Natural history clues to the evolution of bivalved gastropods (Mollusca: Gastropoda: Sacoglossa: Juliidae)

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Abstract

Bivalved gastropods (family Juliidae) are a pan-tropical group of small, green, extraordinary gastropods with a symmetrical clam-like shell. Only a single species of Juliidae has been reported in Malaysia, *Berthelinia singaporensis* Jensen, 2015, feeding on multiple species of siphonous green algae in the genus *Caulerpa*. In spite of a baseline expectation for megadiversity in this region, including 13 recorded species in the genus *Caulerpa* from two small field sites studied here, *B. singaporensis* appears to be a relative generalist without preferences among available *Caulerpa* spp. that occur in suitable (micro)habitats. The life cycle of Juliidae includes univalve, coiled larvae that transform to bivalved adults. In the presence of *Caulerpa*, swimming veligers rapidly settle and begin feeding; in a later, terminal metamorphosis, the larval operculum is shed, and the teleoconch shell field splits to form a second, right valve of the adult bivalved form. By three days after hatching, settled larvae achieve a fully functional bivalved shell with a working hinge. Both veligers and adults produce an unusual elastic mucous tether to maintain attachment to the substratum or food plant. These animals are dependent on *Caulerpa* for their entire life cycle; so, adults occupy environments where dislodgement may be a substantial risk. In the adult, bivalved, form, the gastropod can withdraw completely into its shell and close the valves while maintaining the byssus-like tether. This arrangement, enabling the gastropod to be simultaneously anchored to the food plant and protected within the shell, suggests that being able to use a byssus is the most plausible adaptive explanation for the evolution of the bivalved form within gastropods.

Keywords tropical biodiversity · molluscan evolution · adaptation

Introduction

Over and above the basic principle that there are more species in the tropics, the highest density and diversity of species in shallow marine habitats occur in Southeast Asia (e.g. Edie et al. 2018). This region presents a quandary that is familiar to all who work on its fauna: On the one hand, the biodiversity of SE Asia is globally important in terms of both its

contributions to total world species richness and its pivotal role in understanding large-scale biogeographic and phylogeographic phenomena, such as the global latitudinal diversity gradient (LDG), and insights like Wallace's Sarawak Law (Wallace 1855). On the other hand, at a local scale, this same overwhelming species richness in most clades presents a frustrating taxonomic and natural history challenge to document basic biodiversity. In systems where the fundamental units are poorly identified, it is more difficult to assess emergent patterns. This point has been made repeatedly, about the underestimating of biodiversity in less-studied taxonomic groups and habitats (e.g. Middelfart et al. 2016; Sigwart and Garbett 2018), as well as the downstream cascading problems that arise from analyses based on poor taxonomic resolution and accuracy (Bortolus 2008). Understanding the natural history of marine species in SE Asia is critical to broader scientific aspirations of assessing Earth's species richness, evolutionary innovations, and the drivers and limits of diversity and diversification.

The bivalved gastropod family Juliidae is a pan-tropical clade, noted for their exceptional morphology, with a two-

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part symmetrical shell that strongly resembles a bivalve. Several living species and at least one fossil species in this clade were originally described as bivalves (e.g. Crosse 1875) and only recognised as gastropods from live observations in ca. 1960 (Kawaguti and Baba 1959; Baba 1961). Recognising the convergence of this gastropod shell morphology with a deeply divergent clade of molluscs (bivalves) dramatically expanded the idea of disparity among gastropods and remains critically important to understanding the morphological evolution of molluscs (Sigwart 2017; Runnegar and Pojeta 1985).

Most sacoglossan gastropods, including all members of Juliidae, feed on green algae by piercing the outer membrane with their radula and sucking the internal fluids with a muscular pharynx. Their characteristically green body colour and small body size make many sacoglossans highly camouflaged and easily overlooked by field biologists. Investigations of other Sacoglossa in SE Asia have revealed pseudocryptic sibling species with apparently overlapping niches, sometimes co-occurring and even depending on the same food plant (e.g. Krug et al. 2018).

Species of Juliidae occur throughout the tropics, in the Caribbean, SE Asia, Australia, and Pacific islands, including the type locality of the earliest described species of the genus *Julia* in Hawai'i (Gould 1862; Kay 1962). In Japan (the type locality of the first described species of *Berthelinia* and the region where living Juliidae were first studied, leading to their recognition as gastropods rather than bivalves), there are at least four species in two genera (Jensen 2007). By contrast, in the generally megadiverse fauna of Malaysia and Singapore, only one species of the family Juliidae has been recorded (Jensen 2015; Jensen and Ong 2018).

In the present study, we compiled observations of *Berthelinia singaporensis* Jensen, 2015 from field and lab studies, to illuminate its natural history. The aims of the present paper are to consider what aspects of reproductive biology and autecology may limit the potential for further species divergences within this apparently highly specialised bodyplan, and the evolutionary drivers for the bivalved form.

Material and methods

Live specimens of *Berthelinia singaporensis* were collected from two sites in peninsular Malaysia, on multiple occasions from March 2015 to July 2018 (Fig. 1). The two sites are Blue Lagoon, Melaka (2°24'53.6"N, 101°51'16.8"E), in the Malacca Strait, near the city of Port Dickson (hereafter referred to as the "Port Dickson" population), and a second site at the small island of Pulau Merambong (1°18'53.2"N, 103°36'33.5"E) in the Sungai Pulai Estuary in the Johor Strait between the southern tip of peninsular

Malaysia and the island of Singapore (hereafter the "Merambong" population).

Specimens were obtained by collecting multiple species of green algae in the genus *Caulerpa*, and the gastropods were recovered either by manually examining the algae or by holding live algal samples in small aquaria and observing gastropods crawling on the algae or transparent surface of the aquarium over several days. Animals recovered from Merambong, and animals and *Caulerpa* from Port Dickson were returned to the International Institute of Aquaculture and Aquatic Sciences (I-AQUAS) facility of Universiti Putra Malaysia, in Port Dickson. Multiple species of *Caulerpa* co-occur at both field sites. Our identification of *Caulerpa* species was determined with some caution based on gross morphology and comparison with available regional lists. *Berthelinia* specimens were identified to species by comparison with the original description (Jensen 2015; K. Jensen pers. comm.); analysis of standard barcode marker DNA fragments from *B. singaporensis* specimens from the two study populations indicated no significant divergence (unpublished analyses: McCarthy 2017).

The abundance of *Caulerpa* is variable throughout the year at both sites. To assess potential seasonality of *Caulerpa* availability, we re-analysed existing data for a time series of algal coverage at Merambong. At this site, an ongoing long-term monitoring project has established a 100-m permanent fixed transect, parallel to the shore at approximately chart datum, which is sampled at low spring tides (Wong, unpubl. data). Quadrats at fixed positions at 5 m intervals were each captured as a high-resolution photo-quadrat of 1 m². For analysis, each image is demarcated into a 10 × 10 grid. Each grid area is scored for the presence of algal cover covering at least half of that 10 cm × 10 cm area. Percent cover on the shore is based on a cumulative average of the scores for each of 20 quadrats. This transect was sampled at two-month intervals from April 2015 to April 2017.

For observations of live *Berthelinia*, experimental animals were kept in the I-AQUAS hatchery, housed in static seawater (i.e. not connected to the available flow-through seawater supply) in 500 mL containers with aeration at ambient temperature (typically 27–29 °C), and provided with small amounts of *Caulerpa* spp. Behavioural observations were used to select the most appropriate food, assessing a general tendency of animals to congregate on a particular plant in a volume of mixed *Caulerpa* samples held in aquaria, or observations that animals begin feeding on a single alga within a water basin in preference to continued exploration of the environment. *Caulerpa lentilifera* was primarily used for feeding, but animals laying eggs were transferred to *Caulerpa* spp. with flat surfaces (*C. subserrata*, *C. cf. lamourouxii*) to enable better observation of egg masses.

Fig. 1 Habitat context for *Berthelinia singaporensis*. **a** Regional map, with the area of peninsular Malaysia indicated by a shaded box; **b** Map of peninsular Malaysia showing location of two study sites included here;

c Fluctuating abundance of the siphonous green algae *Caulerpa* spp. and total intertidal algal cover measured in photo quadrats at Pulau Merambong over a two-year period

When egg masses were observed with captive animals, the egg masses were scraped off the alga and moved to Petri dishes with seawater. Daily observations and photographs for developing embryos were used to record the timing of transitions between successive embryonic stages. Embryos were photographed with an Olympus BX43F compound microscope and Olympus DP22 camera with Olympus cellSens Standard V1.14 image processing software.

When the first veliger was observed hatching from an egg mass, the whole remaining egg mass and any free-swimming larvae were transferred to a 250 mL beaker with aerated seawater and provided with a few drops of concentrated *Nannochloropsis* microalgae from established lab cultures. After the majority of larvae were observed swimming, a short stem of *C. cf. lamourouxii* was added to the container to encourage settlement and feeding. Larvae that successfully settled were monitored multiple times daily until they completed the final metamorphosis to the terminal bivalved adult form. Thereafter, juveniles were returned to larger holding tanks (plastic basins of approximately 4 L, with static aerated seawater) to maintain the captive population. Over approximately three years of this study, the captive population was repeatedly re-stocked from both field collection and captive

reproduction and the number of captive specimens varied from a few individuals to up to 50 adults across both populations.

Results

Habitat

We found diverse *Caulerpa* spp. at both field sites; if our identification of *C. lamourouxii* is correct, this expands the number of *Caulerpa* taxa recorded from the Port Dickson site to 9 species (Table 1; Asmida et al. 2017). A previously published list of macroalgae at Merambong Shoal, a seagrass meadow adjacent to our field site, included three species of *Caulerpa*, and we have observed seven other taxa (Table 1; Muta Harah et al. 2014). A regional compilation for SE Asia included seventeen species of *Caulerpa* in Malaysia but noted that many new species have been described recently in the region (Phang et al. 2016). Discrepancies in species lists recorded may relate to the fluctuating populations of green seaweed. The abundance of *Caulerpa* spp. at Merambong varied from 0% cover to over 33% cover in a two-year period (Fig. 1c); general algal coverage fluctuates irregularly and there is

Table 1 Diversity of the siphonous green algae *Caulerpa* in two sites in peninsular Malaysia: (+) recorded in the present study; (1) recorded at Port Dickson by Asmida et al. (2017); (2) recorded at Merambong by Muta Harah et al. (2014); (*) new species in this study or those cited, not included in the *Caulerpa* species list for Malaysia by Phang et al. (2016).

Species	Blue Lagoon, Port Dickson	Merambong, Johor Strait	<i>Berthelinia singaporensis</i> observed feeding
<i>Caulerpa chemnitzia</i> (Esper) J.V. Lamouroux, 1809	+	+	
<i>C. cf. lamourouxii</i> (Turner) C. Agardh, 1817	+*		×
<i>C. lentillifera</i> J. Agardh, 1837	+ ¹	+	×
<i>C. manorensis</i> Nizamuddin, 1964		2*	
<i>C. mexicana</i> Sonder ex Kützinger, 1849		+	
<i>C. microphysa</i> (Weber-van Bosse) Feldmann, 1955	1	+	
<i>C. racemosa</i> (Forsskål) J. Agardh, 1873	+ ¹	+	×
<i>C. racemosa</i> var. <i>macrophysa</i> (Sonder ex Kützinger) W.R. Taylor, 1928		+	
<i>C. serrulata</i> (Forsskål) J. Agardh, 1837	+ ¹	+	×
<i>C. sertularioides</i> (S.G. Gmelin) M.A. Howe, 1905	+ ¹	+ ²	×
<i>C. subserrata</i> Okamura, 1897	1*		
<i>C. taxifolia</i> (M. Vahl) C. Agardh, 1817		2	
<i>C. verticillata</i> J. Agardh, 1847	1		

(N.B. *Caulerpa chemnitzia* listed here as a new record for both sites has formerly been called *C. peltata*; Belton et al. 2014.) The final column shows those that were experimentally verified as food for *Berthelinia singaporensis*

no clear or consistent seasonality to the episodic blooms of *Caulerpa*.

Berthelinia singaporensis does not have a specific dependency on one species of food plant. Adults and egg masses have been found on several species of *Caulerpa* in field and lab conditions, and the animals demonstrate little apparent preference for certain species among their potential food taxa. In captivity, we have found them consuming *Caulerpa racemosa*, *C. lentillifera*, *C. serrulata*, *C. sertularioides*, and *C. cf. lamourouxii*, with active feeding confirmed based on the marks left on the surface of their food plants (Table 1). These notably include *Caulerpa* species with both pinnate fronds and grape-like spherical ramuli. Furthermore, we have not noticed any preference for specific places on the food plant where the gastropods rest in captivity. For *B. singaporensis* on *C. racemosa*, we found feeding radular marks on the tips of the ramuli, between assimilators (branches) and the main stolon of the alga, and on the stolon itself (Fig. 2a).

Life cycle

The size of individuals recovered in field-collected *Caulerpa* ranged from 1.5 to 3 mm in shell length. Individuals held in captivity apparently achieve larger shell sizes, up to a maximum of 5 mm. A group of over 20 adult animals of similar size, collected together in April 2016, maintained apparently good health and

growth in the lab for a period of six months and then all died within a short interval in early October 2016, although the group was divided into independent aquaria (in different buildings with separate seawater supply). We examined a total of 50 egg masses laid by animals kept in our long-term captive colony, to determine the precise number of eggs, and found that the number of egg capsules per egg mass ranged from 8 to 108 with mean 46.6 egg capsules per egg mass (Fig. 3). The diameter of the uncleaved egg within the capsule was approximately 83 µm. Our repeated observations of the developing egg masses produced by animals collected in Merambong all closely followed a consistent developmental calendar (Table 2; Fig. 4). The developing embryos experience a very brief trochophore phase *in ovo* before metamorphosing to a typical gastropod veliger at about 72 h after fertilisation. The larvae experience another secondary and terminal metamorphosis after settlement, to achieve the adult bivalved form.

At approximately 10 days after fertilisation, larvae hatch as swimming gastropod veligers with a small, coiled shell (Fig. 5). The veligers can retract into the shell and produce an elastic mucous tether that they can “glue” to the substratum. In the absence of surface agitation through aeration, the larval protoconch sticks to the surface (Wisely 1962; Grahame 1969; Jensen and Ong 2018), but this is an effect of surface tension, not deliberate adhesion, and the veligers are unable to

Fig. 2 *Berthelinia singaporensis*, with individual animals indicated by arrows. **a** On *Caulerpa* cf. *racemosa*; **b** On *Caulerpa* cf. *sertularioides*. Animal size is approximately 4 mm

escape from the surface and will die there without intervention. This swimming (dispersal) phase lasts for 1–3 days before the animals settle and begin feeding. In some cases, in the presence of healthy *Caulerpa*, swimming lasted for less than 24 h before settlement. Without access to *Caulerpa*, the larvae continue swimming, but, from our preliminary observations, survivorship declines rapidly after 8–9 days even in the presence of suspended microalgae.

At the settlement, within 24 h, the larvae shed the ciliated velum and begin feeding continuously, piercing the algal wall and sucking on the interior juices (Figs. 5, 6). The embryos and larvae are colourless and nearly transparent, and begin to turn visibly green after they have been feeding for several days and complete the second metamorphosis to the bivalved form (Fig. 6c). This final metamorphosis occurs 24–48 h after settlement, when the larva sheds its operculum, and the growing margin of the shell splits to form the second, right valve (Fig. 6). In the next several days after the final metamorphosis, both sides of the shell grow equally (the left shell extends from the coiled protoconch, the right shell extends from the initial flap or “visor” of shell formed at the second, terminal metamorphosis).

Fig. 3 Egg masses of *Berthelinia singaporensis*. **a** New egg mass and mother; note white coiled protoconch visible at top of valve (left valve in view, animal length is approximately 4 mm); **b** Size-frequency of egg masses observed in captivity

Discussion

Habitat and life cycle

Two species of sea grapes, *C. lentillifera* and *C. racemosa*, are commonly harvested for food in parts of SE Asia. The term *latok* in several regional languages refers to either of these two *Caulerpa* species as harvested for food. The broad feeding habits of *B. singaporensis* stand in contrast to other sacoglossan lineages that feed on *Caulerpa* and target a specific place on a specific species of food-plant, such as a preference for feeding by sucking on ramuli (Krug et al. 2018). We speculate that *B. singaporensis* targets *Caulerpa* species based on the abiotic context of the microhabitat, perhaps driven by factors such as temperature or current flow; that is, while this microhabitat may correlate with the preferred habitat of particular *Caulerpa* spp., individual *Berthelinia* do not show clear preference among *Caulerpa* spp. that are growing in appropriate conditions. The morphology of the flat- or pinnate-bladed *Caulerpa* sp. also makes it much easier to find the *Berthelinia* specimens (Fig. 2),

Table 2 Development of *Berthelinia singaporensis*, observed in batches at both 26 and 28 °C

Stage	Day	Approximate hours post-fertilisation	Mean size ($\pm$ s.d.)
Fertilisation	0		
2 cell	0	4 hpf	
8 cell	0	8 hpf	
16 cell	0	11 hpf	
Blastula	1	27 hpf	
Gastrulation	1	33 hpf	92.31 $\pm$ 3.13 μ m, n = 30
Trochophore (swimming)	2	52 hpf	
Early veliger	3	76 hpf	
Shelled veliger	4		
Veliger with organ systems	5		148.88 $\pm$ 9.4 μ m, n = 30
Fully developed veliger, appears ready to hatch	6		Shell length $\sim$ 220 μ m (day 8)
Hatching	10		Shell length 244.11 $\pm$ 12.29 μ m, n = 30
Swimming	10		
Settled on <i>Caulerpa</i> and feeding	12		
Final metamorphosis, bivalved shell and shed operculum	13		

which may slightly confound our impression of preference; however, *B. singaporensis* has been observed feeding on at least five species in laboratory conditions (Table 1). The question of food preferences, as well as larval dispersal, is relevant to issues of diversification, and we will return to this idea below.

Observations of spawning and development of Juliidae have been reported for several species. The development of *B. singaporensis* was also recently described from another population by Jensen and Ong (2018), and our findings corroborate their observations. Details of development have also been reported from *Berthelinia limax* (Kawaguti and Baba, 1959) (Kawaguti and Yamasu 1960), *Berthelinia caribbea* Edmunds, 1963 (e.g. Wisely 1962; Davis 1967), and *Julia japonica* Kuroda and Habe, 1951 (Kawaguti and Yamasu 1966), and some additional observations in the original description of *Berthelinia ganapati* Sarma, 1975. Kawaguti and Yamasu (e.g. 1960) kept long-term captive colonies of *B. limax*; they reported a mean egg mass size of 138 eggs (ranging from 11 to 470 eggs) in a sample of 465 egg masses. Observations of *Berthelinia australis* (Burn, 1960) and several studies on the smaller species *B. caribbea* reported egg masses of less than 100 eggs (Wisely 1962; Edmunds 1963; Davis 1967; Grahame 1969). Published observations of *B. ganapati* were based on a single captive pair, which were kept in the laboratory for only five weeks and laid thousands of eggs at an average of 281 eggs per day, although none of these eggs successfully hatched (Sarma 1975). Interestingly, the first documented observation of *B. singaporensis* included a report of an egg mass of more

than 200 eggs (Jensen 2015). These reports of egg mass sizes from short-term isolated specimens are dramatically larger than observations from healthy captive populations (Kawaguti and Yamasu 1960; herein), and the detailed observations of spawning output in *B. limax* suggested a negative correlation between food quality and egg mass size (Kawaguti and Yamasu 1960). These differing rates of egg laying may be an effect of maternal body size, or increased spawning effort may be potentially associated with stress in these species.

The developmental timetable is substantially shorter in *B. singaporensis* than that reported for *B. limax*, as would be expected in comparing this tropical species to the relatively temperate conditions of other studies in Japan. Kawaguti and Yamasu (1960) noted that the developmental cycle of *B. limax* did not appear seasonal and was not strongly influenced by changes in temperature, including winter temperatures around 10 °C, which agrees with our informal observations over a much smaller range of temperature conditions. Kawaguti and Yamasu (1960) noted that the developmental period (time from spawning to hatching) was shorter in egg masses removed from the food plant ($\sim$ 33 d) compared to those left on the alga ($\sim$ 43 d). We did not repeat this experiment, and all our observations relate to egg masses that were removed for observation.

The developmental timetable we observed for *B. singaporensis* matched the prior reports for *B. singaporensis* (Jensen and Ong, 2018) and was very similar to observations of *B. australis*. At 21 °C, *B. australis* began developing velar lobes at 70 hpf (Wisely 1962). As trochophores and early-stage veligers, the *B. singaporensis* larvae are very active within the egg capsule.

Fig. 4 Some developmental stages of *Berthelinia singaporensis* embryos. **a** First cleavage; **b** Second cleavage; **c** Third cleavage; **d** Early trochophore, with cilia visible; **e** Veliger, with developing organ systems, and eyespots; **f** Fully developed veliger

The veligers grow and actively swim within the egg capsule over the next several days after the first metamorphosis from trochophore to veliger. At about 6 days post-fertilisation, they appear to be fully developed and ready to hatch, have grown to

maximum possible size and can no longer swim or turn within the egg, and become inactive. This was slower in a previous study of the same species, which reported full size and slower-moving intracapsular veligers at 10 days

Fig. 5 Veliger larvae of *Berthelinia singaporensis*. **a** Newly hatched veliger; **b** Swimming veliger, with cells of *Nannochloropsis* visible in the gut through the transparent body; **c** Recently settled veliger on *Caulerpa*

Fig. 6 Second (terminal) metamorphosis of *Berthelinia singaporensis*, transforming from coiled to bivalved form. **a** Larva feeding on *Caulerpa*, noting the veliger operculum (op) still in place, and arrowhead indicating the first “flap” of separated shell material that will expand to form the second (right) valve; **b** Larva immediately after shedding the operculum, visible here (end-on) and as dark oblique line (indicated op); **c** Complete secondary metamorphosis, with two equal teleoconch valves and the coiled protoconch. Asterisks (*) indicate individual cells in a line of cells now visible in the distal edge of the mantle that are green, apparently sequestered from the food plant

(Jensen and Ong 2018). The veligers nonetheless remain within the egg capsule for 3–4 days beyond that point, hatching at 9–10 days post-fertilisation (10–13 days in a prior study; Jensen and Ong 2018). This is similar to *B. australis* hatching at 13 days (Wisely 1962) and considerably accelerated compared to the 30–40 d development reported for *B. limax* (Kawaguti and Yamasu 1960). The following period of 3–4 d to secondary metamorphosis is similar in all reported species: *B. singaporensis* (herein), *B. limax* (Kawaguti and Yamasu 1960), as well as *B. caribbea* (Grahame 1969).

The estimated lifespan of *B. limax* is around 220 days (Kawaguti and Yamasu 1960), and much shorter for the smaller Caribbean species *B. caribbea* (Grahame 1969), and we infer that the lifespan of *B. singaporensis* is likewise less than 1 year.

Bivalved gastropods

The usual form of these gastropods naturally leads to a question about adaptive significance of the bivalved shell form. We can propose several more or less unsatisfactory hypotheses,

which we will discuss below, related to (a) crypsis, (b) mobility, and, most plausibly, (c) tethering.

Sacoglossans are highly camouflaged, matching the colour and often the texture of their preferred algal substrata (e.g. Baumgartner et al. 2009). Captive *B. limax* were observed to turn a darker green shell colour later in life, at about the same size as they apparently reach sexual maturity (Kawaguti and Yamasu 1960). We did not see a clear colour change or gradient in *B. singaporensis*, but we cannot exclude this possibility; individuals do vary in colour, but it is not clear whether this is directly related to age, or food plant species, or intrinsic morphological variation.

Most species of *Caulerpa* have distinct spherical, bubble-like ramuli projecting in clusters from a stem called an “assimilator” (Belton et al. 2014). In some species, these “bubbles” are similar in size to adult *B. singaporensis*. This is most noticeable in the alga *C. racemosa* (Fig. 2a). The verified food algae for *B. singaporensis* include many forms, including those with branching fronds that offer no textural or morphological crypsis (Fig. 2b, Jensen 2015: figure 1F). Another *Caulerpa* species consumed by the gastropods, *C. lentillifera*, has very small ramuli arranged in tightly

packed or grape-like clusters. Another point that refutes the idea of visual camouflage may be that the ramuli of these *Caulerpa* spp. are oblatelately spherical, but the shell of *Berthelinia* is laterally flattened.

Rather than substrate-matching, the sail-like laterally flattened shape of *B. singaporensis* may be advantageous for navigating between these bubble structures or other epibiota on *Caulerpa*. Their flattened body structure is perfect to “sail” and hide between ramuli of *C. lentillifera*, but, again, the gastropods can be found on a wide variety of *Caulerpa* spp. in the field (Table 1). Individuals hidden among the ramuli could, speculatively, have higher survivorship than others. However, this does not provide a strong or plausible adaptive hypothesis. This idea is undermined by the lack of an exclusive preference by *B. singaporensis* for *Caulerpa* with that type of structure, and that they do not stay within those camouflaging structures when they are available. Visual crypsis and issues of mobility do not seem to provide plausible mechanisms for any adaptive significance of the bivalved shell.

Berthelinia singaporensis attaches to the substratum by a thin mucous tether which is secreted by the foot. The distal end of the tether is extremely sticky, but the length of the string is not adhesive. The tether or string is very fine (only a few micrometres wide) and highly elastic; one adult individual with a shell length of 3 mm had a tether that we stretched to 124 mm before breaking. The veliger larvae are able to produce a tether at least as early as settlement and apparently before shedding the velum (i.e. when the larva is still in a coiled form, before the terminal metamorphosis, they can already tether themselves to the substratum). The attachment point of the tether remains adhesive after it is dislodged and after fixation and will stick to anything it contacts. For example, preserved samples of veliger larvae frequently become tethered to the internal wall of a glass pipette. However, this is not unique to *Berthelinia*, at larval or adult stages; it is a feature that Juliidae presumably inherited from an earlier sacoglossan ancestor.

Many other diverse sacoglossan gastropods also produce similar adhesive mucous tethers (e.g. Clark 1982; P. Krug, pers. comm.). Sacoglossans feed on green algae, which are restricted to shallow depths and prone to movement and disturbance from wind, waves, and tidal emersion. The tether is an effective means to avoid dislodgement in a turbulent environment. In the bivalved form, adults and juveniles use this tether to attach to the food plant while feeding or resting. The bivalved shell can close completely while the tether remains attached. We suggest that, inasmuch as the bivalved form may be interpreted as adaptive, the key innovation may be that this form allows the animal to fully retract within a protective shell while maintaining the attachment of the tether. The critical feature of a bivalve form in Juliidae is that a bivalved shell is optimal for attachment with a byssus.

Drivers for species diversity

Much of the earlier research on development in Juliidae was driven by the idea that these animals may represent a “missing link” or transitional form that could indicate the polarity of ancestral forms between gastropods and bivalves (Kawaguti 1959). After a further 60 years of study on molluscan evolution and phylogenetics, the interpretation of the importance of this clade is profoundly different (Sigwart and Lindberg 2014). Interestingly, the existence of bivalved forms of gastropods, a shell form derived independently in Bivalvia, was a key point of evidence used to first recognise the extinct molluscan group Rostroconchia as a separate taxonomic class, as they also have a univalved larval shell but a bivalved adult shell (Pojeta Jr et al. 1972). The bivalved gastropod form should not be dismissed as a unique oddity; rather, it is representative of broader trends within Mollusca and the rapid evolution of highly disparate morphologies (Sigwart 2017).

Sacoglossans are considered a “highly specialised” clade, in reference to the synapomorphies of their pharyngeal morphology and the green body colour, and some have very specific associations with their food alga (e.g. Baumgartner et al. 2009; Jensen 2015). The anatomy of sacoglossan gastropods is very different to other heterobranch gastropods (Jensen 2011), and molecular evidence also indicates Sacoglossa, and, indeed, Juliidae, represents a relatively recently derived and rapidly evolving branch (Schrödl 2014; Krug et al. 2015; Pabst and Kocot 2018). Juliidae may be a fast-evolving branch within that already divergent group, resulting in low signal quality in early molecular phylogenetic analyses (Händeler and Wägele 2007).

Members of Juliidae live in tropical regions all over the world and represent over 22 species with many more undescribed (McCarthy 2017). The genus *Berthelinia* s.l. comprises species in several potentially distinct genera (*Edentellina*, *Midorigai*, *Tamanovalva*), which are currently considered junior synonyms but often still used in identification (Grove and de Little 2014; Wägele and Martin 2014). There is, thus, some modest diversity in the family. Over the history of work on this clade, the largest number of species were found first in Australia and Japan, and the lack of records in other regions with suitable habitat certainly reflects lower sampling effort (Middelfart et al. 2016). At the two study sites examined here, *B. singaporensis* is the only shelled sacoglossan that is obligately feeding on and living on *Caulerpa*. Many species of micromolluscs, and the juveniles of larger species, occupy *Caulerpa* as a habitat and substrate (Sigwart and McLean 2017). The multiple species of *Caulerpa* in these study sites and the coral rubble environment create a complex three-dimensional habitat space that is expansive when considered at the scale of shelled sacoglossans. Although the global species richness of this family is not overwhelming, it is slightly counter-intuitive that after some

significant sampling effort in peninsular Malaysia, we still find only one species of Juliidae.

A large number of sacoglossan species remain undescribed, and ongoing work continues to reveal both new discoveries and cryptic species worldwide (e.g. Carmona et al. 2011; Krug et al. 2013; Espinoza et al. 2014; Krug et al. 2018). Jensen (1997) predicted that there might be a co-evolutionary or correlative relationship between sacoglossan diversity and the evolution of their food plants and that these relationships might have some predictive power about the number of species in genus- or family-level groups within Sacoglossa. Prior reports on a variety of sacoglossan taxa have noted both highly specialised feeding behaviours, other species that utilise a variety of food plants, and some confusion over the difference between algae used as substrata and not ingested (Baumgartner et al. 2009; Händeler and Wägele 2007). *Caulerpa* spp. contain phytotoxins, including caulerpenyne, in concentrations that differ among species (Meyer and Paul 1992). *Berthelinia singaporensis* feeds on multiple species, including those with relatively low (e.g. *C. lentillifera*) and high concentrations of secondary metabolites (*C. sertularoides*; Baumgartner et al. 2009). Some sacoglossan species have more specific associations with their food plant than others, which is concordant with the diversification of a rapidly evolving large clade.

Our results provide direct evidence that *B. singaporensis* will feed on at least five species of *Caulerpa*, while previous records of the food plants for any other *Berthelinia* sp. included one or at most two species of *Caulerpa* (Händeler and Wägele 2007). We note that no prior study with access to a high-quality, long-term captive population of bivalved gastropods has been exposed to the species diversity of *Caulerpa* found in peninsular Malaysia, which limits the ability and relevance to test the preferences of other Juliidae. The fact that they are adapted, apparently, to maintain a byssal attachment to their food plant may explain the broad species ranges, through distribution by rafting. The ability to switch between food plant species is no doubt advantageous in these environments with erratic availability of *Caulerpa*.

The species in tropical ecosystems tend to have specialised niches more frequently than in temperate ecosystems (Stevens 1989; Jablonski et al. 2013). Feeding on one genus of green algae may be relatively specialised, but in the locally relevant context in coastal Malaysia, this hyperdiverse region for shallow marine fauna and flora, the genus *Caulerpa* provides a relatively broad niche. As niche space becomes more crowded in increasingly species-rich habitats, the bounds of what is specialised become narrower and more specialised. Although the form of bivalved gastropods is unusual compared to other gastropods, *B. singaporensis* is functionally a relative generalist, and this may limit the tendency toward sympatric speciation.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval All applicable international, national, and/or institutional guidelines for the care and use of animals were followed by the authors.

Sampling and field studies All necessary permits for sampling and observational field studies have been obtained by the authors from the competent authorities and are mentioned in the acknowledgements, if applicable.

Data availability The datasets generated during and/or analysed during the current study are available from the corresponding author upon reasonable request.

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