



# Ecological speciation by sympatric host shifts in a clade of herbivorous sea slugs, with introgression and localized mitochondrial capture between species

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## ABSTRACT

Host shifting in insect-plant systems was historically important to the development of ecological speciation theory, yet surprisingly few studies have examined whether host shifting drives diversification of marine herbivores. When small-bodied consumers feed and also mate on a preferred host, disruptive selection can split a population into host races despite gene flow. Support for host shifts is notably lacking for invertebrates associated with macroalgae, where the scale of dispersal by planktonic larvae often far exceeds the grain of host patchiness, and adults are typically less specialized than terrestrial herbivores. Here, we present a candidate example of ecological speciation in a clade of sea slugs that primarily consume green algae in the genus *Caulerpa*, including highly invasive species. Ancestral character state reconstructions supported 'sea grapes' (*C. racemosa*, *C. lentillifera*) as the ancestral host for a tropical radiation of 12 *Elysia* spp., with one shift onto alternative *Caulerpa* spp. in the Indo-Pacific. A Caribbean radiation of three species included sympatric host shifts to *Rhipocephalus brevicaulis* in the ancestor of *E. pratensis* Ortea & Espinosa, 1996, and to *C. prolifera* in *E. hamanni* Krug, Vendetti & Valdes 2016, plus a niche expansion to a range of *Caulerpa* spp. in *E. subornata* Verrill, 1901. All three species are broadly sympatric across the Caribbean but are host-partitioned at a fine grain, and distinct by morphology and at nuclear loci. However, non-recombining mtDNA revealed a history of gene flow between *E. pratensis* and *E. subornata*: COI haplotypes from *E. subornata* were 10.4% divergent from *E. pratensis* haplotypes from four sites, but closely related to all *E. pratensis* haplotypes sampled from six Bahamian islands, indicating historical introgression and localized "mitochondrial capture." Disruptive selective likely fueled divergence and adaptation to distinct host environments, indicating ecological speciation may be an under-appreciated driver of diversification for marine herbivores as well as epibionts and other resource specialists.

## 1. Introduction

Traditional models of speciation emphasized drift or differential selection in allopatry as the principal engines generating new marine species, despite the lack of dispersal barriers for pelagic adults or planktonic larvae in the sea (Mayr, 1963; Briggs, 2006; Bird et al., 2012). Contrary to the predictions of those models, molecular studies of diverse marine groups commonly reveal sister taxa that are ecologically differentiated but not geographically separated (González et al., 2018; Holt et al., 2020; Hernández-Hernández et al., 2021). Recent genomic investigations have also supported incipient speciation between marine ecotypes (Johannesson et al., 2017; Teske et al., 2019), or found evidence of divergence with gene flow for reproductively isolated lineages

(Hurt et al., 2013; Titus et al., 2019). These findings suggest ecological speciation may be an important source of marine biodiversity (Bowen et al., 2016; Potkamp and Fransen, 2019; Prada and Hellberg, 2021). However, it remains difficult to falsify alternative models involving complex histories of transient allopatry, secondary contact and admixture (Foote and Morin, 2016; Yang et al., 2017; Foote, 2018; Faria et al., 2021).

Renewed interest in ecological speciation has focused on the role of disruptive selection in splitting populations into reproductively isolated gene pools occupying distinct niches (Gavrilets et al., 2007; Fitzpatrick et al., 2009). Theory supports the emergence of novel ecotypes within a population when a trait under selection affects resource utilization and by pleiotropy, either sexual signaling (Ripa, 2009; van Doorn et al.,

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2009) or habitat use (Fry, 2003). Some traits that could promote divergence or reproductive isolation of ecotypes may be distinctive to marine systems (Krug, 2011). For instance, marine populations may diverge across small-scale gradients in subtidal depth (Crow et al., 2010; Ingram, 2011; Prada and Hellberg, 2020) or intertidal height (Johannesson et al., 2010; Bird et al., 2011). Reproductive traits associated with broadcast spawning or larval development have likewise been implicated in the sympatric divergence of marine taxa, including gamete-recognition proteins (Palumbi and Lessios, 2005), spawning time (Monteiro et al., 2016; González et al., 2018), egg buoyancy (Momigliano et al., 2017), and larval type (Ellingson and Krug, 2016). While studies of such traits are critical to understand patterns of marine diversification, they may not benefit from the large body of theory developed for terrestrial and freshwater faunas.

Ecotypes or sister species may also be partitioned at small spatial scales by habitat or host choice, a process that promotes sympatric divergence across biomes (Hernández-Hernández et al., 2021). When host or habitat specialists spatially segregate into preferred niches, mating preference can be a covariate, allowing assortative mating to evolve in the face of gene flow. Disruptive selection on host or habitat use may thus sunder a population into groups of non-interbreeding specialists that spatially overlap at large scales but are ecologically partitioned at a fine grain. Candidate examples of sympatric divergence with gene flow in reef fish involve selection acting on habitat choice, usually in concert with other sexually selected traits (Munday et al., 2004; Crow et al., 2010; Whitney et al., 2018). In contrast, host choice can be a “matching” trait in small-bodied consumers or sessile epibionts, with individuals sharing host preference alleles being more likely to mate by pleiotropy (Kopp et al., 2018).

In marine systems, proposed cases of sympatric divergence by host shifting include coral epibionts (barnacles: Tsang et al., 2009) and predators (snails: Simmonds et al., 2018; sea slugs: Faucci et al., 2007; Fritts-Penniman et al., 2020), and anemone-associated shrimp (Hurt et al. 2013). Host shifts, long thought to promote speciation in other systems (e.g., insect-plant, parasite-host), may thus be of underappreciated importance to marine biodiversity given the abundance of symbiotic partnerships in the sea (Zann, 1987). Broad phylogenetic patterns in host use have been explored for cladobranch sea slugs (Goodheart et al., 2017), as well as shifts from free-living to symbiotic associations in barnacles (Tsang et al., 2014; Yu et al., 2020) and palaemonid shrimps (Chow et al., 2021). However, even where potential host shifts have been proposed in marine system, few studies have reconstructed ancestral host or habitat use to test whether generalists evolved into specialists, or if specialization predated divergence, in sympatric taxa; more often, host shifts have been inferred from tip states on a phylogeny.

Fritts-Penniman et al. (2020) reconstructed host genus for a radiation of coral-feeding nudibranchs (*Tenellia*); their results were consistent with diverse processes influencing host use and consumer divergence. In *Tenellia* “clade A”, a *Porites* generalist ancestor may have radiated into a clade comprising generalists as well as several taxa with species-specific host use. In “clade B”, host preferences may have diverged in allopatry, while “clade D” provided the clearest example of a sympatric host shift. Examples of host range expansions and contractions were also evident in coral-associated barnacles (Tsang et al., 2014). In the corallivorous snail *Coralliophila violacea*, genome-wide divergence distinguished sympatric host races from different subsets of *Porites* spp. (Simmonds et al. 2018, 2020). Thus, some phylogenetic evidence supports host shifting as a driver of speciation, and genomic data show incipient species may form on different hosts in sympatry, supporting ecological divergence as a potential contributor to the biodiversity of tropical marine systems.

Despite the expansive body of theory and experimental work addressing speciation in terrestrial herbivores, the potential for host shifting by marine herbivores remains largely unexplored. Sea slugs in clade Sacoglossa are among the most specialized marine herbivores (Poore et al., 2008); in many genera, adults feed, mate, and oviposit on a preferred genus or species, usually of green or red algae (Jensen, 1997;

Krug et al., 2016, 2018a, 2018b). Host-specific adaptations include tooth shape (Jensen, 1980; Berriman et al., 2018); sequestration of algal toxins (Cimino and Ghiselin, 2009; Baumgartner et al., 2009); crypsis (Krug et al., 2018b); and retention of functional, diet-derived chloroplasts (Pierce and Curtis, 2012; Pierce et al., 2015). Shifts onto invasive algae suggest ecological divergence can promote rapid host-race formation (Trowbridge, 2004). However, no study has combined phylogenetic, ecological and biogeographical data to evaluate evolutionary scenarios of sympatric divergence via host shift in this group.

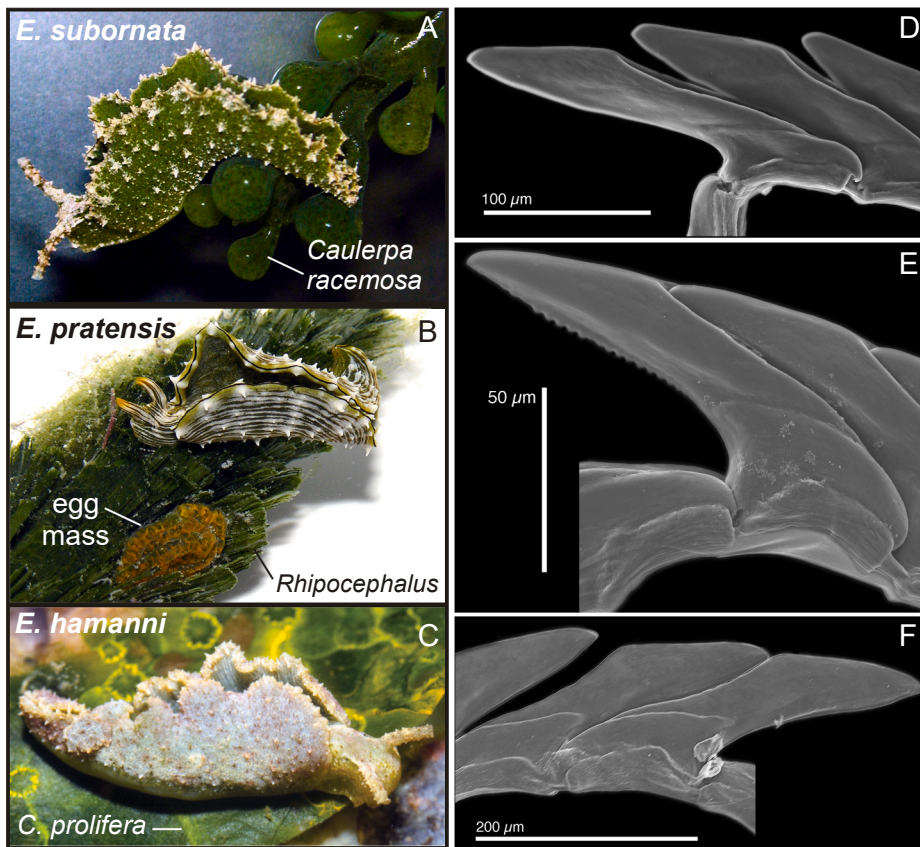
Comprising about a third of named sacoglossan species (>100 spp.), the genus *Elysia* provides a model system for investigating candidate host shifts. Most *Elysia* spp. feed on one or two macroalgal host genera, or a subset of preferred host species (Jensen 1980, 1997). The majority of species (>95%) retain chloroplasts for under 2 weeks, and slugs typically remain physically associated with their host. At least six large-bodied species in the genus *Elysia* (Heterobranchia: Sacoglossa) have been recognized as “*E. tomentosa*” in the tropical Indo-Pacific, consuming uncalcified but chemically defended green algae in genus *Caulerpa* (including highly invasive species). Cryptic species in the *tomentosa* complex are not readily distinguished by external morphology, but are > 9% divergent at the barcoding mitochondrial (mt) cytochrome c oxidase I (COI) gene, and were provisionally named (e.g., “*E. cf. tomentosa* sp.1”) pending formal taxonomic revision (Krug et al., 2013, 2016; Oladi et al., 2018; Medina, 2019). Phylogenetic analyses indicate this species complex also includes six species from the tropical Western Atlantic, three recently described (*E. hamanni*, *E. pawliki*, *E. zemi*; Krug et al., 2016) and three previously recognized (*E. orientalis*, *E. pratensis*, *E. subornata*). Most species in the *tomentosa* complex feed on a subset of *Caulerpa* spp., either ‘sea grapes’ (e.g., *C. racemosa*, or the invasive *C. cylindracea*), or ‘feather’ morph algae (e.g., *C. sertularioides*, or the highly invasive “killer” alga *C. taxifolia*). Notable exceptions include two Caribbean species that were sister taxa in all prior phylogenetic analyses: *E. subornata*, which feeds on diverse *Caulerpa* spp. including both sea grape and feather morphs (Fig. 1A), and *E. pratensis*, reported from *Rhizocephalus* (Fig. 1B) (Krug et al., 2015, 2016). A third Caribbean species, *E. hamanni*, fed solely on *C. prolifera* (Fig. 1C), an alga recently shown to have invasive potential (NOAA Fisheries, 2021). The radular teeth of *E. subornata* (Fig. 1D) are relatively smooth, bearing only fine denticulations, whereas the tooth of *E. pratensis* is markedly serrated (Fig. 1E) as an adaptation to pierce the calcified cell walls of *Rhizocephalus* (Krug et al., 2016). The distinctive host use and associated tooth shape (Fig. 1F) allowed *E. hamanni* to be distinguished from other complex members, but phylogenetic relationships of this species were not previously determined.

Here, we included molecular data for an additional Indo-Pacific species as well as *E. hamanni* in new phylogenetic analyses of the *tomentosa* complex, focusing on relationships, host ecology and gene flow for one Caribbean radiation. We tested the hypothesis that host shifting promoted ecological speciation using ancestral state reconstruction (ASR) to model the evolutionary history of algal associations for slug consumers. Phylogeographic analyses compared population subdivision in *E. subornata* vs. *E. pratensis* to test the hypothesis that species lacking a dispersive larval stage will have a high level of genetic structure. Our findings demonstrate at least two recent host shifts and a niche expansion within one Caribbean clade. Surprisingly, we also found some populations of one species fixed for introgressed mtDNA from another, suggesting historical hybridization and selection on mtDNA.

## 2. Materials and methods

### 2.1. Sample collection and host identification

Specimens of *Elysia pratensis* and *E. subornata* were collected with permission of the State of Florida or host country, except for *E. subornata* from Summerland Key (collected by Y. Gryzmbowski), Bimini (A. Alejandrino), and U.S. Virgin Islands (A. Valdes) (Table 1). Molecular and



**Fig. 1.** Live specimens of *Elysia* spp. feeding on their host algae, and scanning electron micrographs of the leading radular tooth for one exemplar per species. **A**, Live specimen of *E. subornata* on its host *Caulerpa racemosa*; actual size of specimen = 6 mm. **B**, *E. pratensis* on *Rhipocephalus phoenix*, with egg mass; actual size of specimen = 10 mm. **C**, *E. hamanni* on *C. prolifera* showing characteristic ring-like orange feeding scars on the alga; actual size = 18 mm. **D**, Radular SEM of leading tooth from specimen of *E. subornata* from Lee Stocking Island, Bahamas. **E**, Radular SEM of leading tooth from specimen of *E. pratensis* from Lee Stocking Island, Bahamas. **F**, Radular SEM of leading tooth from specimen of *E. hamanni* from Grenada. Images reproduced with permission of Magnolia Press from Krug et al. (2016) except (B), reproduced courtesy of E. Turner. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

**Table 1**  
Collection dates, localities and number of specimens sampled for *Elysia pratensis*, *E. subornata* and *E. hamanni*.

Site	<i>E. pratensis</i> (N)	<i>E. subornata</i> (N)	<i>E. hamanni</i> (N)	Latitude, Longitude	Collection dates
<b>Bahamas</b>					
1. Sweetings Cay (Swe)	13	14		26° 33' 43" N, 77° 51' 15" W	6/2004, 6/2007, 7/2010
2. Great Stirrup Cay (Stir)	8			25° 49' 12" N, 77° 53' 56" W	6/2007
3. Little San Salvador (LSS)	16			24° 34' 30" N, 75° 56' 30" W	6/2004, 6/2007
4. San Salvador (SSal)	10			24° 08' 30" N, 74° 28' 18" W	6/2004, 6/2007, 7/2010
5. Plana Cays (Pla)	8			22° 36' 40" N, 73° 33' 52" W	6/2004, 6/2007
6. Compass Cay (Comp)	4			24° 16' 29" N, 76° 30' 35" W	7/2010
7. New Providence (NPr)	4			25° 00' 35" N, 77° 34' 00" W	7/2010
8. Bimini (Bim)	10	1		25° 44' 19" N, 79° 16' 20" W	7/2010
<b>Florida Keys, U.S. (FL)</b>					
9. Indian River Lagoon <sup>1</sup>		4	21	27° 29' 20" N, 80° 18' 54" W	5/2013 ( <i>E.s.</i> ), 2002 ( <i>E.h.</i> ) <sup>2</sup>
10. Plantation Key <sup>1</sup>		16		24° 59' 27" N, 80° 32' 32" W	20,022
11. Grassy Key <sup>1</sup>		19		24° 45' N, 80° 57' W	20,022, 4/2018
12. Summerland Key <sup>1</sup>	2	6		24° 39' 47" N, 81° 27' 48" W	6/2007, 5/2013
13. Geiger Beach, Key West	6	5		24° 33' 39" N, 81° 40' 39" W	7/2006, 6/2007, 10/2009, 4/2018
14. Jamaica, Discovery Bay (Jam)	1	1		18° 28' 07" N, 77° 24' 53" W	3/2006
15. Curaçao (Cur)		8		12° 21' 05" N, 69° 09' 25" W	1/2009
16. U.S. Virgin Islands, St. Thomas (USVI)		1		18° 21' N, 64° 52' W	4/2006
17. Bermuda (Ber) <sup>1</sup>		11		32° 37' 04" N, 64° 69' 63" W	6/2006
18. Panama, Bocas del Toro (Pan)		7		09° 19' 60" N, 82° 15' 00" W	12/2004, 7/2015
N =	82	93	21		

<sup>1</sup> Data obtained from the NCBI database (1 of 11 samples from Bermuda; otherwise all samples).

<sup>2</sup> Sampling frequency and locality data from Bass (2006).

ecological data were updated for undescribed species in the *tomentosa* complex (Table 2; Krug et al., 2013, 2016). Accessions from the National Center for Bioinformatics (NCBI) database were included for some *E. subornata* and *E. hamanni*. Sampling information for some NCBI accessions were taken from an unpublished Ph.D. dissertation (Bass, 2006) (Table 1). All sequences here attributed to *E. hamanni* were termed

"*E. subornata*" by Bass (2006), but the morphological and ecological details provided confirm her samples were *E. hamanni* and not *E. subornata* (see also: Results). Her specimens were collected within 100 km of the type locality of *E. hamanni* (Indian River lagoon system, FL, U.S.A.) feeding on *C. prolifera*, diagnostic of *E. hamanni*. The similar species *Elysia entredosaguas* Ortea & Bacallado 2014 was described prior

**Table 2**  
Algal host use in *Elysia tomentosa* complex based on field associations and laboratory feeding observations.

species	collection sites	N	algal host <sup>1</sup>
<i>E. hamanni</i> (P)	Indian River Lagoon, FL, USA Banana River, FL, USA	21	<i>C. prolifera</i>
<i>E. orientalis</i> (-)	Florida Keys	1	-
<i>E. pawliki</i> (L)	Sweetings Cay, Bahamas	3	<i>C. racemosa</i>
<i>E. pratensis</i> (R)	Bahamas Florida Keys, FL, USA Jamaica	73 8 1	<i>Rhypocephalus phoenix</i> <i>Rhypocephalus phoenix</i> <i>Rhypocephalus phoenix</i>
<i>E. subornata</i> (SL)	Curaçao  Florida Keys  Bahamas  Jamaica Bermuda  Panama	8  50  15  1 11  8	<i>C. cupressoides</i> , <i>C. paspaloides</i> <i>C. racemosa</i> , <i>C. sertularioides</i> , <i>C. mexicana</i> <i>C. cupressoides</i> , <i>C. racemosa</i> , <i>C. paspaloides</i> <i>C. racemosa</i> <i>C. cupressoides</i> , <i>C. racemosa</i> , <i>C. verticillata</i> <i>C. racemosa</i> , <i>C. sertularioides</i> , <i>C. verticillata</i>
<i>E. zemi</i> (L)	Martinique	3	<i>C. racemosa</i>
<i>E. cf. tomentosa</i> sp.1 (L)	Okinawa, Japan  Mabini, Philippines	3  1	-  <i>C. racemosa</i> <sup>2</sup> or <i>C. lentillifera</i>
<i>E. cf. tomentosa</i> sp.3 (L)	Maui, Hawai'i Toguchi, Okinawa, Japan Calatagan, Philippines Maui, Hawai'i	7 3 1 2	<i>C. racemosa</i> <i>C. racemosa</i> <sup>2</sup> or <i>C. lentillifera</i> <i>C. racemosa</i> <sup>2</sup> <i>C. racemosa</i>
<i>E. cf. tomentosa</i> sp.4 (-)	Okinawa, Japan	1	-
<i>E. cf. tomentosa</i> sp.5 (S)	Lord Howe Island, Australia Mabini, Philippines Calatagan, Philippines Johor, Malaysia Bandar Abbas, Iran	1 2 2 3 6	<i>C. cupressoides</i> <i>C. serrulata</i> <i>C. racemosa</i> <sup>2</sup> , <i>C. serrulata</i> <i>C. taxifolia</i> , <i>C. mexicana</i> <i>C. sertularioides</i>
<i>E. tomentosa</i> sp.6 (L)	Guam  Calatagan, Philippines	1  1	<i>C. racemosa</i> <sup>2</sup> or <i>C. lentillifera</i> <i>C. racemosa</i> <sup>2</sup>
<i>E. cf. tomentosa</i> sp.8 (L)	Calatagan, Philippines	1	<i>C. racemosa</i> <sup>2</sup>
<i>E. cf. tomentosa</i> sp.22 (L)	Lord Howe Island, Australia Maui, Hawai'i	5 2	<i>C. lentillifera</i> <i>C. racemosa</i>

<sup>1</sup> Host character coding: L, 'sea grape' complex: *C. racemosa*, *C. cylindracea*, *C. lentillifera*; P, *C. prolifera*; R, *Rhypocephalus phoenix*; S, feather-morph complex: *C. serrulata*, *C. sertularioides*, *C. taxifolia*; -, missing data.

<sup>2</sup> Possibly *C. cylindracea*, not taxonomically clarified at time of collection (Belton et al., 2014).

to *E. hamanni* from the Yucutan Peninsula (Tulum, Mexico), feeding on *C. prolifera*. However, given the ~1000 km distance between type localities, we retain *E. hamanni* as a valid name until molecular data are available from Mexican *E. entredosaguas* to assess whether the two species are conspecific, given the high prevalence of cryptic species in Caribbean *Elysia* (Krug et al., 2016).

Specimens of *E. subornata* and *E. pratensis* were collected from 14 Caribbean sites (Fig. 2) over a 16-year period, with sampling performed in all seasons (ranging from Jan to Dec). To assess whether *E. subornata* had a preference among *Caulerpa* spp., separate collections of each *Caulerpa* sp. were made at every sampling site. To determine the host of *E. pratensis*, each udotacean green alga was collected separately (0.5–1 kg wet wt) at every site. Algal stipes were carefully pulled from the substrate, placed in a sealed bag, and transferred to an aerated bin of

seawater; slugs were then removed and identified under a stereomicroscope. For other members of the *Elysia tomentosa* complex, host use was inferred from field distributions and confirmed where possible by direct feeding observations in the laboratory. As described above, all suitable chlorophyte hosts were sampled at each collecting site; separate collections were made of each available *Caulerpa* species, minimally including both sea grape and feather-morph *Caulerpa* spp. from all sites. A systematic revision of the Indo-Pacific species, including complete information on radular adaptations and differential host use, will be presented elsewhere (Medina, 2019). In *Elysia*, seasonal host shifts between algal genera have only been noted for one unusually polyphagous species (*E. furvacauda*) that alternated between green and red algae (Brandley, 1984); seasonal shifts among congeneric algal species have occasionally been reported for transiently available algal species in warm-temperate zones (e.g., *Elysia trisinuata* and *Codium* spp.; Trowbridge et al., 2008). Other studies have not reported seasonal changes in diet for host-specialized *Elysia* spp. including any members of the *tomentosa* complex, whose tropical hosts were present year-round in our surveys.

## 2.2. Phylogenetic analyses of interspecific relationships

To resolve the relationships in the *tomentosa* complex, a four-gene phylogeny was inferred using one exemplar each of 12 species. Data for 10 species were taken from Krug et al. (2016), including sequences for one exemplar of *E. subornata* (Esub\_06Jam01) and *E. pratensis* (Epra\_07Pla04); we added new data for the recently collected *E. cf. tomentosa* sp. 8, and for one representative *E. hamanni* from the National Center for Bioinformatics (NCBI) database (Bass, 2006). Sequence data were generated by extracting genomic DNA with DNeasy® kits (Qiagen). For exemplars, portions of four loci were amplified by polymerase chain reaction (PCR) using universal primers and standard reaction conditions: (a) COI; (b) mt large ribosomal subunit (16S) rRNA gene; (c) nuclear histone III (H3) gene; and (d) large ribosomal subunit (28S) rRNA gene; amplification and sequencing were as described (Krug et al., 2015, 2018b). Chromatograms were edited in GeneiousPro 4.8 software and aligned with MAFFT, using models of secondary structure for rRNA genes to remove variable loop regions (Krug et al., 2015, 2018b). Data for the four genes were concatenated, yielding a final aligned matrix comprising 2,802 positions: 658 bp of COI, 404 bp of 16S, 1392 bp of 28S, and 328 bp of H3 (Table S1).

Phylogenetic relationships among species were inferred using Bayesian Inference (BI) with Markov-chain Monte Carlo (MCMC) methods, and by Maximum Likelihood (ML). In each analysis, the out-group was designated as *E. orientalis* Ortea, Moro & Espinosa, 2011, recovered as sister to the *E. tomentosa* clade in an analysis of 102 species in family Plakobranchidae (Krug et al., 2016). The ML analysis was implemented in RAXML v7.2.7 (Stamatakis, 2006) via the CIPRES web portal (Miller et al., 2010), parameterizing a separate GTR +  $\Gamma$  model for each gene. The number of bootstrap pseudoreplicates were determined by the program during the run, and bootstrap support (BS) values  $\geq 70\%$  were taken as significant (Douady et al., 2003).

For BI analyses, mixture models of sequence evolution were implemented in *BayesPhylogenies* to accommodate rate heterogeneity among sites without requiring *a priori* data partitioning by gene or codon position, assigning the best-fit model to each nucleotide position using a likelihood criterion (Pagel and Meade, 2004). The data matrix was analyzed using eight GTR +  $\Gamma$  models, each with four rate classes; the number of models needed to reflect heterogeneous evolutionary rates and base composition across sites was estimated using a reverse-jump implementation of the MCMC sampler. Following Pagel and Meade (2004), we performed four replicate runs of one Markov chain each, lasting  $2 \times 10^7$  generations, saving trees every  $5 \times 10^3$  generations. Posterior distributions were visualized in Tracer v1.7 (Rambaut et al., 2018) to confirm chains reached stationarity and convergence; the first 50% of trees were discarded as burn-in and the remaining tree samples

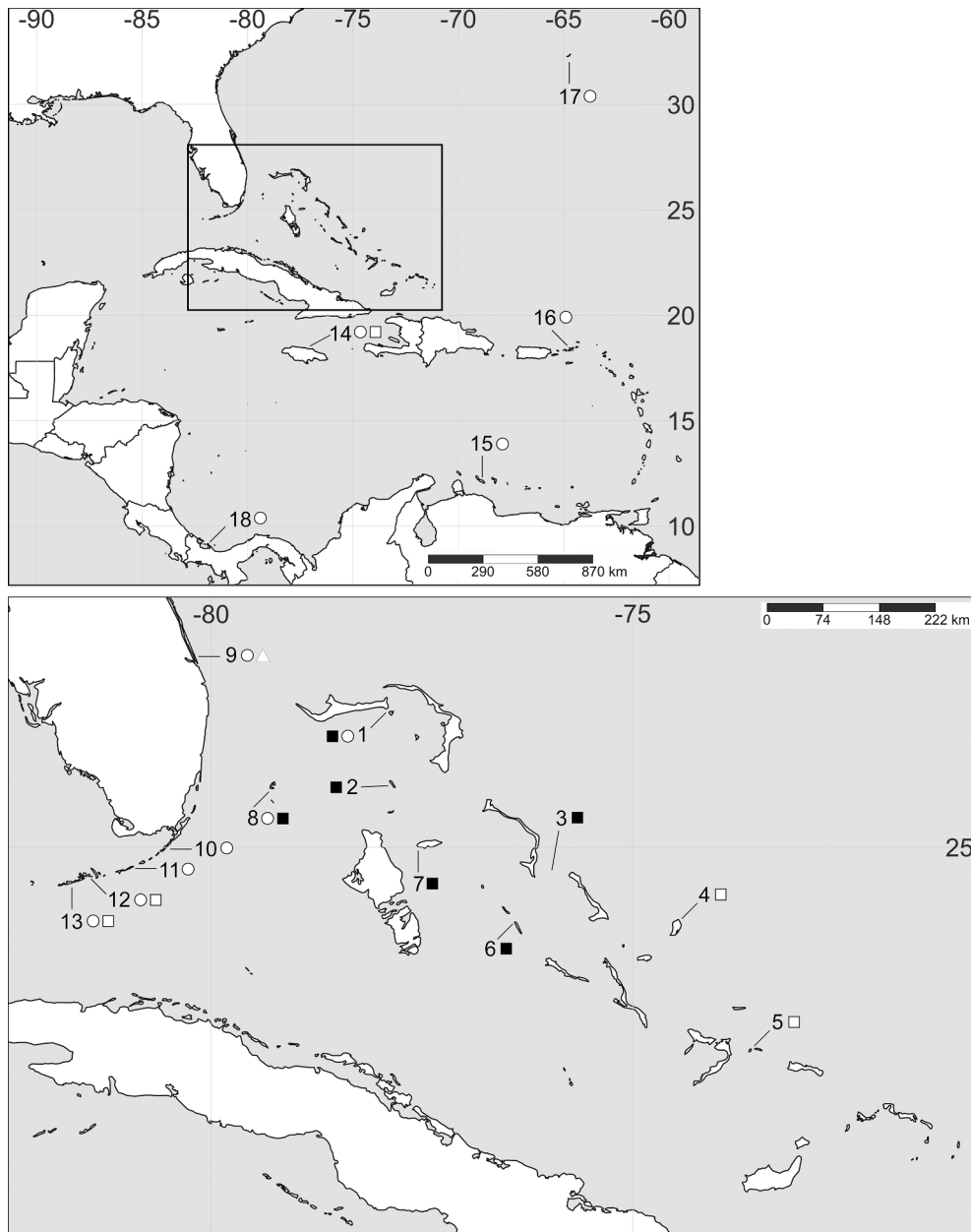


Fig. 2. Sampling locations for sequenced specimens; coordinates and sampling dates given in Table 1. White circle = *E. subornata*; white triangle = *E. hamanni*; white square = *E. pratensis*, native mtDNA; filled square = *E. pratensis*, introgressed mtDNA.

from all runs were combined. A 50% majority-rule consensus tree was generated in *BayesTrees* (<https://www.evolution.reading.ac.uk>) with mean branch lengths, and the posterior probability (Pp) of nodes determined with values  $\geq 90\%$  taken as significant (Huelsenbeck and Ronnquist, 2004; Simmons et al., 2004).

### 2.3. Ancestral character state reconstruction

To test the hypothesis that sympatric host shifts occurred in the *tomentosa* complex, ancestral host use was reconstructed on the four-gene phylogeny using BI algorithms in *BayesTraits* (Pagel et al., 2004). Host use was modeled as a discrete variable with four possible states: (1) *Caulerpa prolifera* (P); (2) ‘sea grapes’ such as *C. racemosa*, *C. lentillifera* or *C. cylindracea* (L); (3) *Caulerpa* spp. with a ‘feather’ morphology of the stipe, such as *C. sertularioides*, *C. serrulata* or *C. taxifolia* (S); and (4) *Rhipocephalus phoenix* (R). Notably, levels of toxic secondary metabolites (oxytoxin, caulerpenyne) are typically lower in ‘sea grape’ species than

‘feather’ morph *Caulerpa* spp. such as the invasive *C. taxifolia* (Baumgartner et al., 2009).

To determine the posterior distribution of host states at each node and transition rates among hosts, continuous-time Markov models of trait evolution were fit to host-use data for extant taxa. The post-burnin sample (8000 trees) from the BI analysis was used to correct for phylogenetic uncertainty. Priors for ASRs and model-fitting tests were drawn from an exponential distribution generated using a hyperprior, with the mean of the exponential drawn from a uniform distribution (0 to 10). A reverse-jump (RJ) MCMC approach was used to reduce the sampled set of model parameters, assigning some transition rates to the zero bin (Pagel and Meade, 2006). MCMC chains were run for  $5 \times 10^7$  iterations, discarding the first 20% as burnin, and sampling rate coefficients, host probabilities and their associated trees every  $10^5$  generations.

Statistical support for an ancestral host was first analysed following Kubo et al. (2019). The Pp distribution of ancestral states was estimated

simultaneously for each node using the 'addNode' command. The analysis was repeated three times, the post burnin samples combined, and the median Pp for each ancestral host plotted. A node was considered confidently reconstructed if the median Pp for one state was  $\geq 50\%$ , twice the null probability of equal support (25%) for each of four possible host states. After ASR, branches connecting two nodes in the same state were color-coded as retaining that state; branches connecting nodes that differed in their inferred state were coded pink ('transitional'), while branches connecting a node for which state could not be confidently inferred were coded grey ('uncertain').

Second, if no host had  $\geq 50\%$  Pp at a given node by ASR, then statistical support for alternative ancestral states was compared using log-Bayes Factor (BF) tests. Each possible host state was sequentially fixed at that node using the 'fossil' command, and the marginal likelihood of that model estimated using the stepping-stone sampler, running 200 stones for  $2 \times 10^5$  iterations. Analyses were repeated three times for each possible host at a given node. Log-BF tests were used to compare model support, taking twice the difference between the log ( $L$ ) marginal likelihood (lk) for alternative models as the BF test statistic, and comparing the median  $L(\text{lk})$  score of three runs; values  $> 2$  were taken as positive evidence (versus 5–10 as strong evidence) favoring one model over another (Raftery, 1996). If one ancestral host was positively supported over all others, then that state was also used in color-coding branches as retaining a state or being transitional between states.

Ancestral nucleotide and amino acid positions for COI were inferred in MEGA X (Kumar et al., 2018) using the ML method (Nei and Kumar, 2000), on the best tree from the RaxML analysis of the four-gene alignment. Ancestral nucleotides were reconstructed for the 658-bp fragment of COI from the four-gene alignment using a GTR +  $\Gamma$  model with four rate classes and all sites. COI sequences were then translated yielding 219 amino acid positions, and analyzed under the JTT matrix-based model (Jones et al., 1992) with uniform rates.

#### 2.4. Comparative phylogeography of *E. subornata* and *E. pratensis*

To determine relationships among mtDNA haplotypes in our focal taxa, tissue was dissected from the posterior end of specimens identified by morphology as *E. subornata* ( $N = 47$ ) and *E. pratensis* ( $N = 82$ ) (Tables 1, S2). For all individuals, COI and H3 loci were amplified as described; a portion of the nuclear 28S gene was also amplified for a subset of specimens (*E. pratensis*,  $N = 51$  slugs, 7 sites; *E. subornata*,  $N = 29$  slugs, 5 sites) using primers 28SF2-5' and 28SR3-3' (Morgan et al., 2002). Sequences were edited to the minimum length obtained for all specimens: 543 bp of COI, 328 bp of H3, and 550 bp of 28S. Chromatograms for the H3 locus were carefully examined for heterozygous positions, and allelic phase resolved by subtracting the common allele from heterozygotes to identify rare alleles (Table S3).

COI haplotypes for additional *E. subornata* specimens ( $N = 35$  slugs, 5 sites) and for *E. hamanni* ( $N = 21$  slugs, 1 site) were obtained from the NCBI database. Most publicly available COI sequences were shorter than the minimum length of COI haplotypes generated for the present study. Therefore, as warranted for a given downstream analysis, we either (a) extended public sequences with question marks to denote missing data (for phylogenetic analyses of COI haplotype relationships); (b) shortened author-generated sequences to the minimum length of public sequences (for AMOVA); or (c) omitted public sequences (for haplotype networks).

Evolutionary relationships among all COI haplotypes were inferred for *E. subornata*, *E. pratensis* and *E. hamanni* by BI and ML methods as described, with the following modifications. Based on analyses of the four-gene matrix, the outgroup specified for the COI gene tree comprised *E. cf. tomentosa* sp. 1, sp. 3, sp. 4, and sp. 6 (see Results). The ML analysis was performed as described, partitioning the data to apply a separate model to the 3rd codon position. For BI analyses, four separate MCMC chains were run for  $2 \times 10^7$  generations, each parameterizing three GTR +  $\Gamma$  mixture models and sampling every  $5 \times 10^3$  generations;

stationarity and convergence among runs were assessed, and the final 25% of each run ( $10^3$  trees) retained as the post-burnin sample. After pooling tree samples from all runs, a 50% majority-rule consensus tree with mean branch lengths was generated and Pp scores calculated.

An unrooted statistical parsimony network was also generated for original COI data (543 bp) using a 95% parsimony criterion in TCS v1.21, to infer mutational connections among extant haplotypes of both species (Clement et al., 2000). A Tamura-Nei (TrN) correction was applied to genetic distances based on jModeltest results (Posada, 2008), calculated in MEGA X.

#### 2.5. Population genetic structure, molecular diversity and selective neutrality

Levels of population subdivision, genetic diversity and selective neutrality of the COI locus were assessed separately for three groups: (1) all *E. subornata* demes, (2) *E. pratensis* demes with native mtDNA, and (3) *E. pratensis* demes with introgressed mtDNA (see Results), due to the independent histories of the two mtDNA lineages in *E. pratensis*. Haplotype frequencies were compared among demes using Slatkin's linearized  $F_{ST}$  in Arlequin 2.0 (Schneider et al., 2000), to test the hypothesis that each sampled deme was genetically distinct. Analysis of molecular variance (AMOVA; Excoffier et al., 1992) was also used to assess the proportion of genetic covariance partitioned among demes by  $\Phi_{ST}$  analysis of TrN distances between haplotypes. Sites with only one sample were excluded from these analyses. Standard diversity indices may not reflect genetic subdivision if markers are polymorphic and haplotypes are not shared among demes (Jost, 2008, 2009). The statistic  $D_{est}$  is an estimator of the among-population component of genetic variation that reflects both the levels of heterozygosity within demes, and the degree to which alleles are shared among demes (Jost, 2008). Overall and pairwise  $D_{est}$  values were therefore estimated for the COI locus for each of the three haplogroups, using 1000 bootstrap pseudoreplicates to generate confidence intervals in SMOGD (Crawford, 2010).

Haplotype and nucleotide diversities at the COI locus were estimated in Arlequin 2.0 for each of the three groups, and also separately for each deme. Tajima's  $D$  (Tajima, 1989) and Fu's  $F_S$  (Fu, 1997) were calculated in Arlequin 2.0 as tests of neutrality and population equilibrium for COI; significant values signal a departure from neutral expectations due to a selective sweep, population bottleneck or expansion (Ford, 2002).

Population-level 28S data were screened for single nucleotide polymorphisms (SNPs) that distinguished the species. Low allelic diversity at the H3 and 28S genes precluded including these loci in analyses of population division, but alleles at both loci were mapped onto the COI gene tree alongside their corresponding haplotype to visualize cytonuclear discordance; more than two H3 alleles were sometimes associated with a haplotype sampled from multiple slugs.

### 3. Results

#### 3.1. Host use in the *Elysia tomentosa* complex

Surveys at 12 sites (Fig. 2, Table 1) supported prior literature reviews reporting *E. subornata* fed on multiple *Caulerpa* spp. Based on field associations and laboratory observations, *E. subornata* included feather-morph species (*C. sertularioides*, *C. taxifolia*, *C. mexicana*) and 'sea grapes' (*C. racemosa*) as well as other algal morphotypes (*C. cupressoides*, *C. paspaloides*, *C. verticillata*) in its diet, confirming prior work that *E. subornata* is a *Caulerpa* generalist (Table 2). However no specimens of *E. subornata* were collected from or observed to feed on *C. prolifera*, or any other algal genus (Table 2). The recently described *E. pawliki* Krug, Vendetti & Valdés, 2016 and *E. zemi* Krug, Vendetti & Valdés, 2016 were both sampled on *C. racemosa*; no host was identified for the poorly known *E. orientalis*. All specimens identified here as *E. hamanni* fed on *C. prolifera* (Bass, 2006). Five Indo-Pacific taxa in the *E. tomentosa*

species complex were sampled from ‘sea grapes’ (*C. racemosa* or *C. lentillifera*). Only *E. cf. tomentosa* sp. 5 fed preferentially on feather-morph *Caulerpa* spp. (Table 2). Host was not identified for only one candidate species (*E. cf. tomentosa* sp. 4), identified by DNA barcoding an egg mass. Host category (feather-morph vs. sea grape) was conserved across distant sampling sites for most Pacific candidate species, including 2–3 sites for most sea-grape feeders, and five sites for *E. cf. tomentosa* sp. 5.

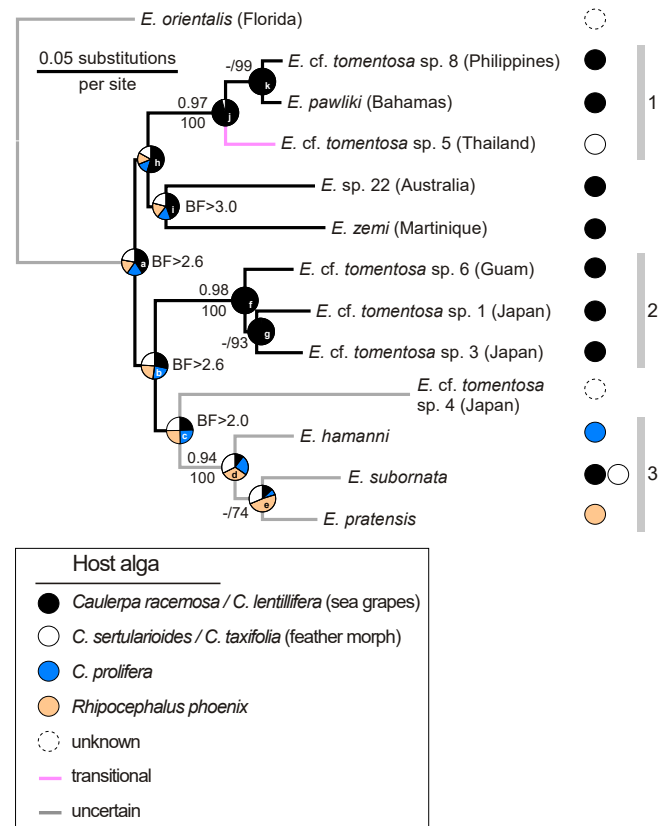
*Elysia pratensis* was only sampled from 11 sites where *Rhipocephalus phoenix* was also found across the Caribbean, and slugs were exclusively associated with this calcified green alga in all seasons. *Rhipocephalus* grows as upright, individual stipes, with tapering rows of overlapping scale-like blades resembling a pinecone (Fig. 1B). Specimens of *E. pratensis* differ from *E. subornata* by having longitudinal white stripes running the length of the body that render them cryptic on *Rhipocephalus* due to the white edges of individual blades on which slugs have fed. Orange extra-capsular yolk ribbons and complete intra-capsular metamorphosis distinguish egg masses of *E. subornata* and *E. pratensis* from other Caribbean sacoglossans (Fig. 1B); juvenile slugs emerge from the egg mass with no pelagic larval swimming period. Egg masses and juveniles of each species were observed only on their respective host algae.

### 3.2. Evolutionary relationships and host shifts in the *Elysia tomentosa* complex

BI and ML analyses of a four-gene data matrix supported three subclades of three species apiece within the *E. tomentosa* complex, but deeper relationships were not resolved (Fig. 3). All pairwise TrN distances between species were > 9% at the COI locus, except *E. pawliki* (Bahamas) and *E. sp. 8* (Philippines) were only 3.6% divergent. Subclade 1 (Pp = 0.97, BS = 100%) comprised two *E. tomentosa*-like species from the Indo-Pacific (sp. 5 and sp. 8) plus *E. pawliki*, which ML analysis recovered as sister to sp. 8. Subclade 2 (Pp = 0.98, BS = 100%) comprised three morphologically similar species from Japan and Guam (sp. 1, sp. 3, sp. 6). Finally, subclade 3 (Pp = 0.94, BS = 100%) comprised a sympatric radiation of three Western Atlantic species: *E. subornata*, *E. hamanni*, and *E. pratensis*. The ranges of *E. pratensis* and *E. hamanni* nest within the more wide-ranging *E. subornata*, which extends north to Bermuda where neither *Rhipocephalus phoenix* nor *E. pratensis* occur (Fig. 2).

Bayesian ASR supported ‘sea grapes’ as the ancestral host of a clade of five species (node h), and of derived subclade 1 (node j), within which a single transition to feather-morph *Caulerpa* spp. was inferred on the branch leading to sp. 5 (Fig. 3). ASR also recovered almost complete Pp support for sea grapes as the ancestral host of subclade 2 (Fig. 3, node f). At deeper nodes, ASR was ambiguous as Pp was more evenly divided among host states. However, L-BF tests supported sea grapes over alternative hosts for the ancestor of the *tomentosa* complex (node a) and for descendant nodes b, c and i (Fig. 3; Supplemental Table S4).

Although sea grapes was the supported host at node c, no host was supported for nodes d or e (Fig. 3). Although the sequence of ancestral host shifting could not be inferred, minimally two host shifts occurred within subclade 3, because the hosts of *E. hamanni* and *E. pratensis* were not used by any other taxon. The minimum sequence of host evolution thus required (1) a shift to *C. prolifera* on the terminal branch leading to *E. hamanni*, and (2) a shift to *R. phoenix* on the branch leading to *E. pratensis* (i.e., two host shifts). Additionally, one host expansion must have occurred to account for the broader host range of *E. subornata* compared to all other species in the *tomentosa* complex; the most parsimonious explanation is a host expansion adding feather-morph algae to an ancestral sea-grapes diet (Fig. 3, Table 2). Alternatively, if either host shift occurred on a branch prior to nodes d or e, then a reversion to *Caulerpa* occurred on the terminal branch leading to *E. subornata* (three host shifts) followed by a niche expansion.



**Fig. 3.** Evolutionary relationships within the *Elysia tomentosa* species complex based on analyses of a four-gene data matrix. Topology and branch lengths are from the ML analysis with significant Pp (above branch, before slash) or BS (below branch, after slash) shown for supported nodes (dash = not significant). Undescribed species delimited by molecular data are indicated by “sp. #” (Krug et al., 2016). Vertical grey boxes highlight three main subclades within the complex. Colored circles indicate the host coding for each taxon (circle with dashed line = missing data), with the corresponding Pp distributions for ancestral host use given on all nodes. Where no ASR yielded > 50% support for one state, results of log-BF tests are given next to nodes if positive evidence favored one ancestral host over all alternative states. Branches are color-coded as retaining their ancestral state unless connecting nodes (or a node + terminal) with different states confidently reconstructed, in which case that branch was colored pink for ‘transitional’. If ancestral host could not be confidently inferred for a node, branches connecting to that node were colored grey for ‘uncertain’. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

### 3.3. Cytonuclear discordance and localized mitochondrial capture

A range-wide phylogeographic survey yielded 34 COI haplotypes from 93 specimens of *E. subornata*, and 38 haplotypes from 81 specimens of *E. pratensis* (Tables 1, S2, S3). Public sequences identified here as *E. hamanni* comprised 10 haplotypes isolated from 21 specimens. Phylogenetic analyses of COI sequences recovered a weakly supported clade (BS = 71%) of *E. hamanni* haplotypes sister to a supported clade (Pp = 0.91, BS = 89%) comprising all specimens of *E. pratensis* and *E. subornata* (Fig. 4). The latter was split into two highly supported sister clades with a mean TrN divergence of 10.2% (net distance = 9.1%). However, mtDNA subclades were not congruent with species identifications based on morphology and host use. One subclade (Pp = 0.97, BS = 99%) contained all *E. pratensis* haplotypes from Florida, Jamaica, and two eastern Bahamas islands (San Salvador, Plana Cays). Most slugs from San Salvador (9 of 10) shared one haplotype up to 4.2% divergent from other haplotypes in this subclade; mean intra-clade divergence was otherwise 1.2%. Two moderately supported subclades comprised

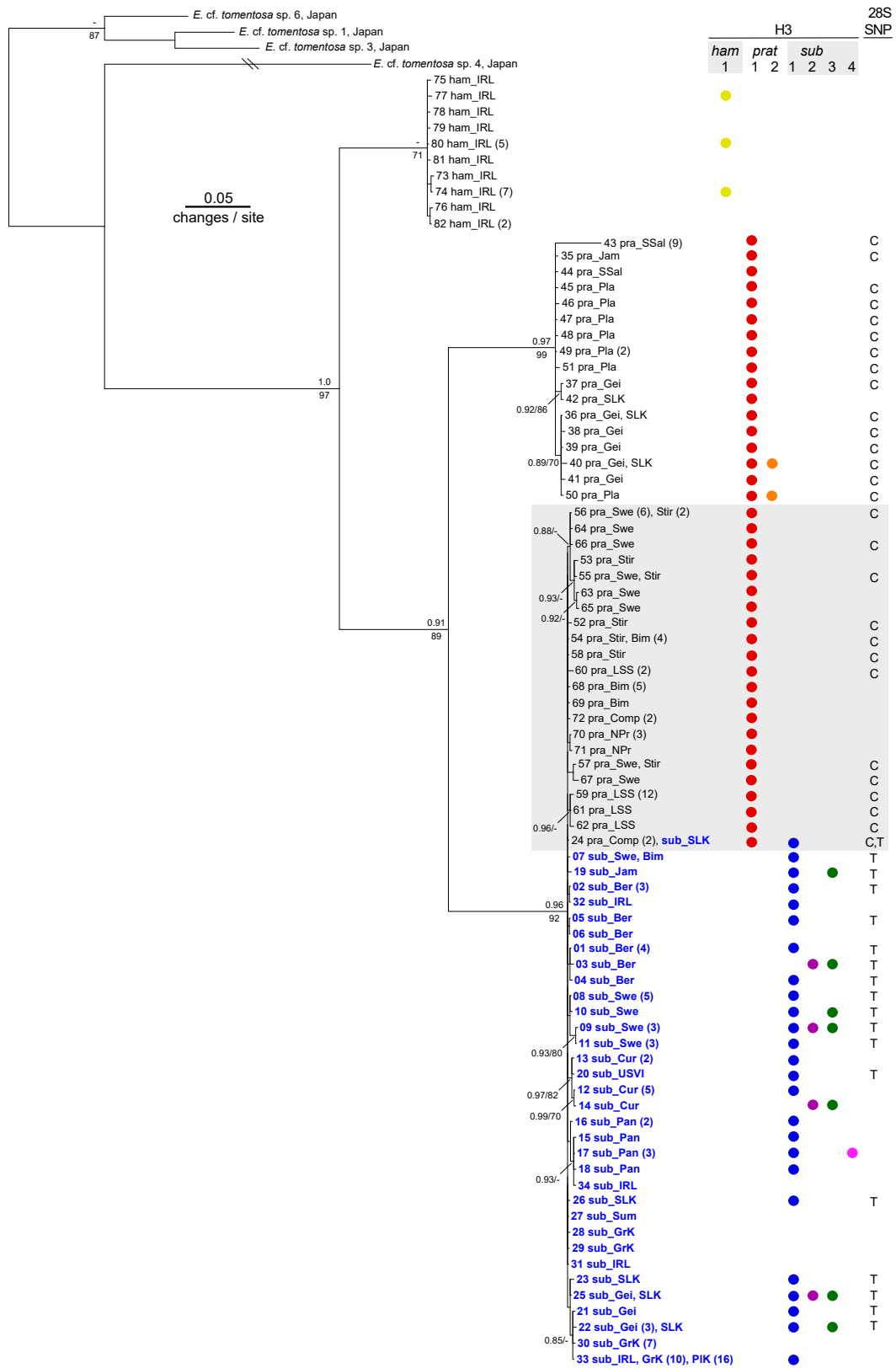


Fig. 4. Phylogenetic relationships among mtDNA lineages in subclade 3 of the *tomentosa* complex. Topology and branch lengths are from the ML analysis of COI haplotypes, with significant Pp (above branch, before slash) or BS (below branch, after slash) shown for supported nodes (dash = not significant). Location codes for haplotypes given in Table 1; numbers in parentheses denote multiple samples yielding the same haplotype at the indicated site. Haplotype names in bolded blue font were from specimens morphologically identified as *E. subornata* and collected on *Caulerpa*. Colored circles indicate H3 allele(s) sampled together with a given COI haplotype; letters (C, T) indicate the SNP from the 28S gene sampled together with a given haplotype. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

haplotypes from Florida plus one Plana haplotype.

The second major subclade ( $P_p = 0.96$ ,  $BS = 92\%$ ) contained all *E. subornata* haplotypes, plus all *E. pratensis* haplotypes from six islands in the northern and central Bahamas (Fig. 4). Mean within-group divergence for this subclade was 1.0%. Moderate phylogeographic structure was evident in BI analyses, with supported subclades comprising (1) most *E. pratensis* haplotypes from Little San Salvador ( $P_p = 0.96$ ); (2) *E. pratensis* haplotypes from Sweetings Cay ( $P_p = 0.93$ ) or Sweetings + Stirrup Cay ( $P_p = 0.93$ ); (3) *E. subornata* haplotypes from Curaçao and the U.S. Virgin Islands ( $P_p = 0.97$ ); and (4) most *E. subornata* haplotypes from Panama ( $P_p = 0.93$ ). A subclade of *E. subornata* haplotypes from Florida was weakly supported ( $P_p = 0.85$ ). Maximum divergence between *E. subornata* haplotypes was 1.7%.

The discordance between (a) mtDNA genealogy, versus (b) slug morphology, host use, and the distribution of nuclear alleles (see below), suggested introgression of *E. subornata* mtDNA into *E. pratensis* followed by near-complete lineage sorting of mtDNA. We hereafter refer to the mtDNA clade sampled only in *E. pratensis* as ‘native’ mtDNA, versus the mtDNA lineage shared by both species as ‘introgressed’ mtDNA in *E. pratensis*. Notably, only a single haplotype (#24) was shared between species, sampled in one *E. subornata* from Florida and two *E. pratensis* from Compass Cay, Bahamas.

The COI haplotypes formed three disconnected statistical parsimony networks under a 95% criterion (Fig. 5). The main network comprised *E. subornata* + putatively introgressed haplotypes sampled in *E. pratensis*. Two smaller, unconnected networks comprised (a) all *E. pratensis* native haplotypes, except (b) the divergent, private *E. pratensis* haplotype sampled at a high frequency in San Salvador, Bahamas. Haplotype #54 from *E. pratensis* was recovered as ancestral to the larger network, but was only 1–2 mutational steps away from numerous *E. subornata* haplotypes sampled from six different sites, and was also only one substitution away from haplotype #24 shared

between the species.

In contrast to mtDNA, the distribution of nuclear alleles was congruent with adult morphology and ecology. Two H3 alleles (one common, one rare) were sampled solely in *E. pratensis*, whereas a different four alleles were sampled only in *E. subornata* (Fig. 4). At the 28S locus, three alleles were sampled from *E. pratensis* and two different alleles were sampled from *E. subornata*. A diagnostic SNP at the 28S locus was congruent with provisional species identifications based on morphology for all scored specimens, with a C fixed in *E. pratensis*, versus a T fixed in *E. subornata* (Fig. 4). The specimens of *E. subornata* and *E. pratensis* that shared a COI haplotype were distinct at the H3 and 28S loci. This cytonuclear discordance was consistent with introgression and localized ‘mitochondrial capture’ in *E. pratensis* populations from the northern and central Bahamas.

### 3.4. Population genetic structure and molecular diversity: *E. pratensis* versus *E. subornata*

As expected for taxa that lack a swimming larval stage, populations in both species were highly subdivided based on  $\Phi_{ST}$  (Table 3) or conventional  $F_{ST}$  values (Table S5). For *E. subornata*, overall structure based on  $\Phi_{ST}$  was exceptionally high and significant (Table 3A), as were all pairwise  $\Phi_{ST}$  estimates (Table 4A); only one pairwise  $\Phi_{ST}$  value was  $< 0.4$ , between two nearby FL sites. All pairwise  $F_{ST}$  values were also high ( $> 0.17$ ) and significant ( $P < 0.01$ ), driven by high-frequency private haplotypes at each Caribbean site and different frequencies of the common FL haplotype among three Floridian sites (Gei, GrK, PIK) (Table 4A).

For *E. pratensis*, separate analyses of ‘native’ and ‘introgressed’ mtDNA were performed due to the marked genetic divergence between clades, which would otherwise inflate  $\Phi_{ST}$  estimates. Among *E. pratensis* populations with native COI haplotypes, population genetic structure

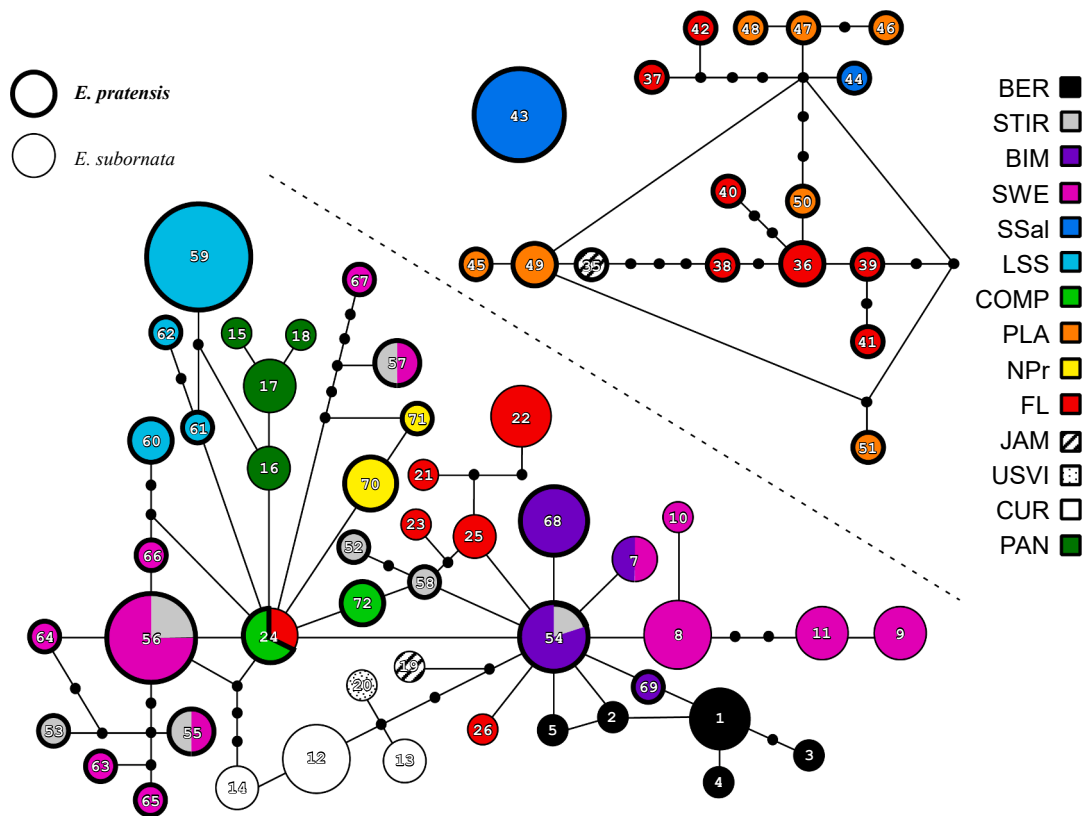


Fig. 5. Statistical parsimony network of COI haplotypes from *Elysia pratensis* and *E. subornata* inferred under a 95% parsimony criterion. Circle area is proportional to the frequency of each haplotype, color-coded by sampling site. Black dots represent unsampled haplotypes, and each line segment indicates one substitution. Haplotype numbers are labeled (Table S3). Thick borders bound *E. pratensis*, thin borders *E. subornata* haplotypes.

**Table 3**

Population genetic structure based on AMOVA for the COI locus ( $\Phi_{ST}$  values computed from TrN-corrected distances) in (A) *Elysia subornata*; (B) *E. pratensis*, native haplotypes; and (C) *E. pratensis*, introgressed haplotypes.

	Source of variation	d. f	Sum of squares	Variance components	% of variation	P
A.	Among Populations	6	115.442	1.607	74.20	>0.00001
	Within Populations	76	42.461	0.559	25.80	
	Total	82	157.903	2.166		
B.	Among Populations	2	86.649	4.800	70.90	>0.00001
	Within Populations	23	45.290	1.969	29.09	
	Total	25	131.939	6.769		
C.	Among Populations	5	48.127	0.969	45.63	>0.00001
	Within Populations	49	56.574	1.155	54.37	
	Total	54	104.701	2.124		

was very high based on  $\Phi_{ST}$  and comparable to *E. subornata* (Table 3B), whereas less (but significant) structure was detected among populations with introgressed haplotypes (Table 3C). Similar overall  $F_{ST}$  values were obtained for *E. pratensis* populations with native and introgressed mtDNA, or by pooling all *E. pratensis* populations (Table S5).

Most pairwise comparisons among *E. pratensis* populations were significant by  $\Phi_{ST}$  and/or  $F_{ST}$  (Table 4B). Exceptions included Sweetings Cay and nearby Stirrup Cay (~80 km apart), which shared three haplotypes and were not differentiated. Also, pairwise  $F_{ST}$  comparisons between Plana, FL-Gei and Stirrup were not significant (Table 4B); however, those sites shared no alleles, none had a private common haplotype, and all had higher mean haplotype diversities ( $0.96 \pm 0.08$ ) than other sites (Table S6). The lack of apparent differentiation by  $F_{ST}$  could thus reflect the lack of sensitivity of this statistic when within-deme diversity is high. Values of Jost's  $D_{est}$ , which corrects for diversity within demes, were comparable for both native-mtDNA and introgressed populations of *E. pratensis*, and both were higher than  $D_{est}$  for *E. subornata* (Table 5). Pairwise  $D_{est}$  values indicated barriers to gene

**Table 4**

Pairwise genetic differences among populations of (A) *Elysia subornata* or (B) *E. pratensis*, based on conventional  $F_{ST}$  using Slatkin's distances (above diagonal), or  $\Phi_{ST}$  values based on Tamura-Nei corrected genetic distances (below diagonal);  $P < 0.05^*$ ;  $P < 0.01^{**}$ ;  $P < 0.001^{***}$ ;  $P \leq 0.00001^{****}$ .

A. <i>E. subornata</i>									
	Cur	Swe	Ber	Pan	FL-Gei	FL-GrK	FL-PIK		
Cur		0.2885****	0.2911****	0.2953***	0.2679****	0.3887****	0.7876****		
Swe	0.6091****		0.2027****	0.1987**	0.1815***	0.3026****	0.6327****		
Ber	0.6262****	0.5121****		0.1956**	0.1778***	0.3056****	0.6724****		
Pan	0.7962****	0.6923****	0.7299****		0.1718**	0.3097***	0.7312****		
FL-Gei	0.5410****	0.5132****	0.4372***	0.6332****		0.2858***	0.6524****		
FL-GrK	0.7355****	0.6785****	0.6622****	0.7913****	0.1983**		0.6732****		
FL-PIK	0.9575****	0.8601****	0.9228****	0.7852****	0.8413****	0.9040****			
B. <i>E. pratensis</i> <sup>1</sup>									
	Swe	Stir	LSS	SSal	Pla	Comp	NPr	Bim	FL-Gei
Swe		0.0000	0.3848****	0.4718****	0.1200*	0.2427**	0.2987**	0.2694****	0.1200*
Stir	0.0180		0.5166****	0.4447***	0.0357	0.1579**	0.2224*	0.1604*	0.0357
LSS	0.5036****	0.3406****		0.6577****	0.3407***	0.4942***	0.5424****	0.4710****	0.3407****
SSal					0.4447****	0.6563**	0.7103**	0.5778****	0.4447***
Pla				0.7842***		0.1579**	0.2224*	0.2031**	0.0357
Comp	0.2318*	0.0415	0.5204**				0.4167*	0.3476**	0.1579**
NPr	0.3612**	0.2604**	0.6120***			0.6680*		0.4061**	0.2224*
Bim	0.4784****	0.2722***	0.6701****			0.6549***	0.7626**		0.2031**
FL-Gei				0.7664***	0.2236**				

<sup>1</sup>Separate  $\Phi_{ST}$  analyses were run on sites with introgressed mtDNA (white background) and native mtDNA (shaded), due to the inflated genetic distances between those sets of haplotypes.

flow among Plana, Florida and Stirrup that were not apparent by  $F_{ST}$  (Table S7).

Overall, COI haplotype diversity was higher in *E. subornata* than in *E. pratensis* with either native or introgressed mtDNA (Table 5). At the population level, there was one notably low-diversity site among *E. pratensis* demes with native mtDNA (San Salvador), and one with introgressed mtDNA (Little San Salvador) (Table S6). Only one population per species showed a departure from neutral expectations: the range-edge Bermuda population of *E. subornata* (Fu's  $F_S$ ,  $P < 0.005$ ), and the San Salvador population of *E. pratensis* (Tajima's  $D$ ,  $P < 0.005$ ) (Table S6).

### 3.5. Selection on mtDNA

When COI data for individual demes were pooled, Fu's  $F_S$  was significant for *E. subornata* and introgressed *E. pratensis*, indicating departures from neutral expectations or demographic equilibrium; such test results are consistent with either selection on mtDNA, or a recent bottleneck and population expansion (Table 5). One possible target for selection on mtDNA included amino acid position 161 (out of 219 complete codons) in our COI alignment. At this position, a fixed difference distinguished *E. hamanni* and native *E. pratensis* (Ser<sub>161</sub>) from all *E. subornata* and introgressed *E. pratensis* (Thr<sub>161</sub>). The inferred ancestral residue for the *tomentosa* complex was Thr<sub>161</sub> (Fig. S1). A conservative change to Ser<sub>161</sub> was supported on one branch (Fig. S1, red arrow). ASR supported a reversion to Thr<sub>161</sub> on the branch leading to *E. subornata* (Fig. S1, blue arrow) and to the putatively introgressed mtDNA found in central Bahamas populations of *E. pratensis*, all specimens of which shared this substitution and the underlying nucleotide change (G → C) at the 2nd position of codon 161 (nucleotide position 483). The relative rate of evolution at nucleotide position 483 was by far the highest for any 2nd codon position in the COI alignment, 37 times higher than the mean rate for all other 2nd position sites. The residue at position 94 was also variable among complex members (Val/Ile), but species in subclades 2 and 3 were all fixed for Val. The remaining nucleotide substitutions in the sequenced COI fragment resulted in synonymous changes for all ingroup species.

**Table 5**

Molecular diversity, selective neutrality and population subdivision in *Elysia subornata* versus *E. pratensis* over the same 543-bp fragment of the COI locus;  $P < 0.005^{**}$ ,  $P < 0.00001^{***}$ .

	<i>E. subornata</i>	<i>E. pratensis</i> <sup>1</sup>	
		native mtDNA	introgressed
$h^2$	0.9583 (0.0112)	0.8917 (0.0547)	0.9178 (0.0216)
$\pi^2$	0.0089 (0.0049)	0.0191 (0.0100)	0.0071 (0.0040)
Tajima's <i>D</i>	-0.6015	1.5880	-0.7597
Fu's $F_S$	-9.0727**	-2.1689	-9.1193**
$\Phi_{ST}$	0.6518***	0.7927***	0.4642***
$F_{ST}$	0.2232***	0.3221***	0.3249***
$D_{est}^3$	0.615 (0.569–0.729)	0.663 (0.501–0.812)	0.678 (0.625–0.726)

<sup>1</sup> Populations of *E. pratensis* with native versus introgressed mtDNA analyzed separately.

<sup>2</sup> COI data corrected for multiple substitutions using the TrN model, with variances for gene and nucleotide diversities given in parentheses.

<sup>3</sup> 95% confidence interval based on 1000 bootstrap pseudoreplicates given in parentheses beneath estimated value.

## 4. Discussion

### 4.1. Ecological speciation by host shifting

Surprisingly few studies have addressed the potential for host shifting by marine herbivores, despite parallels with insect-plant systems often used to test models of divergence with gene flow (Krug, 2011). For marine taxa, most work has focused on host shifts in cnidarian-associated taxa, including nudibranchs (Fritts-Penniman et al., 2020); snails (Simmonds et al., 2020); epibiotic barnacles (Tsang et al., 2014); fish (Munday et al., 2004; Whitney et al., 2018); and anemone shrimp (Hurt et al., 2013). The lack of comparable studies on macroalgae-associated species may be because more herbivores in the sea are generalists (Poore et al., 2008). However, the ubiquity of host-specialized mutualists, epibionts and consumers in marine systems suggests ecological divergence warrants greater attention from phylogenetic and mechanistic perspectives.

Evidence from a growing number of marine systems supports divergence with gene flow for partially isolated ecotypes, or reproductively isolated species (Johannesson et al., 2017; Momigliano et al., 2017; González et al., 2018; Teske et al., 2019; Simmonds et al., 2020; Leder et al., 2021). These studies emphasize the role that disruptive selection may play in the speciation process, splitting one ancestral population into two groups specializing on different habitats, hosts or other resources while also promoting assortative mating (Potkamp and Franssen, 2019; Faria et al., 2021). However, alternative models can be challenging to falsify: gene flow between lineages may occur during initial sympatric divergence, during allopatric intervals, or upon secondary contact (Foote and Morin, 2016; Yang et al., 2017; Foote, 2018; Kopp et al., 2018; Prada and Hellberg, 2020). As a result, the forces that promoted initial diversification (disruptive selection in sympatry, vs. drift or divergent selection in allopatry) can be difficult to distinguish from those that produced final reproductive isolation, such as reinforcement or sexual selection (Nosil et al., 2009; Smadja and Butlin, 2011). Indeed, both geographically isolated populations and disruptive selection on sympatric lineages likely contributed to incipient speciation in model systems such as *Rhagoletis pomonella* (Feder et al., 2003) and crater lake cichlids (Martin et al., 2015). While trait divergence between sympatric sister taxa is often interpreted as evidence for disruptive selection driving ecological speciation, character displacement following secondary contact can be an alternative explanation.

Although prior studies have linked genetic divergence with some ecological difference among marine lineages, ASR has rarely been used to evaluate candidate sympatric host shifts in a phylogenetic context; more commonly, shifts in host or habitat are inferred from alternative

tip states across a tree, or are informally “mapped” onto branches without evaluating statistical support. Formal tests are important to reject alternative hypotheses, such as a generalist ancestor evolving into multiple specialists, and to facilitate tests of ecological fitting versus *de novo* adaptation to novel hosts (Agosta, 2006). Fritts-Penniman et al. (2020) used ASR to infer the genus of host coral used by a specialized clade in the nudibranch genus *Tenellia*, and reported seven cases of sympatric species differing in host use; however, some cases may fit alternative models (e.g., a *Porites* generalist evolving into more host-specialized descendants). However, the sympatric shift from *Pavona* to *Gardineroseris* host corals provides compelling support for a hypothesis of host-driven speciation in this group. In a case of incipient speciation, genomic comparisons of two host races in the snail *Coralliophila violacea* support divergence with gene flow promoted by different selective regimes on alternative coral hosts (Simmonds et al. 2018, 2020). In our study, ASRs supported ‘sea grapes’ as the ancestral *tomentosa* complex host, with one shift on a terminal branch leading to a feather-morph host in sp. 5. Within subclade 3, at least two host shifts occurred among three broadly sympatric lineages, leading to novel hosts in *E. hamanni* (*C. prolifera*) and *E. pratensis* (*R. phoenix*). Thus despite the scarcity of examples in the marine literature, specialized feeding on preferred hosts may favor ecological speciation in the sea as in terrestrial systems.

Notably, 10 of 11 complex members feed on *Caulerpa* spp., an uncalcified alga; only *E. pratensis* feeds on the calcified *R. phoenix*, with resulting adaptations in external and radular morphology. Adult slugs usually remain in close physical association with their host, on which they feed, mate and oviposit. Although *Caulerpa* spp. and *R. phoenix* grow interspersed in many Caribbean habitats, disruptive selection on host preference may drive pre-mating isolation in sea slugs by pleiotropy; host choice is a ‘matching trait’ *sensu* Kopp et al. (2018). Analogously, phytophagous insects can rapidly form new host races when encountering novel plants (Egan and Funk, 2009; Rebar and Rodríguez, 2015).

Nuclear loci were congruent with external morphology and host use for all sampled specimens; moreover, no F1 hybrids were detected. We hypothesize that distinct host algae impose selection on external and internal morphology of their respective slug consumers. For instance, longitudinal stripes on *E. pratensis* are disruptive against the scales of *Rhipocephalus*, and the serrated teeth of *E. pratensis* are typical of slugs that feed on calcified algae compared to the less denticulate, blade-like teeth of *Caulerpa*-feeders (Krug et al., 2016). No other sampled species produces feeding scars similar to *E. hamanni*, suggesting a distinctive feeding strategy evolved to cope with the flat, leaf-like blades of *C. prolifera* which are unlike any other *Caulerpa* spp. The different concentrations of noxious secondary metabolites among *Caulerpa* species (Baumgartner et al., 2009), and between *Caulerpa* versus *Rhipocephalus*, also likely impose divergent selective pressures on herbivore tolerance and preference traits. An improved understanding of host associations for extant species, and how host breadth has evolved in this complex, is also important for evaluating the potential use of *tomentosa* complex members for the proposed biological control of invasive *Caulerpa* spp. (Coquillard et al., 2000).

Ultimately, it remains unclear to what extent host shifting triggers ecological divergence in sympatry, even for well-studied groups such as phytophagous insects (Bolnick & Fitzpatrick, 2007). Genome-wide data are needed to compare models of divergence with gene flow against alternative scenarios (e.g., character displacement in host use). Under the latter model, subclade 3 may have diverged allopatrically during Pleistocene sea level fluctuations; upon secondary contact, *E. pratensis* and *E. hamanni* then shifted onto alternative hosts to reduce competition with *E. subornata*. However, several lines of evidence argue against host shifts as character displacement following secondary contact. First, there was no evidence of host shifts in subclade 2, a sympatric radiation of three species eating ‘sea grapes’ with overlapping distributions in the western Pacific. Thus, host shifting is not necessary for species formation in the *tomentosa* complex, but neither do host shifts necessarily result

from syntopy. Second, the diet of *E. subornata* expanded from the ancestral ‘sea grape’ to encompass additional *Caulerpa* spp., including feather-morph species and other morphologies. The predicted outcome should be a narrowing of niche breadth if secondary contact triggered character displacement in host use, not a host expansion to include many more *Caulerpa* spp. in the ancestor of *E. subornata*.

The scale of dispersal for subclade 3 species represents a third line of evidence favoring host shifts as an adaptive response that could promote divergence. For benthic marine invertebrates, most dispersal occurs during a planktonic larval stage, but both *E. subornata* and *E. pratensis* have encapsulated metamorphosis with juvenile slugs crawling out of the egg mass (Krug, 2009); development in *E. hamanni* is unknown. Both species showed exceptional population subdivision with among the highest  $F_{st}$  and  $\Phi_{ST}$  values reported for marine invertebrates (Bradbury et al., 2008; Weersing and Toonen, 2009; Kelly and Palumbi, 2010; Dawson et al., 2014). Limited dispersal is predicted to increase local adaptation, including to novel hosts, by decreasing the homogenizing effects of gene flow and may thus have favored host shifts in subclade 3 (e.g., compared to subclade 2 where several species have dispersive larvae.).

A fourth line of evidence that favors a role for host shifts during initial divergence is the asymmetric introgression of mtDNA from *E. subornata* into *E. pratensis*, indicating these lineages hybridized and exchanged mtDNA after substantially diverging. Strong premating barriers are likely a prerequisite for ecological character displacement (Aguilée et al., 2011), whereas theory predicts introgression is likely to occur when only weak prezygotic barriers are present (Chan and Levin, 2005). The ability of the species to hybridize is another argument against the character displacement model for differential host use, since strong premating barriers were evidently not present well after the lineages diverged in host use. However, the apparent recency of mtDNA introgression does not directly support a model of divergence with gene flow, compared to coalescent-based evidence for exchange of nuclear alleles during the speciation process (Hurt et al., 2013).

#### 4.2. Mitochondrial capture

Discordance between nuclear and cytoplasmic genomes was consistent with introgression of mtDNA from *E. subornata* into *E. pratensis* prior to the emergence of present-day phylogeographic structure, as most localities in each species had an endemic clade of COI haplotypes. We interpret patterns in the non-recombining mitochondrial genome as evidence for one past hybridization event between *E. subornata* and *E. pratensis*, with *E. subornata* acting as the egg donor; through subsequent rounds of backcrossing, the mtDNA of *E. subornata* entered the nuclear background of *E. pratensis*. The alternative, that incomplete lineage sorting (ILS) distributed ancestral mtDNA lineages from the same clade between *E. subornata* and *E. pratensis*, is implausible given the smaller effective population size of mtDNA; lineage sorting of the two nuclear markers was observed, yet should not occur before mtDNA sorted to reciprocal monophyly. Moreover, *E. subornata* mtDNA occurring within only a subset of *E. pratensis* populations also favors adaptive introgression; divergent lineages should be randomly scattered throughout *E. pratensis* populations in the case of ILS (Toews and Brelsford, 2012). However, ILS may explain the lack of phylogenetic resolution between *E. subornata* mtDNA and introgressed mtDNA in *E. pratensis*, and the presence of a single COI haplotype shared between the species. Sex-biased dispersal, a common driver of cytonuclear discordance in other systems, cannot explain asymmetric introgression in these hermaphroditic sea slugs.

Remarkably, the *E. subornata* cytoplasmic genome became locally fixed in *E. pratensis* from six central Bahamian islands, representing about half of the sampled range of *E. pratensis*. Introgression across a hybrid zone can lead to sweeps of introgressed mtDNA deep into a related species’ range, the ‘introgression conveyor’ model (McGuire et al., 2007). ‘Mitochondrial capture’ is an extreme case of introgression

in which the mtDNA of one species becomes fixed against the nuclear background of another species over a significant portion of its range (Ballard and Whitlock, 2004; Llopart et al., 2014). Although mitochondrial capture has been documented in insects and diverse vertebrates (Berthier et al., 2006; Currat et al., 2008; Plötner et al., 2008; Renoult et al., 2009; Bastos-Silveira et al., 2012; Beysard et al., 2012; Bernal et al., 2017), this may be the first example of population-level mtDNA replacement by a sympatric species in marine invertebrates (Toews and Brelsford, 2012). Recent and ongoing mtDNA introgression has been demonstrated in mussels (Rawson and Hilbish, 1998; Quesada et al., 1999), crabs (Darling, 2011), polar sea urchins and sea stars (Addison and Hart, 2005; Harper et al., 2007); such introgression results in common haplotypes shared between species but in a geographically unstructured manner. Notably, all those invertebrates are gonochorists, and most are broadcast spawners. However, localized fixation of introgressed mtDNA within a large fraction of the range of *E. pratensis* is a rare example of mitochondrial capture in an internally fertilizing marine hermaphrodite.

This phenomenon may be widespread but unappreciated, due to the common reliance on barcoding studies that use only mtDNA markers. For instance, in mimetic nudibranchs, some individuals of multiple species demonstrated cytonuclear discordance; population-level sampling is needed to determine the extent to which native mtDNA is locally replaced in these mimicry-dominated systems where individuals showing hybrid ancestry are surprisingly common (Layton et al., 2020). The findings that mtDNA moves easily between marine heterobranch lineages that are otherwise distinct by nuclear loci, morphology and ecology cautions against an over-reliance on common mt barcoding markers like COI and 16S. Such concerns are important to note for species discovery and delimitation studies on heterobranchs, which harbor a high proportion of cryptic species: individuals may not be reliably assigned to species using only mt genes.

Although fixation could result from genetic drift if population sizes were low and bottlenecks frequent after secondary contact, replacement of mtDNA across a large portion of the sampled range of *E. pratensis* suggests an adaptive explanation (Ballard and Melvin, 2010; Rheindt and Edwards, 2011; Toews and Brelsford, 2012). While mtDNA is typically expected to have a fitness advantage against its own nuclear genetic background (Hutter and Rand, 1995), temperature-dependent selection can favor specific cytonuclear gene interactions (Matsuura et al., 1997; Doi et al., 1999; Willett and Burton, 2003; Dowling et al., 2007, 2008). Introgressed mitochondria may be favored in *E. pratensis* populations if the mitochondria of *E. subornata* are physiologically superior in warmer water, for instance. Water may be consistently warmer at central Bahamas sites where *E. pratensis* with introgressed mtDNA were collected (Sweetings Cay, Little San Salvador, Bimini, Compass Cay), sites in the back of large lagoon systems with restricted circulation; cooler water was commonly experienced at open-coast sites (San Salvador, Plana Cays, FL) where *E. pratensis* had native mtDNA, although site-specific temperature data were not available.

Non-synonymous substitutions in the COI gene may be targets for selection, as functional differences in COI and other cytoplasmic genes can be temperature-dependent (Rawson and Burton, 2002; Willett and Burton, 2003). We noted a fixed difference in amino acid composition between the sequenced portion of the COI gene in introgressed vs. native populations of *E. pratensis*, due to reversion to an ancestral residue (Thr<sub>161</sub>) in *E. subornata*. Other mitochondrial genes could also carry substitutions under selection, however, and non-coding SNPs have also been linked to differences in mitochondrial physiology associated with climatic adaptation (Camus et al., 2017).

The limited dispersal ability of *E. pratensis* may have slowed the spread of introgressed lineages beyond the central Bahamas, coupled with known biogeographical breaks in and around the Bahamas (Debiasse et al., 2016). Such dispersal barriers in other species include the Gulf Stream current, limiting exchange between FL and the Bahamas, and a genetic break north of San Salvador Island, where a

deeply divergent native mtDNA lineage was sampled in *E. pratensis*. However, continued ocean warming may alter the adaptive value of introgressed vs. native mtDNA. If *E. subornata* mtDNA confers a temperature-dependent fitness benefit on an *E. pratensis* cytoplasmic background, warming trends may favor the further spread of introgressed mtDNA at the expense of native mtDNA in *E. pratensis*.

#### 4.3. Systematic implications

Here, we added two new species and range-wide sampling data for *E. subornata* and *E. pratensis* to update prior phylogenetic analyses of the *tomentosa* complex within family Plakobranchidae (Krug et al., 2015, 2016, 2018b). Previous taxonomic hypotheses were supported: five distinct *Elysia* spp. in the Western Atlantic belong to the *tomentosa* complex, differing genetically; in external and radular morphology; in host use; and in reproductive anatomy (Krug et al., 2016). *Elysia* spp. can often be distinguished by a combination of traits including (a) distribution; (b) color and pattern; (c) length of the renopericardial extension on the dorsal surface; (d) the dorsal vessel network; (e) shape and denticulation of radular teeth visualized by scanning electron microscopy (SEM); (f) host use; and (g) larval type. Among Caribbean elysiids, a renopericardium running the whole length of the dorsum is a synapomorphy of subclade 3 shared by *E. subornata*, *E. pratensis* and *E. hamanni*, which we show here are distinct genetically, in radular morphology and host use. In contrast, the renopericardial extension extends less than halfway along the dorsal surface in both *E. pawliki* (Krug et al., 2016: figs. 58–59), and in *E. zemi* with a characteristic dark line at the terminus (Krug et al., 2016: figs. 61–62).

Unfortunately, the systematic status of these species was confused by Ortea et al. (2017) asserting that *E. zemi* and likely *E. pawliki* were junior synonyms of *E. cauze* Er. Marcus, 1957, with no supporting molecular or anatomical data. Numerous authors have recognized *E. cauze* as a junior synonym of *E. subornata* Verrill, 1901 including Clark (1984), Jensen (1993), and Krug et al. (2016); an elongated renopericardium was diagrammed for *E. cauze* by Ev. Marcus (1980), and for *E. subornata* by Clark (1984: fig 14) and Krug et al. (2016: fig 25). Here, we show *E. subornata* is the only genetically cohesive species with an elongated renopericardium found on diverse *Caulerpa* spp. throughout the Caribbean, including the type locality, Bermuda. Moreover, denticles on the teeth of *E. subornata* can be seen by light microscopy and match the description of *E. cauze* (Er. Marcus 1957: figs. 39, 41); the denticles of *E. zemi* and *E. pawliki* are finer and require SEM to visualize, whereas those of *E. pratensis* are much larger. We confirm *E. cauze* is a junior synonym of *E. subornata* as there is no other widespread species with the combination of traits attributed to *E. cauze* (Krug et al., 2016).

Confusingly, Ortea, Moro & Bacallado (2017) assert that Krug et al. (2016) (a) synonymized *E. cauze* and *E. subornata* [a synonymy made by Clark (1984)] and (b) redescribed *E. cauze* as *E. zemi* (type locality: Martinique). Our data show specimens of *E. subornata* (= *E. cauze*) and *E. zemi* from their respective type localities are 18% divergent at COI, and thus are not conspecific. In contrast, material called “*E. cauze*” by Ortea et al. (2017) was collected from the Cape Verde Islands ~ 5,000 km from the original type locality (Island of São Sebastião, São Paulo, Brazil). There is no basis for asserting that *E. zemi* is a synonym of *E. cauze*, itself long established as a junior synonym of *E. subornata*. The material referred to as “*E. cauze*” by Ortea et al. (2017) presumably comprised a mix of (1) an unidentified East Atlantic species; (2) *E. zemi*; and (3) *E. subornata sensu* Verrill, 1901.

The proposed synonymy of *E. pawliki*, *E. zemi* and *E. subornata* (= *E. cauze*) is refuted by our molecular analyses: these three species are polyphyletic and > 17% divergent at COI (Krug et al., 2016). The sole basis for synonymy was that these taxa share colored spots and dark-ringed epidermal glands (Ortea et al., 2017), but related species share traits due to common ancestry; as we show here and elsewhere, the *tomentosa* complex is monophyletic but Caribbean members are not, and thus cannot be one species. Databases such as the World Register of

Marine Species (<https://www.marinespecies.org/>) should follow the preponderance of evidence and reject synonymies that are proposed without visiting type localities, examining type material, following the rules of priority, or considering published phylogenetic and morphological analyses.

#### 4.4. Conclusions: Implications for marine speciation and biodiversity

Marine taxa are increasingly used as model systems to investigate ecological speciation, including cases where microhabitat or host preference may lead to sympatric divergence by partitioning ecotypes at small spatial scales (Whitney et al., 2018; Simmonds et al., 2020). However, the potential for macroalgae-associated species to diverge via sympatric host shifts remains largely unexplored. By modeling ancestral host use, we show here that repeated shifts among algae promoted a sympatric radiation of herbivorous sea slugs, highlighting the role host association may play as a driver of diversification in the sea. We thus add herbivores to the list of marine taxa for which ecological divergence warrants greater attention, from phylogenetic and mechanistic perspectives, to understand fully the origins of reef biodiversity. By highlighting an unusual case of localized but complete mitochondrial capture, our study also cautions against the over-reliance on mtDNA barcoding in species discovery and delimitation, while opening new avenues to study the processes that promote introgression and fixation of foreign mtDNA. In the context of rapid ocean warming, further study is needed to assess the extent to which mitochondrial capture may be temperature-dependent, and to determine if such introgression is more widespread than presently appreciated among marine invertebrates.

#### CRedit authorship contribution statement

**Albert K. Rodriguez:** Formal analysis, Investigation, Funding acquisition, Writing – original draft. **Patrick J. Krug:** Conceptualization, Data curation, Formal analysis, Funding acquisition, Project administration, Visualization, Supervision, Writing – original draft, Writing – review & editing.

#### Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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## Data Accessibility

- DNA sequences: Genbank accession numbers given in Tables S1–S3.
- DNA alignments, Arlequin input files, and output from *BayesTraits* ancestral host reconstructions archived on the Dryad data repository (<https://doi.org/10.5061/dryad.vmcvdcnw>).

## Appendix A. Supplementary material

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.ymp.2022.107523>.

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