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Article

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The phylogenetic position of *Polysiphonia scopulorum* (Rhodomelaceae, Rhodophyta) based on molecular analyses and morphological observations of specimens from the type locality in Western Australia

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Abstract

Considerable uncertainty surrounds the phylogenetic position of *Polysiphonia scopulorum*, a species with an apparently cosmopolitan distribution. Here we report, for the first time, molecular phylogenetic analyses using plastid *rbc*L gene sequences and morphological observations of *P. scopulorum* collected from the type locality, Rottnest Island in Western Australia. Morphological characteristics of the Rottnest Island specimens allowed unequivocal identification, however, the sequence analyses uncovered discrepancies in previous molecular studies that included specimens identified as *P. scopulorum* from other locations. The phylogenetic evidence clearly revealed that *P. scopulorum* from Rottnest Island formed a sister clade with *P. caespitosa* from Spain (JX828149 as *P. scopulorum*) with moderate support, but that it differed from specimens identified as *P. scopulorum* from the U.S.A. (AY396039, EU492915). In light of this, we suggest that *P. scopulorum* be considered an endemic species with a distribution restricted to Australia. Our results showed the existence of several distinct clades among the species of *Polysiphonia sensu lato*, including the clade containing *P. scopulorum* which did not join with the generitype *Polysiphonia stricta* (i.e., *Polysiphonia sensu stricto*). This suggests that the *P. scopulorum* clade might represent a separate genus, however, further studies including multi-gene analyses are recommended before recognizing any segregate taxa.

Key words: Phylogeny, *rbc*L, Rhodophyta, *Polysiphonia scopulorum*, taxonomy

Introduction

The red algal genus *Polysiphonia* Greville (Rhodomelaceae) has a cosmopolitan distribution and encompasses a remarkable diversity, with about 206 currently accepted species (Guiry & Guiry 2017). Recent studies based on morphological and/or molecular analyses have proposed the recognition of several segregate genera (e.g., Kim & Lee 1999; Choi *et al*. 2001; Díaz-Tapia *et al.* 2017), but the taxonomy of *Polysiphonia sensu lato* is seemingly far from settled and further work is needed to clarify the relationships of the included species. Studies are often hampered by uncertainty regarding species discrimination based on morphological analysis alone, which can be a difficult task due to the phenotypic plasticity of specimens (Kim *et al*. 2000). Nevertheless, several studies using morphological features have contributed to our understanding of species diversity around the Pacific Coast (Hollenberg 1968a; Kudo & Masuda 1988; Womersley 2003; Stuercke & Freshwater 2010).

As a consequence of the difficulty in delineating species of *Polysiphonia* based on morphology alone, there have been several studies aimed at verifying species-level taxonomy utilizing molecular evidence (Diaz-Tapia *et al*. 2013; Bustamante *et al*. 2014; Kim & Kim 2014). These studies have highlighted the need for the characterization of species based on specimens collected from the type locality, to enable a critical reassessment of species delimitation using molecular-assisted alpha taxonomy (Mamoozadeh & Freshwater 2012). Recent studies using molecular markers and phylogenetic reconstruction with genetic distance for species identification have provided further clarification of relationships within the genus (Geoffroy *et al*. 2016; Kim *et al*. 2016; Díaz-Tapia *et al.* 2017). Rapid molecular methods to delimit species have been used to circumvent the difficulties of morphology-based identification (Kim & Kim 2016).

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Polysiphonia scopulorum was first described by Harvey (1855: 540) based on specimens collected from "littoral rocks" on Rottnest Island, Western Australia. Later, Womersley (1950: 188) transferred this species to the genus *Lophosiphonia* Falkenberg, although he did not elaborate on the reasons for doing so. Hollenberg (1968a), however, returned the species to the genus *Polysiphonia* based on the absence of a dorsiventral or bilateral apex in all branches, and this was followed by Womersley (1979, 2003). Four varieties of *P. scopulorum* have been described: var. *longinodium* Xiang, var. *macrotrichia* Hollenberg, var. *minima* Hollenberg, and var. *villum* (J.Agardh) Hollenberg, based on variations in thallus length, size of trichoblasts, branching intervals, and the length/diameter ratio of segments (Hollenberg 1968a; Xiang 2004).

As with most species of *Polysiphonia*, *P. scopulorum* can be difficult to identify based on only morphological characters, particularly if reproductive structures are absent, and its taxonomic status has not been explored using molecular analyses, in spite of its apparently cosmopolitan distribution (Guiry & Guiry 2017). Recently, molecular tools for species delimitation have been used for the identification of many red algae (Guillemin *et al.* 2016; Jesus *et al*. 2016), including members of *Polysiphonia sensu lato* (Savoie & Saunders 2015). In this study, we carried out careful morphological observations of *Polysiphonia scopulorum* specimens from the type locality, Rottnest Island in Western Australia, along with molecular analyses using the *rbc*L gene to provide a more complete taxonomic treatment. This study aims to gain insight into the species circumscription of *P. scopulorum* within the classical descriptive taxonomic species concept and to expand our knowledge of the phylogenetic relationships among species of the genus *Polysiphonia*.

Materials and Methods

Samples were collected from the intertidal zone of Rottnest Island in Western Australia on 17 November 2010 and 11 November 2015 (Table 1). Fresh specimens were preserved in silica desiccant for DNA analyses and materials for morphological study were preserved in 3–5% formalin in seawater. For microscopic observations, samples were sectioned with a freezing microtome (NK-101-II, Nippon Optical Works Co., Ltd., Tokyo, Japan). The sectioned preparations were stained with 1% aniline blue acidified with 1% HCL and mounted in 30-40% Karo syrup. Photographs were taken using a digital camera (Canon EOS 600D, Japan) mounted to a microscope (Olympus BX43, Japan). Voucher specimens were mounted on herbarium sheets and lodged at the Herbarium of Jeju National University (JNUB), Jeju, Korea.

TABLE 1. List of species used in this study for *rbc*L analysis with collection information and GenBank accession numbers

Taxa	Collection information	Voucher	*rbc*L	References
***Polysiphonia scopulorum* Harvey**	Rottnest Island, Australia; 11 Nov 2015	15Psc101	MF139308	This study
	Rottnest Island, Australia; 11 Nov 2015	15Psc102	MF139309	This study
	Rottnest Island, Australia; 11 Nov 2015	15Psc103	MF139310	This study
Polysiphonia rudis J.D.Hooker & Harvey	Okiwi Bay, Canterbury, New Zealand; 23 Sep 2013	13rud01	-	This study
***Polysiphonia* sp.**	Longreach bay, Rottnest Island, Australia; 16 Nov 2010	WAL37	MF139312	This study
***Polysiphonia* sp.**	Ricey beach, Rottnest Island, Australia; 17 Nov 2010	WAR50	MF139315	This study
***Polysiphonia* sp.**	Ricey beach, Rottnest Island, Australia; 17 Nov 2010	WAR53	MF139313	This study
***Polysiphonia* sp.**	Ricey beach, Rottnest Island, Australia; 17 Nov 2010	WAR62	MF139314	This study
***Polysiphonia* sp.**	Rottnest Island, Australia; 11 Nov 2015	15Psc201	MF139311	This study
Enelittosiphonia stimpsonii (Harvey) Kudo & Masuda	Akkeshi, Hokkaido, Japan; 9 May 1999	Genbank	JX828126	Bárbara *et al.*(2013)
Fernandosiphonia unilateralis Levring	Chile	Genbank	KX499549	Díaz-Tapia *et al.* (2017)
Lampisiphonia iberica Bárbara, Secilla, Díaz & Choi	Ría de A Coruña, A Coruña, Spain; 30 Apr 2007	GenBank	JX828129	Bárbara *et al*. (2013)

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TABLE 1. (Continued)

Taxa	Collection information	Voucher	*rbc*L	References
Lophosiphonia teges (Womersley) Díaz-Tapia & Maggs	Wallaroo, Adelaide, Australia; 26 Mar 2014	GenBank	KT825865	Bustamante *et al*. (2017)
Melanothamnus somalensis Bornet & Falkenberg	Oman	GenBank	KX499555	Díaz-Tapia *et al*. (2017)
Neosiphonia baliana Bustamante, Won & Cho	Blue Lagoon beach, Padang Bai, Bali, Indonesia; 27 Apr 2012	GenBank	KF146879	Bustamante *et al*. (2013)
Neosiphonia ferulacea (Suhr *ex* Agardh) Guimarães & Fujii	West Limón Bay Jetty, Punta Toro, Colón, Panama; 17 May 2009	GenBank	HM573584	Mamoozdeh & Freshwateri (2011)
Neosiphonia collabens (C. Agardh) Díaz-Tapia & Bárbara	Ártabra, A Coruña, Spain; 14 May 2010	GenBank	JX828157	Bárbara *et al*. (2013)
Neosiphonia flavimarina Kim & Lee	Bangpo, Chungnam, South Korea; 16 Jul 2003	GenBank	DQ787481	Bárbara *et al*. (2013)
Neosiphonia harveyi (J. Bailey) Kim, Choi, Guiry & Saunders	Wembury, Devon, England; 28 Jul 2003	GenBank	DQ787488	Kim & Yang (2006)
Neosiphonia japonica (Harvey) Kim & Lee	Sumiyoshi Harbor, Hakodate, Hokkaido, Japan; 8 Apr 2004	GenBank	DQ787494	Kim & Yang (2006)
Neosiphonia teradomariensis (M. Noda) Kim & Lee	Jukbyun, Gyeongbuk, Korea; 18 Dec 2002	GenBank	JX828136	Bárbara *et al*. (2013)
N. yendoi (Segi) Kim & Lee	Muroran, Hokkaido, Japan; 21 Feb 2014	14yen01	KX265508	Kim *et al*. (2016)
Neosiphonia yongpilii Kim & Kim	Jongdal, Jeju, Korea; 4 Jul 2014	14sim05	KT964454	Kim & Kim (2016)
Polysiphonia amplacapilli Kim & Kim	Udo strait, Jeju, Korea; 6 Jun 2012	GenBank	KF479251	Kim & Kim (2014)
Polysiphonia aterrima Hooker & Harvey	Mataikona, Castle Point, North Island, New Zealand; 16 Nov 2004	GenBank	GU385831	Mamoozadeh & Freshwater (2011)
Polysiphonia atlantica Kapraun & Norris	Parte externa de las Islas de San Pedro, A Coruña, Spain; 12 Mar 2005	GenBank	JX828141	Bárbara *et al*. (2013)
	CORMP Site OB-27, Onslow Bay, NC. USA; 30 Aug 2004	GenBank	EU492910	Mamoozadeh & Freshwater (2011)
Polysiphonia brodiaei (Dillwyn) Sprengel	Portaferry, Down, Ireland; 20 Mar 1998	GenBank	AF342916	McIvor *et al*. (2001)
Polysiphonia caespitosa (M.A. Pocock) Hollenberg	San Ciprián, Lugo, Spain: 25 Apr 2005	GenBank	JX828149	Bárbara *et al*. (2013)
Polysiphonia dokdoensis Bustamante, Won & Cho	Sado, Dokdo, Ulleungdo, Korea	GenBank	KJ407268	Bustamante *et al*. (2014)
Polysiphonia donghaeya Kim & Kim	Dongdo, Dokdo, Gyeongbuk, Korea; 22 Apr 2013	13atl05	KM053380	Kim & Kim (2015)
Polysiphonia elongella Harvey	Pwllheli, Cardigan, Wales; 20 Aug1998	Genbank	AF342913	McIvor *et al*. (2001)
Polysiphonia freshwateri Bustamante, Won & Cho	Yeonji, Uljin, Gyeongbuk, Korea; 5 Oct 2013	GenBank	KJ957812	Bustamante *et al*. (2015)
Polysiphonia kapraunii Stuercke & Freshwater	South Masonboro Inlet Jetty, New Hanover Co., NC, USA; 04 May 2005	GenBank	EU492920	Mamoozadeh & Freshwater (2011)
Polysiphonia macrocarpa (C.Harvey) Sprengel	Punta Gorda, Colón, Panama; 20 May 2009	GenBank	HM573545	Mamoozadeh & Freshwater (2011)
Polysiphonia morroides Kim & Kim	Udo strait, Jeju, Korea; 10 Jun 2012	GenBank	KF479254	Kim & Kim (2014)
Polysiphonia morrowii Harvey	Hakodate, Hokkaido, Japan; 23 Feb 2012	12mo04	KF479258	Kim & Kim (2014)
	Curio Bay, South Island, New Zealand; 28 Oct 2004	GenBank	HM573579	Mamoozadeh & Freshwater (2011)
Polysiphonia muelleriana J.Agradh	Fiordland, Thompson Sound, Deas Cove, New Zealand; 3 Oct 2000	GenBank	AY588412	Fujii *et al*. (2006)/ unpublished
Polysiphonia muninsula Kim & Kim	Munseom, Jeju, South Korea; 13 May 2012	GenBank	KM053372	Kim & Kim (2015)

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TABLE 1. (Continued)

Taxa	Collection information	Voucher	*rbc*L	References
Polysiphonia nigra (Hudson) Batters	Finavarra, Co. Clare, Ireland; 26 Feb 1998	GenBank	JX828164	Bárbara *et al*. (2013)
Polysiphonia nuda Mamoozadeh & Freshwater	Parque de Juventud, Calle Primero, Colon, Panama; 20 May 2009	GenBank	HM573571	Mamoozadeh & Freshwater (2012)
Polysiphonia pacifica Hollenberg	Seal Rock, OR, USA	GenBank	AY396036	Kim *et al*. (2004)
	Seal Rock, OR, USA	GenBank	AY958162	Kim & Yang (2005)
Polysiphonia pacifica Hollenberg	West Bay, AK, USA	GenBank	GQ252565	Carlile & Waaland (2009)
Polysiphonia sabulosia Kim & Kim	Jongdal, Jeju, Korea; 4 Feb 2011	GenBank	KF479248	Kim & Kim (2014)
Polysiphonia schneideri Stuercke & Freshwater	Sebastian Inlet, Indian River County, FL, USA; 26 Feb 2005	GenBank	HM573567	Mamoozadeh & Freshwater (2011)
Polysiphonia scopulorum Harvey	Devil's Punchbowl, OR, USA	GenBank	AY396039	Kim *et al*. (2004)
Polysiphonia scopulorum var. *villum* (J. Agardh) Hollenberg	Masonboro Inlet, New Hanover Co., NC, USA	GenBank	EU492915	Stuercke & Freshwater (2008)
Polysiphonia sertularioides (Grateloup) J.Agardh	Veraguas, Isla Afuera, Panama; 16 Jan 2008	GenBank	HM573547	Mamoozadeh & Freshwater (2012)
Polysiphonia stricta (Dillwyn) Greville	Garron, Larne, Northern Ireland	GenBank	AY958167	Kim & Yang (2005)
	Mare of Romdal, Finnaya, Norway	GenBank	AY958166	Kim & Yang (2005)
	Letete Point, New Brunswick, Canada	GenBank	EU492916	Stuercke & Freshwater (2008)
	Playa de Linorsa, Lugo, Spain; 7 Apr. 2005	GenBank	JX828150	Barbara *et al*. (2013)
Polysiphonia strictissima Hooker & Harvey	Shag point, South Island, New Zealand; 26 Oct 2004	GenBank	HM573582	Mamoozadeh & Freshwater (2011)
Polysiphonia subtilissima Montagne	STRI Research Station, Bocas del Toro, Panama; 03 Sep 2009	GenBank	HM573575	Mamoozadeh & Freshwater (2011)
	Florida, USA	GenBank	JX294917	Lam *et al*. (2013)
	Spain	GenBank	JX294918	Lam *et al*. (2013)
Polysiphonia ulleungensis Bustamante, Won & Cho	Sadongri, Ulleungdo, Korea; 21 Apr. 2013	13atl10	KM114571	Kim & Kim (2015)
Pterosiphonia arenosa Kim & Kim	Gimnyeong, Jeju, Korea; 21 Jan 2012	GenBank	JX857414	Kim *et al*. (2012)
Symphyocladia marchantioides (Harvey) Falkenberg	Black Reef, New Zealand; 11 Mar 2011	GenBank	JF967662	Kim *et al*. (2012)
Vertebrata lanosa (Linneaeus) T.Christensen	Pt. Lepreau, New Brunswick, Canada; 7 Oct 1995	GenBank	EU492914	Stuercke & Freshwater (2008)
Womersleyella setacea (Hollenberg) R.E. Norris	Isolate from Italy, NUIG Marine Algal Culture	GenBank	JX828160	Bárbara *et al*. (2013)

Total genomic DNA samples were extracted using a DNeasy Plant Mini Kit (Qiagen, Hilden, Germany) following the manufacturer's instructions. For Polymerase Chain Reaction (PCR) amplification and sequencing reaction of the *rbc*L gene, primer pairs were used as follows; *rbc*LF7-*rbc*LR898 and *rbc*LJckF2-*rbc*LR1442 (Gavio & Fredericq 2002; Kim *et al.* 2010; Kang & Kim 2013). PCR amplification was carried out with Swift MaxPro thermal cyclers (ESCO, Singapore) with an AccuPower PCR PreMix (Bioneer, Daejeon, Korea). PCR amplification was performed as described in Kim & Kim (2016). PCR products were purified using an AccuPrep PCR purification kit (Bioneer, Daejeon, Korea) and then sequenced commercially (Macrogen, Seoul, Korea). Electropherogram outputs from each samples were edited using Chromas version 1.45 (Technelysium Pty. Ltd., Queensland, Australia).

A total of 62 *rbc*L sequences were aligned using the multiple-sequence editing program BioEdit (Hall 1999) including four outgroups, *Symphyocladia marchantioides, Womersleyella, Lophosiphonia teges* and *Pterosiphonia arenosa* (now *Pterosiphonia spinifera*). Maximum likelihood (ML) phylogenetic analyses were performed using RAxML software (Stamatakis 2006) with a GTR+Γ+I model. To identify the best tree, we used 200 independent tree inferences with the -# option with default –I (automatically optimized Subtree Pruning-Regrafting rearrangement) and –c (25 distinct rate categories) software options. Statistical support for each branch was obtained from 1,000 bootstrap replications using the same substitution model and RAxML program setting.

Results

Molecular analysis:—We sequenced *rbc*L for eight specimens collected from the type locality of *P. scopulorum*, Rottnest Island in Western Australia. Our *rbc*L gene sequences were compared to the sequence data available on GenBank for the *Polysiphonia sensu lato* species complex. The aligned data set consisted of 1250 base pairs with 436 variable positions (34.9%) and 366 phylogenetically informative positions (29.3%). ML analyses of the *rbc*L alignment yielded a phylogenetic tree showing no support for *Polysiphonia sensu lato* as a monophyletic group (Fig. 1). Within *Polysiphonia sensu lato* several clades were revealed with strong bootstrap support values, including a clade containing *P. scopulorum*, a clade containing *Polysiphonia stricta* (Dillwyn) Greville, and a clade containing *Melanothamnus flavimarinus* (M.S. Kim & I.K. Lee) Díaz-Tapia & Maggs (as *Neosiphonia flavimarina* M.S. Kim & I.K. Lee). The eight specimens resolved as five genetic entities, ultimately assigned to *Polysiphonia scopulorum* (n=3) and four unidentified species. Sequences from the three specimens of *P. scopulorum* were essentially identical (1–2 bp, 0.08-0.16% divergence) and formed a sister clade with *Polysiphonia caespitosa* (M.A. Pocock) Hollenberg from Spain (JX828149 as *P. scopulorum*) with moderate bootstrap support (79%). Other GenBank data identified as *P. scopulorum* (AY396039, EU492915) fell into two distinct genetic entities and did not join with the Rottnest Island specimens. These data had only one or two samples and probably represent misidentifications. The interspecific divergence between *P. scopulorum* and *P. caespitosa* was 5.2–5.3% and between *P. scopulorum* and the misidentifications was 6.6–6.7% (EU492915) and 6.9–7.0% (AY396039), respectively.

The clade including *P. scopulorum* from Rottnest Island was distant from the generitype *Polysiphonia stricta* (AY958165) from England with 11.0–11.1% sequence divergence. The divergence between a sequence purportedly of the generitype of *Lophosiphonia*, *L. obscura* (C. Agardh) Falkenberg and *P. scopulorum* was 13.9–14.1% (data not shown), however the former is possibly a misidentification.

Morphological observations

Polysiphonia scopulorum Harvey 1855: 540.

Homotypic Synonyms: *Vertebrata scopulorum* (Harvey) Kuntze 1891: 929; *Lophosiphonia scopulorum* (Harvey) Womersley 1950: 188.

Holotype: TCD (Harvey, Travelling Set no. 187). Note: Specimens included in Harvey's Travelling Set are not available for loan (Parnell *et al.* 2010). However, Womersley visited TCD in 1952 to examine the Harvey herbarium and his account of the species (Womersley 1979) would have incorporated observations of type material. We therefore accept his description as representative of authentic *P. scopulorum*.

Type locality: Rottnest Island, Western Australia (Harvey 1855).

Molecular vouchers: GenBank accession numbers MF139308, MF139309, MF139310.

Specimens examined: AUSTRALIA. Western Australia: Fays Bay, Rottnest Island, 31°59′16″S, 115°31′31″E, 11 November 2015, *151111#24-26* (JNUB).

Description:—Plants form dense tufts, 0.7–1 cm high, epilithic, brownish or yellowish red in color (Fig. 2A), with a prominent prostrate basal system from which erect axes arise endogenously every 4–6 segments of prostrate axes (Fig. 2B, C). Prostrate axes are attached to the substratum by unicellular rhizoids arising from the center of pericentral cells in open connection with them (Fig. 2D, E). Prostrate axes are terete, ecorticate, 60–80 µm in diameter with a segment length/diameter (L/D) ratio of 0.9–1.5 (Fig. 2E). Erect branches are simple or several times forked, terete, ecorticate, 70–80 µm in diameter with each axial cell bearing 4 pericentral cells (Fig. 2F, G & H). Segment L/D is 0.8–1.6 in lower segments, 1.4–2.1 in mid branch and 0.6–0.8 toward apices. A few lateral branches arise exogenously or endogenously from the apex of erect axes (Fig. 2J–M), not associated with trichoblasts. Pericentral cells of erect axes include abundant discoidal rhodoplasts (Fig. 2H, I). Apical cells are dome shaped, 10–13 µm long and 12–13 µm in diameter (Fig. 2N). Vegetative trichoblasts or scar cells are formed one per segment, infrequently on lower parts of erect branches, and more frequent near the apices (Fig. 2G, O, P). Vegetative trichoblasts are 2–3 times dichotomously branched, 400–750 µm long and 25–30 µm in diameter (Fig. 2O). Adventitious branches develop from the mid or lower parts of erect axes (Fig. 2Q). Tetrasporangia form one per segment in upper parts of erect axes, arranged in a slightly spiral series (Fig. 2R,S, U). Tetrasporangia are subspherical, 50–60 µm in diameter, with two presporangial cover cells (Fig. 2R, T). Gametophytes were not observed.

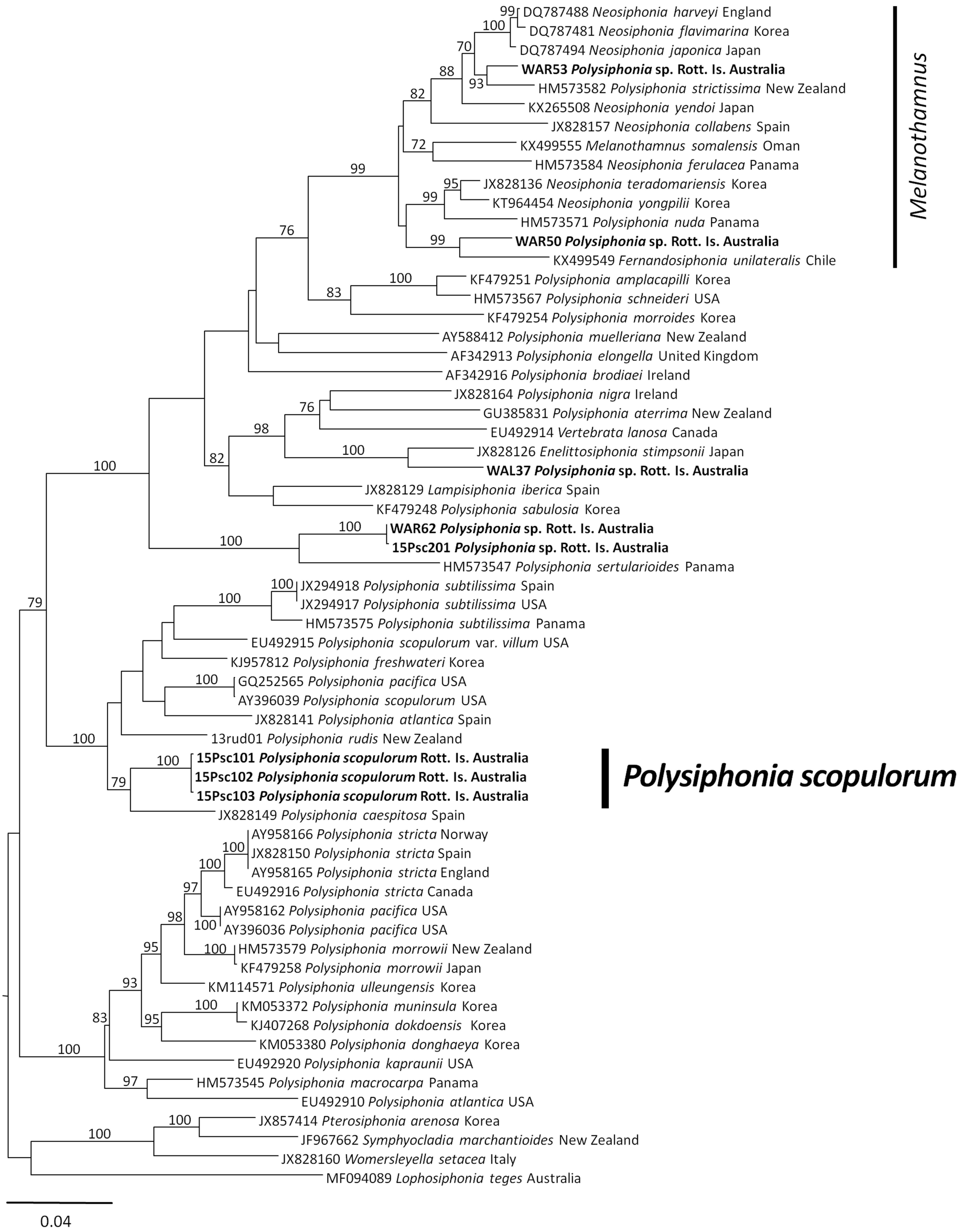

FIGURE 1. Phylogenetic tree of the genus *Polysiphonia sensu lato* and outgroup taxon estimated with Maximum Likelihood (RAxML) analysis of *rbc*L sequences. Samples in bold type represent our data collected from Rottnest Island, Western Australia, while samples in plain type were downloaded from GenBank. Values at nodes indicate ML bootstrap support (BP) and the scale indicates substitutions per site.

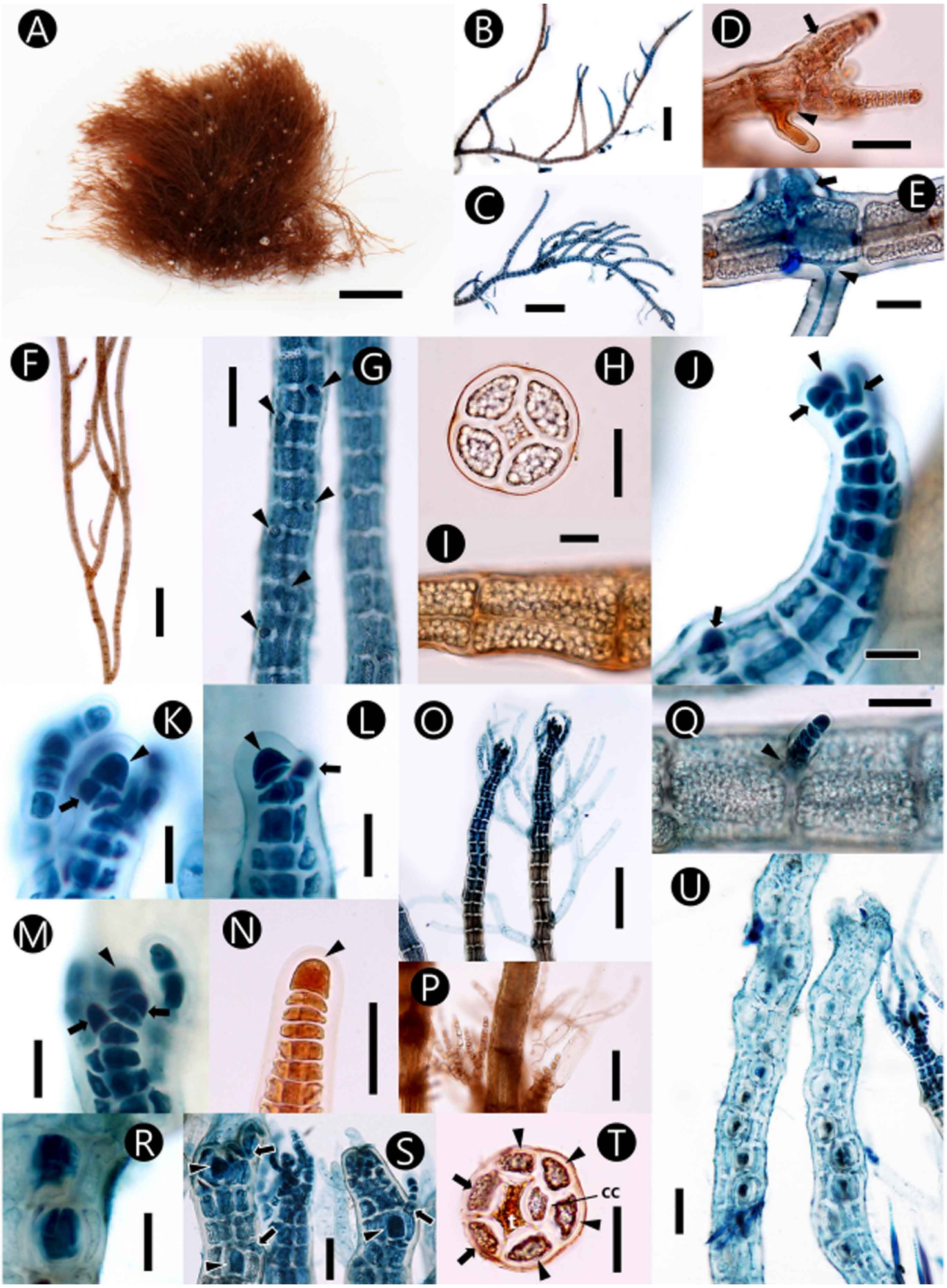

FIGURE 2. *Polysiphonia scopulorum* Harvey. A: Tetrasporophyte (Rottnest Island, Australia; 11 November 2015); B & C: Prostrate axes and numerous erect axes; D: Unicellular rhizoid in open connection (arrowhead) with a pericentral cell and apex of prostrate axes (arrow); E: Unicellular rhizoid in open connection (arrowhead) with pericentral cell and erect axes (arrow); F: Irregular branching pattern; G: Scar cells on erect axes; H: Cross-section of erect axes; I: Discoid rhodoplasts in pericentral cells; J: Apical cell (arrowhead) and endogenous branching (arrows) in erect axes; K–M: Apical cell (arrowhead) and exogenous branching (arrows) in erect axes; N: Apex of erect axes (arrowhead: apical cell); O & P: Trichoblast; Q: Adventitious branch (arrowhead); R: Tetrahedrally divided tetrasporangia; S: Tetrasporangia (arrowheads) and trichoblast or scar cells (arrows); T: Cross-section of tetrasporangial branch (arrowheads: pericentral cells, arrows: presporangial cover cells, t: tetrasporangium, cc: central cell); U: Slightly spiral arrangement of tetrasporangial series (Scale bars: A = 5 mm, B, C, F = 500 µm; D, E, H, I, Q, R, S, T = 50 µm; G, P, U = 100 µm; J–M = 20 µm; N = 30 µm; O = 200 µm).

Distribution:—Womersley (1979) recorded *P. scopulorum* as occurring from Rottnest Island ("probably further north") to Lawrence Rock, Victoria, later (Womersley 2003) broadening this to the Dampier Archipelago in Western Australia and several locations in eastern Australia, north to southern Queensland. The Dampier Archipelago record has been found to represent an undescribed species (Huisman, unpublished observations) and in light of the results of the present study many other records require confirmation. Records from non-Australian locations (see Guiry & Guiry 2017, for sources) should similarly be viewed cautiously.

Habitat:—Our newly collected specimens were growing on rock with sand in the lower intertidal zone, which agrees with Harvey's (1855) "on littoral rocks" description of the species' habitat. According to Womersley (2003), plants occur mainly intertidal on rough-water rock surfaces exposed to surf, or in relatively calm localities, usually lower sublittoral, occasionally sublittoral on older axes of the brown alga *Scaberia agardhii*.

Discussion

Based on morphology, *P. scopulorum* conforms with the genus *Polysiphonia* and can be distinguished from other species in the genus by the following features (in combination): a strongly developed prostrate system attached by rhizoids in open connection with the bearing pericentral cell, the presence of four pericentral cells per segment, erect filaments with short segments and few branches, scar cells and trichoblasts rare or on every segment of well-developed apices, tetrasporangia forming a slightly spiral series in a upper branches, and plants usually forming spreading mats on rock platforms just emergent at low tide (Womersley 2003). As several other species display one or more of these features, potentially leading to misidentifications, we have further characterized the species by undertaking molecular analyses utilizing *rbc*L sequence data. This study documents, for the first time, molecular characterization of *P. scopulorum* based on specimens from the type locality in Rottnest Island, Western Australia and indicates that *P. scopulorum*, which was thought to be a cosmopolitan species, could actually be endemic to Australia.

Our study highlights the difficulty in correctly identifying species of *Polysiphonia* without access to molecular sequencing. *P. scopulorum* from Rottnest Island has 5.2–7.0% sequence divergence from molecular data derived from three specimens identified (at least initially) as *P. scopulorum*: *P. caespitosa* from Spain (JX828149 as *P. scopulorum*) and from the U.S.A. (AY396039), and *P. scopulorum* var. *villum*, also from the U.S.A. (EU492915), these identifications presumably based on morphological features as highlighted in identification keys (Stuercke & Freshwater 2010).

Polysiphonia scopulorum was moved to the genus *Lophosiphonia* by Womersley (1950: 188) but without detailed justification, noting only "the species is clearly a *Lophosiphonia*". The distinguishing features of the genus *Lophosiphonia* are: 1) relatively limited and mostly simple erect branches from permanently prostrate branches; and 2) an adventitiously endogenous origin of all branches (Falkenberg 1901). Hollenberg (1968a), however, regarded the dorsiventral or bilateral apex of all branches as one of the most important characters of *Lophosiphonia*, and thus the species was best placed in *Polysiphonia* rather than *Lophosiphonia*. Subsequently, Womersley (1979, 2003) followed Hollenberg's scheme and included the species under *Polysiphonia*. Our specimens from the type locality differ from *Lophosiphonia* in many respects, including having exogenously ramified and radially constructed branches, in contrast to the endogenously simple and dorsiventrally formed branches in *Lophosiphonia*. A sequence purportedly of the generitype, *L. obscura* (C.Agardh) Falkenb. is available in GenBank (KT825865) and is only remotely related to *P. scopulorum,* with 14% sequence divergence. However, we suspect that this entity might have been misidentified and have not included it in our phylogeny. To represent *Lophosiphonia* we have included a sequence from *Lophosiphonia teges* (Womersley) Díaz-Tapia & Maggs, which is similarly only remotely related to *P. scopulorum* (Fig. 1).

According to Kapraun (1977), there are three types of prostrate axis development in *Polysiphonia sensu lato*: 1) plants initially erect from a discoidal base, but forming a secondary attachment with decumbent branches; 2) plants initially with a horizontal prostrate system derived from an erect apex; and 3) plants consisting of a horizontal prostrate system and apex giving rise to erect exogenous branches. *P. scopulorum* has the third type with a strictly prostrate system. As observed by Womersley (2003) and here, lateral branches can arise endogenously from prostrate axes. This does not contradict Kapraun's definition as exogenous branches can arise only from apices, before the formation of pericentral cells. Non-apical branches arising from a prostrate axis must therefore be endogenous or cicatrigenous (see below). Trichoblasts in *Polysiphonia* species arise mainly near branch apices in erect filaments and they are exogenous and usually deciduous (Hollenberg 1942). In *P. scopulorum*, short trichoblasts arise near the apex of the frond but are generally rapidly shed. Womersley (1979) described scar cells as the basal cell of a trichoblast that remains embedded between the pericentral cells after the trichoblast has been shed. Because of the associations between scar cells and trichoblasts, their occurrence and pattern have been referred to together in most taxonomic keys for *Polysiphonia* (Abbott 1999). In our study, the presence of scar cells producing lateral branches, also known as cicatrigenous branching, was correlated with trichoblasts in a spiral pattern. Womersley (2003) mentioned that lateral branches appear to be largely cicatrigenous, with some exogenous apical branching, and branching from prostrate filaments may be endogenous.

Hollenberg (1968a) described several varieties in *P. scopulorum* from the tropical Pacific (Table 2). In all varieties the tetrasporangia are arranged in straight series, in contrast with the specimens from Australia where the tetrasporangia are arranged in a spiral series (Womersley 1979). Var. *macrotrichia* and var. *minima* have erect branches mostly less than 0.5 cm high. Erect branches arising at intervals of 4–6 segments from the prostrate branches are observed in var. *villum*, which has mostly longer than broad segments in erect branches, whereas var. *scopulorum* (as interpreted by Hollenberg 1968a) shows erect branches arising mostly at intervals of 2–3 segments, with shorter than broad segments in erect branches (Hollenberg 1968a). *P. scopulorum* var. *villum* was found on mangrove roots in the central region of the Belizean barrier reef. The key characteristics distinguishing this variety are: four pericentral cells per segment, branches replacing trichoblasts, rhizoids in open connection with pericentral cells, scar cells absent in decumbent axes, and trichoblasts in erect filaments profusely branched to several orders (Kapraun & Norris 1982). Our specimens of *P. scopulorum* from Rottnest Island share some features with var. *villum*, including branch intervals, but this variety has tetrasporangia in straight series and rare trichoblasts (Stuercke & Freshwater 2008). Our molecular analyses indicate that *P. scopulorum* var. *villum* does not join with *P. scopulorum* and should be recognized as the independent species *P. villum* J. Agardh. Xiang (2004) described *P. scopulorum* var. *longinodium* from China as having being 1 cm height, with few lateral branches, and a sterile tip in spermatangial branches. The validity of all the described varieties of *P. scopulorum* should be tested molecularly.

TABLE 2. A comparison of taxonomic characteristics of *Polysiphonia scopulorum* and several varieties by Hollenberg (1968a).

Characters	*P. scopulorum* Harvey	*P. scopulorum* var. *macrotrichia* Hollenberg	*P. scopulorum* var. *minima* Hollenberg	*P. scopulorum* var. *villum* (J.G.Agardh) Hollenberg
Type locality	Rottnest Island, Western Australia	Oneroa, Raroia Atoll, the Tuamotu Archipelago	North Island, Johnston Island	The Pacific coast of Mexico
Height (cm)	0.7–1	<0.1	0.02–0.03(to 0.1)	0.5–0.8
Substratum	Saxicolous	Epiphytic	Saxicolous	Saxicolous
Type of thallus	Prostrate & erect	Creeping branches	Creeping branches	Prostrate & erect
Type of rhizoid	Open	Open	Open	Open
Branching pattern	4–6 segments	Unbranched or about 8–10 segments	Frequently branched	4–6 segments
Pericentral cells	4	4	4	4
Cortication	Ecorticate	Ecorticate	Ecorticate	Ecorticate
Length/ diameter(width) ratio of segments	0.8–2.1	1	1	Longer than broad
Diameter(width) of segments (μm)	60–80	70(Creeping branches)	35–40	<150
Vegetative trichoblast	400–750 μm long with 2–3 dichotomies	700 μm long with 6–7 dichotomies	750 μm long with 4–6 dichotomies	rare

In our phylogenetic analyses based on the *rbc*L gene, the clade including *P. scopulorum* formed a sister clade with *Polysiphonia caespitosa*, which was described originally as a species of *Falkenbergiella* based on specimens from False Bay, Cape Province, South Africa (Pocock 1953) but transferred to *Polysiphonia* by Hollenberg (1968b). The species grows on sand-covered rocks from sheltered to extremely wave-exposed areas in the mid or low intertidal (Díaz-Tapia & Bárbara 2013). The GenBank sequence (JX828149) of *P. caespitosa* was derived from a specimen from Spain originally attributed to *P. scopulorum*, but referred to *P. caespitosa* by Díaz-Tapia & Bárbara (2013). In Díaz-Tapia *et al.* (2017, fig. 1), JX828149 is attributed (possibly in error) to *P. scopulorum.* As described by Díaz-Tapia & Bárbara (2013), *P. caespitosa* is similar to *P. scopulorum* from Australia in the formation of prostrate and erect branches, endogenous branching, trichoblasts often absent in sterile plants, four pericentral cells per segment, no cortication, and rhizoids in open connection with bearing cells; differences include a larger branch diameter, the size of spermatangia, and the position of endogenous branches. Our specimens of *P. scopulorum* have well developed vegetative trichoblasts, but trichoblasts are absent in *P. caespitosa*.

Our phylogenetic analyses resolved *P. scopulorum* within a clade that includes several species: *P. caespitosa, P. freshwateri, P. subtillissima* etc. and two varieties of *P. scopulorum*. Díaz-Tapia *et al*. (2017) documented two clades *Polysiphonia sensu stricto* 1 and 2 that resolved as separate lineages in *rbc*L analyses but together formed a robust clade in 18S analyses. Relative to their analyses (Díaz-Tapia *et al.* 2017, fig. 1), *P. scopulorum* resolves in *Polysiphonia sensu stricto* 2, whereas the generitype (*P. stricta*) occurs in *Polysiphonia sensu stricto* 1. This divergence from the genus *Polysiphonia*, including the type species, suggests that a separate genus might be appropriate for the *P. scopulorum* clade, which could also be supported on the basis of morphological evidence. However, we agree with Díaz-Tapia *et al*. (2017), who mentioned that these lineages require additional research for a more accurate assessment of relationships. While the phylogeny using the *rbc*L enabled us to identify several clades in *Polysiphonia sensu lato*, further studies incorporating a range of sequences are needed to resolve the relationships among species and clarify the delimitation of genera

In conclusion, our study provides the first molecular evidence for *P. scopulorum* from the type locality in Australia and our phylogenetic results suggest a clear separation between the *P. scopulorum* clade and the *Polysiphonia sensu stricto* clade. Morphological characterization and additional molecular investigations based on multi-locus genome sequences should be undertaken to further clarify their phylogenetic relationships and determine whether a separate genus is warranted.

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