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Molecular analysis identifies a red seaweed common in the Swan Estuary as *Gracilaria transtasmanica* (Gracilariales: Gracilariaceae)

John M. Huisman[1], Giuseppe C. Zuccarello[2] & Showe-Mei Lin[3]

[1]Western Australian Herbarium, Biodiversity and Conservation Science,
Department of Biodiversity, Conservation and Attractions,
Locked Bag 104, Bentley Delivery Centre, Western Australia 6983
[2]School of Biological Sciences, Victoria University of Wellington, PO Box 600, Wellington, New Zealand
[3]Institute of Marine Biology, National Taiwan Ocean University, Keelung City 20224, Taiwcan, R.O.C.
[1]Corresponding author, email: John.Huisman@dbca.wa.gov.au

SHORT COMMUNICATION

Species of the red seaweed *Gracilaria* Grev. (Gracilariales: Gracilariaceae) can often display considerable infra-specific morphological variation, or conversely several cryptic species can be morphologically similar and only confidently identified by molecular methods. This second category includes a group of three species that have been recorded for the Australasian region: *Gracilaria sordida* Nelson, *G. chilensis* Bird, McLachlan & Oliveira and *G. transtasmanica* (M.Preuss, N.Muangmai & Zuccarello) Lyra, Iha, J.M.C.Nunes & C.C.Davis. *Gracilaria sordida* was described by Nelson (1987) to include plants that were often stringy or 'untidy', suggesting that earlier records were incorrectly attributed to forms of *G. secundata* (*sensu* May 1948) or *G. confervoides* (L.) Grev. (= *G. verrucosa* (Huds.) Papenf.). As noted by Nelson, the configuration of the spermatangial conceptacle differentiated *G. sordida* from both species. *Gracilaria sordida* was subsequently placed into synonymy with the earlier *G. chilensis* by Bird *et al.* (1990). Womersley (1996) recorded *G. chilensis* for south-eastern Australia, following Bird *et al.* (1990) in placing *G. sordida* in synonymy. *Gracilaria chilensis* in Australia was described as 'largely confined to river mouths or estuaries, as in Chile and New Zealand' (Womersley 1996: 25).

Molecular studies of New Zealand, Australian and Chilean specimens indicated that two morphologically inseparable species were present, one initially misidentified for New Zealand and Australia as *G. chilensis*, and the second actual *G. chilensis* from Chile and New Zealand, the latter including *G. sordida* (Cohen *et al.* 2004). The former was eventually described in a segregate genus as *Agarophyton transtasmanicum* (Preuss *et al.* 2020) before that genus was subsumed into *Gracilaria* by Lyra *et al.* (2021).

A species of *Gracilaria* is common in the Swan River estuary, Western Australia. This species has been recorded under various names: *G. confervoides* by Thomson (1946: 58) and Royce (1955: 44), *G. confervoides* f. *gracilis* (Stackh.) Grunow by May (1948: 27), *G. verrucosa* by Allender (1981: 22) and *G. comosa* Withell, A.Millar & Kraft by Astill and Lavery (2004). Royce (1955) described the estuary's *Gracilaria* as 'Among the macroscopic forms, *Gracilaria confervoides* is the most abundant, and grows thickly over the river bed at least as far upstream as the Causeway. The thallus is fine, much branched, cartilaginous and terete, or round in cross section, while the branches and branchlets

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are narrowed into fine filiform points.' Specimens in the Western Australian Herbarium date back to 1949 (PERTH 03969010) collected by R.D. Royce from the drift at South Perth. Indeed, *Gracilaria confervoides* was recorded in some of the earliest investigations of Western Australian seaweeds, and noted by Harvey (1855: 550) as 'abundant at Fremantle'. *Gracilaria confervoides* and *G. verrucosa* are both now referred to *Gracilariopsis longissima* (S.G.Gmelin) Steentoft, L.M.Irvine & Farnham, and according to Womersley (1996: 15) 'there now appears no reason to record *G. verrucosa* from southern Australia, and this taxon is probably confined to European coasts'. *Gracilaria confervoides* f. *gracilis* is now treated as *Gracilaria gracilis* (Stackh.) Steentoft, L.M.Irvine & Farnham, another northern hemisphere species, and May's (1948: 27) determination of the Swan Estuary specimens as that taxon is undoubtedly an error.

As morphology is inconclusive in distinguishing this group of species, we have undertaken a molecular analysis using *rbc*L sequences, which clearly places the Swan River entity in *Gracilaria transtasmanica* and not conspecific with any of the species to which it has previously been attributed (Figure 1). While this represents the first record for Western Australia and the Indian Ocean under this name, the species is clearly a long-term component of the native algal flora and should not be considered an introduction. The mitochondrial COI-5P barcode region was also sequenced from a specimen collected from the Swan Estuary but not vouchered. It showed a difference of seven synonymous bp differences to specimens from New Zealand and eastern Australia (Huanel *et al.* 2020; Preuss *et al.* 2020)(data not shown), suggesting a long separation.

Given the species has apparently been present in Western Australia for at least decades, if not centuries, could there be an earlier-named species based on a Western Australian type? One species is clearly closely related, *Gracilaria comosa* Withell, A.Millar & Kraft (in Withell *et al.* 1994). The two species share a similar habit, structure and spermatangial arrangement. According to Withell *et al.* (1994), *G. comosa* is characterised by 'its abundantly divided, terete and very narrow branches and laterals which, except for the larger branches, are slightly to markedly constricted at their bases'. This was also noted in specimens in the Western Australian Herbarium from Jurien Bay (PERTH 08846758, PERTH 08846731) and from Dongara (PERTH 01663887), the latter a paratype from adjacent to the Port Denison type locality. In the description of *G. transtasmanica*, Preuss *et al.* (2020) noted branches and branchlets were 'not, or only slightly, basally constricted'. This was also true of the Swan Estuary specimens. In addition, while tetrasporangia were not recorded by Preuss *et al.* (2020), the Swan Estuary specimens had scattered tetrasporangia, whereas those of *G. comosa* occur in raised nemathecia. While we do not have molecular data for *G. comosa*, we are satisfied that the branches with marked basal constrictions and the disposition of tetrasporangia clearly distinguish this species from *G. transtasmanica*.

Gracilaria transtasmanica belongs to a group of species (the *Agarophyton* of Gurgel *et al.* 2018) that are typically found in estuaries and harbours, and includes the invasive *G. vermiculophylla* (Ohmi) Papenf. that has spread from Japan to numerous locations in the northern hemisphere (Gurgel *et al.* 2018). The distribution of *G. transtasmanica* might suggest similar invasive tendencies, however if this is the case for the Swan Estuary population, given multiple early records, the initial introduction must have been decades, if not centuries ago.

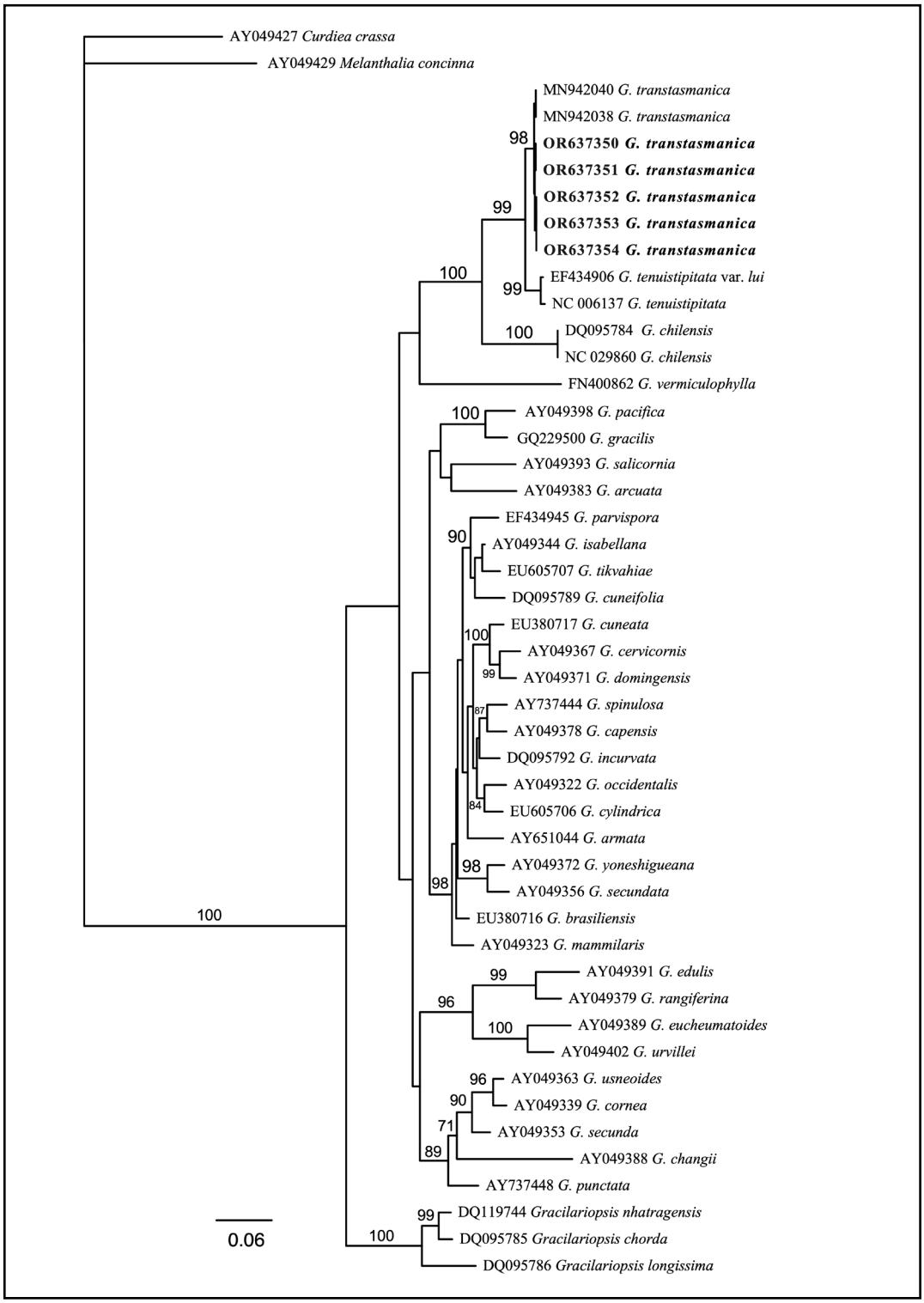

Figure 1. Maximum-likelihood tree of *rbc*L sequences of various *Gracilaria* and *Gracilariopsis* species, with GenBank accession numbers. Swan Estuary specimens in bold. Tree and model selection produced in IQTREE2 (1278bp dataset) (Minh *et al.* 2020). 500 bootstrap replicates in % shown on significant branches. *Curdiea crassa* and *Melanthalia concinna* selected as outgroups.

Gracilaria transtasmanica (M.Preuss, N.Muangmai & Zuccarello) Lyra, Iha, J.M.C.Nunes & C.C.Davis, *Molec. Phylogen. Evol.* 165(107294):10 (2021).

Basionym: *Agarophyton transtasmanicum* M.Preuss, N.Muangmai & Zuccarello, *Phycologia* 59(3): 240 (2020).

Type: Whanganui River mouth, Whanganui, North Island, New Zealand, 18 December 2018, *M. Preuss & G.C. Zuccarello* (*holo*: WELT A033798!; *iso*: WELT A033797!). Genbank Accession numbers: cox1: MN942037, ITS1: MN942043, *rbc*L: MN942038.

[*Gracilaria chilensis auct non* C.J.Bird, McLachlan & E.C.Oliveira: Womersley 1996: 23]

[*Gracilaria confervoides auct non* (L.) Grev.: Thomson 1946: 58; Royce 1955: 44]

[*Gracilaria confervoides* f. *gracilis auct non* (Stackh.) Grunow: May 1948: 27]

[*Gracilaria verrucosa auct non* (Huds.) Papenf.: Allender 1981: 22]

[*Gracilaria comosa auct non* Withell, A.Millar & Kraft: Astill & Lavery 2004: 12, 13]

Description. Thallus greenish to dark red-brown, often drying black, 10–35 cm high, with 1-several fronds arising from a discoid holdfast, irregularly branched with percurrent main axes and numerous lateral branches of varying lengths, apices acute; branches terete, 0.4–1 mm diam., tapering to 0.2–0.4 mm diam. close to the apices, not basally constricted. Structure uniaxial, with a slightly protruding apical cell, pseudoparenchymatous, axial filament not obvious in mature portions, with an abrupt transition in cell size from cortex to medulla; cortex with 2–6 cell layers, outer cells ovoid (often slightly palisade like) and 4–10 µm broad in surface view, L/D 1.5–2.5 in section; medulla 6–12 cells across, inner medullary cells ovoid, 70–270 µm diam., thick walled with numerous secondary pit-connections. Male plants not seen. Cystocarps scattered, protuberant, sessile, hemispherical, conical to globular and basally constricted, 500–800 µm diam.; pericarp 80–100 µm thick, with anticlinal rows of 6–9 cells, ostiolate. Tetrasporangia scattered in the cortex, basally pit-connected, ovoid, 40–50 µm long, 30–36 µm diam., cruciately to almost tetrahedrally divided. (Figure 2)

Diagnostic features. Amongst the local species *G. transtasmanica* can be distinguished by its thallus being irregularly branched with long main branches and numerous laterals of varying lengths that lack basal constrictions, and tetrasporangia scattered in the cortex.

Specimens examined. WESTERN AUSTRALIA: Mouth of Margaret River, drift, 3 Mar. 1965, *B.M. Allender s.n.* (PERTH 06997368); Swan River Estuary, Claremont Jetty, 22 Aug. 1968, *B.M. Allender s.n.* (PERTH 07008287); Swan River Estuary, The Springs, Belmont, 22 Aug. 1968, *B.M. Allender s.n.* (PERTH 07092245); Leschenault Inlet, near Vasse River, [sic, possibly Collie River], 1 m depth in sandy/muddy substrate, 18 Oct. 1976, *M.L. Cambridge s.n.* (PERTH 06997546); Nornalup Inlet, near channel, 2 Apr. 2015, *J.M. Huisman* 2.4.15.1.4 (PERTH 08854351); Canning Bridge, Mount Pleasant, 14 Sep. 2018, *J.M. Huisman* 4.9.18.1 A–D (PERTH 09571027; PERTH 09571108; PERTH 09571140. Genbank OR637350–OR637354); Matilda Bay, Swan River, 30 Mar, 1950, *W. McArthur s.n.* (PERTH 06997406); Swan River, at Guildford Grammar School, abundant, 6 Apr. 1971, *L. Mutton s.n.* (PERTH 07008201); South Perth, drift, 4 Sep. 1949, *R.D. Royce* 347 (PERTH 03969010); Dawesville, Harvey Estuary, 25 Feb. 1972, *G.G. Smith & E.P. Hodgkin s.n.* (PERTH 07040210; PERTH 09605843).

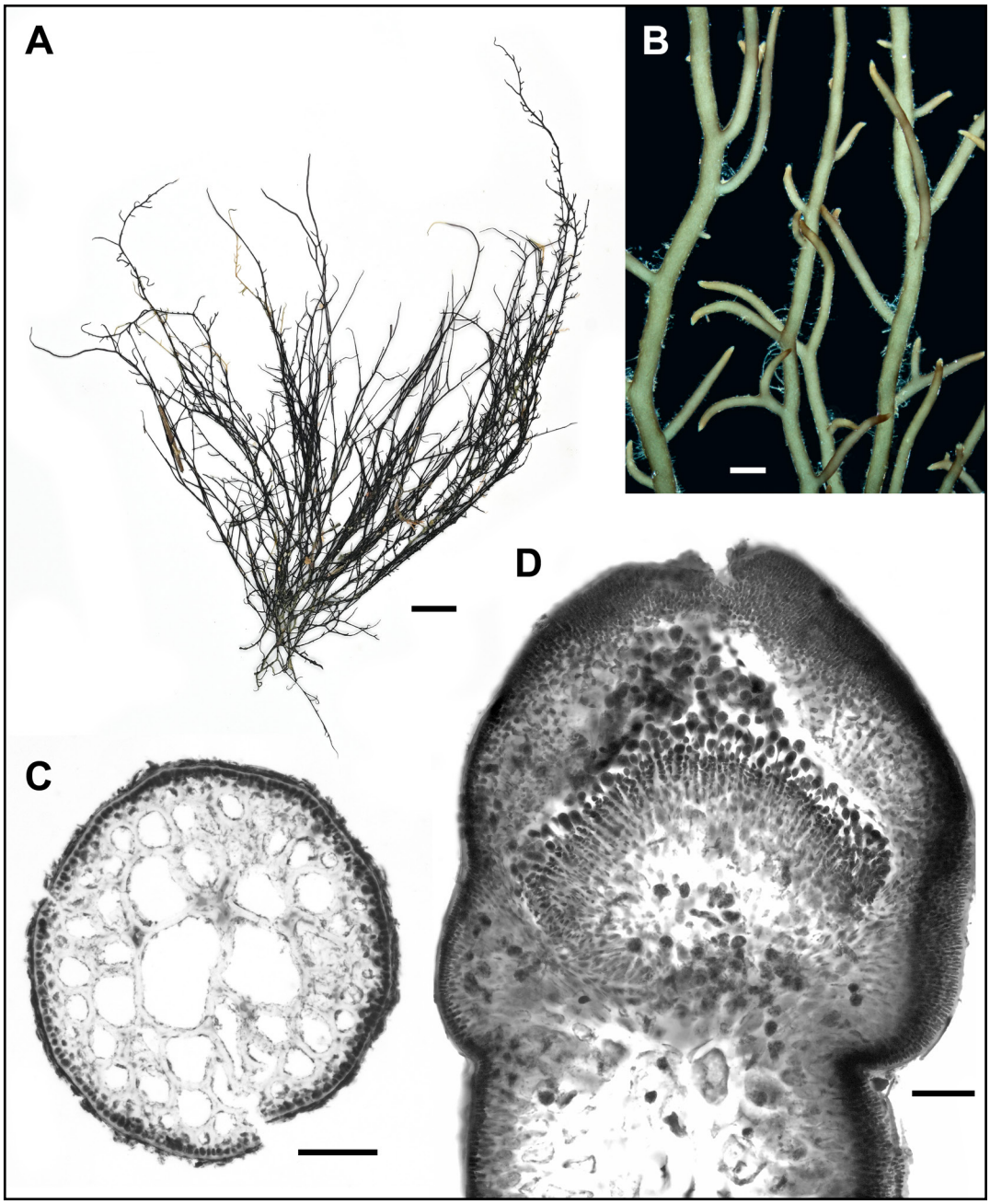

Figure 2. *Gracilaria transtasmanica*. A – pressed specimen showing irregular branching; B – detail of thallus, wet specimen; C – transverse section of branch, showing pseudoparenchmatous structure with abrupt transition between medulla and cortex; D – transverse section of cystocarp. Scale bars = 1 cm (A); 1 mm (B); 100 µm (C, D). Images from PERTH 09571140. Photographs by J.M. Huisman.

Distribution and habitat. North Island of New Zealand (Auckland, Foxton, Whanganui), Tasmania (Kingston Beach, Marion Bay), southern Australia (Glenelg, Hindmarsh Island, Phillip Island), and western Australia (Swan Estuary, Leschenault Inlet, Nornalup Inlet). Typically growing in sandy or muddy estuarine habitats, often attached to shells or loose lying.

Conservation status. This species is clearly widespread in estuarine habitats and is not threatened. Its historical presence in the Swan Estuary suggests it is not an introduction.

Acknowledgements

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