

Morphology and molecular phylogeny of *Hypoglossum inordinatum* sp. nov. (Delesseriaceae, Rhodophyta) from the Gulf of Mexico

John A. West, *School of Biosciences, University of Melbourne, Parkville VIC 3010, Australia*
(correspondence: jwest@unimelb.edu.au)

Susan Loiseaux-de Goër, *11, rue des Moguerou, F-29680 Roscoff, Brittany, France*

Hypoglossum Kützing, 1843 [generitype: *Hypoglossum woodwardii* Kützing, = *Hypoglossum hypoglossoides* (Stackhouse) Collins & Hervey from the NE Atlantic] is a genus of some 32 current species (Guiry & Guiry 2021). It includes species of the subfamily *Delesserioideae*, tribe *Hypoglosseae*, family *Delesseriaceae* with flat branched blades, a midrib and lateral alae (wings) but lacking lateral veins. Growth is from a single transversely dividing apical cell, with the blades developing symmetrically, and with all apical cells of second- and third-order cell rows reaching the margins. Thalli are monostromatic except for the midrib region; intercalary cell divisions are lacking in all cell rows; branching by endogenous initials is restricted to the midrib, or rarely from the mid-region of the alae. Morphological observations have been used traditionally for systematic investigations of species of red algae but cannot be relied upon entirely for assessment any longer. However, molecular phylogeny of the genus is very poorly investigated; data are available for only four *Hypoglossum* species in GenBank (<https://www.ncbi.nlm.nih.gov>). The present investigation, comprising laboratory culture, morphology and some reproduction together with molecular analyses of a *Hypoglossum* collection from the Gulf of Mexico may expand knowledge of the genus. Diaz-Tapia *et al.* (2019) recently published a revision of the order Ceramiales including a revision of the *Delesseriaceae* but no molecular data for *Hypoglossum* were available at the time for inclusion.

Hypoglossum inordinatum J.A. West & Loiseaux, sp. nov.

Holotype: Dried, metabolically inactive material, of JAW 2403, MEL 2476903

Type locality: Puerto Morelos, Quintana Roo, Yucatan, Gulf of Mexico; 27 vii 1980, Sylvia Earle subtidal (20.8495, – 86.8753). Collected only once, never observed again and maintained in culture (as West 2403) for 41 years.

Etymology: The adjectival epithet “*inordinatus*, -a, -um” (discorded, irregular) refers to the distinctive morphology of this species compared to other species of the genus.

Representative culture: JAW 2403.

Description. Blades are to 175 µm wide and to 10 cm long in culture, rarely forming tetrasporangia with tetrahedrally arranged tetraspores in pairs of elongated sori. Released tetraspores grew into tetrasporelings only once for a brief period in 1980 and these sporelings developed no further. Only the tetrasporophyte blades were retained for additional investigations by excising rapidly growing blades when necessary to prevent overcrowding. In low light (1–2 µmol photons m⁻² s⁻¹ LED cool-white lighting, hereafter as “photons”), upright shoots were narrow, mostly with periaxial cells and some flanking cells. Descending filaments adhering to the shoot formed cortication and many uniseriate rhizoid filaments projected away from the main axes. In high light (8–9 photons), narrow blades developed with wings in which most second order cell rows produced third order cell rows. Secondary blades arose from axial cells in main shoots and from intercalary cells of uniseriate rhizoid filaments.

Distribution: Currently known only from the type locality in the Gulf of Mexico.

Culture methods were as described by West (2005). Photography for Figs 1 and 2 below was with a Zeiss GFL bright field microscope and Canon G3 camera. Fig. 1 was prepared with live specimens whilst Fig. 2 was prepared with specimens fixed in 5% Formalin in seawater, washed in distilled water, microwaved for 10 s at 800 mW to shrink cells slightly to show pit connections, then placed

on slides with 1–2 drops of 50% corn syrup with 0.02% aniline blue for stain and 0.1% phenol to prevent fungal growth, a coverslip was lowered carefully over the specimen and excess mounting medium was extracted with bibulous paper strips at the coverslip margins and the coverslip was twice sealed with clear nail-polish. The DNA extraction and sequencing methods of M. Kamiya here are identical to those used by him for similar studies of *H. sabahense* M.J.Wynne, M.Kamiya & J.A.West in Wynne & al. (2020).

In plants grown at 10:14 h daylength (light:dark), low light (1–2 photons) shoots were narrow mostly with only periaxial cells and descending adherent filaments forming cortication. When grown at increased light (8–9 photons) on a shaker (65 rev min⁻¹) blades became wider (to 175 µm wide) and reached 50–100 mm length, often covering bottom of 100 mm diameter deep storage dishes (500 mL) in two months.

In low light, free rhizoids had lower intercalary cells that formed 1–2 apical single cells developed but did not develop further (Fig. 2 D). A week after a transfer from 1–2 photons to 8 photons, the terminal secondary cells of rhizoid intercalary cells formed blade primordia (Figs 2 A, B, asterisk, showed free uniseriate rhizoid laterals developed and 3 intercalary cells directly above was produced a developing blade). In low light, the growth sector of a lower blade showed single flank cell on each periaxial cell (Fig. 2 C, single arrow), but in brighter light, two flank cells on periaxial cells (Fig. 2 C, two arrowheads) were produced in a sector above, followed by cell divisions from upper flank cell, uppermost apical cells fused with lower flank cell of the adjacent upper sector (Fig. 2 C, small asterisks on left and right sides).

At the apex of very slender elongated blades, transverse divisions occurred until the sixth cell of the central axial cell row when each axial cell of each segment cut off two lateral and two transverse periaxial cells (Fig. 2H). The widest blades (to 175 µm) shown in Fig. 1 G showed Type 2 of *Hypoglossum* species in which not all second order rows produced third order rows. Tiny discoid plastids (1.5–3.0 µm in diameter) densely arranged in long linear rows peripherally in periaxial cells (Figs 1 A, D, E, F). The two lateral wing cells cut off primary and secondary rows of flank cells creating a monostromatic blade margin (Fig. 2 H). Younger blades (to 120 µm wide) had no third order cell rows (Fig. 2 H). The blades were loosely attached by scattered uniseriate rhizoids arising from a periaxial or flank cells. Secondary blades appeared to arise from axial cells or periaxial and flank cells (Fig. 1 H, Figs 2 F, G).

Elongate uniseriate rhizoids growing outward had cells 12–20 µm in diameter and of variable length. The filaments were sparsely branched from a cell cut off at the apex or subapex of intercalary rhizoid cells. Often on lower axial sectors of shoots only periaxial cells were seen that produced tightly corticating filaments extended 2–3 axial cell sectors downward (Figs 1 Gm H, vertical lines with midsection arrowhead). Often descending adherent filaments, embedded in the extracellular matrix, were produced by the basal cell of free rhizoids extending away from the shoot (Figs 2 E & F, arrowheads). One or two secondary blade primordia arose from periaxial cells extending outward from the shoots (Figs 1 F & G).

Rhizoids extending from a shoot sometimes contacted another rhizoid stimulating the contact apical cell to divide into short-celled filaments that wrapped firmly around (‘strangling’) an intercalary cell of the other rhizoid (Figs 1 B, C). In rhizoid intercalary cells tiny discoid plastids (1.5–3.5 µm in diameter) were in long irregular rows (Fig. 1 D). Nuclei were usually difficult to see in this strain, (i.e., Figs 1 D, E) except in Fig. 1F in which 3 nuclei were spatially separated in the periphery of each periaxial cell. However, rhizoid intercalary cells were uninucleate (3–4 µm), the

nucleus faintly visible (Fig. 1C, arrowhead). The same extending rhizoid did form a new blade primordium from an intercalary cell (Fig. 1 B, arrowhead).

Comparing *H. inordinatum* and *H. sabahense* cultured at the same time and under the same conditions, the number of nuclei in different cells is different and may result from transfer between neighbouring cells of wings and midribs via secondary cell connections. Uniseriate rhizoidal cells are multinucleate (2–6) in *H. sabahense* and uninucleate in *H. inordinatum*. The multiple nuclear numbers in derived cells of several *Delesseriaceae* were investigated by Goff & Coleman (1990). In species of *Bostrychia* nuclear transfer by conjuncture cells is very obvious and complex in the periaxial and cortical cells. Axial cells remain uninucleate with an increase of DNA level to 4C (Goff & al. 1992). Wynne (pers. comm.) suggested that JAW 2403 might be *H. simulans* M.J.Wynne, I.R.Price & D.L.Ballantine (type locality: Îlet de Pigeon, Malendure, west side of Basse-Terre, Guadeloupe, West Indies), also reported from Puerto Morales (Wynne & al. 1989). However, Wynne in 2005 corrected his vouchers of *H. simulans* from Puerto Morelos (MICH 622850, 622851) to *H. subsimplex* M.J.Wynne. Both these latter species in the field are up to 60 mm long and to 1.5 mm wide, thus quite different, being not as long or narrow as *H. inordinatum* sp. nov. in culture (to 80–100 mm long and to 170 µm wide). *Hypoglossum simulans* and *H. subsimplex* have not been subjected to molecular analyses.

Maximum-likelihood (ML) phylogeny of the *Delesseriaceae* inferred from partial *rbcL* gene sequences (Fig. 3) places *H. inordinatum* closer to *H. hypoglossoides* than *H. sabahense* and *H. anomalum*. The *rbcL* complete gene sequence is given in Supplementary File 1. Fig. 3 was kindly provided by M. Kamiya.

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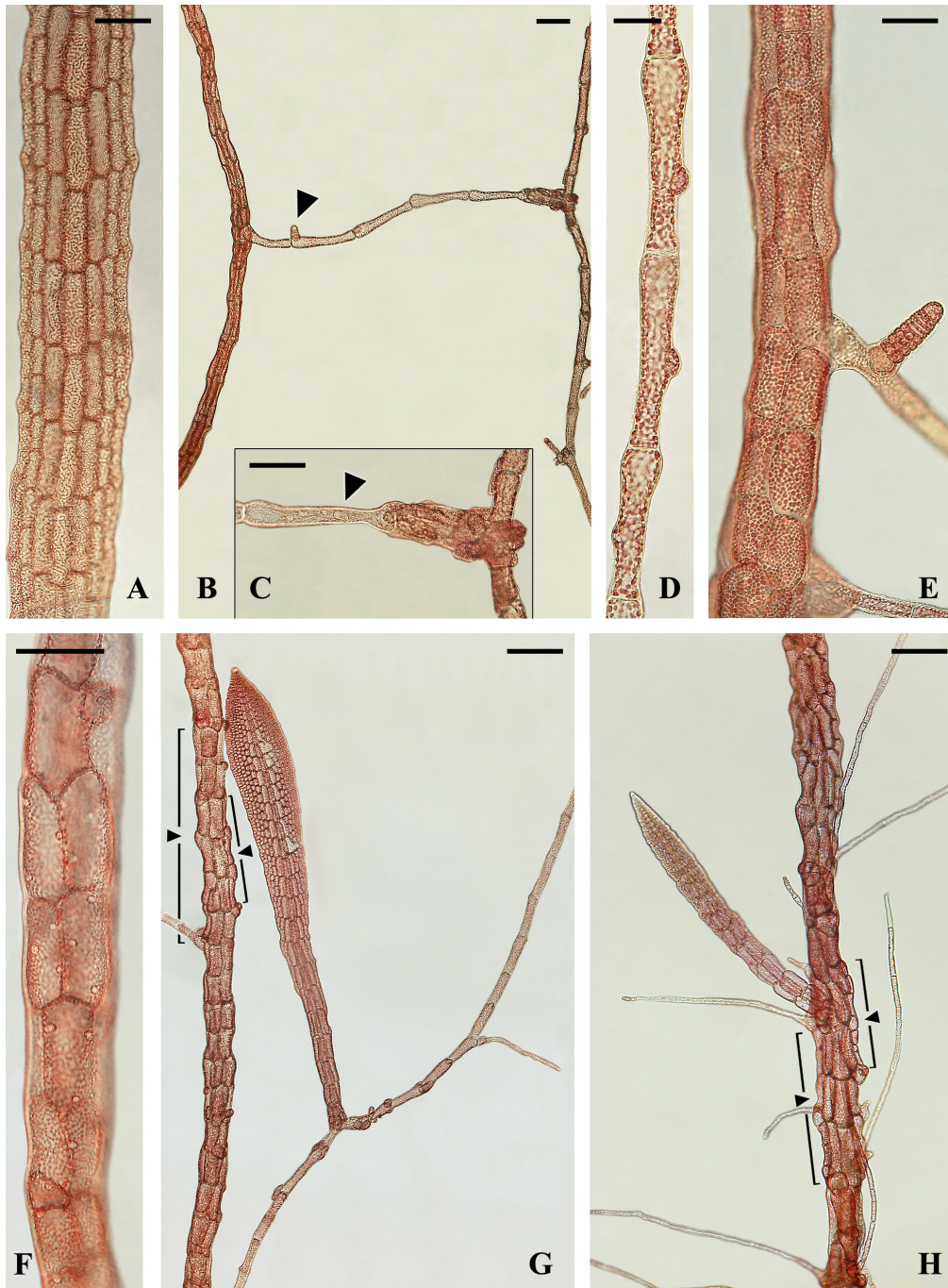


Fig. 1 A–H. *Hypoglossum inordinatum* sp. nov. JAW 2403. (A) Main lower blade 115 μm wide, cells with 1.5–3.0 μm discoid plastids oriented in contorted rows. High light and shaker culture. (B) Free rhizoid grew toward other rhizoid and on contact at tip multiple new tip cells ‘strangle’ the rhizoid filament in low light and stationary culture. Blade primordium (arrowhead) on intercalary cell. (C) Higher magnification showing short filament and complex cell formation at tip wrapping around (‘strangling’) intercalary cell of another rhizoid at a contact site. Elongate intercalary cell with one nucleus (arrowhead) faintly visible. (D) Rhizoid cells with small lateral cells cut off in the centre. These may form either lateral rhizoids or blade primordia. Grown in low light and stationary culture. (E) Blade primordium on rhizoid basal cell near main axis in low light and stationary culture, peripheral plastids compactly placed. (F) Main cylindrical shoot has periaxial cells but lacks flank cells. Three nuclei, enclosed by tiny particles, uniformly distributed against each cell periphery. (G) Shoot at far left with inserted vertical lines interrupted in middle by arrowhead on both sides showing tightly adherent descending filaments along the periaxial cells of narrow main shoot also bearing free rhizoid at left. In middle view a normal wide (170 μm) flat blade with marginal flank cells developed from intercalary free rhizoid in high light and shaker culture. (H) Numerous elongate descending filaments closely adhering along slightly twisted axis (Inserted vertical lines with arrow heads same as in Fig. 1G) and many free rhizoids growing outward, blade evidently arising from periaxial cells. Scale bars represent: A & B, 50 μm ; C, 40 μm ; D, 20 μm ; E, 30 μm ; F, 100 μm ; G & H, 150 μm .

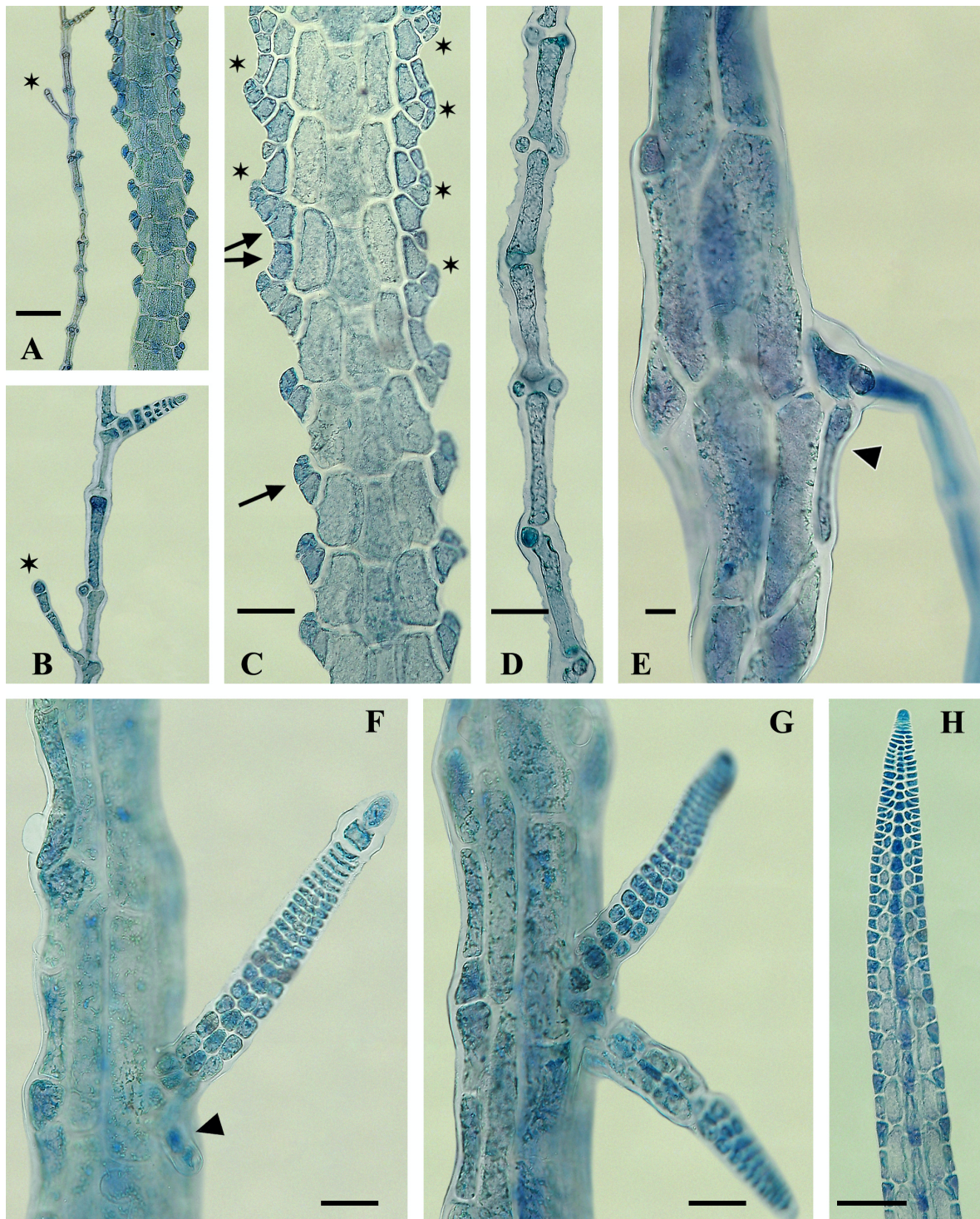


Fig. 2 A–H. *Hypoglossum inordinatum* sp. nov. JAW 2403 (fixed with 5% Formalin in seawater and stained with aniline blue). (A) When light increased to $8\text{--}9\ \mu\text{mol photons m}^{-2}\text{ s}^{-1}$ rhizoidal intercalary cells produced either rhizoid laterals (asterisk) or initiated blade primordia as seen in uppermost cells. (B) Same rhizoid as in A showing rhizoid lateral (asterisk) and upper lateral forming blade; cf. 2 D. (C) Blade in low light has only periaxial cells like 1 E & F but increased light activated flank cell formation, first single flank cell (arrowhead) and then two flank cells with branch cells at tip of upper flank cell fusing (asterisks) with basal flank cell of the sector above. (D) In low light 1–2 single cells developed at the apices of intercalary rhizoid cells but did not form blades or rhizoids; cf. 2 A. (E) Rhizoid basal cells initial cut off a downward growing filament embedded in matrix cell wall polysaccharide as shown in Figs 1 G & 1 H. (F) Secondary blade arising from periaxial cell and descending rhizoid from blade basal cell. (G) Two lateral secondary blades arising from periaxial cells of main shoot, descending embedded filaments visible on left side. (H) Blade apex showing first and second order branching, no third order branching. Scale bars represent: A, 70 μm ; B 20 μm ; C, 35 μm ; D, 30 μm ; E, 20 μm ; F, 40 μm ; G, 70 μm ; H, 120 μm .

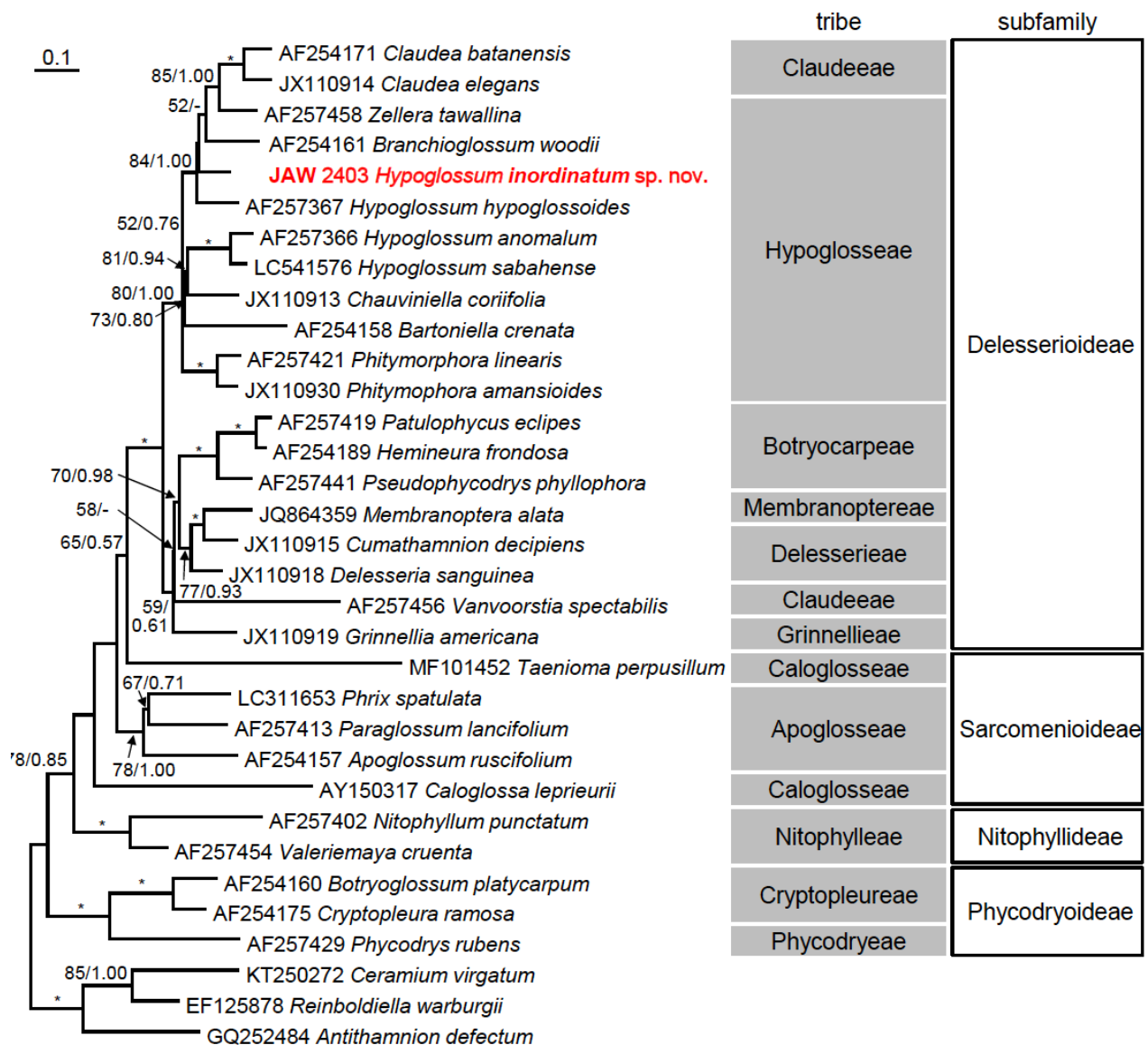


Fig. 3. Maximum-likelihood (ML) phylogeny of the *Delesseriaceae* inferred from partial *rbcL* gene sequences. Bootstrap values for ML (> 50%; left) and posterior probabilities for Bayesian inference (BI) (> 0.5; right) are given on each branch, and robust branches (ML $\geq$ 90% and BI $\geq$ 0.95) are indicated by asterisks. Accession number or strain number is shown before each taxon name. The scale is in units of nucleotide substitutions per site. Kindly provided by M. Kamiya in 2020.