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. periclados* 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 I. New Zealand), *L. hookeriana* (type locality Cape Horn, South America) and *L. periclados*. 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. periclados* mask cryptic diversity. We studied *rbc*L sequences and morphological features of 36 specimens identified as *L. periclados*, 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. periclados* is a complex involving four species and we propose three new species, *L. mutabilis*, *L. nigra* and *L. tasmanica*. Cryptic diversity in *L. periclados* did not align with the previously defined ecotypes and several species were often found at the same site. *Lophurella. periclados*, *L. nigra* and *L. tasmanica* can be distinguished by morphological characters. Conversely, *L. mutabilis* has high morphological plasticity, with characters that overlap with *L. periclados* and *L. nigra*, and can be only distinguished by DNA sequences.

KEYWORDS: cryptic diversity; distribution; molecular systematics; morphology; phenotypic plasticity; new species; phylogeny; Pterosiphonieae; *rbc*L; 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. periclados* (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 the shorter size (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. christosphersenii* 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 on similar morphologies (Verbruggen ~~*et al.*~~, 2014). As a result, in the macroalgae including the family Rhodomelaceae, cryptic diversity is commonly discovered when molecular assisted taxonomy is used for species diversity assessments (e.g. Guillemín *et al.*, 2016; Savoie & Saunders, 2016, 2019; Saunders *et al.*, 2017; 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; ~~Schneider *et al.*, 2017;~~ 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. More surprisingly, 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. periclados* might correspond to different species, because *L. periclados* 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 *rbc*L 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 4ºC 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 *rbc*L was carried out using primers F57/rbcLrevNEW, 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× MyTaqTM reaction buffer, 0.7 µl 10 µM of forward and reverse primers, 0.125 µl 1U μl^{-1} My TaqTM DNA Polymerase (Bioline, London, UK), 17.475

µl MilliQ® water and 1 µl template DNA. The PCR profile consisted of initial denaturation (93ºC for 3 min), 35 cycles of denaturation (94ºC for 30 s), primer annealing (45ºC for 30 s), and extension (74ºC for 90 s) and final extension (74ºC 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 *rbc*L 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 *rbc*L 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~~. Accordingly,~~ and we propose ~~the segregation of~~ *L. pauciramulosa* sp. nov. from New Zealand.

The specimens that we originally identified as *L. periclados* were resolved in four clades (Fig. 1). All eight specimens collected in Port Phillip Bay, Victoria, the type locality of *L. periclados*, 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. periclados* (Fig. 2). In addition to *L. periclados*, 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. periclados* 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. periclados* 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. periclados*, 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. periclados* and the four new species recognized in this study is provided below. Table 1 provides the details of these characters including their measurements in each species. 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. periclados* is based on our collections. The only available detailed description of *L. periclados* 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 periclados* (Sonder) F.Schmitz in Schmitz & Falkenberg, 1897: 441 (Figs 21-27; Figs S1-4, S6-10 and S33-52)**

BASIONYM: *Rhodomela periclados* 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 µm 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 periclados 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. periclados* 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. periclados* that we collected are in agreement with the type. *Lophurella periclados* 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.

HOLOTYPE: MELUA118884a.

TYPE LOCALITY: Blackmans Bay, Tasmania, Australia.

*Rbc*L SEQUENCE OF THE HOLOTYPE: MN149994.

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 more slender than specimens from exposed sites (Figs S11-13 and S19-23). However, this morphological variability was not reflected in the genetic variability in the *rbc*L gene, as haplotypes 2 and 4 were found at both types of sites (Fig. 2). *L. ophurella 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.

HOLOTYPE: MEL2457114.

TYPE LOCALITY: Bastion Point, Mallacoota, Australia

*Rbc*L SEQUENCE OF THE HOLOTYPE: MN149998.

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 *rbc*L 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 ~~Determinate branches~~ that are sparse, 250-300 µm in diameter basally. Trichoblasts absent.

HOLOTYPE: WELT A033737.~~XXX~~.

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

*Rbc*L SEQUENCE OF THE HOLOTYPE: MN150002.

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

404 at apices of erect axes, on every segment. All branch initials developing into
405 determinate branches, 150-230 µm in diameter basally, upwardly incurved when
406 mature. First-order determinate branches producing up to two further orders of
407 determinate branches. Determinate second-order branches reaching a length similar to
408 the parental determinate branch. Trichoblasts restricted to second- and third-order
409 determinate branches.

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411 HOLOTYPE: MELUA118885a.

412 TYPE LOCALITY: Port Arthur, Tasmania, Australia.

413 *Rbc*L SEQUENCE OF THE HOLOTYPE: MN150004.

414 ETYMOLOGY: "*tasmanica*" refers to the type locality of the species.

415

416 *Description*

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

421 Erect axes producing determinate lateral branches on every segment, spirally arranged
422 and upwardly incurved, overtopping the apical cell of the main axes (Figs 47-49).
423 Lateral branches 150-230 µm diameter in basal parts, producing spirally a second-order
424 of determinate branches when young, such branches remaining restricted to basal parts
425 of laterals (Fig. 50). Second-order branches upwardly incurved and reaching a similar
426 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 periclados* 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 *rbc*L gene relative to the previously recognized species in the genus. Sequence divergence was ≥ 1.8% among species, except between *Lophurella tasmanica* and *L. periclados*, 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. periclados*. One of the haplotypes of *L. mutabilis* (H6 in Fig. 1), the New Zealand specimen, was relatively (0.7-0.9 %) divergent from Australian specimens. Likewise, the divergence between the two haplotypes of *L. periclados* (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 either that they should be segregated or that they are single lineages with high genetic variability in the *rbc*L 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 *rbc*L 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 Pterosiphonieae, as they have rhizoids cut off from pericentral cells with 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 and a postsporangial cover cell. Trichoblasts were abundantly found 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 relevant qualitative morphological characters were shared among the species studied here. Nevertheless, some details of 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 green emerald 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. periclados* 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. periclados*. *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 ~~but we did not find relevant morphological characters for its separation from~~ *L. hookeriana*, which has trichoblasts on determinate branches~~based on the available information for this species~~ (Boraso de Zaixso, 2013; EM pers. obs.~~Agardh, 1863; Kylin & Skottsberg, 1919~~).

The other three species here recognized, *Lophurella periclados*, *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~~.~~ periclados* and *L. nigra* are distinct, but *L. mutabilis* is so variable morphologically that some specimens overlap with the morphological characters of both species. *Lophurella~~.~~ periclados* 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 not uncommon 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 reveal morphological differences among them (Walker *et al.*, 2009; Zanolla *et al.*, 2014, Barreto de Jesus *et al.*, 2018). Interestingly, the morphological variability described by Womersley (2003) for *L. periclados* that he related to different levels of wave exposure was observed within *L. periclados* and *L. mutabilis*. Specimens from sheltered sites are more slender and 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* here described 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 here described, that 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-Vicent (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 Pterosiphonieae (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 Pterosiphonieae, which we suggest 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 periclados* 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 periclados* and *L. tasmanica* were only found in mainland Australia and eastern Tasmania, respectively, while *L. nigra* was found in both regions. *Lophurella periclados* 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. 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 *PL. pauciramulosa* and *PL. 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 material within herbarium collections displays considerable morphological variability (WN pers. obs.). The records of *Lophurella periclados* 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 can be correctly referred to *L. mutabilis*.

Interestingly, *Lophurella* was absent from all six sites explored in northern Tasmania. This contrasts with the finding of abundant populations of *Lophurella* spp. in seven of the eight sites sampled in eastern Tasmania. Other red algae and intertidal organisms have similar distribution patterns (Womersley, 2003; Waters, 2008; Díaz-Tapia *et al.*, 2018b). The absence of *Lophurella* in northern Tasmania, as well as the origin of its diversity and the present distribution of the species that we found in southeastern Australia and Tasmania might be related to the combination of palaeogeographical events and contemporary currents in this region. During the Pleistocene, a land bridge was formed between Tasmania and mainland Australia creating an east-west dispersal barrier for marine organisms and facilitating the dispersal of coastal benthic organisms between the mainland and the island (Lewis *et al.*, 2013; Mueller *et al.*, 2018). Subsequently the Bass Strait inundated, but dominant currents in the region contribute to perpetuating the east-west barrier (Waters, 2008;

Mueller *et al.*, 2018). These factors are thought to promote vicariant speciation or genetic differentiation among populations of marine organisms in this region (e.g. Waters, 2008; Mueller *et al.*, 2018) and similar processes might explain the diversity and distribution of *Lophurella* spp. In a wider geographical context, the current distribution of *Lophurella* spp. suggests the occurrence of dispersal events between Australia, New Zealand and South America for the ancestors of the extant species. They were probably mediated by transoceanic dispersal through the Antarctic Circumpolar Current as this current has contributed to the dispersal of other red algae (Boo *et al.*, 2014; Guillemín *et al.*, 2014; Muangmai *et al.*, 2014). Further studies with a wider taxon sampling of South American species and better resolved phylogenies might contribute to understanding the origins and the evolutionary history of the genus *Lophurella*. Two parasites described on *L. hookeriana* from South America have both also been reported in New Zealand. Relationships between parasites and their hosts are often highly specific, and most parasites only grow on one or two host species (Zuccarello & West, 1994; Preuss *et al.*, 2017; Preuss & Zuccarello, 2018). Future work should aim to determine whether parasites from different regions also correspond to different species with different hosts.

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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; H. Verbruggen: original concept, drafting and editing manuscript.

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820

Figure legends

Fig. 1. Maximum likelihood phylogeny of *Lophurella* based on *rbc*L 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 ~~i~~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 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 *rbc*L 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 (µm) of morphological characters for *Lophurella* spp. from Australasia (except *L. caespitosa*).

	L. periclados	*L. mutabilis*	*L. nigra*	*L. pauciramulosa*	*L. tasmanica*
Cortical cells	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	20-25	17-20	20	20	25
Diameter	250-470	(120-) 250-500	250-400	(280-) 350-700	400-500
Rhizoids					
Length	up to 700	up to 700	up to 820	up to 700	up to 1000
Filament diameter	30-80	25-50 µm	30-40	40-60 µm	30-70
Prostrate axes					
Apical cell diameter	17-20	12-20 µm	17-23	20-35	25

Diameter	600-930	(130-) 450-750	330-650	380-850	750-900
Trichoblasts					
Lenghth	up to 850	up to 650	up to 900	-	up to 400
Diameter of basal cell	35-50	25-35	40-50	-	32-50
Spermatangial branches	35-65 × 105-200	45-55 × 125-180	30-50 × 125-165	Unknown	Unknown
Procarps	4-celled	Unknown	4-celled	Unknown	Unknown
Cystocarps	470-500 × 550-600	300-450 × 600-700	430-600 × 400-710	Unknown	Unknown
Carposporangia	15-25 × 105-113	12-18 × 50-100	12-20 × 45-75	Unknown	Unknown
Lenght of tetrasporangial branches	700-1100	500-1800	600-2000	2000	600-900
Tetrasporangia	55-65 × 55-80	40-55 (-70) × 45-63 (-73)	48-65 × 37-75	55-75 × 67-80	40-55 (-70) × 45-63 (-73)

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

	L. tasmanica	*L. pauciramulosa*	*L. nigra*	*L. mutabilis*	*L. periclados*	*L. caespitosa*
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**
Colour	Dark brown	Dark red or brown	Dark brown to black	Light/dark brown, black	Dark brown to black	**Green emerald**
Length of erect axes	Up to 5 cm	**Up to 20 cm**	Up to 5 cm	Up to 15 cm	Up to 10 cm	Up to 5 cm
Trichoblast arrangement	2nd and 3rd order determinate branches	**Absent**	2nd and 3rd order determinate branches	**1st**, 2nd and 3rd order determinate branches	2nd and 3rd 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	**150-230 µm**	250-300 µm	250-400 µm	210-400 µm	300-500 µm	**150-260 µm**
Appearance	**Incurved**	Straight, spiny	Straight, spiny	Straight, spiny	Straight, spiny	Straight, spiny
Length of 2nd 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	New Zealand	Tasmania, Victoria	Tasmania, Victoria, New Zealand	Victoria	New Zealand