

## MORPHOLOGICAL AND MOLECULAR CHARACTERIZATION OF SPECIES OF THE GENUS *CENTROCERAS* (CERAMIACEAE, CERAMIALES), INCLUDING TWO NEW SPECIES<sup>1</sup>

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*Centroceras clavulatum* (C. Agardh) Montagne is widely reported as being a prime example of a cosmopolitan red algal species. Instead, *C. clavulatum* is here determined as restricted to northern Chile, Peru, southern California, southern Australia, and New Zealand. Specimens identified using the current species concept for “*C. clavulatum*” fall into nine morphological groups that correspond to highly supported clades in phylogenetic analyses. Three of these clades correspond to the resurrected species *Centroceras gasparrinii* (Meneghini) Kützing, *C. hyalacanthum* Kützing, and *C. micracanthum* Kützing. Two others are recognized as new species: *Centroceras rodmanii* sp. nov. from southern Chile, which is characterized by hooked spines arranged in a whorl at the node, a spine or flattened gland cell cut off from the first cortical initials, and a single acropetal cortical cell issued from the second cortical initials; and *C. tetrachotomum* sp. nov. from South Africa, which has a tetrachotomous branching pattern, straight spines in a whorl, an acropetal cortical cell and a spine or a flattened gland cell cut off from the first cortical initials, and a two-celled acropetal filament cut off from the second cortical initials. Three additional species from South Africa are also recognized as distinct species. All phylogenetic analyses of the *rbcL* gene, LSU rDNA, and SSU rDNA were consistent with the vegetative and tetrasporangial morphological distinctions, thus supporting the resurrection of three species and the description of two new species.

**Key index words:** algal taxonomy; *C. clavulatum*; *C. gasparrinii*; *C. hyalacanthum*; *C. micracanthum*; *C. rodmanii* sp. nov.; *C. tetrachotomum* sp. nov.; *Centroceras*; Ceramiales; LSU rDNA; molecular systematics; morphology; phylogeny; *rbcL*; Rhodophyta; SSU rDNA

The red algal genus *Centroceras* Kützing (1841: 731) was described in the Ceramiaceae Dumortier (1822: 71, as ‘Ceramieae’) of the Ceramiales Oltmans (1904: 683) but without designation of a generic type. Later, Kylin (1956: 379) lectotypified the genus with *C. clavulatum* (C. Agardh) Montagne (1846: 140; basionym: *Ceramium clavulatum* C. Agardh 1822: 2). Including the generitype *C. clavulatum* (type locality: Callao, Peru), *Centroceras* as currently understood is composed of seven species: *C. corallophiloides* R. E. Norris (1993: 391; type locality: Kaneohe Bay, Island of Oahu, Hawaii), *C. distichum* Okamura (1934: 40; type locality: Wagu, Japan), *C. internitens* Gallagher et Humm (1983: 261; type locality: Florida Keys), *C. japonicum* Itono (1973: 164; type locality: Hateruma I., Ryukyu Archipelago, Japan), *C. minutum* Yamada (1944: 42; type locality: Ant Atoll, near Ponape, Caroline Islands), and recently, *C. secundum* Wynne (2003: 126; type locality: Oman).

Species previously described as *Centroceras* that have been transferred to *Corallophila* by Norris (1993) are *Corallophila apiculatum* (Yamada) R. E. Norris (type locality: Ant Atoll, near Ponape, Caroline Islands; basionym: *Centroceras apiculatum* Yamada 1944); *Corallophila bellum* (Setchell et N. L. Gardner) R. E. Norris (type locality: Guaymas, Mexico; basionym: *Centroceras bellum* Setchell et N. L. Gardner 1924); *Corallophila pignattii* (Giaccone) R. E. Norris (basionym: *Centroceras pignatii* Giaccone 1968); *Corallophila cinnabarina* (Grateloup ex Bory de Saint-Vincent) R. E. Norris [= *Centroceras cinnabarinum* (Grateloup ex Bory de Saint-Vincent) J. Agardh 1851; basionym: *Boryna cinnabarina* Grateloup ex Bory de Saint-Vincent 1822; type locality: l’Étang de Thau, Hérault, France]. *Ceramium eatonianum* (Farlow) De Toni (1903; basionym: *Centroceras eatonianum* Farlow 1875; type locality: Clatsop County, Oregon) was recognized as *Corallophila eatoniana* (Farlow) T. O. Cho, Choi, Hansen et Boo (Cho et al. 2000), and *Centroceras rhizophorum* Montagne was considered as *Ceramium rhizophorum* (Montagne) De Toni (1903).

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*Centroceras clavulatum* has been viewed as highly variable morphologically and a ubiquitous species (Hommersand 1963). More recently, Barros-Barreto et al. (2006) reported that “*C. clavulatum*” may consist of a species complex. Kützing (1841) described six species of *Centroceras* based on segment size, spine shape, and the arrangement of basipetal cortical cells: *C. cryptacanthum*, *C. micracanthum*, *C. leptacanthum*, *C. hyalacanthum*, *C. macracanthum*, and *C. oxyacanthum*. Subsequently, Kützing (1849) described two more species: *C. inerme* and *C. gasparinii*. However, J. Agardh (1851) considered all of the Kützing species of *Centroceras* (1849) as synonyms of *Centroceras clavulatum* (C. Agardh) Montagne (1846). Another species, *Centroceras brachyacanthum* Kützing (1863), was reduced to a variety, *C. clavulatum* var. *brachyacanthum* (Kützing) P. et H. Crouan (in Mazé and Schramm 1878).

Feldmann-Mazoyer (1941) and Hommersand (1963; see also Kützing 1841) broadly characterized *Centroceras clavulatum* as having a pseudo-dichotomous to sometimes tri- or tetrachotomous branching pattern, initiation of three cortical cells per periaxial cell, complete cortication of rectangular cortical cells arranged longitudinally on the main axis and branches, spines produced from periaxial cells at the nodes, and the formation of spermatangial parent cells from periaxial cells.

*Centroceras clavulatum* has been recognized as a representative species with a pantropical to warm-temperate cosmopolitan distribution (van den Hoek and Breeman 1990) inhabiting the southwest Atlantic Ocean (Joly 1965), the eastern Atlantic and Mediterranean Sea (Feldmann-Mazoyer 1941, Lawson and John 1982, Maggs and Hommersand 1993), the Pacific Ocean (Taylor 1945, Abbott and Hollenberg 1976, Itono 1977, Cribb 1983, Boo and Lee 1985), the Indian Ocean (Silva et al. 1996), and the Caribbean Sea (Taylor 1960).

*Centroceras clavulatum* specimens from near its type locality in Peru, and from worldwide collections identified as “*C. clavulatum*,” were investigated. To characterize *C. clavulatum*, chloroplast-encoded *rbcl* gene and nuclear LSU rDNA and SSU rDNA sequences were analyzed and compared to previously overlooked morphological and vegetative characters such as the development of the acropetal cortical filament, shape of gland cells and spines, branching pattern, and the development of tetrasporangia. Fertile specimens were for the most part not available for analysis.

#### MATERIALS AND METHODS

**Morphology.** Samples were collected worldwide and sorted under a dissection microscope (SMZ800, Nikon, Tokyo, Japan). Each identified sample was liquid-preserved in 5% formalin/seawater for morphological observations. Microscope observations were made from materials stained with 1% aqueous aniline blue acidified with 0.1% diluted HCL (Tsuda and Abbott 1985).

**DNA extraction, amplification, and sequencing.** Genomic DNA was extracted using the DNeasy Plant Mini Kit (Qiagen, Valencia, CA, USA) on fresh or silica-gel dried specimens. The *rbcl* gene was amplified using the primer combinations F7-R753 and F645-RrbcSstart as listed in Lin et al. (2001) and sequenced with the primers F7, F645, F993, R376, R753, R1150, RrbcSstart (Freshwater and Rueness 1994, Lin et al. 2001, Gavio and Fredericq 2002). Primers (G01-G10, G02-G14, G04-G13, G06-G07) of SSU rDNA (Saunders and Kraft 1994) were used in this study. Partial fragments of LSU rDNA were amplified using the X and 28F primers (Freshwater et al. 1999). PCR and sequencing protocols are as described in Cho et al. (2003b). Sequences were determined for both forward and reverse strands using the ABI Prism 3100 Genetic Analyzer (PE Applied Biosystems, Foster City, CA, USA) with the ABI Prism BigDye™ Terminator Cycle Sequencing Ready Reaction Kit (PE Applied Biosystems).

**Alignment and phylogenetic analyses.** All generated sequence data of *rbcl*, LSU rDNA, and SSU rDNA were compiled (Table S1 in the supplementary material), and the sequences were manually aligned with Sequencher (Gene Codes Corp., Ann Arbor, MI, USA) and then exported for maximum-likelihood (ML) and maximum-parsimony (MP) algorithms available in PAUP\* (v. 4.0b10, Swofford 2003). *Ceramium horridum* Setchell et N. L. Gardner, *Corallophila eatoniana*, *Microcladia borealis* Ruprecht, and *Reinboldiella schmitziana* (Reinbold) De Toni were selected as the outgroup in the *rbcl* analyses, while only *C. horridum* was included in the SSU rDNA and LSU rDNA analyses; these taxa are closely related to *Centroceras* in global *rbcl* analyses of the Ceramieae (data not shown).

For the ML analyses, the aligned sequences were first analyzed using Modeltest (v. 3.0, Posada and Crandall 1998). Support for the nodes of all ML trees was determined by calculating bootstrap proportion values using 100 replicates. MP trees were constructed with the heuristic search option of PAUP. Support for nodes of on the MP tree was determined by calculating bootstrap proportion values (Felsenstein 1985) using 1,000 replicates and randomizing the input order 500 times.

#### RESULTS

***Centroceras clavulatum*** (C. Agardh) Montagne 1846: 140 (Figs. 1, 2).

**Basionym:** *Ceramium clavulatum* C. Agardh 1822: 2.

**Holotype:** In Herb. Agardh, LD (see: Howe 1911: 509).

**Type locality:** Callao, Peru.

**Representative specimens examined.** Australia: Werribee, Victoria, coll. M. H. Hommersand and F. Hommersand, 6. viii. 1995. Chile: Antofagasta, Northern Chile, coll. E. F. García, 22. i. 2004. New Zealand: Ahipara Bay, coll. M. H. Hommersand, 13. xi. 2004. Peru: near type locality, Punta La Cruz, Ancon, Lima, coll. N. Arakaki, 30. viii. 2003. USA: California: Lovers Point, Pacific Grove, Monterey, coll. T. O. Cho, 10. xii. 1999; Crystal Cove, Orange Co., coll. T. O. Cho and S. Murray, 5. xii. 1999; Ocean Beach, San Diego, coll. S. Fredericq, 17. vii. 2000; Pescadero, San Mateo Co., coll. T. O. Cho and B. Y. Won, 3. vii. 2003.

**Morphology.** Thalli are rose pink and 7–10 cm high, form dense tufts, and consist of erect and prostrate axes (Fig. 1a). The overall branching pattern is subdichotomous (Fig. 1, a–c). Branching takes place at intervals of 9–10 (average  $9.7 \pm 0.5$ )

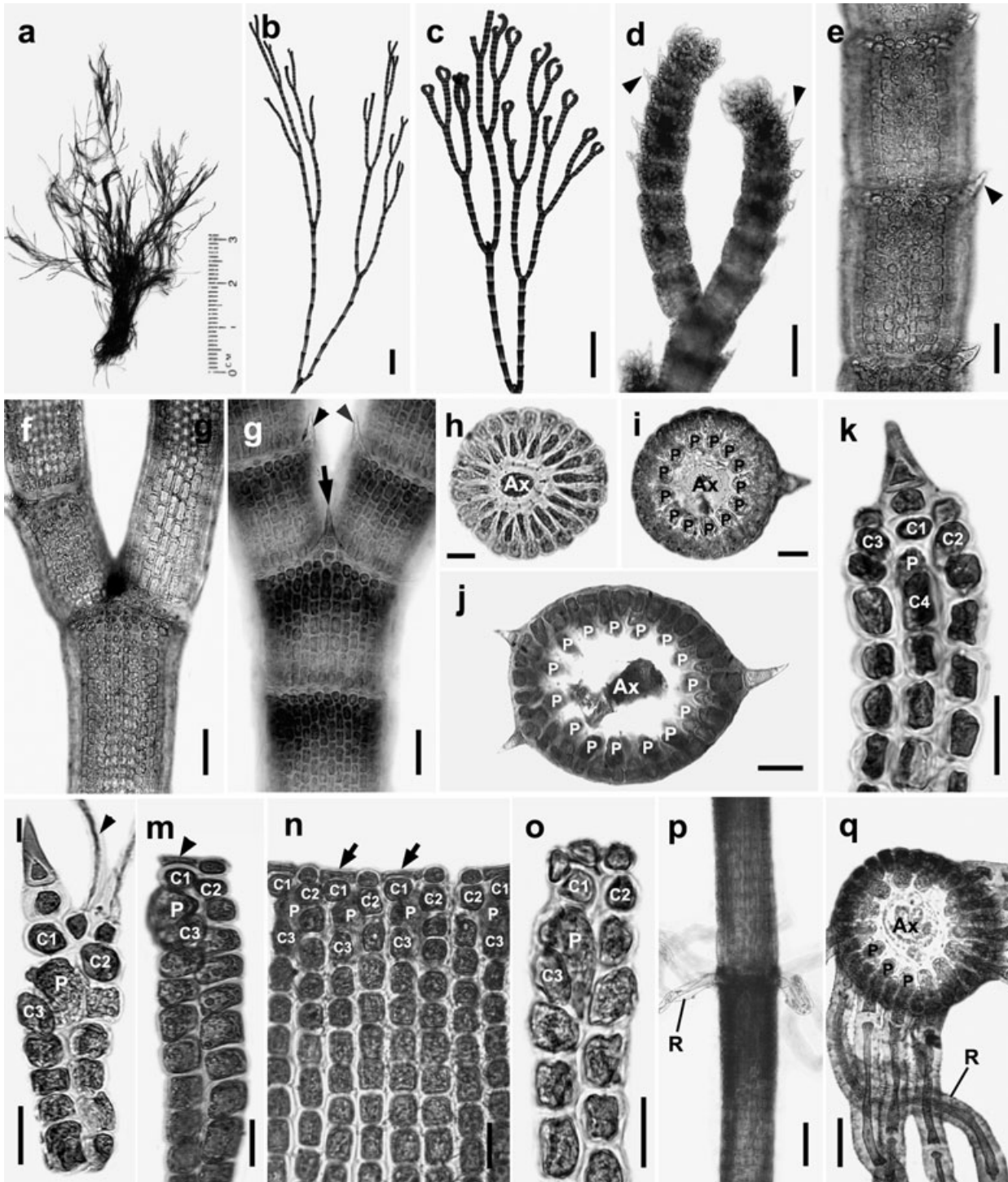


FIG. 1. *Centroceras clavulatum* (C. Agardh) Montagne (LAF-30-viii-03-1-1; slides 1, 2, 4, and 6) from near the type locality; Punta La Cruz, Ancon, Lima, Peru. (a) Vegetative specimen (LAF-30-viii-03-1-1). (b) Upper thallus part. Scale bar, 1 mm. (c) Upper thallus showing pseudodichotomous branching. Scale bar, 1 mm. (d) Apical region showing pseudodichotomous branching and abaxial spines (arrowheads). Scale bar, 100  $\mu$ m. (e–g) Spines (arrowheads and arrow) in upper (e) and middle (f) thallus parts, and at branching point (g). Scale bars, 50  $\mu$ m. (h–j) Cross-section views through cortical nodes at upper (h) and middle (i and j) parts. Scale bar, 10  $\mu$ m (h) and 20  $\mu$ m (i and j). (k) Cortical unit showing the first periaxial cell on abaxial side with four cortical initials, with first one bearing a spine. Scale bar, 20  $\mu$ m. (l) Cortical unit showing the first cortical initial bearing a spine and an acropetal cortical cell with hair-like extension (arrowhead). Scale bar, 20  $\mu$ m. (m) Cortical unit showing the first cortical initial with a flattened gland cell (arrowhead). Scale bar, 20  $\mu$ m. (n) Mature cortication showing flattened gland cells (arrows). Scale bar, 20  $\mu$ m. (o) Cortical unit showing the first cortical initial bearing two cortical cells. (p) Cortical node with rhizoids at lower part. Scale bar, 100  $\mu$ m. (q) Cross-section view showing multicellular rhizoids produced from periaxial cells. Scale bar, 50  $\mu$ m. Ax, axial cell; C1–4, sequence of cortical initials; P, periaxial cell; R, rhizoid.

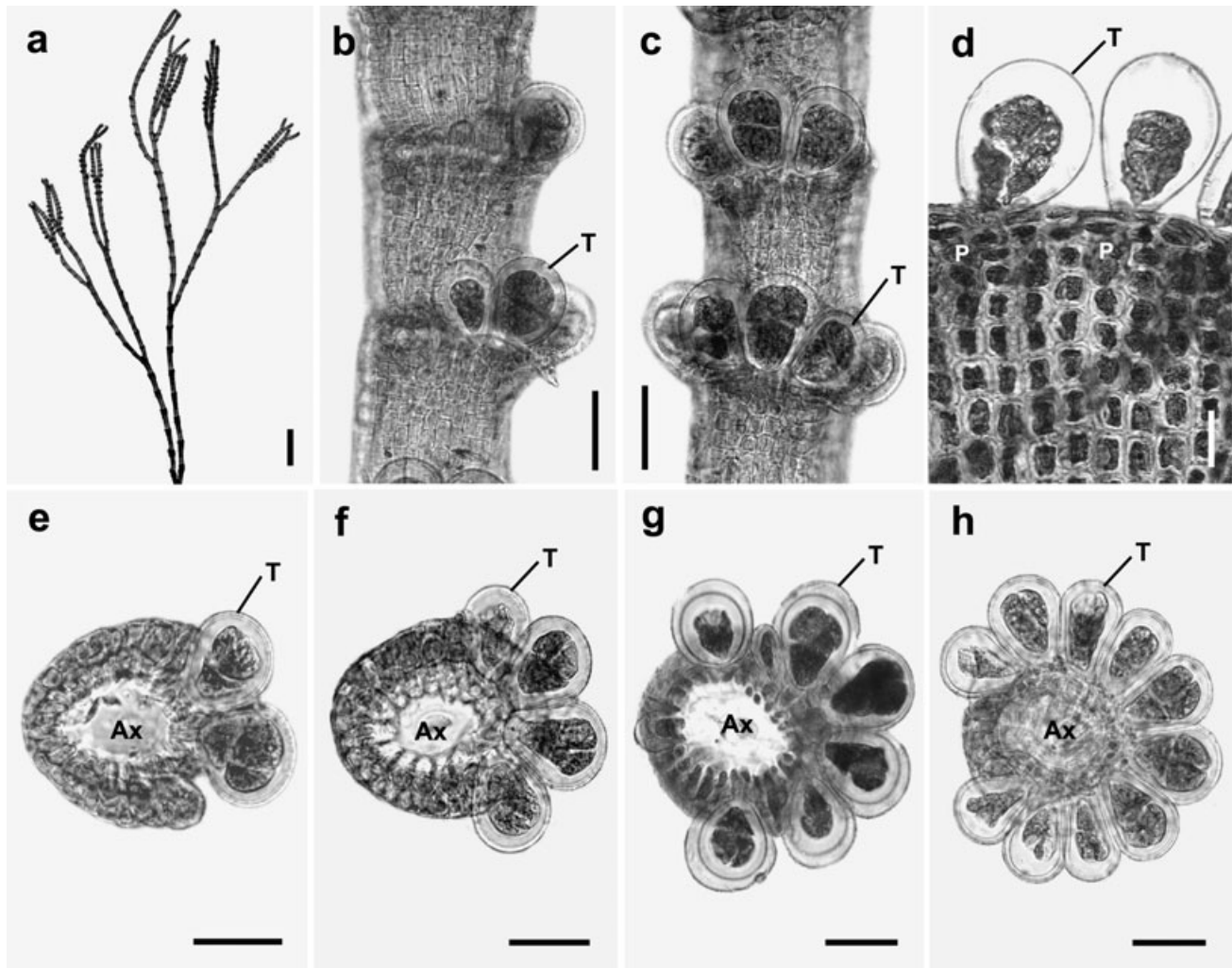


FIG. 2. *Centroceras clavulatum* (C. Agardh) Montagne (LAF-30-viii-03-1-2; slides 8 and 9) from near the type locality; Punta La Cruz, Ancon, Lima, Peru. (a) Tetrasporangial thallus. Scale bar, 1 mm. (b) Upper part of tetrasporophyte showing abaxial arrangement of tetrasporangia. Scale bar, 50  $\mu$ m. (c) Middle thallus part with tetrasporangia in whorl at node. Scale bar, 50  $\mu$ m. (d) Cortication, with tetrasporangia produced from periaxial cells. Scale bar, 20  $\mu$ m. (e-h) Cross-section views at nodes showing tetrasporangia produced in alternate sequence. Scale bars, 50  $\mu$ m. Ax, axial cell; P, periaxial cell; T, tetrasporangium.

axial cells in the main axes, and at intervals of 6–9 (average  $7.5 \pm 0.7$ ) axial cells in the lateral axes. In addition, adventitious branches develop from periaxial cells in the lower thallus part.

Erect axes have a forcipulate, slightly inrolled apex (Fig. 1d) beset with spines oriented at a  $45^\circ$  angle (Fig. 1g). Erect axes are 110–130  $\mu$ m in diameter. Twelve to 16 periaxial cells are cut off obliquely from the upper part of each axial cell and remain at the nodes after axial cell elongation (Fig. 1, h–j). All periaxial cells produce the cortical initials that divide into cortical filaments contributing to the cortex. The first periaxial cell on the abaxial side cuts off four cortical initials, the first three from the upper end and the fourth from its lower end (Fig. 1k). The first cortical initial produces a spine upon further division (Fig. 1k). The second and third cortical initials each cut off one cell acropetally and a filament basipetally by transverse division

(Fig. 1k); the fourth cortical initial cuts off a filament basipetally (Fig. 1k). The other periaxial cells each cut off three cortical initials, the first two from the upper end and the third from its lower end (Fig. 1l). In this case, the first cortical initial cuts off a spine and a cortical cell (Fig. 1l), a flattened gland cell (Fig. 1m), or two cortical cells (Fig. 1o). The second cortical initial cuts off one cell acropetally and a filament basipetally by transverse division (Fig. 1, l–o). The third cortical initial cuts off a filament basipetally (Fig. 1l). The mature cortex is complete. The acropetal cortical filaments are two cells long including the cortical initial (Fig. 1n), and the basipetal cortical filaments are 18–25 cells in length.

Spines occur as a row on the abaxial side throughout the length of the thallus (Fig. 1, d–f) or in a whorl at each node (Fig. 1g). Each spine is straight and composed of three cells, including the cortical initial (Fig. 1, k and l). A refractive, flattened gland

cell is produced horizontally from the first cortical initial (Fig. 1, m and n). Numerous uniseriate and multicellular rhizoids are produced from periaxial cells (Fig. 1, p and q) and terminate in a pad.

In tetrasporic thalli, tetrasporangia distributed along several upper cortical nodes of the erect axes (Fig. 2a) are produced initially from the first-formed periaxial cell on the abaxial side, then on the adaxial side in an alternate sequence resulting in a whorl around the axis (Fig. 2, b, c, and e–h). A single tetrasporangium develops per periaxial cell (Fig. 2d). Mature tetrasporangia are tetrahedrally divided, spherical to ellipsoidal, and average  $98 \pm 7 \mu\text{m} \times 71 \pm 5 \mu\text{m}$  excluding the sheath and  $115 \pm 5 \mu\text{m} \times 96 \pm 4 \mu\text{m}$  with the sheath, and almost completely project from the cortical node (Fig. 2c).

Male and female thalli were not found in our collections.

*Centroceras gasparrinii* (Meneghini) Kützing 1849: 689 (Figs. 3; 4; and 11, a–d).

*Basionym*: *Ceramium gasparrinii* Meneghini 1844: 186.

*Isotype*: L-0193946 (#940265-102)!

*Type locality*: Palermo, Sicily.

*Heterotypic synonym*: *Centroceras inerme* Kützing 1849: 688, 1863: 7, pl. 17: figs. e–g [L-0193931 (#938316-447)]!

*Representative specimens examined*. Antigua: Jolly Beach, coll. A. Renoux, 26. xii. 2002 and 28. xii. 2002. Brasil: Praia Rasa, Buzios, Rio de Janeiro, coll. C. F. D. Gurgel, 22. xi. 2003. Japan: Koinoura, Tsuyazaki Fukuoka, coll. T. O. Cho and S. Kawaguchi, 17. iii. 1999. Korea: Gampo, Kyeongsangbuk-do, coll. T. O. Cho and S. M. Boo, 27. ix. 1998; Guryongpo, Pohang, Kyeongsangbuk-do, coll. T. O. Cho, 7. vii. 1999. Madagascar: Port de Fort Dauphin, N Fort Dauphin, coll. E. Coppejans, D. Douterlungne & I. Razanakoto, 1. ix. 2002. Mexico: near Algodones Beach, Guaymas, Sonora, Gulf of California, coll. M. H. Hommersand and J. Hughey, 27. ii. 1998; El Tecolote, Baja California Sur, coll. T. O. Cho and R. Riosmena-Rodriguez, 20. v. 2000; Sargento, La Rentana, Baja California Sur, coll. T. O. Cho and R. Riosmena-Rodriguez, 13. v. 2000. Caribbean Panama: Galeta, STRI, Galeta Lab, coll. B. Wysor, 20. vi. 1999; West Portobelo, coll. B. Wysor, 4. ii. 1999. Pacific Panama: Isla Culebra, coll. B. Wysor, 16. iv. 1999. South Africa: Mzamba, coll. T. O. Cho and S. Fredericq, 21. viii. 2005; Trafalgar, KwaZulu-Natal, coll. T. O. Cho and S. Fredericq, 23. viii. 2005. Sri Lanka: W. coast, coll. E. Coppejans, 19. i. 1997. USA: California: Point Loma, San Diego Co., coll. M. H. Hommersand, 7. vii. 1996; La Jolla, San Diego Co., coll. M. H. Hommersand, 18. vii. 1996; Florida: Sand Key State Park, St. Petersburg, coll. T. O. Cho, 19. ix. 2002, and coll. B. Y. Won and T. O. Cho, 3. iii. 2003; Smathers Beach, Key West, coll. B. Y. Won and T. O. Cho, 30. x. 2003; St. Lucie State Park, St. Lucie Co., coll. B. Y. Won

and T. O. Cho, 4. iii. 2003; Hawaii: Ka'ala'wai, Island of O'ahu, coll. P. Vroom, 13. viii. 2003; Kawaikui Beach Park, O'ahu, coll. P. Vroom, 19. iii. 2004; Kewalo Basin, O'ahu, coll. P. Vroom, 18. iii. 2004; Kupikipikio Point, O'ahu, coll. P. Vroom, 19. iii. 2004; Texas: Port Aransas, coll. W. Schmidt, 20. v. 2003, coll. S. Fredericq, 19. iv. 1999, and coll. T. O. Cho and B. Y. Won, 8. vii. 2003.

*Morphology*. Thalli are rose pink and 3–6 cm high, form dense tufts, and consist of erect and prostrate axes (Figs. 3a and 11a). Branching is pseudodichotomous to trichotomous (Fig. 3b). Branches are formed at intervals of 10–12 (average  $11.3 \pm 0.5$ ) axial cells in the main axes and at intervals of 10–12 (average  $11.0 \pm 0.8$ ) axial cells in the lateral axes. In addition, adventitious branches develop from periaxial cells in the lower thallus part.

Erect axes have forcipulate, slightly inrolled apices beset with spines oriented at a  $45^\circ$  angle on the upper part of each node (Fig. 3, b and c) and 140–160  $\mu\text{m}$  in diameter. Twelve to 19 periaxial cells are cut off obliquely from the upper part of each axial cell and remain at the nodes after axial cell elongation (Fig. 3, g and h). All periaxial cells produce corticating initials that divide into cortical filaments contributing to the cortex. The first periaxial cell on the abaxial side cuts off four cortical initials, the first three from its upper end and the fourth from its lower end (Fig. 3i). The first cortical initial cuts off a spine and a cortical cell (Fig. 3i). The second and third cortical initials cut off one cell acropetally and a filament basipetally by transverse division (Fig. 3i). The fourth cortical initial cuts off a filament basipetally (Fig. 3i). The other periaxial cells cut off three cortical initials, the first two from their upper end and the third from their lower end (Fig. 3, j–m). The first cortical initial cuts off a spine and a cortical cell (Fig. 3j), a gland cell and a cortical cell (Fig. 3k), or two cortical cells (Fig. 3m). The second cortical initial cuts off one cell acropetally and a filament basipetally by transverse division (Fig. 3, j–m). The third cortical initial cuts off a filament basipetally (Fig. 3, j–m). The mature cortex is complete. Acropetal cortical filaments are two cells long, including the cortical cell initial, and the basipetal cortical filaments are 13–23 cells long.

Whorled spines occur at each node throughout the length of the thallus (Fig. 3, c–f). Each spine is straight and three cells long (Figs. 3, i–j, and 11c) including the cortical initial. A gland cell is obliquely cut off from the first cortical initial and is ovoid in shape (Fig. 3, k and l). Numerous uniseriate and multicellular rhizoids are produced from periaxial cells and terminate in a pad (Fig. 3, n and o).

In tetrasporic thalli, tetrasporangia distributed along several upper cortical nodes of the erect axes and adventitious branches (Fig. 4a) are produced initially from the first-formed periaxial cell on the abaxial side, then on the adaxial side in an alternate sequence resulting in a whorl around the axis

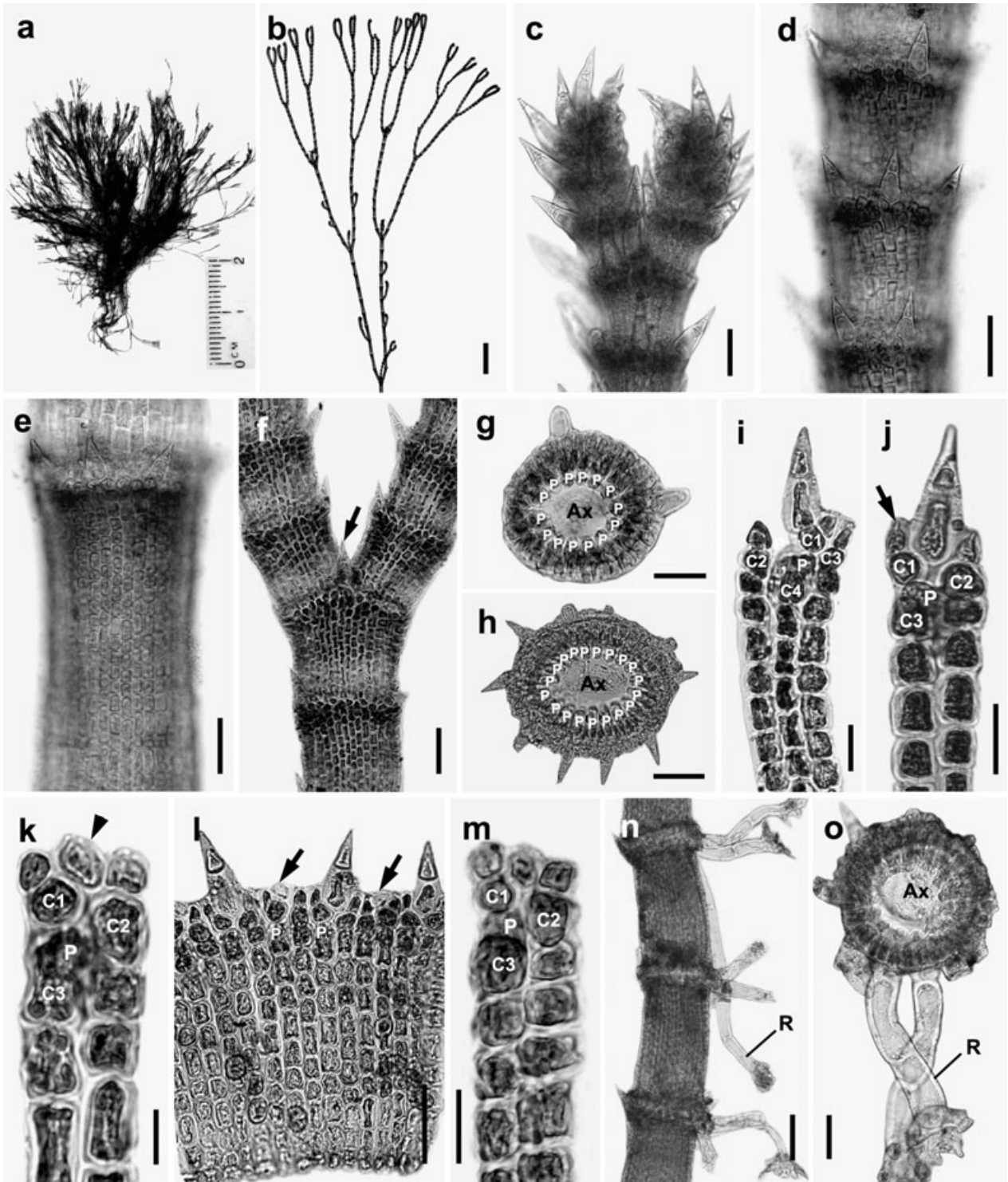


FIG. 3. *Centroceras gasparrinii* (Meneghini) Kützing (LAF-30-x-03-1-1; slides 1, 2, 3, and 5) from Smathers Beach, Key West, FL, USA. (a) Vegetative specimen (LAF-30-x-03-1-1). (b) Upper thallus part. Scale bar, 1 mm. (c) Apical region. Scale bar, 50  $\mu$ m. (d–e) Whorled arrangement of spines in middle (d) and lower (e) thallus parts. Scale bars, 50  $\mu$ m. (f) Spine (arrow) at branching point. Scale bar, 50  $\mu$ m. (g, h) Cross-section views through cortical nodes at midthallus. Scale bars, 50  $\mu$ m. (i) Cortical unit showing the first periaxial cell on abaxial side linked to four cortical initials. Scale bar, 20  $\mu$ m. (j) Cortical unit showing the first cortical initial bearing a spine and a cortical cell (arrow). Scale bar, 20  $\mu$ m. (k) Cortical unit showing the first cortical initial bearing a gland cell (arrowhead) and a cortical cell. Scale bar, 20  $\mu$ m. (l) Mature cortication showing the spines and ovoid gland cells (arrows). Scale bar, 40  $\mu$ m. (m) Cortical unit showing the first cortical initial bearing two cortical cells. Scale bar, 20  $\mu$ m. (n) Creeping thallus part with rhizoids. Scale bars, 100  $\mu$ m. (o) Cross-section view showing multicellular rhizoids with digitate tips extending from periaxial cells. Scale bar, 50  $\mu$ m. Ax, axial cell; C1–4, sequence of cortical initials; P, periaxial cell; R, rhizoid.

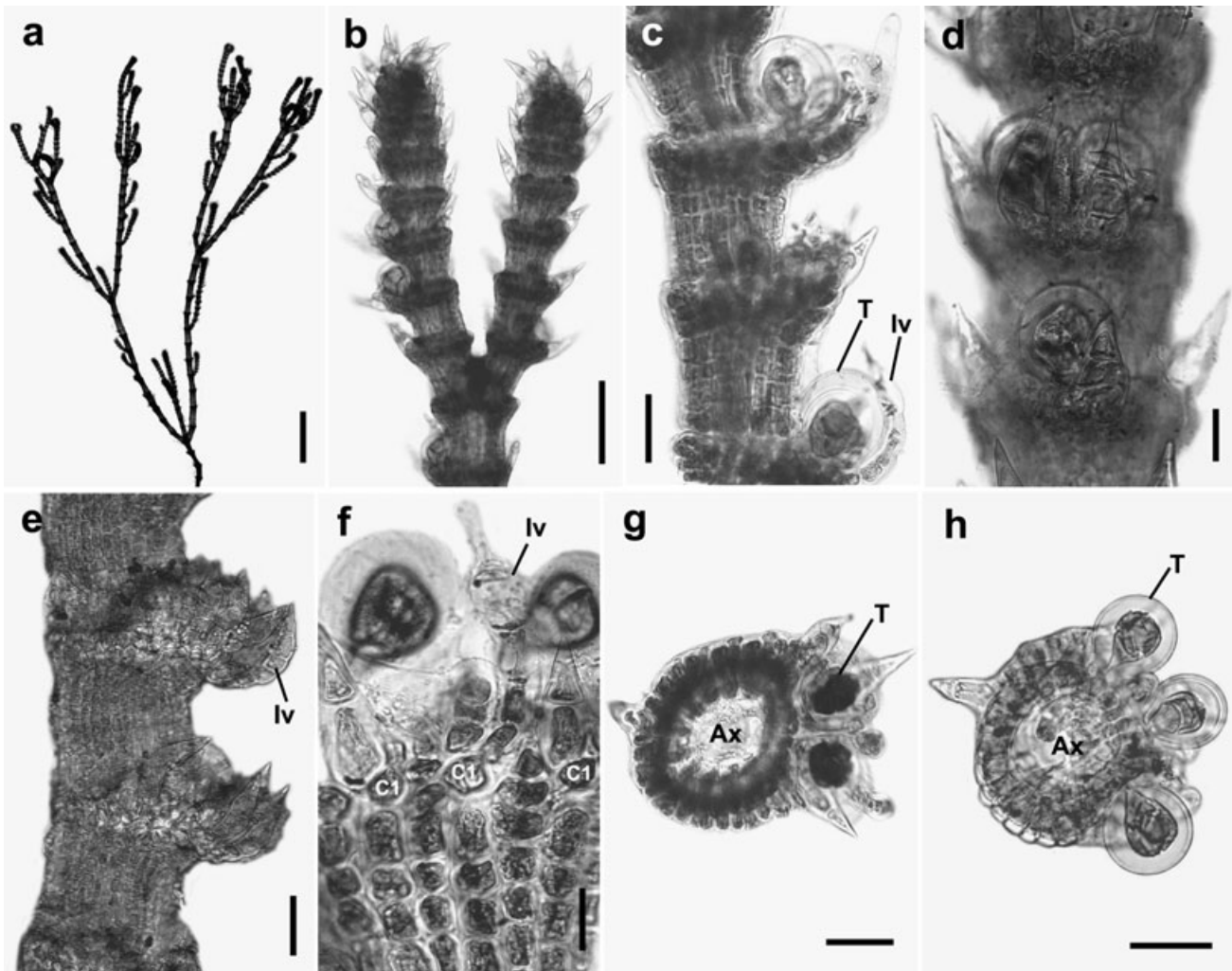


FIG. 4. *Centroceras gasparrinii* (Meneghini) Kützing (LAF-30-x-03-1-2; slides 1, 2, and 3) from Smathers Beach, Key West, FL, USA. (a) Tetrasporangial thallus. Scale bar, 1 mm. (b–e) Apical region with tetrasporangia at cortical nodes (b), produced in a whorl around the axis from the abaxial to adaxial side, and surrounded by involucre branchlets, as shown at upper (c), middle (d), and lower (e) parts. Scale bars, 50  $\mu\text{m}$  (b, c, e) and 20  $\mu\text{m}$  (d). (f) Cortication, tetrasporangia, and involucre branchlets. Scale bar, 20  $\mu\text{m}$ . (g, h) Cross-section views through node showing tetrasporangia produced in alternate sequence. Scale bars, 50  $\mu\text{m}$ . Ax, axial cell; C1, the first cortical initials; Iv, involucre branchlets; T, tetrasporangium.

(Fig. 4, b–d). A single tetrahedral tetrasporangium, spherical to ellipsoidal, and averaging  $40 \pm 1 \mu\text{m} \times 29 \pm 2 \mu\text{m}$  excluding the sheath, and  $59 \pm 1 \mu\text{m} \times 54 \pm 6 \mu\text{m}$  with the sheath, develops periaxial cell (Fig. 4, g and h) and is surrounded by four- to six-celled involucre branchlets that are produced from the first cortical initials or are transformed from spines (Fig. 4, e and f).

Male and female thalli were not found in our collections.

**Remarks.** Meneghini (1844: 186) in describing *Ceramium gasparrinii* placed it in *Ceramium* subgen. *Centroceras*, clearly recognizing its affinities to *Centroceras*. It was subsequently transferred to the genus *Centroceras* by Kützing (1849).

*Centroceras hyalacanthum* Kützing 1841: 742 (Figs. 5; 6; and 11, e–h).

*Centroceras hyalacanthum* Kützing 1841: 742, 1849: 689, 1863: 7, pl. 19: figs. d–f.

**Lectotype:** L-0193933 (#939285-73)! (designated hererin).

**Type locality:** “Wahrscheinlich aus Westindien” (Kützing 1841: 742) [=West Indies]; Antilles, French West Indies.

**Heterotypic synonyms:** *Centroceras brachyacanthum* Kützing 1863: 8, pl. 20: figs. d–f [L-0193935 (#939285-71)]! Type locality: Antilles. *Centroceras oxyacanthum* Kützing 1841: 742, 1849: 689, 1863: 7-8, pl. 20: figs. a–c [L-0193936 (#939285-79)]! Type locality: Cuba.

**Representative specimens examined.** French West Indies: Guadeloupe, Anse Babin, coll. A. Renoux, 17. xii. 2003. USA: Florida: Merritt Island, Brevard Co., coll. B. Y. Won and T. O. Cho, 5. iii. 2003;

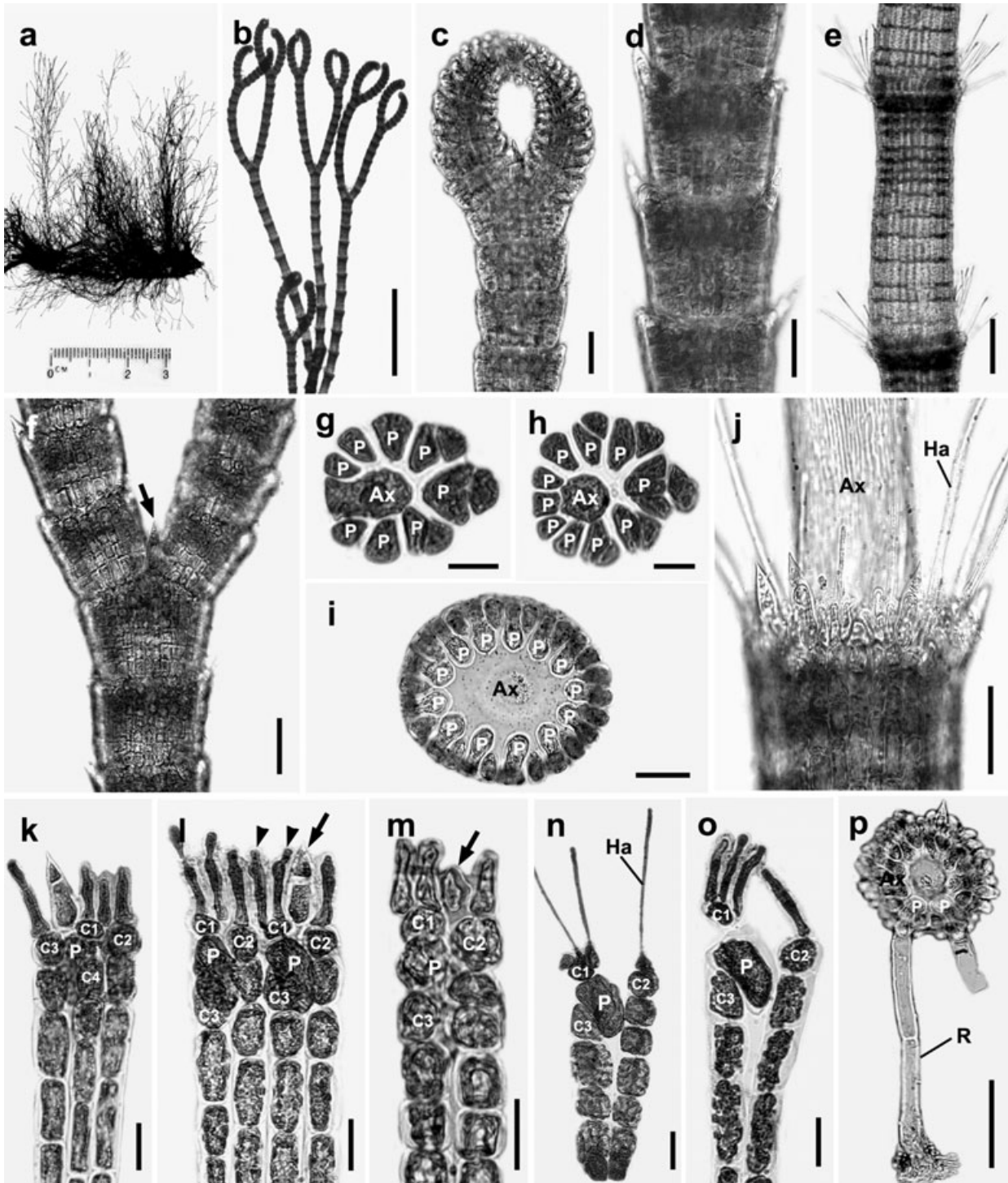


FIG. 5. *Centrocercus hyalacanthum* Kützinger (LAF-3-iii-03-1-1; slides 2, 3, 5, 6, and 8) from North Skyway, St. Petersburg, Pinellas Co., FL, USA. (a) Vegetative specimen (LAF-3-iii-03-1-1). (b) Upper thallus part. Scale bar, 1 mm. (c) Apical region. Scale bar, 50  $\mu$ m. (d–e) Whorled arrangement of spines in middle (d) and lower (e) thallus parts. Scale bars, 50  $\mu$ m (d) and 100  $\mu$ m (e). (f) Branching point with spine (arrow). Scale bar, 50  $\mu$ m. (g–i) Cross-section views through cortical nodes of upper (g, h) and middle (i) thallus parts. Scale bars, 10  $\mu$ m (g and h) and 40  $\mu$ m (i). (j) Mature cortication showing spines and hair cells at axial node. Scale bar, 40  $\mu$ m. (k) Cortical unit showing the first periaxial cell on abaxial side bearing four cortical initials. Scale bar, 20  $\mu$ m. (l) Two cortical units showing the first cortical initial bearing a spine (arrow) and two elongated cortical cells (arrowheads), or two elongated cortical cells. Scale bar, 20  $\mu$ m. (m) Cortical units showing the first cortical initial bearing an ovoid gland cell (arrow) and two elongated cortical cells. Scale bar, 20  $\mu$ m. (n–o) Young (n) and mature (o) cortical units showing the first cortical initial bearing three elongated cortical cells. Scale bar, 20  $\mu$ m. (p) Cross-section view showing multicellular rhizoids with digitate tip initiated from periaxial cells. Scale bar, 100  $\mu$ m. Ax, axial cell; C1–4, sequence of cortical initials; Ha, hair cell; P, periaxial cell; R, rhizoid.



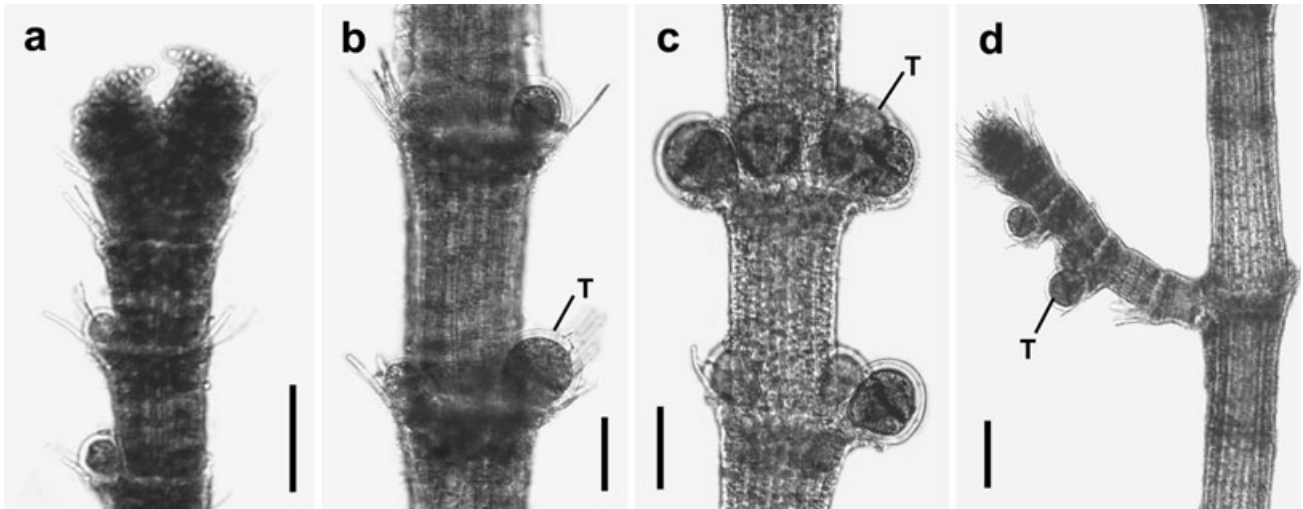


FIG. 6. *Centroceras hyalacanthum* Kützing (LAF-3-iii-03-1-2; slides 1, 3, and 4) from North Skyway, St. Petersburg, Pinellas Co., FL, USA. (a–c) Cortical nodes with tetrasporangia in upper (a), middle (b), and lower (c) thallus parts, arranged in a whorl around the axis from the abaxial to adaxial sides. Scale bars, 50  $\mu\text{m}$ . (d) Adventitious branch with tetrasporangia at node. Scale bar, 100  $\mu\text{m}$ . T, tetrasporangium.

Satellite Beach, Brevard Co, coll. B. Y. Won and T. O. Cho, 5. iii. 2003; North Skyway, St. Petersburg, Pinellas Co., coll. B. Y. Won and T. O. Cho, 3. iii. 2003.

**Morphology.** Thalli are rose pink and 2–5 cm high, form dense tufts, and consist of erect and prostrate axes (Figs. 5a and 11e). Branching is pseudodichotomous (Fig. 5b); branches develop at intervals of 9–10 (average  $9.3 \pm 0.7$ ) axial cells in the main axes and at intervals of 8–11 (average  $9.0 \pm 1.2$ ) axial cells in the lateral axes. In addition, adventitious branches develop from periaxial cells in the lower part of the thallus.

Erect axes have forcipulate, slightly inrolled apices beset with spines oriented at a  $45^\circ$  angle on the upper part of each node (Figs. 5, b and c, and 11f) and measure 140–165  $\mu\text{m}$  in diameter. Ten to 13 periaxial cells are cut off obliquely from the upper part of each axial cell and remain at the nodes after axial cell elongation (Fig. 5, g–i). All periaxial cells produce the cortical initials that divide into cortical filaments contributing to the cortex. The first periaxial cell on the abaxial side cuts off four cortical initials, the first three from their upper end and the fourth from their lower ends (Fig. 5k). The first cortical initial cuts off a spine and two elongated cortical cells (Fig. 5k). The second and third cortical initials cut off one elongated cortical cell acropetally and a filament basipetally by transverse division (Fig. 5k). The fourth cortical initial cuts off a filament basipetally (Fig. 5k). The other periaxial cells each cut off three cortical initials, the first two from their upper end and the third from their lower end (Fig. 5, l–o). The first cortical initial cuts off a spine and two elongated cortical cells (Fig. 5l), a gland cell and two elongated cortical cells (Fig. 5, m and n), or three elongated cortical cells (Figs. 5o and 11h). The second cortical initial cuts off one elongated cortical cell acropetally and a filament basipe-

tally by transverse division (Fig. 5, l–o). The third cortical initial cuts off a filament basipetally (Fig. 5, l–o). The mature cortex is complete. The acropetal cortical filaments are two cells long including the cortical initial, and the basipetal cortical filaments are 15–20 cells long.

The whorled spines occur at each node throughout the thallus (Figs. 5, c–f, and 11, f and g). Each spine is straight and composed of three cells (Fig. 5, k and l) including the cortical initial. Ovoid gland cells are obliquely cut off from the first cortical initial (Fig. 5m). Numerous uniseriate and multicellular rhizoids are produced from periaxial cells and terminate in a pad (Fig. 5p).

In tetrasporic thalli, tetrasporangia, produced initially from the first-formed periaxial cell on the abaxial side, then on the adaxial side in an alternate sequence resulting in a whorl around the axis (Fig. 6, a–c), are distributed at the upper cortical nodes of the erect axes (Fig. 6, a–c) and on adventitious branches (Fig. 6d). A single tetrasporangium develops per periaxial cell. Mature spherical to ellipsoidal tetrasporangia, 45–55  $\mu\text{m}$  in diameter, almost completely project from the cortical nodes.

Male and female thalli were not found in our collections.

*Centroceras micracanthum* Kützing 1841: 741 (Figs. 7 and 11, i–l).

*Centroceras micracanthum* Kützing 1841: 741, 1849: 688, 1863: 7, pl. 18: figs. a–d.

*Lectotype*: L-0193939 (#939285-83)! (designated herein)

*Type locality*: Rio de Janeiro, Brazil.

*Heterotypic synonyms*: *Centroceras macracanthum* Kützing 1841: 741, 1849: 689, 1863: 7, pl. 19: figs. a–c [L-0193934 (#939285-77)]! *Type locality*: Brazil. *Centroceras leptacanthum* Kützing 1841: 741, 1849: 689,

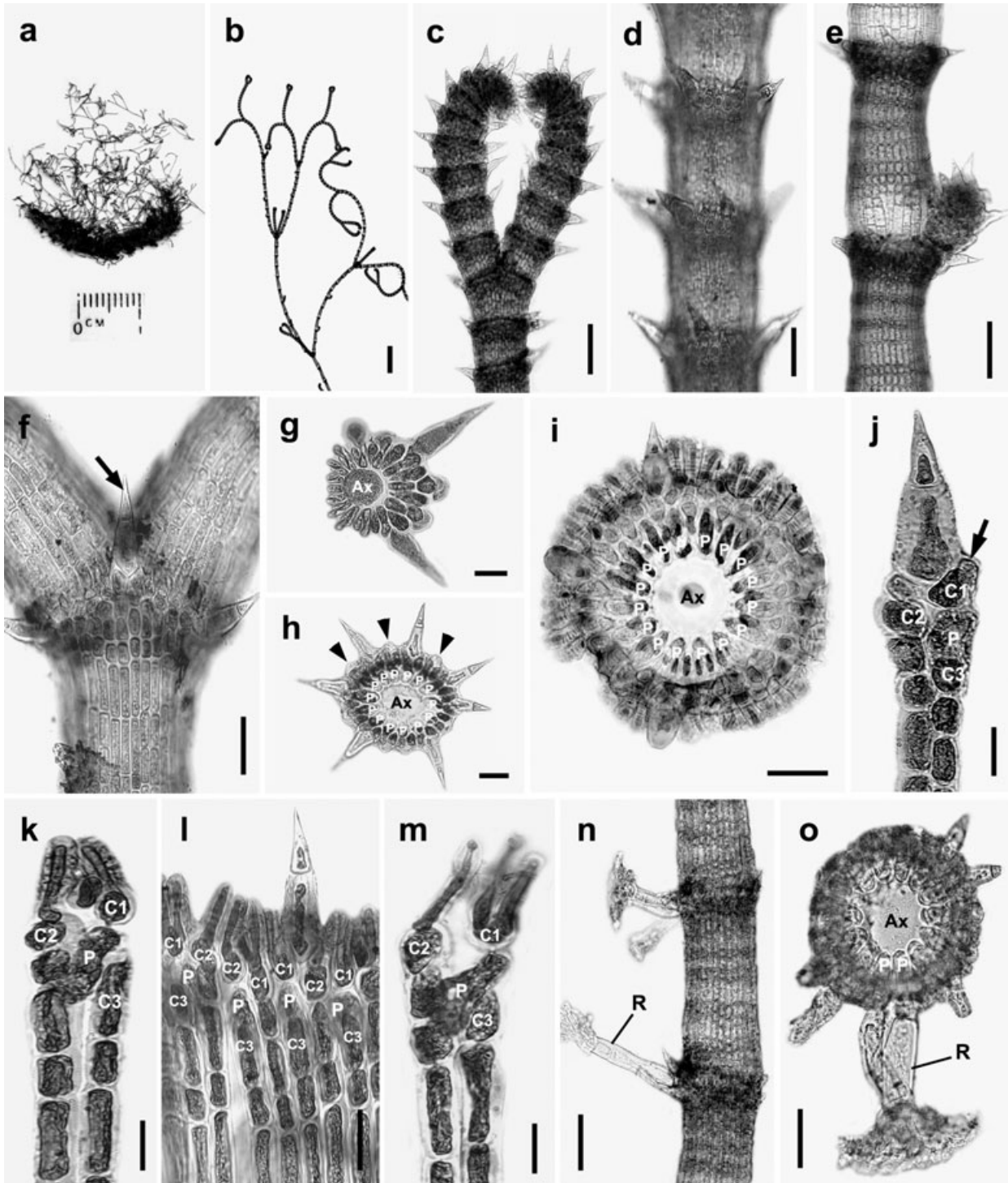


FIG. 7. *Centroceras micracanthum* Kützing (LAF-30-x-03-1-1; slides 1, 2, 4, 5, and 7) from Smathers Beach, Key West, FL, USA. (a) Vegetative specimen. (b) Thallus. Scale bar, 1 mm. (c) Apical region. Scale bar, 100  $\mu$ m. (d–e) Whorled arrangement of spines in middle (d) and lower (e) thallus parts. Scale bars, 50  $\mu$ m (d) and 100  $\mu$ m (e). (f) Branching point with spine (arrow). Scale bar, 50  $\mu$ m. (g–i) Cross-section views through cortical nodes on upper (g), middle (h), and lower (i) parts of thallus showing spines, gland cells (arrowheads), and elongated cortical cells. Scale bars, 20  $\mu$ m (g) and 50  $\mu$ m (h and i). (j) Young cortical unit showing the first cortical initial with a spine and a young cortical cell (arrow). Scale bar, 20  $\mu$ m. (k) Cortical unit showing the first cortical initial with an ovoid gland cell and an elongated acropetal cell. Scale bar, 20  $\mu$ m. (l) Mature cortication showing the spine and elongated cortical cells. Scale bar, 20  $\mu$ m. (m) Cortical unit showing the first cortical initial bearing two elongated cortical cells. Scale bar, 40  $\mu$ m. (n) Creeping thallus part with rhizoids. Scale bars, 100  $\mu$ m. (o) Cross-section view showing multicellular rhizoids with digitate tip, produced from periaxial cells. Scale bar, 50  $\mu$ m. Ax, axial cell; C1–3, sequence of cortical initials; P, periaxial cell; R, rhizoid.

1863: 7, pl. 18: figs. e–g [L-0193945 (#910167-101)]! Type locality: “Genua” (Kützing 1841: 741) [=Genoa, Liguria, Italy]. *Centroceras cryptacanthum* Kützing 1841:741, 1849, 689; Kützing 1863: 7, pl. 17: figs. a–d [L-0193944]. Type locality: Peru.

*Representative specimens examined.* Caribbean Panama: Punta Galeta, vic. STRI Galeta Lab, coll. B. Wysor, 21. ix. 1999 and 1. x. 1999. USA: Florida: Bridge to Key Biscayne, Miami-Dade Co., coll. M. H. Hommersand, J. Hughey and M. Volovsek, 8. iii. 1997; Smathers Beach, Key West, coll. B. Y. Won and T. O. Cho, 30. x. 2003.

*Morphology.* Thalli are rose pink and 2–3.5 cm high, form dense tufts, and consist of erect and prostrate axes (Figs. 7a and 11i). Branching is pseudodichotomous, rarely tri- or tetrachotomous (Fig. 7b); branches develop at intervals of 11–13 (average  $11.8 \pm 0.6$ ) axial cells in the main axes and at the intervals of 11–12 (average  $11.3 \pm 0.5$ ) axial cells in the lateral axes. In addition, adventitious branches develop from periaxial cells in the lower thallus part.

Erect axes have forcipulate, slightly inrolled apices beset with spines oriented at a  $45^\circ$  angle on the upper part of each node (Fig. 7c), and 160–190  $\mu\text{m}$  in diameter. Fourteen to 17 periaxial cells are cut off obliquely from the upper part of each axial cell and remain at the nodes after axial cell elongation (Fig. 7, g–i). All periaxial cells produce the cortical initials that divide into cortical filaments contributing to the cortex. The first periaxial cell on the abaxial side cuts off four cortical initials, the first three from their upper end and the fourth from their lower ends. The other periaxial cells each cut off three cortical initials, the first two from their upper end and the third from their lower end (Figs. 7, j–m, and 11l). The first cortical initial cuts off a spine and one elongated cortical cell (Figs. 7, j and l, and 11l), a gland cell and one elongated cortical cell (Fig. 7k), or two elongated cortical cells (Figs. 7m and 11l). The second cortical cell initial cuts off one elongated cortical cell acropetally and a filament basipetally by transverse division (Figs. 7, j–m, and 11l). The third cortical initial cuts off a filament basipetally (Figs. 7, j–m, and 11l). The mature cortex is complete. The acropetal cortical filaments are two cells long including the cortical initial, and the basipetal cortical filaments are 15–20 cells long.

Whorled spines occur at each node throughout the length of the thallus (Figs. 7, c–f, and 11, j and k). Each spine is straight and composed of three cells (Fig. 7, j and l) including the cortical initial. An ovoid gland cell is obliquely cut off from the first cortical initial (Fig. 7k). Numerous uniseriate and multicellular rhizoids are produced from periaxial cells and terminate in a pad (Fig. 7, n and o).

Male, female, and tetrasporangial thalli were not found in our collections.

*Centroceras rodmanii* Won, T. O. Cho et Fredericq sp. nov. (Figs. 8 and 9).

Thalli epiphytic, erecti, 2–5 cm alti, 120–140  $\mu\text{m}$  in diam., partim prostrati partim erecti, pseudodichotome ramosi. Apices forcipulati, leviter incurvati. Periaxiales cellulae 10–12, omnes formatae initia corticalia tres; initium corticale primum formans spinam aut cellulam glandularem; initium corticale secundum formans unicam cellulam corticalem filumque basipetale; initium corticale secundum formans solum filumque basipetale. Internodi omnino tecti unico strato cellularum corticalium. Spinae 3–4-cellulosae, uncinatae, formatae in verticillum in quoque nodo. Cellulae glandulosae complanatae. Multicellularia rhizoidea formata cellulis periaxialibus cells in magis veteribus ramis, affixa pulvino ad apicem. Tetrasporangia tetraeda,  $54 \pm 9 \mu\text{m} \times 34 \pm 6 \mu\text{m}$ , a cellulis periaxialibus, distributa in ramis ad ramificationis puncta projecta. Thalli feminei masculinique ignoti.

Thalli epiphytic, erect, 2–5 cm high, 120–140  $\mu\text{m}$  in diameter, partly prostrate and partly erect, pseudodichotomous branched. Apices forcipulate, slightly inrolled. Periaxial cells 10–12, each producing three cortical initials: the first cortical initial produces a spine or gland cell; the second cortical initial produces a single acropetal cortical cell and a basipetal filament; the third cortical initial produces only a basipetal cortical filament. Internodes completely covered with one layer of cortical cells. Spines 3–4 celled, hooked, born in a whorl at each node. Gland cells flattened. Multicellular rhizoids produced from periaxial cells on older branches, attached with a pad at the tip. Tetrasporangia tetrahedral,  $54 \pm 9 \mu\text{m}$  by  $34 \pm 6 \mu\text{m}$ , produced from periaxial cells, distributed in branches produced at branching points and projecting. Carposporangial and spermatangial thalli unknown.

*Holotype.* LAF-1-i-96-1-2 (TC435), Cocholgue, Bahía Concepción, Nuble, Chile, coll. M. H. Hommersand, 1. i. 1995; isotype: US Alg. Coll.

*Type locality.* Cocholgue, Bahía Concepción, Nuble, Chile ( $36^\circ 36' \text{S}$ ,  $72^\circ 57' \text{E}$ ).

*Etymology.* The epithet *Centroceras rodmanii* is chosen to honor Dr. James Rodman, former NSF Systematic Biology Program Director, for championing taxonomic research on neglected or esoteric groups of organisms.

*Representative specimens examined.* Chile: Cocholgue, Bahía Concepción, Nuble, coll. M. H. Hommersand, 1. i. 1995 (holotype and isotypes); Desembocadura Bio Bio, Concepción, southern Chile; coll. N. Arakaki, ix. 1998 (paratype).

*Morphology.* Thalli are rose pink, 3–5 cm high, form dense tufts, and consist of erect and prostrate axes (Fig. 8a). The branching pattern of the thalli is pseudodichotomous (Fig. 8b). Branches formed at intervals of 7–9 (average  $8.6 \pm 0.9$ ) axial cells in the main axes, and at intervals of 6–8 (average  $7.8 \pm 0.6$ ) axial cells in the lateral axes. In addition,

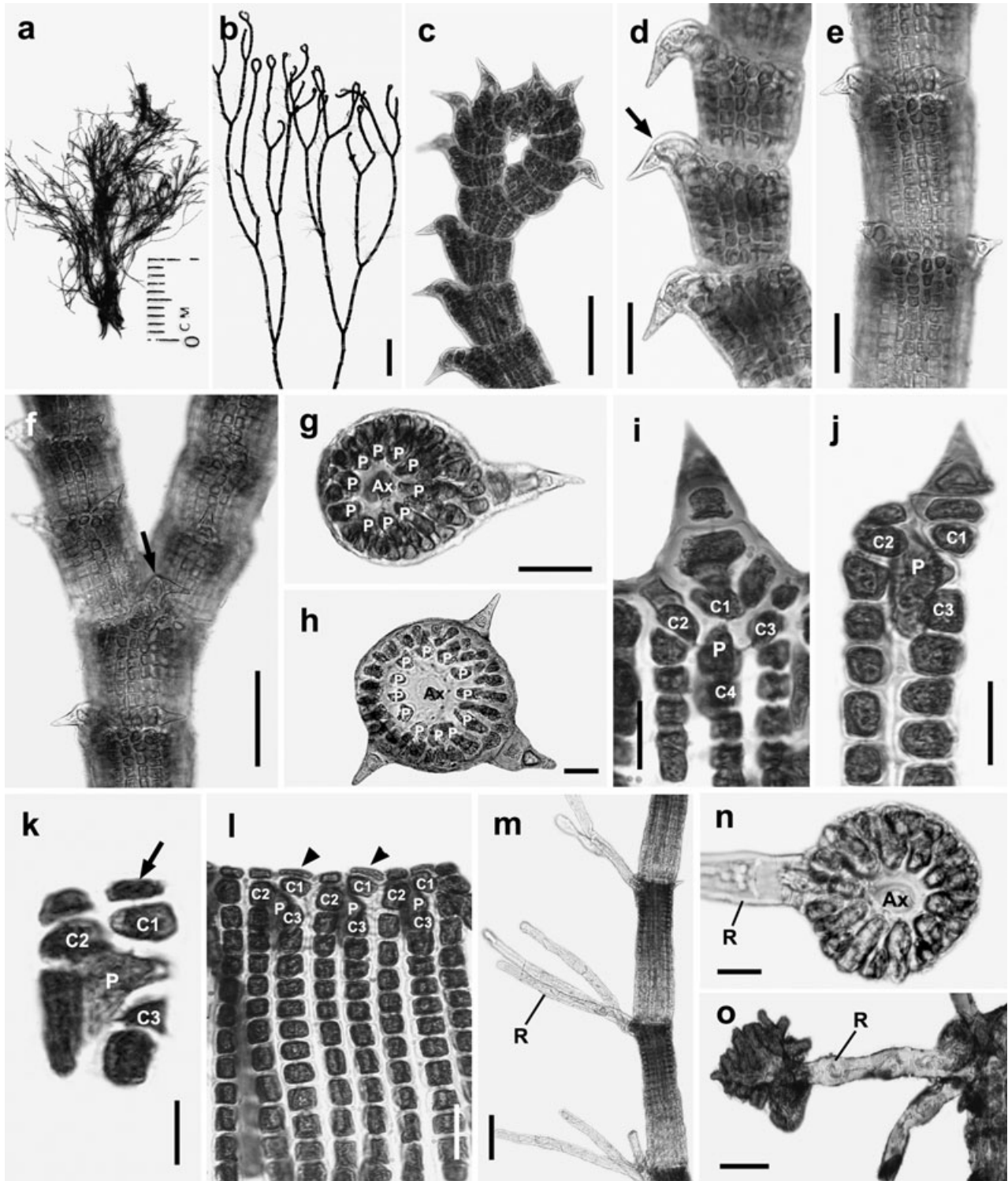


FIG. 8. *Centroceras rodmanii* sp. nov. (LAF-1-i-96-1-1; slides 1, 2, 4, 6, 7, and 9) from Cocholgue, Bahía Concepción, Chile. (a) Vegetative specimen. (b) Thallus. Scale bar, 1 mm. (c) Apical region. Scale bar, 100  $\mu$ m. (d–e) Whorled arrangement of hooked spines (arrow) in upper (c) and middle (d–e) parts of thallus. Scale bars, 50  $\mu$ m. (f) Branching point with spine (arrow). Scale bar, 100  $\mu$ m. (g, h) Cross-section views through cortical nodes of upper (g) and middle (h) parts of thallus. Scale bars, 50  $\mu$ m. (i) Cortical unit showing the first periaxial cell on abaxial side bearing four cortical initials. Scale bar, 20  $\mu$ m. (j) Cortical unit showing the first cortical initial bearing a spine. Scale bar, 20  $\mu$ m. (k) Young cortical unit showing the first cortical initial with a flattened gland cell (arrow). Scale bar, 20  $\mu$ m. (l) Mature cortication showing the flattened gland cells (arrowheads). Scale bar, 40  $\mu$ m. (m) Creeping part of thallus with rhizoids. Scale bars, 25  $\mu$ m. (n) Cross-section view showing rhizoid produced from periaxial cells. Scale bars, 100  $\mu$ m. (o) Digitate tip of rhizoid. Scale bar, 100  $\mu$ m. (o). Ax, axial cell; C1–4, sequence of cortical initials; P, periaxial cell; R, rhizoid.

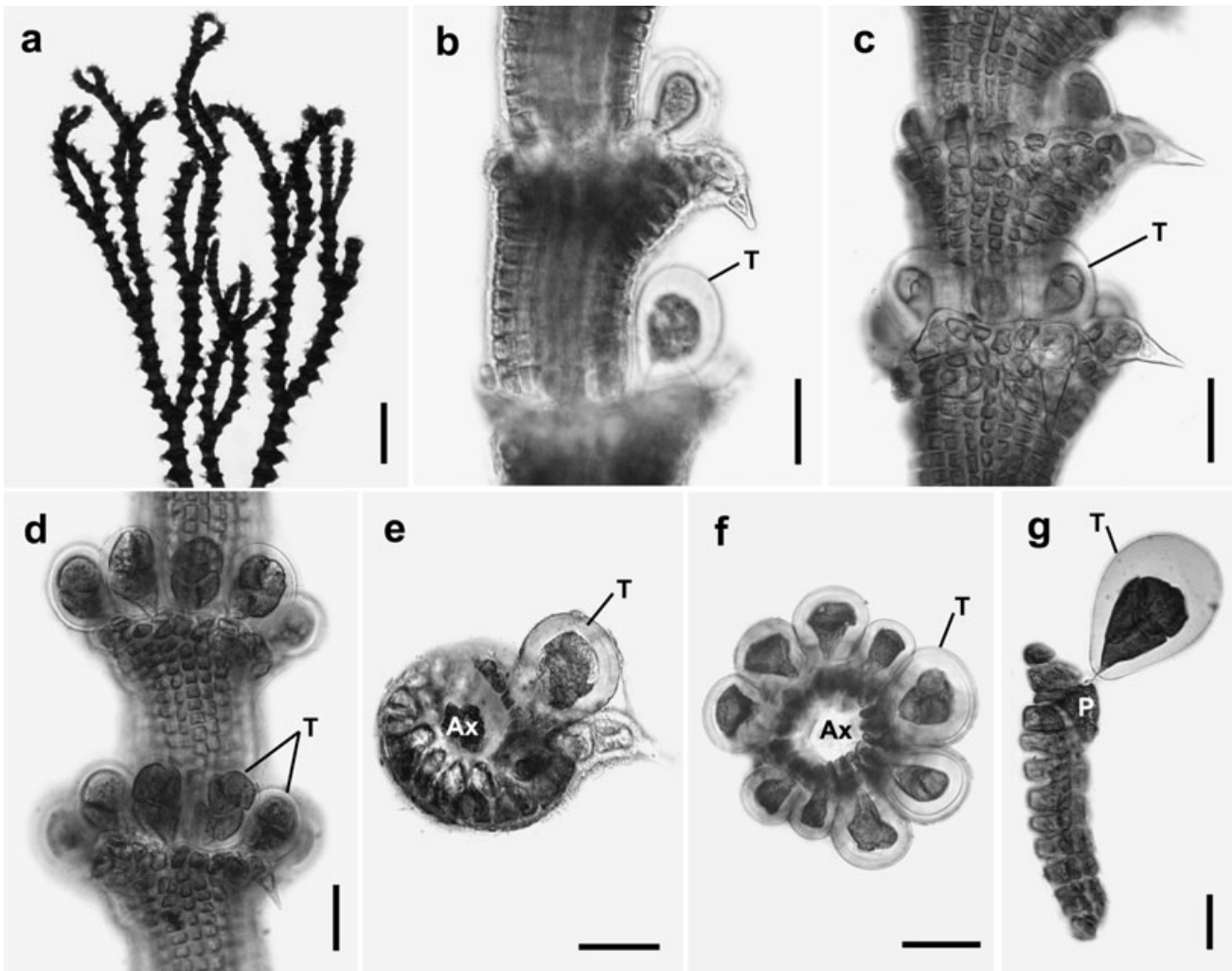


FIG. 9. *Centroceras rodmanii* sp. nov. (LAF-1-i-96-1-2; slides 2, 4, 5, and 7) from Cocholgue, Bahía Concepción, Chile. (a) Tetrasporangial thallus. Scale bar, 0.5 mm. (b–d) Cortical nodes with tetrasporangia cut off from the abaxial side to adaxial side in a whorl around the axis, and involucre branchlets, in upper (b), middle (c), and lower (d) parts. Scale bars, 50  $\mu$ m. (e–f) Cross-section views of the nodes showing tetrasporangia produced in alternate sequence. Scale bars, 40  $\mu$ m (e) and 50  $\mu$ m (f). (g) Cortical unit showing a tetrasporangium produced from a periaxial cell. Scale bar, 20  $\mu$ m. Ax, axial cell; P, periaxial cell; T, tetrasporangium.

adventitious branches develop from periaxial cells in the lower thallus part.

Erect axes, 120–140  $\mu$ m in diameter, have forcipulate, strongly inrolled apices beset with spines (Fig. 8c). Ten to 12 periaxial cells are cut off obliquely from the upper part of each axial cell and remain at the nodes after axial cell elongation (Fig. 8, g and h). All periaxial cells produce the cortical initials that divide into cortical filaments contributing to the cortex. The first periaxial cell on the abaxial side cuts off four cortical initials; the first three from their upper end and the fourth from their lower end (Fig. 8i). The first cortical initial cuts off a spine and a cortical cell (Fig. 8i). The second and third cortical initials cut off one cortical cell acropetally and a filament basipetally by transverse division (Fig. 8i). The fourth cortical initial cuts off a filament basipetally (Fig. 8i). The

other periaxial cells cut off three cortical initials; the first two from their upper end and the third from their lower end (Fig. 8, j–l). The first cortical initials cut off a single spine (Fig. 8j) or a gland cell (Fig. 8k). The second cortical initial cuts off one cell acropetally and a filament basipetally by transverse division (Fig. 8, j–l). The third cortical initial cuts off a filament basipetally (Fig. 8, j–l). The mature cortex is complete. The acropetal cortical filaments are two cells long including the cortical initial, and the basipetal cortical filaments 15–22 cells long.

Spines occur at each node in a whorl on the upper and lower parts of the thallus (Fig. 8, c–f). Each spine is hooked (Fig. 8, c–f) and is composed of 3–4 cells (Fig. 8, i and j) including the cortical initial. A flattened gland cell is produced horizontally from the first cortical initial (Fig. 8, k and l).

Numerous uniseriate and multicellular rhizoids are produced from periaxial cells and terminate in a pad (Fig. 8, m–o).

In tetrasporic thalli, tetrasporangia, distributed along the upper cortical nodes of the erect axes (Fig. 9a), are produced initially from the first-formed periaxial cell on the abaxial side, then on the adaxial side in an alternate sequence resulting in a whorl around the axis (Fig. 9, b–f). A single tetrahedral tetrasporangium develops per periaxial cell (Fig. 9g). Mature tetrasporangia, spherical to ellipsoidal, average  $54 \pm 9 \mu\text{m}$  by  $34 \pm 6 \mu\text{m}$  excluding the sheath, and  $67 \pm 7 \mu\text{m}$  by  $51 \pm 6 \mu\text{m}$  with the sheath, almost completely project from the cortical node (Fig. 9d).

Male and female thalli were not found in our collections.

*Centroceras tetrachotomum* Won, T. O. Cho et Fredericq sp. nov. (Fig. 10).

Thalli epiphytici, erecti, 1.5–3.2 cm alti, 150–185  $\mu\text{m}$  in diam., partim prostrati partim erecti, tetrachotome ramosi. Apices forcipulati, valde incurvati. Periaxiales cellulae 15–19, omnes formatae initia corticalia tres; initium corticale primum vel ramosum dichotomum vel aramosum; initium corticale secundum formans filum acropetale filumque basipetale; initium corticale tertium formans solum filumque basipetale. Internodi omnino tecti unico strato cellularum corticalium isodiametrarum. Spinae 2–3-cellulosae, rectae, formatae initiis corticalibus primis. Cellulae glandulosae complanatae, formata initiis corticalibus primis. Multicellularia rhizoidea formata cellulis periaxialibus cells in magis veteribus ramis, affixa pulvino ad apicem. Tetrasporangia tetraeda,  $46 \pm 4 \mu\text{m}$  by  $37 \pm 4 \mu\text{m}$ , a cellulis periaxialibus, distributa in ramis plerumque ad ramificationis puncta projecta. Thalli feminei masculinique ignoti.

Thalli epiphytic, erect to about 1.5–3.2 cm high, 150–185  $\mu\text{m}$  in diameter, partly prostrate and partly erect, and tetrachotomously branched. Apices forcipulate, strongly inrolled, 15–19 periaxial cells, each producing three cortical initials: the first cortical initial is either dichotomously branched or unbranched; the second cortical initial produces a two-celled acropetal filament and a basipetal filament; and the third cortical initial produces only a basipetal filament. Internodes completely covered with one layer of isodiametric to rectangular cortical cells. Spines 2–3 celled, straight, produced from the first cortical initial, arranged in a row on the abaxial side or rarely in a whorl. Gland cells flattened, produced from the first cortical initials. Multicellular rhizoids develop from periaxial cells on older branches, multicellular, attached with a pad at the tip. Tetrasporangia tetrahedral,  $46 \pm 4 \mu\text{m}$  by  $37 \pm 4 \mu\text{m}$ , produced from periaxial cells, and project from branches usually produced at branching points. Female and male plants unknown.

*Holotype*: LAF-25-i-01-1-1 (TC415), Lambert Bay, Western Cape, South Africa, coll. M. H. Hommersand, 25. i. 2001; isotype: US Alg. Coll.

*Type locality*: South Africa: Lambert Bay, Western Cape ( $32^{\circ}05' \text{S}$ ,  $18^{\circ}17' \text{E}$ ).

*Etymology*: The species name *tetrachotomum* reflects on the tetrachotomous branching pattern characteristic of this species.

*Representative specimens examined*. South Africa: Lambert Bay, Western Cape, coll. M. H. Hommersand, 25. i. 2001 (holotype and isotypes).

*Morphology*. Thalli are rose pink, 1.5–3.2 cm high, form dense tufts, and consist of erect and prostrate axes (Fig. 10a). Branching is tetrachotomous (Fig. 10b) with branches developing at intervals of 8–9 (average  $8.6 \pm 0.5$ ) axial cells in the main axes, and at intervals of 7–8 (average  $7.3 \pm 0.5$ ) axial cells in the lateral axes. In addition, adventitious branches develop from periaxial cells in the lower thallus part.

Erect axes, 150–185  $\mu\text{m}$  in diameter, have forcipulate, slightly inrolled apices beset with spines oriented at a  $45^{\circ}$  angle on the upper part of each node (Fig. 10, c and d). Fifteen to 19 periaxial cells are cut off obliquely from the upper part of each axial cell and remain at the nodes after axial cell elongation (Fig. 10, f and g). All periaxial cells produce cortical initials that divide into cortical filaments contributing to the cortex. The first periaxial cell on the abaxial side cuts off four cortical initials, the first three from their upper end and the fourth from their lower ends. The other periaxial cell cuts off three cortical cell initials each, the first two from their upper end and the third from their lower end. The first cortical initial cuts off a spine and one cortical cell (Fig. 10h), the gland cell (Fig. 10i), or two cortical cells (Fig. 10k). The second cortical initial cuts off two celled cortical filaments acropetally and a filament basipetally by transverse division (Fig. 10, h–k). The third cortical initial cuts off a filament basipetally (Fig. 10, h–k). The mature cortex is complete. The acropetal cortical filaments are three cells long including the cortical initial, and the basipetal cortical filaments are 20–25 cells long.

Spines are arranged in a row on the abaxial side or rarely in whorl in the upper and lower parts of the thallus (Fig. 10, c–e). Each spine is straight and composed of three cells long including the cortical cell initial (Fig. 10h). A flattened refractive gland cell is produced horizontally from the first cortical initial (Fig. 10, i and j). Numerous uniseriate and multicellular rhizoids are produced from periaxial cells terminating in a pad.

Tetrasporangia, distributed along several upper cortical nodes of the erect axes adventitious branches (Fig. 10, b, l, and m), are produced initially from the first-formed periaxial cell on the abaxial side, then on the adaxial side in an alternating sequence resulting in a whorl around the axis

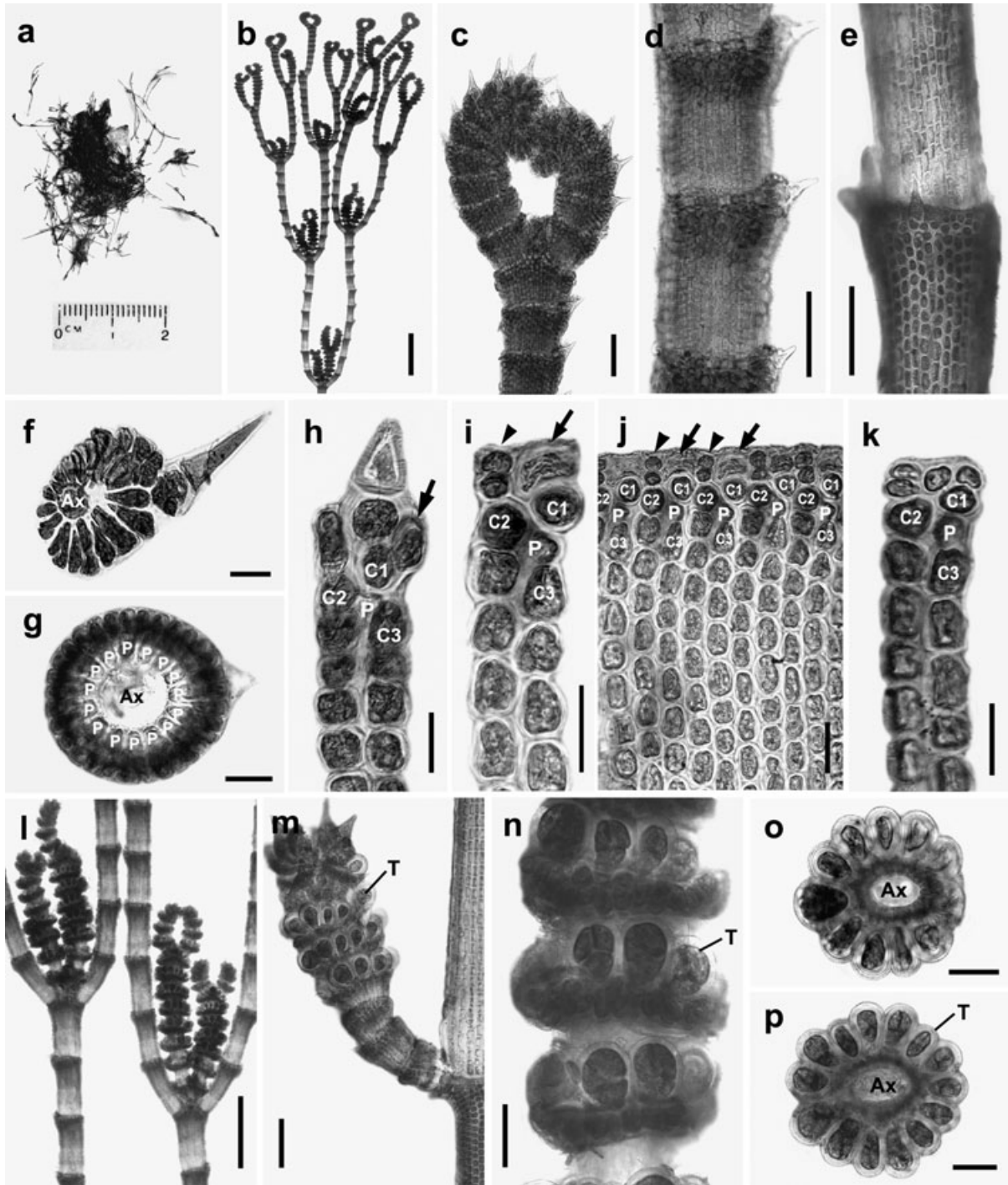


FIG. 10. *Centroceras tetrachotomum* sp. nov. (LAF-25-i-01-1-1; slides 1, 2, 4, 5, 6, and 9) from Lambert Bay, Western Cape, South Africa. (a) Tetrasporic specimen. (b) Tetrasporophyte showing tetrachotomous branching. Scale bar, 1 mm. (c) Apical region. Scale bar, 100  $\mu$ m. (d–e) Whorled arrangement of spines in middle (d) and lower (e) parts. Scale bars, 100  $\mu$ m. (f–g) Cross-section views through cortical nodes of upper (f) and middle (g) thallus parts. Scale bars, 20  $\mu$ m (f) and 50  $\mu$ m (g). (h) Cortical unit showing the first cortical initial bearing a spine and a cortical cell (arrow). Scale bar, 20  $\mu$ m. (i) Cortical unit showing the first cortical initial bearing a flattened gland cell (arrow) and the second cortical initial bearing a two-celled filament (arrowhead). Scale bar, 20  $\mu$ m. (j) Mature cortication showing the flattened gland cells (arrows) borne on first cortical initials and the two-celled cortical filaments (arrowheads) borne on second cortical initials. Scale bar, 20  $\mu$ m. (k) Cortical unit showing the branched first cortical initial bearing two cortical cells. Scale bar, 20  $\mu$ m. (l) Determinate axes with tetrasporangia. Scale bar, 500  $\mu$ m. (m) Adventitious branch with tetrasporangia at the node. Scale bar, 100  $\mu$ m. (n) Cortical nodes with tetrasporangia in a whorl around the axis. Scale bars, 50  $\mu$ m. (o, p) Cross-section views of the nodes showing tetrasporangia produced in alternate sequence. Scale bars, 50  $\mu$ m. Ax, axial cell; C1–3, sequence of cortical initials; P, periaxial cell; T, tetrasporangium.

(Fig. 10, n–p). One tetrahedral tetrasporangium develops per periaxial cell. Mature tetrasporangia, spherical to ellipsoidal, average  $46 \pm 4 \mu\text{m}$  by  $37 \pm 4 \mu\text{m}$  excluding the sheath and  $62 \pm 2 \mu\text{m} \times 46 \pm 5 \mu\text{m}$  with the sheath, almost completely project from the cortical node.

Male and female thalli were not found in our collections.

We examined Kützing's type material located in the Leiden Herbarium (L) for comparison with recently collected material (Fig. 11 and Table 1).

Key to the species previously known as "*Centroceras clavulatum*":

1. Gland cells at nodes flattened ..... 2
1. Gland cells at nodes ovoid ..... 4
2. Spines at nodes hooked ..... *C. rodmanii*
2. Spines at nodes straight ..... 3
3. Tetrachotomous branching; second cortical cell initial bearing a two-celled cortical filament ..... *C. tetrachotomum*
3. Pseudodichotomous or trichotomous branching; second cortical initial bearing a single cortical cell ..... *C. clavulatum*
4. Terminal acropetal cortical cells elongated ..... 5
4. Terminal acropetal cortical cells ovoid ..... *C. gasparrinii*
5. First cortical cell initial bearing two cortical cells ..... *C. micracanthum*
5. First cortical cell initial bearing three cortical cells ..... *C. hyalacanthum*

*Phylogenetic analyses.* A 1,396 bp portion of the 1,467 bp *rbcL* gene (95.16% nucleotides sequenced) was sequenced that included 297 parsimony informative sites, excluding the outgroup. Interspecific pairwise comparison of the *rbcL* sequences reveals 3.7%–8.2% sequence divergence among species within *Centroceras*. The SSU rDNA data set was analyzed based on a 1,694 bp portion that includes 49 informative sites, excluding the outgroup. Pair-wise comparison of SSU sequences among species reveals 0.7%–5.2% sequence divergence. Pair-wise comparison of LSU sequences among species reveals a 4.1%–11.9% sequence divergence.

All phylogenetic analyses of *rbcL*, LSU rDNA, and SSU rDNA are consistent with the morphological distinctions that are here used to resurrect *C. gasparrinii*, *C. micracanthum*, and *C. hyalacanthum* and to define *C. rodmanii* sp. nov. and *C. tetrachotomum* sp. nov. as new species. ML phylogenetic trees were obtained from the alignment of the separate *rbcL* (Fig. 12), SSU rDNA, and LSU rDNA (Fig. 13) sequences. The topologies of ML and MP of the chloroplast encoded *rbcL* and the nuclear encoded LSU rDNA and SSU rDNA are congruent except for the phylogenetic position of *C. rodmanii* and *C. minutum* (Figs. 12 and 13). All ML trees of the *rbcL*, SSU, and LSU rDNA sequences distinguish the *Centroceras clavulatum* complex as comprising nine distinct species with robust bootstrap support,

namely, *C. clavulatum*, *C. gasparrinii*, *C. hyalacanthum*, *C. micracanthum*, *C. rodmanii* sp. nov., *C. tetrachotomum* sp. nov., *C. sp.-1*, *C. sp.-2*, and *C. sp.-3*. MP and neighbor-joining (NJ) analyses of *rbcL*, SSU, and LSU rDNA produced trees of identical topology to the ML results. The partition homogeneity test was performed and the *P*-value was 0.01. This value did not support combining the *rbcL*, SSU, and LSU rDNA data sets.

#### DISCUSSION

The molecular and morphological analyses indicate that specimens identified and reported as *Centroceras "clavulatum"* are often misidentified and in fact are nine discrete species. Prior to this study, the species concept was too broadly defined and thus contained distinct taxa that were incorrectly considered synonyms or overlooked or undescribed species. Our morphological evidence has shown that *Centroceras clavulatum* (C. Agardh) Montagne should be restricted to specimens with pseudo- or trichotomous branching pattern, straight spines, and flattened gland cells. Careful morphological study of all type material of species of *Centroceras* described by Kützing (1841, 1849) and deposited in the Leiden Herbarium (L) has revealed three validly published species (Table 1), *Centroceras gasparrinii* (Meneghini) Kützing, *C. hyalacanthum* Kützing, and *C. micracanthum* Kützing, which are here recognized as distinct species. Two other species are recognized and herein described, *Centroceras rodmanii* sp. nov. and *Centroceras tetrachotomum* sp. nov. Additionally, three others (*C. sp.-1*, *C. sp.-2*, and *C. sp.-3*) from South Africa are also recognized as distinct species.

*Centroceras gasparrinii*, *C. hyalacanthum*, and *C. micracanthum* have rarely been reported since Kützing (1841, 1849, 1863), and these names have long been considered as synonyms of *C. clavulatum* (e.g., Silva et al. 1996). However, the present study clearly shows that these taxa can be separated vegetatively from *C. clavulatum* (C. Agardh) Montagne, based on the comparative development of the acropetal cortical filaments. The two ovoid acropetal cortical cells cut off by the first cortical initial in *C. gasparrinii*, and the elongated terminal acropetal cortical cells in *C. hyalacanthum* and *C. micracanthum* clearly represent important distinguishing features characterizing these species. Recent collections of these species agree well in their habit, branching pattern, and cortical cell arrangement with that of type material of Kützing (1841, 1849). *Centroceras hyalacanthum* is similar to *C. micracanthum* in having elongated acropetal cortical cells, but *C. hyalacanthum* is distinguished by having up to three, and *C. micracanthum* two elongated cortical cells cut off from the first cortical initial (Table 2). Our observations of type material indicate that *Centroceras inermis* Kützing (1849) is conspecific with *C. gasparrinii*



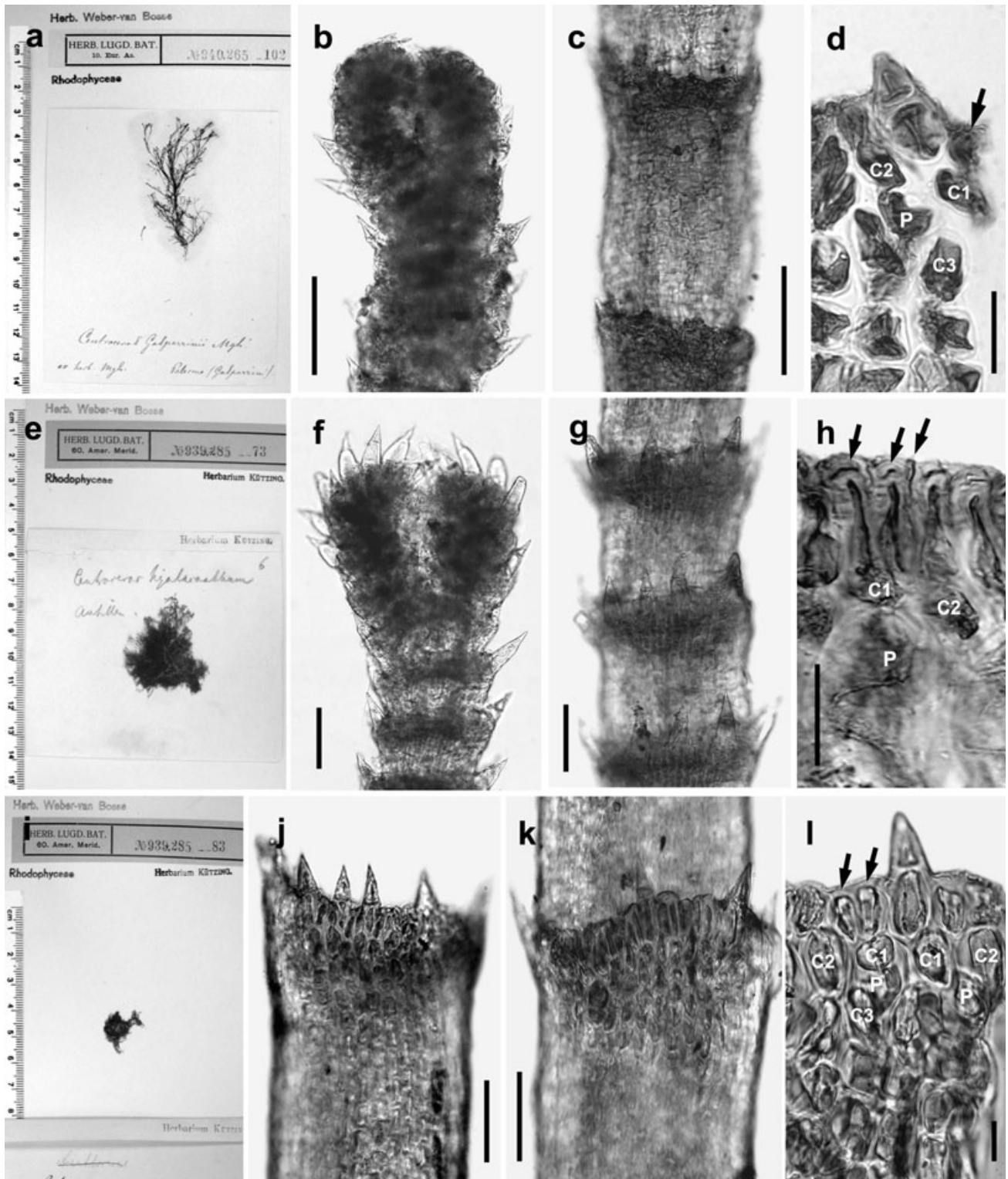


FIG. 11. (a–d) Type specimens (L0193946) of *Centroceras gasparrinii*. (a) Habit. (b) Apical region. Scale bar, 100  $\mu$ m. (c) Cortical nodes, with whorled spines. Scale bar, 100  $\mu$ m. (d) Cortical unit showing the first cortical initial bearing a spine and cortical cell (arrow). Scale bar, 20  $\mu$ m. (e–h) Type specimens (L0193933) of *C. hyalacanthum*. (e) Habit. (f) Apical region. Scale bar, 100  $\mu$ m. (g) Cortical nodes, with whorled spine. Scale bar, 100  $\mu$ m. (h) Cortical unit showing the first cortical initial bearing three elongated cortical cells (arrows). Scale bar, 20  $\mu$ m. (i–l) Type specimens (L0193939) of *C. micracanthum*. (i) Habit. (j, k) Cortical nodes, with whorled spines. Scale bars, 40  $\mu$ m. (l) Cortical unit showing the first cortical initial bearing two elongated cortical cells (arrows). Scale bar, 4.5  $\mu$ m. C1–3, sequence of cortical initials; P, periaxial cell.

TABLE 1. Historical *Centroceras* specimens Kützing: L(1).

Species	Barcode	Described as	Collecting information (specimen number; location; collector)
<i>Centroceras clavulatum</i>	L-0193938	<i>C. cryptocanthum</i> var. <i>longianticulatum</i> Kützing 1841: 742	Weber-van Bosse Herb. #939285-69; Pacific Peru; Bartling
<i>C. gasparrinii</i>	L-0193931	<i>C. inermis</i> Kützing 1849: 688	Weber-van Bosse Herb. #938316-447; Côtes de Guinée; Suringar, W. F. R.
	L-0193932	<i>C. inermis</i> Kützing 1849: 688	Weber-van Bosse Herb. #938316-442; Guinée; Kützing, F. T.
	L-0193946	<i>Centroceras gasparrinii</i> (Meneghini) Kützing Meneghini Hab. 1844: 186	Weber-van Bosse Herb. #940265-102; Palermo, Sicily; Weber-van Bosse, A. A.
	L-0193933	<i>C. hyalacanthum</i> Kützing 1841: 742	Weber-van Bosse Herb. #939285-73; Anülles (F.W.I.); Kützing, F. T.
<i>C. hyalacanthum</i>	L-0193935	<i>C. brachyacanthum</i> Kützing 1863: 688	Weber-van Bosse Herb. #939285-71; Anülles (F. W. I.); Kützing, F. T.
	L-0193936	<i>C. oxyacanthum</i> Kützing 1841: 741	Weber-van Bosse Herb. #939285-79; Cuba; Kützing, F. T.
	L-0193937	<i>C. hyalacanthum</i> Kützing 1841: 742	Weber-van Bosse Herb. #939285-75; Cuba Mont.; Montagne hb.
	L-0193940	<i>C. hyalacanthum</i> Kützing 1841: 742	Weber-van Bosse Herb. #940254-2; Vera Cruz, Mexico GOM; Kützing, F. T.
	L-0193934	<i>C. macracanthum</i> Kützing 1841: 741	Weber-van Bosse Herb. #939285-77; Brazil; Kützing, F. T. Kunth
	L-0193939	<i>C. micracanthum</i> Kützing 1841: 741	Weber-van Bosse Herb. #939285-83; Rio de Janeiro. Brazil; Kützing, F. T.
	L-0193941	<i>C. leptacanthum</i> Kützing 1841: 741	Weber-van Bosse Herb. #940265-52; Genoa 27. Aug. 1828 Von Hrn. V. Martens; Kützing, F. T.
<i>C. micracanthum</i>	L-0193942	<i>C. leptacanthum</i> Kützing 1841: 741	Weber-van Bosse Herb. #940265-51; Messina; Nagel; Kützing, F. T.
	L-0193943	<i>C. leptacanthum</i> Kützing 1841: 741	Weber-van Bosse Herb. #940265-49; Dalmatic; Kützing, F. T.
	L-0193944	<i>C. cryptocanthum</i> Kützing 1841: 741	Weber-van Bosse Herb. #940265-47; Kützing, F. T.
	L-1093945	<i>C. leptacanthum</i> Kützing 1841: 741	Weber-van Bosse Herb. #910167-101; Genua Italy; Martens

(Meneghini) Kützing (1849), and that *C. oxyacanthum* Kützing (1841) and *C. brachyacanthum* Kützing (1863) are conspecific with *C. hyalacanthum* Kützing (1841). Furthermore, *C. macracanthum* Kützing (1841), *C. leptacanthum* Kützing (1841), and *C. cryptocanthum* Kützing (1841) are also conspecific with *C. micracanthum* Kützing (1841).

*Centroceras rodmanii* sp. nov. is distinguished by having several hooked spines, one present in each node of every individual thallus, and flattened gland cells. Although Grunow (1868) described *C. clavulatum* var. *uncinatum* from Chile, and Hoffmann and Santelices (1997) reported this species as "*C. clavulatum*" with detailed spine drawings, *C. rodmanii* sp. nov. is here recognized as a new species in the genus *Centroceras*. *Centroceras tetrachotomum* sp. nov. is distinguished from other species of *Centroceras* by its characteristic tetrachotomous branching pattern and by the second cortical cell bearing a two-celled cortical filament; the second cortical initial in all other *Centroceras* species produces only a single acropetal cortical cell (see Table 2).

The characters used to distinguish species in this study are primarily cortical filament morphology: shape of the acropetal cortical cells, number of acropetal cortical cells, shape of gland cells, and shape of spines (Fig. 14). The development of the cortical filaments has been accepted as one of the important features for recognizing species in the tribe Ceramieae (Womersley 1978). Most characters related to acropetal cortication, as advanced in this study, have been overlooked and not used before in the taxonomy of *Centroceras*. These characters may allow for easy identification of the different species when reproductive stages are lacking.

The acropetal cortical filaments develop from the first and second cortical initials. Acropetal cortical cells produced from the first cortical initial may have more systematic information than those produced from the second cortical initial, because the first cortical initial bears cortical cells, spines, and/or gland cells, which have different shapes and numbers that are specific to the various species (Fig. 14). Two different shapes of acropetal cortical cells are here recognized: ovoid in *C. clavulatum*, *C. gasparrinii*, *C. tetrachotomum* sp. nov., and *C. rodmanii* sp. nov. versus elongated in *C. hyalacanthum* and *C. micracanthum*. Three groups based on the number of cortical cells (including spines) from the first cortical initials are here recognized: only one in *C. rodmanii* sp. nov.; two in *C. clavulatum*, *C. gasparrinii*, *C. micracanthum*, and *C. tetrachotomum* sp. nov.; and three in *C. hyalacanthum* (Fig. 14). Specimens of *Centroceras rodmanii* sp. nov. may bear a spine or a gland cell on the first cortical initial, while other species studied herein possess a spine and cortical cell, a gland cell and cortical cell, or only a gland cell (Fig. 14). Although the shape of their acropetal cortical cells is similar, *Centroceras*

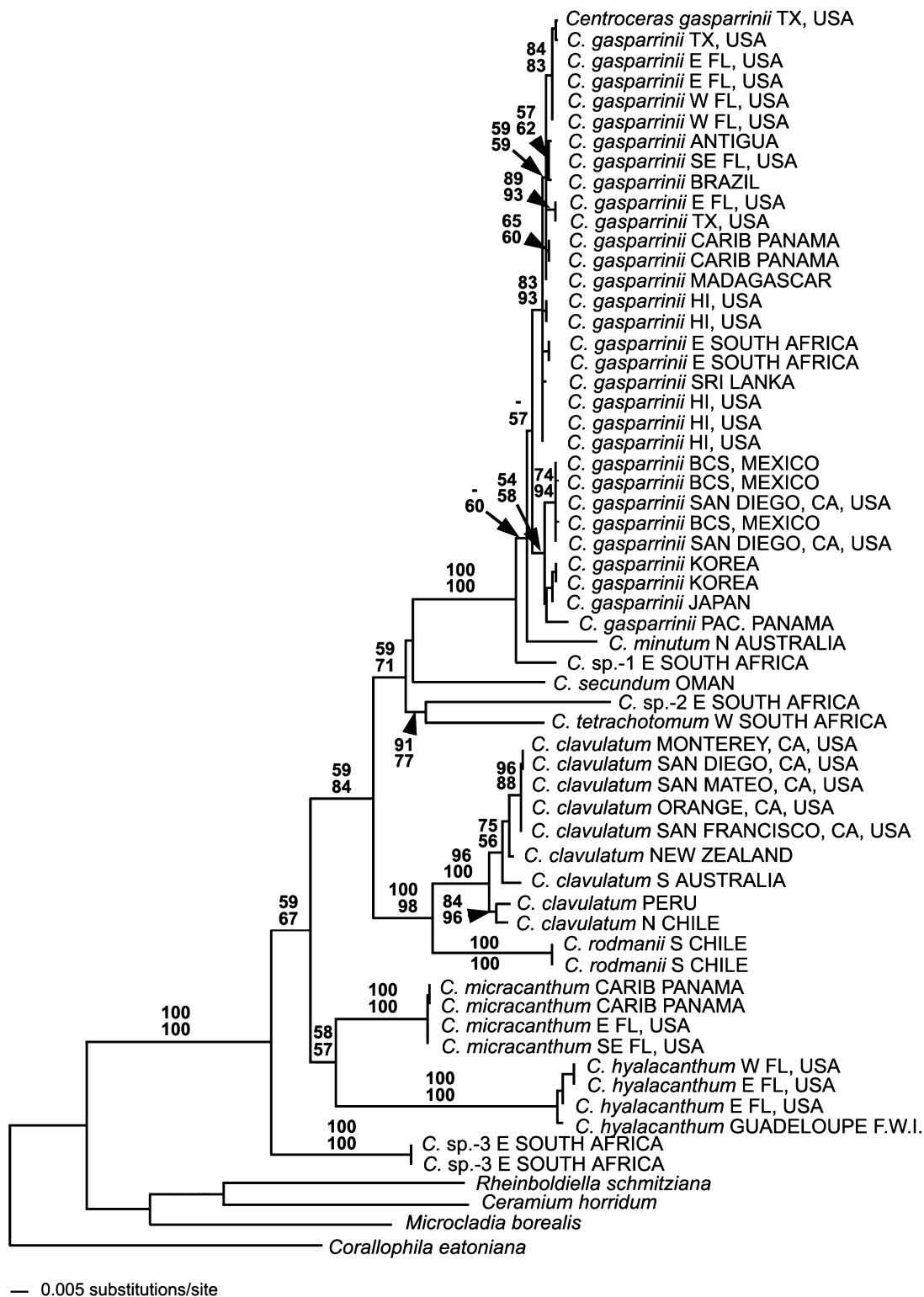


FIG. 12. Phylogeny of the *Centroceras* based on the *rbcL* sequences inferred from maximum-likelihood analyses using the GTR (General Time Reversible model, Rodriguez et al. 1990) +I (Invariable sites) +G (Gamma distribution). The parameters were as follows: assumed nucleotide frequencies A = 0.3207, C = 0.1326, G = 0.2047, T = 0.3419; substitution rate matrix with A-C substitutions = 1.6487, A-G = 5.0018, A-T = 2.3218, C-G = 1.3394, C-T = 21.3468, G-T = 1.0000; proportion of sites assumed to be invariable = 0.6095 and rates for variable sites assumed to follow a gamma distribution with shape parameter = 2.3126. Bootstrap proportion values (>50%) for ML (100 replicates, upper) and MP (1,000 replicates, lower) are shown at the nodes.



TABLE 2. Comparison of morphological features among *Centroceras* species.

	Cortical cell number from first cut off cortical initial	Terminal cell shape	Spine shape	Spine presence/absence; arrangement	Gland cell; shape	Cortical cell number from second cortical initial	Branching pattern	Tetrasporangial cover	Periaxial cell number	Distribution	Reference
<i>C. clavulatum</i> (collected near type locality)	2	Ovoid	Straight	Abaxial/whorled	Flattened	1	Subdichotomous	No involucre; projecting	13–16	Australia, New Zealand, Peru, and N. Chile	This study
<i>C. gasparinii</i> (type material!)	2	Ovoid	Straight	Whorled	Ovoid	1	Di-trichotomous	Involucre; covered	13–19	Atlantic, Gulf of Mexico, Madagascar, Pacific S. Africa, Sri Lanka, and Caribbean	This study
<i>C. hyalacanthum</i> (type material!)	3	Elongated	Straight	Whorled	Ovoid	1	Dichotomous	No involucre; projecting	10–13	French West Indies and Florida	This study
<i>C. micracanthum</i> (type material!)	2	Elongated	Straight	Whorled	Ovoid	1	Dichotomous	–	14–16	Panama and Florida	This study
<i>C. rodmanii</i> sp. nov.	–	Ovoid	Hooked	Whorled	Flattened	1	Dichotomous	No involucre; projecting	10–12	Florida Chile	This study
<i>C. tetrachotomum</i> sp. nov.	2	Ovoid	Straight	Whorled	Flattened	2	Tetrachotomous	No involucre; projecting	15–19	S. Africa	This study
<i>C. internitens</i>	2	Elongated	No spine	–	Ovoid	1	Alternate	No involucre; projecting	16–20	Florida	Gallagher and Humm (1983)
<i>C. distichum</i>	2	Elongated	Straight	–	–	–	Alternate	No involucre; immersed in cortex	10–12	Japan, S. Africa	Okamura (1934), Itono (1977) Stegenga et al. (1997)
<i>C. japonicum</i>	–	–	No spine	–	–	–	Subdichotomous	No involucre;	Unknown	Ryukyu Island	Itono (1973)
<i>C. minutum</i>	2	Ovoid	–	–	Ovoid	1	Alternate	2-cell involucre; covered	6–8	Indo-Pacific	Yamada (1944), this study
<i>C. secundum</i>	2	Ovoid	Straight	Whorled	–	–	Alternate	–	8–11	Oman	Wynne (2003)
<i>C. corallophiloides</i>	2	–	No spine	–	–	2	Irregular	No involucre; projected	6–8	Hawaii	Norris (1993)

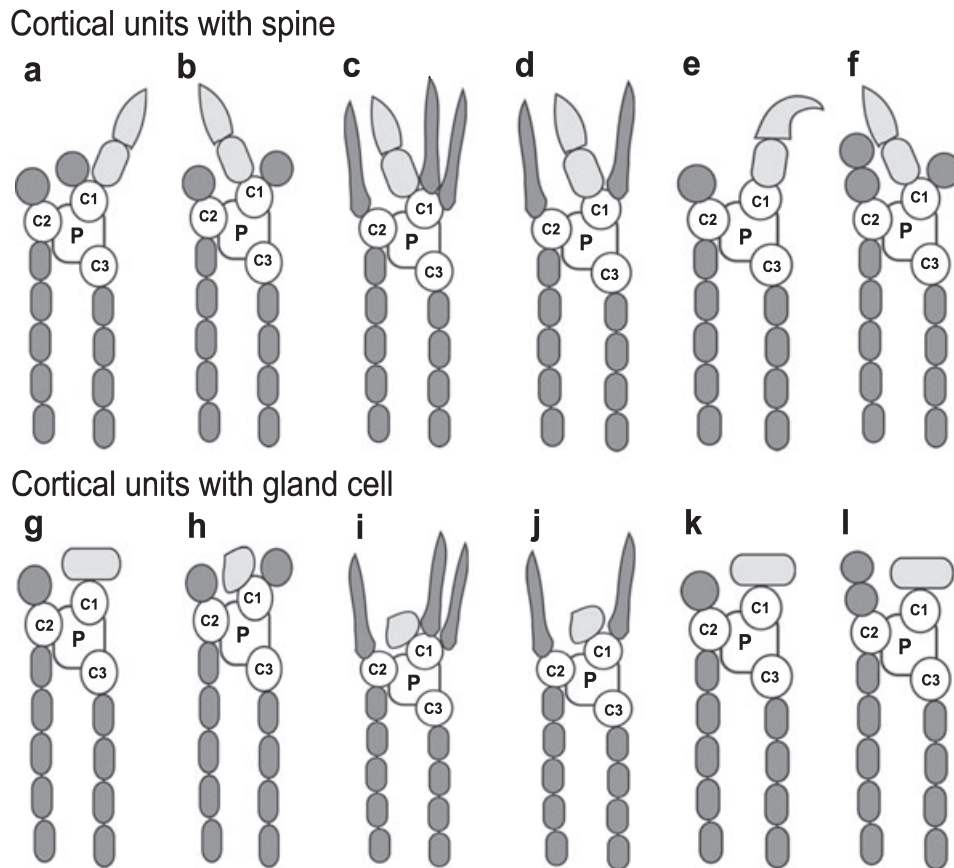


FIG. 14. Diagram for cortical units, with spines (a–f) or gland cells (g–l) in *Centroceras* species studied in this study: (a, g) *C. clavulatum*; (b, h) *C. gasparrinii*; (c, i) *C. hyalacanthum*; (d, j) *C. micracanthum*; (e, k) *C. rodmanii* sp. nov.; (f, l) *C. tetrachotomum* sp. nov. C1–3, sequence of cortical initials; P, periaxial cell.

*rbcl* sequence divergence, *Centroceras tetrachotomum* sp. nov. from South Africa is distinguished by 5.7%–8.1% gene sequence divergence from the other species, and forms a clade distinct from the other South African species: *Centroceras gasparrinii*, *C. sp.-1*, *C. sp.-2*, and *C. sp.-3*. *Centroceras rodmanii* sp. nov. is distinguished by 3.8%–7.8% gene sequence divergence from the other species of *Centroceras*. These ranges of sequence divergence among *Centroceras* species are larger than in those of other genera, such as *Ceramium* (Cho et al. 2003a,b, 2008) within the same tribe.

*Centroceras clavulatum* is widely reported as being a prime example of a cosmopolitan species (van den Hoek and Breeman 1990). Instead, based on comparative morphological and molecular analyses of specimens going under the name *C. "clavulatum"* worldwide, the distribution range of *C. clavulatum* is herein determined as restricted to the Pacific: northern Chile, Peru, Southern California, S. Australia, and New Zealand. Although there is low gene sequence divergence, 1.0%–1.2%, between populations around the type locality (Peru and northern Chile) and the other Pacific areas (California,

Australia, and New Zealand), they show a different arrangement of the spines. *Centroceras gasparrinii* is distributed in the Pacific, Atlantic, and Indian oceans, the Gulf of Mexico, and the Caribbean Sea. There may be two groups on the west and east sides of North America based on *rbcl* gene sequence divergence (0.7%–1.4%), although there are no obvious differences in morphology. One group of *Centroceras gasparrinii* is distributed on the west coast of the American continent: Pacific Panama, Korea, and Japan; the other on the east coast: the Atlantic Ocean, the Gulf of Mexico (from Florida to Texas), the Caribbean, and the Indian Ocean (Sri-Lanka, South Africa, and Madagascar). However, the latter also includes Hawaiian vouchers. *Centroceras hyalacanthum* is distributed in Florida and the French West Indies; *C. micracanthum*, in Florida and Panama. *Centroceras rodmanii* sp. nov. is distributed in southern Chile. *Centroceras tetrachotomum* sp. nov. and the unidentified *C. sp.-1*, *C. sp.-2*, and *C. sp.-3* are distributed in South Africa. A separate paper coauthored by several additional investigators will be dedicated to the phylogeography of *Centroceras* worldwide, and three

undescribed South African Species will be discussed in a separate publication.

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### Supplementary Material

The following supplementary material is available for this article:

**Table S1.** Taxa and collecting information of samples used in the analyses of the *rbcL*, SSU, and LSU rDNA with their GenBank accession numbers.

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