16"W		
13	Geiger Beach, Key West (Gei)	24° 33' 59" N, 81° 40' 13" W	7/2006, 6/2007, 10/2009	PJK
14	Dry Tortugas (Dry)	24° 37' 47" N, 82° 52' 26" W	2010/10/1	P.J.K., C.B.
15	**Bermuda**, St. George's Harbor (Ber)	32° 21' 57" N, 64° 42' 30 "W	6/2006	PJK, RAE
16	**Jamaica,** Discovery Bay (Jam)	18° 28' 07" N, 77° 24' 53" W	3/2006	PJK, RAE
17	**U.S. Virgin Islands**, St. Thomas (USVI)	18° 21' N, 64° 52' W	4/2006	AV
		18° 22' N, 64° 55' W	2004-2006	AB
18	**Antigua** (Ant)			
	English Harbor	17° 07' 04" N, 61° 50' 42" W	4/2008	DAW
	Parham Harbor	17° 06' 43" N, 61° 46' 01" W	4/2008	DAW
19	**Dominica,** Secret Bay (Dom)	15° 32' 59" N, 61° 28' 22" W	9/2006, 6/2007, 4/2008	DAW
20	**St. Lucia**, Sou Friere Bay (StL)	13° 51' 27" N, 61° 03' 45" W	3/2008	DAW
21	**Bonaire** (Bon)	12° 9' 21" N, 68° 16' 55" W	5/2012	SKP
22	**Curaçao**, Spanish Waters (Cur)	12° 04' 51" N, 68° 51' 15" W	1/2009	PJK
23	**Panama,** Bocas del Toro (Pan)	09° 19' 60" N, 82° 15' 00" W	12/2004	PJK, RAE, DAW
24	**Belize** (Blz)	17° 30' N, 88° 15' W	2004-2006	AB
	Turneffe Atoll	17° 10' 30" N, 87° 54' 59" W	6/1991	MH

PJK—Patrick J. Krug; AB—Anna Bass; AV—Angel Valdes; RAE—Ryan A. Ellingson; DAW—Demian A. Willette; SKP—Skip K. Pierce; YG—Yvonne Gryzymbowski; CB—Carole Bewley; ADP—Anne DuPont; MH—Michael Hellberg.