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Reassessment of the genus *Lophurella* (Rhodomelaceae, Rhodophyta) from Australia and New Zealand reveals four cryptic species

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Running title: Cryptic diversity in *Lophurella*

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ABSTRACT

Cryptic diversity is common in the red algae and is often discovered when comparing specimens from distant locations or different morphotypes of species with high phenotypic plasticity. The genus *Lophurella* includes seven species from the cold-temperate coasts of the Southern Hemisphere. *Lophurella pericladus* is the only species reported from Australia where two morphotypes were identified in relation to levels of wave exposure. In New Zealand, three species of *Lophurella* have been reported – the endemic *L. caespitosa* (type locality Parimahu, North Island of New Zealand), *L. hookeriana* (type locality Cape Horn, South America) and *L. pericladus*. We reassessed species diversity of *Lophurella* in Australia and New Zealand with the aim of determining (1) whether New Zealand and South American specimens of *L. hookeriana* actually represent a single species, and (2) if the morphotypes of *L. pericladus* mask cryptic diversity. We studied *rbcL* sequences and morphological features of 36 specimens identified as *L. pericladus*, one specimen of *L. caespitosa*, and five samples of *L. hookeriana*, three from New Zealand and two from Cape Horn. Molecular analyses revealed that *L. hookeriana* from New Zealand and South America are distinct species and the new species *L. pauciramulosa* is described from New Zealand. *Lophurella pericladus* is a complex involving four species and we propose three new species, *L. mutabilis*, *L. nigra* and *L. tasmanica*. Cryptic diversity in *L. pericladus* did not align with the previously defined ecotypes and several species were often found at the same site. *Lophurella pericladus*, *L. nigra* and *L. tasmanica* can be distinguished by morphological characters. Conversely, *L. mutabilis* has high morphological plasticity, with characters that overlap with *L. pericladus* and *L. nigra*, and can be only distinguished by DNA sequences.

KEYWORDS: cryptic diversity; distribution; molecular systematics; morphology; phenotypic plasticity; new species; phylogeny; Pterosiphoniae; *rbcL*; red algae.

Introduction

The genus *Lophurella* F.Schmitz (in Schmitz & Falkenberg, 1897) includes seven recognized species (Guiry & Guiry, 2019). It differs from other genera in the Rhodomelaceae by the following combination of characters: thalli consist of prostrate and erect terete axes, with axes having 4 or 7 pericentral cells that are completely corticated from close to the apices, bearing radially arranged determinate branches (Falkenberg, 1901; Womersley, 2003). Based on these features, *Lophurella* was originally placed in the tribe Polysiphonieae (Falkenberg, 1901; Hommersand, 1963; Womersley, 2003). However, it was recently transferred to the Pterosiphonieae using molecular and morphological evidence (Díaz-Tapia *et al.*, 2017). The rhizoids of *Lophurella* have multicellular haptera and differ from the unicellular haptera characteristic of the Polysiphonieae and Streblocladieae (Díaz-Tapia *et al.*, 2017).

Lophurella is restricted to the cold-temperate Southern Hemisphere, with species reported from Australia, New Zealand, South America and Tristan da Cunha (Guiry & Guiry, 2019). *Lophurella pericladus* (Sonder) F.Schmitz, the generitype, is common in the low intertidal in Southern Australia, Victoria and Tasmania and also found in New South Wales (Millar & Kraft, 1993; Womersley, 2003). It is the only member of the genus in Australia, its type locality (Port Phillip Bay, Victoria), and is easily distinguished from other members of the Rhodomelaceae (Womersley, 2003). It has also been reported in New Zealand where it differs from congeners by having scarcely branched main erect axes that bear abundant determinate branches (Adams, 1994; Womersley, 2003). *Lophurella caespitosa* (Harvey) Falkenberg is endemic to New Zealand and is characterized by its emerald green colour and being smaller (up to 5 cm) than its congeners in the region (Adams, 1994; Nelson, 2013). The third member of the genus recorded in New Zealand is *L. hookeriana* (J.Agardh) Falkenberg (type locality Cape Horn, South America), characterized by long erect axes (up to 15 cm) that are more profusely branched and with fewer determinate branches than other species (Adams, 1994). Three species have been recorded only in South America: *L. patula* (J.D.Hooker & Harvey) De Toni, *L. gaimardii* (Gaudichaud ex C.Agardh) De Toni and *L. comosa* (J.D.Hooker & Harvey) Falkenberg. Finally, *L. christospherseni* Baardseth is only known in Tristan da Cunha (Baardseth, 1941).

Species delimitation based on morphological characters is often difficult in marine macroalgae that exhibit high morphological plasticity or converge to present similar morphologies (Verbruggen, 2014). As a result, cryptic diversity is commonly discovered when molecular assisted taxonomy is used for species diversity assessments (e.g. Guillemín *et al.*, 2016; Saunders *et al.*, 2017), including in the family Rhodomelaceae (Savoie & Saunders, 2016, 2019; Díaz-Tapia *et al.*, 2018a). New cryptic species have been detected as the result of comparing sequence data for specimens of the presumed same species from distant locations (e.g. Bustamante *et al.*, 2014; Díaz-Tapia *et al.*, 2018a; Schneider *et al.*, 2018). This led us to hypothesize that the records of *L. hookeriana* from New Zealand and South America might actually correspond to different species. Cryptic diversity is also common within a geographical region (e.g. Guillemín *et al.*, 2016; Savoie & Saunders, 2016, 2019). Phenotypic plasticity is often recognized in red algal species with morphological variation in relation to environmental conditions. However, the use of sequence data has shown that this plasticity often masks cryptic species (Milstein & Saunders, 2012; Zanolla *et al.*, 2014; Barreto de Jesús *et al.*, 2019). We suspected that the morphotypes of *L. pericladus* might correspond to different species, because *L. pericladus* is known to exhibit morphological variability in Tasmania associated with different levels of wave

exposure (Womersley, 2003). The aim of this work is to test these hypotheses, re-assessing species diversity of the genus *Lophurella* in Australia and New Zealand using *rbcL* plastid gene sequences and detailed morphological studies of the specimens.

Materials and methods

Material of *Lophurella* spp. was collected during surveys of the family Rhodomelaceae in Victoria and eastern Tasmania (Australia) and New Zealand (Table S1). Regions adjacent to the known range of the genus in Australia, the York Peninsula (Southern Australia) and the northern coast of Tasmania were explored without finding *Lophurella*. We also obtained two samples of *L. hookeriana* from Cape Horn (Chile), its type locality. Materials for DNA extraction were dried in silica gel desiccant. Plants for morphological study were preserved in 4% formalin seawater at 4°C and stored in the dark. Some specimens were mounted in 20% Karo® Syrup (ACH Foods, Memphis, TN, USA). Sections for microscopic observations were made by hand using a razor blade. Voucher specimens were deposited in the University of Melbourne Herbarium (MELU), the National Herbarium of Victoria (MEL) and Museum of New Zealand Te Papa Tongarewa (WELT).

DNA was extracted from silica gel-dried material following Saunders & McDevit (2012) or an adapted cetyltrimethylammonium bromide (CTAB) protocol (Doyle & Doyle, 1987). PCR amplification of *rbcL* was carried out using primers F57/*rbcL*revNEW, F2/R1008, F2/R1464 and F2/R1452 (Saunders & Moore, 2013; Díaz-Tapia *et al.*, 2018a). Reactions were performed in a total volume of 25 µl, consisting of 5 µl 5× MyTaq™ reaction buffer, 0.7 µl 10 µM of forward and reverse primers, 0.125 µl 1U µl⁻¹ My Taq™ DNA Polymerase (Bioline, London, UK), 17.475 µl MilliQ® water and 1 µl template DNA. The PCR profile consisted of initial denaturation (93°C for 3 min), 35 cycles of denaturation (94°C for 30 s), primer annealing (45°C for 30 s), and extension (74°C for 90 s) and final extension (74°C for 5 min). The PCR products were purified and sequenced by Macrogen (Korea) or the sequencing service of the University of A Coruña.

39 new *rbcL* sequences were analysed together with the four sequences available in GenBank (Table S1). One of the GenBank sequences (KT825866) was originally misidentified as *Womersleyella pacifica* Hollenberg. However BLAST searches revealed its close similarity to *Lophurella* and we included it in our dataset. Sequences were aligned using Muscle in Geneious 6.1.8 (Kearse *et al.*, 2012). The alignment was 1424 nucleotides long in total, and sequence lengths were 665-1464 bp.

To obtain a species-level phylogeny of the genus a maximum likelihood (ML) phylogeny was inferred. This phylogeny includes a single sequence per haplotype, selected according to quality in terms of length (i.e. the longest sequence). The phylogenetic tree for *rbcL* was estimated with Maximum Likelihood (ML) using RAxML 8.1.X (Stamatakis, 2014). GTR-Gamma was used as the nucleotide model and branch support was estimated with 100 bootstrap replicates. Two species of *Echinothamnion* were selected as outgroup, as it is the closest sister genus based on phylogenetic analyses of the tribe Pterosiphonieae (Savoie & Saunders, 2016).

Results

Molecular identification and phylogeny

RAxML analyses of the 43 sequences of *Lophurella* specimens resolved seven lineages that we consider to represent species, three previously recognized and four new species (Fig. 1). Sequence divergence within each lineage was 0-0.9% (0-12 bp), and among lineages 1.0-3.4% (15-44 bp) (Table S2). The previously recognized species *L. caespitosa* sampled in New Zealand (its type locality) and *L. hookeriana* from Cape Horn (Chile), also its type locality, were clearly separated from other species by 1.9-3.4% sequence divergence. Two sequences of *L. hookeriana* from Chile differed by 0.6% from a sequence from the Falkland Islands. Our molecular data showed that all three specimens that we collected and identified as *L. hookeriana* in New Zealand differed from the topotype specimens by 1.9-2.3% sequence divergence and we propose *L. pauciramulosa* sp. nov. from New Zealand.

The specimens that we originally identified as *L. pericladus* were resolved in four clades (Fig. 1). All eight specimens collected in Port Phillip Bay, Victoria, the type locality of *L. pericladus*, and nearby areas formed a highly supported clade with two haplotypes that diverged by 0.8% (11 bp). Accordingly, we concluded that our collections of topotype material correspond to *L. pericladus* (Fig. 2). In addition to *L. pericladus*, the only species of the genus previously recorded in Australia, three other species were identified in Australia, one also being present in New Zealand (Fig. 2), and we propose the erection of three new species. *Lophurella tasmanica* sp. nov. was closely related, with high support, to *L. pericladus* and sequence divergence between them was 1-1.1% (13-15 bp). The clade corresponding to *L. nigra* sp. nov. included six sequences, five identical and one that diverged by 0.1% (1 bp). The clade corresponding to *L. mutabilis* sp. nov. consisted of 18 sequences and six haplotypes. The five Australian haplotypes (H1-5) diverged by 0-0.4% (up to 3 bp) and the New Zealand haplotype (H6) diverged by up to 0.9% (12 bp) from Australian haplotypes.

Relationships among the species that we identified in the genus *Lophurella* were not resolved in our phylogenetic analysis (except for grouping the sister species *L. pericladus* and *L. tasmanica*).

Morphological observations

Of the 40 specimens of *Lophurella* spp. collected during our sampling surveys of the family Rhodomelaceae in Victoria, Tasmania (Australia) and New Zealand, 36 were morphologically identifiable as *L. pericladus*, three as *L. hookeriana* and one as *L. caespitosa* (which is distinctive in colour, thallus length and branching pattern). A description of the characters shared among *L. pericladus* and the four new species recognized in this study is provided, with the details of these characters including their measurements in each species (Table 1). We also include a diagnosis of each new species, as well as a summary of the morphological characters that differ among the species here studied (Table 2). The description of *L. pericladus* is based on our collections. The only available detailed description of *L. pericladus* was provided by Womersley (2003) but considering the distribution of the selected specimens used by Womersley and our results, his description was most probably based on a mixture of species.

Morphology of *Lophurella* spp. from Australasia (except *L. caespitosa*)

Vegetative morphology

Thallus formed of prostrate and erect axes (Fig. 3), habit varying among species. Axes consisting of a small axial cell and four pericentral cells, heavily corticated from close to the apices. In cross-section, pericentral cells of young branches covered by a layer of cortical cells (Figs 4-5). In old parts of thalli, pericentral cells surrounded by one to four layers of little-pigmented pseudoparenchymatous cells and a layer of deeply pigmented cortical cells (Figs 6-7). Cortical cells in surface view rounded to elongate-polygonal. Plastids elliptical to irregular (Fig. 8).

Prostrate axes (Fig. 9) growing from a dome-shaped apical cell, increasing in diameter in older parts. Axes lacking trichoblasts, forming a branch initial on every segment or at intervals of several segments, spirally arranged, from which endogenous branches arise, also on every segment or at intervals of several segments. Lateral and ventral branches producing further prostrate axes or remaining as short laterals; dorsal branches producing erect axes. Several rhizoids usually formed on every segment, cut off from cortical cells, consisting of a unicellular filament terminating in a multicellular discoid pad (Fig. 10). Haptera initially formed by cells cut off from the basal part of the rhizoidal filament, subsequently branching dichotomously for up to two orders (Fig. 11).

Erect axes growing from a dome-shaped apical cell (Fig. 12), increasing in diameter in mid and basal parts. Branching pattern, abundance and arrangement of determinate branches and trichoblasts varying among species. Trichoblasts, when present, initially short and pigmented, later enlarging and becoming unpigmented, dichotomously branched up to five orders, with uninucleate cells (Figs 13-14). They were deciduous and left conspicuous scar cells when shed.

Reproductive morphology

Gametophytes dioecious. Spermatangial branches formed on determinate lateral branches, replacing trichoblasts, in dense clusters arranged spirally on every segment (Fig. 15). Spermatangial branches cylindrical, often incurved, with one or two apical sterile cells when mature (Fig. 16). Procarps formed on modified trichoblasts, consisting of a supporting cell bearing a four-celled carpogonial branch, a basal sterile cell and two lateral sterile cells (Fig. 17). Cystocarps formed on determinate branches in mid-parts of the thallus, ovoid and with an apical ostiole (Fig. 18). Carposporangia clavate.

Tetrasporangia formed in mid-parts of the thallus on determinate branches that were more profusely branched than vegetative laterals. One tetrasporangium formed per segment, arranged in densely compacted long spiral series (Fig. 19). Tetrasporangia subspherical, with two presporangial and one postsporangial cover cells that remained ecorticate (Fig. 20).

***Lophurella pericladus* (Sonder) F.Schmitz in Schmitz & Falkenberg, 1897: 441 (Figs 21-27; Figs S1-4, S6-10 and S33-52)**

BASIONYM: *Rhodomela pericladus* Sonder, 1855.

SYNONYMS: *Rhodomela simpliciuscula* Harvey *nom. nudum*.

LECTOTYPE: MEL 612898 (Womersley, 2003; Fig. S1).

ISOLECTOTYPES: MEL 612897, 612899, 612900 (Womersley, 2003; Figs S2-4).

TYPE LOCALITY: Port Phillip Bay, Victoria, Australia.

Description

Thallus dorsiventral, consisting of a prostrate system that bears rhizoids ventrally, erect axes dorsally and produces further prostrate axes laterally (Figs 21-23). Erect axes up to 10 cm in length, with main axis unbranched or pseudodichotomously branched up to four orders (Figs 21-23). Axes densely clothed with short determinate branches spirally arranged throughout the length of the main axes, sometimes denuded in basal parts (Figs 21-23). Thalli dark red to black in colour, with a rigid to flaccid texture.

At apices of erect axes, branch initials produced on every segment in a spiral sequence; endogenous determinate lateral branches also developing on every segment (Fig. 24). Determinate branches incurved when young (Fig. 24), soon becoming straight and acquiring a spiny appearance (Fig. 25), 300-500 μ m in diameter basally. First-order determinate laterals producing branch initials on every segment; only some initials developing further, producing second and third-orders of endogenous determinate laterals (Fig. 26). Second- and third-order determinate laterals remaining short, often unilaterally arranged (Fig. 26). Basal parts of the erect axes lacking determinate laterals in some specimens, usually unbranched when present. Determinate laterals either overtopping apical cell of the main axis or the apical cell protruding beyond the branches. Trichoblasts absent on main axes and first-order determinate laterals (Figs 24 and 26), borne on second- and third-order determinate laterals, on every segment (Figs 26-27).

Distribution, habitat and morphological variability of our collections and type material

Lophurella pericladus was commonly found in Port Phillip Bay and on nearby open coasts and is the only member of the genus that we identified in this region (Fig. 2). It was also collected at Mallacoota, the easternmost reefs in Victoria. It formed turfs in the intertidal zone of wave-exposed reefs, where specimens were robust, with a rigid texture, short (up to 5 cm in length) and scarcely branched (Figs S6-9). These specimens correspond to haplotype 2 (H2 in Fig. 1). A sequence from Robe (Southern Australia) that we downloaded from GenBank was identical to H2. The second haplotype (H1 in Fig. 1) corresponded to specimens collected in the drift or in a marina at Queenscliff, more sheltered locations inside Port Phillip Bay (Victoria). These specimens were more flaccid, more profusely branched and longer (up to 10 cm in length) (Fig. S10). The morphological variability observed in our specimens is similar to the conspicuous variability in habit of the type material. The type collection of *L. pericladus* is housed at MEL and includes four specimens. Two specimens were short (6 cm) and scarcely branched (Figs S3-4), while the remaining two were longer (10 cm) and more profusely branched (Figs S1-2). Among them, Womersley (2003) designated MEL612898 as the lectotype (Fig. S1). The specimens of *L. pericladus* that we collected are in agreement with the type. *Lophurella pericladus* was absent in our collections from Tasmania and New Zealand.

***Lophurella mutabilis* Díaz-Tapia, sp. nov. (Figs 28-35, S11-23 and S53-69)**

Diagnosis: Thalli dorsiventral, with a prostrate system that bears rhizoids ventrally, erect axes dorsally and produces further prostrate axes laterally. Erect axes with main axes unbranched or pseudodichotomously to irregularly branched, clothed with determinate branches, usually on every segment and spirally arranged but occasionally sparse. Axes with four pericentral cells. Erect axes growing by divisions of apical cell that protrudes above the lateral determinate branches, branch initials forming at apices on every segment or several segments apart. Some or all branch initials developing into determinate branches, 210-400 µm in diameter basally, straight and spine-like when mature. First-order determinate branches producing up to two further orders of branches that remain short. Trichoblasts restricted to determinate branches. *RbcL* sequence of the holotype: MN149994.

HOLOTYPE: MELUA118884a.

TYPE LOCALITY: Blackmans Bay, Tasmania, Australia.

ETYMOLOGY: “*mutabilis*” refers to the high variability observed among specimens of this species in habit and other morphological characters.

Description

Thalli dorsiventral, consisting of a prostrate system that bears rhizoids ventrally, erect axes dorsally and produces further prostrate axes laterally (Figs 28-29). Habit variable, ranging from small (5 mm in length) pseudodichotomously branched specimens with sparse determinate branches (Fig. 28) to large specimens (up to 15 cm in length); main axes branching irregularly alternately or pseudodichotomously, profusely, to up to four orders, with axes clothed by abundant determinate laterals arranged spirally or unilaterally (Figs 29-31). Light to dark red or black in colour, with a rigid to flaccid texture.

Erect axes producing branch initials on every segment in a spiral sequence or, more rarely, several segments apart, all or only some developing into lateral determinate branches (Fig. 32). Determinate branches usually abundant and spirally arranged, clothing the main axes, 210-400 µm in diameter in basal parts and upwardly incurved when young, later becoming straight, spine-like (Figs 33-34). Determinate laterals producing one or two orders of short determinate branches, arranged spirally or unilaterally. Trichoblasts usually present at the apices of first- and higher order determinate branches, formed on every segment in a spiral arrangement (Fig. 35), absent from the apices of main axes and, in some specimens, also from first-order determinate branches (Fig. 32).

Distribution, habitat, and morphological variability

Lophurella mutabilis was abundant in eastern Tasmania (Fig. 2), forming turfs in the low intertidal of moderately to strongly wave-exposed sites. *Lophurella mutabilis* was highly variable in habit (Figs S11-23); specimens from sheltered locations (Tinderbox and Southport, Figs S15-18) were more profusely branched and slenderer than

specimens from exposed sites (Figs S11-13 and S19-23). However, this morphological variability was not reflected in the genetic variability in the *rbcL* gene, as haplotypes 2 and 4 were found at both types of sites (Fig. 2). *Lophurella mutabilis* was also collected at a site in western Victoria where a single small (5 mm in length, Fig. 28) male specimen was found epiphytic on *Cystophora* sp. Genetically, this specimen corresponded to H1 in Fig. 1. In New Zealand, *L. mutabilis* H6 (Fig. 1) was collected in the low intertidal of a site on Stewart Island.

***Lophurella nigra* Díaz-Tapia, sp. nov. (Figs 36-41, S24-28 and S70-88)**

Diagnosis: Thalli dorsiventral, with a prostrate system that bears rhizoids ventrally, erect axes dorsally and produces further prostrate axes laterally. Erect axes pseudodichotomously or irregularly branched, bearing sparse determinate branches. Axes with four pericentral cells. Branch initials formed on every segment at the apices of the erect axes. Some branch initials developing into determinate branches, 250-400 µm in diameter basally, straight and spine-like when mature. Trichoblasts restricted to second or higher orders of determinate branches. *RbcL* sequence of the holotype: MN149998.

HOLOTYPE: MEL2457114.

TYPE LOCALITY: Bastion Point, Mallacoota, Australia

ETYMOLOGY: “*nigra*” refers to the black colour of the thallus.

Description

Thalli dorsiventral, consisting of an extensive prostrate system that bears rhizoids ventrally, erect axes dorsally and produces further prostrate axes laterally (Figs 36-38). Erect axes up to 5 cm in length, irregularly branched up to three orders, either with one main axis and lateral determinate branches or pseudodichotomously branched, with several main axes, that bear sparse and irregularly or unilaterally arranged determinate laterals (Figs 36-39). Thalli dark red to black in colour, with a rigid texture.

Erect axes producing branch initials on every segment, of which only some develop lateral endogenous branches. Determinate laterals unbranched or producing one or two orders of further determinate laterals, often unilaterally arranged (Fig. 39). Determinate laterals 250-400 µm in diameter basally. Trichoblasts formed on second- and third-order determinate laterals, spirally arranged on every segment, but absent from main axes and first-order determinate laterals (Figs 40-41).

Distribution, habitat and morphological variability

Lophurella nigra was collected in eastern Victoria where it formed turfs in the low intertidal of wave-exposed sites. It was also collected in the same habitat in northeastern Tasmania, as well as in the subtidal (5 m depth). Victorian specimens were short (up to 7 mm) and robust, while Tasmanian ones were longer (up to 5 cm) and more slender. This variability in habitat and distribution did not correspond with the genetic

variability found in the *rbcL* gene. The two haplotypes were detected at a single sampling site and most specimens, independent of habitat and distribution, corresponded to haplotype 2 (Fig. 1, Table S1).

***Lophurella pauciramulosa* Díaz-Tapia, sp. nov. (Figs 42-45, S29-31 and S89-101)**

Diagnosis: Thalli predominantly erect, attached by a short prostrate system that bears rhizoids ventrally, erect axes dorsally and produces further prostrate axes laterally. Erect axes pseudodichotomously branched up to seven orders, bearing sparse determinate branches. Axes with four pericentral cells. Branch initials formed on every segment at the apices of the erect axes. Some branch initials developing into determinate branches that are sparse, 250-300 µm in diameter basally. Trichoblasts absent. *RbcL* sequence of the holotype: MN150002.

HOLOTYPE: WELT A033737.

TYPE LOCALITY: Green Island, South Island, New Zealand.

ETYMOLOGY: “*pauciramulosa*” refers to the scarcity of determinate branches compared with most other members of the genus.

Description

Thalli predominantly erect (Fig. 42), attached to the substratum by a short prostrate system that bears rhizoids ventrally and produces further prostrate axes laterally. Erect axes up to 20 cm in length, branched pseudodichotomously up to seven orders, producing series of unilaterally arranged short determinate laterals at irregular intervals (Fig. 43). Thalli dark dull purple red in colour, drying black, with a firm texture.

Erect axes producing determinate endogenous lateral branches at irregular intervals (Figs 44-45). Lateral branches 250-300 µm basally, unbranched or once-branched in vegetative thalli. Trichoblasts absent.

Habitat and distribution

This species was collected in the subtidal (2-10 m depth) from the south east coast of South Island and Stewart Island, New Zealand. *Lophurella pauciramulosa* is often infected by the parasites *Sporoglossum lophurellae* Kylin and *Colacopsis lophurellae* Kylin.

***Lophurella tasmanica* Díaz-Tapia, sp. nov. (Figs 46-50, S32 and S102-119)**

Diagnosis: Thalli dorsiventral, with a prostrate system that bears rhizoids ventrally, erect axes dorsally and produces further prostrate axes laterally. Erect axes with unbranched main axes clothed with spirally arranged determinate branches formed on every segment. Axes with four pericentral cells. Erect axes growing by the division of an apical cell that is overtopped by lateral determinate branches. Branch initials formed at apices of erect axes, on every segment. All branch initials developing into determinate branches, 150-230 µm in diameter basally, upwardly incurved when

mature. First-order determinate branches producing up to two further orders of determinate branches. Determinate second-order branches reaching a length similar to the parental determinate branch. Trichoblasts restricted to second- and third-order determinate branches. *RbcL* sequence of the holotype: MN150004.

HOLOTYPE: MELUA118885a.

TYPE LOCALITY: Port Arthur, Tasmania, Australia.

ETYMOLOGY: “*tasmanica*” refers to the type locality of the species.

Description

Thallus dorsiventral, consisting of a prostrate system that bears rhizoids ventrally, erect axes dorsally and produces further prostrate axes laterally (Fig. 46). Erect axes up to 5 cm in length with unbranched main axes clothed by spirally arranged determinate laterals. Thalli dark red in colour, with a rigid texture.

Erect axes producing determinate lateral branches on every segment, spirally arranged and upwardly incurved, overtopping the apical cell of the main axes (Figs 47-49). Lateral branches 150-230 μ m diameter in basal parts, producing spirally a second-order of determinate branches when young, such branches remaining restricted to basal parts of laterals (Fig. 50). Second-order branches upwardly incurved and reaching a similar length to the parental first-order determinate branch (Fig. 50). A third-order of determinate laterals remained as short branches (Fig. 50). Determinate laterals in basal parts of the thalli less profusely branched, probably denuded. Trichoblasts formed on second- and third-order determinate branches in a spiral arrangement on every segment but absent from the apex of the main axes and the first-order determinate branches (Fig. 50).

Habitat and distribution

Only known from the type locality, in southeastern Tasmania (Fig. 2), where it was collected in the low intertidal of a moderately wave-exposed site.

Discussion

We found that 36 specimens initially identified as *Lophurella pericladus* from Australia and New Zealand represented a complex of four cryptic or semi-cryptic species for which we propose three new species, *L. mutabilis*, *L. nigra* and *L. tasmanica*. Moreover, we found that *L. hookeriana* from New Zealand differs from specimens from the type locality in Chile, requiring the description of the new species *L. pauciramulosa* from New Zealand.

The new species are distinguished by their sequence divergence in the *rbcL* gene relative to the previously recognized species in the genus. Sequence divergence was $\geq$ 1.8% among species, except between *Lophurella tasmanica* and *L. pericladus*, which were 1.0-1.1% divergent. Although sequence divergence for this pair of species is less than for the other species here described, they can be morphologically distinguished

(see discussion below) and we recognize them as separate species. This contrasts with the recognition of a single species for the six haplotypes we found in *L. mutabilis* and the two haplotypes of *L. pericladus*. One of the haplotypes of *L. mutabilis* (H6 in Fig. 1), the New Zealand specimen, was relatively highly (0.7-0.9%) divergent from Australian specimens. Likewise, the divergence between the two haplotypes of *L. pericladus* (0.8%) was relatively high and they might be considered as separate species. However, in the absence of relevant morphological characters for distinguishing these highly divergent haplotypes, we do not recognize them as distinct species at present. Future work with larger sampling sizes across the distribution range of these lineages as well as additional molecular markers might reveal whether they should be segregated or that they are single lineages with high genetic variability in the *rbcL* gene. Species boundaries based on sequence data are often based on comparable divergence values among sister species assuming that interspecific divergence is higher than intraspecific variability (Leliaert *et al.*, 2014). However, the establishment of boundaries based on sequence divergence is not always straightforward and different species, even if closely related, may have experienced different evolutionary histories resulting in different levels of intraspecific variability (Díaz-Tapia *et al.*, 2018a; Phillips *et al.*, 2019). In our *Lophurella* spp. dataset, there was no large difference between intra- and interspecific variability in the *rbcL* gene, and therefore we also took morphological characters into account when delineating the species.

All the species described here accord with the concept of the tribe Pterosiphoniae, as rhizoids are cut off from pericentral cells and have multicellular haptera (Díaz-Tapia *et al.*, 2017). Likewise, they fit the definition of the genus *Lophurella* (Womersley, 2003): the thallus consists of prostrate and erect axes, axes have four pericentral cells and are heavily corticated from close to the apices, spermatangial branches replace trichoblasts and have apical sterile cells, cystocarps are globose and tetrasporangia form spiral series. Moreover, all the studied species had tetrasporangia with two presporangial cover cells and a postsporangial cover cell. Trichoblasts were found abundantly in most species here studied (except *L. pauciramulosa*) and their arrangement was unusual when compared with other Rhodomelaceae. Trichoblasts in this family are usually produced at the apexes of main axes and branches (Maggs & Hommersand, 1993; Womersley, 2003; Díaz-Tapia *et al.*, 2013). However, in *Lophurella*, trichoblasts were absent from the main axes and restricted to second or higher order determinate branches. Womersley (2003) noted this particular character in his description of the genus.

Most of the taxonomically informative qualitative morphological characters that are employed for distinguishing species in the Pterosiphoniae were shared among the species studied here (Maggs & Hommersand, 1993; Womersley, 2003; Savoie & Saunders, 2016). Nevertheless, some other detailed morphological features can contribute to species identification. Table 2 summarizes the main characters that we found useful for distinguishing the species of *Lophurella* in Australia and New Zealand. They include vegetative morphology, habitat, and the presence or absence of parasites (*Colacopsis lophurellae* and *Sporoglossum lophurellae*). The reproductive structures when known were virtually uniform among species and were not informative for species delimitation, as is often the case in the Rhodomelaceae (Díaz-Tapia & Bárbara, 2011; García-Redondo *et al.*, 2016). Morphologically, *L. caespitosa*, *L. pauciramulosa* and *L. tasmanica* can be distinguished from other congeners from Australia and New Zealand. The most conspicuous characters of *L. caespitosa* are its emerald green colour, the absence of trichoblasts and the branching pattern of erect axes that are denuded

below, with abundant branches in upper parts bearing tufts of short determinate laterals at the apices (Adams, 1994; Nelson, 2013; PD, pers. obs.). The other species, by contrast, are dark red to black in colour, have trichoblasts (except *L. pauciramulosa*) and the erect axes have a different branching pattern (Table 2). *Lophurella tasmanica* is morphologically similar to *L. pericladus* and some specimens of *L. mutabilis* which have the main axes clothed with short determinate branches. *Lophurella tasmanica* differs from this pair of species mainly because its determinate laterals are thinner, more profusely branched, with longer second-order determinate branches, and determinate laterals are incurved at maturity. As a result, the main axes are densely covered by determinate laterals that lack the spiny appearance of *L. mutabilis* and *L. pericladus*. *Lophurella pauciramulosa* is mainly distinguished from the other species studied here by having long (up to 20 cm) and predominantly erect thalli, scarce production of determinate branches, complete absence of trichoblasts, as well as the subtidal habitat and the common presence of parasites (*C. lophurellae* and *S. lophurellae*). *Lophurella pauciramulosa* is morphologically distinct from Australian and New Zealand congeners and it can be also distinguished from *L. hookeriana*, which has trichoblasts on determinate branches (Boraso de Zaixso, 2013; EM, pers. obs.).

The other three species here recognized, *Lophurella pericladus*, *L. nigra* and *L. mutabilis*, are examples of cryptic species as they cannot be distinguished by morphological characters and DNA sequences are required for their identification. *Lophurella pericladus* and *L. nigra* are distinct, but *L. mutabilis* is so variable morphologically that some specimens overlap with the morphological characters of both species. *Lophurella pericladus* has spirally arranged determinate laterals formed on every segment while *L. nigra* bears sparse determinate laterals in an irregular pattern. We observed specimens of *L. mutabilis* with both of these habits and other morphological details are also shared with the other two species. Therefore, the high morphological plasticity of *L. mutabilis* prevents reliable morphological identification of these three species. This scenario is frequently encountered in the red algae and similar problematic morphological delineations have been discussed in other groups (Milstein & Saunders, 2012; Carro *et al.*, 2014; Verbruggen, 2014). However, more commonly, phenotypic plasticity explains the high levels of cryptic diversity detected in the red algae but detailed morphological studies of the species delineated based on DNA data can reveal morphological differences among them (Walker *et al.*, 2009; Zanolla *et al.*, 2014, Barreto de Jesus *et al.*, 2018). The morphological variability described by Womersley (2003) for *L. pericladus* that he related to different levels of wave exposure was observed within *L. pericladus* and *L. mutabilis*. Specimens from sheltered sites are slenderer and more elongate than those from wave-exposed coasts. Therefore, the cryptic diversity that we detected did not correspond to the morphotypes noted by Womersley (2003).

In addition to the species of *Lophurella* described here and included in our molecular analyses, another four species are currently recognized. *Lophurella comosa*, from South America, is clearly distinguished from the rest of the genus by having seven pericentral cells (Hooker & Harvey, 1845; Harvey, 1847) whereas all other species have four. *Lophurella patula* and *L. gaimardii*, both also from South America, resemble *L. pauciramulosa* in exceeding 10 cm in length and having sparse determinate branches (Hooker & Harvey, 1845; De Toni, 1905). They differ from the other three species described here, which have shorter erect axes (up to 10 cm, except *L. mutabilis*) and/or the erect axes are clothed with short determinate branches. *Lophurella patula* has main axes with alternate branches (Hooker & Harvey, 1845; Kylin & Skottsberg, 1919),

differing from *L. pauciramulosa* that is pseudodichotomously branched. *Lophurella gaimardii* has only been reported from the type locality, the Falkland Islands, and according to the original description (Agardh, 1822, as *Rhodomela*), this species has trichoblasts (“*ad apicem ramentorum racemosa, pellucida*”) which appear to be shown in the illustration in Bory de Saint-Vincent (1826). Therefore, *L. gaimardii* differs in this respect from *L. pauciramulosa*, which lacks trichoblasts. Finally, *Lophurella christophersenii* from Tristan da Cunha differs from other species because lateral determinate branches are shed from the older parts of the thallus, resulting in a long stem bearing determinate branches only in the upper parts (Baardseth, 1941). Moreover, it has spermatangial branches on the first dichotomy of trichoblasts (Baardseth, 1941), while spermatangial branches completely replace trichoblasts in other congeners when known. Indeed, this character is uniform in all the genera included at present in the tribe Pterosiphoniae (Womersley, 2003; Díaz-Tapia & Bárbara, 2011; García-Redondo *et al.*, 2016; Díaz-Tapia *et al.*, 2017). This leads us to question the placement of *L. christophersenii* in the genus *Lophurella* and the tribe Pterosiphoniae, and we suggest that this taxon should be re-evaluated using molecular data.

Species of *Lophurella* have restricted distributions and our study showed that their range is even narrower than that indicated in previous diversity assessments based on morphological identifications. *Lophurella hookeriana* was the only species with a transoceanic recorded distribution (Adams, 1994; Guiry & Guiry, 2019), but our data show that the New Zealand and South American populations represent different species. *L. hookeriana* and *L. pauciramulosa* are endemic to South America and New Zealand, respectively. *Lophurella pericladus* was previously reported from southeastern Australia, Tasmania and New Zealand, but our study showed that it is a complex of four species with different but overlapping distribution patterns (Fig. 2). *Lophurella pericladus* and *L. tasmanica* were only found in mainland Australia and eastern Tasmania, respectively, while *L. nigra* was found in both regions. *Lophurella pericladus* has been also recorded in New South Wales (Millar & Kraft, 1993) and the identity of these specimens needs to be reassessed using molecular data. *Lophurella mutabilis* has the widest distribution, including southeastern Australia, Tasmania and New Zealand. The present distribution of *Lophurella* spp. suggests that their evolutionary history might have been affected by palaeobiogeographical processes, such as dispersal events between Australasia and South America or the land-bridge formed between Tasmania and mainland Australia during the Pleistocene, in the same way that these processes might have shaped the current distribution of other red algae (Guillemin *et al.*, 2014; Díaz-Tapia *et al.*, 2018b; Mueller *et al.*, 2018). Further studies including wider taxon sampling might contribute to better understanding how these processes have contributed to the evolutionary history of the Australasian red algae.

Given the level of cryptic speciation discovered in the genus in Australia further research in New Zealand is required, including determining the distributional ranges of *L. pauciramulosa* and *L. mutabilis*. It is likely that there is further diversity within what has been known as *L. hookeriana* in New Zealand: this species has been reported from the northern North Island through to the New Zealand subantarctic, and specimens within herbarium collections display considerable morphological variability (WN, pers. obs.). The records of *Lophurella pericladus* in New Zealand also have to be re-examined: this species has been reported from Cook Strait south to Stewart Island as well as from the Chatham Islands, and it is not clear that all of the specimens are correctly referred to *L. mutabilis*.

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Author contributions

P. Díaz-Tapia: original concept, morphological and molecular analyses, drafting and editing manuscript; C.A. Maggs: original concept, drafting and editing manuscript; W. Nelson: providing specimens, morphological analyses, editing manuscript; E.C. Macaya: providing specimens, morphological analyses; H. Verbruggen: original concept, drafting and editing manuscript.

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780

Figure legends

Fig. 1. Maximum likelihood phylogeny of *Lophurella* based on *rbcL* sequences. Species names at the tip branches indicate the original identification based on morphological characters; the vertical bars and their corresponding names indicate the reassessed species diversity based on morphological characters and molecular data. Names of new taxa are printed in bold. Values at the nodes represent bootstrap support, only shown if > 80.

Fig. 2. Distribution of *Lophurella* spp. and their respective haplotypes (see Fig. 1) in Australia (left) and New Zealand (right).

Figs 3-14. *Lophurella* spp.: vegetative morphology. **Fig. 3.** Habit of a specimen with prostrate and erect axes (*L. nigra*). **Figs 4-7.** Cross-section of an axis in the upper thallus (Figs 4-5) and the mid-thallus (Figs 6-7), with an axial cell (a), four pericentral cells (p), cortical cells (c) and, only in Figs 6-7, pseudoparenchymatous cells (ps). **Fig 8.** Cortical cells showing plastids. **Fig. 9.** Apex of a prostrate axis. **Figs 10-11.** Prostrate axes with rhizoids cut off from cortical cells and with multicellular haptera. **Fig. 12.** Apex of an erect axis with initials on every segment (arrowheads). **Fig. 13.** Apex of an erect axis bearing trichoblasts on the second-order determinate branches. **Fig. 14.** Determinate branch bearing spirally arranged trichoblasts. Figs 3, 9, 12 and 13, *L. nigra*; Figs 4 and 6, *L. tasmanica*; Figs 5, 7, 10 and 14, *L. mutabilis*; Fig. 8, *L. pauciramulosa*, Fig. 11, *L. periclados*. Scale bars: Fig. 3, 2.5 mm; Figs 4 and 12, 40 µm; Fig. 5, 30 µm; Fig. 6, 400 µm; Fig. 7, 200 µm; Fig. 8, 15 µm; Figs 9 and 13, 350 µm; Figs 10 and 11, 100 µm; Fig. 14, 150 µm.

Figs 15-20. *Lophurella* spp.: reproductive morphology. **Fig. 15.** Spermatangial branches densely clustered on second-order determinate branches. **Fig. 16.** Spermatangial branches with one or two sterile apical cells (arrows). **Fig 17.** Procarp showing the supporting cell (su), a four-celled carpogonial branch (1-4) and a basal sterile cell (st). **Fig. 18.** Cystocarp with an apical ostiole (arrow). **Fig. 19.** Determinate branches with spirally arranged tetrasporangia. **Fig. 20.** Tetrasporangia with two presporangial (arrows) and a postsporangial (arrowhead) cover cells. Figs 15 and 16, *L. periclados*; Figs 17, 18 and 20, *L. nigra*; Fig. 19, *L. mutabilis*. Scale bars: Fig. 15, 300 µm; Fig. 16 and 18, 70 µm; Fig. 17, 10µm; Fig. 19, 100 µm; Fig. 20, 25 µm.

Figs 21-27. *Lophurella periclados*. **Figs 21-23.** Habit of specimens PD2746, PD772, PD4787, respectively. **Fig. 24.** Apex of the thallus, the arrow showing the apical cell. **Fig. 25.** Axis with determinate branches. **Fig. 26.** First-order determinate branch lacking trichoblasts (arrow) and bearing second and third-order branches with trichoblasts. **Fig. 27.** Apex of a third-order determinate branch bearing spirally arranged trichoblasts. Scale bars: Figs 21 and 22, 8 mm; Fig. 23, 15 mm; Fig. 24, 150 µm; Figs 25 and 26, 850 µm; Fig. 27, 100 µm.

Figs 28-35. *Lophurella mutabilis*. **Figs 28-31.** Habit of specimens PD1111, PD3411, PD3483 and PD3106, respectively. **Fig. 32.** Apical part of an erect axis, forming

determinate branches several segments below the apex. **Figs 33-34.** Thallus clothed with determinate branches. **Fig. 35.** Apex of a third-order determinate branch bearing spirally arranged trichoblasts. Scale bars: Fig. 28, 450 μm ; Fig. 29, 7 mm; Fig. 30, 2 cm; Fig. 31, 4 cm; Fig. 32 and 34, 700 μm ; Fig. 33, 4 mm; Fig. 35, 150 μm .

Figs 36-41. *Lophurella nigra*. **Figs 36-8.** Habit of specimens PD3555, PD2736, and PD2741, respectively. **Fig. 40.** Apex of an erect axis bearing two orders of determinate branches, the second-order bearing trichoblasts. **Fig. 41.** Determinate branch, lacking trichoblasts, bearing an order of determinate branches with young trichoblasts. Scale bars: Fig. 36, 5 mm; Figs 37 and 38, 2.5 mm; Fig. 39, 1 mm; Fig. 40, 350 μm ; Fig 41, 250 μm .

Figs 42-45. *Lophurella pauciramulosa*. **Fig. 42.** Habit of the holotype (specimen WELT A033737 (ASR166)). **Fig. 43.** Apical part of an erect axis with determinate branches. **Fig. 44.** Apex of an erect axis. **Fig. 45.** Determinate branch lacking trichoblasts. Scale bars: Fig. 42, 35 mm; Fig. 43, 6 mm; Fig. 44, 60 μm ; Fig. 45, 400 μm .

Figs 46-50. *Lophurella tasmanica*. **Fig. 46.** Habit of specimen PD3584. **Fig 47.** Apex of an erect axis with apical cell indicated (arrow). **Fig. 48.** Apical part of an erect axis densely clothed with determinate branches. **Fig 49.** Mid-part of an erect axis with basally branched determinate laterals. **Fig. 50.** Determinate branch bearing two orders of branches, of which the third-order branches bear trichoblasts. Scale bars: Fig. 46, 8 mm; Fig. 47, 100 μm ; Fig. 48, 2.5 mm; Figs 49-50, 800 μm .

Supplementary information

Table S1. Collection information or publication and GenBank accession numbers of the sequences used in phylogenetic analyses.

Table S2. Intra- and interspecific sequence divergence in the *rbcL* gene in the genus *Lophurella*.

Figs S1-32. Habit of specimens of *Lophurella* spp. from Australasia.

Figs S33-119. Details of morphological characters of *Lophurella* spp. from Australasia (except *L. caespitosa*).

Table 1. Measurements of morphological characters for *Lophurella* spp. from Australasia (except *L. caespitosa*).

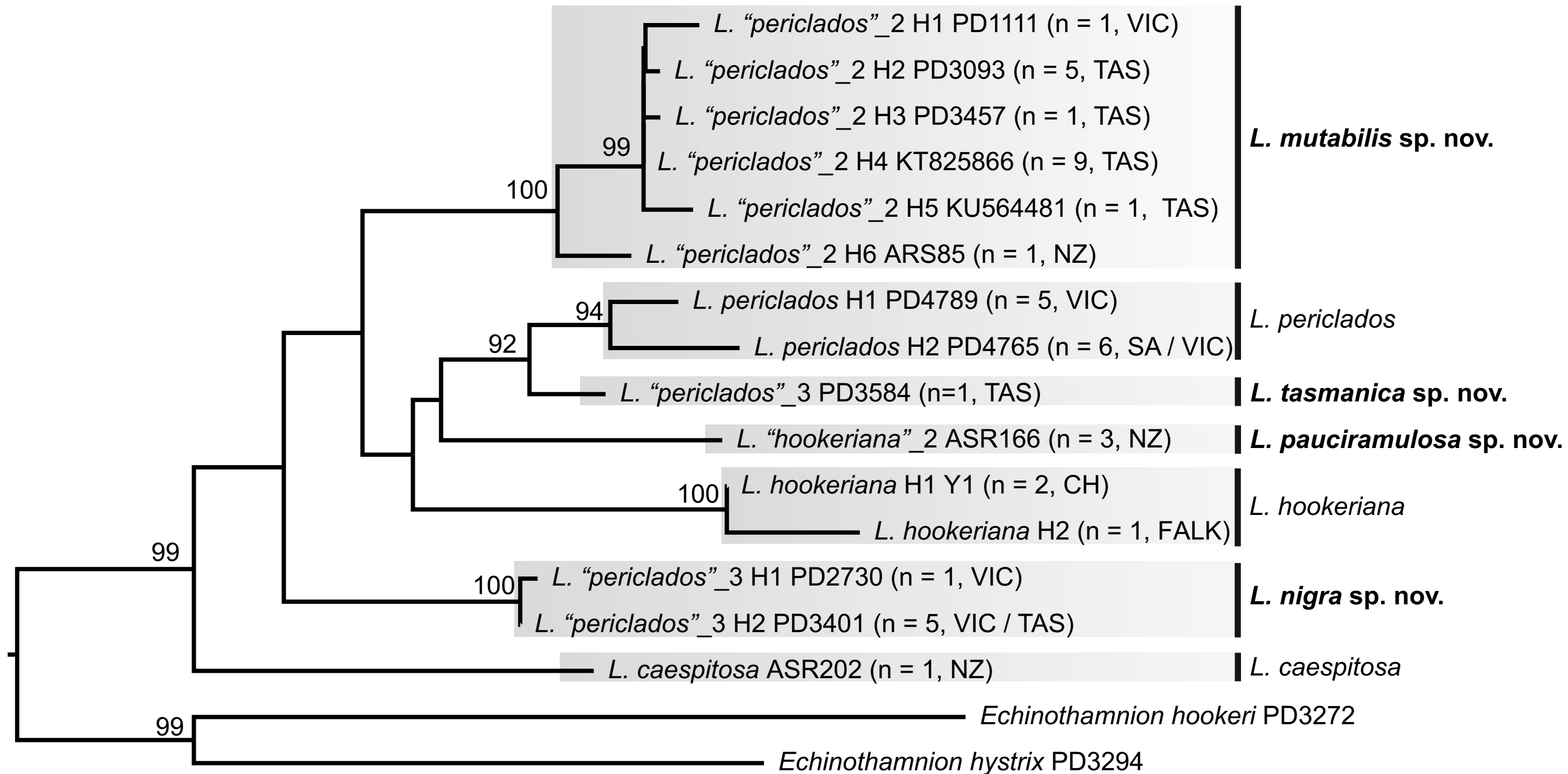
	<i>L. pericladus</i>	<i>L. mutabilis</i>	<i>L. nigra</i>	<i>L. pauciramulosa</i>	<i>L. tasmanica</i>
Cortical cells (μm)	10-23 × 12-55	7.5-30 × 10-75	10-25 × 12-38	7.5-20 × 7.5-43	12-50 × 35-100
Prostrate axes					
Apical cell diameter (μm)	20-25	17-20	20	20	25
Diameter (μm)	250-470	(120-) 250-500	250-400	(280-) 350-700	400-500
Rhizoids					
Length (μm)	up to 700	up to 700	up to 820	up to 700	up to 1000
Filament diameter (μm)	30-80	25-50 μm	30-40	40-60 μm	30-70
Erect axes					
Apical cell diameter (μm)	17-20	12-20 μm	17-23	20-35	25
Diameter (μm)	600-930	(130-) 450-750	330-650	380-850	750-900
Trichoblasts					
Length (μm)	up to 850	up to 650	up to 900	-	up to 400
Diameter of basal cell (μm)	35-50	25-35	40-50	-	32-50
Spermatangial branches (μm)	35-65 × 105-200	45-55 × 125-180	30-50 × 125-165	Unknown	Unknown
Procarys	4-celled	Unknown	4-celled	Unknown	Unknown
Cystocarys (μm)	470-500 × 550-600	300-450 × 600-700	430-600 × 400-710	Unknown	Unknown
Carposporangia (μm)	15-25 × 105-113	12-18 × 50-100	12-20 × 45-75	Unknown	Unknown

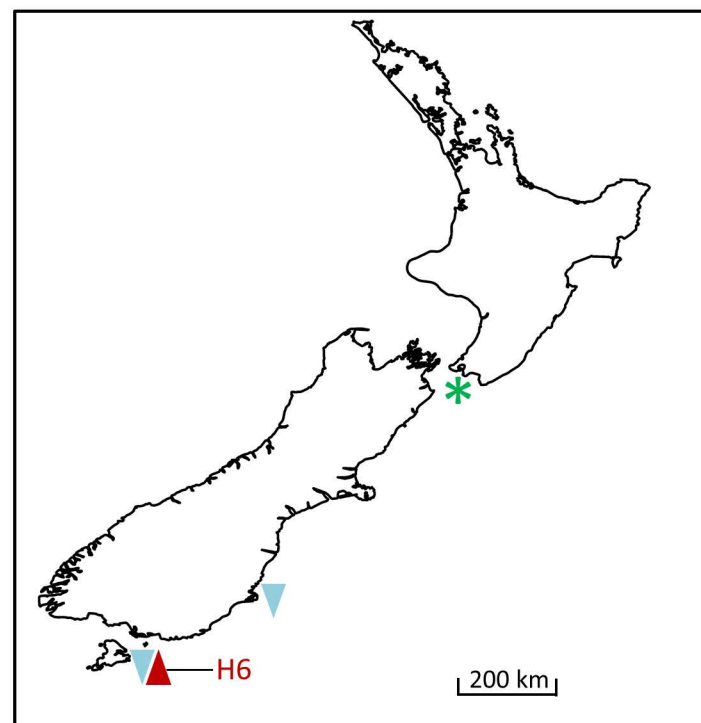
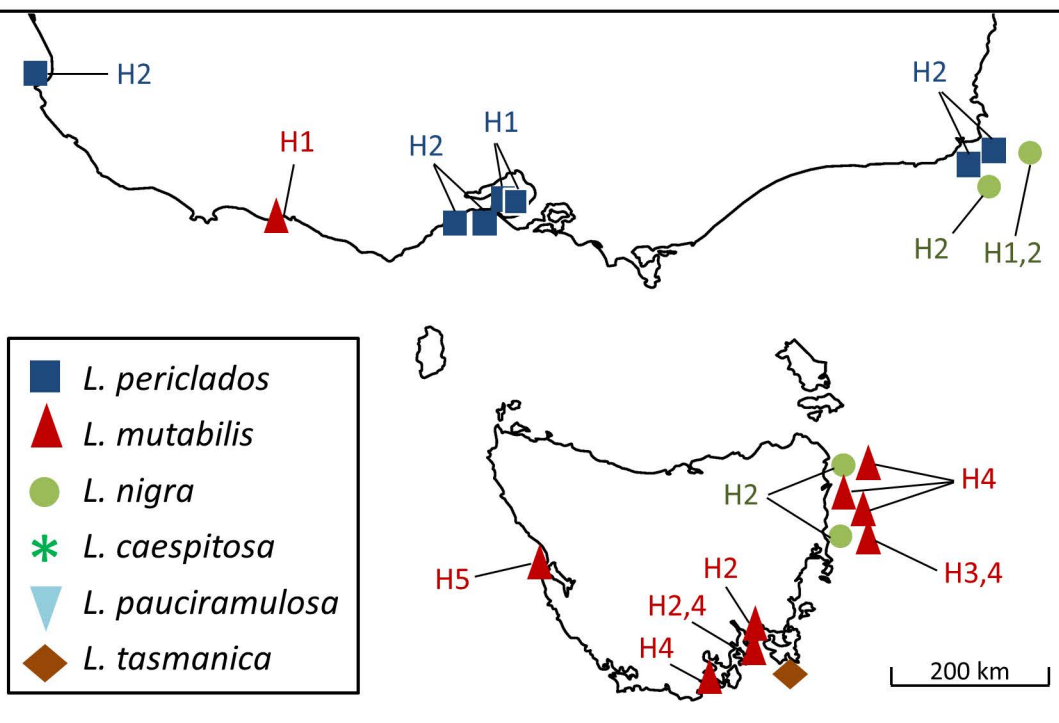
Length of tetrasporangial branches (μm)	700-1100	500-1800	600-2000	2000	600-900
Tetrasporangia (μm)	55-65 $\times$ 55-80	40-55 (-70) $\times$ 45-63 (-73)	48-65 $\times$ 37-75	55-75 $\times$ 67-80	40-55 (-70) $\times$ 45-63 (-73)

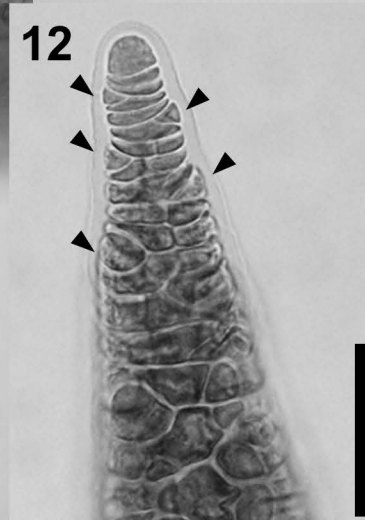
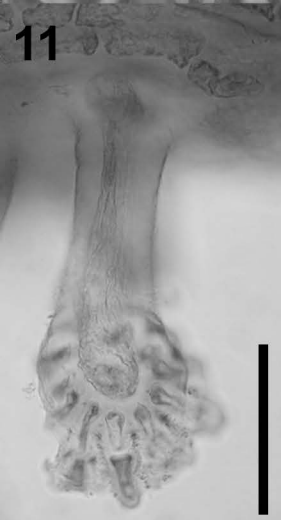
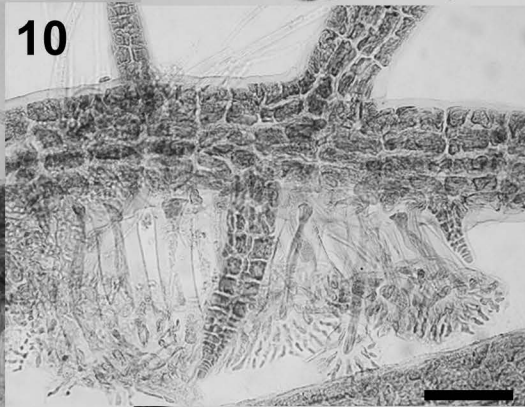
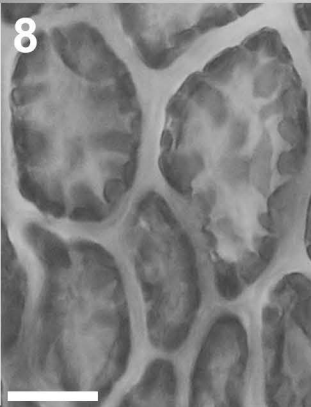
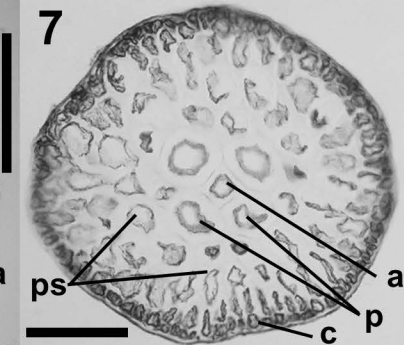
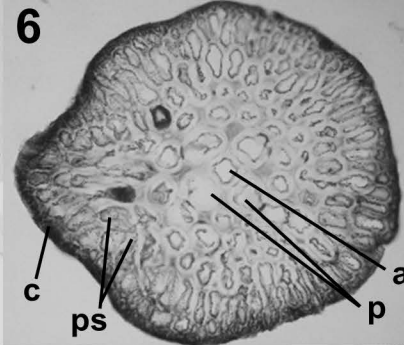
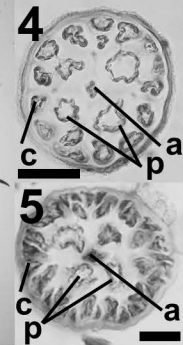
Table 2. Comparison of selected morphological characters useful for species delineation and distributions between the species of *Lophurella* from Australia and New Zealand. Characters printed in bold are key for distinguishing the species.

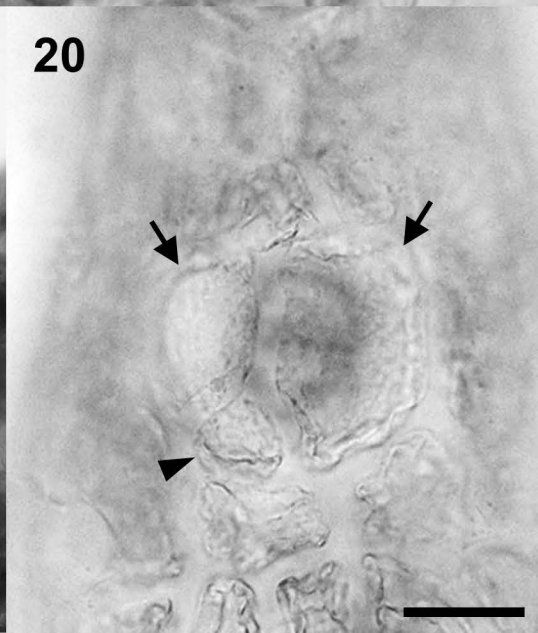
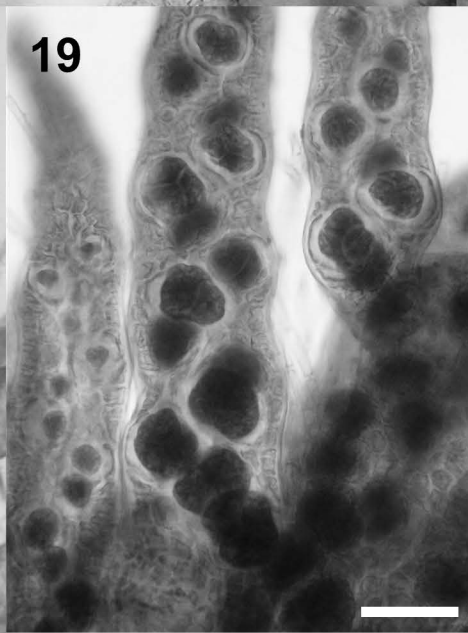
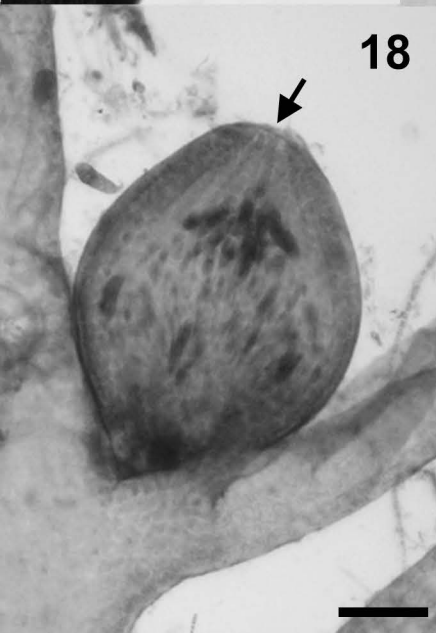
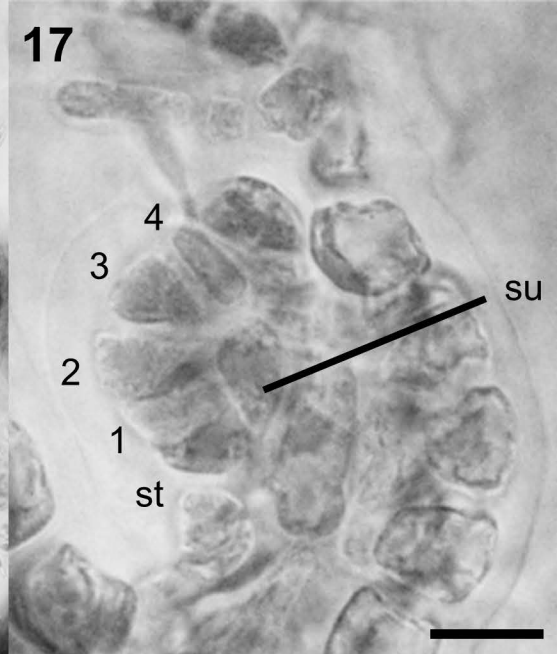
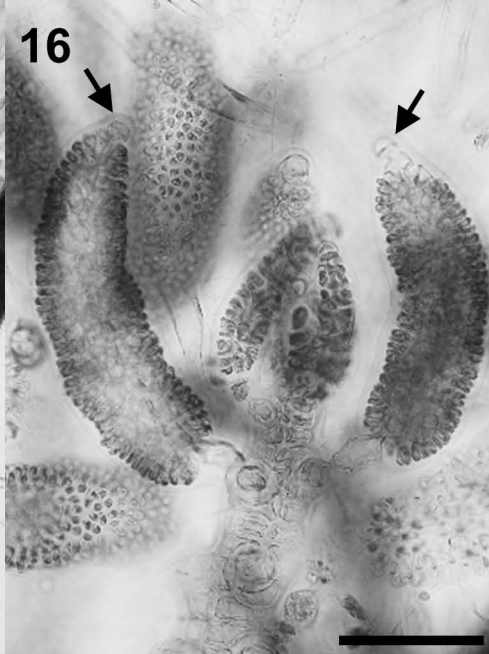
	<i>L. tasmanica</i>	<i>L. pauciramulosa</i>	<i>L. nigra</i>	<i>L. mutabilis</i>	<i>L. pericladus</i>	<i>L. caespitosa</i>
Habit	Dorsiventral	Predominantly erect	Dorsiventral	Dorsiventral	Dorsiventral	Dorsiventral
Erect axes branching pattern	Unbranched	Pseudodichotomous up to 7 orders	Pseudodichotomous or irregular, up to 3 orders	Unbranched axes, pseudodichotomous or irregular up to 4 orders	Unbranched or pseudodichotomous up to 4 orders	Denuded below, abundant branches in upper parts Lower thallus Deep red/black, upper axes green emerald
Colour	Dark brown	Dark red or brown	Dark brown to black	Light/dark brown, black	Dark brown to black	
Length of erect axes (cm)	Up to 5	Up to 20	Up to 5	Up to 15	Up to 10	Up to 5
Trichoblast arrangement	2 nd and 3 rd order determinate branches	Absent	2 nd and 3 rd order determinate branches	1 st , 2 nd and 3 rd order determinate branches	2 nd and 3 rd order determinate branches	Absent
Determinate branches						
Arrangement	On every segment	Scarce	Scarce	Scarce/On every segment	On every segment	Tufted at apices of branches
Diameter (µm)	150-230	250-300	250-400	210-400	300-500	150-260
Appearance	Incurved	Straight, spiny	Straight, spiny	Straight, spiny	Straight, spiny	Straight, spiny
Length of 2 nd order determinate branches	Similar to 1st order	Short	Short	Short	Short	Short
Apical cell	Overtopped by determinate branches	Protruding above determinate branches	Protruding above determinate branches	Protruding above determinate branches	Overtopped or protruding above determinate branches	Overtopped by determinate branches
Habitat	Intertidal	Subtidal	Intertidal or subtidal	Intertidal	Intertidal	Intertidal
Parasites	Absent	Present	Absent	Absent	Absent	Absent

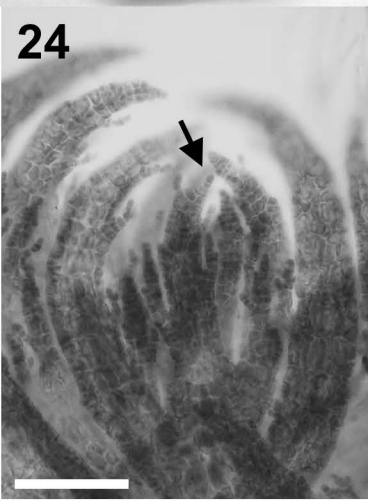
Distribution	Tasmania (Australia)	New Zealand	Tasmania and Victoria (Australia)	Tasmania and Victoria (Australia); New Zealand	Victoria (Australia)	New Zealand
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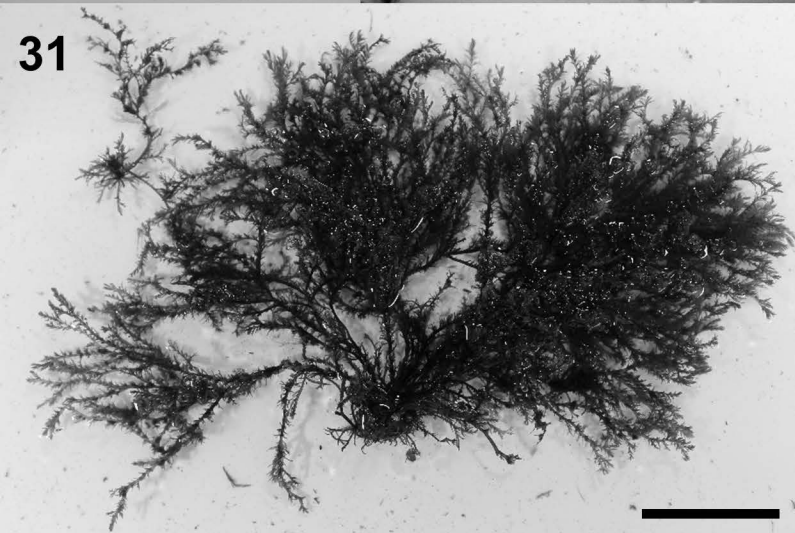
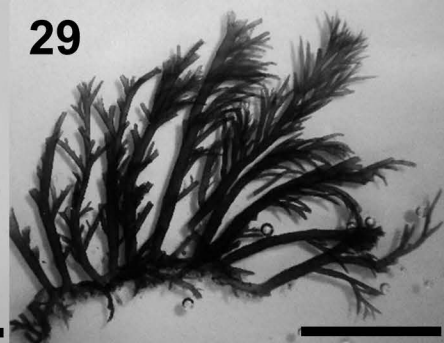
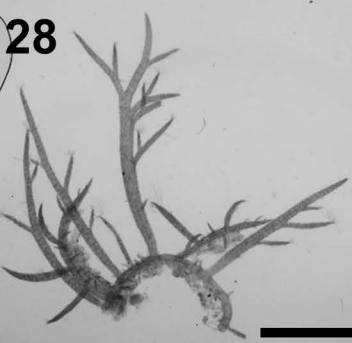






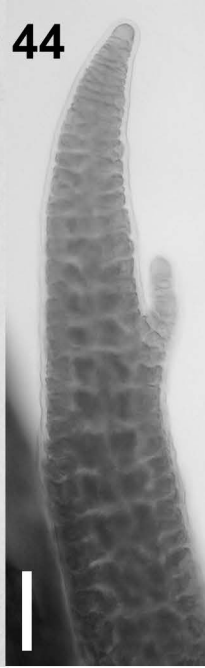
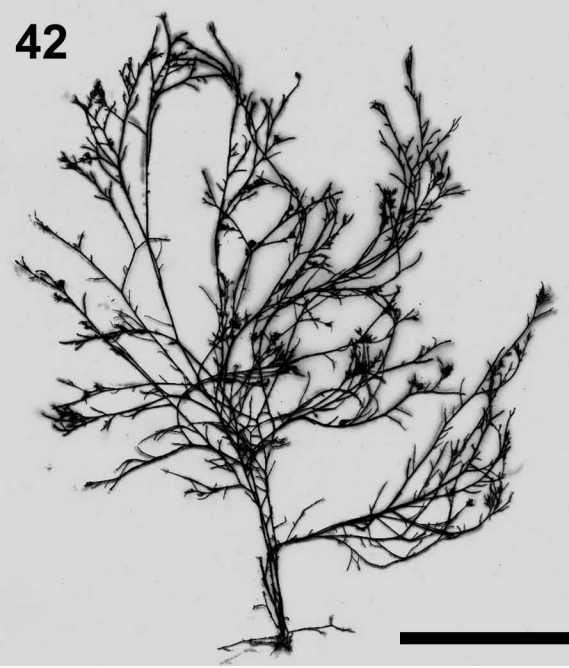






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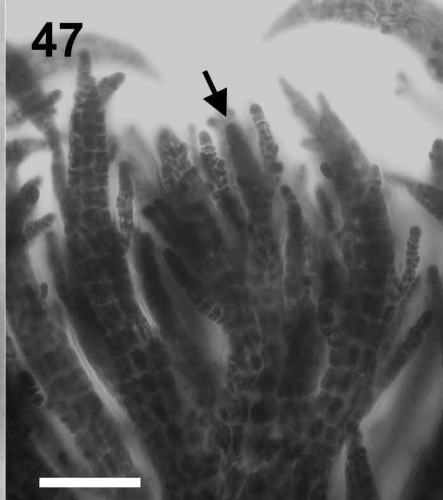


Table S1. Collection information or publication and GenBank accession numbers of the sequences used in phylogenetic analyses.

		Specimen	Haplotype	GenBank Accession Number <i>rbc L</i>
Collection site, habitat, date & collectors / Reference				
		ASR202 (WELT A033734)	-	MN149978
<i>Lophurella caespitosa</i>	Arthur's Nose, Wellington South coast, North Island, New Zealand; mid-intertidal; 21.vi.2017; WN			
<i>Lophurella hookeriana</i>	Yendegaia, Magellan Region, Southern Patagonian, Chile; subtidal (1 m); 22.x.2016; ECM	Y1	H1	MN149974
	Yendegaia, Magellan Region, Southern Patagonian, Chile; subtidal (1 m); 22.x.2016; ECM	Y2	H1	MN149975
	Falkland Islands / García-Soto & López-Bautista 2018	-	H2	MH388599
<i>Lophurella pericladus</i>	Robe, Southern Australia / García-Soto & López-Bautista 2018	-	H1	KU564481
	13th beach, Victoria, Australia; intertidal; 9.xi.2014; tetrasporophyte; PD	PD772	H1	MN150010
	Galaneuse Reef, Victoria, Australia; intertidal; 6.xii.2014; female gametophyte; PD	PD2915	H1	MN150014
	Queenscliff, Port Phillip Bay, Victoria, Australia; drift; 27.xi.2018; PD	PD4765	H2	MN150013
	Queenscliff, Port Phillip Bay, Victoria, Australia; on pontoons in the marina; 30.xi.2018; PD	PD4787	H2	MN150005
	Queenscliff, Port Phillip Bay, Victoria, Australia; on pontoons in the marina; 30.xi.2018; PD	PD4789	H2	MN150009
	Queenscliff, Port Phillip Bay, Victoria, Australia; on pontoons in the marina; 30.xi.2018; PD	PD4790	H2	MN150008
	Queenscliff, Port Phillip Bay, Victoria, Australia; on pontoons in the marina; 30.xi.2018; PD	PD4791	H2	MN150007
	Queenscliff, Port Phillip Bay, Victoria, Australia; on pontoons in the marina; 30.xi.2018; PD	PD4792	H2	MN150006
	Pebbly Beach, Mallacoota, Victoria, Australia; intertidal; 9.xi.2016; PD, KD & HV	PD2746	H1	MN150012
	Shipwreck Creek, Mallacoota, Victoria, Australia; intertidal; 13.xi.2016; male gametophyte; PD, KD & HV	PD2866	H1	MN150011
<i>Lophurella nigra</i> sp. nov.	Bastion Point, Mallacoota, Victoria, Australia; subtidal (1 m); 8.xi.2016; female gametophyte; PD, KD & HV	PD2730	H1	MN149995
	Bastion Point, Mallacoota, Victoria, Australia; subtidal (1 m); 8.xi.2016; tetrasporophyte and female gametophyte; PD, KD & HV	PD2736	H2	MN149998
	Pebbly Beach, Mallacoota, Victoria; intertidal; 9.xi.2016; tetrasporophyte; PD, KD & HV	PD2741	H2	MN150000
	The Gardens, Tasmania, Australia; intertidal; 7.xi.2017; male gametophyte; PD & JFC	PD3401	H2	MN149999
	Bicheno, Tasmania, Australia; intertidal; 9.xi.2017; PD & JFC	PD3475	H2	MN149997
	Bicheno, Tasmania, Australia; intertidal; 9.xi.2017; PD & JFC	PD3555	H2	MN149996
<i>Lophurella mutabilis</i> sp. nov.	Killarnei, Victoria, Australia; intertidal; 27.xii.2014; male gametophyte; PD	PD1111	H1	MN149980
	Hells Gates, Tasmania, Australia / Savoie & Saunders 2016	GWS016008	H5	KU564481
	Falmouth, Tasmania, Australia / Bustamante et al. 2016, as <i>Womersleyella pacifica</i>	CUK10968	H4	KT825866
	Blackmans Bay, Tasmania, Australia; intertidal; 30.x.2017; PD & JFC	PD3072	H2	MN149993
	Blackmans Bay, Tasmania, Australia; intertidal; 30.x.2017; tetrasporophyte; PD & JFC	PD3075	H2	MN149994
	Blackmans Bay, Tasmania, Australia; intertidal; 30.x.2017; tetrasporophyte; PD & JFC	PD3079	H2	MN149992
	Blackmans Bay, Tasmania, Australia; intertidal; 30.x.2017; female gametophyte; PD & JFC	PD3093	H2	MN149991

	Collection site, habitat, date & collectors / Reference	Specimen	Haplotype	GenBank Accession Number
	Tinderbox, Tasmania, Australia; subtidal (1 m); 31.x.2017; PD & JFC	PD3139	H2	MN149990
	Tinderbox, Tasmania, Australia; subtidal (1 m); 31.x.2017; PD & JFC	PD3106	H4	MN149984
	Southport, Tasmania, Australia; intertidal; 1.xi.2017; tetraspophyte; PD & JFC	PD3164	H4	MN149983
	Southport, Tasmania, Australia; intertidal; 1.xi.2017; tetraspophyte; PD & JFC	PD3172	H4	MN149982
	Binalong Bay, Tasmania, Australia; intertidal; 8.xi.2017; tetraspophyte; PD & JFC	PD3411	H4	MN149986
	Binalong Bay, Tasmania, Australia; intertidal; 8.xi.2017; PD & JFC	PD3423	H4	MN149987
	Bicheno, Tasmania, Australia; intertidal; 9.xi.2017; tetrasporophyte; PD & JFC	PD3478	H4	MN149989
	Bicheno, Tasmania, Australia; intertidal; 9.xi.2017; tetrasporophyte; PD & JFC	PD3483	H4	MN149988
	Bicheno, Tasmania, Australia; intertidal; 9.xi.2017; female gametophyte; PD & JFC	PD3457	H3	MN149981
	The Gardens, Tasmania, Australia; intertidal; 7.xi.2017; PD & JFC	PD3376	H4	MN149985
		ASR85		
	East of Bishop, Stewart Island, New Zealand; low intertidal; 1.iv.2017; RD	(WELT A033735)	H6	MN149979
		ASR102		
<i>Lophurella pauciramulosa</i> sp. nov.	East of Bishop, Stewart Island, New Zealand; subtidal (10 m); 1.iv.2017; RD	(WELT A033736)	-	MN150003
		ASR166		
	Green Island, South Island, New Zealand; subtidal (8 m); 31.v.2017; tetrasporophyte; RD & BT	(WELT A033737)	-	MN150002
		ASR175		
	Green Island, South Island, New Zealand; subtidal (2 m); 31.v.2017; tetrasporophyte; MD & AK	(WELT A033738)	-	MN150001
<i>Lophurella tasmanica</i> sp. nov.	Port Arthur, Tasmania, Australia; intertidal; 13.xi.2017; tetraspophyte; PD & JFC	PD3584	-	MN150004
Outgroup				
<i>Echinothamnion hookeri</i>	Wynyard, Tasmania, Australia; drift; 4.xi.2017; PD & JFC	PD3272	-	MN149976
<i>Echinothamnion hystrix</i>	Wynyard, Tasmania, Australia; drift; 4.xi.2017; PD & JFC	PD3294	-	MN149977

Collectors. BT: B. Twist; ECM: Erasmo Macaya; HV: Heroen Verbruggen; JFC: Joana F. Costa; KD: Kyatt Dixon; MD: M. Desmond; PD: P. Díaz-Tapia; RD: Roberta D'Archino; WN: Wendy Nelson

References

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- Savoie, A.M. & Saunders, G.W. (2016). A molecular phylogenetic and DNA barcode assessment of the tribe Pterosiphonieae (Ceramiales, Rhodophyta) emphasizing the Northeast Pacific. *Botany*, **94**: 917-939.

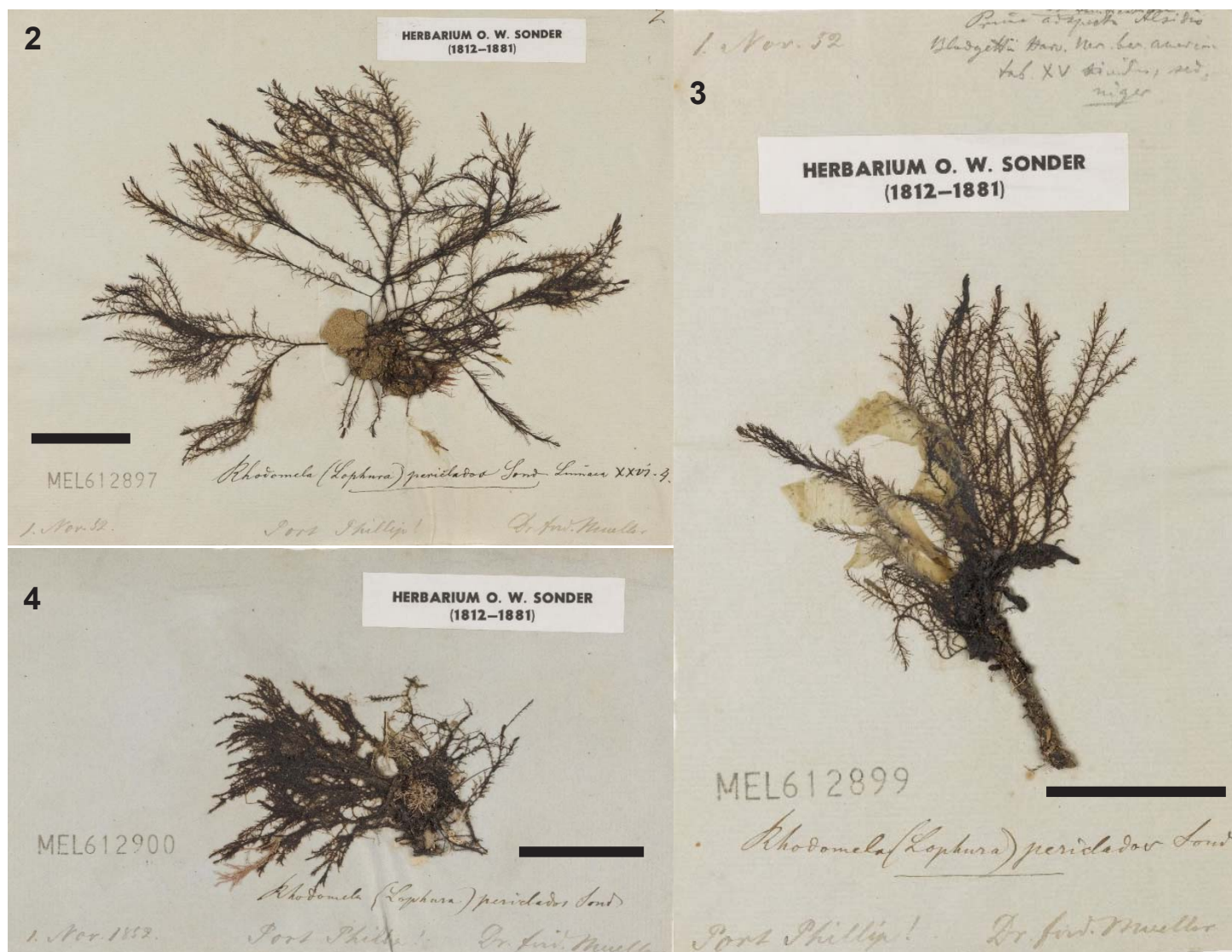
Reassessment of the genus *Lophurella* (Rhodomelaceae, Rhodophyta) from Australia and New Zealand reveals four cryptic species

European Journal of Phycology. Díaz-Tapia, P., Maggs, C.A., Nelson, W., Macaya, E.C., Verbruggen, H.

Figs S1-32. Habit of specimens of *Lophurella* spp. from Australasia.



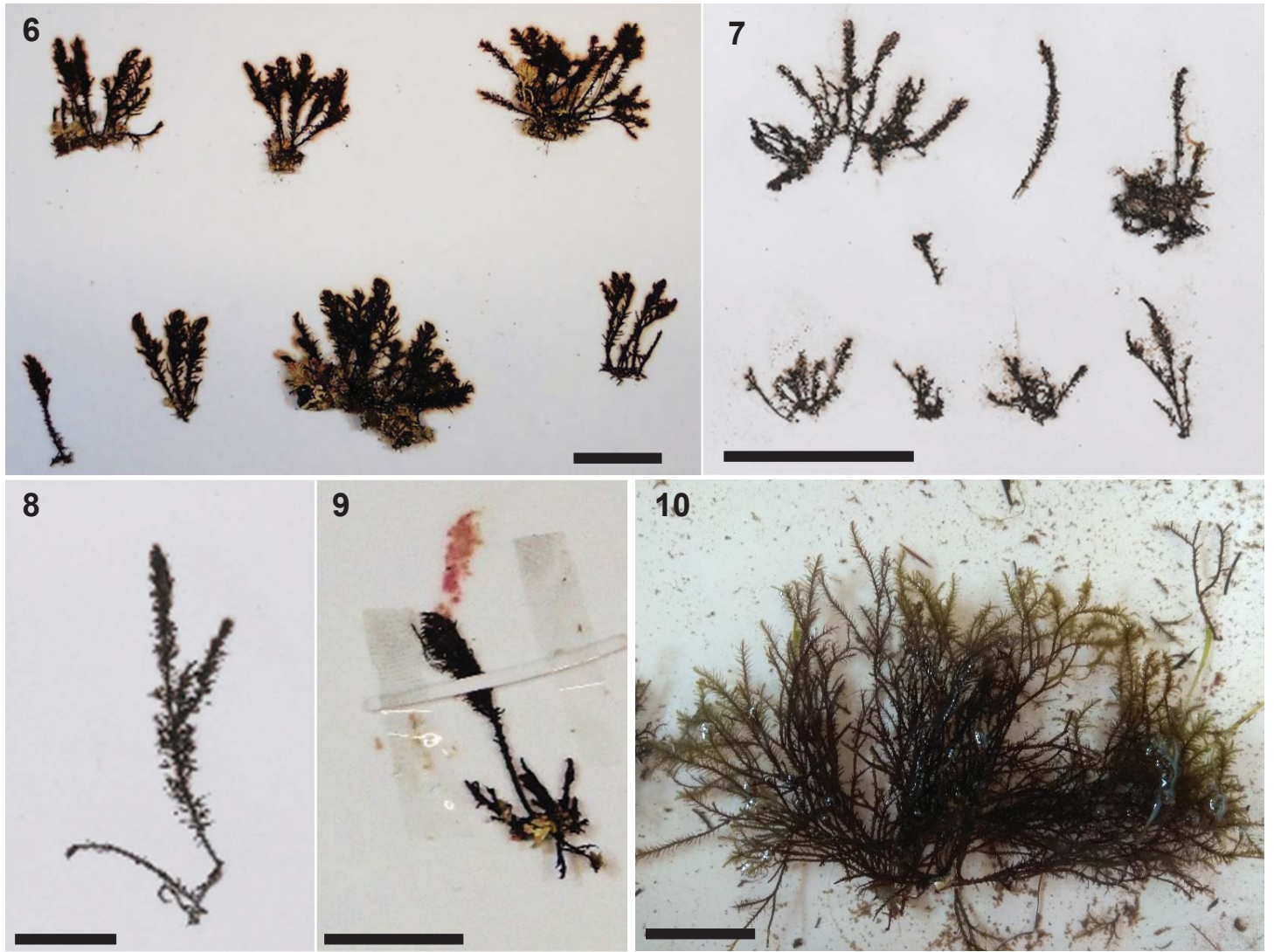
Fig. S1. *Lophurella periclados*. Lectotype. Scale bar: 3 cm. Reproduced with permission from the Royal Botanic Gardens Victoria.



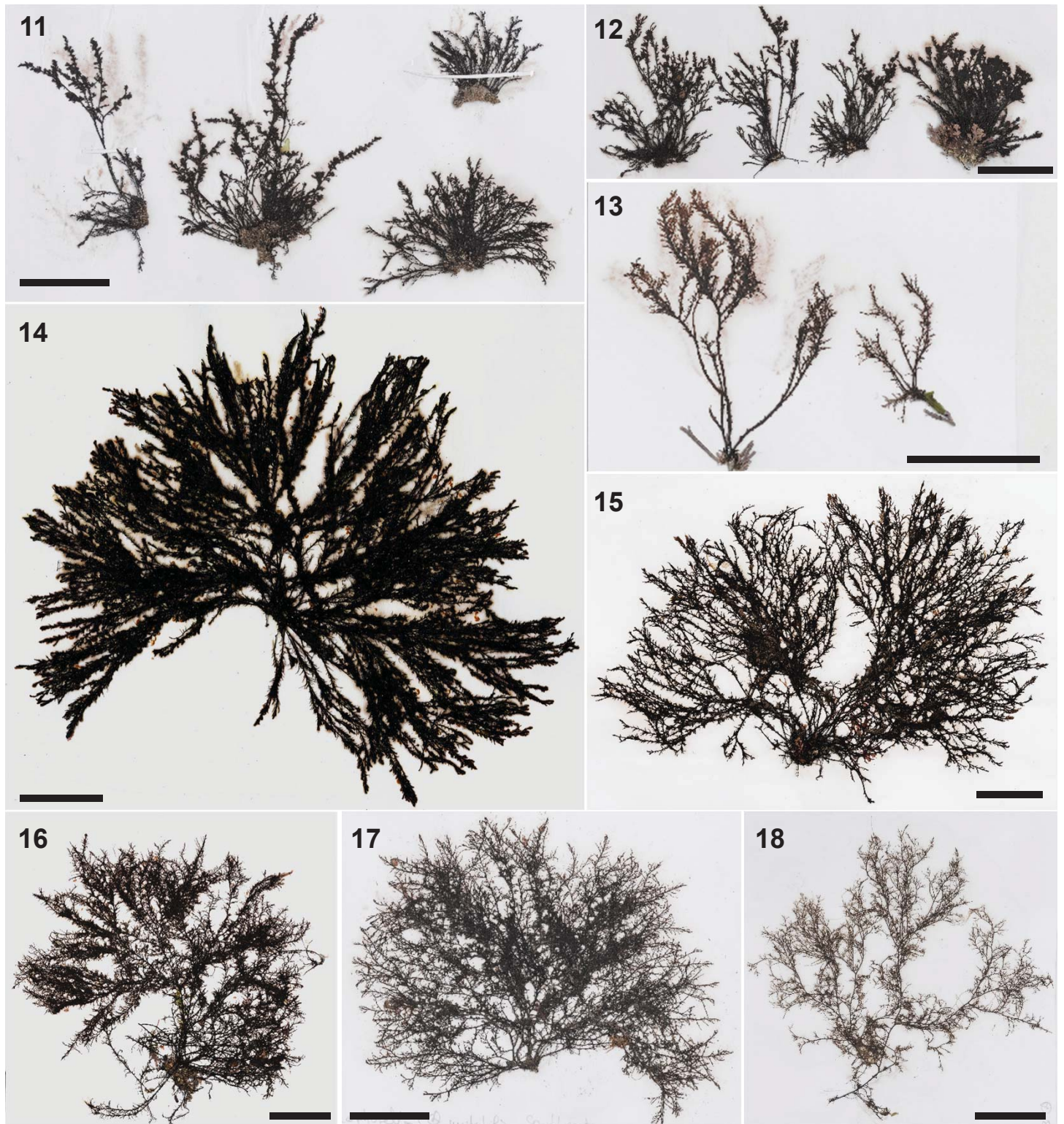
Figs S2-4. *Lophurella pericladus*. Isolectotypes. Scale bars: 3 cm. Reproduced with permission from the Royal Botanic Gardens Victoria.



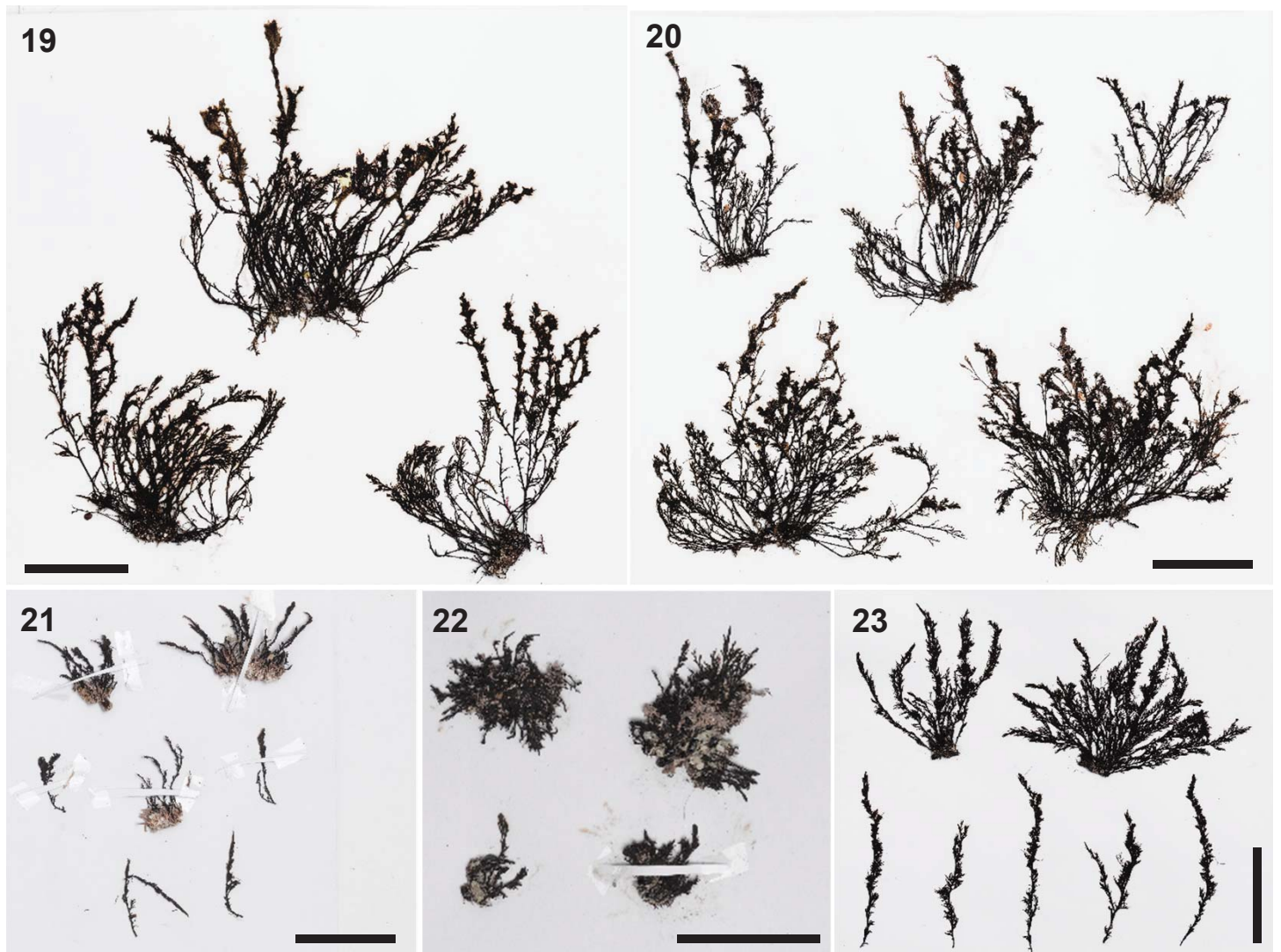
Fig. S5. *Lophurella caespitosa*. Specimen ASR202 (WELT A033734). Scale bar: 3 cm.



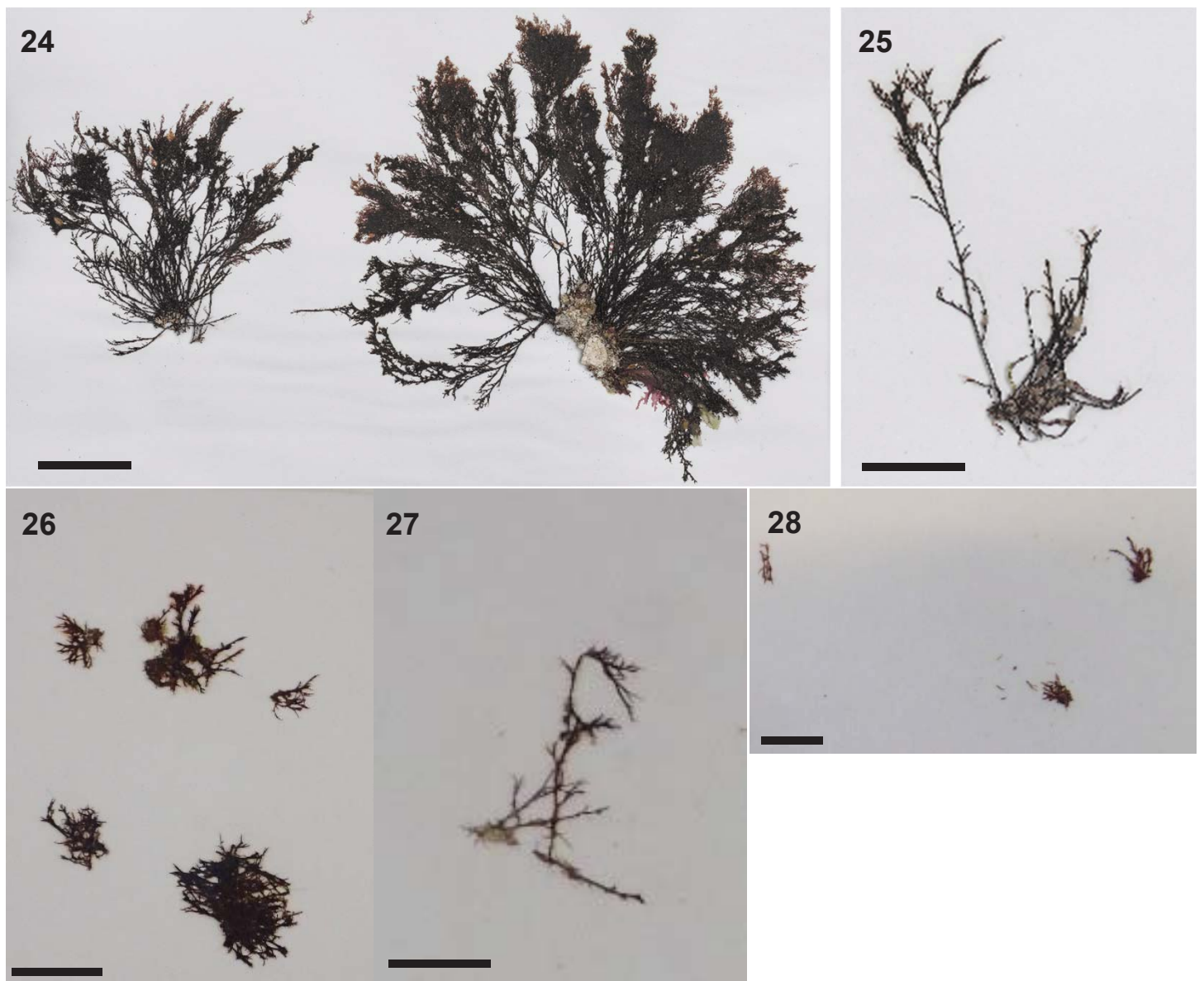
Figs S6-10. *Lophurella pericladus*. **Fig S6.** Specimen PD2866. **Fig S7.** Specimen PD772. **Fig S8.** Specimen PD2915. **Fig S9.** Specimen PD2746. **Fig S10.** Specimen PD4790. Scale bars: Fig S6, 1.5 cm; Figs S7 and S10, 3 cm; Figs S8 and 9, 1 cm.



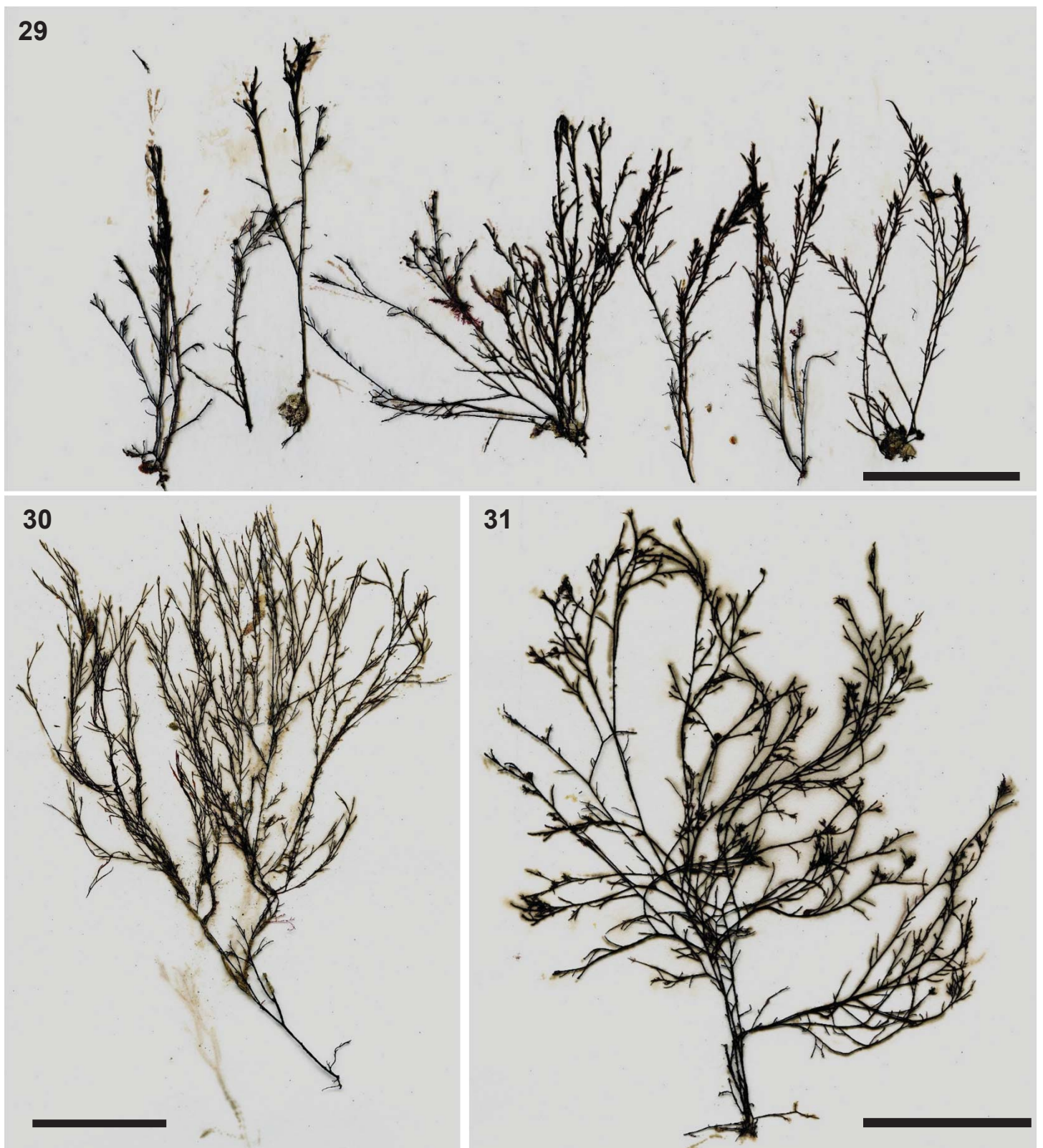
Figs S11-18. *Lophurella mutabilis*. **Fig. S11.** Specimen PD3075 (Holotype, MELUA118884a). **Fig. S12.** Specimen PD3079. **Fig. S13.** Specimen PD3093. **Fig. S14.** Specimen ASR85. **Fig. S15.** Specimen PD3106. **Fig. S16.** Specimen PD3139. **Fig. S17.** Specimen PD3164. **Fig. S18.** Specimen PD3172. Scale bars: 3 cm.



Figs S19-23. *Lophurella mutabilis*. **Fig. S19.** Specimen PD3483. **Fig. S20.** Specimen PD3478. **Fig. S21.** Specimen PD3376. **Fig. S22.** Specimen PD3411. **Fig. S23.** Specimen PD3457. Scale bars: 3 cm.



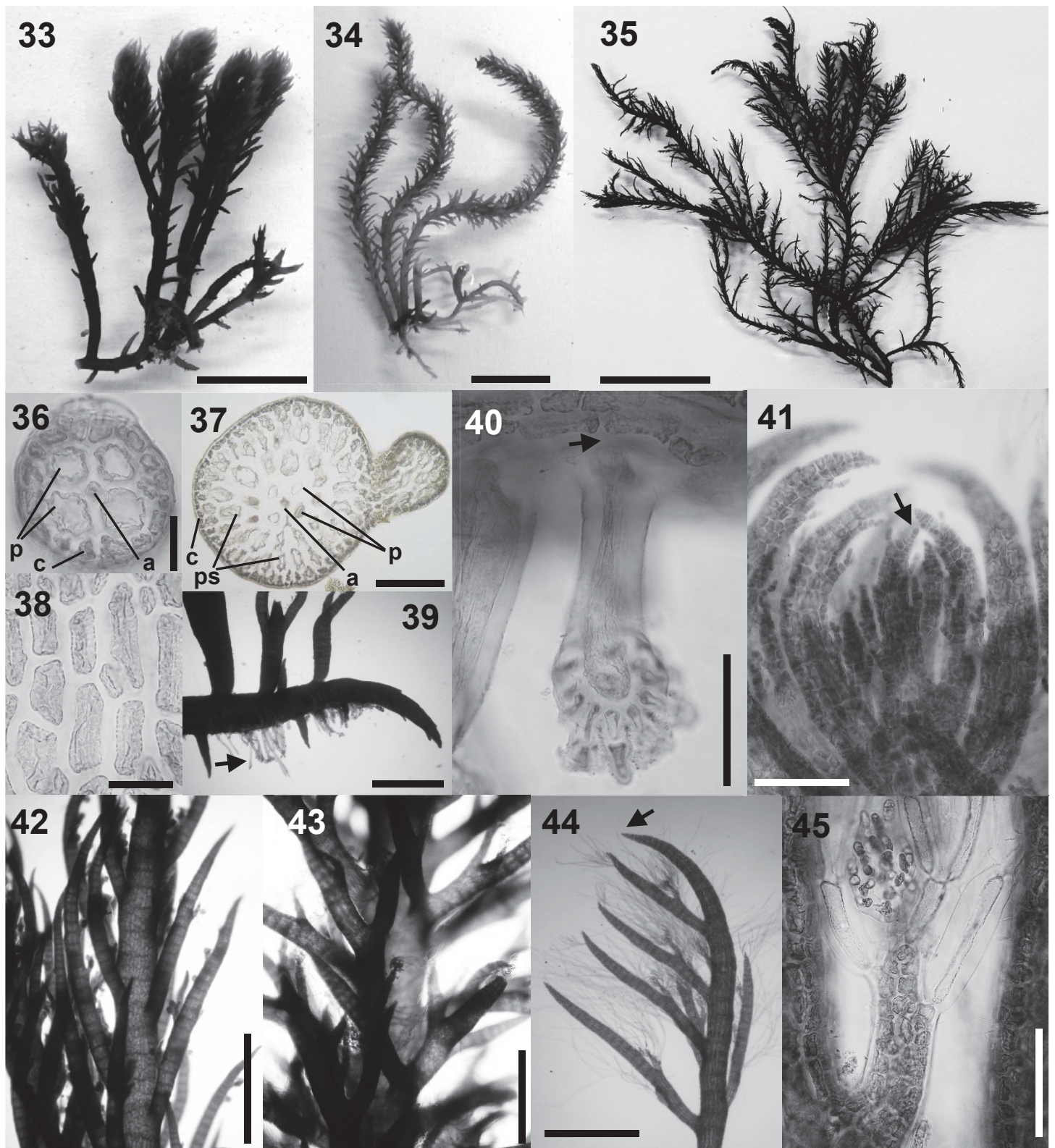
Figs S24-28. *Lophurella nigra*. Fig. S24. Specimen PD3475. **Fig. S25.** Specimen PD3401. **Fig. S26.** Specimen PD2736 (Holotype, MEL2457114). **Fig. S27.** Specimen PD2741. **Fig. S28.** Specimen PD2730. Scale bars: Fig. S24, 3 cm; Fig. S25, 1.5 cm; Fig. S26, 1 cm; Figs S27 and S28, 5 mm.



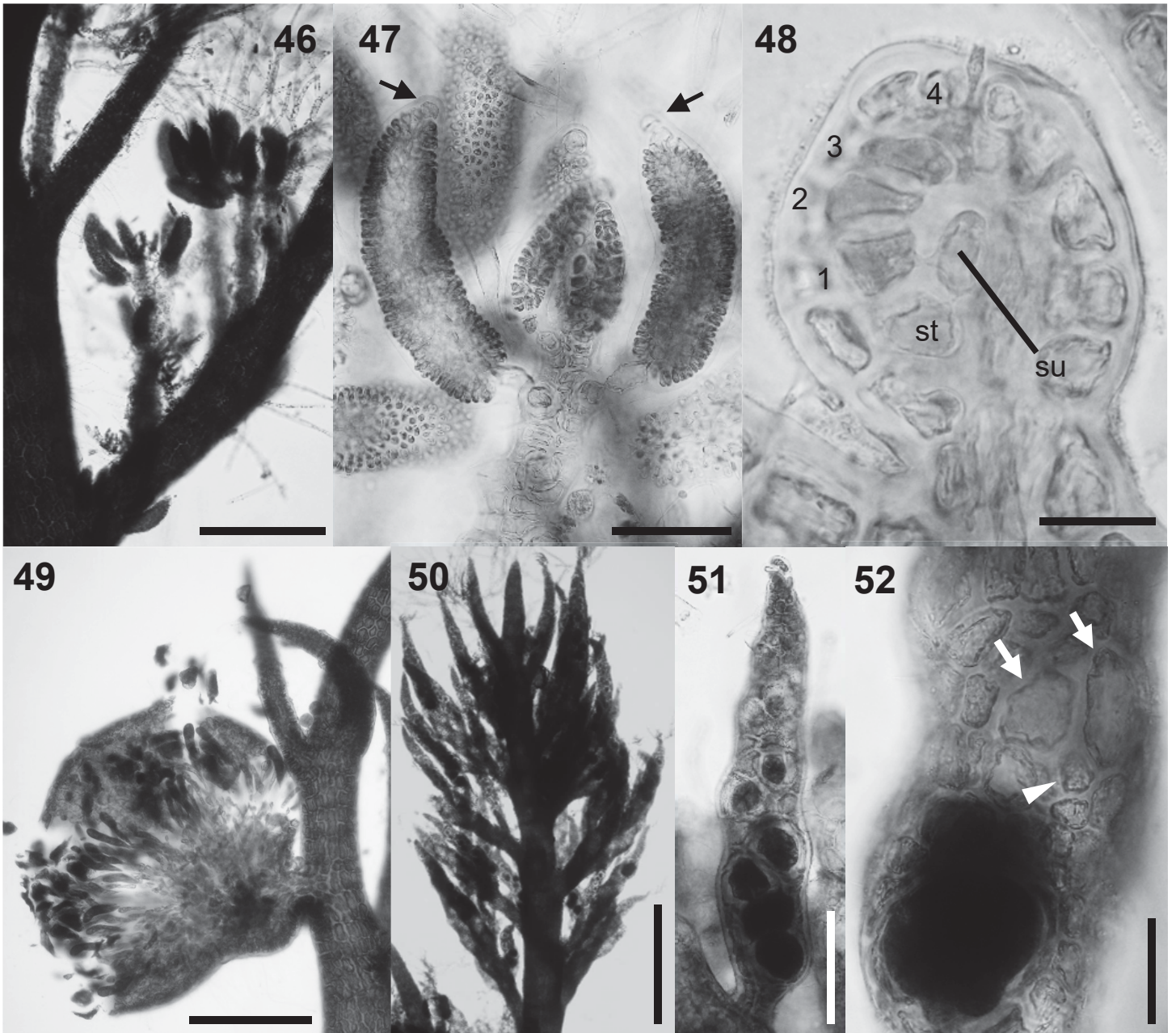
Figs S29-31. *Lophurella pauciramulosa*. **Fig. S29.** Specimen ASR175. **Fig. S30.** Specimen ASR 102. **Fig. S31.** Specimen ASR 166 (Holotype, WELT A033737). Scale bars: Figs S29 and S30, 4 cm; Fig. S31, 3.5 c m .



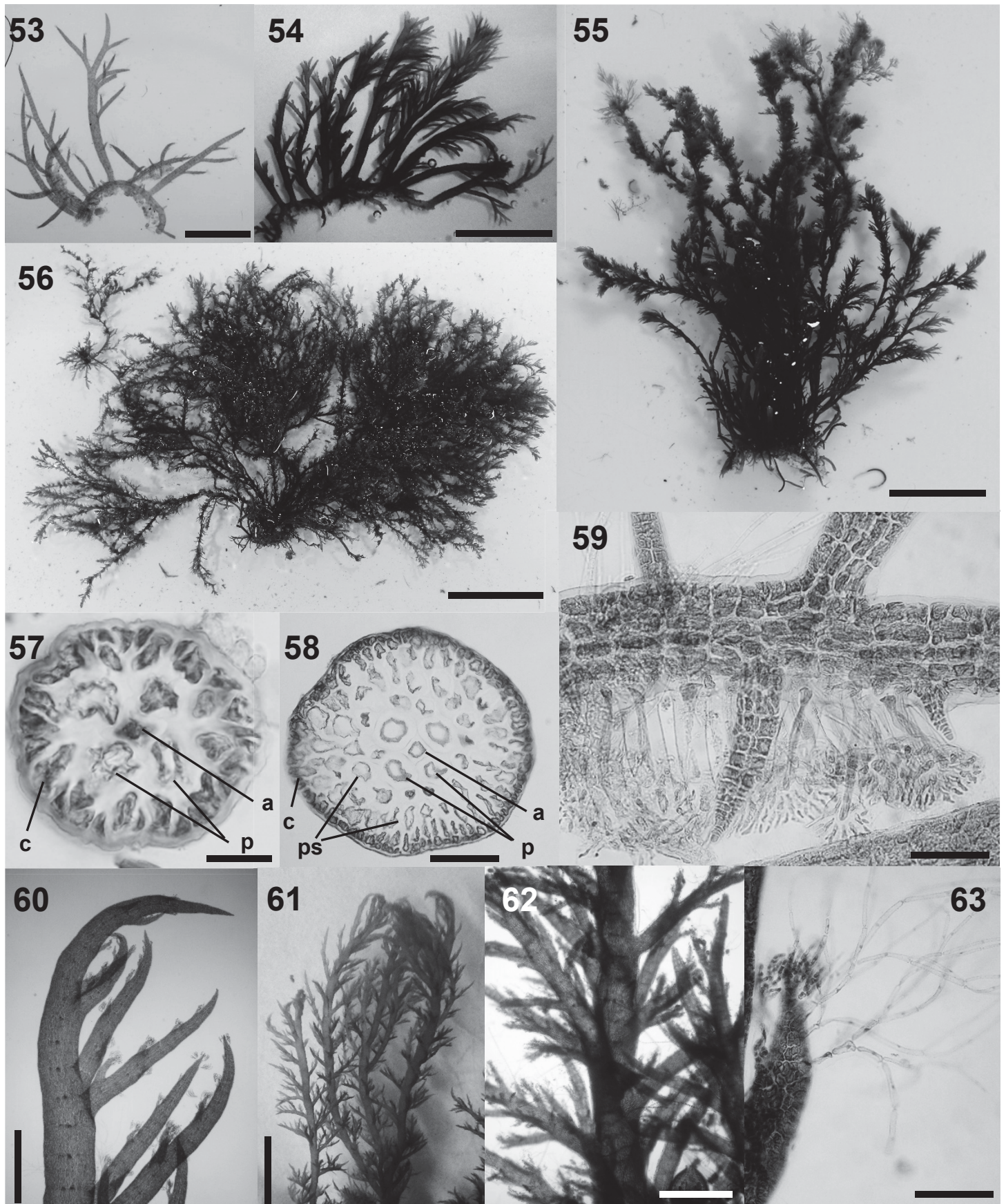
Fig. S32. *Lophurella tasmanica*. Specimen PD3584 (Holotype, MELUA118885a). Scale bar: 3 cm.



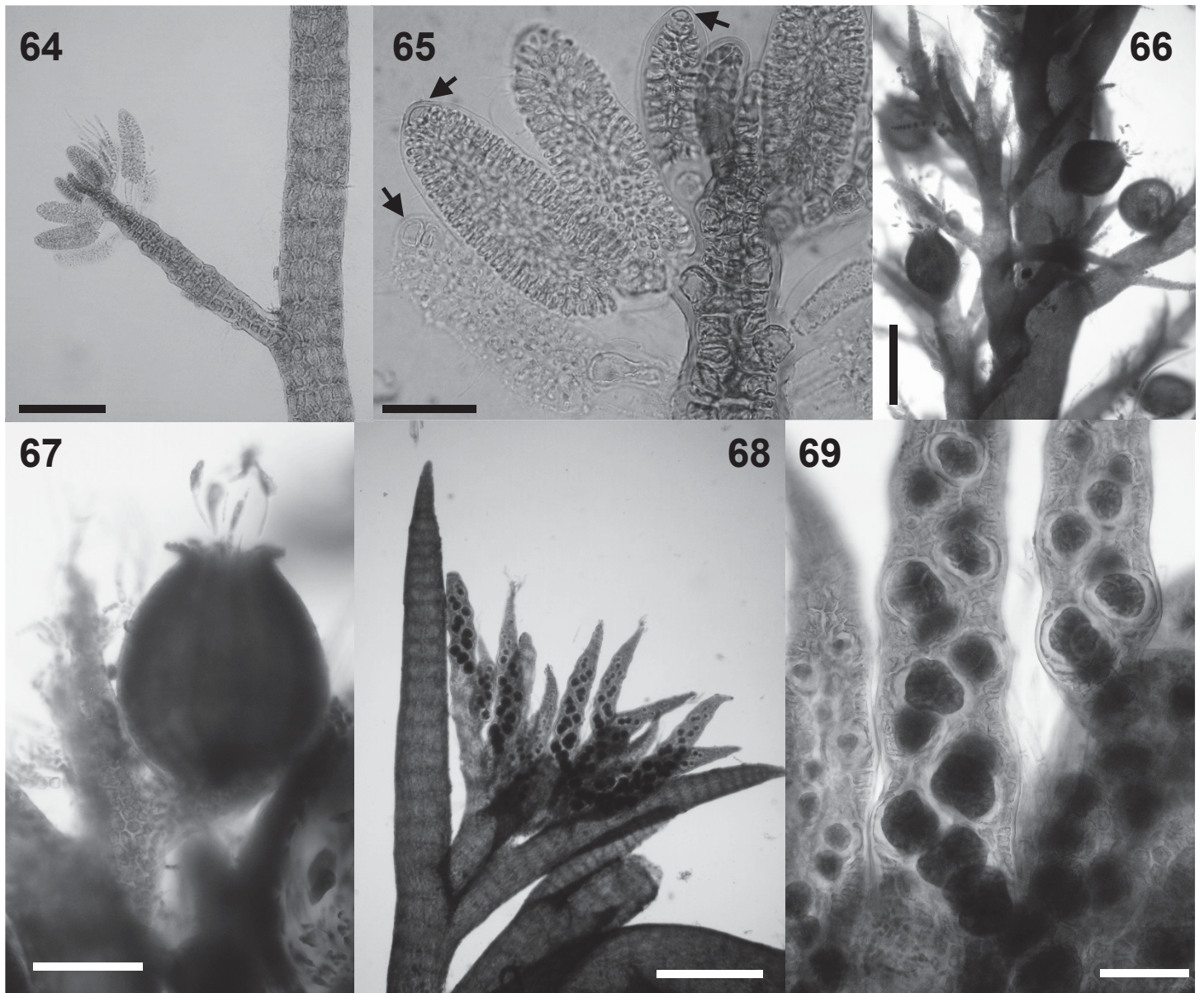
Figs S33-45. *Lophurella pericladus*: vegetative morphology. **Figs 33-35.** Habit of specimens PD2746, PD772, PD4787, respectively. **Figs 36-37.** Cross-section of a thallus in the apical part (Fig. 36) and the mid part (Fig. 37), with an axial cell (a), four pericentral cells (p), cortical cells (c) and, only in Fig. 37, pseudoparenchymatous cells (ps). **Fig. 38.** Cortical cells in surface view. **Fig. 39.** Prostrate axes with rhizoids (arrow). **Fig. 40.** Rhizoid cut off (arrow) from cortical cells and with multicellular haptera. **Fig. 41.** Apex of the thallus, the arrow showing the apical cell. **Figs 42-43.** Axes with determinate branches that are simple in the apical parts (Fig. 42) and branched in mid parts (Fig. 43). **Fig. 44.** First-order determinate branch lacking trichoblasts (arrow) and bearing second and third-order branches with trichoblasts. **Fig. 45.** Apex of a third-order determinate branch bearing spirally arranged trichoblasts. Scale bars: Figs 33 and 34, 8 mm; Fig. 35, 15 mm; Fig. 36, 50 μ m; Fig. 37, 300 μ m; Fig. 38, 35 μ m; Figs 39 and 42-44, 850 μ m; Figs 40 and 45, 100 μ m; Fig. 41, 150 μ m.



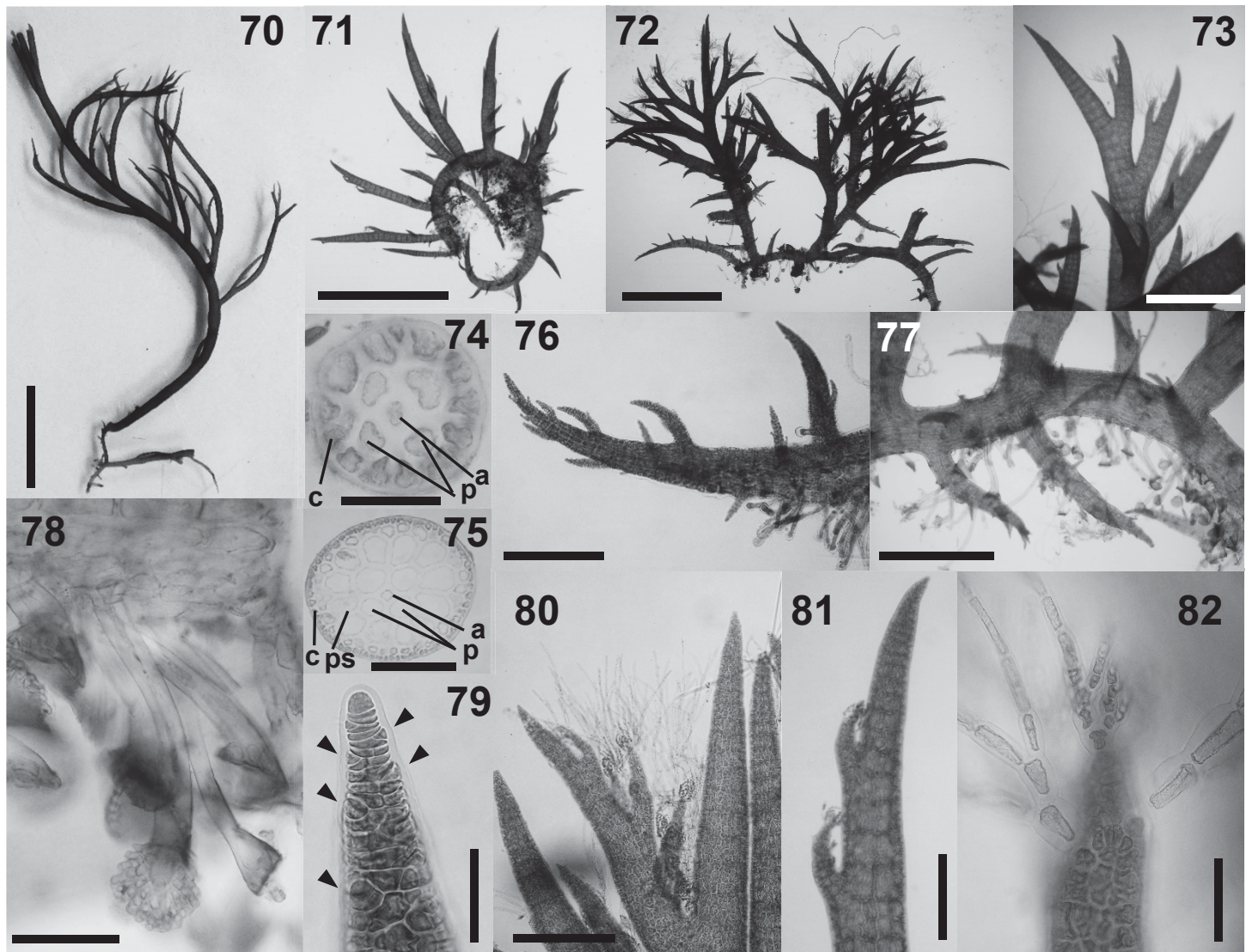
Figs S46-52. *Lophurella pericladus*: reproductive morphology. **Fig. 46.** Spermatangial branches densely clustered on second-order determinate branches. **Fig. 47.** Spermatangial branches with one or two sterile apical cells (arrows). **Fig. 48.** Procarp showing the supporting cell (su), a four-celled carpogonial branch (1-4) and a basal sterile cell (st). **Fig. 49.** Cystocarp. **Fig. 50.** Determinate branches bearing tetrasporangia. **Fig. 51.** Determinate branch with spirally arranged tetrasporangia. **Fig. 52.** Tetrasporangia with two presporangial (arrows) and a postsporangial (arrowhead) cover cells. Scale bars: Figs 46 and 49, 300 μm ; Fig. 47, 70 μm ; Fig. 48, 20 μm ; Fig. 50, 800 μm ; Fig. 51, 150 μm ; Fig. 52, 50 μm .



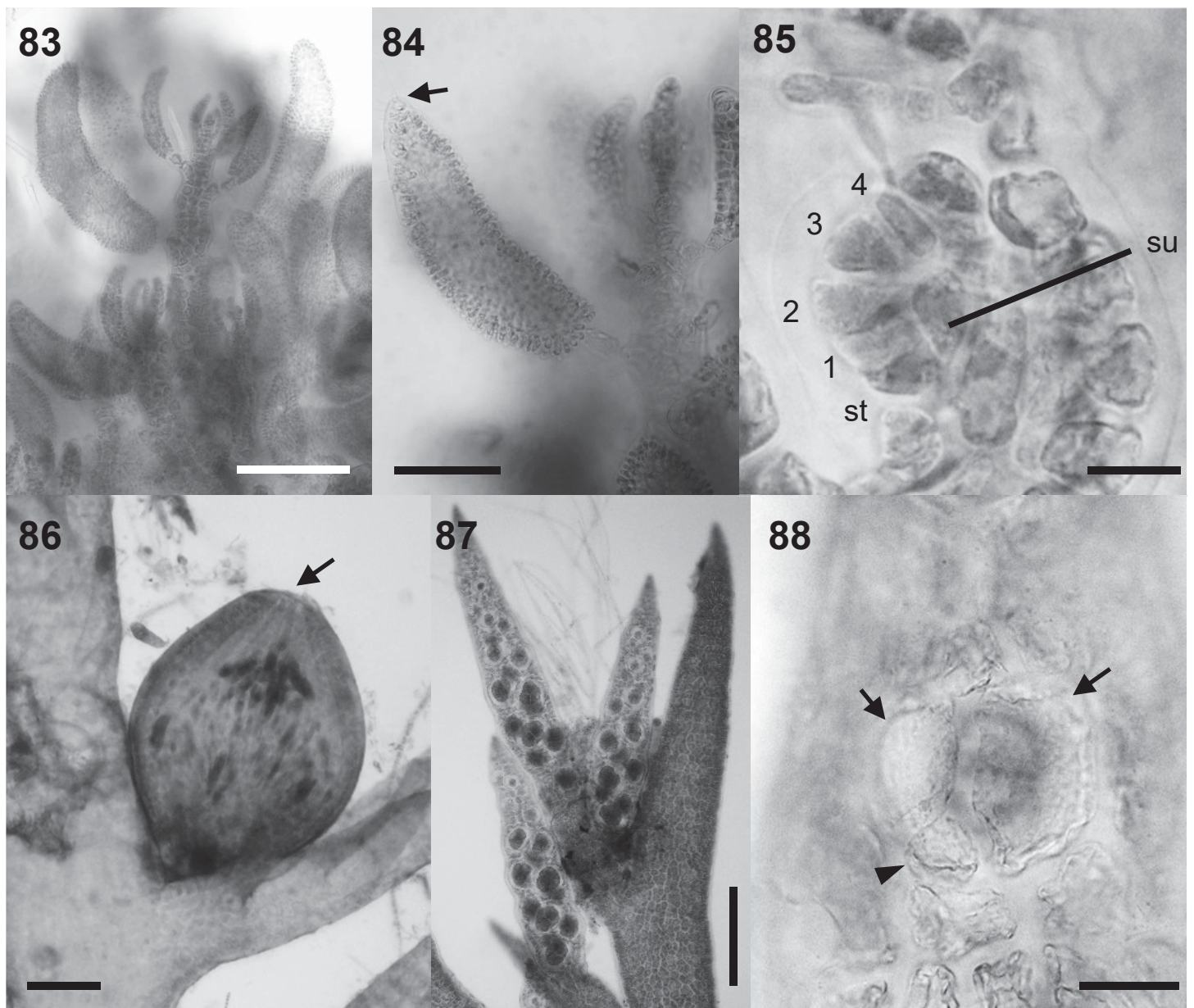
Figs S53-63. *Lophurella mutabilis*: vegetative morphology. **Figs 53-56.** Habit of specimens PD1111, PD3411, PD3483 and PD3106, respectively. **Figs 57-58.** Cross-section of axes in the apical part (Fig. 57) and the mid part (Fig. 58) of thallus, showing an axial cell (a), four pericentral cells (p), cortical cells (c) and, only in Fig. 28, pseudoparenchymatous cells (ps). **Fig. 59.** Prostrate axes with rhizoids cut off from cortical cells and with multicellular haptera **Fig. 60.** Apical part of an erect axis, forming determinate branches several segments below the apex. **Figs 61-62.** Thallus clothed with determinate branches. **Fig. 63.** Apex of a third-order determinate branch bearing spirally arranged trichoblasts. Scale bars: Fig. 53, 450 μ m; Fig. 54, 7 mm; Fig. 55, 2 cm; Fig. 56, 4 cm; Fig. 57, 30 μ m; Fig. 58, 200 μ m; Fig. 59, 100 μ m; Figs 60 and 62, 700 μ m; Fig. 61, 4 mm; Fig. 63, 150 μ m.



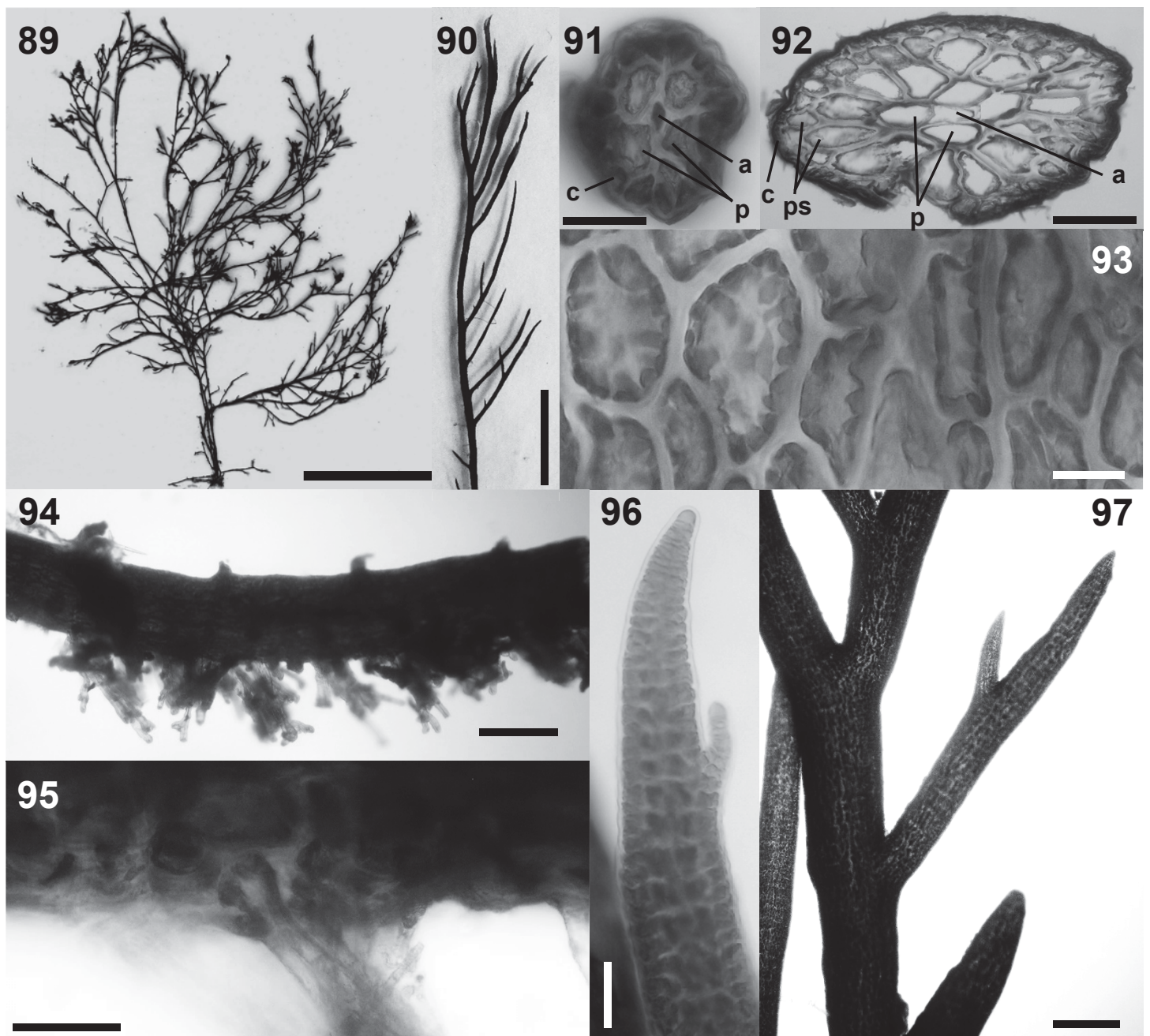
Figs S64-69. *Lophurella mutabilis*: reproductive morphology. **Fig. 64.** Spermatangial branches densely clustered on determinate branches. **Fig. 65.** Spermatangial branches with apical sterile cells (arrows). **Figs 66-67.** Cystocarps. **Figs 68-69.** Determinate branches bearing spirally arranged tetrasporangia. Scale bars: Figs 64 and 67, 200 μm ; Figs 65 and 69, 100 μm ; Figs 66 and 68, 600 μm .



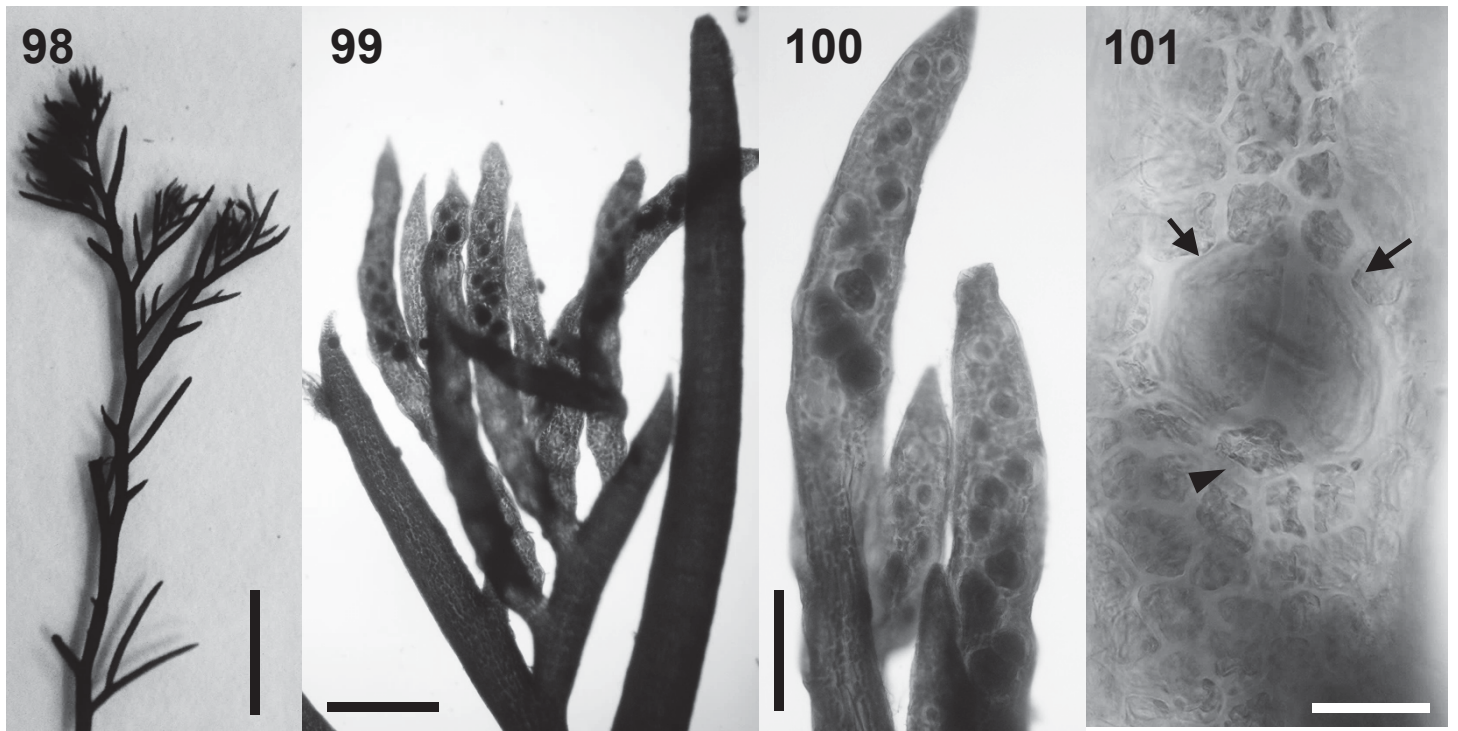
Figs S70-82. *Lophurella nigra*: vegetative morphology. **Figs 70-72.** Habit of specimens PD3555, PD2736, and PD2741, respectively. **Fig 73.** Apical part of an erect axis with determinate branches. **Figs 74-75.** Cross-section of axes in the apical part (Fig. 74) and the mid part (Fig. 75) of thallus, showing an axial cell (a), four pericentral cells (p), cortical cells (c) and pseudoparenchymatous cells (ps). **Figs 76-78.** Prostrate axes with rhizoids cut off from cortical cells and with multicellular haptera. **Fig 79.** Apex of an erect axis with initials on every segment (arrowheads). **Fig. 80.** Apex of an erect axis bearing two orders of determinate branches, the second-order bearing trichoblasts. **Fig. 81.** Determinate branch, lacking trichoblasts, bearing an order of determinate branches with young trichoblasts. **Fig. 82.** Apex of a second-order determinate branch bearing spirally arranged trichoblasts. Scale bars: Fig. 70, 5 mm; Figs 71 and 72, 2.5 mm; Figs 73 and 77, 1 mm; Figs 74 and 82, 70 μ m; Figs 75 and 81, 250 μ m; Figs 76 and 80, 350 μ m; Fig. 78, 150 μ m; Fig. 79, 40 μ m.



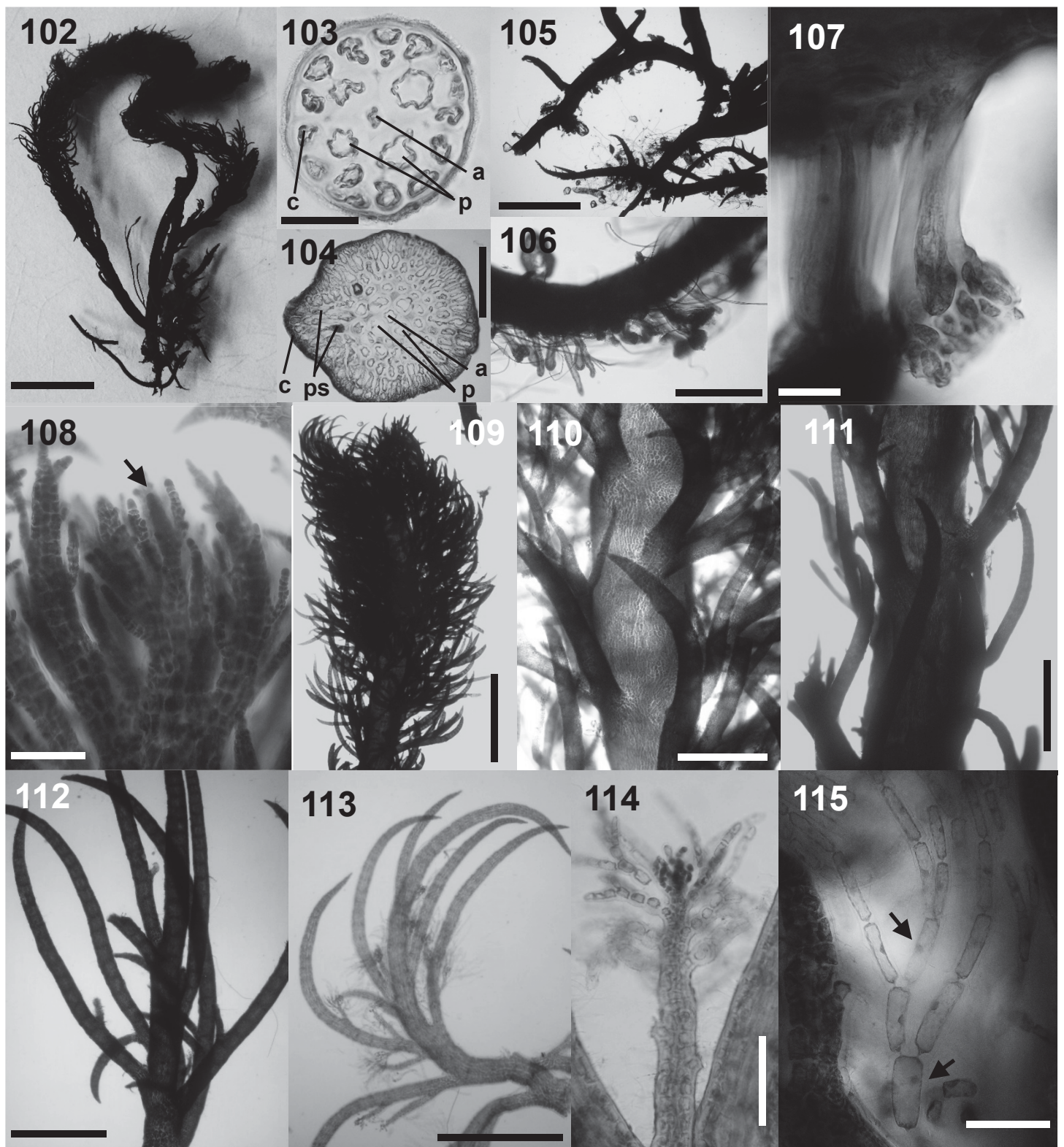
Figs S83-88. *Lophurella nigra*: reproductive morphology. **Fig. 83.** Spermatangial branches densely clustered on determinate branches. **Fig. 84.** Spermatangial branch with an apical sterile cell (arrow). **Fig. 85.** Procarp showing the supporting cell (su), a four-celled carpogonial branch (1-4) and a basal sterile cell (st). **Fig. 86.** Cystocarp with an apical ostiole (arrow). **Fig. 87.** Determinate branches bearing tetrasporangia. **Fig. 88.** Tetrasporangia with two presporangial (arrows) and a postsporangial (arrowhead) cover cells. Scale bars: Fig. 83, 150 μm ; Figs 84 and 86, 70 μm ; Fig. 85, 10 μm ; Fig. 87, 250 μm ; Fig. 88, 25 μm .



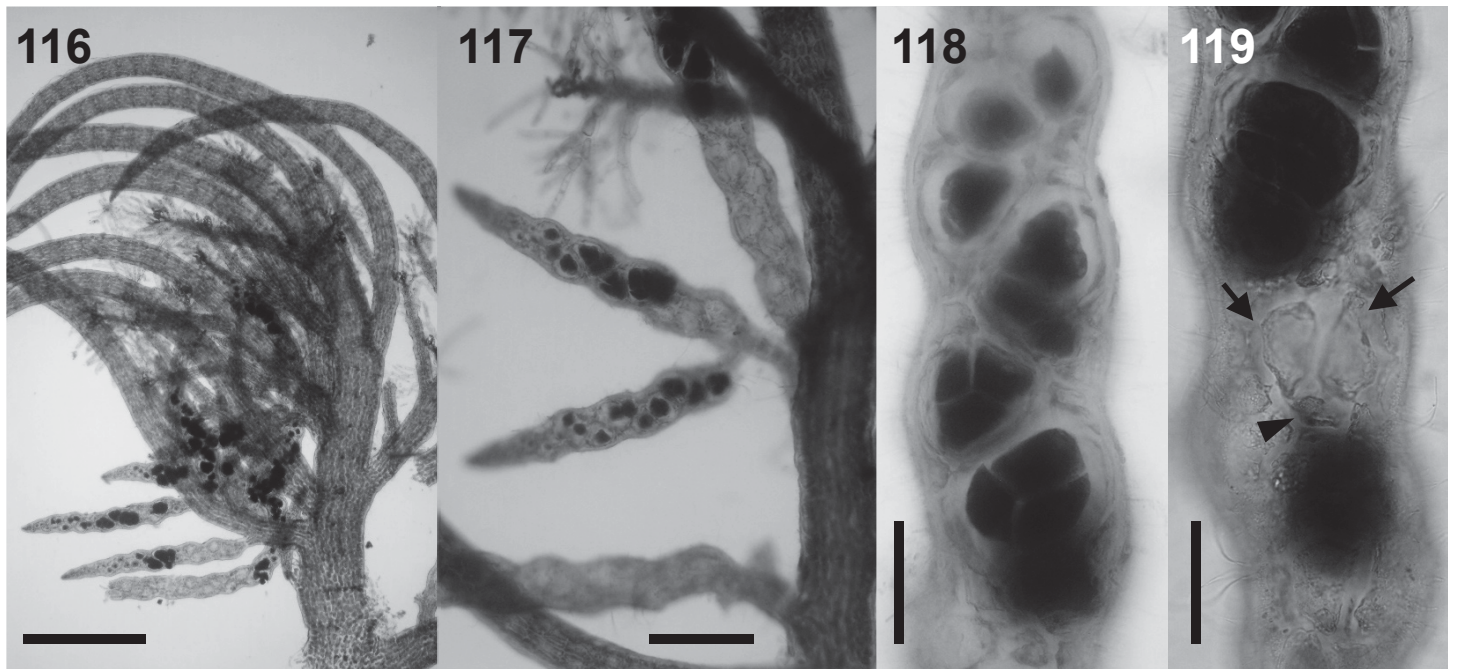
Figs S89-97. *Lophurella pauciramulosa*: vegetative morphology. **Fig. 89.** Habit of the holotype (specimen ASR166). **Fig. 90.** Apical part of an erect axis with determinate branches. **Figs 91-92.** Cross-section of axes in the apical part (Fig. 91) and the mid part (Fig. 92) of thallus, showing an axial cell (a), four pericentral cells (p), cortical cells (c) and, only in Fig. 92, pseudoparenchymatous cells (ps). **Fig. 93.** Cortical cells showing plastids. **Figs 94-95.** Prostrate axes with rhizoids. **Fig 96.** Apex of an erect axis. **Fig. 97.** Determinate branch lacking trichoblasts. Scale bars: Fig. 89, 35 mm; Fig. 90, 6 mm; Figs 91 and 95-96, 60 μ m; Fig. 92, 250 μ m; Fig. 93, 15 μ m; Figs 94 and 97, 400 μ m.



Figs S98-101. *Lophurella pauciramulosa*: reproductive morphology. **Fig. 98.** Apex of an axis with densely clustered tetrasporangial branches. **Figs 99-100.** Determinate branches bearing tetrasporangia. **Fig. 101.** Tetrasporangium with two presporangial (arrows) and a postsporangial (arrowhead) cover cells. Scale bars: Fig. 98, 5 mm; Fig. 99, 500 μ m; Fig. 100, 200 μ m; Fig. 101, 50 μ m.



Figs S102-115. *Lophurella tasmanica*: vegetative morphology. **Fig. 102.** Habit of specimen PD3584. **Figs 103-104.** Cross-section of axes in the apical part (Fig. 103) and the mid part (Fig. 104) of thallus showing axial cell (a), four pericentral cells (p), cortical cells (c) and, only in Fig. 104, pseudoparenchymatous cells (ps). **Figs 105-107.** Prostrate axes with rhizoids cut off from cortical cells and with multicellular haptera. **Fig. 108.** Apex of an erect axis with apical cell indicated (arrow). **Fig. 109.** Apical part of an erect axis densely clothed with determinate branches. **Figs 110-111.** Mid-part of an erect axis with unbranched or basally branched determinate laterals. **Figs 112-113.** Determinate branches bearing an order of determinate branches with (Fig. 113) or without (Figs 112) trichoblasts. **Fig. 114.** Apex of a second-order determinate branch bearing spirally arranged trichoblasts. **Fig. 115.** Trichoblast with uninucleate cells (arrows). Scale bars: Fig. 102, 8 mm; Figs 103 and 107, 40 μ m; Figs 104 and 106, 400 μ m; Fig. 105, 3 mm; Fig. 108, 100 μ m; Fig. 109, 2.5 mm; Figs 110-113, 800 μ m; Fig. 114, 150 μ m; Fig. 115, 70 μ m.



Figs S116-119. *Lophurella tasmanica*: reproductive morphology. **Figs 116-117.** Determinate lateral branches with densely clustered tetrasporangial branches. **Fig. 118.** Tetrasporangia spirally arranged. **Fig. 119.** Tetrasporangia with two presporangial (arrows) and a postsporangial (arrowhead) cover cells. Scale bars: Fig. 116, 600 μm ; Fig. 117, 200 μm ; Figs 118 and 119, 60 μm .