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 synonymsies 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), transferred one species from *Bosellia* (*Elysia marcus*), and described six new species (*Elysia pavilki* 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 velatimus* 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 (Dionisio *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 Valdès *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; Valdès *et al.* 2006; Garcia *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 (Muséum 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). Types and voucher specimens from the following institutions 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 ± 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 MgCl₂, 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 an 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 interspecific (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. papilloosa 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 Tratthen (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 taxae 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, Kears 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.

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</table>

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.

1. 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.

2. Fully 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.
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 + $\Gamma$ 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 $\geq$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 + $\Gamma$ 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 $\geq$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 $\sim$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. marcusii); 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. crispat a, which has relatively non-dispersive larvae. Notably, all E. crispat a and nominal E. clarki morphotypes were recovered as a single species by ABGD. Moreover, H3 alleles were shared among nominal specimens of E. crispat a and E. clarki.
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. 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 Plakobranchiidae: (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 pleiomorphic in *Elysia*; stylets are present in *Thuridilla* and *Plakobranchus*, 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


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

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

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

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

*Tridachia* Deshayes 1857: 142 (Type species: *Elysia schrammi* Möhr, 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).
Systematics of Caribbean Elysia

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

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

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

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

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

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


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 Hydropsycye for this species if it is later found that it does not belong to any known genus. Pease (1860) described the genus Pterogasteron for two species collected in the Hawaiian Islands, Pterogasterorn ornament Pease, 1860 and Pterogasterorn bellum Pease, 1860. No type species was indicated. Pease’s illustrations were published in Bergh (1881: pl. G, fig. 18–19), who transferred Pterogasterorn ornament 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, Hydropsycye, 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 *Elysia* for *Elysia pusilla* Bergh, 1871 and distinguished it from *Elysia* because of the short tentacles and the carinated side of the head. The name *Elysia 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 *Elysia* 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) *Elysia* Verrill, 1872 was published earlier and therefore has priority, thus *Elysia* Bergh, 1872 is unavailable. Jensen & Wells (1990) and Jensen (1992; 1997b) considered *Elysia* 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, *Elysia stylifera* Jensen, 1997. However, *Elysia* is paraphyletic with respect to *Elysia* in both morphological (Gosliner 1995; Jensen 1997a) and molecular (Händeler *et al.* 2009; Krug *et al.* 2015) phylogenetic analyses. Thus, *Elysia* 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.


**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).
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.

**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

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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.


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


*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) in *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 (MNHN).

**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.
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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

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).
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).


**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.

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 Curacao 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 teniissima, 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*” 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*, 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 teniissima*, 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 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. 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_10NPt02, 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)
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 *D. diomedeas* (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 *D. diomedeas* from Caribbean populations, but ecological and life-history data on *E. ellenae* are needed to formulate hypotheses regarding its divergence from *E. crispata*.


**Remarks.** Orsted & Mörch in Mörch (1863) described *Elysia (Tridachia) crispata* in a brief Latin description based on unpublished drawings by Anders Sandoe Orsted, 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)


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 renopercardiac (Fig. 15A–D). Renopercardiac extension only slightly posterior of the pericardium, short. Wide, clear dorsal vessels emerging asymmetrically from renopercardiac 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 $\frac{1}{5}$ total tooth length (Fig. 17B). Base of tooth approximately $\frac{1}{4}$ 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.

**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.


**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 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


*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* *n. syn.* (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,
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).

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 for different specimens ranging from 0.01 to 0.05.
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 ⅔ 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 *Rhipephalus 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 *Udeacea* 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*, *Rhipephalus*, *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 elysids, 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.

**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, Curacao, 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 identity 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, 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 serrated 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)


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


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 blotsches 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.


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).
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).


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)


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

**Material examined.** Anse d u 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.
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 ⅕ 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
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.


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.


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. hamamini* 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*.


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

*Elysia pruvotifoliae* 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.

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. incrassata.*

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
latter 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 bennettiae* Thompson, 1973, *Elysia degeneri* Ostergaard, 1955, and two undescribed species (*E. cf. bennettiae*, *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

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 Mörlch” by Verrill (1900), which she argued were different from the true Tridachia crispatula. 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 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). Ortega & 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 intraspecific 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.

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)


**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.
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)


_Type material._ _Elysia serca_—untraceable, not at MZSP (Siqueira Dornellas & Simone 2011); _Elysia clena_—untraceable, not at USNM or MZSP (Siqueira Dornellas & Simone 2011).


_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 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.
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*.


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 stippled 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)


**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.
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, Ellington, 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).


**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 eysiiids, 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)


**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.


**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_.

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