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***Agarophyton transtasmanicum* sp. nov., from Australia and New Zealand**

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Running title: *Agarophyton transtasmanicum sp. nov.*

## **ABSTRACT**

The Gracilariaceae is a species rich family, with a number of members having high commercial value as sources of agar. Members of this family are also known for their phenotypic plasticity and convergent morphologies resulting in considerable taxonomic confusion. Over the past two decades, two species of *Agarophyton* (previously part of *Gracilaria*) have been recognized in New Zealand with very similar morphologies and growth habits, and they have been incorrectly grouped as *Agarophyton chilense*. *Agarophyton chilense* is distributed in Chile and New Zealand and is genetically distinct from *Agarophyton* sp. from Australia and New Zealand. We name this new species *A. transtasmanicum* sp. nov. Morphologically *A. transtasmanicum* has fewer medullary cell layers and a more abrupt transition in cell size between cortex and medulla than *A. chilense*. The *cox1* and *rbcL* dataset grouped *A. transtasmanicum* as sister to *A. tenuistipitatum* with high support. Clarifying the distinctions between *A. chilense* and *A. transtasmanicum* will enable further research, including investigating differences in distribution patterns, physiology and ecology, and chemical composition between these two *Agarophyton* species.

**KEYWORDS:** *cox1*, ITS1; Phylogeny; *rbcL*; Rhodophyta; Systematics; Taxonomy

## INTRODUCTION

The Gracilariaceae is one of the most species-rich and commercially important red algal families. Some Gracilariaceae members are known for their phenotypic plasticity, relatively simple and convergent morphologies. Due to their commercial importance and classification challenge, numerous taxonomic studies have been done on the group (e.g., Abbott *et al.* 2004). Some genera have been proposed and later discarded, especially lineages related to *Gracilaria*. The stability and utility of the genus *Hydropuntia* has been controversial, with authors both accepting (Gurgel & Fredericq 2004; Gurgel *et al.* 2018) and not accepting this change (Iha *et al.* 2018; Lyra *et al.* 2015), plus a name was suggested, but not formally proposed, for a lineage containing one of the most important commercial *Gracilaria*'s, *Gracilaria chilensis* C.J.Bird, McLachlan & E.C.Oliveira, and a well-known invasive alga, *Gracilaria vermiculophylla* Ohmi (Gurgel & Fredericq 2004). A recent taxonomy has accepted both *Hydropuntia*, and formally described a new genus, *Agarophyton*, for the lineage containing *Gracilaria chilensis* (Guiry *et al.* 2018; Gurgel *et al.* 2018).

*Agarophyton chilense* (C.J.Bird, McLachlan & E.C.Oliveira) Gurgel, J.N.Norris & Fredericq and a sister species, *A. tenuistipitatum* (C.F.Chang & B.-M.Xia) Gurgel, J.N.Norris & Fredericq, are the second most intensively cultivated red algal group world-wide with 3.9 million tonnes harvested per year (after *Eucheuma* with 10.2 million tonnes, Ferdouse *et al.* 2018). Plus, the invasive nature of *A. vermiculophyllum* (Ohmi) Gurgel, J.N.Norris & Fredericq has been well studied (Bippus *et al.* 2018; Hu & Juan 2014; Krueger-Hadfield *et al.* 2016, 2017). Many members of the Gracilariaceae have remarkable applications including for human and animal consumption, in the pharmaceutical industry, and as medical agar (Ferdouse *et al.* 2018). Also some species are being cultivated for bioremediation in polyculture as part of multi-trophic aquaculture (Pereira & Yarish 2008).

The similar morphological characters of some Gracilariaceae species has led to problems in their taxonomy. *Gracilaria chilensis* (now *Agarophyton chilense*) in New Zealand and Chile was initially described as *Gracilaria sordida* (Nelson 1987) in New Zealand. *Gracilaria sordida* was later synonymised with *G. chilensis*, a species that was described a year earlier (Bird *et al.* 1986), based on similarities in morphology and molecular data (Bird *et al.* 1990). Later a molecular analysis of a sample, incorrectly, identified as *A. chilense* from the Manukau Harbour, Auckland, was grouped as sister to *A. tenuistipitatum* (Goff *et al.* 1994). ITS restriction fragment length polymorphism demonstrated that while *A. chilense* is present in New Zealand, another species was found in Manukau Harbour (Candia *et al.* 1999). The difference between these two species in New Zealand was further demonstrated by the presence of gigartinine (5-(3-amidinoureido)-2-aminovaleric acid) (Ito & Hashimoto 1966) in the Manukau Harbour samples and not in *A. chilense* (Wilcox *et al.* 2001, 2007). Further phylogenetic studies demonstrated that the specimen sequenced by Goff *et al.* (1994) was identical with material from Australia (Byrne *et al.* 2002) and therefore was also placed erroneously in *A. chilense*. A phylogenetic study incorporating specimens from Australia, Chile and New Zealand clearly showed that there are two genetically different species incorrectly placed under the same name: *A. chilense* from Chile and New Zealand, and an undescribed species from Australia and New Zealand (Clade B) (Cohen *et al.* 2004).

The aim of this study is to formally describe the vegetative and reproductive morphology of this new species, currently known as *A. sp.* clade B from New Zealand and Australia, and compare its morphological characteristics and phylogenetic position to *A. chilense* and the other two *Agarophyton* species, *A. tenuistipitatum* and *A. vermiculophyllum*. This present work extends our understanding of the genus *Agarophyton* in New Zealand providing a number of opportunities for future research in their phylogeography, eco-physiology and biochemistry.

## MATERIALS AND METHODS

**Algal sampling.** Algae were collected during low tide from Australia and New Zealand (Table S1). Algal samples were pressed onto herbarium sheets and subsamples stored in silica gel. Type and voucher herbarium specimens have been deposited in the herbarium of the Museum of New Zealand Te Papa Tongarewa, Wellington, New Zealand (WELT - Thiers 2019). For morphological observation, sections were made by hand using a stainless-steel razor blade and stained with 1% aniline blue in 40% Karo® corn syrup. Photographs were obtained by microscope digital camera Olympus DP-70 (Olympus, Tokyo, Japan).

**Molecular analyses.** DNA extraction followed the Chelex-based technique (Zuccarello *et al.* 1999). A mitochondrial (the cytochrome-c-oxidase subunit I, *cox1*), a nuclear (internal transcript spacer 1, ITS1) marker and a plastid (the ribulose 1,5-bisphosphate carboxylase/oxygenase gene, *rbcL*) marker were chosen according to previous systematic studies of Gracilariaceae (Cohen *et al.* 2004; Gurgel *et al.* 2018). Genomic DNA was amplified by PCR using primer combinations for *cox1* (Saunders 2005), for ITS1 (White *et al.* 1990), and for *rbcL* (Freshwater & Rueness 1994; Kim *et al.* 2010). The PCR conditions for ITS1 amplification were as follows: initial denaturation at 94°C for 5 mins, followed by 16 cycles of 94°C for 1 min, 62.5°C for 30sec and 72°C for 1 min, followed by 25 cycles of 94°C/55°C/72°C for 1 min each and a final step at 72°C for 10 min. The PCR profile for *cox1* and *rbcL* were as follows: 94°C for 5 min; 36 cycles of 94°C/45°C/72°C for 1 min each and then a final step of 5 min for extension at 72°C. The PCR amplifications were examined by electrophoresis in 1% agarose gel, and PCR products were cleaned with ExoSAP-IT (USB product, Affymetrix, Santa Clara, California, USA). Purified PCR products were Sanger sequenced commercially (Macrogen Inc., Seoul, South Korea).

All sequences were assembled, edited and aligned using Geneious 10.2.6 (<https://www.geneious.com>). Additional sequences of Gracilariaceae species included in the *cox1*, ITS1 and *rbcL* dataset are presented in Table S2. TCS statistical parsimony network in PopArt 1.7 (<http://popart.otago.ac.nz>) was used to analyze genetic diversity of ITS1. Maximum likelihood (ML) and Bayesian phylogenetic analyses were performed with all three codons partitioned for *cox1* and *rbcL* sequences. ML analysis was performed using the General Time Reversible + gamma model and hill-climbing algorithm. 1000 non-parametric bootstrap replicates were inferred from 10 distinct alternative runs (Felsenstein 1985) in RAxML 7.2.8 (Stamatakis 2006). Bayesian phylogenetic analysis was conducted using MrBayes 3.2.0 (Ronquist *et al.* 2012). Two independent sets of four Markov chain Monte Carlo (MCMC) chains were run for three million generations with sampling every 1000 generations. The two independent MCMC chains were converged when split frequency was below 0.01. The first 25% of sampled trees as a ‘burn-in’ was discarded and 25,000 trees were saved to construct the consensus tree.

## RESULTS

**Molecular phylogeny.** The 1467 bp *rbcL* alignment contained 37 taxa representing the new species and all known *Agarophyton* species. This dataset strongly supported the new species as sister to *A. tenuistipitatum* with high bootstrap (100%) and posterior probability (1.0) and as member of the genus *Agarophyton* (Fig. 1).

The *cox1* alignment, with a length of 654 bp, contained the holotype of the new species and representatives of all other *Agarophyton* species. The *cox1* alignment grouped the new species as sister to *A. tenuistipitatum* with strong support (Fig S1).

The 383 bp ITS1 alignment contained 13 samples of the new species and no indels were detected. Five different ribotypes were found that differed by a maximum of 2.9% (11



bp; A3 and A5). Lineage A1 (n=7, including Genbank Accession no. AY131297) was the most common ribotype and was found in Australian and New Zealand. Ribotypes A2 (AY131295) and A3 (AY131295) occurred in Australia. Lineage A4 (n=3) was the most common ribotype exclusive to Australia. Ribotype A5 (AF034265) was only present in New Zealand (Fig. 2).

The *cox1* and *rbcL* markers showed that the new species belongs within the genus *Agarophyton* and is genetically distinct from *A. chilense* and *A. tenuistipitatum*. In addition, the ITS1 marker showed that samples from Australia previously identified as *A. chilense*, were similar to New Zealand sequences and, therefore, belong to this new species. We proposed the new species here:

***Agarophyton transtasmanicum* M.Preuss, N.Muangmai & Zuccarello sp. nov.**

**Figs. 3-12**

DIAGNOSIS: Plants slender, 5–17 cm tall, and cylindrical 0.5–0.7 mm in diameter. Plants irregularly branched, sometimes alternatively. Short branchlets form around axis and branches. Thallus in cross section consists of 1–2 layers of cortical cells and 6–8 layers of medullary cells. Cell transition from cortex to medulla abrupt. Spermatangia formed in solitary or confluent shallow pits (textorii-type conceptacle). Mature cystocarps globose, pericarps rather thin. Tetrasporophytes were not observed.

TYPE LOCALITY: 39°56'44.5"S, 174°59'50.8"E, Whanganui River Mouth, Whanganui, North Island, New Zealand.

HOLOTYPE: WELT A033798, male gametophyte (Fig. 3), collected 18 December 2018 by M. Preuss and G.C. Zuccarello, deposited in the Herbarium of the Museum of New Zealand Te Papa Tongarewa. Genbank Accession numbers: *cox1*: MN942037, ITS1: MN942043, *rbcL*: MN942038.

ISOTYPE: WELT A033797, female gametophyte (Fig. 4), collected 18 December 2018 by M. Preuss and G.C. Zuccarello, deposited in the Herbarium of the Museum of New Zealand Te Papa Tongarewa.

GENBANK ACCESSION NUMBERS: ITS1: MN942042-MN942047; *rbcL*: MN942038-MN942041.

ETYMOLOGY: *transtasmanicum* refers to the distribution of the species in Australia and New Zealand across the Tasman Sea.

DISTRIBUTION: Specimens were found in the North Island of New Zealand (Auckland, Foxton, Whanganui) and Tasmania (Kingston Beach, Marion Bay) and southern Australia (Glenelg, Hindmarsh Island, Phillip Island).

DESCRIPTION: Plants attached to rocks and shells or unattached, and usually occurring in the intertidal zone in estuaries. Plants were slender, 5–17 cm tall, and cylindrical, 0.5–0.7 mm in diameter, growing in tufts or solitary (Figs. 3–4), and dark brownish red to black in colour. Branches were numerous, alternate, mostly irregular, up to 4 or 5 orders (Fig. 3). Short filiform branchlets distributed around axes and branches (Figs. 3–4). Branches and branchlets were not, or only slightly, basally constricted. Axes and branches were tapered toward the

tips, and branch tips were often hooked (Figs. 3–4). In transverse section the thallus consisted of 1 or 2 layers of pigmented cortical cells, and 6–8 layers of medullary cells, 50–100  $\mu\text{m}$  in diameter (Fig. 5). There was an abrupt transition in cell size from the medulla to the cortex (Fig. 5).

Male gametophytes were more branched and larger than female thalli (Figs. 3–4).

Spermatangial conceptacles were scattered throughout the thallus (Fig. 6), forming shallow pits (textorii-type), 20–25  $\mu\text{m}$  deep, 20–40  $\mu\text{m}$  wide (Figs. 7–8). Spermatangia formed solitary (Fig. 7) to confluent conceptacles (Fig. 8). Cystocarps were scattered over the thallus surface.

Mature cystocarps were depressed spherical, 700–900  $\mu\text{m}$  in diameter, with a rostrum and constricted base (Fig. 9). Gonimoblast filaments consisted of ovoid to elongate cells, and produced outer unbranched chains of carposporangia, 5–25  $\mu\text{m}$  in diameter (Fig. 10).

Nutritive filaments connected to the base of the gonimoblasts (Fig. 11). Pericarps were thin, 70–110  $\mu\text{m}$  thick, and consisted of 6–10 cell layers (Fig. 12). Pericarp tissue was an outer layer of 1–3 elongate cells grading into an inner layer of 4–8 oblate to elongate branched cells (Fig. 12).

## DISCUSSION

This study used morphological and molecular data to distinguish this new species, from New Zealand and Australia. Molecular data (*cox1*, *rbcL*) clearly indicate that our samples from New Zealand and Australia are distinct from all other *Agarophyton* species, and thus we propose a new species, *Agarophyton transtasmanicum* sp. nov. In addition, our molecular data (ITS1) have shown that our recently collected samples are conspecific with samples previously sequenced from Australia and New Zealand (Byrne *et al.* 2002; Goff *et al.* 1994),

indicating that these sequences had been misidentified when placed in *Agarophyton chilense*, as previously suggested by Cohen *et al.* (2004).

Within the genus *Agarophyton*, *A. transtasmanicum* and *A. chilense* are morphologically very similar, and their similarity has led to the confusion of the two species in the field (Bird *et al.* 1986; Byrne *et al.* 2002; Nelson 1987), especially in the region of overlapping distribution range (Wilcox *et al.* 2007). *Agarophyton transtasmanicum* shares several characteristics with *A. chilense*, including branching pattern, spermatangial conceptacles, cystocarp shape, the position of nutritive filaments and pericarp arrangement (Table 1), but they may be distinguished by the number of medullary layers (6–8 cells in *A. transtasmanicum* and 8–10 cells in *A. chilense*), and cortex-to-medulla transition (abrupt in *A. transtasmanicum*, but relatively gradual in *A. chilense*). In the field, *A. transtasmanicum* is generally shorter than *A. chilense* and has hooked apices on several branch tips (Byrne *et al.* 2002). Despite their morphological similarity, molecular data (*rbcL*) clearly distinguish between *A. transtasmanicum* and *A. chilense*. The phylogenetic relationship of *A. transtasmanicum* and *A. tenuistipitatum* as sister species was also shown using the *cox2-3* spacer and RUBISCO spacer (Cohen *et al.* 2004). Both species, *A. transtasmanicum* and *A. chilense*, can be found in the same locations in New Zealand, and based on currently available data only *A. transtasmanicum* is present in Australia.

Our phylogenetic analyses of *rbcL* data demonstrated that *A. transtasmanicum* is closely related to *A. tenuistipitatum*. Morphologically, both *A. transtasmanicum* and *A. tenuistipitatum* possess often an irregular branching pattern with several branchlets and abrupt cell transition (Chang & Xia 1976, 1988; Lewmanomont 1994; Ohno *et al.* 1999). The differences between the two are thallus length, the number of medullary cells and pericarp thickness (Table 1). Thalli of *A. transtasmanicum* are smaller in length and diameter than *A. tenuistipitatum* (Chang & Xia 1988; Lewmanomont 1994). The main axis of *A.*

*transtasmanicum* contains more medullary cell layers (6-8 cells) than *A. tenuistipitatum* (4–6 cells, Chang & Xia 1988; Lewmanomont 1994). The mature cystocarps of *A.*

*transtasmanicum* have more pericarp cell layers (6-10 cells) than *A. tenuistipitatum* (4–5 cells, Chang & Xia 1988; Lewmanomont 1994). The distribution range of the two do not overlap.

*Agarophyton transtasmanicum* is currently known to occur in southeastern Australia and New Zealand whereas *A. tenuistipitatum* is mostly found in China, Southeast Asia and North America (Chang & Xia 1988; Lewmanomont 1994; Ohno *et al.* 1999).

*Agarophyton transtasmanicum* also shares a variety of common morphological features with *A. vermiculophyllum*, for example, thallus shape, branching pattern, cystocarp shape and anatomy (Table 1). The greatest differences between these two species is that the spermatangial conceptacle is of the *textorii*-type in *A. transtasmanicum*, and the *verrucosa*-type (deep pot-shaped) in *A. vermiculophyllum* (Rueness 2005; Terada & Yamamoto 2002). These species do not overlap in distributional ranges: *A. vermiculophyllum* is native to China, Korea and Japan, and introduced to the North Atlantic and north west Pacific (Bellorin *et al.* 2004; Kim *et al.* 2010; Krueger-Hadfield *et al.* 2018; Terada & Yamamoto 2002).

The genus *Agarophyton* contains four species, including our new species, *A. transtasmanicum*. The genus was originally established on the basis of several characters, including the abrupt transition between cortex and medulla (Gurgel *et al.*, 2018). However, the significance of the characteristic transition between cortex-medulla cells is uncertain at the genus level as it occurs as abrupt transition in *A. transtasmanicum* and *A. tenuistipitatum*, and gradual transition in *A. chilense* and *A. vermiculophyllum* (Table 1). Accordingly, we conclude that the diagnosis of cortex-medulla cell transition is flawed as a genus level character, but appears to be useful at the species level.

Our knowledge of *A. transtasmanicum* is still limited. As a result, further studies on the genetic diversity and population connectivity across Tasman Sea of *A. transtasmanicum*

are needed to shed light on the species history, and demographic patterns. In addition, the ability of *Agarophyton transtasmanicum* to produce *gigartinine*, serving as a means of nitrogen storage, has raised interest in the possible commercial attributes of this species (Wilcox *et al.* 2007). Therefore *A. transtasmanicum* could be an ideal candidate for use in bioremediation in nitrogen loaded environments such as polluted rivers and in polyculture as part of multi-trophic aquaculture. Future physiological studies are needed to understand more about the responses of *A. transtasmanicum* to environmental conditions.

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**Table 1.** Comparison of some morphological characteristics of *Agarophyton transtasmanicum* sp. nov. with all other *Agarophyton* species.

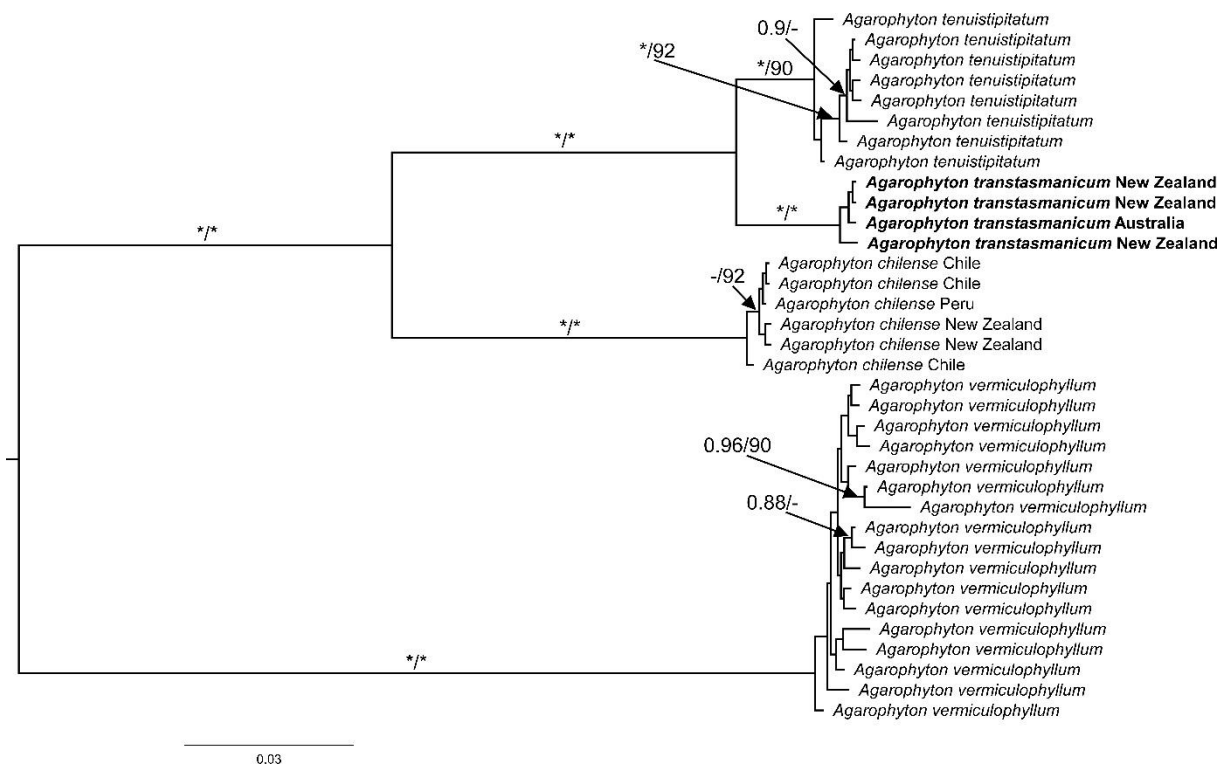
| <b>Characters</b>          | <i>Agarophyton transtasmanicum</i> sp. nov. <sup>1,2</sup> | <i>Agarophyton chilense</i> <sup>3,4,5</sup>        | <i>Agarophyton tenuistipitatum</i> <sup>6,7,8,9</sup> | <i>Agarophyton vermiculophyllum</i> <sup>10,11,12,13,14,15</sup> |
|----------------------------|--|---|---|--|
| <b>Thallus length (cm)</b> | 5–20   | Usually 10–60, sometimes reaches 1.5 m              | Usually 20–40, sometimes reaches 1 m                  | Usually 10 – 30, sometimes reaches to 1 m                        |
| <b>Thallus width (mm)</b>  | 0.5–0.9  | 0.5–2.0   | 0.5–1.5   | 1–2 mm, sometimes reaches to 5                                   |
| <b>Branching pattern</b>   | Mostly irregular, sometimes alternate, up to 5 orders,     | Variable, irregular or alternate, up to 4 orders    | Mostly irregular, sometimes alternate, up to 3 orders | Alternate or irregular, sometimes second or subdichotomous       |
| <b>Branchlets</b>          | Many around axis and branches                              | Some around lower portion of axis and main branches | Many around axis and branches                         | Some around axis and branches                                    |
| <b>Cortical layer</b>      | 1–3 cells  | 1–2 cells   | 1–2 cells   | 1–2 cells  |

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|                               |  |   |  |   |
|-------------------------------|--|---|--|---|
| <b>Medullary layers</b>       | 6–8 cells  | 8–10 cells  | 4–6 cells  | 6–13 cells  |
| <b>Cell transition</b>        | Abrupt   | Gradual   | Abrupt   | Gradual   |
| <b>Male structure</b>         | <i>textorii</i> -type  | <i>textorii</i> -type   | <i>textorii</i> -type                                | <i>verrucosa</i> -type  |
| <b>Cystocarp shape</b>        | Depressed sphere, with a rostrate ostiole and constricted base | Depressed sphere, with a slight rostrate ostiole and constricted base | Sphere, with a rostrate ostiole and constricted base | Sphere, with a slight rostrate ostiole and constricted base                                       |
| <b>Gonimoblast cell shape</b> | Round to elongate  | Round to isodiametric   | Round to isodiametric                                | Mostly round  |
| <b>Pericarp layers</b>        | 6–11 cells   | 6–8 cells   | 4–5 cells  | Approximately 9 cells   |
| <b>Distribution</b>           | Southern Australia and New Zealand                             | South America and New Zealand   | China, Southeast Asia and North America              | Japan, Korea and China, and introduced to some locations in North Atlantic and North West Pacific |

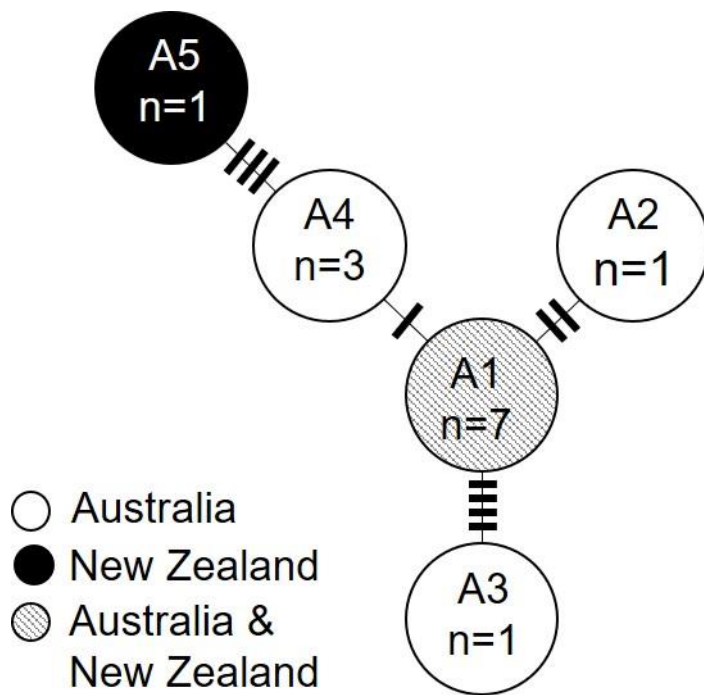
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<sup>1</sup>This study; <sup>2</sup>Byrne *et al.* 2002; <sup>3</sup>Bird *et al.* 1986; <sup>4</sup>Nelson 1987 (as *Gracilaria sordida*); <sup>5</sup>Bird *et al.* 1990; <sup>6</sup>Chang & Xia 1976; <sup>7</sup>Chang & Xia 1988;  
<sup>8</sup>Lewmanomont 1994; <sup>9</sup>Ohno *et al.* 1999; <sup>10</sup>Ohmi 1956 (as *Gracilariopsis vermiculophylla*); <sup>11</sup>Terada & Yamamoto 2002; <sup>12</sup>Bellorin *et al.* 2004;  
<sup>13</sup>Rueness 2005; <sup>14</sup>Kim *et al.* 2010; <sup>15</sup>Krueger-Hadfield *et al.* 2018.



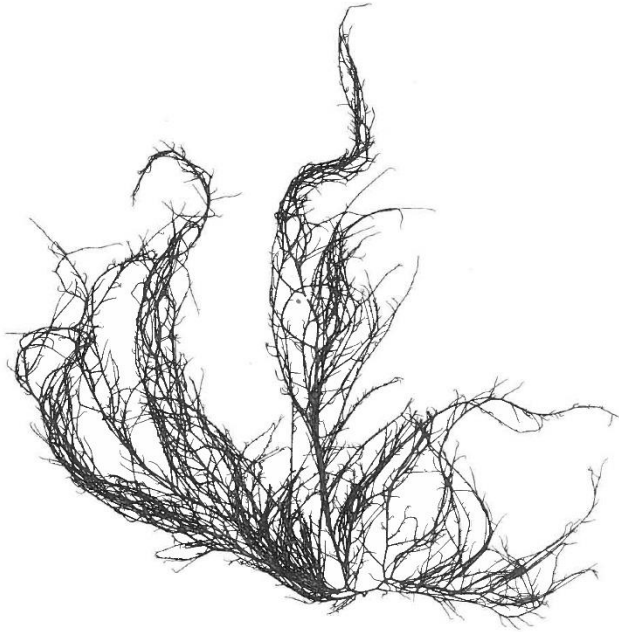
**Fig. 1.** Bayesian topology of *rbcL* sequences for *Agarophyton transtasmanicum* sp. nov., *A. chilense*, *A. tenuistipitatum* and *A. vermiculophyllum* (Table S2). Specimens of *A. transtasmanicum* are highlighted in bold. Country of collections are given for *A. transtasmanicum* and *A. chilense*. Asterisks indicate posterior probability value of 1.00 and bootstrap value of 100%. Values <0.85 posterior probability and <85% ML bootstrap not shown. *Crassiphycus changii* and *Crassiphycus firmus* were used as outgroups but removed to facilitate representation.



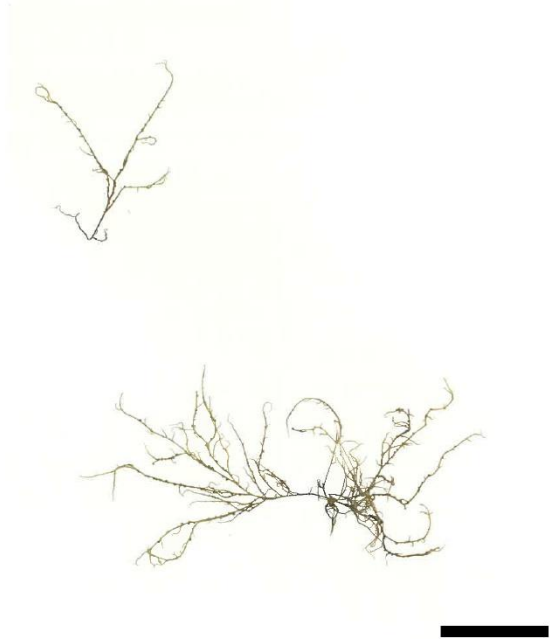


**Fig. 2.** ITS1 ribotype network of *Agarophyton transtasmanicum* *sp. nov.* with five different ribotypes represented (A1-A5). Cross lines represents one mutational step. Number of samples (n) with ribotype indicated and location of ribotypes shown: Australia (white), New Zealand (black), and Australia and New Zealand (cross-hatch).

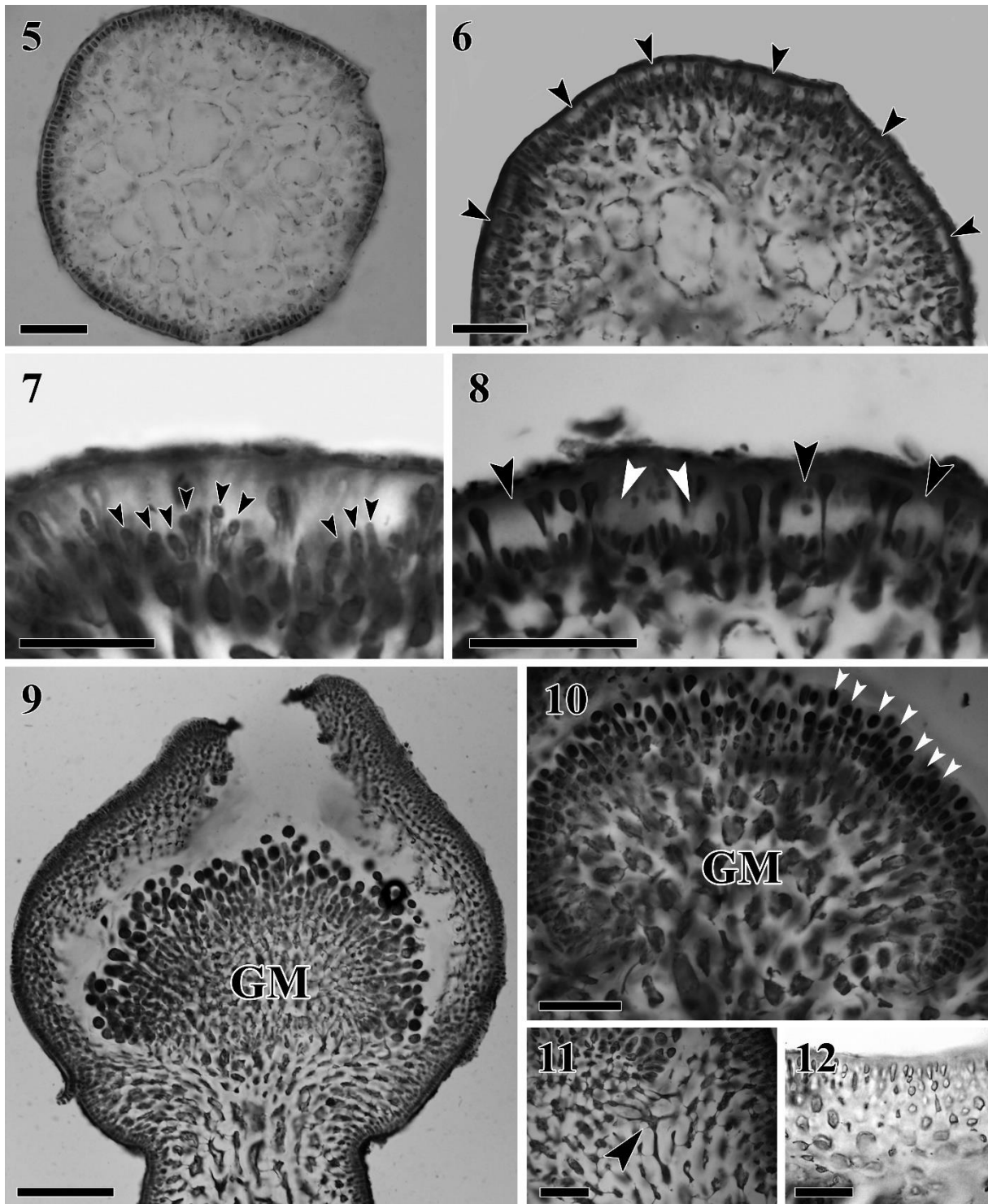
3



4



**Figs. 3–4.** Habits of *Agarophyton transtasmanicum* sp. nov. from Whanganui River Mouth, New Zealand. **Fig. 3.** Holotype, male gametophyte (WELT A033798). Scale bar = 4 cm. **Fig. 4.** Isotype, female gametophyte (WELT A033797). Scale bar = 3 cm.



**Figs. 5–12.** Detailed morphological feature of *Agarophyton transtasmanicum* sp. nov. **Fig. 5.** Cross-section of main axis showing abrupt transition from cortex to medulla. Scale bar = 150  $\mu$ m. **Fig. 6.** Cross-section of male gametophyte showing textorii-type spermatangial conceptacles (arrowheads). Scale bar = 100  $\mu$ m. **Fig. 7.** A young spermatangial conceptacle

bearing spermatia (arrowheads). Scale bar = 40  $\mu\text{m}$ . **Fig. 8.** Mature confluent (white arrowheads) and solitary spermatangial conceptacles (black arrowheads). Scale bar = 50  $\mu\text{m}$ . **Fig. 9.** Cross-section of mature cystocarp, with rostrum and constricted base, showing gonimoblast filament (GM). Scale bar = 200  $\mu\text{m}$ . **Fig. 10.** Close-up view of gonimoblast (GM) consisting of ovoid to elongate cells with the outer unbranched chains of carposporangia (arrowheads). Scale bar = 100  $\mu\text{m}$ . **Fig. 11.** Cystocarp base showing nutritive filament (arrowhead) connecting the gonimoblast to the pericarp. Scale bar = 40  $\mu\text{m}$ . **Fig. 12.** Pericarp tissue consisting of outer elongate cell and inner roundish cells. Scale bar = 40  $\mu\text{m}$ .

**Table S1.** Samples collected for molecular and morphological analysis of *A. transtasmanicum* in Australia and New Zealand. Genbank Accession no. indicated in sequenced regions.

| Species                   | Date                        | Location                  | Coordinates   | Collector        | cox1             | ITS1     | rbcL     |          |
|---------------------------|-----------------------------|---------------------------|---------------|------------------|------------------|----------|----------|----------|
| <i>A. transtasmanicum</i> | 18.12.2018                  | Whanganui River,          | 39°56'44.5"S, | M. Preuss &      | MN942037         | MN942043 | MN942038 |          |
|                           |                             | Whanganui, North Island,  | 174°59'50.8"E | G. C. Zuccarello |                  | MN942044 | MN942040 |          |
|                           |                             | New Zealand               |               |                  |                  |          | MN942041 |          |
|                           | 18.12.2018                  | Manawatu River, Foxton,   | 40°28'12.5"S, | M. Preuss &      |                  | MN942045 |          |          |
|                           |                             | North Island, New Zealand | 175°13'57.0"E | G. C. Zuccarello |                  |          |          |          |
|                           | 21.11.2018                  | Hindmarsh Is., Mundoo     |               |                  | G. C. Zuccarello |          | MN942042 | MN942039 |
|                           |                             | Channel, site 2           |               |                  |                  |          |          |          |
|                           | 21.11.2018                  | Hindmarsh Is., Mundoo     |               |                  | G. C. Zuccarello |          | MN942046 |          |
| Channel. Site 1           |                             |                           |               |                  |                  |          |          |          |
| 22.11.2018                | Glenelg River Mouth,        |                           |               | G. C. Zuccarello |                  | MN942047 |          |          |
|                           | Nelson, Victoria, Australia |                           |               |                  |                  |          |          |          |

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|          |                        |               |           |        |
|----------|------------------------|---------------|-----------|--------|
| 10.02.19 | Browns River, Kingston | 42°58'39.9"S, | M. Preuss | XXXXXX |
|          | Beach, Tasmania,       | 147°19'42.4"E |           |        |
|          | Australia              |               |           |        |

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**Table S2.** List of species and Genbank Accession numbers for *cox1*, ITS1 and *rbcL* sequences from Genbank used in molecular analyses.

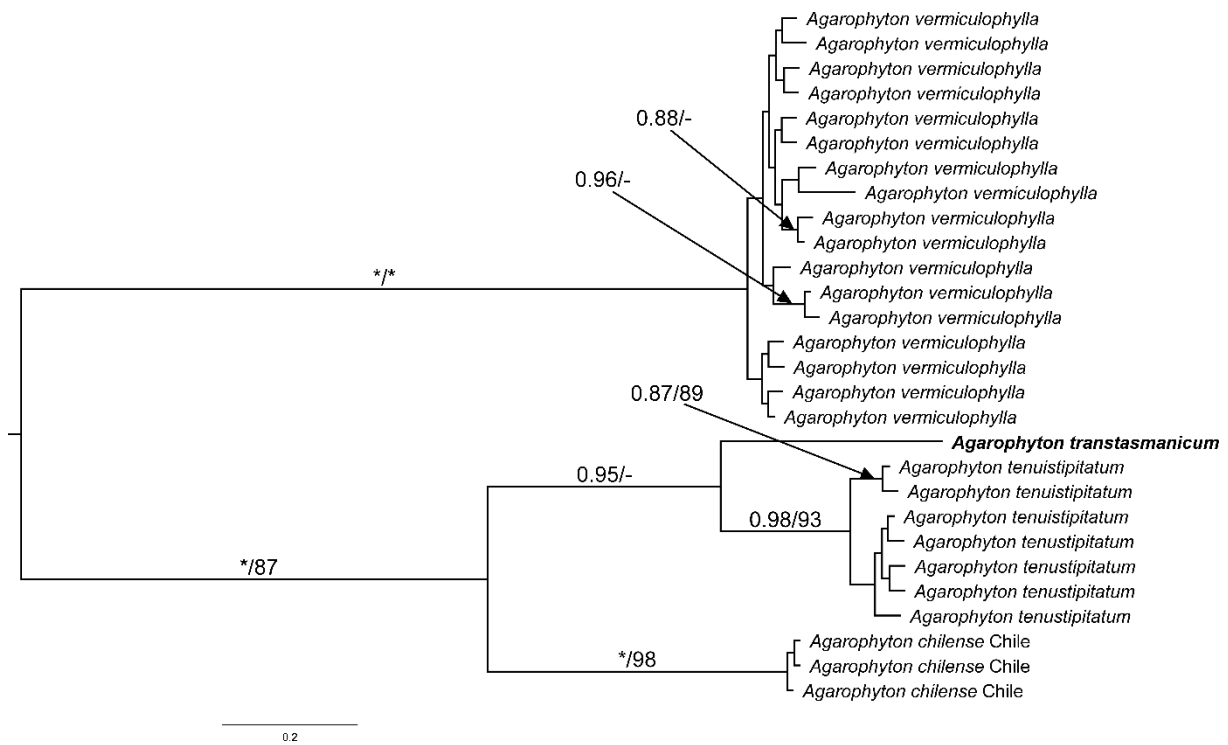
| Species                                     | Genbank Accession numbers |             |                    |
|---|---------------------------|-------------|--------------------|
|   | <u><i>cox1</i></u>        | <u>ITS1</u> | <u><i>rbcL</i></u> |
| <i>Agarophyton chilense</i>                 | KP728466                  |             | AY049396           |
|   | MF41962                   |             | DQ095784           |
|   | NC 026831                 |             | HQ998843           |
|   |                           |             | HQ998848           |
|   |                           |             | KP857578           |
|   |                           |             | MH760419           |
| <i>Agarophyton transtasmanicum</i> sp. nov. |                           | AF034265    |                    |
|   |                           | AY131295    |                    |
|   |                           | AY131296    |                    |
|   |                           | AY131297    |                    |
| <i>Agarophyton tenuistipitatum</i>          | JQ026074                  |             | DQ119743           |
|   | JQ026076                  |             | EF434906           |
|   | JQ407638                  |             | EU380718           |
|   | JQ407639                  |             | JN605793           |
|   | JQ407641                  |             | KF214701           |
|   | JQ407649                  |             | MH760420           |
|   | JQ407653                  |             | MH760421           |
|   |                           |             | MH760425           |
| <i>Agarophyton vermiculophyllum</i>         | GQ292865                  |             | AY049314           |
|   | HQ3220449                 |             | AY725172           |
|   | HQ322048                  |             | DQ095822           |

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|                             |          |          |
|-----------------------------|----------|----------|
|                             | HQ322086 | EF434907 |
|                             | HQ412552 | EU600293 |
|                             | JQ407598 | EU605702 |
|                             | JQ407609 | JQ407698 |
|                             | JQ619143 | JQ407699 |
|                             | JQ736334 | JQ407701 |
|                             | JQ794749 | JQ407704 |
|                             | JQ794751 | JQ728687 |
|                             | JQ794756 | JQ768762 |
|                             | JQ794757 | JQ768764 |
|                             | JQ794759 | JQ768767 |
|                             | KF367746 | JQ768769 |
|                             | KF789527 | JQ768770 |
|                             | KJ526627 | HQ880644 |
| <i>Crassiphycus changii</i> | KY009863 | AY049388 |
| <i>Crassiphycus firmus</i>  | KY315284 | DQ119739 |

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**Fig. S1.** Bayesian topology of partial *cox1* sequences for *Agarophyton transtasmanicum* sp. nov., *A. chilense*, *A. tenuistipitatum* and *A. vermiculophyllum* (Table S2). The holotype of *A. transtasmanicum* is highlighted in bold. Asterisks indicate posterior probability value of 1.00 and bootstrap value of 100%. Values <0.85 posterior probability and <85% ML bootstrap not shown. *Crassiphycus changii* and *Crassiphycus firmus* were used as outgroups but removed to facilitate representation.