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The *Caloglossa leprieurii* complex (Delesseriaceae, Rhodophyta) in the Americas: the elucidation of overlooked species based on molecular and morphological evidence

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Abstract – The red algal genus *Caloglossa* (Delesseriaceae, Ceramiales) in the New World is represented by *Caloglossa leprieurii sensu lato*. The earliest name for any species in the genus, other than the basionym *Delesseria leprieurii*, is *Apiarium apiculum*. On the basis of comparative morphological data, and *rbc*L and LSU rDNA sequence analyses, the *C. leprieurii* complex in the New World is found to include four species: *C. leprieurii*, *C. apomeiotica*, *C. apicula* comb. nov., and *C. ruetzleri* sp. nov. The four species can be distinguished by subtle vegetative characters, including rhizoid morphology, the degree of constriction at the thallus nodes, presence or absence of adventitious branches, number of cell rows cut off from the first axial cell of the main axis, and the position of the cystocarp on the blades. A dichotomous key highlights the diagnostic vegetative characters to separate species of the *C. leprieurii* complex that occur in the Americas. *Caloglossa leprieurii* is a tropical species from the Caribbean Sea, the Indian Ocean and the tropical eastern Pacific Ocean. *Caloglossa ruetzleri* is apparently restricted to the Caribbean Sea and the Gulf of Mexico, *C. apomeiotica* to the eastern Pacific and southern Atlantic Oceans, and C. apicula the Western Atlantic Ocean, Gulf of Mexico and Indian Ocean.

Atlantic Ocean / *Caloglossa* / Ceramiales / Delesseriaceae / Gulf of Mexico / LSU rDNA / *rbc*L / Rhodophyta / systematics

Résumé – Le complexe *Caloglossa leprieurii* (Delesseriaceae, Rhodophyta) dans les Amériques : élucidation des espèces incluses sur la base de critères morphologiques et moléculaires. Le genre d'algues rouges *Caloglossa* (Delesseriacées, Ceramiales) dans le nouveau monde est représenté *sensu lato* par le *C. leprieurii*. Le premier nom d'une espèce d'écrite dans le genre est l'*Apiarium apiculum*. À partir de l'analyse morphologique comparative et du séquençage des genes *rbc*L et LSU rADN, le complexe *C. leprieurii*

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s'avère inclure quatre espèces : *C. leprieurii*, *C. apomeiotica*, *C. apicula* comb. nov., et *C. ruetzleri* sp. nov. Les quatre espèces peuvent être distinguées par des caractères végétatifs subtils, y compris la morphologie du rhizoïde, le degré de la constriction nodale, la présence ou absence des branches adventices, le nombre de rangées de cellules venant de la première cellule axiale de l'axe principal, et la position des cystocarpes sur le thalle. Une clef dichotomique précise les caractères diagnostiques végétatifs pour séparer les espèces du complexe *C. leprieurii* en Amérique. *C. leprieurii* est une espèce tropicale de la mer des Caraïbes, de l'Océan Indien et de l'Océan Pacifique Est tropical ; *C. ruetzleri* est apparemment limité à la mer des Caraïbes et au Golfe du Mexique ; *C. apomeiotica* au Pacifique Est et l'Océan Atlantique Sud ; et *C. apicula* à l'Océan Atlantique Ouest, le Golfe du Mexique et l'Océan Indien.

***Caloglossa* / Ceramiales / Delesseriaceés / Golfe du Mexique / LSU rADN / Océan Atlantique / *rbc*L / Rhodophyta / systématique**

INTRODUCTION

The red algal genus *Caloglossa* (Harvey) G. Martens (1869) was based on *Delesseria leprieurii* Montagne (1840) from Sinnamary, northwest of Cayenne, northern French Guiana (Lipkin & Silva, 2002), with the generitype *Caloglossa leprieurii* (Montagne) G. Martens placed in the Delesseriaceae Bory de Saint-Vincent (1828) of the Ceramiales Oltmanns (1904). Illustrations of *C. leprieurii* show a flat blade-like thallus (Montagne, 1840, pl. 5: Fig. 1), up to 2.5 cm in length, monostromatic on either side of a polystromatic midrib, and with pseudodichotomous or exogenous branching (e.g., Nägeli, 1855 as *Hypoglossum leprieurii*; Kylin, 1923; Papenfuss, 1961).

Caloglossa leprieurii sensu lato has been considered to be a widespread pantropical to temperate species, having been reported from the coastline of eastern North America as far as 41 degrees N and the coastline of New Zealand as far as 41 degrees S. It inhabits a narrow depth range of high intertidal to very shallow subtidal habitats and has wide salinity tolerance of brackish to freshwater, and is recorded in coastal mangroves, salt marshes, lagoons, and riverine habitats, up to 150 miles inland (King & Puttock, 1994; Kamiya, 2004; Sheath, 2003).

Caloglossa leprieurii has been regarded as highly polymorphic (King & Puttock, 1994; Kamiya *et al.*, 1998; Kamiya, 2004). Post (1936), for example, recognized *C. leprieurii* var. *leprieurii* with five forms and *C. leprieurii* var. *hookeri* (Harvey) Post. King & Puttock (1994) elevated two of these forms to species level, namely, *C. continua* (Okamura) R. J. King et Puttock and *C. triclada* (Post) R. J. King *et* Puttock, and suggested that the remaining variation in *C. leprieurii* required further study. Kamiya *et al.* (1995, 1998) undertook an investigation of the *C. leprieurii* complex and recognized three morphotypes within this complex throughout the world, each based on the number of cell rows produced from the first axial cell of the main axis and blade width (Kamiya *et al.*, 1995, table 3). One of the three morphotypes from the western Pacific, New Zealand and Australia differed from the others based on molecular phylogenetic analysis, and Kamiya *et al.* (2003) recognized it as a distinct species, *C. vieillardii* (Kützing) Setchell, which had been considered to be a synonym of *C. leprieurii* var. *hookeri* by Post (1936). The evolutionary distinction of the other two morphotypes, however, remained uncertain (Kamiya, 2004; Kamiya *et al.*, 2004).

West *et al.* (1994) described a new species, *Caloglossa apomeiotica* J.A. West et Zuccarello, previously identified as *C. "leprieurii"* from Pacific México, based primarily on its asexual mode of reproduction by apomictic tetrasporangia. Kamiya *et al.* (2003) considered *C. apomeiotica* a synonym of *C. leprieurii sensu stricto* since they could not otherwise distinguish these species based on their molecular or morphological data.

The purpose of this study was to re-evaluate and characterize the specimens of the *Caloglossa leprieurii* complex in the northern Gulf of Mexico (Cox, 1901; Taylor, 1928, 1960; Humm & Caylor, 1957; Kapraun, 1974; Yarish & Edwards, 1982; Dawes & Mathieson, 2008) and other specimens from the Americas that had been previously considered conspecific with *C. leprieurii.* This investigation used both *rbc*L and LSU rDNA sequence analysis and morphological characters to elucidate the diversity of species that have previously been called *C. leprieurii* in the Americas.

MATERIALS AND METHODS

Molecular data. Silica gel-dried, alcohol-preserved specimens and extracted DNA samples are deposited at the University of Louisiana at Lafayette (LAF). Protocols for DNA extraction followed the procedure in Gavio & Fredericq (2002). Gene amplification and sequencing of the *rbc*L and the LSU rDNA followed Gavio & Fredericq (2002) and Lin *et al.* (2001), respectively. Primers used for gene amplification and sequencing of the *rbc*L are listed in Freshwater & Rueness (1994) and additional primers used are presented in Gavio & Fredericq (2002) and Lin *et al.* (2001). Gene amplification and sequencing of the middle segment of LSU was used the primers presented in Freshwater *et al.* (1999). PCR and cycle sequence reaction procedures followed Gavio & Fredericq (2002).

Alignment and partition homogeneity test. The sequenced samples analyzed are listed in Table 1 and deposited in GenBank. Two data sets were produced. The *rbc*L and LSU rDNA data sets was compiled with Sequencher 4.1 (Gene Codes Corp., Ann Arbor, MI, USA), then imported into MacClade v4.0 (Maddison & Maddison, 2000) and PAUP* 4.0b10 (Swofford, 2003) for alignment. The LSU sequence data was first aligned using ClustalX 1.8 (Thompson *et al.*, 1997) before it was imported into MacClade v4.0 and PAUP* for added manual alignment. For the combined *rbc*L-LSU data set a Partition (= Homogeneity) test [the incongruence length difference (ILD) test of Farris *et al.* (1994)], as implemented in PAUP*, was conducted to determine if the two genes could be combined with 1000 addition sequence replicates. The concatenated dataset had a P value = 0.359 showing that they can be combined.

Phylogenetic Analysis. Phylogenetic analyses were conducted using Maximum Parsimony (MP) and Maximum Likelihood (ML) algorithms as implemented in PAUP* and PHYML (Guindon & Gascuel, 2003), respectively. Bayesian inference was performed in MrBayes 3.0 (MB) (Huelsenbeck & Ronquist, 2001). *Apoglossum ruscifolium* (Turner) J. Agardh was used as the outgroup for the *rbc*L data set based on phylogenetic results indicating that it is closely related to *Caloglossa* (Lin *et al.*, 2001). The combined *rbc*L-LSU sequence data used *Centroceras gasparrinii* (Meneghini) Kützing, a member of the Ceramiaceae, a family allied to the Delesseriaceae (Lin *et al.*, 2001)] as the outgroup.

Parsimony trees obtained under the Fitch criterion of equal weights for all substitutions (Fitch, 1971) were inferred from a heuristic search, excluding uninformative characters, with 1000 random sequence additions holding 10 trees at each step, and the tree-bisection-reconnection (TBR) swapping algorithm. Support for nodes in the MP analysis were assessed by calculating bootstrap proportion (BP) values (Felsenstein, 1985) as implemented in PAUP* by generating 1000 bootstrap data sets, from resampled data, with 1000 random sequence additions.

Optimal models of sequence evolution to fit the data alignment estimated by hierarchical likelihood ratio tests were performed by Modeltest v.3.6 (Posada & Crandall, 1998). The model of sequence evolution chosen for both data files was the GTR+I+G (General Time Reversible model with variable base frequencies, symmetrical substitution matrix). A ML phylogenetic tree was generated for each of the two data sets, using the substitution model, gamma distribution and proportion of invariable sites determined by the model. For each data file, the ML tree and ML bootstrap values (generated from 1,000 bootstrap trees) were inferred by PHYML 3.0, using the Nearest Neighbor Interchange (NNI) branch swapping method.

For the Bayesian analysis the optimal model of sequence evolution obtained for each data set was used to set up the Markov Chain Monte Carlo (MCMC) search for the Bayesian analyses. Four chains of the (MCMC) were run, sampling one tree every 100 generations for 2 X 10^6 generations starting with a random tree for each of the two data files. Phylogenetic inferences were based on the trees sampled after the "burn in" point. A 50% majority rule consensus, as implemented by PAUP*, was computed from those saved trees. This frequency corresponds to the posterior probability of the clades.

Morphological studies. Herbarium specimens and live collections were used in this study (Table 1). For light microscope observations specimens were stained with 1% aqueous aniline blue acidified with 0.1% diluted HCl, and mounted in 25-40% Karo© syrup (Englewood Cliffs, NJ, USA). Voucher specimens were liquid-preserved in 5% buffered Formalin/seawater, and/or pressed and air-dried on herbarium sheets, and deposited in the herbarium of the University of Louisiana at Lafayette (LAF), and the Algal Collection of the U.S. National Herbarium (US).

RESULTS

Molecular analyses

The *rbc*L and LSU sequences included in this study represent a wide sampling of the *Caloglossa leprieurii* species complex, with a strong emphasis on the Americas. The *rbc*L dataset consisted of 41 samples (1467 bp, 286 parsimony informative sites including the outgroup, *Apoglossum ruscifolium*), MP generated eight trees (646 steps) and the topology of the MP trees did not differ significantly from that of the ML and MB trees. The combined *rbc*L-LSU dataset utilized 27 samples (2484 bp with 316 parsimony informative sites), and MP generated one tree (805 steps). The MP tree topology did not differ significantly from those of the ML and MB trees and only the ML trees are presented (Figs 1-2).

Table 1. Source of species used in this study of the *Caloglossa leprieurii*-complex.

Species	*coll. id.*	*Strain num.*	*Location*	*Date*	*Collector*	*GenBank accession num.*
C. apicula comb. nov.	662	–	Galveston, TX, USA	2 ii 2003	D. Krayesky	–
C. apicula	K1	–	Galveston, TX, USA	20 iii 2003	Tae Oh Cho	*rbc*L: HM775440 LSU: HM775485
C. apicula	K2	–	Marsh Is., LA, USA 1st collection	16 iv 1970	D. Kapraun	–
C. apicula	K3	–	Marsh Is., LA, USA 2nd collection	16 iv 1970	D. Kapraun	–
C. apicula	K8	–	Muscle Shoals, (NW) AL, USA	2 vi 2003	P.G. Davison	*rbc*L: HM775441 LSU: HM775484
C. apicula	K13	–	Port Fourchon, LA, USA	27 vi 2003	D. Krayesky	*rbc*L: HM775443 LSU: HM775490
C. apicula	K27	WEST 3174	Stamford, CT, USA	13 viii 1991	J.A. West	*rbc*L: HM775444 LSU: HM775482
C. apicula	K29	WEST 2290	Mullica River Estuary, NJ, USA	17 vii 1973	C. Yarish	*rbc*L: HM775445 LSU: HM775486
C. apicula	K43	–	Marsh Is., LA, USA	17 ix 2003	D. Krayesky	*rbc*L: HM775449
C. apicula	K49	–	Pass Christian, MS, USA	1 x 2003	D. Krayesky	*rbc*L: HM775438 LSU: HM775488
C. apicula	K62	–	James I., SC, USA	17 vi 1994	J.A. West	*rbc*L: HM775447
C. apicula	–	WEST 3407	James I., SC, USA	17 vi 1994	J.A. West	*rbc*L: AY150319 LSU: AF522223
C. apicula	K99	–	Poguosom, VA, USA	10 viii 2004	W. Schmidt	–
C. apicula	K118	–	Mobile, AL, USA	3 iv 2005	B.Y. Won	*rbc*L: HM775450
C. apicula	K119	–	Mobile, AL, USA	30 iii 2005	B.Y. Won	*rbc*L: HM775439 LSU: HM775483
C. apicula	K141	–	Petit Bois Is., MS, USA	12 vii 2004	J. López-Bautista	*rbc*L: HM775448
C. apicula	K142	–	Amrat, nr. Ocean Springs, MS, USA	12 vii 2004	J. López-Bautista	–
C. apicula	K262	–	nr. Ocean Springs, MS, USA	24 v 2009	D. Krayesky	–
C. apicula	K168	–	New Iberia, LA, USA	25 iv 2006	S. Fredericq	rbcL: HM775437 LSU: HM775487
C. apicula	K171	–	Point Washington, FL, USA	1 v 2006	Tae Oh Cho	*rbc*L: HM775442 LSU: HM775489
C. apicula	K221	–	Cobhams Wharf, VA, USA	?	F. Ott	*rbc*L: HM775446 LSU: HM775481
C. apicula	(US00296143)	–	Fort Lee, NJ, USA	s.d.	H. Averill	–
C. apicula	(US00296172)	–	St. Augustine, FL, USA	v 1879	G.A. Hall	–
C. apicula	(US00091713)	–	Jersey City, NJ, USA	xii 1850?	C.F. Durant	–
C. apicula	(MICH *Durant* 27)	–	Jersey City, NJ, USA	xii 1850?	C.F. Durant	–

Table 1. Source of species used in this study of the *Caloglossa leprieurii*-complex. *(continued)*

Species	*coll. id.*	*Strain num.*	*Location*	*Date*	*Collector*	*GenBank accession num.*
C. apomeiotica J.A. West *et* Zuccarello	K64	–	Isla Perico, Balboa, Panama	4 iv 1999	B. Wysor	*rbc*L: HM775458
C. apomeiotica	–	–	I. Espiritu Santo, B.C.S., Mexico	19 iii 1992	J.A. West	LSU: AF522201
C. apomeiotica	K81	WEST 3033	Bahia Balandra, B.C.S. Mexico	6 i 1990	J.A. West	*rbc*L: HM775456 LSU: HM775476
C. apomeiotica	K73	–	Mangaratiba city, Brazil	15 xi 2003	F. Gurgel	*rbc*L: HM775459
C. apomeiotica	–	–	Isle of Palms, SC, USA	4 x 1995	J.A. West	LSU: AF522203
C. apomeiotica	K54	–	El Manchon, Guatemala	23 iii 1993	J.A. West	*rbc*L: HM775457 LSU: HM775475
C. apomeiotica	US-00266161	–	Bahia Jiquilisco, nr. Salinas de Mapachin and Isla Madresal, El Salvador	9 ix 1960	E.Y. Dawson	–
C. apomeiotica	US-00296163	–	Gulfo de Fonseca, nr. La Union, El Salvador	6 ix 1960	E.Y. Dawson	–
C. intermedia Kamiya *et* J.A. West	K16	–	James Is., SC, USA	10 vii 2003	D.R. Wiseman	*rbc*L: HM775468
C. leprieurii (Mont.) G. Martens	K26	WEST 3147	Isla Margarita, Venezuela	13 iv 1991	J.A. West	*rbc*L: HM775463
C. leprieurii	K34	WEST 3201	Durban, KwaZulu-Natal, S. Africa	4 x 1991	A. Critchley	*rbc*L: HM775465
C. leprieurii	K47	WEST 4289	Puthen I., Kerala, India	17 ii 2003	J.A. West	*rbc*L: HM775467
C. leprieurii	K48	WEST 4198	British Guyana	30 i 2002	M. Kuertzen	*rbc*L: HM775464 LSU: HM775473
C. leprieurii	K50	WEST 3387	Isla Magueyes, La Parguera, Puerto Rico	1 v 1993	J.A. West	*rbc*L: HM775461 LSU: EU349106
C. leprieurii	–		Isla Magueyes, La Parguera, Puerto Rico	6 iii 1994	J.A. West	LSU: AF522221
C. leprieurii	K53	WEST 4304	Ifaty, Madagascar	21 iv 2003	J.A. West	*rbc*L: HM775466
C. leprieurii	K61	–	Basse Terre, Guadeloupe, F.W.I	16 i 2003	A. Renoux	–
C. leprieurii	K143	–	KwaZulu-Natal, South Africa	24 viii 2005	C. Boédeker	*rbc*L: HM775460 LSU: HM775474
C. leprieurii	K144	–	nr. Cayenne, French Guyana	19 vii 2005	J. López-Bautista	*rbc*L: HM775462
C. leprieurii	(L0055717)	–	Sinnamary, NW of Cayenne, Fr. Guiana	?	Leprieur	–

Table 1. Source of species used in this study of the *Caloglossa leprieurii*-complex. *(continued)*

Species	*coll. id.*	*Strain num.*	*Location*	*Date*	*Collector*	*GenBank accession num.*
C. leprieurii	(US00296151)	–	St. George's Island, Bermuda	24 ii 1949	W.R. Taylor & A.J. Brnatowicz	–
C. leprieurii	(US00296165)	–	Paramaribo, British Guyana	1951	D.C. Guijskes	–
C. leprieurii	(US00296156)	–	Bahia Salinas, Costa Rica	4 ii 1959	E.Y. Dawson	–
C. monosticha Kayima	K130	MK 892	Derby, Australia	2 x 1991	M. Kamiya	*rbc*L: HM775469 LSU: HM775469
C. ogasawaraensis Okamura	K38	WEST 3969	Nusa Lembongan, Bali, Indonesia	25 iv 1999	J.A. West	*rbc*L: HM775470 LSU: HM775471
C. ogasawaraensis	–	GZ61	Georges Hall, NSW, Australia	5 iv 1997	G. Zuccarello	*rbc*L: AY150325
C. ruetzlerii sp. nov.	K146	–	Cudjoe Key, FL USA	23 xi 2005	Tae Oh Cho	*rbc*L: HM775452 LSU: HM775478
C. ruetzlerii	K169	–	Twin Cays, Belize	18 v 2006	S. Fredericq	*rbc*L: HM775453 LSU: HM775479
C. ruetzlerii	K170	–	Twin Cays, Belize	12 v 2006	S. Fredericq	*rbc*L: HM775454 LSU: HM775480
C. ruetzlerii	K74	–	Summerland Key, FL, USA	4 ii 2004	Tae Oh Cho	*rbc*L: HM775451 LSU: HM775477
C. ruetzlerii	–	–	Isla Morada, FL, USA	15 vi 1994	J.A. West	*rbc*L: AY150316 LSU: AF522225
C. ruetzlerii	K240	–	Isla Solarte, Bocas del Toro, Panama	17 i 2007	B. Wysor & D.W. Freshwater	*rbc*L: HM775455
Caloglossa sp. 1	–	–	Bali, Indonesia	13 iv 1999	M. Kawachi	*rbc*L: AY150322 LSU: AF522226
Caloglossa sp. 2	–	–	Shimajiri, Miyako Is., Japan	31 v 1991	M. Kamiya	*rbc*L: AY150323 LSU: AF522227
C. vieillardii (Kütz.) Setchell	–		Garden Island, Adelaide Australia	22 i 1993	J.A. West	*rbc*L: AY150329 LSU: AF522205
Hypoglossum pygmaeum G. Martens	(L0055708)		Calcutta, India	?	?	–
Apoglossum ruscifolium (Turner) J. Agardh	–	–	France	2000	?	*rbc*L: AF312310
Centroceras gasparrinii (Meneghini) Kütz.	TC407	–	Baja, Mexico	27 ii 1998	M. Hommersand	*rbc*L: DQ374317 LSU: DQ374358

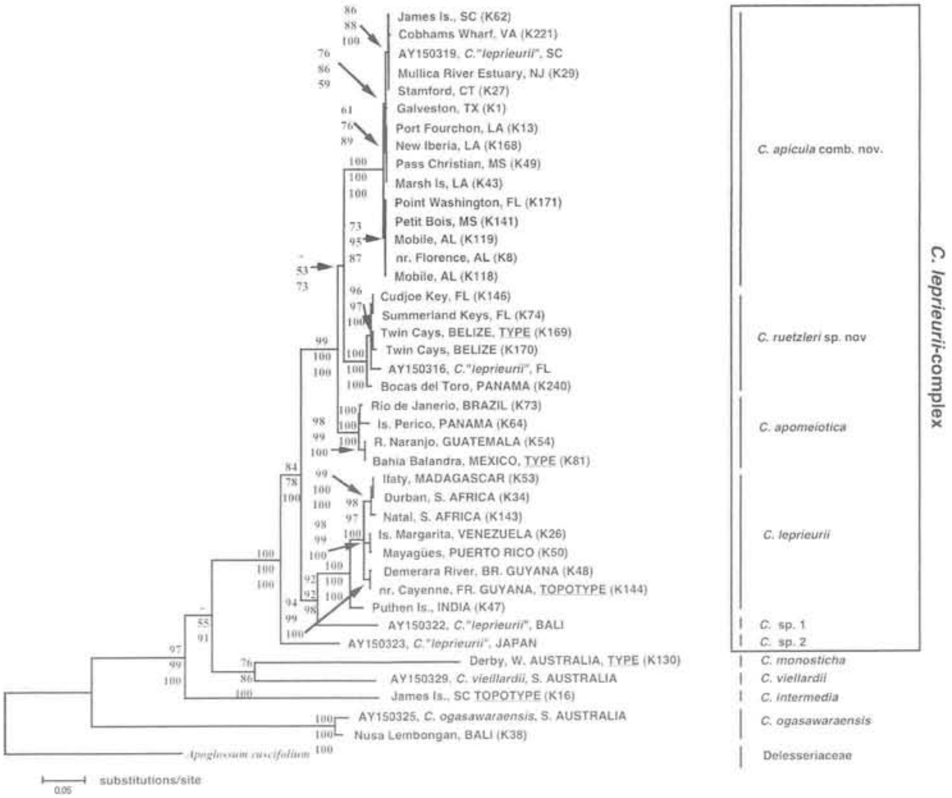

Fig. 1. Maximum-likelihood tree of *rbc*L sequence data (-ln L 5868.545136) showing relationship of *Caloglossa leprieurii* complex and other *Caloglossa* species. Three tiers of numbers at each node; top numbers are MP bootstrap values, middle numbers are ML bootstrap values and bottom numbers are MB posterior probabilities (in %).

Specimens identified as *Caloglossa leprieurii* in the past form a well-supported monophyletic group, which includes six clades (= species) (Figs 1-2). Within the *C. leprieurii* complex each clade demonstrates an interclade *rbc*L sequence divergence ranging from 2.7%-6.2% and an intraclade *rbc*L sequence divergence range between 0%-1.5%. When other clades representing *Caloglossa* species are included (e.g. *C. intermedia* Kamiya et J. A. West and *C. ogasawaraensis* Okamura), the sequence divergence amongst species varies from 2.7-14.1%. *Caloglossa* sp. 1 and *Caloglossa* sp. 2 represent two taxa from the Indian and northwestern Pacific Ocean (Figs 1-2), respectively, that are sister lineages to all other clades whose taxa were identified before this study as *C. leprieurii*.

Morphological analysis

Previous investigations have explained the morphological differences seen in the thallus of *Caloglossa leprieurii* as phenotypic plasticity in a polymorphic species. When looking at the morphology of individuals within what has been previously identified as *C. leprieurii,* subtle but distinct differences can be seen in terms of branching pattern, number of cell rows derived from the FMA, rhizoid

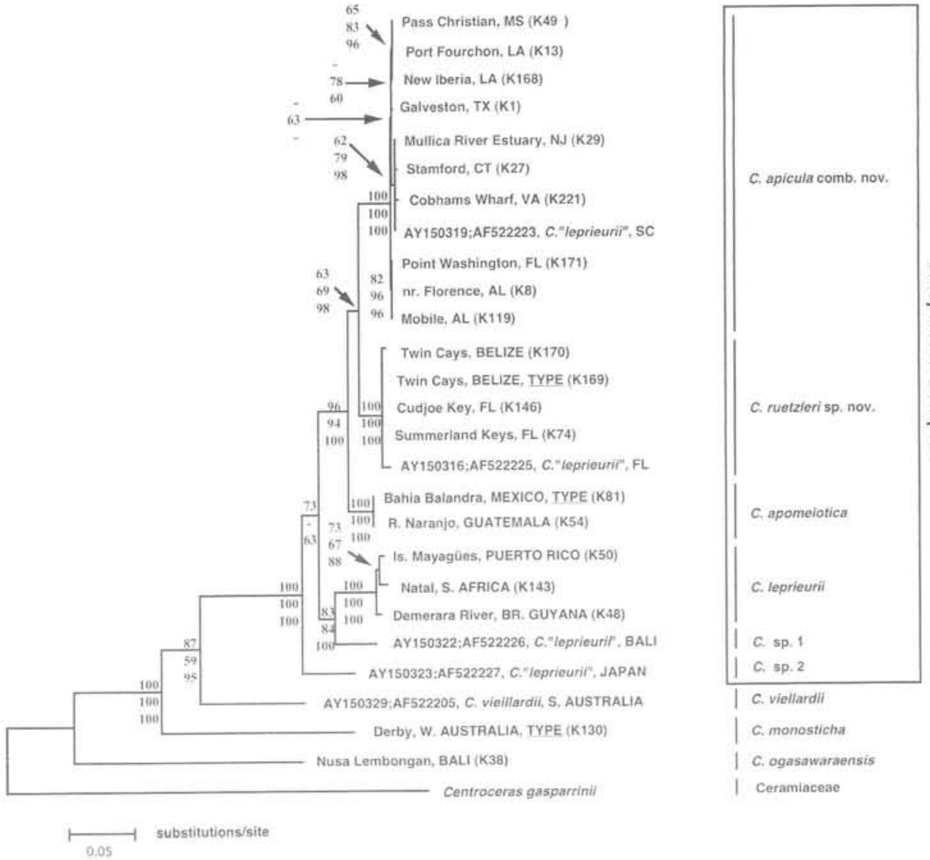

Fig. 2. Maximum-likelihood tree of *rbc*L-LSU sequence data (-ln L= 8602.800829) showing relationship of *Caloglossa leprieurii* complex and other *Caloglossa* species. Three tiers of numbers at each node; top numbers are MP bootstrap values, middle numbers are ML bootstrap values and bottom numbers are MB posterior probabilities (in %).

morphology and blade width and length (Table 2). Molecular data (Figs 1-2) corroborate the morphological differences that specimens identified as *C. leprieurii* in the Americas actually encompass four distinct species, three of which differ from *C. leprieurii* and thus are in need of taxonomic clarification. A dichotomous key is here provided to highlight diagnostic features among taxa previously going under the name *C. leprieurii*; the authors of each species provided in the key is shown in the Taxonomic Treatment following the key.

Key to the species of the *Caloglossa leprieurii* complex in the Americas

1a. Basal rhizoidal cells loosely arranged, not forming a cortical pad (Fig. 15) .... *C. apicula*

1b. Basal rhizoidal cells tightly adherent, forming a cortical pad (Fig. 7) 2

  2a. Only one cell row derived from the first axial cell of the main axis opposite the lateral branch; thallus highly constricted at nodes over long interval, with internodes forming elliptical "blades" (Figs 34, 35) ...... *C. ruetzleri*

2b. Usually more than one cell row derived from the first axial cell of the main axis opposite the lateral branch; thallus not highly constricted at nodes, strap-like throughout (Figs 3, 4) 3

3a. Number of cell rows derived from the first axial cell of the main axis opposite the lateral branch (1-) 2-5; thallus width at median internode region 0.9-2.5 (-4) mm . . . . *C. apomeiotica*

3b. Number of cell rows derived from the first axial cell of the main axis opposite the lateral branch (2-) 3-7; thallus width at median internode region 0.5-1.4 mm . . . . *C. leprieurii*

Table 2. Morphological vegetative characters of species of the *Caloglossa leprieurii*-complex in the Americas. The definition of rhizoid distribution, FLA, NA, FMA follows Kamiya *et al.* (2003).

	C. apicula comb. nov.	*C. apomeiotica*	*C. leprieurii*	*C. ruetzleri* sp. nov.
Endogenous branching	Present	Present	Present	Present
Adventitious branching	Present-occasionally	Absent	Absent	Absent
Number of rhizoids per cell	Multiple	Multiple	Multiple	Multiple
Rhizoid distribution	Type F	Type F	Type F	Type F
Rhizoids tightly adhering at base (corticated pad)	Absent	Present	Present	Present
Adaxial cell row derived from the FLA[1]	Absent	Absent	Absent	Absent
Number of cell rows derived from the NA[2]	1-2	1-2	1-2	1
Number of cell rows derived from the FMA[3]	1-3	(1-)2-5	(2-)3-7	1
Constriction at node	Slightly	Slightly	Slightly	Strongly (long interval)
Middle blade width (mm)	0.1-1.0	0.9-2.5(-4)	0.5-1.4	1.1-2.0
Blade length (mm)	1.0-3.0	1.5-4.0	1.5-4.0	3.0-6.0
Thallus length (cm)	0.3-1.2	0.3-1.7	0.8-2.0	0.5-1.0

1. First axial cell from lateral axis.
2. Nodal axial cell opposite lateral branch.
3. First axial cell at main axis opposite lateral branch.

Silva (2004) called attention to the fact that the widely accepted generic name *Caloglossa* was predated by the validly published name *Apiarium* Durant (1850). Described as a new genus and a new species, *A. apicula* from "Jersey City" (New York Bay) by Durant (1850: 18), *Apiarium* was obviously unknown to Martens (1869) when he validated the generic name *Caloglossa*. Today the two names are considered congeneric, *Apiarum* Durant having priority. On the primary basis that *Caloglossa* has been widely used for more than 150 years, Silva (2004) proposed it for conservation over *Apiarum*, a proposal that has been subsequenty accepted (McNeill *et al.*, 2006). Silva (2004) further stated that "The alga named *Apiarum apicula* by Durant is unequivocally representative of *Caloglossa* *leprieurii* (or at least of *Caloglossa*)." Our morphological observations based on the isolectotypes of *Apiarum apicula* (US Alg. Coll 0917213; US Alg. Coll. 31224; and MICH, Fig. 18) show that it is a species of *Caloglossa* distinguishable from *C. leprieurii* primarily by its basal rhizoids cells that are not tightly adherent to one another. Furthermore, the isolectotypes of *Apiarum apicula* are morphologically identical to our collections of *Caloglossa* from localities that collectively form a molecular lineage distinct from that of *C. leprieurii*. Therefore, we name this lineage *C. apicula*.

Taxonomic Treatment

Caloglossa leprieurii (Montagne) G. Martens, 1869: 234, 237. **(Figs 3-12)**

Basionym: *Delesseria leprieurii* Montagne 1840: 196-197, pl. 5: fig. 1.

Synonymy: *Hypoglossum leprieurii* (Montagne) Kützing 1849: 875.

Lectotype: *Lectotype* selected by Kamiya *et al.* (2003): Sinnamaria [=Sinnamary], NW of Cayenne, northern French Guiana; #L-0055717. However, if following Recommendation 9A4 of the ICBN one could consider the lectotype to be PC. Herb. Thuret-Bornet TA 22509/22510 (selected by Silva, 2004, p. 556). Silva (2004) did not comment on or reject the isotype selection of #L-0055717 by Kamiya *et al.* (2003), when designating his chosen lectotype (PC); therefore, we follow the selection of Kamiya *et al.* (2003), as it was validly designated.

Distribution: *Western Atlantic Ocean*: Bermuda to Venezuela; probably more widespread in tropical waters of the Western Atlantic Ocean. *Eastern Pacific Ocean*: Costa Rica. *Indian Ocean*: India, Madagascar, and South Africa.

Habitat: Epiphytic on mangrove pneumatophores and prop roots; intertidal to shallow subtidal.

Specimens studied: *Western Atlantic Ocean:* Bermuda: St. George's Island (*W. R. Taylor & A. J. Bernatowicz*, 24 ii 1949, US Alg. Coll. 00296151). Guadeloupe, French West Indies: Grande-Terre (A. Renoux, s.n., 16 i 2003). Puerto Rico: Isla Magüeyes, La Parguera (*J. A. West*, 1 v 1993). Venezuela: Isla Margarita (*J. A. West*, 13 iv 1991). Guyana: West Branch, Demerara River (*M. Kuertzen*, 30 i 2002); and, Paramaribo (*D. C. Guijskes*, 1951, US Alg. Coll. 00296165). French Guiana: Sinnamary, NW of Cayenne (*Leprieur*, s.d., L 0055717); and, vic. Cayenne (*J. López-Bautista*, topotype, 19 vii 2005). *Eastern Pacific Ocean*: Costa Rica: Bahía Salinas (*E. Y. Dawson*, 4 ii 1959, US Alg. Coll. 296156). *Indian Ocean:* South Africa: Durban, KwaZulu-Natal (*A. Critchley*, 4 x 1991); and KwaZulu-Natal (*C. Boédeker*, 24 viii 2005). Madagascar: Ifaty (*J. A. West*, 21 iv 2003). India: Puthen I., Kerala (*J. A. West*, 17 ii 2003).

Thalli flat (Fig. 3), light-brown when fresh (air-dried herbarium material pale brown to light pink to dark red violet), subdichotomously branched, 0.8-2.0 cm long; consisting of midrib of two transverse and two lateral periaxial cells, and one axial cell series, with monostromatic wings to either side (Figs 3, 4). Blades slightly or occasionally strongly constricted at nodes, 1.5-4.0 mm long and 0.5-1.4 mm wide at median internode region (Fig. 4). Endogenous branching present at node. Adventitious branching absent. Rhizoids tightly adhering at base of blade to form a mound of tightly adhering cells, appearing stipe-like (Figs 7-11). Adaxial cell row derived from the first axial cell from lateral axis absent (Fig. 6). One or two cell rows derived from the nodal axial cell opposite lateral branch. Number of cell rows derived from the first axial cell at main axis opposite lateral branch (2-) 3-7 (Fig. 5).

Cystocarps are formed near apices of the fertile blades. Male reproductive structures were not observed. Tetrasporangia are 55-65(-80) µm in length by 35-56 µm in width (Fig. 12).

Remarks: Caloglossa leprieurii can be easily separated from *C. apicula* by its lowermost rhizoidal cells that are tightly adherent to each other at their base and form a corticated pad (Figs 7-11), a structure first observed by King & Puttock (1994) in *C. leprieurii sensu* King and Puttock (= *C. vieillardii*, Puttock pers. comm.). *Caloglossa leprieurii* is confirmed for tropical coastlines of the western Atlantic, Indian, eastern Pacific Oceans. Molecular data support that the previous reports of *C. leprieurii* from North America are actually other species of *Caloglossa* (Figs 1-2). *Caloglossa leprieurii* reported from Japan (GenBank: AY150323), previously regarded as the multiple slender morphotype of *C. leprieurii* (Kamiya *et al.*, 1995, 1998), may represent another species of *Caloglossa* on the basis of *rbc*L and LSU data (*Caloglossa* sp. 2, Figs 1-2). Molecular data also suggest a *C. "leprieurii"* reported from Bali (GenBank: AY150322) is distinct from *C. leprieurii* (*Caloglossa* sp. 1, Figs 1-2).

Caloglossa apicula (Durant) Krayesky, Fredericq *et* J.N. Norris, *comb. nov.* **(Figs 13-24)**

Basionym: *Apiarium apicula* Durant 1850: 18.

Lectotype: on *Fucus versiculous* Linneaus, low tide, late October 1850, coll. Charles F. Durant; #*C. F. Durant 27* [*Lectotype* selected by Silva (2004) NYBG]. *isolectotypes*; US Alg. Type Coll. 091713 (single specimen, mounted in Durant 1850, p. [16].); US Alg. Type Coll. 31224 (two specimens on the herbarium sheet); and MICH).

Type locality: UNITED STATES: Jersey City, northwest side of Upper New York Bay, New Jersey.

Heterotypic synonymy: *Caloglossa leprieurii* f. *pygmaea* (G. Martens) Post 1936: 49 (in part: only her description of North American specimens: not the type specimen]; (non *Hypoglossum pygmaea* G. Martens, 1871: 172. *Type*: salt lakes, Calcutta, *Kurz*-3039, s.d.; [*lectotype* selected by Wynne & Kraft (1985), # L - 0055708!]; *isolectotype*: BM)

Caloglossa leprieurii sensu Humm *et* Caylor, 1957; Taylor, 1960, in part; Kapraun, 1974; Yarish & Edwards, 1982; Kamiya *et al.*, 2000, in part.

Distribution: *Eastern United States*: Connecticut to Florida. *Northern Gulf of Mexico*: Texas to Florida.

Figs 3-12. *Caloglossa leprieurii* (Montagne) G. Martens. **3.** Habit of *Caloglossa leprieurii* (Kwa-Zulu-Natal, South Africa). Scale bar, 2.0 mm. **4.** Topotype specimen. Node of thallus showing constriction at node, and cell row lineages. Scale bar, 0.5 mm. **5.** Topotype specimen. Node of thallus showing constriction at node, and cell row lineages. Short straight arrow points to the nodal axial cell; long straight arrow points to the first axial cell of main axis; arrowhead points to the first axial cell of the lateral axis; and the line with arrow parallel to the thallus margin at right highlights the cell row lineage derived from the nodal axial cell and the first axial cell of the main axis opposite the lateral branch, respectively (vic. Cayenne, French Guiana). Scale bar, 100 µm. **6.** Node of thallus illustrating absence of an adaxial cell row lineage derived from the first axial cell of lateral axis; arrowhead points to the first axial cell of the lateral axis (Isla Margarita, Venezuela). Scale bar, 100 µm. **7-9.** Mature rhizoids with stipe-like corticated pad. Mound of tightly adhering cells at base of rhizoids at thallus bifurcation (Basse Terre, Guadeloupe, F. W. I.). Scale bars, 100 µm. **10-11.** Lectotype of *Delesseria leprieurii* Montagne (L-0055717). Rhizoids forming stipe-like mound of tightly adhering cells at their base (type locality: Sinnamary, NW of Cayenne, French Guiana). Dashed line represents contour of mound of tightly adhering cells. **12.** Blade of *Caloglossa leprieurii* with tetrasporangia (Isla Margarita, Venezuela). Scale bar, 100 µm.

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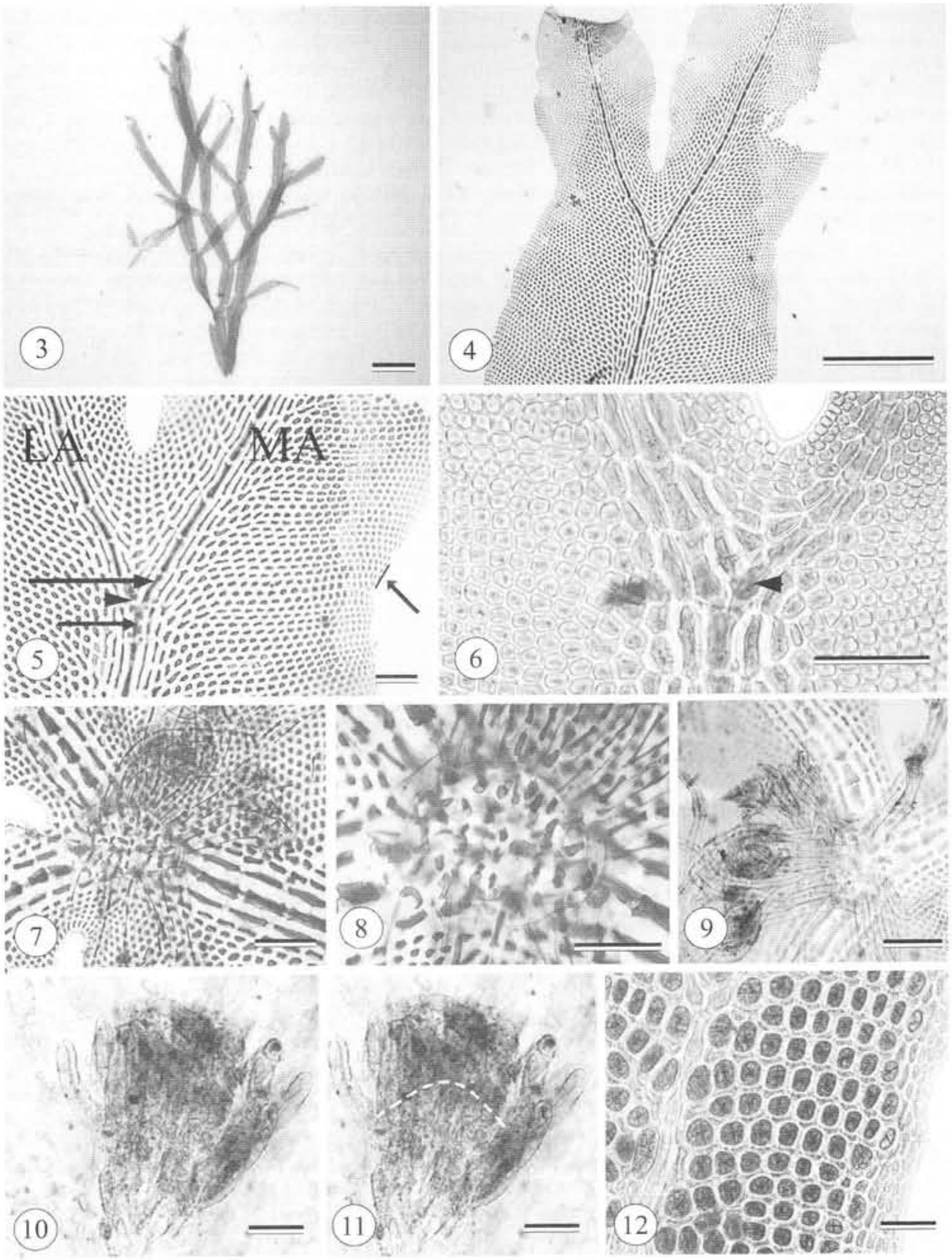

Habitat: Intertidal; confirmed growing on rocks, or epiphytic on *Spartina alterniflora* Loisel., pneumatophores of *Avicennia germinans* (L.) L., and *Fucus versiculosus* L.; in salt marshes, lagoons and mangroves; also occasionally in freshwater streams and rivers.

Specimens studied: *Gulf of Mexico*: Texas: Galveston, Galveston Is. (*D. M. Krayesky*, 2 ii 2003), and (*T. O. Cho*, 20 iii 2003). Louisiana: Port Fourchon, Lafourche Parish (*D. M. Krayesky*, 27 vi 2003); Marsh Is., Iberia Parish (*D. M. Krayesky*, 17 ix 2003); New Iberia, Iberia Parish (*S. Fredericq*, 25 iv 2006). Mississippi: Pass Christian, Harrison County (*D. M. Krayesky* 1 × 2003); Petit Bois Is. (barrier island), Jackson County (*J. López-Bautista,* 12 vii 2004); near Ocean Springs, Biloxi Bay, Jackson County (*J. López-Bautista,* 12 vii 2004), and (*D. M. Krayesky*, 24 v 2009). Alabama: Muscle Shoals, Colbert County (*P. G. Davison*, 2 vi 2003); Mobile, Mobile County (*B. Y. Won,* 30 iii 2005); Mobile (*B. Y. Won,* 3 iv 2005). Florida: Point Washington, Walton County (*T. O. Cho*, 1 v 2006).

Eastern United States: Connecticut: Stamford, Fairfield County (*J. A. West*, 13 viii 1991). New Jersey: Mullica River-Great Bay Estuary, Ocean and Atlantic Counties (*C. Yarish*, 17 vii 1973); Fort Lee, Bergen County (*H. Averill*, s.d., US Alg. Coll. 00296143); Jersey City, Hudson County (*C. F. Durant, 27* × 1850; *Apiarium apicula*, isolectotypes US Alg. Coll. 91713, US Alg. Coll. 31224, and MICH. Virginia: Poquoson, York County (*W. Schmidt*, 10 viii 2004). South Carolina: James Is., Charleston County (*J. A. West*, 17 vi 1994). Florida: St. Augustine (*G. A. Hall*, v 1879, US Alg. Coll. 00296172).

Additional species studied: *Indian Ocean* - India: Calcutta, *Caloglossa leprieurii* f. *pygmaea* (G. Martens) Post (≡ *Hypoglossum pygmaeum* G. Martens 1871; lectotype, *Kurz.* s.d.; L 0055708; *selected by* Wynne & Kraft (1985).

Thalli flat, light brown to brown, when fresh (air-dried herbarium material pale brown to light pink to dark red-violet) subdichotomously branched, narrow blades (Fig. 13), 0.3-1.2 cm long,. Blades only slightly constricted at nodes, 1.0-3.0 mm long and 0.1-1.0 mm wide at median internode region; consisting of a midrib region with monostromatic wings to either side (Figs 13-14). Endogenous branching present at node (Fig. 21). Adventitious branching occasional from node (Fig. 19) and internode regions (Fig. 20). Rhizoidal filaments forming regularly on ventral thallus surface, loosely arranged and not tightly adhering to each other at their base, no stipe-like structure formed (Figs 15-18). Adaxial cell row derived from the first axial cell from lateral axis absent (Fig. 14). One or two cell rows derived from the nodal axial cell opposite lateral branch. Number of cell rows derived from the first axial cell at main axis opposite lateral branch 1-3 (Fig. 14).

Cystocarps up to 300 µm in diameter, formed near apices to median regions of internode blades (Figs 22-23) on mature thalli. Male reproductive structures were not observed. Tetrasporangia 40-48 µm long, 35-43 µm in width (Fig. 24).

Figs 13-25. *Caloglossa apicula* comb. nov. (13-24) and *Hypoglossum pygmaeum* G. Martens (25). **13.** Habit of *Caloglossa apicula* (Galveston, Texas). Scale bar, 1.0 mm. **14.** Internode of *C. apicula*. Short straight arrow points to the nodal axial cell; long straight arrow points to the first axial cell of main axis; arrowhead points to the first axial cell of the lateral axis; arrows at left point to cell row lineages derived from the nodal axial cell and the first axial cell of the main axis opposite the lateral branch (Poquoson, Virginia). Scale bar, 100 µm. **15-17.** *C. apicula*, showing loose arrangement of rhizoids at their base (James Is., South Carolina). Scale bars, 100 µm. **18.** Isolectotype of *Apiarium apicula*: showing loose arrangement of rhizoids at their base (Jersey City, New Jersey; *C. F. Durant 27*, US-Alg. Type Coll. 91713). Scale bar, 100 µm. **19.** *Caloglossa apicula* (Galveston, Texas). Adventitious branching along nodal region. Scale bar, 0.5 mm. **20.** *Caloglossa apicula* (Galveston, Texas). Adventitious branching along internodal region. Scale bar, 0.25 mm. **21.** Endogenous branch at node (Poquoson, Virginia). Scale bar, 40 µm. **22.** Formation of cystocarps in mature internodal regions of thallus. Scale bar, 0.5 mm. **23.** Cystocarp forming near thallus apex in *C. apicula* (near Ocean Springs, Mississippi). Scale bar, 0.3 mm. **24.** Blade with tetrasporangia. Scale bar, 40 µm. **25.** Lectotype of *Hypoglossum pygmaeum* G. Martens, rhizoids at node. Tightly adherent rhizoids forming a stipe-like stalk near their base (arrow) and a holdfast-like structure near their tips (arrowheads) (Calcutta, India; L 0055708). Scale bar, 100 µm.

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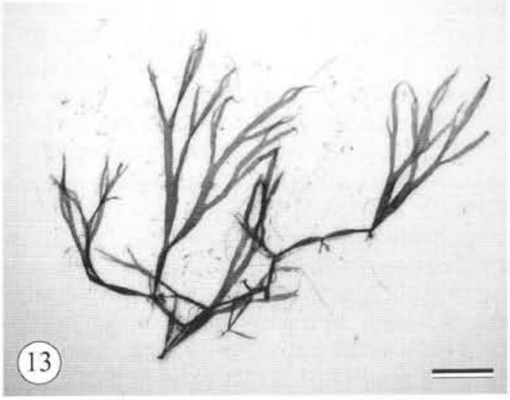
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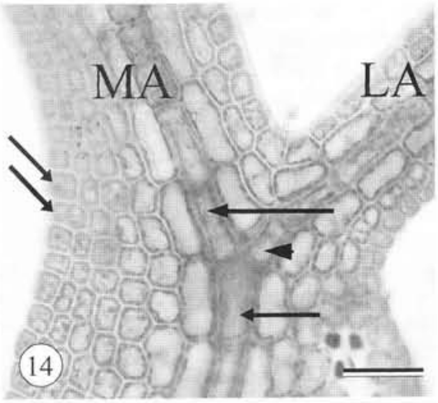
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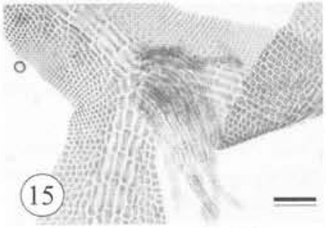
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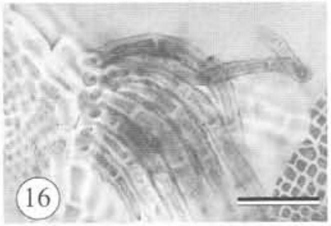
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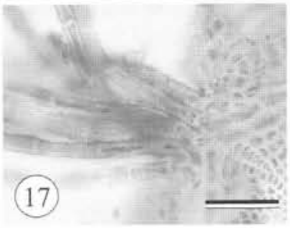
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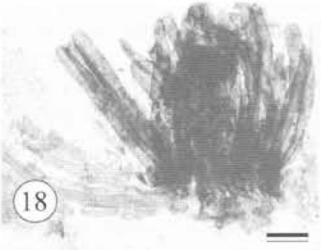
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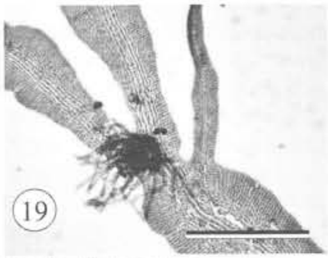
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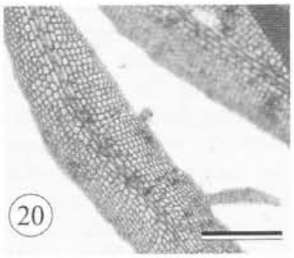
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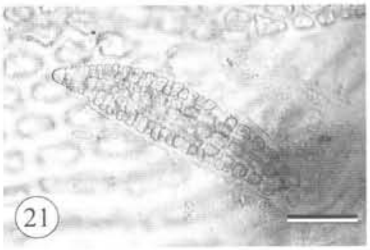
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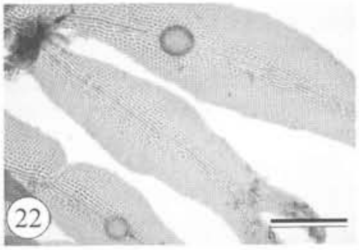
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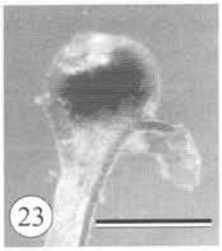
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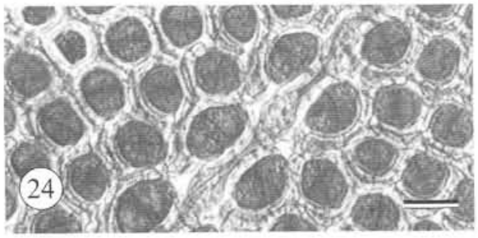
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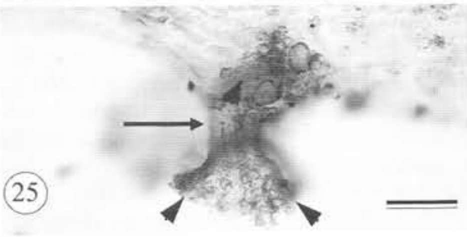
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Remarks: *Caloglossa apicula* was previously identified as *C. leprieurii* by all investigators (e.g., Humm & Caylor, 1957; Taylor 1960, in part; Kapraun, 1974; Yarish & Edwards, 1982; Kamiya *et al.*, 2000). *Caloglossa apicula* is different from *C. leprieurii* in rhizoid morphology, thallus structure, and the tendency to produce adventitious branches (Figs 19-20). Whereas *C. leprieurii* has rhizoids that adhere to form a corticated pad (Figs 17-19), *C. apicula* rhizoids are loosely arranged at their point of origin and are not associated with corticating cells (Figs 15-17). Kamiya *et al.* (1995) found that the cortex of rhizoids at the base (*i.e.* corticating cells) to be a stable morphological character found in *C. leprieurii*.

The cystocarps in *C. apicula* develop near growing branch apices (Fig. 23) or in middle regions of the thallus away (Fig. 22), whereas they are located near the tips in *C. leprieurii* (Montagne 1840, pl. 5: fig. 1b). This suggests that a procarps-bearing, fertile branch in *C. apicula* may continue growing after carpogonia have been fertilized, whereas in *C. leprieurii* the growth of fertile branches in length may cease in that their cystocarps appear only at the apices of the branches. *Caloglossa apicula* is apparently restricted to warm to cold temperate localities of eastern North America, whereas *C. leprieurii* is widely distributed in tropical regions, i.e. the Caribbean Sea, Indian Ocean (KwaZulu-Natal, South Africa to India) and the tropical eastern Pacific Ocean (Costa Rica).

Although sharing some similarities with *C. triclada* (Post) King *et* Puttock and *C. rotundata* Kamiya in their production of endogenous and occasional adventitious branching, *C. apicula* is distinct from these species. Blades are strap-like in *C. apicula* and *C. triclada* and elliptical to rotund in *C. rotundata*. In *C. apicula* adventitious branching can occur from internodal regions unlike in *C. triclada* and *C. rotundata*, where such branches are restricted to the nodal regions of the thalli. *Caloglossa apicula* and *C. triclada* can be also separated from *C. rotundata* in rhizoid morphology: the former two species possess "Type F" rhizoids, which are positioned at the nodes (*sensu* Kamiya *et al.*, 2003), whereas the latter species has the "Type C" (*sensu* Kamiya *et al.*, 2003). Within the "Type F" condition, *C. apicula* and *C. triclada* are different as *C. triclada* is reported (King & Puttock, 1994) to have a corticated pad, a character also seen in *C. leprieurii* (Figs 17-19), but absent in *C. apicula* (Figs 15-17). Although collections of *C. triclada* and *C. rotundata* are limited, both taxa appear to be tropical species in contrast to the temperate distribution of *C. apicula*.

When Post (1936) studied *C. leprieurii*, she also noted morphological differences between some specimens (including *C. apicula*) and the generitype, *C. leprieurii*, and considered them to be a new *forma*, *C. leprieurii* f. *pygmaea* (G. Martens) Post [basionym: *Hypoglossum pygmaeum* G. Martens 1871]. However, our study of the lectotype of *H. pygmaeum* (L 0055708, Calcutta, India, *Kurz*, *s.d.*) reveals that *H. pygmaeum* is also morphologically distinct from *C. apicula* (Figs 13-24). The rhizoidal filaments in *H. pygmaeum* tightly adhere to one another coalescing in a stipe-like stalk (different from the stipe-like cortical pad referred to earlier e.g. *C. leprieurii*), whose tips form a holdfast-like structure (Fig. 25); such rhizoidal holdfasts have not been observed in *C. apicula* (Figs 15-18). In addition, *H. pygmaeum* lacks the tendency to produce adventitious branches, and the endogenous branching appears to be more prolific than in *C. apicula* (Fig. 21). The taxonomic status of *C. leprieurii* f. *pygmaea* needs to be further tested with DNA sequence analysis.

Female gametophytes (Figs 22-23) and sporophytes with tetrasporangia (Fig. 24) were observed. These reproductive specimens were in collections from Texas and Louisiana made in February and March, 2003. Additionally, a sporophytic culture isolate (*J. A. West 3174*; Univ. Melbourne) was found to reproduce asexually by means of bisporangia, with diploid bispores that give rise to new sporophytes (J. A. West, *pers. obs.*). According to Dixon (1973: 134) another red alga, *Crouania attenuata* (C. Agardh) J. Agardh (Ceramiaceae), produced bisporangia on sporophytes during winter months in the North Atlantic and Pacific Oceans, followed by the formation of tetrasporangia on the same alga during the summer months. It is possible that *C. apicula* is also dependent upon seasonal specific biotic/abiotic factors that are in part responsible for the formation of either bisporangia or tetrasporangia by the sporophytes. This could also be tested with life history experiments in culture.

Caloglossa apomeiotica J. A. West *et* Zuccarello *in* West *et al.* 1994: 383, figs 1-15. **(Figs 26-33)**

Holotype: Intertidal, growing on prop roots of *Rhizophora mangle* L.; Puerto San Carlos, Bahía Magdalena, Baja California Sur, Pacific México (West 3025, 6. i. 1990, UC 1199678).

Distribution: *Eastern Pacific Ocean*: Baja California Sur, México to Panamá. Here recorded for the first time from the *Western Atlantic Ocean:* South Carolina, USA, to Brazil.

Habitat: Associated with mangroves, growing on pneumatophores of *Avicennia germinans* (L.) L. and *Laguncularia racemosa* (L.) Gaertn. f., and prop roots of *Rhizophora mangle* L.

Specimens studied: *Eastern Pacific Ocean:* México: Bahía Balandra, Baja California Sur (*J.A. West*, 6 i 1990, Paratype). Guatemala: El Manchon (*J.A. West*, 23 iii 1993). El Salvador: Bahía Jiquilisco, vic. Salinas de Mapachin and Isla Madresal (*E.Y. Dawson*, 9 ix 1960, US Alg. Coll. 00266161); and, vic. La Union, Gulfo de Fonseca (*E.Y. Dawson*, 6 ix 1960, US Alg. Coll. 00296163). Panamá: Isla Perico, Balboa (*B. Wysor*, 4 iv 1999). *Western Atlantic Ocean*: Brazil: Mangaratiba, Rio de Janeiro (state) (*C.F.D. Gurgel*, 15 xi 2003).

Thalli flat (Fig. 26), light brown when fresh (air-dried herbarium material pale brown to light pink to pale violet red, subdichotomously branched, 0.3-1.7 cm long: consisting of a midrib of two transverse and two lateral periaxial cells and one axial cell series, with monostromatic wings to either side (Figs 26-27). Blades slightly or occasionally strongly constricted at nodes, 1.5-4.0 mm long and 0.9-2.5 (-4.0) mm wide at median internode region (Fig. 28). Endogenous branching present. Adventitious branching absent. Rhizoidal filaments forming on ventral thallus surface, filaments tightly appressed at base to form mound of tightly adhering cells appearing stipe-like (Figs 30-32). Adaxial cell row derived from the first axial cell from lateral axis lacking. One to two cell rows derived from the nodal axial cell opposite lateral branch. Number of cell rows derived from the first axial cell at main axis opposite the lateral branch (1-) 2-5 (Fig. 29). Only tetrasporophytic plants were observed. Tetrasporangia 50-56 (-60) µm tall, width 40-49 µm in diameter (Fig. 33). Asexual reproduction apomictic.

Remarks. West *et al.* (1994) described *C. apomeiotica* as a new species based on material from Pacific Mexico that had previously been identified as *C. leprieurii*, primarily on the basis of its asexual mode of reproduction by apomictic bi- and tetrasporangia. Subsequently Kamiya *et al.* (2003) considered it a taxonomic synonym of *C. leprieurii*, on the basis that the character of asexual reproduction alone was not sufficient to separate *C. apomeiotica* from *C. leprieurii*.

Caloglossa apomeiotica is resurrected as a distinct species on the basis of newly found morphological and molecular data. The number of cell rows derived from the first axial cell of the main axis opposite the lateral branch is 2-5 in *C. apomeiotica* (Fig. 29) versus 3-7 in *C. leprieurii* (Fig. 28). Thalli of

C. apomeiotica are usually somewhat more robust (Fig. 26) than those of *C. leprieurii* (Fig. 3). *Caloglossa apomeiotica* is shown to be molecularly distinct from *C. leprieurii* (Figs 1-2).

Initially, West *et al.* (1994) observed cultured sporophytes of *C. apomeiotica* to reproduce asexually by both bisporangia and tetrasporangia; however, only the bispores germinated to generate the sporophyte phase. Recently an isolate culture (*J. A. West 3376*, Univ. Melbourne) was discovered to reproduce sexually as well. Our molecular data place this sample within the *C. apomeiotica* clade (Fig. 1). It is possible that *C. apomeiotica* also reacts to environmental cues that allow a sporophyte to produce viable bisporangia or tetrasporangia under certain conditions. Because only herbarium specimens from Pacific Panamá, El Salvador, and Brazil were available for this study, we were unable to test the reproductive strategy of *C. apomeiotica* in culture to further elucidate its life history.

Caloglossa ruetzleri Krayesky, Fredericq *et* J.N. Norris **sp. nov.** **(Figs 34-40)**

Holotype: Intertidal; epiphytic on mangrove pneumatophores and prop roots, Twin Cays, [16°48’N; 88°05’W], Belize Barrier Reef, Belize, 18 v 2006, coll. S. Fredericq, J. N. Norris, C. F. D. Gurgel & R. H. Sims, *D. M. Krayesky 169* (US Alg. Type Coll.0210477); *isotype*: LAF.

Homotypic synonymy: *Caloglossa leprieurii sensu* Norris and Bucher, 1982: 206 [non *C. leprieurii* (Montagne) G. Martens, 1869: 234].

Distribution: Caribbean Sea: Belize and Panamá. Gulf of Mexico: Florida Keys, Monroe County, Florida, USA.

Habitat: Intertidal; growing on red mangrove pneumatophores and prop roots.

Etymology: The species epithet honors Dr. Klaus Ruetzler, Research Zoologist, Curator, and Director of the *Smithsonian Institution Caribbean Coral Reef Ecosystems Program* (CCRE), National Museum of Natural History, in recognition of long-term support of CCRE, his numerous research contributions, and encouragement of our marine botanical research on the Belizean Barrier Reef. His ongoing research at Twin Cays, Belize, led to our identification of a previously unknown species and has enabled long-term observations on the second largest barrier reef system in the world.

Specimens studied: *Caribbean Sea:* Panama: Isla Solarte, Bocas del Toro (*B. Wysor & D.W. Freshwater*, 17 i 2007). Belize: Twin Cays, Belize Barrier Reef (*S. Fredericq, J. N. Norris & C. F. D. Gurgel*, 12 v 2006). *Gulf of Mexico*: Florida Keys: Summerland Key, Monroe County (*T. O. Cho*, 4 ii 2004); and Cudjoe Key, Monroe County (*T. O. Cho*, 23 xi 2005).

Thalli plani, modice aut modo fusci, materia herbarii pallida fusca ad pallidam roseam ad pallidam violaceorubram, subdichotomi ramosi, 0.5-1.0 cm long, constantes ex costae regione alis monostromaticis alteruter latere. Regio costae

Figs 26-33. *Caloglossa apomeiotica* J. A. West et Zuccarello. **26.** Habit (Mangaratiba City, Brazil). Scale bar, 1.5 mm. (27-33) Specimens from Isla Perico, Balboa, Panama. **27.** Juvenile node. Scale bar, 100 µm. **28.** Mature node, showing the main axis and lateral axis. Scale bar, 100 µm. **29.** Main axis at thallus node; arrowhead points to the first axial cell of the lateral axis. Short arrow points to the nodal axial cell; long arrow points to the first axial cell of main axis; arrows at right point to cell row lineages derived from the nodal axial cell and the first axial cell of the main axis opposite the lateral branch. Scale bar, 100 µm. **30.** Corticated pad of rhizoids. Rhizoid initials forming mound of tightly adhering cells. Arrow points to outline of mound. Scale bar, 40 µm. **31-32.** Mature rhizoids with mound of tightly adhering cells at base of rhizoids. Scale bars, 100 µm. **33.** Tetrasporangia. Scale bar, 40 mm.

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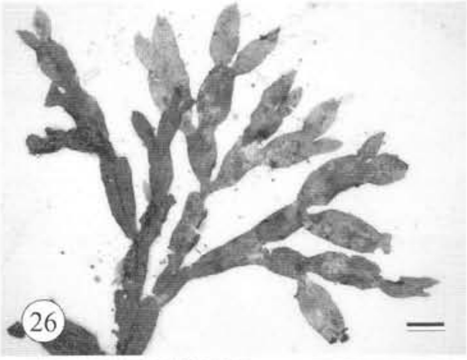
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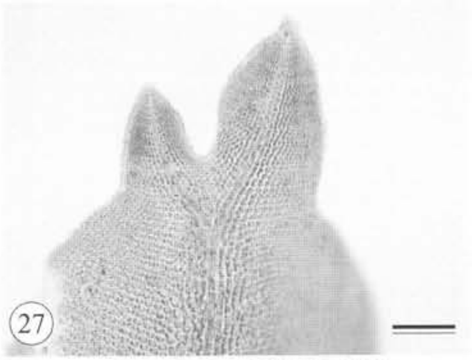
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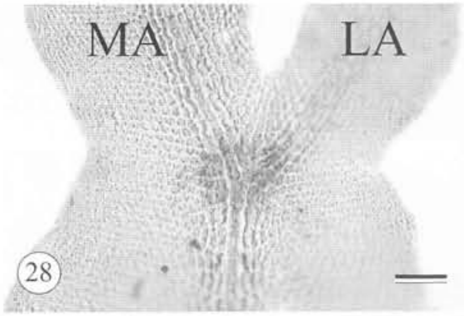
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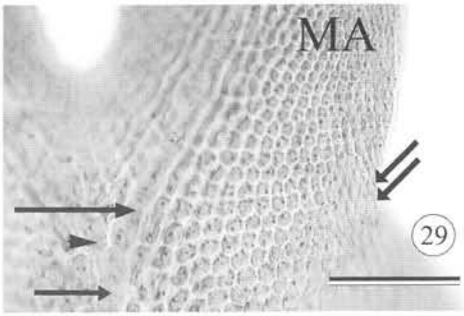
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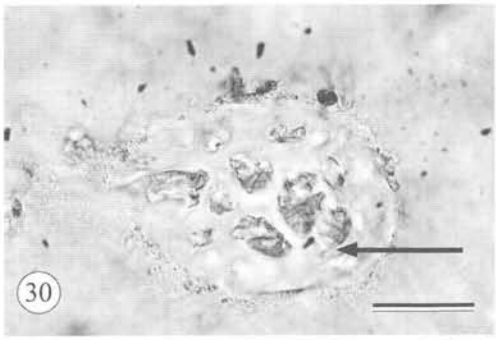
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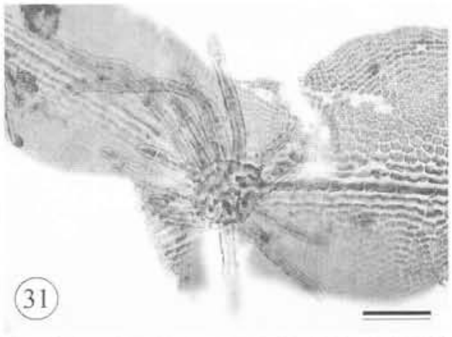
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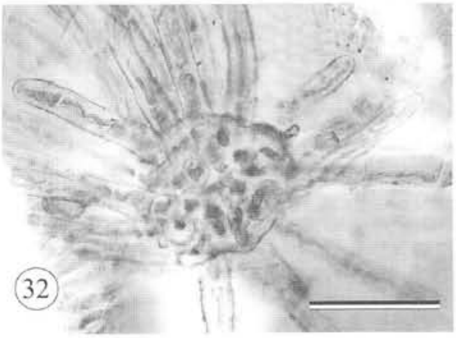
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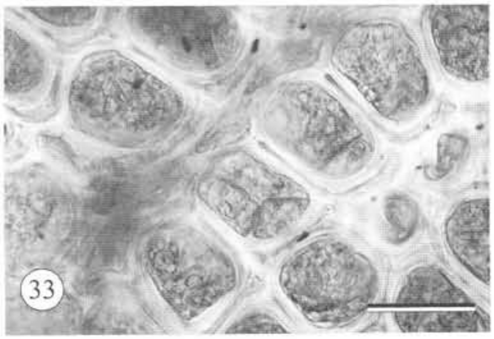
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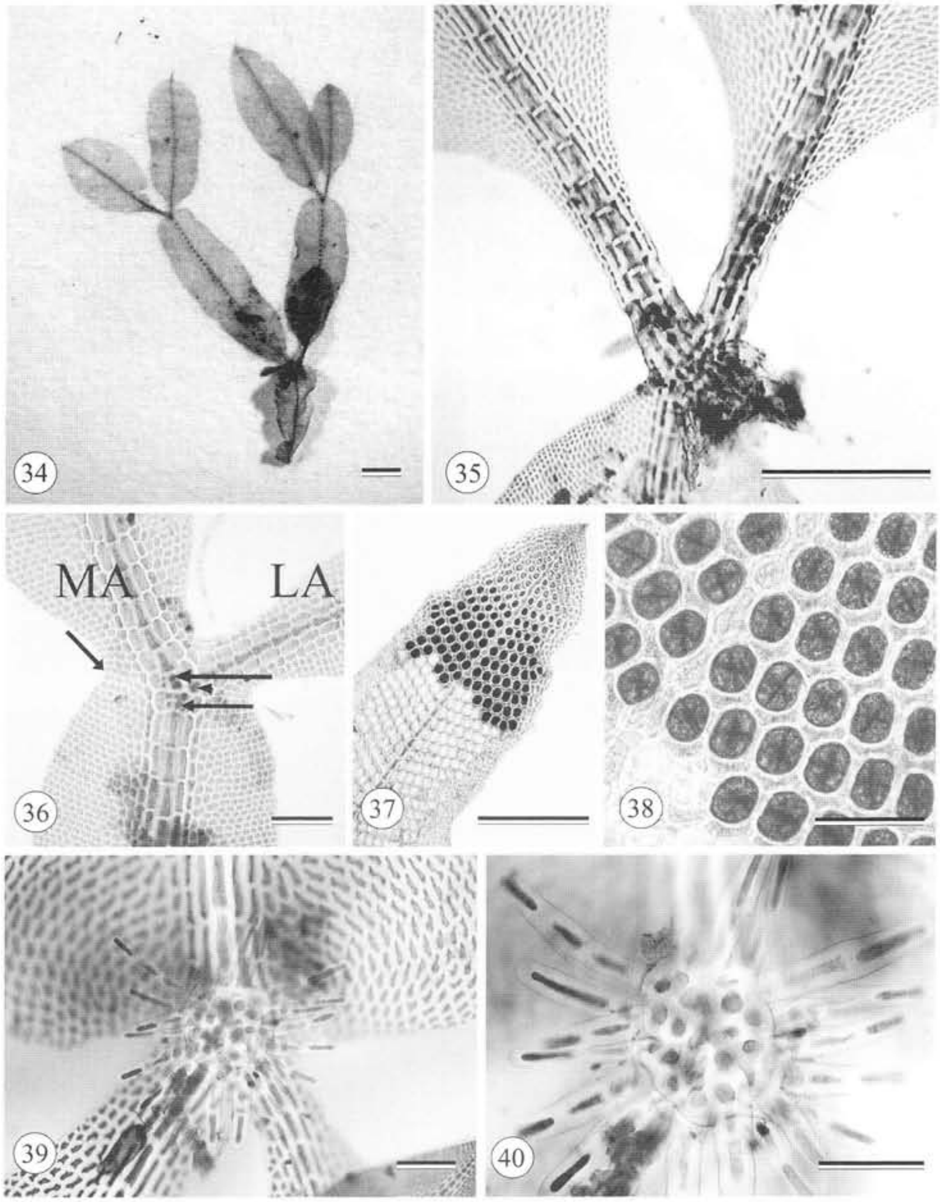

Figs 34-40. *Caloglossa ruetzleri* sp. nov. (34-36, 39-40) Holotype of *C. ruetzleri* (Twin Cays, Belize). **34.** Habit of holotype (*D. M. Krayesky 169*, US Alg. Type Coll. 0210477). Scale bar, 1.5 mm. **35.** Strongly constricted node. Scale bar, 0.5 mm. **36.** Nodal region of thallus. Short straight arrow points to the nodal axial cell; long straight arrow points to the first axial cell of main axis; arrowhead points to the first axial cell of the lateral axis; and the inclined arrow points to cell row lineage derived from the nodal axial cell and the first axial cell of the main axis opposite the lateral branch, respectively. Scale bar, 100 μm. **37.** Blade with tetrasporangia (Isla Solarte, Bocas del Toro, Caribbean Panama). Scale bar, 0.5 mm. **38.** Tetrasporangia (Isla Solarte, Bocas del Toro, Caribbean Panama). Scale bar, 100 μm. **39-40.** Mature rhizoids with mound of tightly adhering cells at base of rhizoids. Scale bars, 100 μm.

constans ex duo cellulis transversalibusque duo cellulis lateralibus periaxialibus, serie axiali cellulis. Laminae constrictae valde ad nodos, regio nodalis filiformis, 3.0-6.0 mm longaeque 1.1-2.0 mm latae internodi medianam regionem. Internodi thalli aut "laminae" ovato usque ad subovatos. Ramificatio endogenea praesens. Ramificatio adventitia absens. Fila rhizoidea formantia regulatim paginam ventralem thalli, arcte appressa basem formantia statum stipitis. Series cellularum adaxialis oriunda e prima axiali cellula lateralis axis absens. Numerus serierum cellularum oriundarum e cellula axiali nodalis opposita ramum laterale 1. Numerus serierum cellularum oriundarum e prima cellula axiali in axem principalem opposita ramum laterale 1. Tetrasporangia (35-) 42-44 µm lat., 46-49(-55) µm long. Diversae structurae reproductivae non visae.

Thalli flat (Fig. 34), light brown to brown when fresh (air-dried herbarium material pale brown to light pink to pale violet red), subdichotomously branched, 0.5-1.0 cm long, consisting of midrib of two transverse and two lateral periaxial cells and one axial cell series, with monostromatic wings to either side. Blades strongly constricted at nodes (Figs 34-35), 3.0-6.0 mm long and 1.1-2.0 mm wide at median internode region; internodes (or "blades") ovate to subovate (Fig. 34). Endogenous branching present at nodes. Adventitious branching absent. Rhizoid filaments forming regularly on the ventral thallus surface, filaments tightly adhering at base to form a stipe-like structure (Figs 39-40). Adaxial cell row from first axial cell from lateral axis absent. A single cell row is derived from both the nodal axial cell opposite lateral branch and from the first axial cell at the main axis opposite lateral branch (Fig. 36). Tetrasporangia 46-49 (-55) µm tall, (35-) 42-44 µm in diameter (Figs 37-38). Other reproductive structures not observed.

Remarks: Caloglossa ruetzleri was previously identified as *C. leprieurii* from Islamorada, Florida Keys, USA (GenBank: AF522225) by Kamiya *et al.* (2003). The combination of molecular and morphological data reveal that *C. ruetzleri* is a distinct species. Unlike the blades of *C. apomeiotica* (Figs 27-28), *C. leprieurii* (Fig. 4) and *C. apicula* (Fig. 14), *C. ruetzleri* is highly constricted at the nodes over a long interval of 5-6 axial cells (Fig. 35), with elliptical blades at the internodes (Fig. 13). In contrast, the habits of *C. apomeiotica*, *C. apicula* and *C. leprieurii* are more strap-like, with usually only slight constriction at the nodes (Figs 3, 13, 26). Members of the *C. leprieurii* complex have a "Type F" rhizoid position at the nodes (*sensu* Kamiya *et al.*, 2003); all the species described herein have a "Type F" rhizoid position (Table 2). The arrangement of the basal rhizoidal cells in *C. ruetzleri*, *C. apomeiotica* and *C. leprieurii* (Figs 7-9) are tightly adherent to one another at their base to form a stipe-like structure (Figs 39-40). All four species are also characterized by a lack of an adaxial cell row derived from the first axial cell from lateral axis (Figs 6, 14, 29, 36). *Caloglossa ruetzleri* differs from the other three species in the range of the number of cell rows derived from the first axial cell of the main axis opposite to the lateral branch as it contains only a single cell row (Fig. 36), whereas *C. apicula* possesses one to three, *C. apomeiotica* usually has two to five, and *C. leprieurii* generally produces three to seven cell rows. The tetrasporangia of *Caloglossa ruetzleri* (Figs 37-38) are similar in size to those of *C. apicula* (Fig. 24), but are smaller than those of *C. apomeiotica* (Fig. 33) and *C. leprieurii* (Fig. 22). *Caloglossa ruetzleri* grows on the pneumatophores and prop roots of mangroves and appears to be relatively uncommon, in comparison to other tropical species of the *C. leprieurii* complex, such as *C. leprieurii* and *C. apomeiotica*.

DISCUSSION

Previous investigations have reported the *Caloglossa leprieurii* species complex as occurring pantropically, as well as in some temperate regions (e.g., Post, 1936; Kamiya, 2004). *Caloglossa leprieurii* specimens from subtropical to cold-temperate regions of eastern North America are shown to be morphologically and molecularly distinct from tropical representatives of the Caribbean and the western Atlantic. This study demonstrates that the *C. leprieurii* complex, although monophyletic, is in fact composed of several distinct species, four of them, *C. apomeiotica*, *C. apicula*, *C. leprieurii*, and *C. ruetzleri*, occurring in various temperate to tropical regions of the Americas. These four species are molecularly distinct, based on *rbc*L and LSU rDNA sequence data; the clades are well supported and the interclade sequence divergence ranges from 2.7%-5.5% for *rbc*L. The interspecific *rbc*L sequence divergence of *Caloglossa* species exhibits a wider range when compared to some groups of red algae. Within other genera of the Ceramiales there are *rbc*L sequence divergence ranges between 3.8%-8.1% in Centroceras (Won *et al.*, 2009) and 1.2%-4.3% in *Ceramium* (Cho *et al.*, 2003a, b). In the Gelidiales, interspecific *rbc*L sequence divergence reported in *Geldium* varies from 1.2-7.3% (Freshwater & Rueness, 1994). The Rhodymeniales exhibits similar interspecific *rbc*L sequence divergence ranging from 5.6-9.8% for specific *Botryocladia* species (Gavio & Fredericq, 2003). In some instances interspecific *rbc*L sequence divergence is less than the examples cited above as a 0.55% divergence separates *Irvinea boergesenii* (Feldmann) R.J. Wilkes, L.M. McIvor et Guiry from *I. ardreana* (Brodie et Guiry) Guiry (Wilkes *et al.*, 2006). Likewise, the adequate degree of interspecific *rbc*L sequence divergence amongst *C. apomeiotica*, *C. apicula*, *C. leprieurii*, and *C. ruetzleri* demonstrates that these taxa are distinct at the species level.

The four newly circumscribed *Caloglossa* species can be separated by subtle but distinct morphological characters (Table 2). Although distinguishable character-states are not readily evident, a consistent difference between *C. apomeiotica* and *C. leprieurii* is that the cell row derived from the first cell of the main axis opposite the lateral branch can generate two to five cell rows of the second order in the former versus three to seven in the latter. Using a phylogenetic species concept (*sensu* Wheeler & Platnick, 2000), morphological evidence and molecular data demonstrate that these four species are distinct and can furthermore be identified based their respective morphologies.

Molecular data suggest that *Caloglossa apomeiotica*, *C. apicula* and *C. ruetzleri* appear more closely related to each other than to *C. leprieurii*, a western Atlantic, eastern Pacific and Indian Ocean species. The *rbc*L and LSU sequence data also suggest that the samples previously identified as *C. leprieurii* from Bali and Japan (Kamiya *et al.*, 2003, 2004) are distinct from the *C. leprieurii* clade (Figs 1-2). Future studies will be necessary to elucidate the identity of these western Pacific *C. leprieurii*. Kamiya (2004) reported three western Pacific morphotypes within the *C. leprieurii* complex. The four species reported herein from the Americas are within the "multiple/broad" morphotype.

Whereas the *C. leprieurii* complex might superficially be considered to be comprised of cryptic species, i.e., species that cannot be distinguished based on morphology (see Verbruggen *et al.*, 2009; Leliaert *et al.*, 2009; for other groups of algae), a main point of this paper is that this is not the case in that we have found morphological characters that separate *C. leprieurii* from *C. apicula*, *C. ruetzleri*, and *C. apomeiotica*. The term pseudocryptic (*sensu* Knowlton, 1993) implies that morphological characters differentiating species might have been overlooked at first; this was certainly the case with the *C. leprieurii* complex before our study. In this investigation morphological characters were first identified to delimit species followed by utilizing molecular data to further corroborate *C. apicula*, *C. ruetzleri*, and *C. apomeiotica* as species distinct from *C. leprieurii*. A comprehensive analysis of material of the different morphotypes occurring in the western Pacific will be necessary to address whether these taxa are pseudocryptic or not.

This investigation has shown *Caloglossa leprieurii* to have a more narrow distribution than previously reported. Our data reveal that *C. leprieurii* is strictly a tropical to subtropical species, and that *C. apicula* is the only species of the *C. leprieurii* complex that is exclusively subtropical to temperate in distribution. While *C. ruetzleri* is a tropical species, *C. apomeiotica* occurs in warm-temperate and tropical localities as well. Furthermore, of these four species of the *C. leprieurii* complex, all but *C. leprieurii* are restricted to the Americas (Fig. 41).

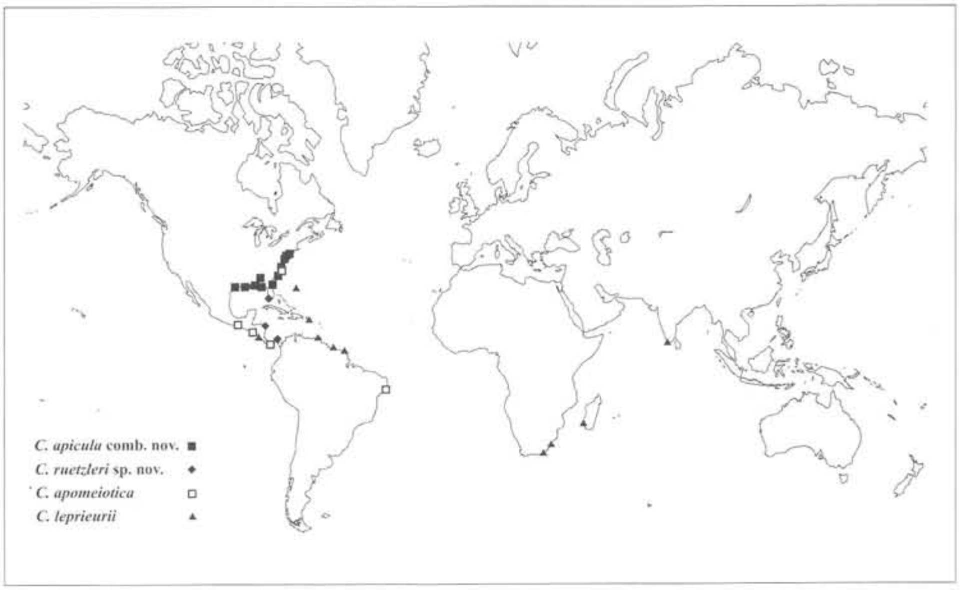

Fig. 41. Distribution map of *Caloglossa leprieurii*, *C. apicula*, *C. ruetzleri*, and *C. apomeiotica*.

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