J. Phycol. **51,** 236–246 (2015)

DOI: 10.1111/jpy.12264

A MOLECULAR INVESTIGATION OF THE GENUS *ECKLONIA* (PHAEOPHYCEAE, LAMINARIALES) WITH SPECIAL FOCUS ON THE SOUTHERN HEMISPHERE[1]

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Brown algae of the order Laminariales, commonly referred to as kelps, are the largest and most productive primary producers in the coastal inshore environment. The genus *Ecklonia* (Lessoniaceae, Phaeophyceae) consists of seven species with four species in the Northern Hemisphere and three in the Southern Hemisphere. It was recently transferred to the family Lessoniaceae based on phylogenetic analyses of nuclear and chloroplastic markers, though the type of the genus was not included and its relationship with allied genera *Eckloniopsis* and *Eisenia* remained unresolved. The present study is the first to produce a phylogeny focussed on the genus *Ecklonia*. It included sequences from nuclear, mitochondrial, and chloroplastic DNA, for most of the distribution range of the three current Southern Hemisphere species (*Ecklonia radiata*, *Ecklonia maxima*, and a sample of a putative *Ecklonia brevipes* specimen), sequences for East Asiatic species (*Ecklonia cava*, *Ecklonia kurome*, and *Ecklonia stolonifera*), as well as the closely related genera *Eckloniopsis* and *Eisenia*. Results confirmed *E. radiata* and *E. maxima* as two distinct species in South Africa, *E. radiata* as a single species throughout the Southern Hemisphere (in South Africa, Australia, and New Zealand) and East Asiatic species as a distinct lineage from the Southern Hemisphere clade. Results further pointed out a close sister relationship between *Eckloniopsis radicosa* and two *Eisenia* species (including the type species: *Eisenia arborea*) to the genus *Ecklonia* suggesting that the genera *Eckloniopsis* and *Eisenia* are superfluous.

Key index words: **atp8; *Ecklonia*; ITS; kelp forests; Lessoniaceae; phylogeny; *rbcL*; trnWI**

***Abbreviations*: atp8, adenosine tri-phosphate dehydrogenase subunit 8; GTR, general-time-reversible; ITS, internal transcribed spacer; RuBisCO, ribulose-1,5-bisphosphate carboxylase/oxygenase; trnWI (section between *trnW* and *trnI* genes), t-RNAs**

Ecklonia Hornemann is a genus of brown seaweed in the family Lessoniaceae (previously Alariaceae) of the Laminariales (Lane et al. 2006). *Ecklonia* is distributed in both Hemispheres (Steneck et al. 2002, Steneck and Johnson 2013, Guiry and Guiry 2014),

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Editorial Responsibility: C. Lane (Associate Editor)

but largely limited to East Asia in the Northern Hemisphere, with only small populations recorded in cooler deep water habitats in Oman and off the north-west coast of Africa (Sheppard et al. 1992, Guiry and Guiry 2014). In the Southern Hemisphere, it is found in South Africa, Australia, and New Zealand (Lüning 1990, Stegenga et al. 1997, Bolton 2010, Guiry and Guiry 2014). According to Guiry and Guiry (2014), of a total 23 species and infraspecific names available for the genus, only 10 *Ecklonia* species (and three forms) are currently accepted taxonomically. However, on the basis of morphology, Bolton and Anderson (1994) considered *Ecklonia fastigiata* (Endlicher & Diesing) Papenfuss, and *Ecklonia richardiana* J. Agardh to be synonymous with *Ecklonia radiata* (C. Agardh) J. Agardh. They further included *Ecklonia biruncinata* (Bory de Saint-Vincent) Papenfuss in *E. radiata*, following the treatments of Womersley (1967) and Novaczek (1980), thus leading to a final count of seven current species. Four of those species occur exclusively in the Northern Hemisphere: *Ecklonia muratii* Feldmann in Mauritania (Northeastern Atlantic), *Ecklonia cava* Kjellman, *Ecklonia stolonifera* Okamura, and *Ecklonia kurome* Okamura in Japan and Korea, with *E. kurome* also occurring in China (Bolton 2010, Guiry and Guiry 2014). Two species occur exclusively in the Southern Hemisphere: *Ecklonia maxima* (Osbeck) Papenfuss (Fig. 1a) and *Ecklonia brevipes* J. Agardh. *E. brevipes* was described from northern New Zealand (Lindauer et al. 1961, Adams 1994), but was also tentatively recorded from Hamelin Bay, Western Australia (Huisman 2000), while *E. maxima* forms large kelp forests along the west coast of southern Africa (Stegenga et al. 1997, Guiry and Guiry 2014). The latter has also been reported from several islands in the south Atlantic, Indian and Pacific Oceans: St. Helena, Tristan da Cunha, Falkland Island, St. Paul Island, and Auckland Island (Guiry and Guiry 2014). However, except for St. Paul Island, which has a number of other seaweed species in common with South Africa, Papenfuss (1942) considered these reports doubtful, and they have not subsequently been substantiated.

Ecklonia radiata (Fig. 1b) is the most widely distributed species, occurring in the Southern Hemisphere in South Africa, Australia and New Zealand, but also reported from Madagascar, and in the Northern Hemisphere from Oman and the central-eastern Atlantic Ocean (Mauritania, Senegal, the Canary and Cape Verde Islands; Stegenga et al. 1997, John et al. 2004, Wing et al. 2007).

The characters separating species of *Ecklonia* are almost entirely based on external morphology, particularly stipe and holdfast characteristics. As Bolton and Anderson (1994) pointed out, the morphological distinction between some species remains unclear, aside from the very different modes of growth of *E. stolonifera* (spreading stolon-like holdfast) and *E. brevipes* (forming new holdfasts from the tips of blades), and the very long, hollow stipe of *E. maxima*. *Ecklonia radiata* is particularly polymorphic (Fig. 1, j–m; Wernberg et al. 2003). In Australia, Wernberg and Vanderklift (2010) described variations in rugosity, spinosity, stipe length, frond thickness, and frond densities which they linked to wave exposure. In New Zealand, Wing et al. (2007) also measured morphological variations of *E. radiata* (frond length, width, thickness and number, as well as stipe length and diameter) and found some of these to depend on light levels related to wave exposure. Morphological variations in South African populations of *E. radiata* have also been observed (Fig. 1, b–e), including a range of frond morphologies (spiny to smooth), frond colors (striped to plain), and marginal serration (Fig. 1, f–g). Differences such as these have contributed to taxonomic confusion in the past, and the description of a number of different species and subspecies, now considered synonymous (see Bolton and Anderson 1994).

Bolton and Anderson (1994) noted that *E. radiata*, *E. cava*, *E. kurome*, and *E. muratii* are difficult to tell apart based on morphological descriptions. They also noted that *E. muratii*, as described by Feldmann (1973), was similar to plants from Oman under the name *E. radiata* and reassigned it to an "*E. radiata* complex" including the four species mentioned above. Moreover, the main morphological character used to separate *E. maxima* from *E. radiata* is the morphology of the stipe, which in the former is long (sometimes up to 10 m) and hollow, and in the latter is shorter and solid. However, because intermediate morphologies have been observed, the distinction between these species also is not clear in some populations (M. Rothman, personal observation).

Consequently, the taxonomic confusion surrounding the genus *Ecklonia* has been evident for a long time (Bolton and Anderson 1994). While a few sequences have been published, no study has yet assessed *Ecklonia* species molecularly or examined their phylogenetic relationships. Using small subunit rDNA sequences, Boo et al. (1999) confirmed the placement of *E. cava* in the order Laminariales. Subsequently, Boo and Yoon (2000), using internal transcribed spacer (ITS) and *rbcL* sequence data sets, constructed a scheme of the Laminariales which grouped *Ecklonia*, *Eckloniopsis* (Kjellman) Okamura, and *Eisenia* Areschoug in a clade that was later upheld by Lane et al. (2006). Based on phylogenetic analyses of the large subunit rDNA, ITS, the ribulose-1,5-bisphosphate carboxylase/oxygenase (RuBisCO) operon, and the NADH dehydrogenase subunit 6 (nad6) regions, Lane et al. (2006) further proposed the transfer of *Ecklonia*, *Eckloniopsis*, and *Eisenia* from the family Alariaceae to the Lessoniaceae. The authors stressed the need to include a sequence of *E. maxima*, the type species of the genus *Ecklonia*, to elucidate the status of *Eckloniopsis* and

FIG. 1. (a) A mature *Ecklonia maxima* sporophyte; (b–e) different morphologies of *Ecklonia radiata* at De Hoop Nature Reserve, South Africa; (f–h) indicate different frond morphologies of *E. radiata* at De Hoop, South Africa, (i) an *E. radiata* with haptera-like protrutions from Hamelin Bay, Australia, (j–m) different morphologies of *E. radiata* at different sites in Australia; (j) Ballina, New South Wales, Australia, (k) Abrolhos Island, Western Australia, (l) Albany, Western Australia, (m) Jervis Bay, New South Wales, Australia.

Eisenia. Finally, using ITS, Wing et al. (2007), confirmed that polymorphic populations of *E. radiata,* from 14 different fjords in New Zealand, formed a single species.

The aim of the present study was to produce the first phylogeny of *Ecklonia*, to answer three questions: (i) are *E. radiata* and *E. maxima* two distinct species? (ii) is *E. radiata* from South Africa and Australia/New Zealand a single species?; and (iii) what are the relationships between Northern and Southern Hemisphere species of *Ecklonia* and other genera of the Lessoniaceae?

MATERIALS AND METHODS

Sample collection and identification. Ecklonia maxima and E. radiata samples were collected from southern Africa and Australia along their distribution ranges from a total of 13 and 6 localities, respectively; *E. cava*, *E. kurome*, and *E. stolonifera* were collected from six localities around Japan. For a detailed sampling list see Table 1. Voucher specimens are lodged at the Seaweed Unit, Department of Agriculture Forestry and Fisheries, South Africa. Samples from mature sporophytes were collected to represent observed morphological variability within populations at the various sites either using SCUBA, snorkeling, or walking at low tide. Whenever possible, photographs of the specimens were taken. Tissue samples (3–5 cm^2) of each specimen were collected from the youngest part of the frond of the first secondary blade and stored in silica gel for later DNA extraction (Chase and Hills 1991). Specimens were identified based on characters as described in Okamura (1927), Lindauer et al. (1961), Womersley (1987), Adams (1994), Stegenga et al. (1997), and Yoshida (1998).

DNA extraction, amplification, and sequencing. DNA extraction and PCR amplification were done in the Systematics laboratory of the Department of Biological Sciences of the University of Cape Town (South Africa). Silica gel-dried mate-

TABLE 1. Collection details or references, sample and GenBank accessions for species used in this study.

Species	Location, Collector	Sample no.	*rbcL*	ITS	atp8	trnWI
Ecklonia cava	Goza-Shirahama, JP, K. Kogame	D1744			KM575758	KM575814
	Goza-Shirahama, JP, K. Kogame	D1744			KM575758	KM575814
Ecklonia cava	Zushi, JP, S. Uwai	D1751			KM575765	KM575820
Ecklonia cava	Zushi, JP, S. Uwai	D1752			KM575751	KM575809
Ecklonia cava	Martin 2011, JP	NA	GU593873	GU593773	GU593723	GU593923
Ecklonia cava	Yoon et al. 2001, KR	NA	AF318967	AF319009		
Ecklonia kurome	Hatozu, Saeki, JP, S. Uwai	D1745			KM575746	KM575806
Ecklonia kurome	Hatozu, Saeki, JP, S. Uwai	D1746			KM575762	KM575812
Ecklonia kurome	Ohmi, Itoi-gawa, JP, M. Okaji	D1747			KM575763	KM575824
Ecklonia kurome	Ohmi, Itoi-gawa, JP, M. Okaji	D1748			KM575749	KM575808
Ecklonia kurome	Hatozu, Saeki, JP, S. Uwai	D1774			KM575760	KM575823
Ecklonia kurome	Feng et al., unpublished data, CN	NA	EF407572	EF407574		
Ecklonia maxima	Doring Bay, ZA, M. Rothman	D1728			KM575766	KM575798
Ecklonia maxima	Doring Bay, ZA, M. Rothman	D1729	KM575789	KM575775	KM575756	KM575800
Ecklonia maxima	De Hoop, ZA, R. Anderson	D1730	KM575782	KM575769	KM575757	KM575829
Ecklonia maxima	De Hoop, ZA, R. Anderson	D1731			KM575729	KM575825
Ecklonia maxima	De Hoop, ZA, R. Anderson	D1732	KM575792	KM575778	KM575733	KM575826
Ecklonia maxima	De Hoop, ZA, R. Anderson	D1733	KM575783	KM575770	KM575734	KM575827
Ecklonia maxima	De Hoop, ZA, R. Anderson	D1734	KM575793	KM575779	KM575735	KM575828
Ecklonia maxima	Lüderitz, NM, A. Plos	D1753			KM575748	KM575807
Ecklonia maxima	Lüderitz, NM, A. Plos	D1754			KM575754	KM575799
Ecklonia maxima	Jacobs Bay, ZA, M. Rothman	D1757			KM575745	–
Ecklonia maxima	Port Nolloth, ZA, M. Rothman	D1764	KM575788	KM575774		
Ecklonia maxima	Port Nolloth, ZA, M. Rothman	D1765	KM575786	KM575772		
Ecklonia maxima	Port Nolloth, ZA, M. Rothman	D1766			KM575752	KM575797
Ecklonia maxima	Stanford's Cove, ZA, M. Rothman	D1767			KM575761	KM575822
Ecklonia maxima	Oudekraal, ZA, M. Rothman	D1772			KM575755	KM575795
Ecklonia maxima	Oudekraal, ZA, M. Rothman	D1773			KM575767	KM575810
Ecklonia maxima	Feng et al., unpublished data, ZA	NA	EF407573	EF407575		
Ecklonia radiata	Hamlin Bay, AU, M. Mohring	D1723			KM575740	KM575796
Ecklonia radiata	Abrolhos, AU, M. Mohring	D1724	KM575784	KM575781	KM575741	KM575821
Ecklonia radiata	Abrolhos, AU, M. Mohring	D1725			KM575743	KM575817
Ecklonia cf. *radiata/maxima*	Buffels Bay, ZA, D. Kemp	D1726			KM575736	KM575830
Ecklonia cf. *radiata/maxima*	Buffels Bay, ZA, R. Anderson	D1727	KM575785	KM575771	KM575753	KM575805
Ecklonia radiata	De Hoop, ZA, C. Boothroyd	D1736	KM575790	KM575776		
Ecklonia radiata	De Hoop, ZA, C. Boothroyd	D1737			KM575738	KM575801
Ecklonia radiata	Hamlin Bay, AU, M. Mohring	D1739			KM575730	–
Ecklonia radiata	Hamlin Bay, AU, M. Mohring	D1740			KM575737	KM575815
Ecklonia radiata	Hamlin Bay, AU, M. Mohring	D1741[a]			KM575739	KM575802
Ecklonia radiata	Hamlin Bay, AU, M. Mohring	D1742			KM575731	–
Ecklonia radiata	Jurien Bay, AU, M. Mohring	D1743	KM575787	KM575773	KM575747	KM575803
Ecklonia radiata	Kei Mouth, ZA, D. Kemp	D1759			KM575732	KM575831
Ecklonia radiata	Kei Mouth, ZA, D. Kemp	D1760	KM575791	KM575777		
Ecklonia radiata	Kei Mouth, ZA, D. Kemp	D1762			KM575768	KM575804
Ecklonia radiata	Hluleka, ZA, C. Boothroyd	D1769			KM575742	KM575813
Ecklonia radiata	Hluleka, ZA, C. Boothroyd	D1770			KM575744	KM575818
Ecklonia radiata	Hluleka, ZA, C. Boothroyd	D1771			KM575759	KM575816
Ecklonia radiata	Lane et al. (2006), AU	NA	AY851552	AY857898		
Ecklonia radiata	Martin (2011), NZ	NA	GU593874	GU593774	GU593724	GU593924
Ecklonia stolonifera	Oma, JP, S. Uwai	D1749			KM575764	KM575819
Ecklonia stolonifera	Oma, JP, S. Uwai	D1750			KM575750	KM575811
Eckloniopsis radicosa	Yoon et al. (2001), JP	NA	AF318969	AF319011		
Eisenia arborea	Lane et al. (2006), CA	NA	AY851550	AY857899		
Eisenia bicyclis	Yoon et al. (2001), KR	NA	AF318963	AF319012		
Laminaria digitata	Lane et al. (2006), CA	NA	AY857886	AY851559		
Laminaria digitata	Yoon et al. (2001), UK	NA	AF318971	AF319014		
Laminaria hyperborea	Yoon et al. (2001), FR	NA	AF318972	AF319015		
Laminaria pallida	Doring Bay, ZA, M. Rothman	D1768	KM575794	KM575780		
Lessonia adamsiae	Martin (2011), NZ	NA			GU593749	GU593949

TABLE 1. (continued)

Species	Location, Collector	Sample no.	*rbcL*	ITS	atp8	trnWI
Lessonia brevifolia	Martin (2011), NZ	NA			GU593753	GU593953
Lessonia corrugata	Lane et al. (2006), AU	NA	AY851545	AY857902		
Lessonia corrugata	Martin (2011), AU	NA			GU593744	GU593944
Lessonia nigrescens	Lane et al. (2006), CL	NA	AY851544	AY857901		
Lessonia nigrescens	Martin (2011), CL	NA			GU593925	GU593725
Lessonia tholiformis	Martin (2011), NZ	NA			GU593746	GU593946
Lessonia trabeculata	Martin (2011), CL	NA			GU593733	GU593933
Lessonia vadosa	Martin (2011), FK	NA			GU593736	GU593936

AU, Australia; CA, Canada; CL, Chile; CN, China; FK, Falkland Islands; FR, France; JP, Japan; KR, Republic of Korea; NM, Namibia; NZ, New Zealand; UK, United Kingdom; ZA, South Africa.
[a]Specimen originally identified as *Ecklonia brevipes*.

rial (0.2–0.3 g) was ground in a Retsch mixer mill MM 4000 (Retsch GmbH, Haan, Germany). Two extraction protocols were used to extract the DNA. The first method used a Qiagen plant DNA extraction kit following the manufacturer's protocol, but success was limited, probably due to high levels of polysaccharides, tannins, and phenols in the samples. A higher success rate was obtained using a combination of CTAB and SDS method as described by Maeda et al. (2013). Genomic DNA was then purified using the GENECLEAN® III Kit (MP Biomedical, LLC, Illkrirch-Graffenstaden, France) following the manufacturer's protocol.

The purified DNA was used for the PCR amplification in 25 μL volumes in a 2700 GeneAmp PCR System (Applied Biosystems, Foster City, CA, USA). The reaction mix contained 1 μL DNA, 20.25 μL ultra-pure water, 2.5 μL KAPA *Taq* buffer (Kapa Biosystems, Cape Town, South Africa), 0.5 μL KAPA dNTP Mix, 0.25 μL of each primer, and 0.25 μL KAPA *Taq* DNA Polymerase Ready Mix or SuperTherm*Taq* DNA Polymerase (Roche, Mannheim, Germany).

Four genetic markers were amplified: (i) two mitochondrial intergenic spacer regions: spacer between the t-RNAs for tryptophan and isoleucine genes (trnWI) and between the adenosine tri-phosphate dehydrogenase subunit 8 (atp8) and t-RNA serine genes (hereafter mentioned as atp8) using primers from Voisin et al. (2005); (ii) one nuclear marker covering the ITS1, 5.8S gene, was amplified with the primer pair LB1 and LB2 from Yoon et al. (2001); and (iii) one chloroplastic marker: the large subunit of the RuBisCO (*rbcL*) using primers KL2 and KL8 from Lane et al. (2006).

The thermal profile for PCR amplification of *rbcL* was as follows: an initial denaturation cycle of 95°C for 1 min, followed by 35 cycles of 95°C for 30 s, 45°C for 30 s, and 72°C for 1 min. A final annealing step at 72°C was extended for 10 min. The thermal profile for PCR amplification for ITS was as follows: an initial denaturation cycle of 94°C for 5 min, followed by 35 cycles of 94°C for 1 min, 53°C for 1 min, and 72°C for 1 min. A final annealing step at 72°C was extended for 4 min. For both trnWI and atp8-trnS, the thermal profile for PCR amplification was as follows: an initial denaturation cycle of 95°C for 2 min, followed by 32 cycles of 95°C for 30 s, 50°C for 30 s, and 72°C for 30 s. A final annealing step at 72°C was extended for 5 min. All PCR products were purified using the NucleoFast membrane (Machery-Nagel, Düren, Germany) on a Freedom Evo robot (Tecan Group Ltd., Männedorf, Switzerland) and sequenced with PCR primers using the BigDye Terminator v3.1 sequencing kit (Life Technologies, Johannesburg, South Africa). Purifying and sequencing was done by the Sequencing Unit at the University of Stellenbosch (South Africa).

Sequences were edited, manually aligned, and concatenated in Bioedit (Hall 1999). Sequences representing as many species of *Ecklonia*, *Eisenia*, and *Eckloniopsis* as possible from as many localities as possible were downloaded from Genbank and added to the alignments. Although many sequences were available for all four markers for *Ecklonia*, no atp8 and trnWI sequences were available for *Eisenia* and *Eckloniopsis*. As a consequence, two different data sets were analyzed: a concatenated atp8/trnWI data set for studying the relationships of species within the genus *Ecklonia*, and a concatenated *rbcL*/ITS data set for studying the relationships of taxa within the family Lessoniaceae.

The best-fit models were estimated for each individual alignment with FindModel (available at: http://hiv.lanl.gov) and were all found to be general-time-reversible (GTR) plus gamma. A Bayesian inference was applied to a concatenated data sets consisting of atp8 + trnWI and *rbcL* + ITS using BEAST v.1.7.5 (Drummond et al. 2012) through the online CIPRES Science Gateway Platform (Miller et al. 2010). The analysis used estimated base frequencies, 4 T categories to model among-site rate heterogeneity, a relaxed log-normal molecular clock, a coalescent tree prior with a randomly generated starting tree, and run for 50 million generations. A tenth of the tree was discarded as burn-in. Convergence of the Markov chains was checked using Tracer v.1.4 (Rambaut and Drummond 2007). The maximum likelihood analysis was performed using RAxML-HPC2 on XSEDE using a GTR-MIX evolutionary model (Stamatakis 2006). A multiparametric bootstrap resampling with 1,000 iterations provided bootstrap supports. The concatenated atp8/trnWI tree was rooted with sequences of *Lessonia* Bory de Saint-Vincent species (downloaded from GeneBank), with the aim of looking into relationships between species of *Ecklonia*, because *Lessonia* is closely related to *Ecklonia* (Lane et al. 2006). The concatenated *rbcL*/ITS tree was rooted with *Laminaria* J.V. Lamouroux species (one sequence from this study and the rest downloaded form GeneBank), a species from the closely related family, Laminariaceae (Lane et al. 2006), with the aim of looking into relationships within the Lessoniaceae family.

RESULTS

A total of 44 samples were successfully sequenced (Table 1), including 24 from South Africa (14 *E. maxima*, 10 *E. radiata*), two from Namibia (both *E. maxima*), eight from Australia (seven *E. radiata* and one *E. brevipes*), and 10 from Japan (three *E. cava*, five *E. kurome* and two *E. stolonifera*). Sequences

previously published and available on GenBank were used for: *E. cava* (Japan), *E. kurome* (Japan), *E. radiata* (New Zealand and Australia), *E. stolonifera* (Japan), *Eckloniopsis radicosa* (Kjellman) Okamura (Japan), *Eisenia arborea* Areschoug (Canada), and *Eisenia bicyclis* (Kjellman) Setchell (Korea). For the outgroup of the concatenated atp8/trnWI tree: *Lessonia adamsiae* C.H. Hay (New Zealand), *L. brevifolia* J. Agardh (Chile), *L. nigrescens* Bory de Saint-Vincent (Chile), *L. tholiformis* C.H. Hay (New Zealand), *L. trabeculata* Villouta & Santelices (Chile), and *L. vadosa* Searles (Falkland Islands) sequences were downloaded from GenBank. For the outgroup of the concatenated *rbcL*/ITS analysis: *Laminaria digitata* (Hudson) J.V. Lamouroux (United Kingdom and Canada), and *L. hyperborea* (Gunnerus) Foslie (France) sequences were downloaded from GenBank, while one sequence was obtained during the present study for *Laminaria pallida* Greville (South Africa). Final alignments included 28 sequences for the *rbcL*/ITS and 48 for atp8/trnWI and were 1,367 and 538 base pairs long including gaps, respectively.

The concatenated atp8/trnWI analysis (Fig. 2) recovered two clades: a Southern Hemisphere clade (posterior probabilities [PP] = 0.99; bootstrap [B] <60%) and an Asiatic one (PP = 1; B = 81%). The Southern Hemisphere clade was further subdivided into two subclades representing *E. radiata* (PP = 1, B = 78%, sequence divergence <1%) and *E. maxima* (PP = 1; B = <65%, sequence divergence <1%). The *E. radiata* subclade included sequences from South Africa, Australia, and New Zealand, while the *E. maxima* subclade was represented by South African and Namibian sequences only (with an interspecific sequence divergence of 1.2%–2.7%). One specimen, from Hamelin Bay, Western Australia, which had been tentatively identified as *E. brevipes* (specimen D1741) was recovered in the "*E. radiata* clade." The Northern Hemisphere clade was further subdivided in two subclades, one containing the available sequences for *E. cava* (with an intraspecific sequence divergence <1%), and the rest of the Asiatic species (with a sequence divergence of 0.2%–1.4%). Only the second clade was significantly supported (PP = 1, B = 65%).

The *rbcL*/ITS analysis (Fig. 3) produced two fully supported clades, one representing the genus *Lessonia* (PP = 1; B = 99%–100%) and the other one representing mainly the genus *Ecklonia* but also containing the available sequences for *Eckloniopsis* and *Eisenia* (PP = 1; B = 99%). The *Ecklonia* clade was further subdivided into two subclades, one representing the Southern Hemisphere species of *Ecklonia* as well as a sequence for *E. arborea* (PP = 0.82; B = 62%), and one containing the Asiatic species of *Ecklonia* as well as sequences for *E. radicosa* and *E. bicyclis* (PP = 0.93; B = 99%). The Southern Hemisphere clade was subdivided into two groups representing *E. maxima* (PP = 0.99, B = 70%, intraspecific sequence divergence <1%) and *E. radiata* (PP = 0.94; B < 65%, intraspecific sequence divergence <1%), with the sequence for *E. arborea* standing alone. Interspecific divergence between *E. radiata* and *E. maxima* ranged from 3% to 7%. The *E. radiata* group included sequences from South Africa, Australia, and New Zealand, while the *E. maxima* group contained only sequences from southern Africa (South Africa and Namibia). The Northern Hemisphere clade did not produce any well-supported subclades and sequence divergence ranged from 0% to 1.4%. Interestingly, one *Ecklonia* specimen (D1727) from Bordjiesrif, (near Cape Point, South Africa) produced atp8 and trnWI sequences matching with the *E. radiata* clade (Fig. 2), while *rbcL* and ITS sequences matched the *E. maxima* clade (Fig. 3). The sample was re-extracted and sequenced again, but results remained the same.

DISCUSSION

Our phylogeny of *Ecklonia* included sequences for most of the distribution range of the three current Southern Hemisphere species (*E. radiata*, *E. maxima*, and a specimen tentatively identified as *E. brevipes*), sequences for East Asiatic species (*E. cava*, *E. kurome*, and *E. stolonifera*) as well as closely related genera *Eckloniopsis* and *Eisenia*. Our results confirmed *E. radiata* and *E. maxima* as two distinct species in South Africa, *E. radiata* as a single species throughout the Southern Hemisphere (in South Africa, Australia, and New Zealand) and East Asiatic species as a lineage distinct to the Southern Hemisphere clade. We further showed the close sister relationship between *E. radicosa*, two *Eisenia* species (including the type species *E. arborea*), and the genus *Ecklonia,* suggesting the two former genera are superfluous and should be subsumed in *Ecklonia*.

Diversity and distribution of Ecklonia *in the Southern Hemisphere*. Both the concatenated *rbcL*/ITS and the concatenated atp8/trnWI analyses recovered *E. maxima* and *E. radiata* as two distinct species with comparable intraspecific divergence (<1%).

Sporophytes of *E. maxima* can grow a stipe of up to 10 m long and usually form extensive kelp forests along the South African west coast. *E. maxima* is distributed from Koppie Alleen in De Hoop Nature Reserve, South Africa (34°28′ 42.55″ S 20°30′ 37.23″ E; Bolton et al. 2012) westwards to north of Lüderitz, Namibia (26°37′ 52.56″ S 15°09′ 06.31″ E; Stegenga et al. 1997). In the south, it dominates shallower inshore waters, forming near homogeneous stands of floating kelp forests, from the subtidal fringe down to 5–10 m deep which are gradually replaced northward by another kelp, *L. pallida*.

Ecklonia radiata sporophytes are, in South Africa, generally less than 1 m long (Stegenga et al. 1997), but in some sites in Australasia they can reach 2 m long (see Fig. 1j and Wernberg et al. 2003), consist-

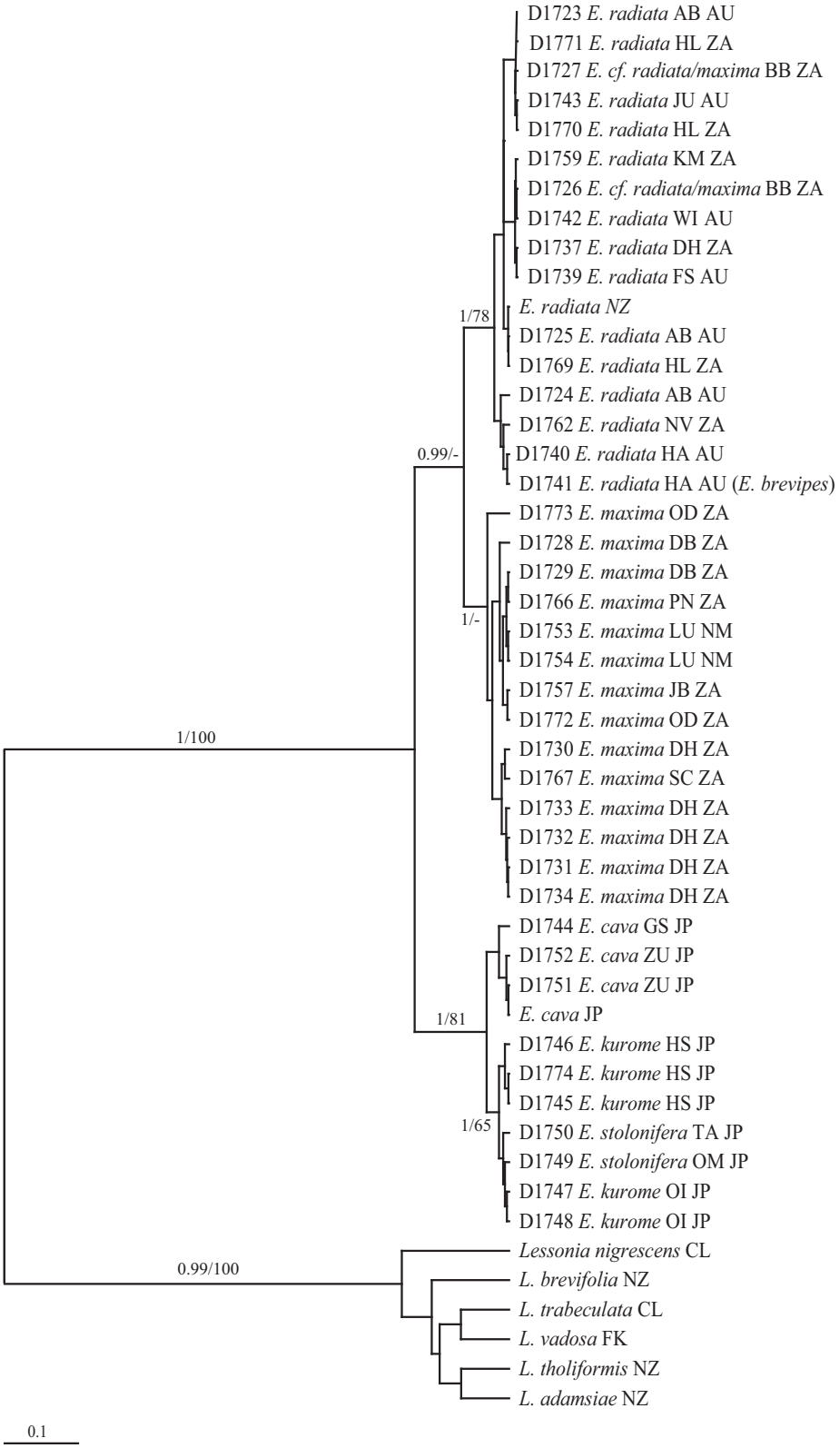

FIG. 2. Bayesian phylogeny based on the concatenated atp8/trnWI alignment. Posterior probabilities below 0.8 and bootstrap less than 60% represented by "–". Branch numbers indicate Baysian analysis; Maximum likelihood.

ing of a primary blade with secondary lateral fronds, a stipe, and holdfast. Unlike *E. maxima*, *E. radiata* sporophytes have solid stipes, fronds that are sometimes spiny, rugose or smooth, or varying combinations on a single sporophyte (Wernberg et al. 2003). In South Africa, *E. radiata* occurs inshore on the south and east coasts of South Africa, from Koppie Alleen in De Hoop Nature Reserve eastwards to

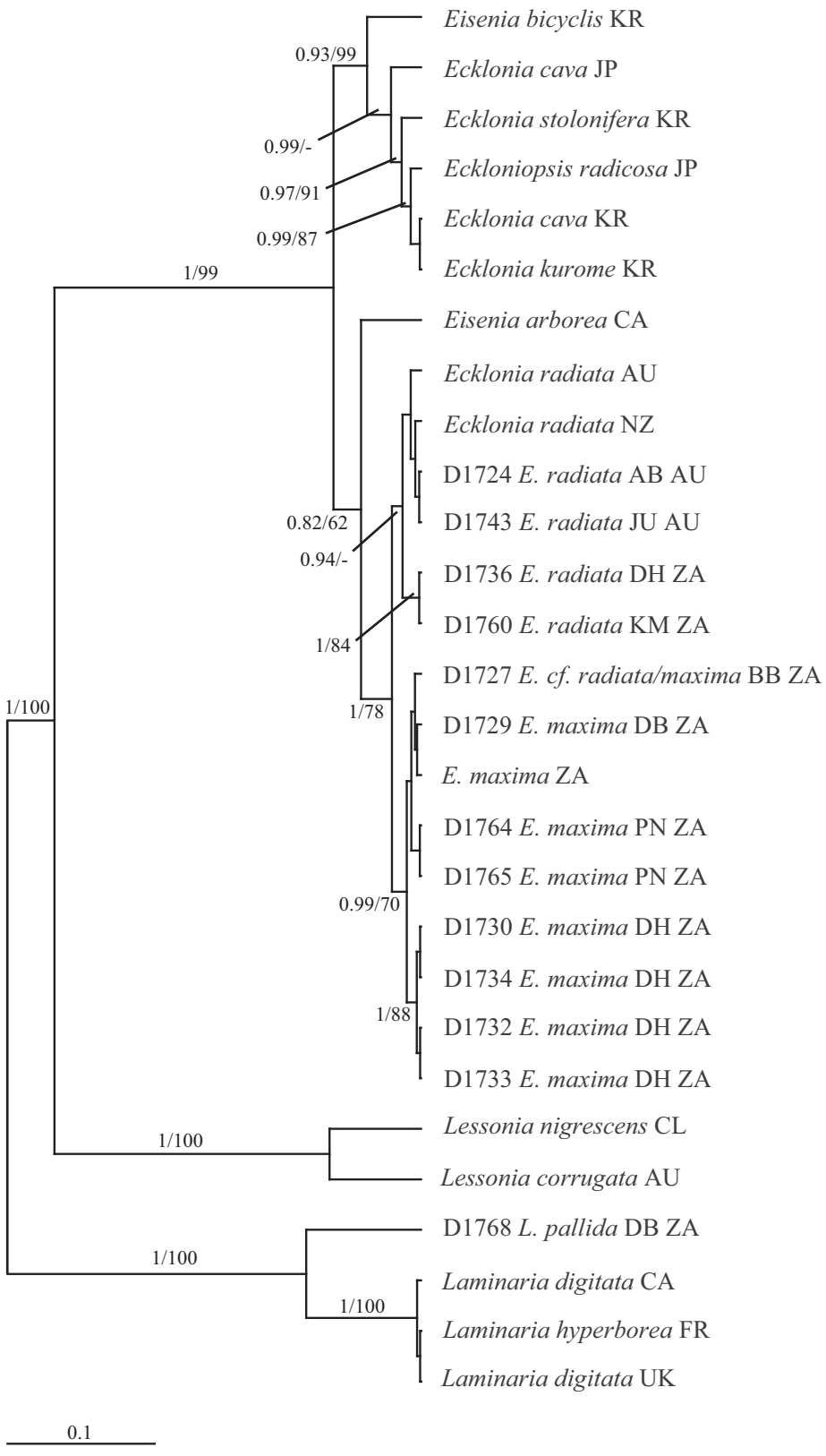

FIG. 3. Bayesian phylogeny based on the concatenated *rbcL*/ITS alignment. Posterior probabilities below 0.8 and bootstrap less than 60% represented by "–". Branch numbers indicate Bayesian analysis; maximum likelihood.

Port Edward (31°3′ 24″ S 30°13′ 24″ S). It is far less abundant than *E. maxima* and mostly occurs in the sublittoral fringe or very shallow subtidal. However, there are also records from further east than Port Edward, but all from deeper subtidal habitats, to at least 40 m deep (De Clerck et al. 2005). The

recording of subtidal *E. radiata* at Bordjiesrif (South Africa) by Stegenga et al. (1997) extends the species distribution ~200 km further west than its generally accepted distribution limit at Koppie Alleen. However, no sporophytes have been found in the region between Koppie Alleen and Bordjiesrif, and results of our molecular analyses could not confirm the presence of this species at Bordjiesrif. Specimens collected from Bordjiesrif had a morphology intermediate between *E. radiata* and *E. maxima* (general *E. maxima*-like appearance with a solid stipe and broad, rugose fronds with serrated edges), while molecular analyses also provided mixed results. Hence, the specimens sequenced clustered within the *E. maxima* clade in the *rbcL*/ITS analysis (D1727) and within the *E. radiata* clade in the atp8/trnWI analysis (D1726 & D1727; Figs. 2 and 3). The presence of hybrids between the two species at Bordjiesrif requires further and more detailed studies.

In Australia, *E. radiata* is distributed around the western and southern coasts from Kalbarri and the Abrolhos Island on the west coast around southern Australia and Tasmania to Caloundra in Queensland (Womersley 1987, Huisman 2000, Wernberg et al. 2003). Upon studying the description of *E. radiata* (Lindauer et al. 1961, Hommersand 1986, Adams 1994), it is clear that aside from the Australasian *E. radiata* sometimes having a slightly longer stipe, *E. radiata* in the Southern Hemisphere has many overlapping morphological characters (Wernberg et al. 2003). *Ecklonia radiata* is further distributed throughout New Zealand and on many New Zealand Islands: Three Kings Island/Manawatawhi, Stewart Island/Rakiura, Snares Island/Tini Heke (Guiry and Guiry 2014).

The third Southern Hemisphere *Ecklonia* species, *E. brevipes*, was described by J. Agardh (1877) from the Bay of Islands in New Zealand. Since then it has been recorded from this locality, Stewart Island (Lindauer et al. 1961, Adams 1994), Bay of Islands (North Island), Fiordland (South Island) (Adams 1994), and "tentatively" from Hamelin Bay in Western Australia (Huisman 2000). *E. brevipes* was described as having blades that are flabellate or erratically arranged lobes angled in any direction without order, and with marginal teeth that can develop into adventitious attachment organs. Its habitat is sublittoral, in shallowish water, on somewhat loose substratum of sand and corallines (Agardh 1877, Lindauer et al. 1961). Adams (1994) also describe its habitat as subtidal on a muddy substratum in sheltered, turbid areas and mentioned that colonies of *E. brevipes* can develop from detached, sunken plants and that they can increase by fragmentation. During the present study, one specimen collected from Hamelin Bay was tentatively identified as *E. brevipes* (Fig. 1i) following the morphological description and illustration provided by Huisman (2000). It was similar to *E. radiata* except for the presence of haptera-like protrusions at the frond tips that appeared to be reattachment organs, but was different from the descriptions of specimens of *E. brevipes* from the type locality (Agardh 1877, Lindauer et al. 1961, Adams 1994). Results of our molecular analysis further indicated that this specimen was molecularly similar to *E. radiata* (Fig. 3), thus not supporting the presence of *E. brevipes* as a distinct species in Australia. Similar reattachment organs to those observed on specimens from Hamelin Bay, although not as pronounced, have been observed from the margins of the secondary fronds in *E. maxima* sporophytes from southern Africa (Bolton and Anderson 1994). We believe that *E. brevipes* does not occur in Australia, and material from the type locality needs to be assessed molecularly to establish whether this species is indeed separate from *E. radiata*. The specimen from Hamelin Bay might be an *E. radiata* exhibiting an ecotype or abnormality that is amplified under specific environmental conditions conducive to vegetative propagation.

The East Asiatic clade. Phylogenies produced during the present study supported the East Asiatic sequences as a lineage independent from the Southern Hemisphere clade. In the concatenated atp8/trnWI analysis, *E. cava* formed a clade (which was however not supported) separate from *E. kurome* and *E. stolonifera*. Okamura (1927) pointed out the difficulty in distinguishing *E. cava* from *E. kurome*, but stated that the central rachis of *E. kurome* varies while that of *E. cava* does not, and that the former is palatable while the latter is not. Results of the atp8/trnWI analysis indicate that these species could be distinct, but the corresponding clade was not significantly supported nor did it appear in the *rbcL*/ITS analyses indicating that more sequences from these species are required to fully resolve their statuses. Similarly, previous studies by Yoon et al. (2001) and Lane et al. (2006) could not differentiate between *Eckloniopsis cava* and *E. stolonifera*, raising questions about whether they are distinct taxa.

Our results further showed that the available sequences for *Eckloniopsis radicosa* and *E. bicyclis* are part of the East Asiatic *Ecklonia* clade. *E. radicosa* was first described by Kjellman et al. (1885) as *Laminaria radicosa* Kjellman, but was later transferred to *Ecklonia* as *Ecklonia radicosa (*Kjellman) Okamura (Okamura 1892). Okamura (1927) re-examined the species and decided it was sufficiently different from *Ecklonia* to belong to a distinct and new monospecific genus which he named *Eckloniopsis*. The two major differences (as compared to *Ecklonia*), on which Okamura based the description of his new genus, were its frond morphology and the absence of secondary blades arising from the meristem. There are however examples of *Ecklonia* species showing similar characters, for example *E. stolonifera* lacks secondary fronds and substantial ecotypic frond plasticity has been observed in *E. radiata* (Wernberg and Thomsen 2005, Fowler-Walker et al.

2006, Wernberg and Vanderklift 2010). The results of the present study resolved the available sequences for *E. radicosa* within the *Ecklonia* clade and highlighted its sister relationship with Japanese species (Fig. 3). On the basis of this result, we consider *Eckloniopsis* as superfluous and propose to transfer the species "*radicosa*" back to the genus *Ecklonia* and therefore consider *E. radicosa* (Kjellman) Okamura (Okamura 1927) as an homotypic synonym of *Ecklonia radicosa* (Kjellman) Okamura (Okamura 1892). More sequences are needed to clarify its relationship with the other Asiatic species of *Ecklonia*. The situation of *E. bicyclis* is discussed below.

Ecklonia *and* Eisenia. The genus *Eisenia* was described based on *E. arborea* (type from San Francisco, USA) by Areschoug (1876). *E. bicyclis* (Kjellman) Setchell was first described as *Ecklonia bicyclis* by Kjellman et al. (1885), but was transferred to *Eisenia* by Setchell (1905), because of the splitting of the meristem at the top of the stipe which is characteristic of the genus. Yendo (1911) later proposed *E. bicyclis* as a form of *E. arborea*, but the name is not currently accepted (Guiry and Guiry 2014). Aside from the split in the meristem at the top of the stipe, there is little morphological difference between *Ecklonia* and *Eisenia*.

Based on *rbcL* and ITS, Boo and Yoon (2000) produced a systematic scheme of the Laminariales, placing *Ecklonia*, *Eckloniopsis*, and *Eisenia* in an *Ecklonia* group. This group was later confirmed by Yoon et al. (2001) who placed *Ecklonia*, *Eckloniopsis*, and *Eisenia* in a robust group with a 100% bootstrap. Furthermore, similar results were obtained by Lane et al. (2006) who also doubted that *Eisenia*, based on their molecular analysis, is a distinct genus. However, because their analysis did not include the type of *Ecklonia* (*E. maxima*), they were reluctant to make this modification.

Results of the concatenated *rbcL*/ITS phylogeny presented in our study, including sequences for *E. maxima*, did not support *Eckloniopsis* (discussed above) nor *Eisenia* as distinct from *Ecklonia*. The sequences for *E. bicyclis* from Korea grouped in a well-supported clade with Asiatic *Ecklonia* species, while the sequence for the type species of *Eisenia* (*E. arborea*) collected from Canada clustered with the Southern Hemisphere *Ecklonia* species (Fig. 3). On the basis of our results and the discussions of previous authors, we believe that *Eisenia* is superfluous and propose to transfer the species *E. arborea* and *E. bicyclis* to *Ecklonia*. We therefore consider *E. arborea* Areschoug and *E. bicyclis* (Kjellman) Setchell to be homotypic synonyms of *Ecklonia arborea* (Areschoug) Rothman, Mattio & Bolton *comb. nov.* (see below), and *E. bicyclis* Kjellman, respectively. Okamura (1927) believed that the other *Eisenia* species might one day be proved to be one and same species, or at least varieties of a single species. We find it likely that remaining species of *Eisenia* also belong to *Ecklonia*, but the status of these species needs to be assessed using molecular data.

The present study shows that *Ecklonia* is divided into two independently evolving lineages, both including species with split (formerly *Eisenia*) and nonsplit meristems. One of the lineages appeared centered in the East Asiatic region (Japan, Korea and China), whereas the other lineage appeared more widespread with species occurring in the Pacific, Atlantic, and Indian Oceans, but mostly in southern Africa, Australia, and New Zealand and along the west coast of the Americas. Where and when the genus originated is difficult to determine, but our results suggest that split or nonsplit meristems have evolved twice independently. We believe that the analysis of sequences for other species of *Eisenia* and a calibrated phylogeny are necessary to further discuss this hypothesis.

Taxonomic conclusions. From the data presented here, we conclude that two distinct *Ecklonia* species occur in southern Africa (*E. maxima* and *E. radiata*), and confirm *E. radiata* as a single species throughout the Southern Hemisphere in South Africa and Australasia. On the basis of our chloroplastic and nuclear analysis, we resurrect *E. radicosa* (Kjellman) Okamura and *E. bicyclis* Kjellman, and consider *E. arborea* synonymous with the new combination *E. arborea* (Areschoug) Rothman, Mattio & Bolton *comb. nov.*

Ecklonia arborea (Areschoug) Rothman, Mattio & Bolton **comb. nov.** Basionym: *Eisenia arborea* Areschoug *In* De tribus Laminarieis (*Egregia* Areschoug, *Eisenia* Areschoug, *Nereocystis*) et de *Stephanocystide osmundacea* (Turner). Trevisan Observationes praecursorias offert. *Botaniska Notiser* 1876:69.

This study was supported by the Department of Agriculture, Forestry and Fisheries, South Africa, the University of Cape Town, the National Research Foundation (NRF), and the South African National Biodiversity Institute (SeaKeys). The Japanese link was part of the NRF/JST international collaboration. T. Wernberg was supported by the Australian Research Council and a UWA Research Collaboration Award. Our sincere thanks to all who contributed taxa for analysis: K. Kogame, M. Okaji and T. de Bettignies. The authors thank T. Horiguchi and K. Kogame for translating the original Japanese descriptions. Thank you to T. Hedderson and M. Muasya for their assistance during this project. We are grateful to T. Silberfield and C. Lane for providing their published sequence alignments. Lastly thank you to C. Boothroyd, D. Kemp, M. Noffke, A. Plos, and S. Conrad for assisting with field work and sample collections.

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