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RESEARCH NOTE

Observations of reproduction in *Rhipidosiphon javensis* (Halimedineae, Bryopsidales) in Hawaii

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Recent field-based studies in the Bryopsidales have provided a wealth of reproductive information for several ecologically dominant genera, yet inconspicuous members of the order often remain poorly described. Here we report reproductive events for aquarium-grown populations of *Rhipidosiphon javensis* and provide statistical size ranges for vegetative and reproductive features for the first time. Hawaiian representatives of the genus develop gametangia over a three-day period, and follow the typical reproductive pattern found in other members of the suborder Halimedineae, being holocarpic and releasing gametes in predawn hours.

INTRODUCTION

Despite the recent success of studies documenting reproductive strategies in structurally large and ecologically dominant Bryopsidalean taxa (Clifton 1997; Hay 1997a; Clifton & Clifton 1999), the gametangia of almost half the 32 currently recognized genera in the Bryopsidales still remain undescribed at even the simplest level (Vroom *et al.* 1998). These poorly understood genera are often small, inconspicuous algae with low abundance in reef systems. In Hawaii and elsewhere in the Pacific, and in the Indian Ocean and the Caribbean, the genus *Rhipidosiphon* is rarely encountered in field studies (Doty 1969; Littler & Littler 1990) and, when observed, individuals are small and population numbers are extremely low (see 'Frondose Algae of Waikiki' at http://www.botany.hawaii.edu/reefalgae, and Doty 1969; 'Algal foray at Hanauma Bay' at http://128.171.207.10/botany/guide/field3a.htm, and Bernatowicz 1972). Additionally, reproductive individuals are seldom seen; they have never been observed in Hawaiian field collections during the past 50 years (I.A. Abbott, personal communication) and were only observed once by us during five years of culture studies. This study reports observations of gametangial structures in *Rhipidosiphon* from aquarium-grown Hawaiian populations.

Rhipidosiphon is a member of the tropically cosmopolitan suborder Halimedineae (Order Bryopsidales, see Hillis-Colinvaux 1984, Vroom *et al.* 1998). This suborder contains some of the most ecologically important calcified genera found in reef systems (e.g. Hillis-Colinvaux 1980; Fork & Larkum 1989; Meinesz & Hesse 1991; Purnachandra Rao *et al.* 1994; Sant *et al.* 1996; Garbary *et al.* 1997; Hay 1997b, c; Hillis 1997). Recent research (Clifton 1997; Clifton & Clifton 1999) has expanded knowledge of reproduction in the Halimedineae by revealing the types of gametangia found in several genera and has provided observations of gamete formation, gamete morphology, and the timing of gamete release. As a result, genera can be reevaluated and some past taxonomic confusion resolved.

Many traditional botanical classification systems use reproductive characters to group taxa (Linneaus 1753, Abbott 1999). Without knowledge of reproductive characters in the Halimedineae, researchers have been forced to rely on vegetative characters when proposing evolutionary affinities (Gepp & Gepp 1911; Silva 1982; Hillis-Colinvaux 1984). Recently, as more reproductive characters within the Halimedineae have been observed, it has become necessary to reassess the value of vegetative characters, which have evidently grouped taxa on the basis of similarities that are only superficial (Meinesz 1980a; Vroom *et al.* 1998).

Although reproductive structures have been observed before in *Rhipidosiphon javensis* Montagne (Meinesz 1980b; Littler & Littler 1990), previous observations were of only one or two individuals. Our study is the first to provide time course and statistical data from individuals of several reproductively mature populations. In addition, reproductive traits can be used to develop new hypotheses of evolutionary affinities within the Halimedineae.

MATERIAL AND METHODS

Rubble bearing visible blades of *R. javensis* or microscopic plants was collected from shaded outdoor, flowing seawater tables at the Pacific Biomedical Research Center at Kewalo Basin, Honolulu, Oahu, in December 1995; from Kahaluu and Puako, Hawaii, in January 1999; and from Paiko Lagoon, Oahu, in March 1999 (Fig. 1). Rubble was transported in seawater to the University of Hawaii at Manoa and kept in tanks with approximately four litres of recirculating seawater (gravity forced with turbulent mixing) at room temperature (20–

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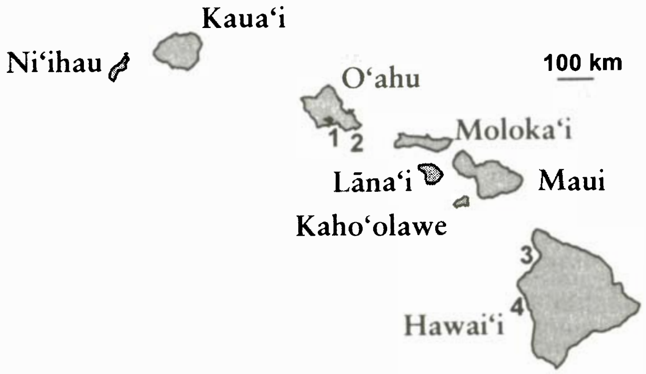

Fig. 1. Map of Hawaii showing collection sites for *Rhipidosiphon javensis* populations. (1) Kewalo Basin, Oahu; (2) Paiko Lagoon, Oahu; (3) Puako, Hawaii; and (4) Kahaluu, Hawaii.

25°C), with ambient north lighting. Individuals from different populations were maintained in separate aquaria that were monitored daily. Once reproductive events began, individual thalli were observed with an Olympus SZX12 dissecting microscope and photographed using an Olympus C-35AD-4 camera. After release, gametes were obtained by pipette from the water surrounding the adult thalli and observed using an Olympus BH-2 compound microscope and measured with an ocular micrometer. Flagella were not included in measurements. Analysis of variance (ANOVA) was used to test for differences between gametangia and gametes released from different thalli.

OSBERVATIONS AND DISCUSSION

R. javensis exhibits the simplest morphology of the 10 flabellate genera within the Halimedineae. Plants consist of monosiphonous stipes attached to monostromatic, fan-shaped blades. Siphons in the blade divide dichotomously and are clearly visible under low magnification (Fig. 2A). The calcified blades of Hawaiian populations may attain heights of 3 cm under calm water conditions, although mature individuals in this study had an average blade height (excluding stipe) of only 1.06 cm (n = 27, $S_{\bar{x}} \pm 0.06$).

Immature gametangia were first observed in all four populations (Fig. 1) at 1:45 PM on 28 June 1999, the first full moon after the summer solstice. On that day, 43.3% of the blades (n = 280) started to develop gametangia. By the next day, 56.8% of individuals were fertile. Fertile blades (excluding the stipe) were 5–21 mm long (n = 32, mean = 10.43, $S_{\bar{x}} \pm 0.55$). Extended tubular gametangia of *R. javensis* developed terminally on vegetative siphons over a three-day period (Fig. 3). Although vegetative siphons commonly exhibit constrictions above each dichotomy, little or no constriction was observed at the junction between the gametangia and the siphons that bear them. Gametangial growth was exponential during the first 10 hours of observation (Fig. 3). The pigmentation of immature gametangia was similar to that of the vegetative thallus (Fig. 2B). After the initial exponential phase, the rate of growth slowed (Fig. 3) and gametangia became densely pigmented as they filled with cytoplasm transported from the vegetative portions of the plant. Because of the calcium carbonate sheath surrounding each siphon, the majority

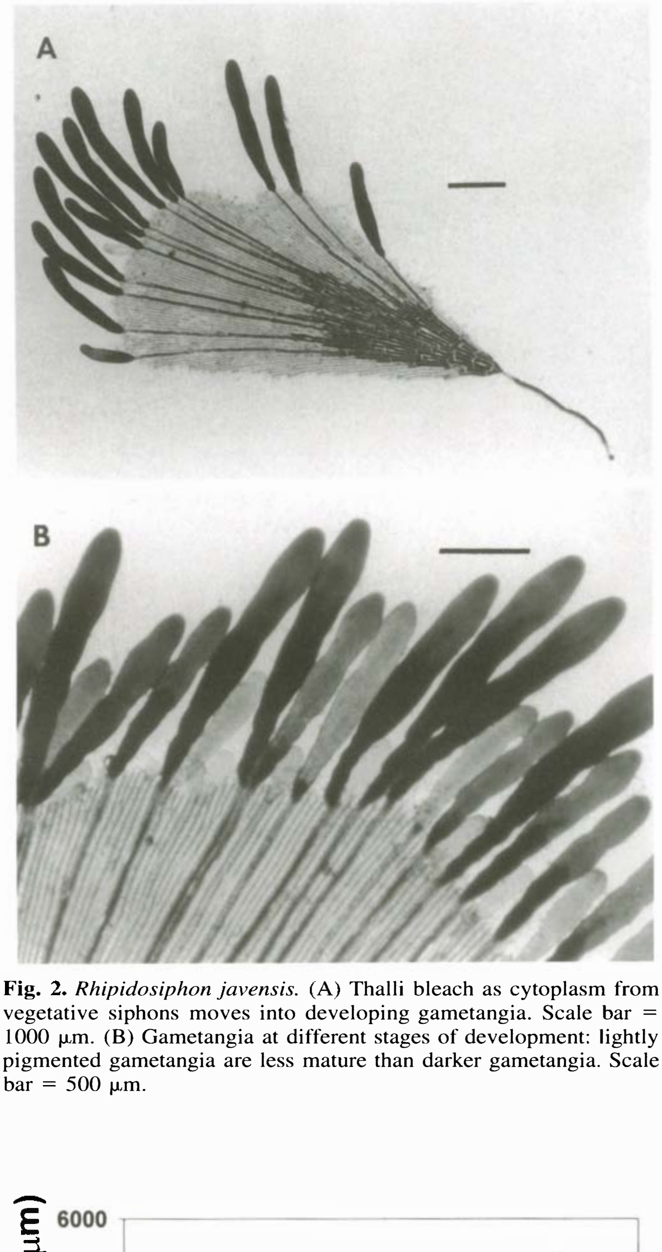

Fig. 2. *Rhipidosiphon javensis.* (A) Thalli bleach as cytoplasm from vegetative siphons moves into developing gametangia. Scale bar = 1000 μm. (B) Gametangia at different stages of development: lightly pigmented gametangia are less mature than darker gametangia. Scale bar = 500 μm.

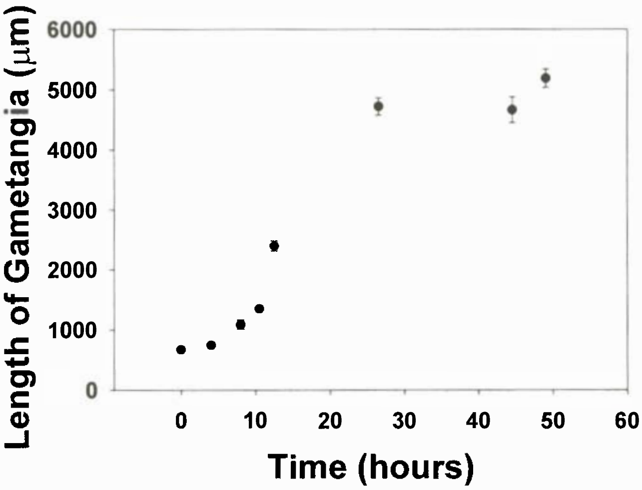

Fig. 3. Increase of gametangial length during their three-day period of development. Bars represent standard error. Time 0 indicates the first observations of already developing gametangia at 1:45 PM on 28 June 1999.

of the thallus turned white as the siphons emptied. However, the vegetative siphons on which gametangia formed remained darkly pigmented after the rest of the thallus had bleached (Fig. 2A). Mature gametangia averaged 5.19 mm ($S_{\bar{x}}$ ± 0.15, $n = 10$) in length and 0.36 mm ($S_{\bar{x}}$ ± 0.01) in width.

Gamete release from most thalli occurred during the hours before dawn on 1 July 1999, leaving behind dead, whitened thalli for this holocarpic genus. All reproductive individuals that did not release gametes on 1 July did so during predawn hours of 2 July. All gametes were biflagellate. ANOVA results suggest Hawaiian *R. javensis* may be anisogamous (Table 1) with dioecious thalli ($n = 80$, $F = 41.32$, $P < 0.001$), but fusion was not observed between putative male and female gametes. Unlike studies on other flabellate blades in the Bryopsidales (Clifton & Clifton 1999), no morphological or pigmentation differences between putative male and female thalli ($n = 159$) were observed with the *R. javensis* populations. Thus, because the observed gametes were relatively similar in size and no fusion was seen, it is also possible that all the gametes observed were of one sex and that the small (but significant) size differences between gametes are brought about through being produced by different adult thalli. Recent research on *Caulerpa taxifolia* (Vahl) C. Agardh (Žuljević & Antolić 2000) has shown that Mediterranean populations only produce male gametes, and it is possible that our *Rhipidosiphon* cultures were similar.

The genus *Rhipidosiphon* was first described by Montagne (1842) but was soon sunk by most authors into the morphologically similar genus *Udotea* Lamouroux (for instance, Gepp & Gepp 1911). In 1990, Littler & Littler re-established the genus because of the simple anatomy of *Rhipidosiphon* species, compared with the morphological complexity of *Udotea.* Because recent investigations of reproduction in the Halimedineae have found divergent patterns of reproduction among *Udotea* species (Clifton & Clifton 1999), and because phylogenies inferred from sequence data suggest that *Udotea* is paraphyletic (W.H.C.F. Kooistra, personal communication), the entire *Rhipidosiphon–Udotea* complex may need revision. Attention to gametangia and gametes in these genera seems warranted.

Past studies attempting to determine cues that trigger gametangia formation offer contradictory views on the importance of lunar cycles on reproductive strategies in the Halimedineae (Clifton 1997; Hay 1997a; Clifton & Clifton 1999). Because *R. javensis* was not observed to produce gametangia during other full moons, it is unknown whether moon phase, day length, a combination of the two, or other factors helped trigger reproductive events. Additionally, the flow-through nature of the circulating seawater system does not preclude the possibility of chemical signalling.

The biflagellate gametes and unmodified gametangia of *U. flabellum* (Table 1) suggest a more primitive state than the highly specialized female gametes and external gametangia found in other *Udotea* species (see Clifton & Clifton 1999). Because *R. javensis* exhibits biflagellate gametes produced from external gametangia, *Rhipidosiphon* may represent a transitional step between *U. flabellum* and the *U. abbottiorum, U. caribaea* and *U. cyathiformis* complex. The wide divergence of reproductive traits in these taxa makes it clear that taxonomic revision of the *Udotea–Rhipidosiphon* complex is

Table 1. Comparison of gametangial type and gamete size among selected species of *Rhipidosiphon* and *Udotea*[1]. Statistical analysis of *Rhipidosiphon javensis* gametes suggests anisogamy.

		Macrogametes		Microgametes	
	Type of gametangia	Length means ± $S_{\bar{x}}$ (range)	Width means ± $S_{\bar{x}}$ (range)	Length means ± $S_{\bar{x}}$ (range)	Width means ± $S_{\bar{x}}$ (range)
Rhipidosiphon javensis[2]	external, terminal	6.20 ± 0.08 (5.46–7.64)	3.50 ± 0.04 (2.96–4.06)	5.39 ± 0.08 (4.68–6.86)	3.28 ± 0.04 (2.81–3.90)
R. floridensis	?	?	?	?	?
Udotea flabellum	internal, unspecialized	10.4 ± 0.1 (9.8–12.8)	2.8 ± 0.1 (2.3–3.0)	6.6 ± 0.1 (6.0–6.8)	2.4 ± 0.1 (2.3–3.0)
U. abbottiorum	external, terminal	185.2 ± 1.2 (168.0–222.0)	76.6 ± 1.0 (60.0–102.0)	6.4 ± 0.1 (6.0–6.8)	2.5 ± 0.1 (2.3–3.0)
U. caribaea	external, terminal	245.9 ± 2.3 (180.0–288.0)	120.8 ± 1.2 (102.0–168.0)	6.3 ± 0.1 (5.3–6.8)	2.4 ± 0.1 (2.3–3.0)
U. cyathiformis	external, terminal	248.4 ± 1.7 (198.0–288.0)	125.5 ± 1.3 (102.0–156.0)	6.5 ± 0.1 (5.3–6.8)	2.6 ± 0.1 (2.3–3.0)

[1] From this study, Littler & Littler (1990), Clifton & Clifton (1999).

[2] Data based on 80 observations.

needed; future molecular studies, combined with traditional characters, may help resolve the present uncertainties.

Understanding the diversity in reproduction in a variety of species is essential in ancient groups such as the Bryopsidales (which are at least 500 million years old: see Knoll 1994). The simple morphologies of many taxa may well be adaptations to the environments in which they live, and convergent evolution of morphology may greatly underestimate the amount of genetic diversity present among members of such ancient lineages. The many flabellate morphologies found in the Halimedineae suggest this may be the ancestral state for the group, and differences in reproductive strategies and subtle life-history traits may be among the only morphological characters able to reveal lineages and relationships.

As relationships among flabellate genera become better understood, genera that exhibit unique modifications of the bladelike morphology may be pivotal in understanding evolution in the group. For instance, the calcified genus *Tydemania* expresses two distinct morphologies: an upright, segmented thallus and a flabellate blade. If reproductive structures are discovered to occur on bladelike portions of the thallus, their position (either terminal as seen in *Rhipidosiphon,* or else internal as seen in *U. flabellum*) may offer insight into the lineage from which segmented, calcified genera such as *Halimeda* eventually arose.

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