

# CENTROCERAS CLAVULATUM (CERAMIACEAE, RHODOPHYTA): A Cosmopolitan Species?



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## ABSTRACT

*Centroceras clavulatum* (C. Agardh) Montagne (1846), originally described from Callao, Peru, is currently known as a widely distributed species worldwide. Over 40+ vouchers referred to in the literature as *C. clavulatum* were investigated on the basis of chloroplast-encoded *rbcl* and LSU rDNA sequence analyses, and comparative morphology. Eight well-supported clades were identified. *Centroceras "clavulatum"* encompasses at least seven additional species that are either new or have been placed under its synonymy. The eight clades under study are distinct from *C. intermitens* Gallagher & Humm, *C. secundum* Wynne and *C. minutum* Yamada, and include, respectively, representative taxa with the following distribution: (1) Peru, (2) California, Gulf of California, Korea, Japan, Pac. Panama, S. Australia, (3) E. Florida & Caribbean Panama, (4) California, (5) W. and E. Florida, (6) S.W. South Africa, (7) Chile, and (8) E. & W. Florida, Texas, Caribbean Sea. Four clades correspond to validly described species described by Kützing that have been placed in synonymy with *C. clavulatum*, and that are herein resurrected.

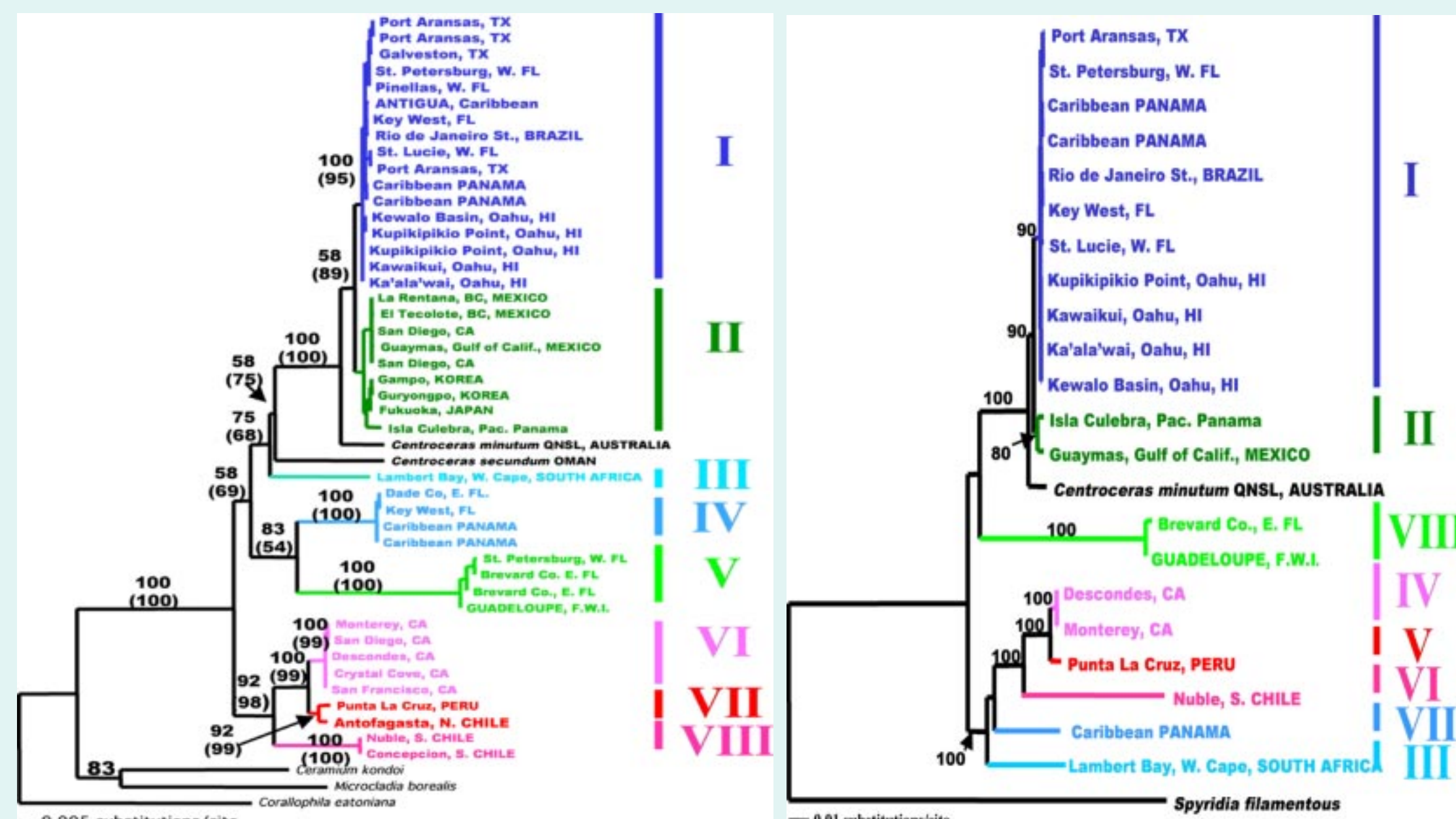


Fig 1. Maximum Likelihood tree inferred from *rbcl* sequences of *Centroceras "clavulatum"*

Fig 2. Maximum Likelihood tree inferred from concatenated *rbcl* and LSU rDNA sequences of *Centroceras "clavulatum"*

## INTRODUCTION

The genus *Centroceras* was established by Kützing (1841) and included 6 new species based on segment size, spine shape and arrangement of basipetal cortical cells; no type species was designated. Montagne (1846) added a species, *C. clavulatum*, based on *Ceramium clavulatum* C. Agardh (1822). In 1849, Kützing reported 2 more species of *Centroceras*, but J. Agardh (1851) synonymized all of Kützing's 8 species with *Centroceras clavulatum* (Ag.) Montagne. Kützing (1863) kept his species as distinct taxa, and described an additional species. Kylin (1956) lectotyped the genus with *C. clavulatum* (Ag.) Montagne, but only recognized *C. clavulatum*.

Seven species worldwide are currently recognized in the phylogenetic literature: *C. clavulatum* (C. Agardh) Montagne (1846), *C. distichum* Okamura (1934), *C. intermitens* Gallagher & Humm (1983), *C. minutum* Yamada (1944), *C. japonicum* Itono (1973), *C. corallophilloides* R. E. Norris (1993), and *C. secundum* (Wynne 2003).

## MATERIAL AND METHODS

**Morphology.** Samples were collected worldwide and sorted according to morphology under a stereomicroscope. Each identified sample was preserved in 4% Formalin/seawater for morphological observations. Microscope observations were made from materials stained with 1% aqueous aniline blue acidified with 0.1% diluted HCL.

**DNA extraction, amplification and sequencing.** Genomic DNA was extracted using either the DNeasy Plant Mini Kit (QIAGEN, Valencia, CA, USA) on fresh or silica-gel dried specimens, or a modified CTAB method (Doyle and Doyle 1987) as described in Cho *et al.* (2003).

Chloroplast encoded *rbcl* and nuclear encoded LSU rDNA were selected for inferring phylogenetic relationships. *RbcL* amplification primer combinations included F7-R753, F645-RrbcSstart (Cho *et al.* 2003) and LSU rDNA amplification primers comprised F28-X (Fredericq *et al.* 2003); sequence primers for *rbcl* included F7, F645, F993, R337, R753, R1150, RrbcSstart, and for LSU rDNA included F28 and X. Sequences were determined for both forward and reverse strands using anABI Prism 3100 Genetic Analyzer with the ABI Prism BigDye™ Terminator Cycle Sequencing Ready Reaction Kit.

**Alignment and phylogenetic analyses.** All generated *rbcl* and LSU rDNA sequence data were compiled and the sequences were manually aligned with Sequencher, then exported separately and combined for maximum parsimony (MP) and maximum likelihood (ML) analyses (PAUP\* v. 4.0b10, Swofford 2002).

## RESULTS

**Molecular analysis :** We collected +40 samples of *C. clavulatum* worldwide based on common morphological characters. We included 1400/1467 bp of *rbcl* and 900 bp of LSU rDNA in the phylogenetic analyses, resulting in eight distinct clades referred to as *C. clavulatum* in the literature, including a population from near the type locality (Callao, Peru) (Figs 1, 2).

**Morphological analysis:** Diagnostic species characters include type of branching pattern and cortication, number of corticating filaments, spine presence/absence. To figure out the different morphological characters in each clade, we observed samples based on following characters: the developmental pattern of first cortical filament, gland cell shape, spine shape and arrangement, and branching pattern.

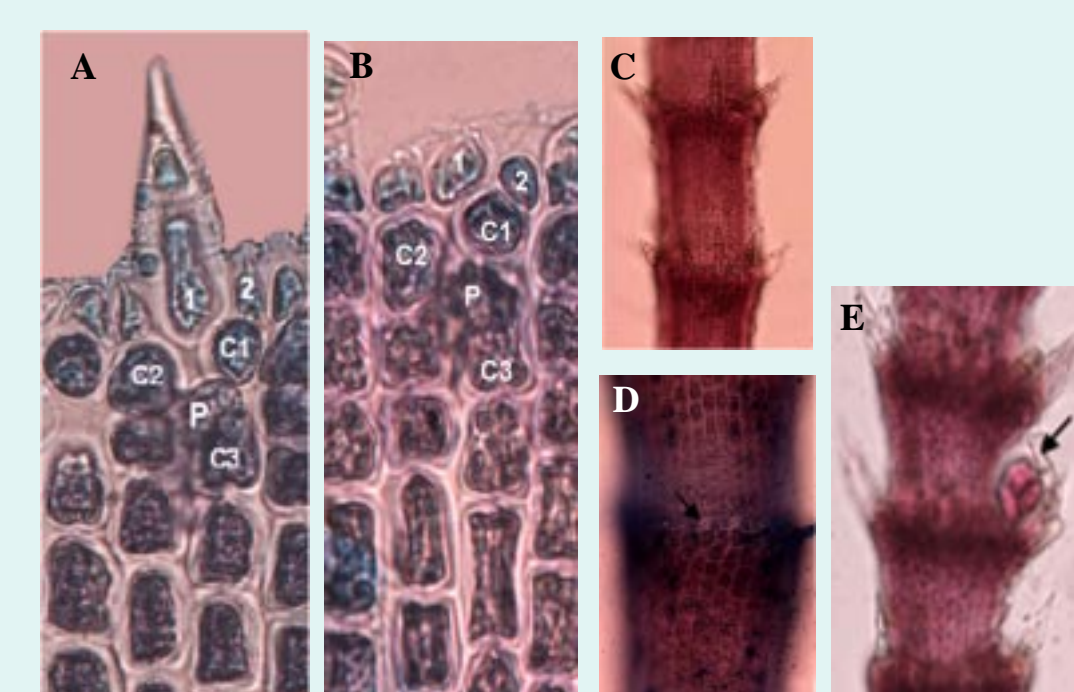


Fig 2. Representative taxon of clade I. A. Straight spine from first cortical initial. B. 2 cortical cells from first cortical initial. C. Cortex. D. Ovoid gland cell. E. Tetrasporangium with involucrel branch.

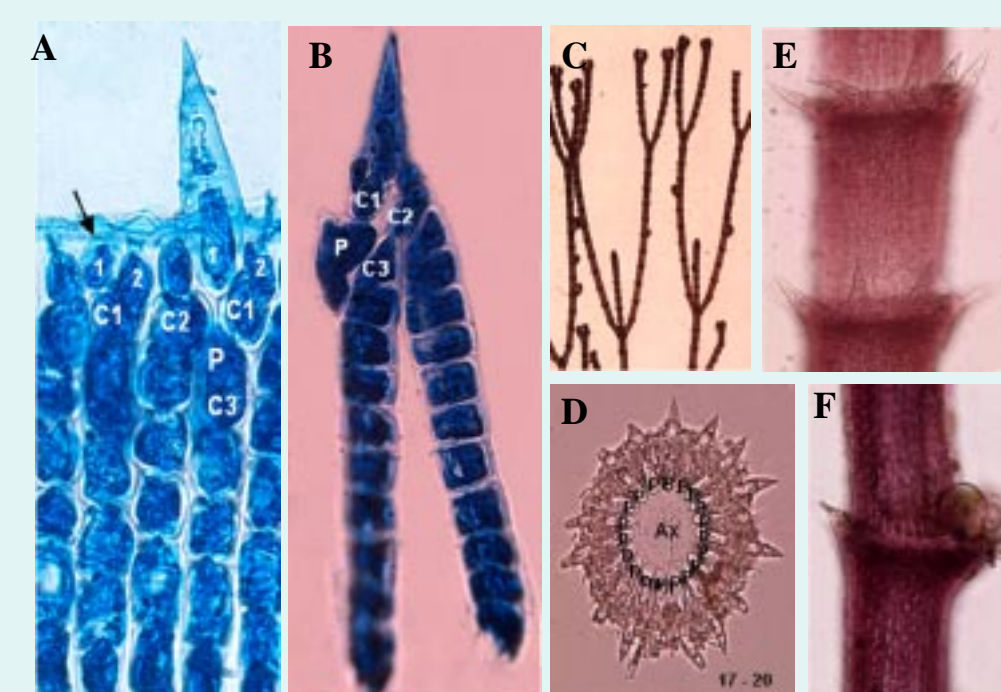


Fig 3. Representative taxon of clade II. A. 2 cortical cells from first cortical initial. B. Straight spine. C. Trichotomous branching pattern. D. Cross section view. E. Without involucrel branches associated with tetrasporangia.

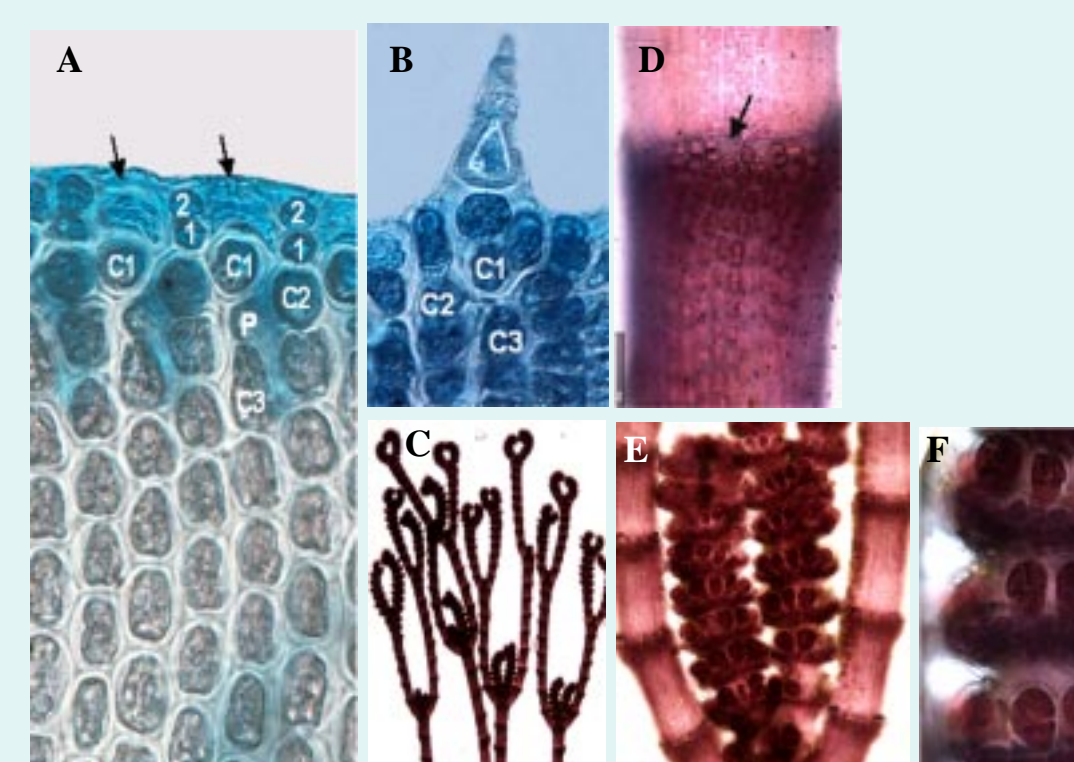


Fig 4. Representative taxon of clade III. A. Flattened gland cell from first cortical initial. B. Straight spine. C. Tetraochotomous branching pattern. D. Cortex. E & F. Tetrasporangia without involucrel branches

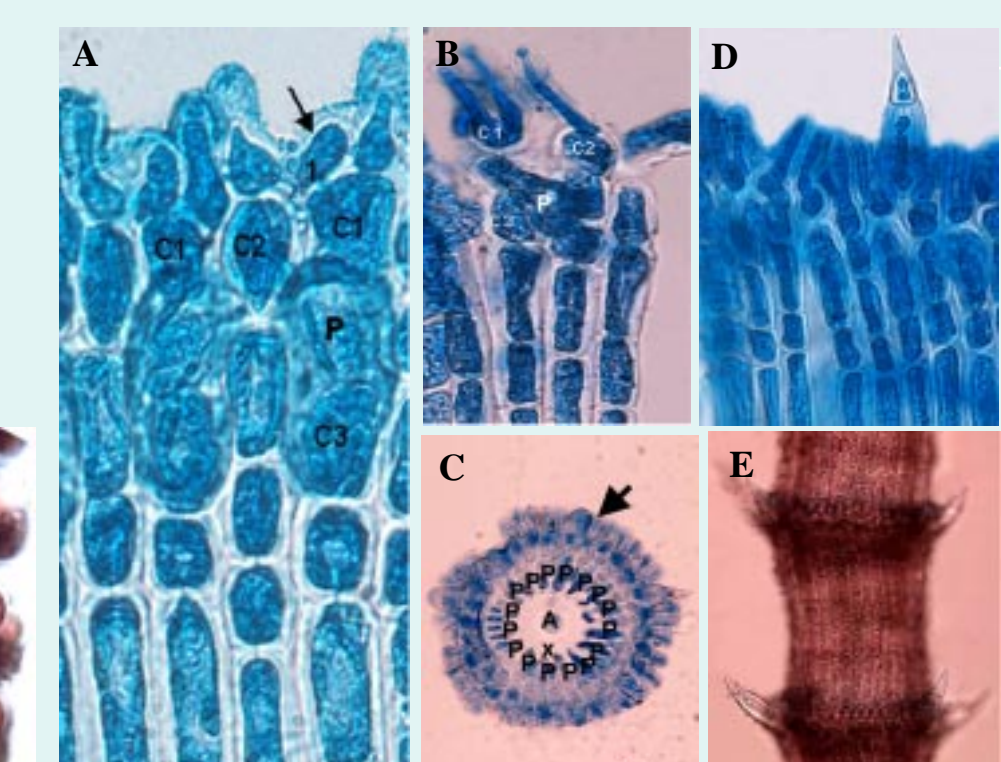


Fig 5. Representative taxon of clade IV. A & B. 2 elongated cortical cells from first cortical initial. C. Cross section. D. Whorled spine arrangement.

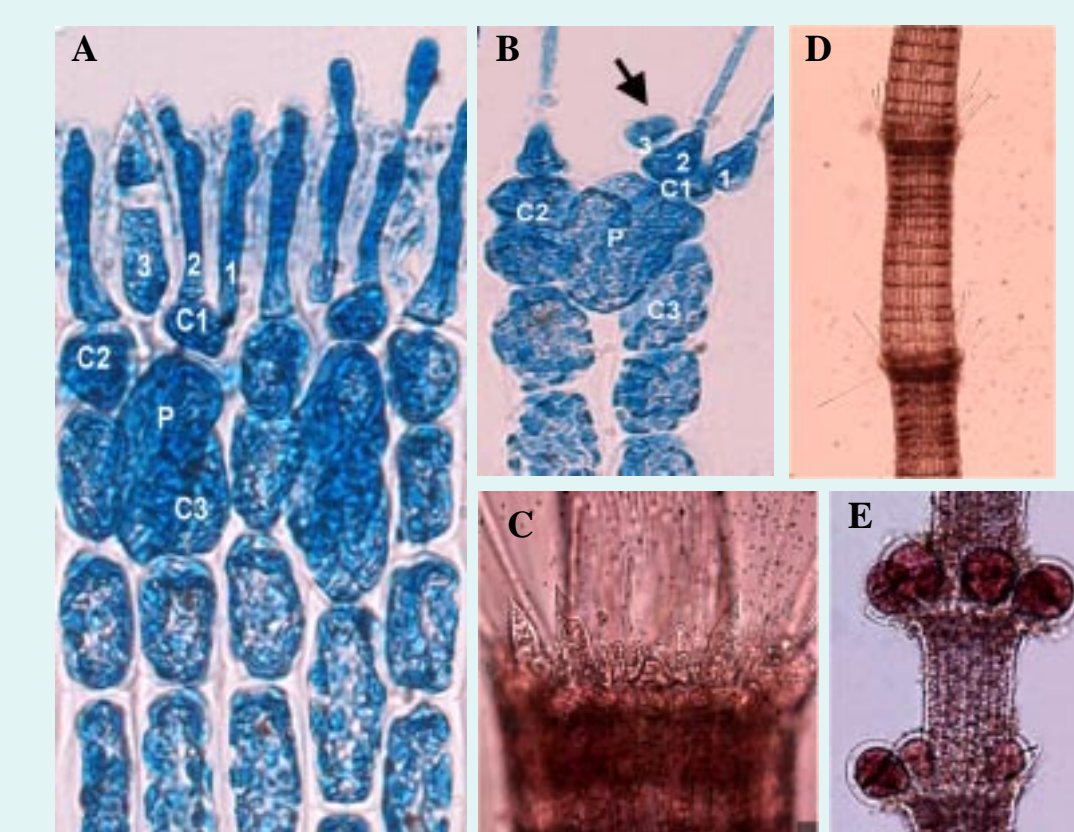


Fig 6. Representative taxon of clade V. A & B. 3 elongated cortical cells from first cortical initial. C. Whorled spine arrangement. D. Cortex. E. Tetrasporangia without involucrel branches.

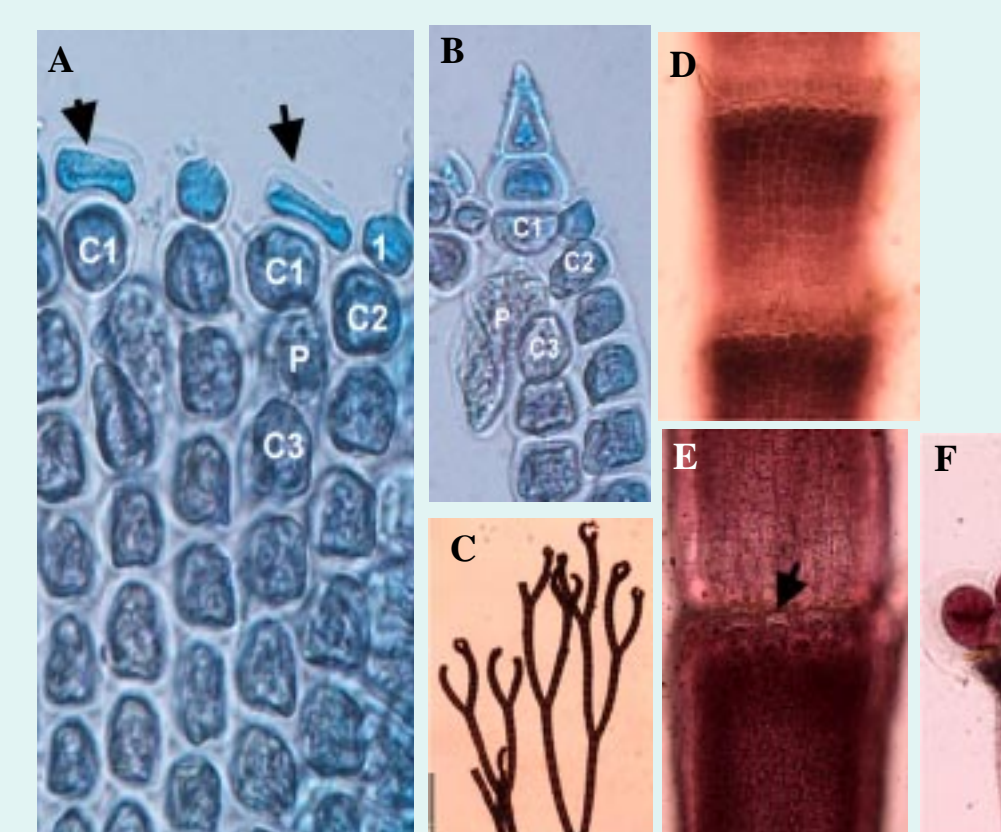


Fig 7. Representative taxon of clade VI. A. Flattened gland cell from first cortical initial. B. Straight spine. C. Dichotomous branching pattern. D & E. Cortex. F. Tetrasporangia without involucrel branches.

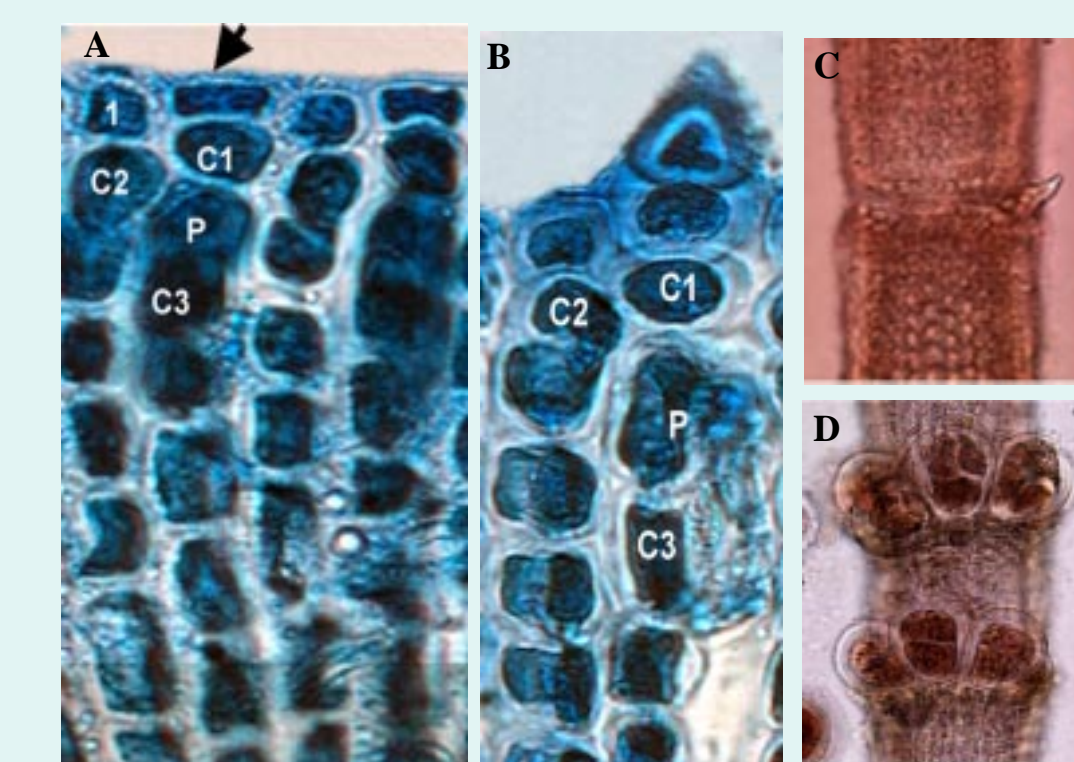


Fig 8. Representative taxon of clade VII. A. Flattened gland cell from first cortical initial. B. Straight spine. C. Abaxial spine arrangement. D. Tetrasporangia without involucrel branches.

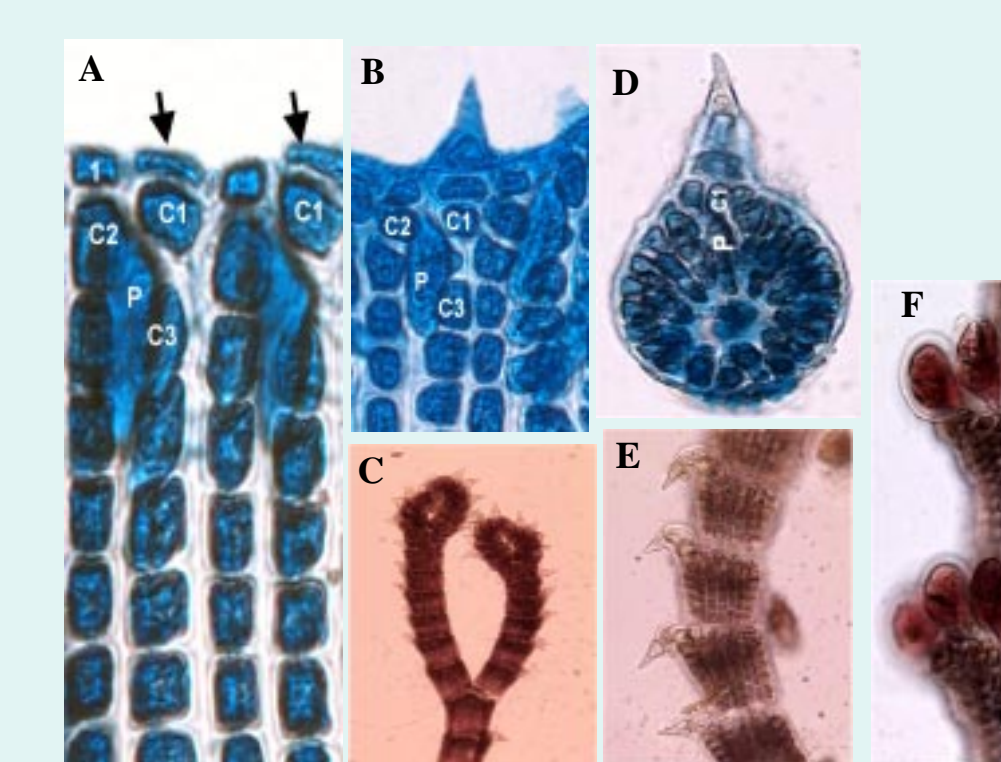


Fig 9. Representative taxon of clade VIII. A. Fattened gland cell from first cortical initial. B. One cortical cell from first cortical initial. C. Whorled spine arrangement. D. Cross section. E. Hooked spine. F. Tetrasporangia without involucrel branches.

Table 1. Comparison of morphological features among the *Centroceras "clavulatum"*.

	I	II	III	IV	V	VI	VII	VIII
gland cell shape	ovoid	ovoid	flattened	ovoid	ovoid	flattened	flattened	flattened
cortical cells per first cortical initial	2	2	1	2	3	1	1	1
shape of cortical cell	oblique	oblique	oblique	elongated	elongated	oblique	oblique	oblique
Spine shape	straight	straight	straight	straight	straight	straight	straight	hooked
Spine arrangement	whorled	whorled	whorled	whorled	whorled	whorled	abaxial	whorled
Branching pattern	di-tri	di-tri	tetra	di	di	di	di	di
Involuter branches & tetrasporangia	present	absent	absent	absent	absent	absent	absent	absent

**Kützing's type material:** Type specimens of Kützing' validly described *Centroceras* species, deposited in the Leiden herbarium (L!) were studied for comparative purposes.

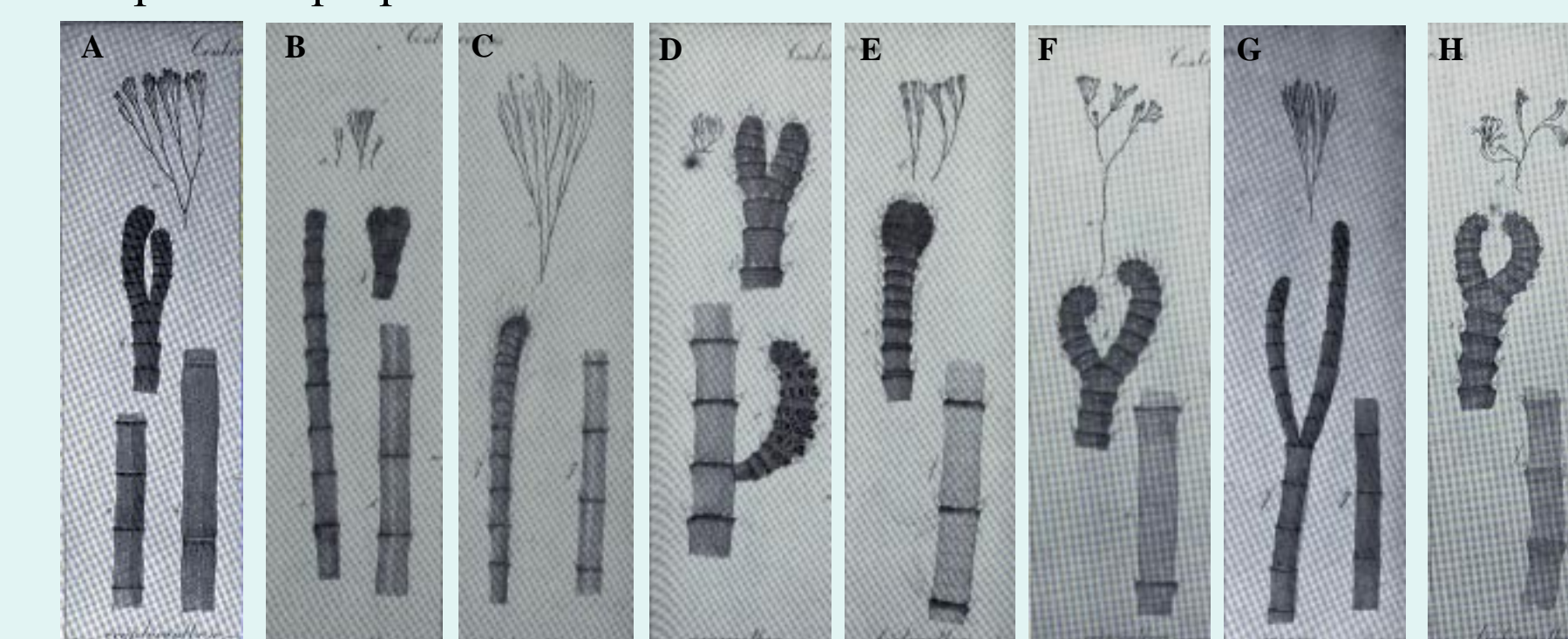


Fig 10. Kützing' 1863 drawings of: A. *Centroceras erythacanthum* Kg. 1841 B. *Centroceras micracanthum* Kg. 1841 C. *Centroceras leptacanthum* Kg. 1841 D. *Centroceras macracanthum* Kg. 1841 E. *Centroceras hyalacanthum* Kg. 1841 F. *Centroceras oxyacanthum* Kg. 1841 G. *Centroceras inerme* Kg. 1849 H. *Centroceras brachyacanthum* Kg. 1863

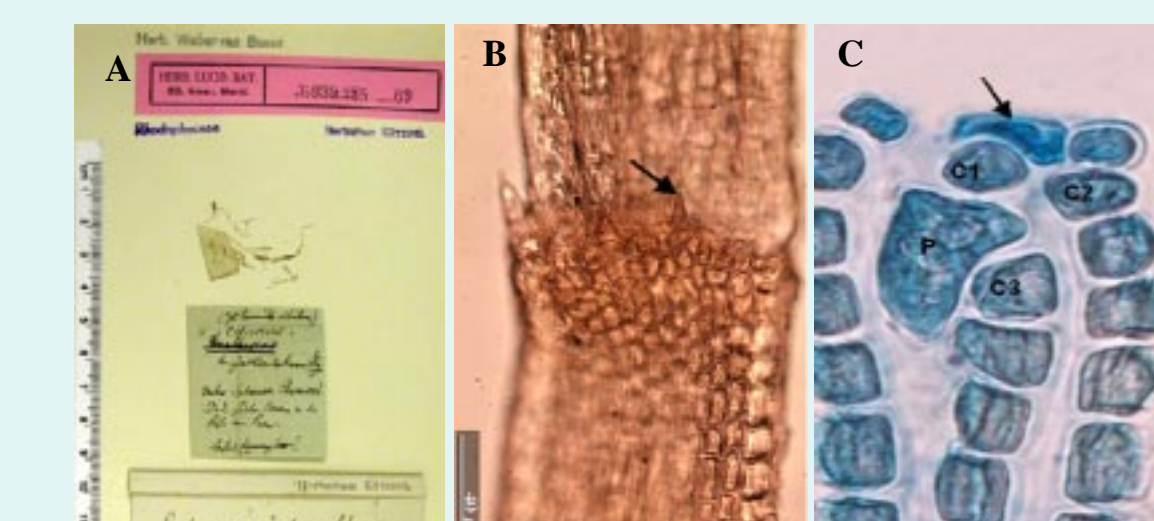


Fig 11. Type specimen of *Centroceras cyptacanthum* var. *longiarticulatum* Kg. (Clade VII) A. Habit B. Cortex C. Cortical filament

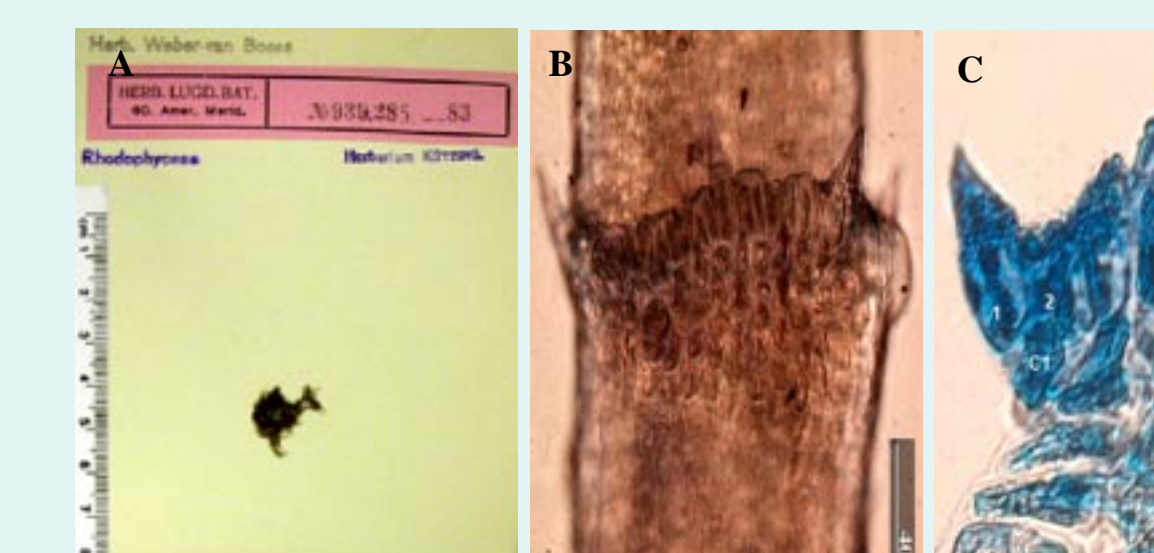


Fig 12. Type specimen of *Centroceras micracanthum* Kg. (Clade IV) A. Habit B. Cortex C. Cortical filament

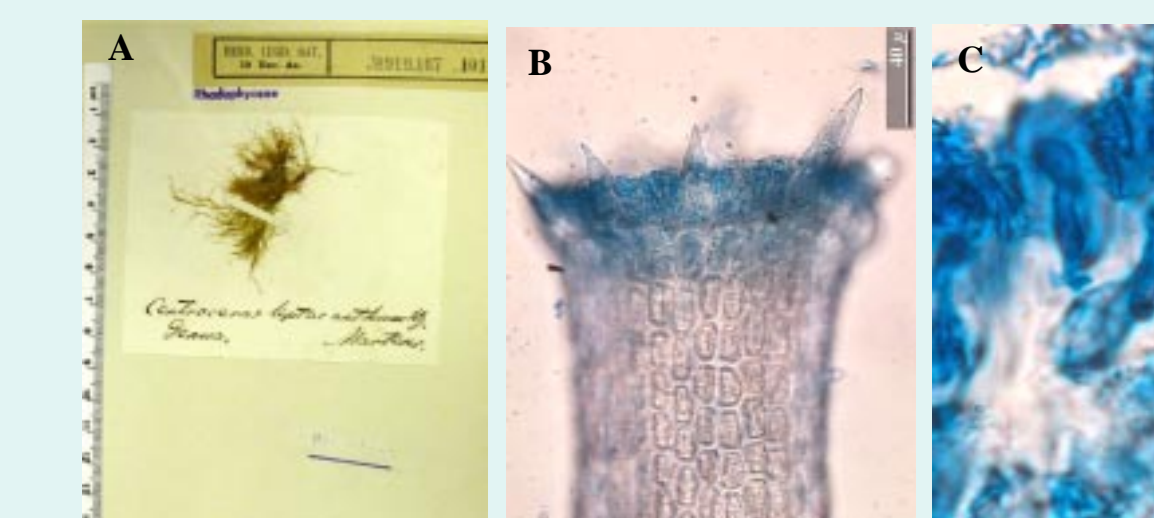


Fig 13. Type specimen of *Centroceras leptacanthum* Kg. (Clade IV) A. Habit B. Cortex C. Cortical filament

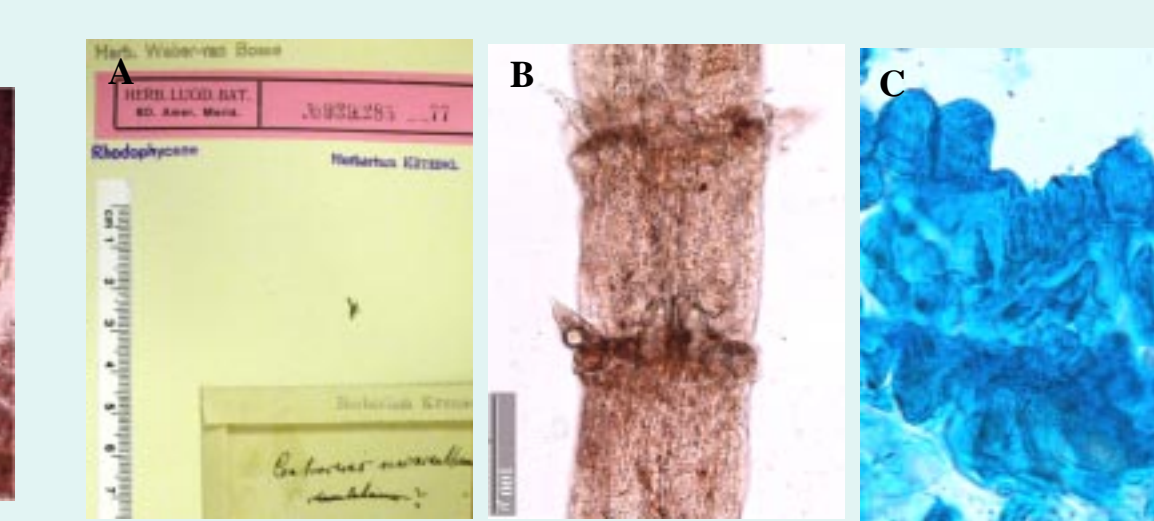


Fig 14. Type specimen of *Centroceras macracanthum* Kg. (Clade IV) A. Habit B. Cortex C. Cortical filament

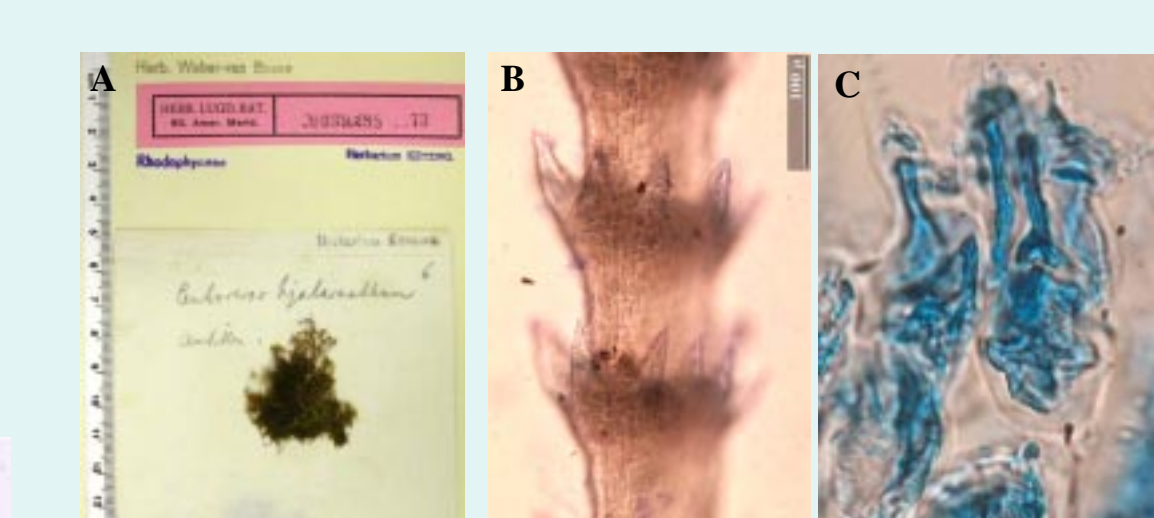


Fig 15. Type specimen of *Centroceras hyalacanthum* Kg. (Clade V) A. Habit B. Cortex C. Cortical filament

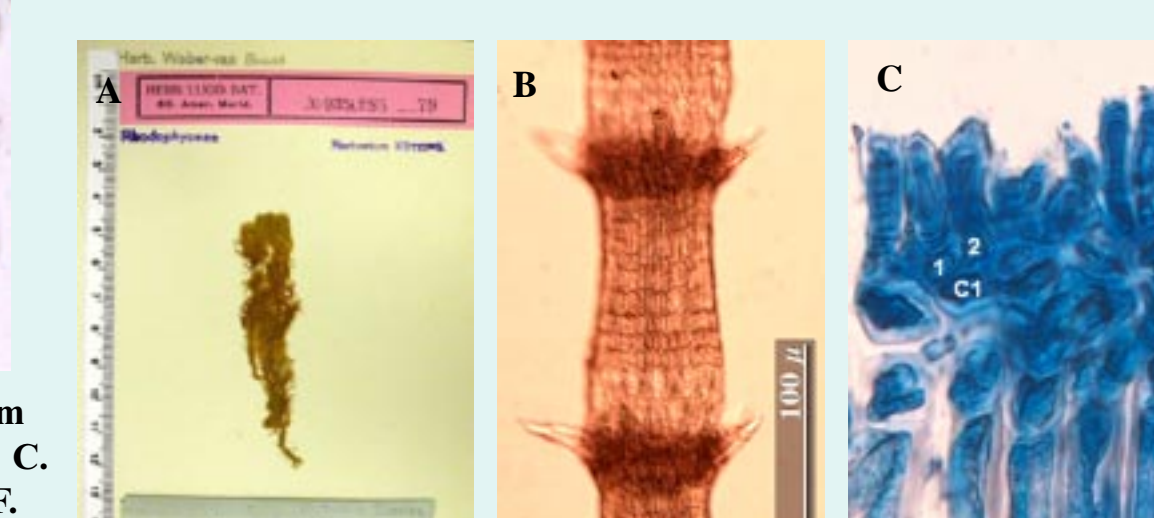


Fig 16. Type specimen of *Centroceras oxyacanthum* Kg. (Clade V) A. Habit B. Cortex C. Cortical filament

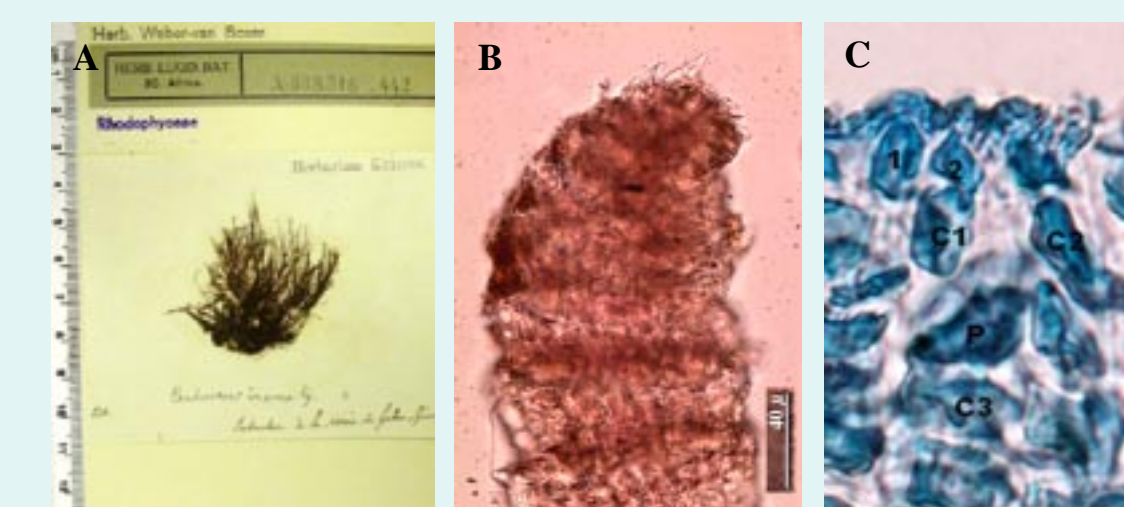


Fig 17. Type of *C. inerme* (Clade I). A. Herbarium. B. Cortex. C. Cortical filament

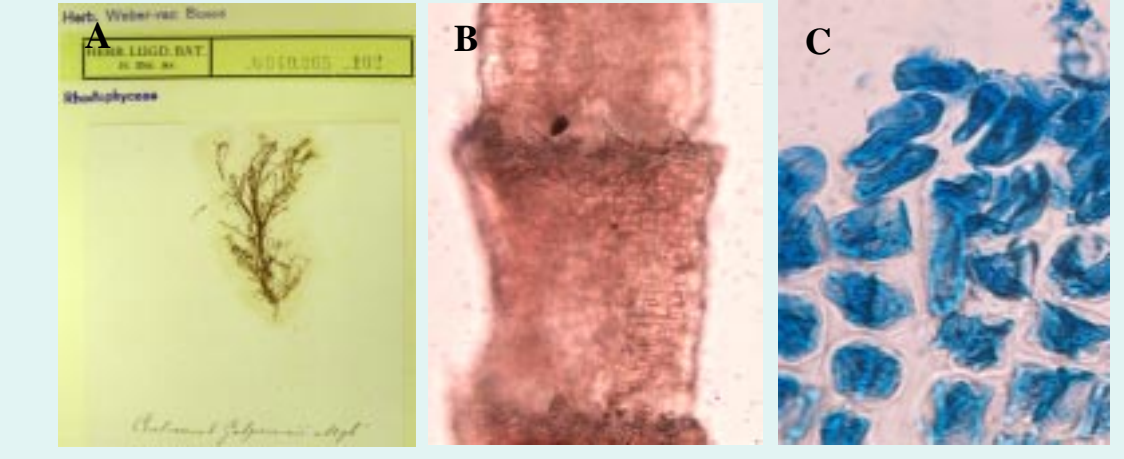


Fig 18. Type of *C. gasparrinii* (Clade I). A. Herbarium. B. Cortex. C. Cortical filament

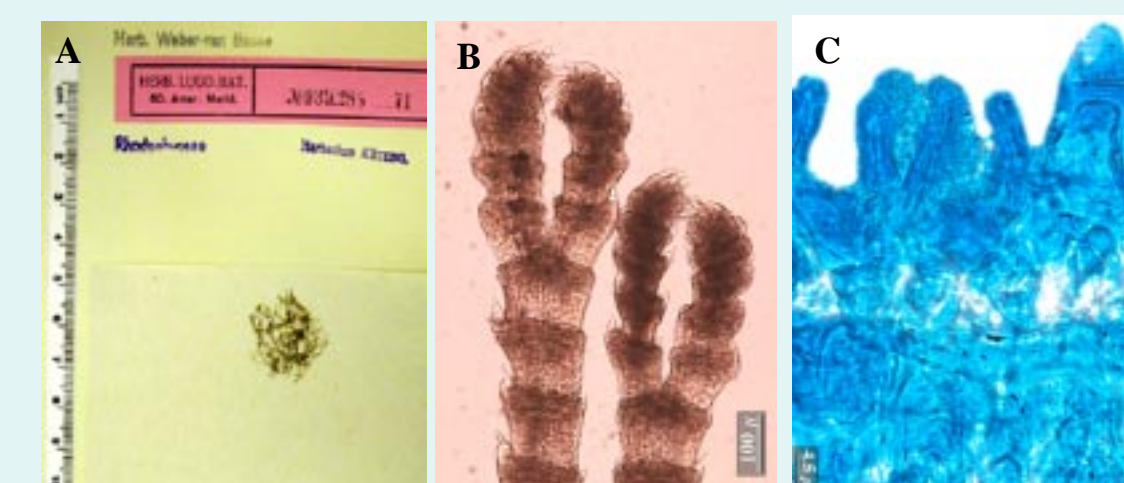
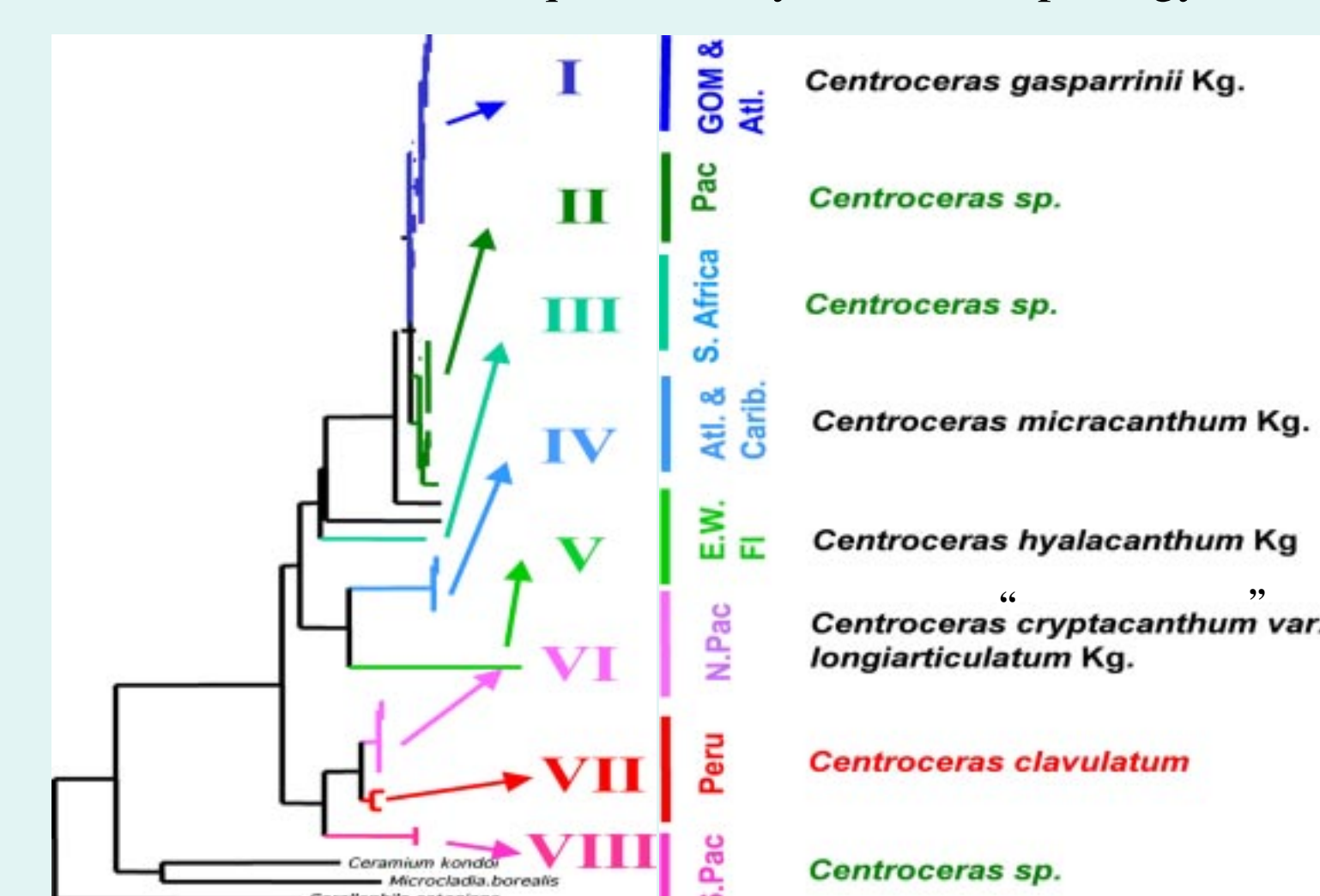


Fig 19. Type of *C. brachyacanthum* (Clade I). A. Herbarium. B. Cortex. C. Cortical filament

## CONCLUSIONS

1. *Centroceras "clavulatum"* includes eight distinct clades corresponding to eight distinct species based on comparative *rbcl* and LSU rDNA sequence analyses and morphology.



2. These groups are characterized by branching pattern, spine arrangement and shape, number of cortical cells per first cortical cell initials, shape of cortical cells and gland cells, and +/- involucrel cells associated with tetrasporangia.

3. In Florida there are 3 *Centroceras* species: *C. gasparrinii* Kg., *C. micracanthum* Kg., *C. leptacanthum* Kg.

In Panama: 3 species: *C. gasparrinii* Kg., *C. micracanthum* Kg., *C. leptacanthum* Kg., *C. sp. #1*.

In Peru: 1 species: *C. clavulatum* (C. Agardh) Montagne.

In Chile: 2 species: *C. clavulatum* (C. Agardh) Montagne, *C. sp. #3*.

In Brazil: 2 species: *C. gasparrinii* Kg., and *C. micracanthum* Kg.

4. The clades correspond to biogeographic regions: NE Pacific, Pacific, Gulf of Mexico-Atlantic-Caribbean, with some overlap, and recent introductions.

5. We have been able to resurrect 4 names: *C. gasparrinii* Kg., *C. micracanthum* Kg., *C. hyalacanthum* Kg., *C. cryptacanthum* var. *longiarticulatum* Kg..

6. The erection of new species is ongoing

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## REFERENCES

- Agardh C.A. 1822. *Species algarum*. Vol. 1, pp. 169-531. Lund.  
 Agardh J. G. 1851. *Species, genera et ordines algarum*. Vol. 1, pp. 113-150. Lund.  
 Cho, T. O., Fredericq, S. & Boo, S. M. (2003). *Ceramium inkuyui* sp. nov. (Ceramiales, Rhodophyta) from Korea: A new species based on morphological and molecular evidence. *J. Phycol.*, 39: 236-247.  
 Doyle, J. J. & Doyle, J. L. 1987. A rapid DNA isolation procedure for small quantities of fresh leaf tissue. *Phytochem. Bull.* 19:11-5.  
 Fredericq S., Aderson R.J. & Lopez-Bautista J. (2003). Circumscription of the Phylloporaceae (Gigartinales, Rhodophyta) from the Cape region, South Africa, based on molecular evidence. *Proc. Vllth Int. Seaweed Symp., Oxford Univ. Press*, pp. 263-4.  
 Gallagher S.B. & Humm H.J. (1983). *Centroceras intermitens* n. sp. (Rhodophyceae, Ceramiales) from the western tropical North Atlantic Ocean. *J. Phycol.* 19:261-268.  
 Itono H. (1973). Notes on marine algae from Hateruma Island, Pyukyu. *Bot. Mag. (Tokyo)* 86:155-168.  
 Kylin H. (1956). *Die Gattungen der Rhodophyceen*. Lund. 673 pp.  
 Kützing F.T. (1841). Ueber *Ceramium* Ag. *Linnaea* 15: 727-746.  
 Kützing F.T. (1849). *Species algarum*. Lipsiae. vi + 922p.  
 Kützing F.T. (1863). *Tabulae Phycologicae...* Vol. XIII. 31pp., 100 pls. -- Nordhausen.  
 Montagne C. (1846). *Phyceae*. In, M.C. Durieu de Maisonneuve, *Exploration scientifique de l'Algerie...* Botanique (Flora d'Algerie), Cryptogamie. Paris. 197 pp.  
 Norris R.E. (1993). Taxonomic studies on Ceramiales (Ceramiales, Rhodophyta) with predominantly basi petal growth of corticating filaments. *Bot. Mar.* 36:389-398.  
 Okamura K. (1935). *Icones of Japanese algae*. Vol. VII(5), pls. 321-325. Tokyo.  
 Setchell W. A. & Gardner N. L. (1924). New marine algae from the Gulf of California. *Proc. Calif. Acad. Sci.*, 4th ser., 12: 695-949, pls. 12-88, map.  
 Swofford, D. L. 2002. PAUP\*: Phylogenetic Analysis Using Parsimony (\* and many Other Methods). Version 4.0b10. Sinauer, Sunderland, MA.  
 Wynne M.J. (2004). *Centroceras secundum* sp. nov. (Ceramiales, Rhodophyta) from the sultanate of Oman. *Nova Hedwigia*. 77:125-137.  
 Yamada Y. (1944). A list of the marine algae from the Atoll of Ant. *Sci. Pap. Inst. Agr. Res. Pac. Sci. Hokkaido Imp. Univ.* 3: 31-45, 2 pls.