



The genera *Melanothamnus* Bornet & Falkenberg and *Vertebrata* S.F. Gray constitute well-defined clades of the red algal tribe Polysiphonieae (Rhodomelaceae, Ceramiales)

Pilar Díaz-Tapia, Lynne McIvor, D. Wilson Freshwater, Heroen Verbruggen, Michael J. Wynne & Christine A. Maggs

To cite this article: Pilar Díaz-Tapia, Lynne McIvor, D. Wilson Freshwater, Heroen Verbruggen, Michael J. Wynne & Christine A. Maggs (2017) The genera *Melanothamnus* Bornet & Falkenberg and *Vertebrata* S.F. Gray constitute well-defined clades of the red algal tribe Polysiphonieae (Rhodomelaceae, Ceramiales), *European Journal of Phycology*, 52:1, 1-30, DOI: [10.1080/09670262.2016.1256436](https://doi.org/10.1080/09670262.2016.1256436)

To link to this article: <http://dx.doi.org/10.1080/09670262.2016.1256436>

 View supplementary material 

 Published online: 01 Feb 2017.

 Submit your article to this journal 

 Article views: 181

 View related articles 

 View Crossmark data 

The genera *Melanothamnus* Bornet & Falkenberg and *Vertebrata* S.F. Gray constitute well-defined clades of the red algal tribe Polysiphonieae (Rhodomelaceae, Ceramiales)

Pilar Díaz-Tapia^{a,b,c}, Lynne McIvor^d, D. Wilson Freshwater^e, Heroen Verbruggen^{id}^e, Michael J. Wynne^f and Christine A. Maggs^{id}^{b,d}

^aBioCost Research Group, Universidad de A Coruña, 15071, A Coruña, Spain; ^bFaculty of Science and Technology, Bournemouth University, Talbot Campus, Poole, Dorset BH12 5BB, UK; ^cSchool of BioSciences, University of Melbourne, Victoria 3010, Australia; ^dSchool of Biological Sciences, Queen's University Belfast, Medical Biology Centre, BT9 7BL, Northern Ireland; ^eCenter for Marine Science, University of North Carolina at Wilmington, 5600 Marvin Moss Lane, Wilmington, NC 28409, USA; ^fDepartment of Ecology and Evolutionary Biology and Herbarium, University of Michigan Ann Arbor, MI 48109, USA

ABSTRACT

Polysiphonia is the largest genus of red algae, and several schemes subdividing it into smaller taxa have been proposed since its original description. Most of these proposals were not generally accepted, and currently the tribe Polysiphonieae consists of the large genus *Polysiphonia* (190 species), the segregate genus *Neosiphonia* (43 species) and 13 smaller genera (< 10 species each). In this paper, phylogenetic relationships of the tribe Polysiphonieae are analysed, with particular emphasis on the genera *Carradoriella*, *Fernandosiphonia*, *Melanothamnus*, *Neosiphonia*, *Polysiphonia sensu stricto*, *Streblodadia* and *Vertebrata*. We evaluated the consistency of 14 selected morphological characters in the identified clades. Based on molecular phylogenetic (*rbcL* and 18S genes) and morphological evidence, two speciose genera are recognized: *Vertebrata* (including the type species of the genera *Ctenosiphonia*, *Enelittosiphonia*, *Boergeseniella* and *Brongniartella*) and *Melanothamnus* (including the type species of the genera *Fernandosiphonia* and *Neosiphonia*). Both genera are distinguished from other members of the Polysiphonieae by synapomorphic characters, the emergence of which could have provided evolutionarily selective advantages for these two lineages. In *Vertebrata* trichoblast cells are multinucleate, possibly associated with the development of extraordinarily long photoprotective trichoblasts. *Melanothamnus* has 3-celled carpogonial branches and plastids lying exclusively on radial walls of the pericentral cells, which similarly may improve resistance to damage caused by excessive light. Other relevant characters that are constant in each genus are also shared with other clades. The evolutionary origin of the genera *Melanothamnus* and *Vertebrata* is estimated as 75.7–95.78 and 90.7–138.66 Ma, respectively. Despite arising in the Cretaceous, before the closure of the Tethys Seaway, *Melanothamnus* is a predominantly Indo-Pacific genus and its near-absence from the north-eastern Atlantic is enigmatic. The nomenclatural implications of this work are that 46 species are here transferred to *Melanothamnus*, six species are transferred to *Vertebrata*, and 13 names are resurrected for *Vertebrata*.

ARTICLE HISTORY Received 1 October 2016; Revised 28 October 2016; Accepted 30 October 2016

KEYWORDS Biogeography; evolution; molecular systematics; morphology; phylogeny; *Polysiphonia*; red algae; time calibration

Introduction

The Rhodomelaceae Areschoug is the largest family of red algae, currently including more than 1000 species (Guiry & Guiry, 2016). It consists of the tribes Amansieae Schmitz (1889), Bostrychieae Falkenberg (1901), Chondrieae Schmitz & Falkenberg (1897), Herposiphonieae Schmitz & Falkenberg (1897), Heterocladieae Falkenberg (1901), Laurencieae Schmitz (1889), Lophothalieae Schmitz & Falkenberg (1897), Neotenophyceae Kraft & I.A. Abbott (2002), Pleurostichidiaceae (Hommersand, 1963), Polysiphonieae Schmitz (1889), Polyzonieae Schmitz & Falkenberg (1897), Pterosiphonieae Falkenberg (1901), Rhodomeleae Schmitz & Falkenberg (1897), Sonderelleae L.E. Phillips (2001) and Streblodadiaceae nom. nud. (Hommersand, 1963; Kraft & Abbott, 2002; Womersley, 2003), of which

the most speciose is the Polysiphonieae with over 300 species in 15 currently recognized genera (Guiry & Guiry, 2016).

Within the Polysiphonieae the genus *Polysiphonia* Greville (1824), nom. cons., has representatives throughout the world, in the majority of photic marine benthic habitats including brackish ones (e.g. Hollenberg, 1942, 1944, 1968a, 1968b; Womersley, 1979; Maggs & Hommersand, 1993; Lam *et al.*, 2013). *Polysiphonia* is poorly circumscribed, and has remained in a state of taxonomic flux since its original description. Numerous schemes for subdividing this large and morphologically diverse genus into smaller and more manageable groups have been proposed (e.g. Segi, 1951; Hollenberg, 1968a, 1968b), based mostly on the number of periaxial cells, either four (subgenus *Oligosiphonia*) or more than four

(subgenus *Polysiphonia*). These schemes have generally been rejected and several generic names (e.g. *Orcasia* Kylin (1941), based on *Polysiphonia senticulosa* Harvey) are currently regarded as synonyms of *Polysiphonia*. However, despite having been subsumed within *Polysiphonia* in most classification schemes, *Vertebrata* S.F.Gray (1821) is currently recognized as a monospecific genus containing only the type species, *V. lanosa*.

The segregate genus *Neosiphonia* M.-S.Kim & I.K. Lee (Kim & Lee, 1999) has been widely accepted and is now the second largest in the Polysiphonieae (Guiry & Guiry, 2016). *Neosiphonia* (type species: *N. flavimarina* from Korea) is characterized by the following features: (1) thalli erect with a main axis bearing branches; (2) branches or trichoblasts formed on every segment; (3) rhizoids cut off from pericentral cells; (4) carpogonial branches 3-celled; (5) spermatangial branches formed on a branch of modified trichoblasts; (6) tetrasporangia in spiral arrangement (Kim & Lee, 1999). These features contrast markedly with the key characters of *Polysiphonia sensu stricto*, exemplified by the type species *P. stricta*: prostrate axes with rhizoids in open connection with pericentral cells; carpogonial branches 4-celled; spermatangial branches borne directly on axes; tetrasporangia in straight rows (Kim *et al.*, 2000). In addition to describing the new species *N. flavimarina*, Kim & Lee (1999) also transferred 11 species of *Polysiphonia* to *Neosiphonia*, all based on material from Korea, and there are 43 currently recognized species (Guiry & Guiry, 2016), not all of which exhibit the six key characters of *Neosiphonia* listed above.

Kim & Lee (1999) considered *Neosiphonia* (also referred to as the ‘*Polysiphonia japonica* complex’ *sensu* Yoon (1986)) to be related to *Fernandosiphonia* Levring, which was erected for *F. unilateralis* from the Juan Fernández Islands off Chile on the basis of its unilateral development of ultimate branches (Levring, 1941) and which currently consists of three species. They reported that *Neosiphonia* differed from *Fernandosiphonia* principally in its branching pattern, the origin of spermatangial branches, and the 3-celled carpogonial branches. Kim & Lee (1999) did not comment, however, on the possible relationship between *Fernandosiphonia* and *Streblocladia* F.Schmitz (in Schmitz & Falkenberg, 1897). Hommersand (1963) and Norris (1994) compared *Fernandosiphonia* (trichoblasts formed spirally) with *Streblocladia* (trichoblasts borne only adaxially). Choi *et al.* (2001) drew attention to the relationship in their 18S tree between *N. japonica* and *Polysiphonia virgata*, the type species of *Carradoriella* P.C.Silva (Kylin, 1956, as *Carradoria*; Silva *et al.*, 1996), and suggested that *Neosiphonia* might either be subsumed into *Carradoriella* or be resolved as a sister to it. Recent searches of DNA sequence databases unexpectedly showed a possible

relationship between *Neosiphonia* species and *Melanothamnus somalensis*, the type species of the genus *Melanothamnus* Bornet & Falkenberg, which was regarded as *incertae sedis* (Falkenberg, 1901).

Given the taxonomic and nomenclatural complexity within the Polysiphonieae, our aims were to re-evaluate the morphological features of *Neosiphonia* and *Vertebrata* in relation to those of *Fernandosiphonia*, *Streblocladia*, *Carradoriella*, *Melanothamnus* and *Polysiphonia sensu stricto* within a phylogenetic analysis of the Polysiphonieae using sequences of the plastid-encoded *rbcL* gene and the ribosomal DNA 18S gene (SSU). We surveyed within the Polysiphonieae the distribution of a striking characteristic of the ‘*Polysiphonia japonica* complex’, the position of plastids on radial walls of the periaxial cells and their absence from the outer walls such that nuclei are clearly visible after staining (Maggs & Hommersand, 1993; McIvor *et al.*, 2001). Likewise, we analysed the multinucleate *vs.* uninucleate character of trichoblast cells, which seems to be taxonomically significant (Maggs & Hommersand, 1993).

Materials and methods

Field collections, morphological studies and literature review

Samples of Polysiphonieae (Table S1) were collected from European Atlantic coasts, New Zealand, Australia, Taiwan, Japan, Chile, USA, South Africa and Oman and processed fresh, desiccated in silica gel or preserved in ethanol.

Type material of *Fernandosiphonia unilateralis* was obtained from the Herbarium, Botanical Museum, Göteborg, Sweden (GB) by correspondence with the curator. It consisted of four permanent slides, a herbarium sheet and liquid-preserved material. Furthermore, we studied recent collections from Juan Fernández Islands, the type locality. We also studied the type material in US and TCD of several species currently assigned to *Neosiphonia* (Table S2; herbarium abbreviations as in Thiers, 2016) for which the key morphological characteristics (Table 1) could not be clearly ascertained from published literature, in order to determine their correct generic assignment. For this purpose, we exclusively considered the descriptions provided for material from type localities or near them. To ensure the accuracy of our interpretation of the genera, our concept of them is based on material of their type species obtained from their type localities. For *Streblocladia*, we used material of, and sequences from, the type species *S. glomerulata* from New Zealand. *Carradoriella* (i.e. *Polysiphonia virgata*) was obtained from the type locality in South Africa, and the type species of *Vertebrata* and *Melanothamnus* came from Ireland and Oman respectively.

Table 1. Comparison of selected morphological characteristics among the *Polysiphonia sensu stricto* 1 and 2, *Vertebrata*, *Carradoriella*, *Strebloladia*, '*Polysiphonia*' *scheideri* and *Melanthamnus* clades.

Feature	<i>Polysiphonia sensu stricto</i> 1 and 2	<i>Vertebrata</i>	<i>Carradoriella</i> clade	<i>Strebloladia</i> clade	' <i>Polysiphonia</i> ' <i>scheideri</i> clade	<i>Melanthamnus</i>
Thallus habit	Erect; decumbent; prostrate	Erect; decumbent; prostrate	Erect	Erect	Erect; decumbent	Erect; decumbent
Rhizoid connection	Open	Cut off	Cut off	Cut off	Cut off	Cut off
Pericentral cells	4 (6–8 in <i>Bryocladia cuspidata</i> , clade 2)	6–24	5–16	4–12	4–7	4–9
Cortication	Absent	Absent/Present	Present	Present	Absent	Absent/Present
Plastid arrangement	Scattered	Scattered	Scattered	Scattered	Scattered	Radial walls
Branch/trichoblast arrangement	With naked segments	On every segment or with naked segments	On every segment or with naked segments	With naked segments	With naked segments	On every segment or with naked segments
Branches in trichoblast axils	No	Yes/No	Yes/No	No	Yes/No	No
Trichoblast cell nuclei and pigmentation	Uninucleate; pigmentation absent	Multinucleate; pigmentation absent (present)	Uninucleate; pigmentation absent	Trichoblasts absent	Uninucleate; pigmentation absent	Uninucleate; pigmentation absent
Branching pattern	Spiral, pseudodichotomous	Spiral, pseudodichotomous, dorsiventral	Pseudodichotomous	Dorsiventral, spiral, pseudodichotomous	Spiral, pseudodichotomous	Dorsiventral, spiral, pseudodichotomous
Spermatangial branches	Replacing trichoblasts (or on a trichoblast branch in <i>P. devoniensis</i> and <i>P. kapraunii</i>), with or without sterile apical cells	On a branch of trichoblasts (replacing them in <i>V. lanosa</i>), with/without sterile apical cells	On a branch of trichoblasts, with sterile apical cells	Replacing trichoblasts, without sterile apical cells	On a branch of trichoblasts, with/without sterile apical cells	On a branch of trichoblasts, with/without sterile apical cells
Carpogonial branch	4-celled	4-celled	4-celled	Unknown	4-celled	3-celled
Cystocarp morphology	Globular; ovoid; urceolate	Globular; ovoid	Ovoid	Ovoid	Globular	Globular; ovoid
Cells of the ostiole	Similar to the cells below	Similar to the cells below	Similar to the cells below	Larger than cells below	Similar to the cells below	(Similar to) Larger than cells below
Tetrasporangial rows	Straight (slightly spiral)	Straight or spiral (two per segment in <i>Ctenosiphonia</i>)	Straight or spiral	Straight or spiral	Straight or spiral	(Straight) Spiral
References	This work, 5, 6, 9, 15, 16, 18, 22	This work, 1, 2, 3, 6, 7, 8, 16, 18, 20, 21, 24	This work, 16, 21	This work, 2, 3	This work, 7, 13, 18, 22	This work, 2, 3, 4, 6, 9, 10, 11, 12, 14, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25

References: ¹Abbott & Hollenberg (1976); ²Adams (1991); ³Adams (1991); ⁴Bustamante *et al.* (2013b); ⁵Dawes & Mathieson (2008); ⁶Diaz-Tapia & Bárbara (2013); ⁷Diaz-Tapia *et al.* (2013a); ⁸Diaz-Tapia *et al.* (2013b); ⁹Hollenberg (1942); ¹⁰Hollenberg (1968a); ¹¹Hollenberg & Norris (1977); ¹²Kim & Lee (1999); ¹³Kim & Kim (2014); ¹⁴Kim & Kim (2016); ¹⁵Kim *et al.* (1994); ¹⁶Maggs & Hommersand (1993); ¹⁷Mamoozadeh & Freshwater (2011); ¹⁸Mamoozadeh & Freshwater (2012); ¹⁹Muangmai *et al.* (2014); ²⁰Segi (1949); ²¹Stegenga *et al.* (1997); ²²Stuercke & Freshwater (2010); ²³Uwai & Masuda (1999); ²⁴Womersley (2003); ²⁵Yoon (1986).

Table 2. Genus *Vertebrata* with new combinations resulting from the present study printed in bold, followed by basionyms and taxonomic synonyms.

Binomial in <i>Vertebrata</i> Basionym Synonyms	Type material Type locality
<i>Vertebrata constricta</i> (Womersley) Díaz-Tapia & Maggs, comb. nov. <i>Polysiphonia constricta</i> Womersley (1979, 497–498; Southern Australian species of <i>Polysiphonia</i> Greville (Rhodophyta). <i>Australian Journal of Botany</i> , 27: 459–528)	Holotype: AD A32927 Kangaroo I., South Australia; 21. xi.1968
<i>Vertebrata foetidissima</i> (Cocks ex Bornet) Díaz-Tapia & Maggs, comb. nov. <i>Polysiphonia foetidissima</i> Cocks ex Bornet (1892, pp. 314–315; Les algues de P. K. A. Schousboe. <i>Mémoires de la Société Nationale des Sciences naturelles et Mathématiques de Cherbourg</i> , 28: 165–376)	Lectotype (Maggs & Hommersand, 1993): PC 0146017 Plymouth, England; undated
<i>Vertebrata isogona</i> (Harvey) Díaz-Tapia & Maggs, comb. nov. <i>Polysiphonia isogona</i> Harvey (in Hooker 1855, p. 231; <i>The botany of the Antarctic voyage</i> Reeve, London)	Lectotype (Womersley, 1979): BM 1082304 Blind Bay, Cook's Straits, New Zealand; viii.1849
<i>Vertebrata lobophoralis</i> (N.R.Mamoozadeh & D.W.Freshwater) D.W.Freshwater, comb. nov. <i>Polysiphonia lobophoralis</i> N.R.Mamoozadeh & D.W.Freshwater (2012, pp. 331–333; <i>Polysiphonia sensu lato</i> (Ceramiales, Florideophyceae) species of Caribbean Panama including <i>Polysiphonia lobophoralis</i> sp. nov. and <i>Polysiphonia nuda</i> sp. nov. <i>Botanica Marina</i> , 55: 317–347)	Holotype: US 217938 Bocas del Toro, Panama; 6.viii.2010
<i>Vertebrata nigra</i> (Hudson) Díaz-Tapia & Maggs, comb. nov. <i>Conferva nigra</i> Hudson (1762, p. 481; <i>Flora anglica</i> Prostant venales apud J. Nourse in the Strand & C. Moran in Covent-Garden, London) <i>Polysiphonia nigra</i> (Hudson) Batters	Neotype (Maggs & Hommersand, 1993): BM 1067621 Marsden, Durham, England; 12.vi.1971
<i>Vertebrata reptabunda</i> (Suhr) Díaz-Tapia & Maggs, comb. nov. <i>Hutchinsia reptabunda</i> Suhr (1831, p. 684; Beschreibung einiger neuen Algen. <i>Flora</i> 14: 673–687, 709–716, 725–731) <i>Lophosiphonia reptabunda</i> (Suhr) Kylin	Holotype: L 955.62.97 Biarritz, Pyrénées-Atlantiques, France

Fresh material and herbarium samples were prepared as squashes, either unstained or stained with aqueous aniline blue, post-fixed in 1% HCl, and mounted in 80% Karo corn syrup (Bestfoods Inc., NJ, USA). Permanent slide mounts were prepared as vouchers and deposited in: BM, MICH, SANT, WNC and MEL.

A systematic review was carried out to identify relevant phycological literature from around the world from which to assess for each species of Polysiphonieae the 14 vegetative and reproductive features relevant to *Neosiphonia* and *Vertebrata*.

Nomenclatural authorities for the species mentioned in the manuscript are provided in Tables 2–5 and S1–S2.

DNA extraction, PCR amplification and sequencing

This was carried out in four different laboratories using different protocols as described below.

At Queen's University Belfast, DNA was extracted from fresh, silica gel-dried or ethanol-preserved material using the Qiagen DNeasy Plant Mini Kit (Qiagen GmbH, Hilden, Germany), according to the manufacturer's instructions, or by a CTAB method, modified after Doyle & Doyle (1987). For PCR amplification, a PTC-200 DNA Engine (MJ Research Inc.) was used. Except for material of *Fernandosiphonia unilateralis*, all PCR amplifications were carried out using *rbclFC* as the forward primer, and *rbclRD* as the reverse primer (Nam *et al.*, 2000; McIvor *et al.*, 2001). All reactions contained 200 µM each of dATP, dCTP, dGTP and dTTP, 0.3 µM of each primer, 2.5 mM MgCl₂, and 1.6 units of BiotaqTM DNA

polymerase (Bioline, UK). The PCR amplification followed Nam *et al.* (2000) and McIvor *et al.* (2001). About 1250 base pairs (bp) of the *rbcl* gene were amplified and the PCR fragments for sequencing were purified using the High Pure PCR Product Purification Kit (Roche Diagnostics Ltd, Lewes, UK), according to the manufacturer's instructions. The PCR products were directly sequenced commercially by MWG-Biotech, Ebersberg, Germany.

Type material of *Fernandosiphonia unilateralis* had been preserved in formalin by Leving (1941) prior to long-term storage in ethanol (A. Athanasiados, personal communication). At the Leiden herbarium, various protocols for retrieving DNA from formalin-preserved specimens were attempted (Kirby & Reid, 2001); the most successful was to soak and wash the material repeatedly in clean sterile water, prior to DNA extraction using using a Chelex-100 (Biorad, Hercules, California) protocol (Goff & Moon, 1993; Zuccarello *et al.*, 1999). Applying a strategy for amplifying degraded 'ancient' DNA (Provan *et al.*, 2008), primers were designed from an alignment of *Neosiphonia harveyi* and related species in order to amplify 100-bp fragments. We used the primers F183 (5' TGCAGGTGAATCTTCTACAGCT 3') and R383 (5' ACGTTACCAATAATTGAAGCTGTT 3').

At the University of Melbourne, DNA was extracted from silica gel-dried material following Saunders & McDevit (2012). PCR amplification was carried out for *rbcl* using the primers F7/RbclStart or F57/rbclrevNEW (Freshwater & Rueness, 1994; Saunders & Moore, 2013) and for 18S using the primers F47 (5' AGCCATGCAAGTGCCTGTAT 3') and R1867 (5' CGCAGGTCACCTACGAAA 3').

Table 3. Genus *Vertebrata* with resurrected names resulting from the present study and *V. lanosa* printed in bold, followed by basionyms and taxonomic synonyms.

Binomial in <i>Vertebrata</i> Basionym Synonyms	Type material Type locality
<i>Vertebrata aterrma</i> (J.D.Hooker & Harvey) Kuntze <i>Polysiphonia aterrma</i> J.D.Hooker & Harvey	Probable syntypes: TCD 12786-8, BM 1067593-6 and BM 1067598 New Zealand
<i>Vertebrata australis</i> (C.Agardh) Kuntze <i>Cladostephus australe</i> C.Agardh <i>Brongniartella australis</i> (C.Agardh) F.Schmitz	Lectotype (Parsons, 1980): PC Western Australia
<i>Vertebrata byssoides</i> (Goodenough & Woodward) Kuntze <i>Fucus byssoides</i> Goodenough & Woodward <i>Brongniartella byssoides</i> (Goodenough & Woodward) F.Schmitz	Lectotype (Maggs & Hommersand, 1993): BM Christchurch, England; 1794
<i>Vertebrata fruticulosa</i> (Wulfen) Kuntze <i>Fucus fruticulosus</i> Wulfen <i>Boergeseniella fruticulosa</i> (Wulfen) Kylin	Lectotype (Maggs & Hommersand, 1993): Wulfen (1789), pl. 16, fig. 1 Trieste [Tergestum], Adriatic
<i>Vertebrata fucooides</i> (Hudson) Kuntze <i>Conferva fucooides</i> Hudson <i>Polysiphonia fucooides</i> (Hudson) Greville	Neotype (Maggs & Hommersand, 1993): BM 807101 Unlocalized, undated
<i>Vertebrata furcellata</i> (C.Agardh) Kuntze <i>Hutchinsia furcellata</i> C.Agardh <i>Polysiphonia furcellata</i> (C.Agardh) Harvey	Lectotype (Maggs & Hommersand, 1993): LD 40907 Brittany, France; undated
<i>Vertebrata hypnoides</i> (Welwitsch) Kuntze <i>Polysiphonia hypnoides</i> Welwitsch <i>Ctenosiphonia hypnoides</i> (Welwitsch) Falkenberg	Holotype: LD Agardh's herbarium no. 39346 Lisbon, Portugal
<i>Vertebrata lanosa</i> (Linnaeus) T.A.Christensen <i>Fucus lanosus</i> Linnaeus <i>Polysiphonia lanosa</i> (Linnaeus) Tandy	Holotype: LINN 1274.23 Iceland, undated
<i>Vertebrata simulans</i> (Harvey) Kuntze <i>Polysiphonia simulans</i> Harvey	Lectotype (Maggs & Hommersand, 1993): BM-K Devon, England; 20.v.1831
<i>Vertebrata stimpsonii</i> (Harvey) Kuntze <i>Polysiphonia stimpsonii</i> Harvey <i>Enelittosiphonia stimpsonii</i> (Harvey) Kudo & Masuda	Holotype: TCD 11956 Hakodate Bay, Japan
<i>Vertebrata subulifera</i> (C.Agardh) Kuntze <i>Hutchinsia subulifera</i> C.Agardh <i>Polysiphonia subulifera</i> (C.Agardh) Harvey	Lectotype (Maggs & Hommersand, 1993): LD 41607 Venice, Italy; undated
<i>Vertebrata thuyoides</i> (Harvey) Kuntze <i>Polysiphonia thuyoides</i> Harvey <i>Boergeseniella thuyoides</i> (Harvey) Kylin	Lectotype (Maggs & Hommersand, 1993): TCD Milltown Malbay, Ireland; 1831
<i>Vertebrata tripinnata</i> (J.Agardh) O.Kuntze <i>Polysiphonia tripinnata</i> J.Agardh (1842, p. 142; <i>Algae maris Mediterranei et Adriatici</i> , observationes in diagnosis specierum et dispositionem generum. Apud Fortin, Masson et Cie, Paris) [Kuntze transferred ' <i>Polysiphonia tripinnata</i> Harvey' to <i>Vertebrata</i> , presumably a typographical error as the basionym is <i>P. tripinnata</i> J.Agardh (1842)]	Lectotype (Díaz-Tapia <i>et al.</i> , 2013b): LD J. Agardh's Herbarium 40938 Trieste, Italy
<i>Vertebrata urbana</i> (Harvey) Kuntze <i>Polysiphonia urbana</i> Harvey	Probable holotype: TCD 186 Table Bay, Cape Province, South Africa

Note: The positions of *Polysiphonia paniculata* in the *rbcl* and 18S trees are not congruent. This suggests that these two sequences, generated from samples from Chile and California, respectively (Table S1), were obtained from different species. The assignment of this species to the genus *Vertebrata* therefore requires further study to clarify the identity of the two published sequences. The type locality is Peru.

Reactions were performed in a total volume of 25 µl, consisting of 5 µl 5× MyTaqTM reaction buffer, 0.7 µl 10 µM of forward and reverse primers, 0.125 µl 1U µl⁻¹ My TaqTM DNA Polymerase (Bioline), 17.475 µl MilliQ[®] water and 1 µl template DNA. The PCR profile consisted of initial denaturation (93°C for 3 min); 35 cycles of denaturation (94°C for 30 s), primer annealing (45°C for 30 s), and extension (74°C for 90 s); and final extension (74°C for 5 min). The PCR products were purified and sequenced commercially by MacroGen.

At A Coruña, *Melanothamnus* from Oman was extracted using the CTAB protocol (Doyle & Doyle, 1987) and *rbcl* was amplified using the primers F7-

R753 and F57-*rbcl*revNEW (Freshwater & Rueness, 1994; Saunders & Moore, 2013). The PCR products were purified and sequenced commercially by the sequencing service of the University of A Coruña.

DNA extraction, amplification and sequencing at UNCW were as described by Stuercke & Freshwater (2008).

Sequence alignment and phylogenetic analysis

A total of 65 *rbcl* and 48 18S sequences were downloaded from GenBank and 25 new *rbcl* and 10 new 18S sequences were generated in this study. The

Table 4. Genus *Melanothamnus* with new combinations resulting from the present study printed in bold, followed by basionyms and taxonomic synonyms.

New combination (if any) Basionym Synonyms	Type material Type locality, collection date	Notes
<i>Melanothamnus somalensis</i> Bornet & Falkenberg	Lectotype (here designated): PC 584992 Somalia	Figs 3–8
<i>Melanothamnus afaqhusainii</i> M.Shameel	Holotype: KUH-SW SAH-127 Pakistan	
<i>Melanothamnus unilateralis</i> (Levring) Díaz-Tapia & Maggs, comb. nov.	Holotype: GB Juan Fernández Islands, Chile	Figs 9–18
<i>Fernandosiphonia unilateralis</i> Levring (1941, pp. 660–662; Die Meeresalgen der Juan Fernandez-Inseln. Die Corallinaceen der Juan Fernandez-Inseln. In: <i>The natural history of Juan Fernandez and Easter Island</i> (Skottsberg, C., editor) Vol. 2, 601–670; 753–757. Almqvist & Wiksells Boktryckeri, Uppsala)		
<i>Melanothamnus apiculatus</i> (Hollenberg) Díaz-Tapia & Maggs, comb. nov.	Holotype: US 48522 O'ahu Island, Hawai'i; 30.vii.1959	3-celled carpogonial branches (Kim & Abbott, 2006); plastid character (Hollenberg 1968a; fig. 9)
<i>Polysiphonia apiculata</i> Hollenberg (1968a, p. 61; An account of the species of <i>Polysiphonia</i> of the central and western tropical Pacific ocean. II <i>Oligosiphonia</i> . <i>Pacific Science</i> , 22: 56–98)		
<i>Neosiphonia apiculata</i> (Hollenberg) Masuda & Kogame		
<i>Melanothamnus bajacali</i> (Hollenberg) Díaz-Tapia & Maggs, comb. nov.	Holotype: AHFH Isla Guadalupe, Baja California, Mexico; 18.xii. 1949	'Cell walls hyaline' (Hollenberg, 1961). Molecular data available from Yucatan, Mexico (Mamoozadeh & Freshwater, 2011)
<i>Polysiphonia bajacali</i> Hollenberg (1961, pp. 347–348; Marine red algae of Pacific Mexico, Part 5: The genus <i>Polysiphonia</i> . <i>Pacific Naturalist</i> , 2: 345–375)		
<i>Neosiphonia bajacali</i> (Hollenberg) N.R.Mamoozadeh & D.W. Freshwater		
<i>Melanothamnus balianus</i> (D.E.Bustamante, B.Y. Won & T.O.Cho) Díaz-Tapia & Maggs, comb. nov.	Holotype: CUK 7937 Blue Lagoon beach, Padang Bai, Karangasem, Bali, Indonesia; 27.iv. 2012.	Molecular data from the type locality (Bustamante <i>et al.</i> , 2013b)
<i>Neosiphonia baliana</i> D.E.Bustamante, B.Y.Won & T.O.Cho (2013b, pp. 516–518; <i>Neosiphonia baliana</i> sp. nov. and <i>N. silvae</i> sp. nov. (Rhodomelaceae, Rhodophyta) from Bali, Indonesia. <i>Botanica Marina</i> , 56: 515–524)		
<i>Melanothamnus blandii</i> (Harvey) Díaz-Tapia & Maggs, comb. nov.	Lectotype (Womersley, 1979): TCD Brighton, Port Phillip, Victoria, Australia	3-celled carpogonial branches; plastid character. Molecular data available from the type locality (this work)
<i>Polysiphonia blandii</i> Harvey (1862, pl. 184; <i>Phycologia australica</i> Vol. 4. Lovell Reeve & Co, London)		
<i>Melanothamnus cheloniae</i> (Hollenberg & J.N. Norris) Díaz-Tapia & Maggs, comb. nov.	Holotype: US 160602 Sonora, Gulf of California, Mexico; 21.i.1974	Plastid character (Hollenberg & Norris, 1977; fig. 4B)
<i>Polysiphonia sphaerocarpa</i> var. <i>cheloniae</i> Hollenberg & J.N.Norris (1977, p. 16–17; The red alga <i>Polysiphonia</i> (Rhodomelaceae) in the Northern Gulf of California. <i>Smithsonian Contributions to the Marine Sciences</i> , 1: 1–21)		
<i>Neosiphonia cheloniae</i> (Hollenberg & J.N.Norris) J.N. Norris		
<i>Melanothamnus collabens</i> (C.Agardh) Díaz-Tapia & Maggs, comb. nov.	Syntypes: LD Agardh herbarium 40885–40887 and 40890–40898; Cádiz, Spain	3-celled carpogonial branches; plastid character; molecular data available from the type locality (Díaz-Tapia & Bárbara, 2013)
<i>Hutchinsia collabens</i> C.Agardh (1824, p. 153; <i>Systema algarum</i> . Berlinginiana, Lundae)		
<i>Polysiphonia collabens</i> (C.Agardh) Kützing		
<i>Streblocladia collabens</i> (C.Agardh) Falkenberg		
<i>Neosiphonia collabens</i> (C.Agardh) Díaz-Tapia & Bárbara		
<i>Melanothamnus concinnus</i> (Hollenberg) Díaz-Tapia & Maggs, comb. nov.	Holotype: US 61210; La Jolla, California, USA; 26. xii.1936	Plastid character observed in our study of the type material
<i>Polysiphonia concinna</i> Hollenberg (1944, pp. 474–475; An account of the species of <i>Polysiphonia</i> on the Pacific coast of North America. II. <i>Polysiphonia</i> . <i>American Journal of Botany</i> , 31: 474–483)		
<i>Polysiphonia johnstonii</i> var. <i>concinna</i> (Hollenberg) Hollenberg		
<i>Neosiphonia concinna</i> (Hollenberg) J.N.Norris		

(Continued)

Table 4. (Continued).

New combination (if any) Basionym Synonyms	Type material Type locality, collection date	Notes
Melanothamnus decumbens (T.Segi) Díaz-Tapia & Maggs, comb. nov. <i>Polysiphonia decumbens</i> T.Segi (1951, p. 218; Systematic study of the genus <i>Polysiphonia</i> from Japan and its vicinity. <i>Journal of the Faculty of Fisheries, Prefectural University of Mie</i> , 1: 169–272) <i>Neosiphonia decumbens</i> (T.Segi) M.-S.Kim & I.K.Lee	Holotype: SAP 25880; Mihonoseki, Shimane Prefecture, Japan; vi.1948	3-celled carpogonial branches; plastid character (Kim, 2003, fig. 5F). Molecular data available from Korea (Kim & Yang, 2006)
Melanothamnus ecorticatus (R.E.Norris) Díaz-Tapia & Maggs, comb. nov. <i>Fernandosiphonia ecorticata</i> R.E.Norris (1994, p. 434; Some cumophytic Rhodomelaceae (Rhodophyta) occurring in Hawaiian surf. <i>Phycologia</i> 33: 434–443)	Holotype: BISH 630042 Keokea Bay, Hawai'i; v.1990	Plastid character; ostiolar cells larger than other pericarpial cells (Abbott, 1999)
Melanothamnus eastwoodiae (Setchell & N.L. Gardner) Díaz-Tapia & Maggs, comb. nov. <i>Polysiphonia eastwoodiae</i> Setchell & N.L.Gardner (1930, p. 161, as <i>P. eastwoodae</i> ; Marine algae of the Revillagigedo Islands expedition in 1925. <i>Proceedings of the California Academy of Sciences</i> , 4: 109–215) <i>Neosiphonia eastwoodae</i> (Setchell & N.L.Gardner) Xiang Si-duan	Holotype: CAS 173674 Islas Revillagigedo	Plastid character observed in type material
Melanothamnus ferulaceus (Suhr ex J.Agardh) Díaz-Tapia & Maggs, comb. nov. <i>Polysiphonia ferulacea</i> Suhr ex J.Agardh (1863, p. 980; <i>Species Genera et Ordines Algarum</i> C.W.K. Gleerup, Lundae) <i>Neosiphonia ferulacea</i> (Suhr ex J.Agardh) S.M. Guimaraes & M.T.Fujii	Type materials are in LD, J.Agardh's Herbarium (not seen) Atlantic Mexico; North America; Guadeloupe; Hawaiian Islands; Marquesas Islands; Australia	Plastid character in Panama and Brazil. Molecular data from Panama (Guimaraes <i>et al.</i> , 2004; Mamoozadeh & Freshwater, 2012)
Melanothamnus fibrillosus (Okamura) Díaz-Tapia & Maggs, comb. nov. <i>Pterosiphonia fibrillosa</i> Okamura (1912, p. 172; <i>Icones of Japanese Algae. Vol. II (10)</i> . Privately published, Tokyo) <i>Kintarosiphonia fibrillosa</i> (Okamura) S. Uwai & Masuda	Lectotype (Uwai & Masuda, 1999): SAP Shirahama, Chiba Prefecture, Japan; undated	3-celled carpogonial branches, plastid character, cells surrounding the ostiole much larger than the cells below (Uwai & Masuda, 1999, figs 18, 19).
Melanothamnus flavimarinus (M.-S.Kim & I.K.Lee) Díaz-Tapia & Maggs, comb. nov. <i>Neosiphonia flavimarina</i> M.-S.Kim & I.K.Lee (1999, p. 272; <i>Neosiphonia flavimarina</i> gen. et sp. nov. with a taxonomic reassessment of the genus <i>Polysiphonia</i> (Rhodomelaceae, Rhodophyta). <i>Phycological Research</i> , 47: 271–281)	Holotype: SNU IBA001 Bangpo, Anmyondo, Korea; 17. vii.1988.	3-celled carpogonial branches; plastid character (Kim & Lee, 1999, fig. 5). Molecular data available from the type locality (Kim & Yang, 2006)
Melanothamnus forfex (Harvey) Díaz-Tapia & Maggs, comb. nov. <i>Polysiphonia forfex</i> Harvey (1859, pl. XCVI; <i>Phycologia Australica</i> Vol. 2. Lovell Reeve & Co, London)	Lectotype (Womersley, 1979): TCD 15353-4 Rottneest Island, Western Australia	3-celled carpogonial branches; plastid character, molecular data available from the type locality (this work)
Melanothamnus gorgoniae (Harvey) Díaz-Tapia & Maggs, comb. nov. <i>Polysiphonia gorgoniae</i> Harvey (1853, p. 39; <i>Nereis boreali-americana</i> <i>Smithsonian Contributions to Knowledge</i> , 5: [i–ii], [1]–258, pls XIII–XXXVI) <i>Neosiphonia gorgoniae</i> (Harvey) S.M.Guimaraes & M. T.Fujii	Syntypes: TCD 12801-4, NY 900637-8 Key West, Florida, USA	3-celled carpogonial branches observed in Brazil (Guimaraes <i>et al.</i> , 2004); plastid character observed in type material (this work)
Melanothamnus harlandii (Harvey) Díaz-Tapia & Maggs, comb. nov. <i>Polysiphonia harlandii</i> Harvey (1860, p. 330; <i>Characters of new algae</i> <i>Proceedings of the American Academy of Arts and Sciences</i> , 4: 327–335) <i>Neosiphonia harlandii</i> (Harvey) M.S.Kim & I.K.Lee	Probable syntypes: TCD 11955, US 56848 Hong Kong	3-celled carpogonial branches. Molecular data available from Korea (Kim, 2003; Kim & Yang, 2006)
Melanothamnus harveyi (Bailey) Díaz-Tapia & Maggs, comb. nov. <i>Polysiphonia harveyi</i> Bailey (1848, p. 38; Continuation of the list of localities of algae in the United States. <i>Proceedings of the American Academy of Arts and Sciences</i> , 4: 327–335) <i>Neosiphonia harveyi</i> (Bailey) M.-S.Kim, H.-G.Choi, Guiry & G.W.Saunders	Lectotype (Maggs & Hommersand, 1993): TCD 12810 Bailey; Stonington, Connecticut, USA; vii.1847	3-celled carpogonial branches (this work); plastid character. Molecular data available from the type locality (McIvor <i>et al.</i> , 2001)

(Continued)

Table 4. (Continued).

New combination (if any) Basionym Synonyms	Type material Type locality, collection date	Notes
Melanothamnus hawaiiensis (Hollenberg) Díaz-Tapia & Maggs, comb. nov. <i>Polysiphonia hawaiiensis</i> Hollenberg (1968a, pp. 66–67; An account of the species of <i>Polysiphonia</i> of the central and western tropical Pacific ocean. II <i>Oligosiphonia. Pacific Science</i> , 22: 56–98) <i>Neosiphonia hawaiiensis</i> (Hollenberg) M.-S.Kim & I. A. Abbott	Holotype: US 48524 Waikiki beach, O'ahu Island, Hawai'i; 21.i.1963	3-celled carpogonial branches (Kim & Abbott, 2006). Plastid character (Abbott, 1999, fig. 122C)
Melanothamnus incomptus (Harvey) Díaz-Tapia & Maggs, comb. nov. <i>Polysiphonia incompta</i> Harvey (1847, p. 44; <i>Nereis australis</i> Reeve Brothers, London)	Probable holotype: TCD 192 False Bay, Cape Province, South Africa	Plastid character. Molecular data available from the type locality (this work)
Melanothamnus japonicus (Harvey) Díaz-Tapia & Maggs, comb. nov. <i>Polysiphonia japonica</i> Harvey (in M.C. Perry 1857, p. 331; <i>Account of the Botanical specimens</i> . (Gray, A., editor) <i>Narrative of the expedition of an American squadron to the China Seas and Japan</i> Senate of the Thirty-third Congress, Second Session, Executive Document. House of Representatives, Washington) <i>Neosiphonia japonica</i> (Harvey) M.S.Kim & I.K.Lee	Lectotype (Masuda <i>et al.</i> , 1995): TCD 11905 Hakodate, Japan; v.1854	Plastid character (this work); 3-celled carpogonial branches. Molecular data available from the type locality (Kim & Yang, 2006)
Melanothamnus johnstonii (Setchell & Gardner) Díaz-Tapia & Maggs, comb. nov. <i>Polysiphonia johnstonii</i> Setchell & Gardner (1924, p. 767; XXIX Expedition of the California Academy of Sciences to the Gulf of California in 1921. <i>The Marine Algae. Proceeding of the California Academy of Science, Series 4</i> 12: 695–949) <i>Neosiphonia johnstonii</i> (Setchell & N.L.Gardner) J.N. Norris	Holotype: CAS1361 San Esteban Island, Gulf of California; iv.1921	Plastid character. Molecular data available from the type locality (this work). The sequence from California (KX756670) is only 0.1–0.2% divergent in its <i>rbcL</i> sequence from <i>M. collabens</i> from Spain. Further studies are needed to clarify the possible synonymy between these two species that share the unusual character of having (5–) 6 pericentral cells
Melanothamnus nudus (N.R.Mamoozadeh & D.W. Freshwater) D.W.Freshwater, comb. nov. <i>Polysiphonia nuda</i> N.R.Mamoozadeh & D.W. Freshwater (2012, p. 335; <i>Polysiphonia sensu lato</i> (Ceramiales, Florideophyceae) species of Caribbean Panama including <i>Polysiphonia lobophoralis</i> sp. nov. and <i>Polysiphonia nuda</i> sp. nov. <i>Botanica Marina</i> , 55: 317–347)	Holotype: US 211334 Parque de Juventud, Calle Primero, Colon, Caribbean coast of Panama; 20.v.2009	Molecular data available from the type locality (Mamoozadeh & Freshwater, 2012)
Melanothamnus peruviansis (D.E.Bustamante, B.Y. Won, M.E.Ramirez & T.O.Cho) Díaz-Tapia & Maggs, comb. nov. <i>Neosiphonia peruviansis</i> D.E.Bustamante, B.Y.Won, M.E.Ramirez & T.O.Cho (2012, p. 360; <i>Neosiphonia peruviansis</i> sp. nov. (Rhodomelaceae, Rhodophyta) from the Pacific coast of South America. <i>Botanica Marina</i> , 55: 359–366)	Holotype: CUK 7976 Lagunillas, Pisco, Ica, southern coast of Lima, Peru; 21.viii.2008	Plastid character. Molecular data available from the type locality (Bustamante <i>et al.</i> , 2012, fig. 10)
Melanothamnus platycarpus (Børgesen) Díaz-Tapia & Maggs, comb. nov. <i>Polysiphonia platycarpa</i> Børgesen (1934, p. 23; Some Indian Rhodophyceae especially from the shores of the Presidency of Bombay-IV. <i>Bulletin of Miscellaneous Information, Royal Botanic Gardens, Kew</i> , 1934: 1–30)	Probable syntypes: BM 1067681 and 106760 Bombay, India; 19.xii.1927	3-celled carpogonial branches; plastid character (Iyengar & Balakrishnan, 1949, fig. 1)
Melanothamnus pseudovillum (Hollenberg) Díaz-Tapia & Maggs, comb. nov. <i>Polysiphonia pseudovillum</i> Hollenberg (1968a, p. 73; An account of the species of <i>Polysiphonia</i> of the central and western tropical Pacific ocean. II <i>Oligosiphonia. Pacific Science</i> , 22: 56–98)	Holotype: US 61232; North Island, Johnston Islands; 22.vi.1965	Cell walls 'hyaline' (Hollenberg, 1968a). Molecular data available from Panama (Mamoozadeh & Freshwater, 2011)
Melanothamnus ramireziae (D.E.Bustamante, B.Y. Won & T.O.Cho) Díaz-Tapia & Maggs, comb. nov. <i>Neosiphonia ramirezii</i> D.E.Bustamante, B.Y.Won & T.O.Cho (2013a, <i>Neosiphonia ramirezii</i> sp. nov. (Rhodomelaceae, Rhodophyta) from Peru. <i>Algae</i> , 28: 73–82)	Holotype: CUK 6511 Lagunillas, Pisco, Ica, Peru; 21.viii.2008	Plastid character, 3-celled carpogonial branches. Molecular data available from the type locality (Bustamante <i>et al.</i> , 2013a, fig. 1f)
Melanothamnus silvae (D.E.Bustamante, B.Y.Won & T.O.Cho) Díaz-Tapia & Maggs, comb. nov. <i>Neosiphonia silvae</i> D.E.Bustamante, B.Y.Won & T.O.Cho (2013b, pp. 518–520; <i>Neosiphonia baliana</i> sp. nov. and <i>N. silvae</i> sp. nov. (Rhodomelaceae, Rhodophyta) from Bali, Indonesia. <i>Botanica Marina</i> , 56: 515–524)	Holotype: CUK 7976 Geger, Nusadua, Bali, Indonesia; 26.iv.2012	Plastid character. Molecular data available from the type locality (Bustamante <i>et al.</i> , 2013b, figs 22–23)

(Continued)

Table 4. (Continued).

New combination (if any) Basionym Synonyms	Type material Type locality, collection date	Notes
Melanothamnus simplex (Hollenberg) Díaz-Tapia & Maggs, comb. nov. <i>Polysiphonia simplex</i> Hollenberg, (1942, p. 782; An account of the species of <i>Polysiphonia</i> on the Pacific coast of North America. I. Oligosiphonia. <i>American Journal of Botany</i> , 29: 772–785) <i>Neosiphonia simplex</i> (Hollenberg) Y.-P.Lee	Holotype: US 61238 Laguna Beach, Orange County, California, USA; 14.v.1937	Plastid character. Molecular data available from the type locality (this work). <i>RbcL</i> sequence not included in our phylogeny because it is only 1% divergent from <i>N. ramirezii</i>
Melanothamnus sphaerocarpus (Børgesen) Díaz-Tapia & Maggs, comb. nov. <i>Polysiphonia sphaerocarpa</i> Børgesen (1918, p. 271; The marine algae of the Danish West Indies. Part 3. Rhodophyceae (4). <i>Dansk Botanisk Arkiv</i> , 3: 241–304) <i>Neosiphonia sphaerocarpa</i> (Børgesen) M.-S.Kim & I. K.Lee	Isotypes: US, C Store Nordsidebugt, St. Thomas, Virgin Islands	Plastid character. Molecular data available from Florida (Mamoozadeh & Freshwater, 2011, fig. 18)
Melanothamnus strictissimus (J.D.Hooker & Harvey) Díaz-Tapia & Maggs, comb. nov. <i>Polysiphonia strictissima</i> J.D.Hooker & Harvey (1845, p. 538; <i>Algae Novae Zelandiae</i> <i>London Journal of Botany</i> , 4: 521–551)	Probable syntype: BM 561312 New Zealand	Plastid character (this work). Molecular data available from the type locality (Stuercke & Freshwater, 2010)
Melanothamnus thailandicus (N.Muangmai & C. Kaewsuralikhit) Díaz-Tapia & Maggs, comb. nov. <i>Neosiphonia thailandica</i> N.Muangmai & C. Kaewsuralikhit (2014, pp. 460–461; The new species <i>Neosiphonia thailandica</i> sp. nov. (Rhodomelaceae, Rhodophyta) from the Gulf of Thailand. <i>Botanica Marina</i> , 57: 459–467)	Holotype: KUMF-SRC 03-011-1 Sri Racha Harbor, Chon Buri, Thailand; 11.iii.2011	3-celled carpogonial branches. Molecular data available from the type locality (Muangmai <i>et al.</i> , 2014)
Melanothamnus yongpili (B.Kim & M.-S.Kim) Díaz-Tapia & Maggs, comb. nov. <i>Neosiphonia yongpili</i> B.Kim & M.-S.Kim (2016, pp. 324–325; <i>Neosiphonia yongpili</i> sp. nov. (Rhodomelaceae, Rhodophyta), known as <i>Neosiphonia simplex</i> from Korea, with an emphasis on cystocarp development. <i>Phycologia</i> , 55: 323–332)	Holotype: JNUB 140704-101 Jongdal, Jeju Island, Korea; 04. iv.2014	3-celled carpogonial branches; plastid character. Molecular data available from the type locality (Kim & Kim, 2016, fig. 14)

Note: Here we include only species that we can confidently assign to this genus (i.e. we have examined type material or suitable pictures of the type material showing the plastid character and/or sequences are available from the type locality or nearby).

sequences and their corresponding GenBank accession numbers are listed in Table S1.

Sequences were aligned using Muscle in Geneious 6.1.8 (Kearse *et al.*, 2012). Identical sequences and those that diverged by less than 1.1% were removed from the *rbcL* analysis, except for *Neosiphonia flavimarina* and *N. harveyi* (0.4% divergence), the two selected representatives of the ‘*N. japonica* complex’ (Kim & Lee, 1999), which also includes *N. decumbens*, *N. harlandii* and *P. akkeshiensis* Segi (McIvor *et al.*, 2001; Kim & Yang, 2006; Bárbara *et al.*, 2013; Savoie & Saunders, 2015). Identical sequences were also removed from the 18S analysis. The sequences included in the final alignment were selected after considering their quality in terms of both length and the presence of ambiguous bases. Phylogenetic trees for *rbcL* and 18S were estimated with Maximum likelihood (ML) using RAxML 8.1.6 (Stamatakis, 2014). GTR-Gamma was selected as the best nucleotide model; branch support was estimated with 100 bootstrap replicates. Three species of *Symphycladia* Falkenberg (in Schmitz & Falkenberg, 1897) were selected as the outgroup in the *rbcL* phylogeny and one species each of *Symphycladia*, *Xiphosiphonia* Savoie & Saunders (2016) and *Herposiphonia* Nägeli

(1846) were selected as outgroups for the 18S analysis. This outgroup selection was based on our phylogenomic analyses of the major lineages of the Rhodomelaceae which resolve a clade formed by the Herposiphonieae and Pterosiphonieae as sister to the Polysiphonieae (Díaz-Tapia *et al.*, 2015).

We used MrBayes v.3.2.2 for Bayesian phylogenetic inference (Ronquist *et al.*, 2011). The *rbcL* alignment was analysed using a single (unpartitioned) GTR+Γ+I as well as completely unlinked GTR+Γ+I for each codon position. We used a single GTR+Γ+I model for 18S. All analyses were run for 5 million generations, sampling every 1000th generation and using two independent runs each consisting of four incrementally heated Metropolis-coupled (MCMC) chains. Convergence and stationarity of runs were evaluated with Tracer v.1.6.0 (Rambaut *et al.*, 2013), resulting in the use of a burn-in of 500k generations for all analyses. Post-burn-in trees were summarized with the sump command in MrBayes, using the all-compatible-groups consensus type.

Trees were calibrated in geological time using relaxed molecular clock analyses. The calibration was derived from node ages inferred by Yang *et al.* (2016), which estimated the earliest split in

Table 5. New combinations in *Melanothamnus* made for formal reasons (because the current genus is here placed in synonymy with *Melanothamnus*) although type material has not been examined. They are printed in bold, followed by basionyms and taxonomic synonyms.

Binomial in <i>Melanothamnus</i> (if any) Basionym Synonyms	Type material Type locality	Notes
<i>Melanothamnus hancockii</i> (E.Y.Dawson) Díaz-Tapia & Maggs, comb. nov. <i>Polysiphonia hancockii</i> E.Y.Dawson (1944, pp. 331–332; The marine algae of the Gulf of California. Allan Hancock Pacific Expeditions 3: 189–432) <i>Fernandosiphonia hancockii</i> (E.Y.Dawson) R.E.Norris	Holotype: LAM EYD629c Baja California, Mexico; 16.ii.1940	Plastid character. Molecular data from Japan (this work)
<i>Melanothamnus masonii</i> (Setchell & N.L.Gardner) Díaz-Tapia & Maggs, comb. nov. <i>Polysiphonia masonii</i> Setchell & Gardner (1930, p. 160; Marine algae of the Revillagigedo Islands expedition in 1925. <i>Proceedings of the California Academy of Sciences</i> , 4: 109–215) <i>Neosiphonia masonii</i> (Setchell & N.L.Gardner) J.N.Norris	Holotype: CAS 173618 Isla Guadalupe, México; iv.1925	
<i>Melanothamnus minutissimus</i> (Hollenberg) Díaz-Tapia & Maggs, comb. nov. <i>Polysiphonia minutissima</i> Hollenberg (1942, p. 781, An account of the species of <i>Polysiphonia</i> on the Pacific coast of North America. I. Oligosiphonia. <i>American Journal of Botany</i> , 29: 772–785)	Holotype: US 66797 Punta Banda, Baja California, Mexico; 17. xii.1938	Plastid character. Molecular data from Japan (this work)
<i>Melanothamnus nanus</i> (A.J.K.Millar) Díaz-Tapia & Maggs, comb. nov. <i>Fernandosiphonia nana</i> A.J.K.Millar (1990, p. 439; Marine red algae of the Coffs Harbour region, northern New South Wales. <i>Australian Systematic Botany</i> , 3: 293–593)	Holotype: MELU AM752 Coffs Harbour, New South Wales; 27. viii.1980	
<i>Melanothamnus notoensis</i> (Segi) Díaz-Tapia & Maggs, comb. nov. <i>Polysiphonia notoensis</i> Segi (1951, p. 266; Systematic study of the genus <i>Polysiphonia</i> from Japan and its vicinity. <i>Journal of the Faculty of Fisheries, Prefectural University of Mie</i> , 1: 169–272) <i>Neosiphonia notoensis</i> (Segi) M.S.Kim & I.K.Lee	Holotype: SAP 025894 Shibagaki, Ishikawa Prefecture, Japan; 9. vii.1947	Plastid character in Korea (Nam & Kang, 2012; fig. 47E)
<i>Melanothamnus polyphysus</i> (Kützing) Díaz-Tapia & Maggs, comb. nov. <i>Polysiphonia polyphysa</i> Kützing (1863, p. 20; <i>Tabulae phycologicae; oder, Abbildungen der Tange. Vol. XIII.</i> Gedruckt auf kosten des Verfassers (in commission bei W. Köhne), Nordhausen) <i>Neosiphonia polyphysa</i> (Kützing) Skelton & G.R.South	Holotype: L 4082747 Vieillard; New Caledonia; undated	
<i>Melanothamnus porrectus</i> (T.Segi) Díaz-Tapia & Maggs, comb. nov. <i>Polysiphonia porrecta</i> Segi (1951, p. 260; Systematic study of the genus <i>Polysiphonia</i> from Japan and its vicinity. <i>Journal of the Faculty of Fisheries, Prefectural University of Mie</i> , 1: 169–272) <i>Neosiphonia porrecta</i> (Segi) Y.-P. Lee	Holotype: SAP 025867 Henashi, Nishitsugaru-gun, Aomori Prefecture, Japan; 19. vi.1948.	Plastid character in Korea (Lee, 2008, p. 314, fig. C)
<i>Melanothamnus savatieri</i> (Hariot) Díaz-Tapia & Maggs, comb. nov. <i>Polysiphonia savatieri</i> Hariot (1891, p. 226; Liste des algues marines rapportés de Yokoska (Japon) par M. le Dr Savatier. <i>Mémoires de la Société nationale des sciences naturelles de Cherbourg</i> , 27: 211–230) <i>Neosiphonia savatieri</i> (Hariot) M.S.Kim & I.K.Lee	Lectotype (Kim, 2005): PC 0011879 Yokosuka, Kanagawa Prefecture, Japan	3-celled carpo gonial branches in Malaysia (Masuda <i>et al.</i> , 2001). Molecular data available from Korea (Phillips <i>et al.</i> , 2000)
<i>Melanothamnus sparsus</i> (Setchell) Díaz-Tapia & Maggs, comb. nov. <i>Lophosiphonia sparsa</i> Setchell (1926, p. 103; Tahitian algae collected by W.A. Setchell, C.B. Setchell and H.E. Parks. <i>University of California Publications in Botany</i> , 12: 61–142) <i>Polysiphonia sparsa</i> (Setchell) Hollenberg <i>Neosiphonia sparsa</i> (Setchell) I.A.Abbott	Holotype: UC 261144; Arue Reef, Tahiti; 27. vi.1922	Plastid character in Vietnam (Abbott <i>et al.</i> , 2002; fig. 27)
<i>Melanothamnus teradomariensis</i> (M.Noda) Díaz-Tapia & Maggs, comb. nov. <i>Polysiphonia teradomariensis</i> M.Noda (in Noda, M. & Kitami, T. 1971, 47; Some species of marine algae from Echigo Province facing the Japan Sea. <i>Scientific Reports Niigata University, Ser. D. (Biology)</i> , 8: 35–52) <i>Polysiphonia japonica</i> var. <i>teradomariensis</i> (M.Noda) H. Y.Yoon <i>Neosiphonia teradomariensis</i> (M.Noda) M.-S.Kim & I.K. Lee	Holotype: Herbarium Niigata University Echigo Province, Japan; 28.xi.1968	Molecular data available from Korea (Bárbara <i>et al.</i> , 2013)

(Continued)

Table 5. (Continued).

Binomial in <i>Melanothamnus</i> (if any) Basionym Synonyms	Type material Type locality	Notes
<i>Melanothamnus tongatensis</i> (Harvey ex Kützing) Díaz-Tapia & Maggs, comb. nov. <i>Polysiphonia tongatensis</i> Harvey ex Kützing, (1864, p. 14; <i>Tabulae phycologicae; oder, Abbildungen der Tange</i> . Vol. XIV. Gedruckt auf kosten des Verfassers (in commission bei W. Köhne), Nordhausen) <i>Neosiphonia tongatensis</i> (Harvey ex Kützing) M.-S.Kim & I.K.Lee	Holotype: L 4083619 Tonga, Friendly Islands; undated	Molecular data available from Panama (Mamoozadeh & Freshwater, 2011)
<i>Melanothamnus upolensis</i> (Grunow) Díaz-Tapia & Maggs, comb. nov. <i>Polysiphonia upolensis</i> Grunow (1874, p. 49; <i>Algen der Fidschi-, Tonga- und Samoa-Inseln, gesammelt von Dr. E. Graeffe. Journal des Museum Godeffroy</i> , 3: 23–50) <i>Neosiphonia upolensis</i> (Grunow) M.S.Kim & Boo	Syntypes: W Upolu, Western Samoa	Molecular data available from Hawaii (Sherwood <i>et al.</i> , 2010)
<i>Melanothamnus yendoii</i> (T.Segi) Díaz-Tapia & Maggs, comb. nov. <i>Polysiphonia yendoii</i> Segi (1951, p. 211; Systematic study of the genus <i>Polysiphonia</i> from Japan and its vicinity. <i>Journal of the Faculty of Fisheries, Prefectural University of Mie</i> , 1: 169–272) <i>Neosiphonia yendoii</i> (Segi) M.-S.Kim & I.K.Lee	Holotype: SAP 0258883 Muran, Hokkaido, Japan; 30.iv.1935	Molecular data available from Korea (Bárbara <i>et al.</i> , 2013)
<i>Neosiphonia saccorhiza</i> (F.S.Collins & Hervey) J.M.C. Nunes & S.M.Guimarães, nom. inval. <i>Lophosiphonia saccorhiza</i> F.S.Collins & Hervey <i>Polysiphonia saccorhiza</i> (F.S.Collins & Hervey) Hollenberg	Isotypes: NY, Collins Herbarium Gibbet Island, Bermuda	Transfer to <i>Melanothamnus</i> is not made here as <i>N. saccorhiza</i> is an invalid combination (the basionym was not cited), and the phylogenetic affinities of <i>Lophosiphonia saccorhiza</i> are unknown.

Ceramiales (between *Spyridia* Harvey (1833) and the remaining Ceramiales) to be 292 Ma old (st dev \approx 24.6 Ma). After adding the *rbcL* sequences of Ceramiales from the Yang *et al.* (2016) study to our alignment and setting *Spyridia* as the outgroup, node ages were inferred with two Bayesian methods. The first analysis used an autocorrelated model of molecular evolutionary rate change (Thorne & Kishino, 2002) as implemented in PhyloBayes v.3.3f (Lartillot *et al.*, 2009). The MCMC chain was run for 50k cycles, stationarity was assessed with Tracer, and the node ages summarized with the readdiv command, discarding the first 25k cycles as burn-in. The second analysis used uncorrelated rates of evolution sampled from a lognormal distribution (Drummond *et al.*, 2006) as implemented in BEAST v.1.8.2. The MCMC chain was run for 10 million generations, used a Yule tree prior, and an unpartitioned GTR+ Γ +I model of sequence evolution. Stationarity was assessed with Tracer. A maximum clade credibility tree and median node heights were inferred with TreeAnnotator, discarding the first 1 million generations as burn-in and using a posterior probability limit of zero.

Results

DNA sequences and alignments

DNA extraction and PCR amplification of type material of *Fernandosiphonia unilateralis* that had been initially formalin/seawater fixed and then stored in ethanol for several decades yielded a 95 bp partial

rbcL sequence with seven ambiguous nucleotides. The sequence was unique by comparison with other taxa sequenced either at QUB or in Leiden, confirming that there had been no contamination.

Twenty-five new *rbcL* sequences and ten 18S sequences were obtained from members of the Polysiphoniae (Table S1), including an *rbcL* sequence from *Melanothamnus somalensis*, and four *rbcL* and two 18S sequences from new collections of *F. unilateralis* from the type locality. Alignments for the *rbcL* were unambiguous, with no insertions or deletions.

Phylogenetic analyses

The ML *rbcL* tree (Fig. 1) has three strongly supported major clades within the Polysiphoniae: *Polysiphonia sensu stricto* 1 (including *P. stricta*, the type of the genus), *Polysiphonia sensu stricto* 2 (with morphological features corresponding to those defining *Polysiphonia sensu stricto*: Kim *et al.*, 2000) and a third clade grouping all the other taxa. The third clade comprised a large number of lineages, many with low or intermediate support. The two most speciose lineages, here named *Vertebrata* and *Melanothamnus*, however, are both robustly supported (Fig. 1). The *Vertebrata* clade includes *V. lanosa*, the current name for the type species of *Vertebrata*, *V. fastigiata* S.F.Gray (1821), as well as the type species of several other genera: *Brongniartella* Bory (1822), *Boergesenella* Kylin (1956), *Enelittosiphonia* Segi (1949) and *Ctenosiphonia* Falkenberg (in Schmitz & Falkenberg,

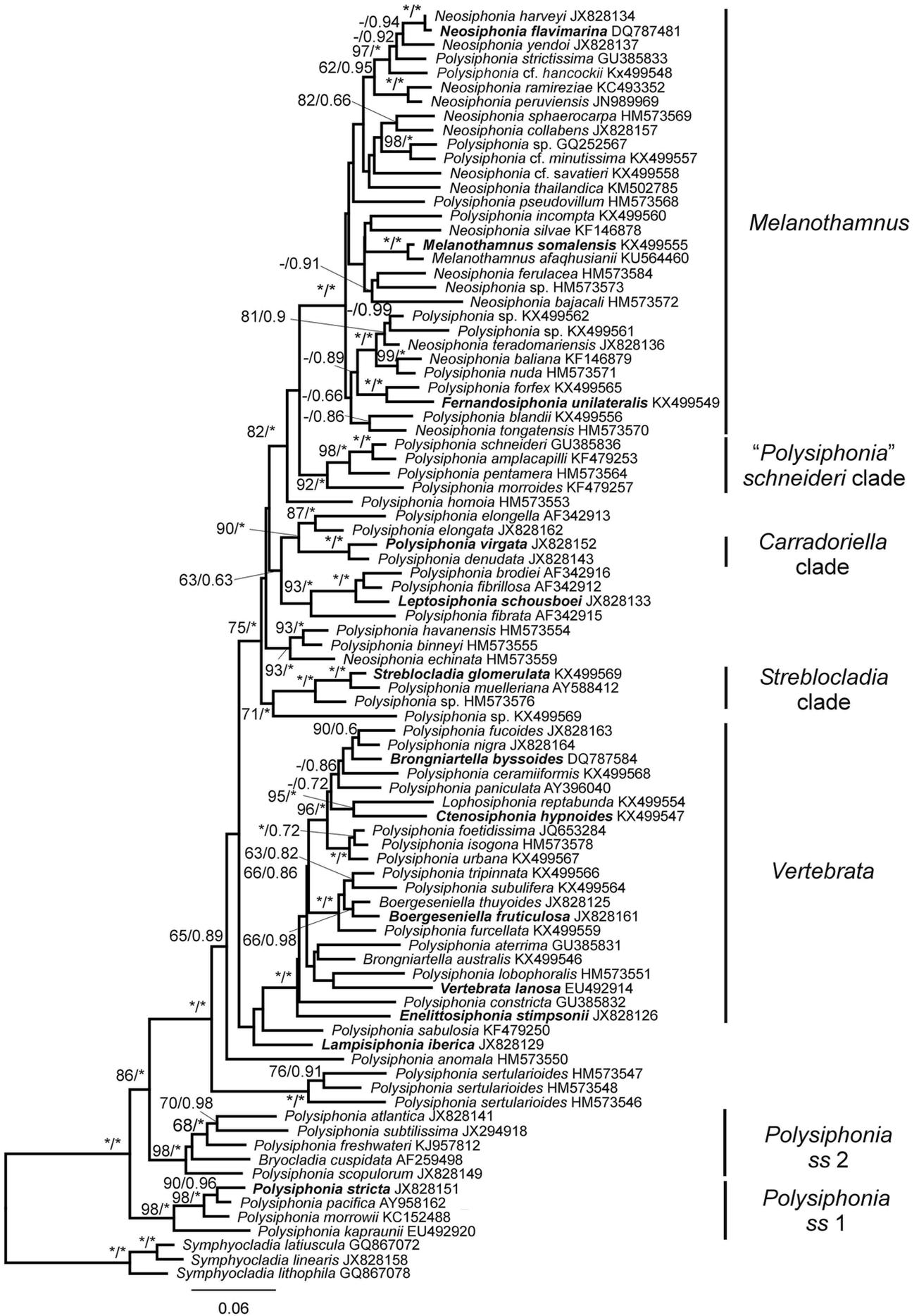


Fig. 1. Phylogenetic tree estimated with ML analysis of *rbcL* sequences. Values at nodes indicate bootstrap support (BP)/posterior probability (PP) (only shown if > 60/0.6). Branches marked with an asterisk received 100% (BP)/1.00 (PP) support. Species names printed in bold correspond to type species of genera.

1897). The *Melanothamnus* clade includes *Fernandosiphonia unilateralis*, *Neosiphonia flavimarina* and *M. somalensis*, the type species of their corresponding genera. In addition to these two large clades, six other lineages containing 3–4 species are highly supported (BP/PP > 90/0.95); among these are the *Carradoriella* clade including *Polysiphonia virgata*, the type species of *Carradoriella*, and the *Strebloladia* clade, which includes the type species *S. glomerulata*. Our phylogenetic tree also resolved five individual species as sisters to the other clades with low support.

The *Melanothamnus* clade receives support of 100/1.00 (Fig. 1). In addition to *F. unilateralis*, *N. flavimarina* and *M. somalensis*, this clade includes 27 other species currently assigned to *Neosiphonia* and *Polysiphonia*. The 95 bp sequence obtained from the type material of *F. unilateralis* analysed separately showed that this sequence was positioned unequivocally within the *Melanothamnus* clade, but sequence ambiguities due to the quality of the DNA made it impossible to determine its precise position.

The phylogenetic relationships among species within the *Melanothamnus* clade are generally poorly resolved, with a few exceptions. Although the lineage formed by '*Polysiphonia*' *schneideri*, '*P.*' *amplacapilli*, '*P.*' *pentamera* and '*P.*' *morroides* is very weakly positioned as sister to the *Melanothamnus* clade in the *rbcL* tree, in 18S analyses this position is robustly supported (see below).

The RAxML 18S tree (Fig. 2) has a similar topology to the *rbcL* phylogeny, with three strongly supported major clades: *Polysiphonia sensu stricto* 1 and 2 and a third clade with all the other taxa. *Polysiphonia sensu stricto* clades 1 and 2 are placed robustly together (99/1.00). Within the third clade, the *Vertebrata* clade receives full support, while support is lower for *Melanothamnus* (82/1.00). The sister relationship between the *Melanothamnus* and '*P.*' *schneideri* clades is strongly supported in the 18S phylogeny. In addition, the *Carradoriella* and *Strebloladia* clades are highly supported.

The time-calibrated phylogenies (Figs S1, S2) estimated the divergence in *Vertebrata* to be more ancient than in *Melanothamnus* (90.7–138.66 vs 75.7–95.78 Ma). Furthermore, the radiation of major lineages in *Vertebrata* and *Melanothamnus* was gradual and took place over periods of c. 20 and 12 Ma, respectively.

Morphological observations

An overview of the distribution of selected morphological characters within clades of the Polysiphonieae is shown in Table 1.

Habit: There is considerable variation within and among clades (Table 1), with the exception of the *Carradoriella*

and *Strebloladia* clades, in which all species are erect. True prostrate axes giving rise to erect axes, as opposed to decumbent axes that themselves become erect, are confined to *Polysiphonia sensu stricto* and *Vertebrata*. Most species of the *Melanothamnus* clade are completely erect or have a very short prostrate system. However, some taxa are decumbent (e.g. *Polysiphonia blandii*, *P. simplex*), forming extensive prostrate systems with rhizoids in the basal parts of the erect axes. Members of the Polysiphonieae are typically smaller than 10 cm. As an exception, *M. afaqhusainii* can exceed 1 m in length.

Rhizoids: The connection between the rhizoids and the pericentral cells from which they originate is a uniform character within each clade, so far as it can be observed (Table 1). Rhizoids are in open connection with pericentral cells in *Polysiphonia sensu stricto* (Fig. 19), while they are cut off from pericentral cells in the other clades (Figs 20–24). Observations on rhizoids cannot be made in mature specimens of some species, such as *Vertebrata lanosa* which is an obligate hemi-parasite that lacks rhizoids, and *Fernandosiphonia unilateralis*, *Strebloladia glomerulata* and *Melanothamnus somalensis*, which all have compact basal discs without individual rhizoids.

Pericentral cells and cortication: The number of pericentral cells and the presence of cortication are variable in most of the clades (Table 1). All species in the *Vertebrata* clade have six or more pericentral cells, while members of the *Polysiphonia sensu stricto* clades have four pericentral cells, with the exception of *Bryocladia cuspidata* (6–8 pericentrals). Cortication is uniformly absent in the *Polysiphonia sensu stricto* and '*P.*' *schneideri* clades. Cortication is variable within the other clades, absent or slight in small species of *Fernandosiphonia* but very heavy in *Melanothamnus*, and absent or slight in most species of *Vertebrata* with the exception of *Boergesenella*, in which cortication is elaborate.

Plastid arrangement: The arrangement of plastids in the cells is a synapomorphy for the *Melanothamnus* clade. The species in this clade have the plastids lying exclusively on radial walls of pericentral cells so the outer walls appear transparent (Table 1, Figs 8, 13, 34–39). This particular arrangement of the plastids can be easily observed under the microscope as the cells show a dark flank when observed in detail (Figs 14, 35, 37), as well as a transparent halo when the pericentral cells are observed in a suitable position (Fig. 38). All the other taxa of the Polysiphonieae have plastids against all the cell walls including the outer wall (Table 1, Figs 25–33). The revision of the type materials listed in Table S2, currently assigned to *Neosiphonia*, allowed us to verify the plastid character in the species *Polysiphonia concinna*, *P. eastwoodiae*, *P. gorgoniae*, *P. harlandii* and *P. johnstonii*. Conversely, the species *Lophosiphonia mexicana*, *P.*

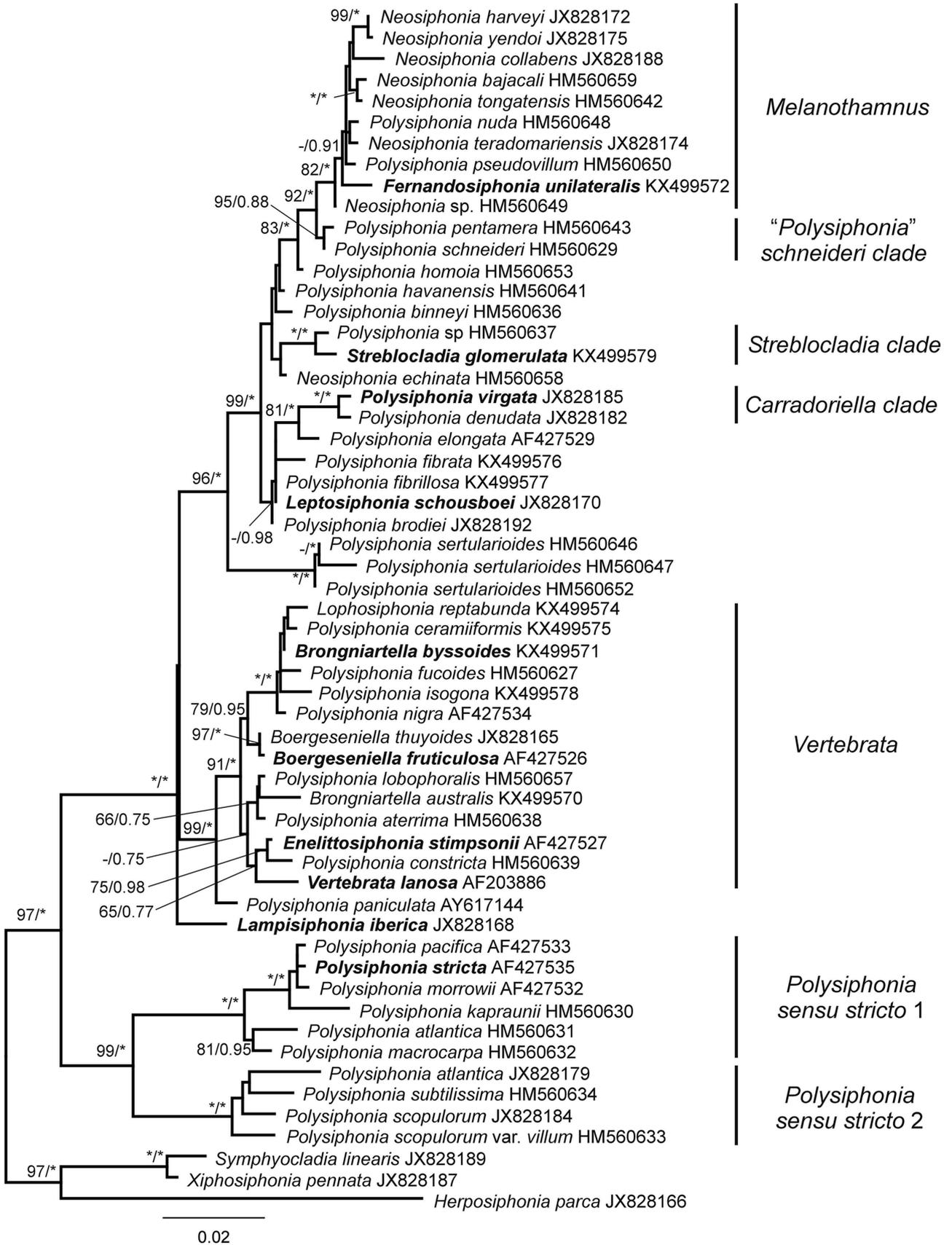
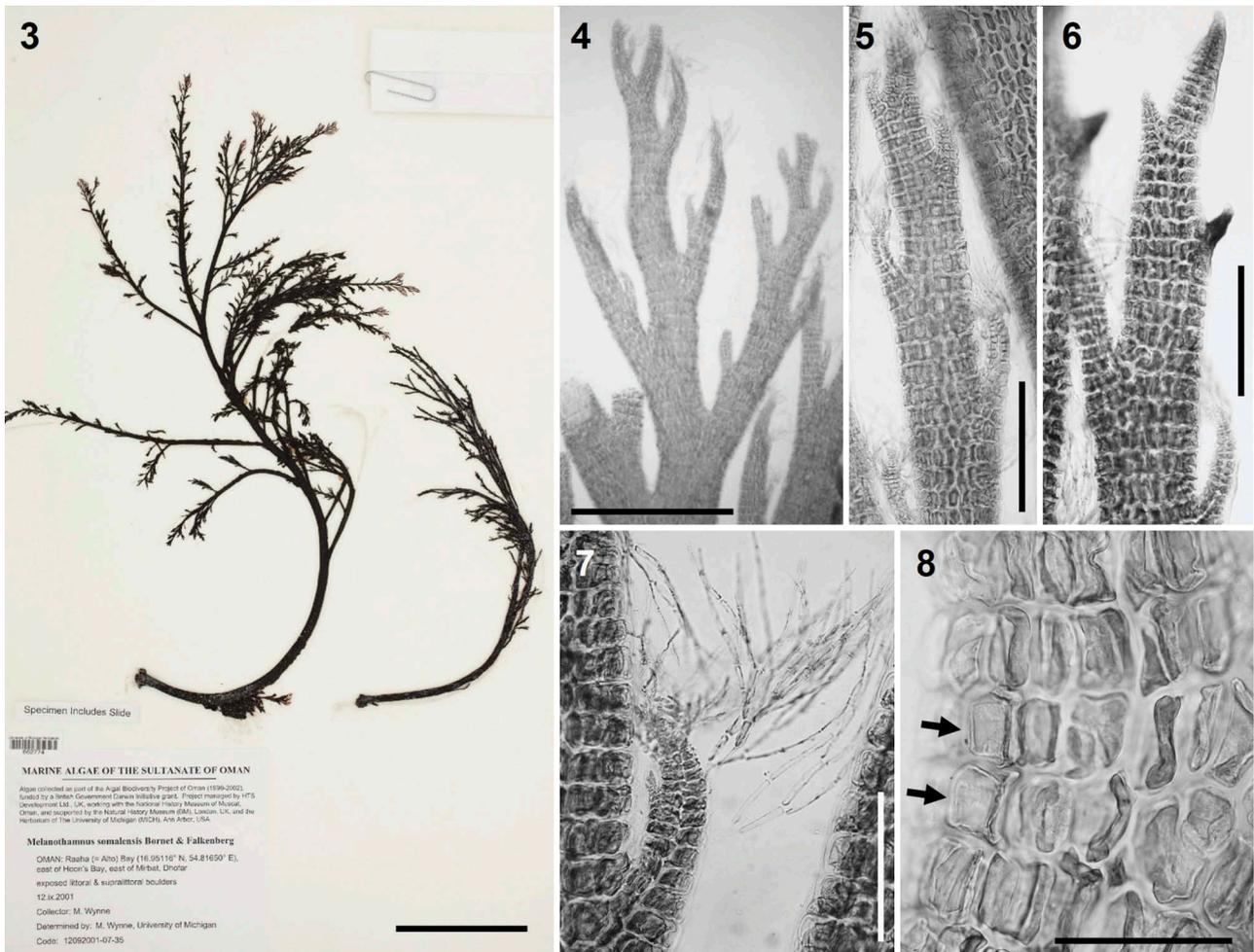


Fig. 2. Phylogenetic tree estimated with ML analysis of 18S sequences. Values at nodes indicate bootstrap support/posterior probability (only shown if > 60%/0.6 PP). Branches marked with an asterisk received 100%/1.00 PP support. Species names printed in bold correspond to type species of genera.



Figs 3–8. *Melanothamnus somalensis*, the type species of *Melanothamnus*. **Fig. 3.** Herbarium specimen MICH 662774. **Fig. 4.** Apical part of a specimen with alternately arranged branches. **Figs 5–6.** Apices of branches with (Fig. 5) or without (Fig. 6) abundant trichoblasts. **Fig. 7.** Apex of a lateral branch with trichoblasts. **Fig. 8.** Surface view of cells with the plastids lying exclusively on radial walls while the outer walls appear transparent (arrows). Scale bars: Fig. 3, 6 cm; Fig. 4, 1 mm; Figs 5 and 6, 350 µm; Fig. 7, 200 µm; Fig. 8, 100 µm.

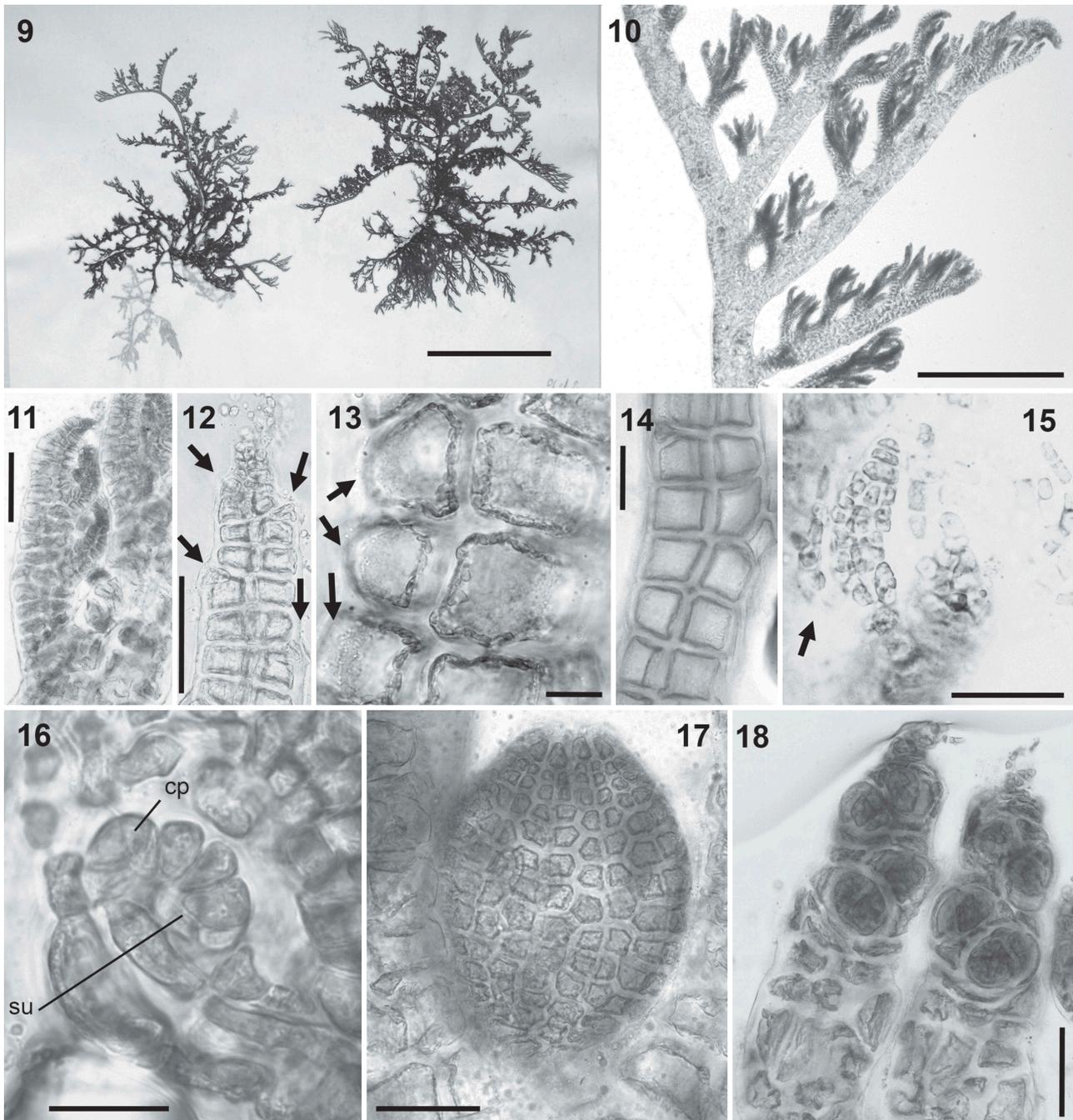
beaudettei, *P. confusa*, *P. poko*, *P. profunda* and *P. rubrorhiza* have the plastids scattered within the cells, including against the outer wall cells.

Branch/trichoblast arrangement: Whether trichoblasts and/or branches are formed on every segment or are separated by naked segments is variable in three clades, *Melanothamnus*, *Carradoriella* and *Vertebrata*. Most species of the *Melanothamnus* clade have branches or trichoblasts on every segment, which is a key feature of *Neosiphonia*. However, there are exceptions in this clade such as *Neosiphonia colabens*, *Polysiphonia nuda* and *P. pseudovillum* from Panama, in which there are interspersed naked segments. Conversely, the formation of trichoblasts/branches with naked segments between them is a uniform character in the *Streblocladia*, '*Polysiphonia*' *schneideri* and *Polysiphonia sensu stricto* clades (Table 1). Within all clades except *Melanothamnus*, branches may form in a position axillary to trichoblasts, but although constant at the species level, this

character is variable within clades. In the *Melanothamnus* clade this character is absent, and branches are never axillary.

Trichoblast nuclei: The proximal cells of trichoblasts are multinucleate in the *Vertebrata* clade (Table 1, Figs 41–43), with up to 8 or more nuclei in the basal cell and decreasing in number towards the apices, which can be uninucleate. The nuclei are uniformly distributed inside the cells, each appearing to have a domain within the cell. Conversely, all the cells of trichoblasts are uninucleate in other clades of the Polysiphonieae (Figs 40, 44–46). The only known exception is *Leptosiphonia schousboei*, which sometimes has two nuclei in the trichoblast cells.

Branching pattern: Despite the great significance previously placed on dorsiventral vs radial branching in the Rhodomelaceae, this character varies within all our clades. A primary dorsiventral branching pattern

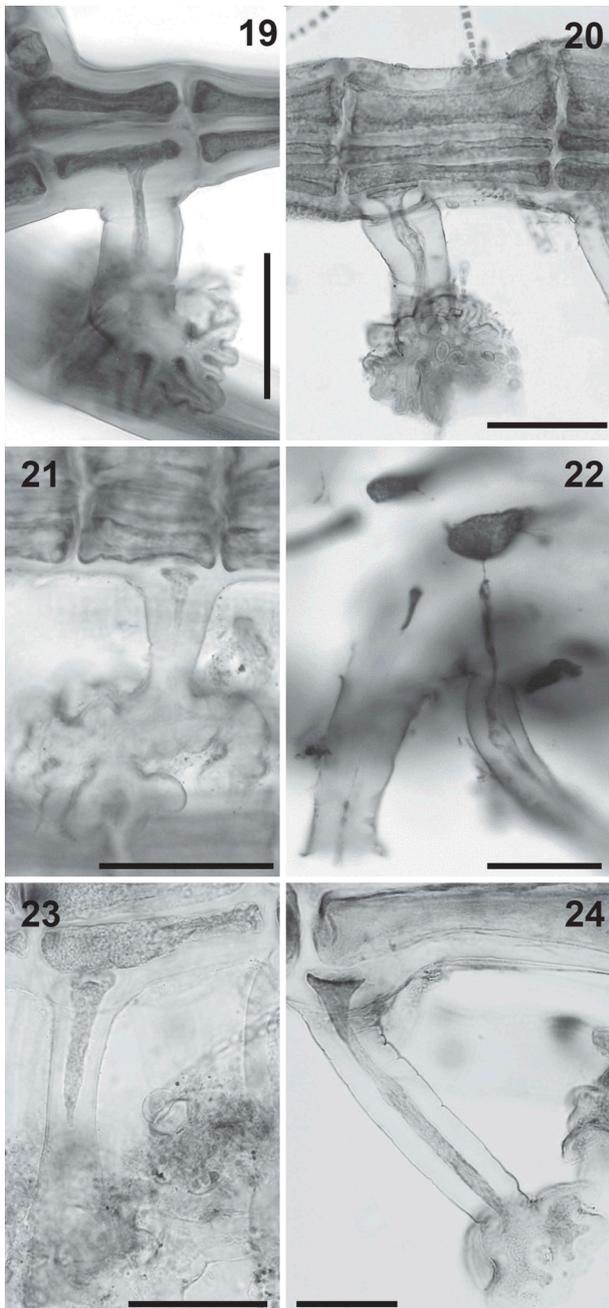


Figs 9–18. *Fernandosiphonia unilateralis* type material, the type species of *Fernandosiphonia*. **Fig. 9.** Herbarium specimen. **Figs 10–11.** Branches unilaterally arranged. **Fig. 12.** Axis with scar cells of trichoblasts (arrows). **Figs 13–14.** Surface view of pericentral cells with plastids lying only on the radial walls, so that the outer walls appear transparent (Fig. 13, arrows) and cells have a dark flank (Fig. 14). **Fig. 15.** Young spermatangial branch formed on the first dichotomy of a trichoblast, the other branch remaining vegetative (arrow). **Fig. 16.** Procarp (su = supporting cell; cp = carpogonium). **Fig. 17.** Cystocarp. **Fig. 18.** Tetrasporangia arranged in short spiral series. Scale bars: Fig. 9, 3 cm; Fig. 10, 2 mm; Fig. 11, 450 μ m; Figs 12, 14, 17 and 18, 100 μ m; Figs 13 and 15, 40 μ m; Fig. 16, 20 μ m.

characterizes some species of the clades *Melanothamnus* (*F. unilateralis* and *N. collabens*; Figs 10, 11), *Streblocladia* (*S. glomerulata*) and *Vertebrata* (*Ctenosiphonia hypnoides*) (Table 1). However, this characteristic is not significant in delineating these three genera, as our phylogenetic tree reveals that each of these four species is closely related to others that have spirally or pseudodichotomously arranged branches. For example, the branching pattern of members of the *Streblocladia* clade varies from

dorsiventral in *S. glomerulata*, the type species, to spiral or pseudodichotomous in *Polysiphonia muelleriana* and *Polysiphonia* sp. Likewise, the dorsiventral *Neosiphonia collabens* is related to species with spiral or pseudodichotomous branching patterns.

Spermatangial branches: Whether spermatangial branches replace trichoblasts completely or replace only one branch of a dichotomously branched trichoblast is a constant character in all clades (Table 1). In



Figs 19–24. Rhizoid anatomy in the Polysiphoniae. In open connection with pericentral cells in *Polysiphonia stricta* (Fig. 19, *Polysiphonia sensu stricto* clade 1). Cut off from pericentral cells in *P. foetidissima* (Fig. 20, *Vertebrata* clade), *P. denudata* (Fig. 21, *Carradoriella* clade), *Polysiphonia* sp. (Fig. 22, *Streblacladia* clade), *P. schneideri* (Fig. 23, '*P.*' *schneideri* clade) and *P. incompta* (Fig. 24, *Melanothamnus* clade). Scale bars: Figs 19–23, 100 μm ; Fig. 24, 500 μm .

the two *Polysiphonia sensu stricto* and *Streblacladia* clades, spermatangial branches almost uniformly replace trichoblasts (Fig. 47). In the other clades, they are formed on the first dichotomy of modified trichoblasts (Figs 15, 49–52), with the exception of *Vertebrata* (Fig. 48) as *V. lanosa* has no trichoblasts in male thalli – they can be observed only occasionally in females. The presence or absence of apical sterile cells on spermatangial branches is variable

within clades, except for *Carradoriella* in which they are present (Figs 47–52, Table 1).

Carpogonial branches: The *Melanothamnus* clade is characterized by having 3-celled carpogonial branches (Table 1). In our study, we observed this character in *N. harveyi*, *N. collabens*, *P. blandi* and *P. forfex* (Figs 57, 58). By contrast, the other Polysiphoniae uniformly have 4-celled carpogonial branches like the majority of the Rhodomelaceae (Table 1, Figs 53–56).

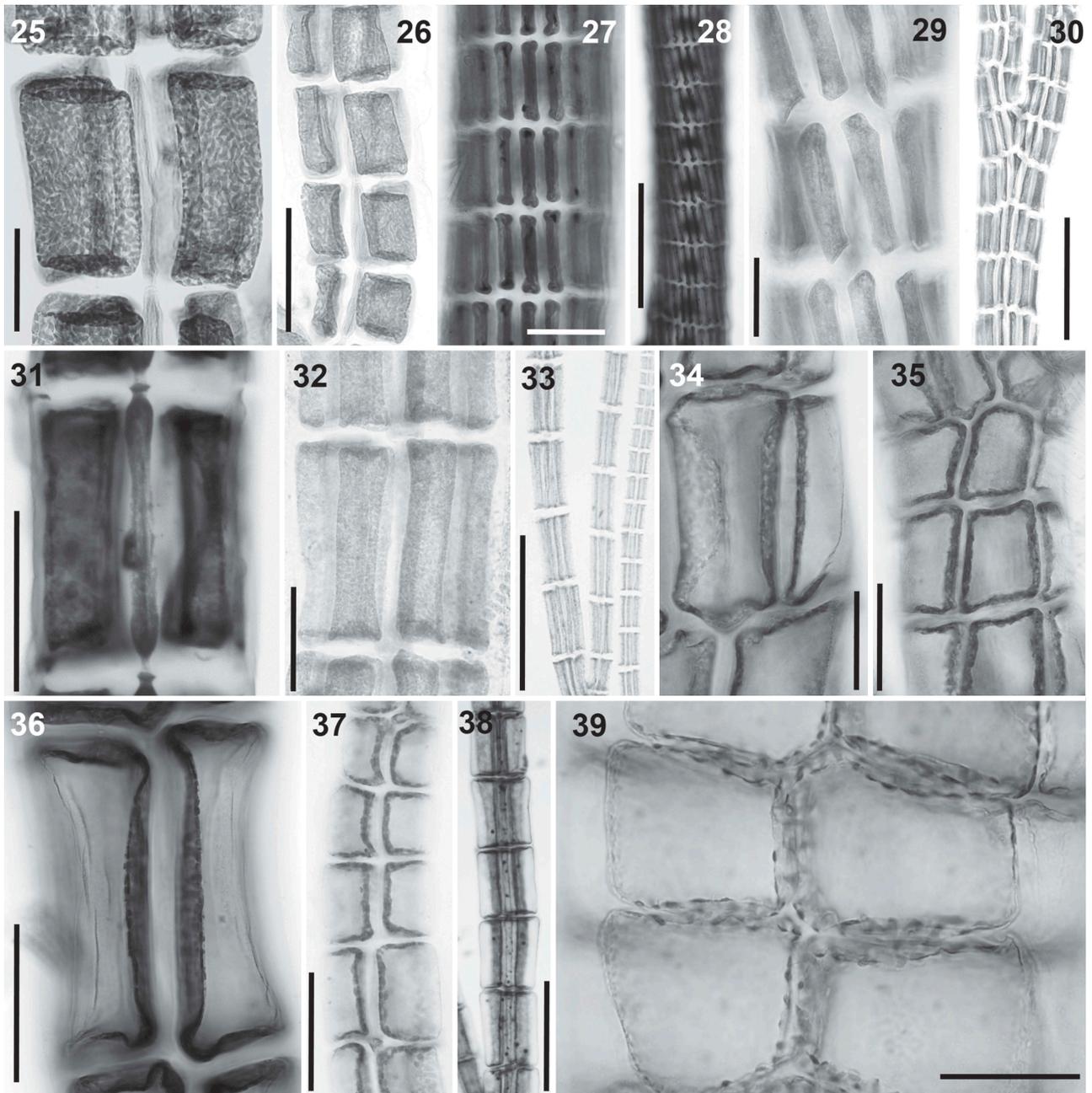
Cystocarps: The outline morphology of cystocarps varies from globose to ovoid in all the clades analysed here (Table 1, Figs 60–64). Urceolate cystocarps are exclusive to the *Polysiphonia sensu stricto* clades (Table 1, Fig. 59). Cells around the ostiole are conspicuously larger than (more than twice the size of) the cells of the pericarp immediately below in most species of the *Melanothamnus* clade (Fig. 70). However, *Neosiphonia harveyi* is an exception, and the cells of the ostiole in this species are only slightly larger than the other cells of the pericarp. This character is also seen in *Streblacladia glomerulata* (Fig. 68). Conversely, the cells of the ostiole in the other four clades are uniformly similar to the cells below (Figs 65–67, 69).

Tetrasporangia: The formation of tetrasporangia in straight or spiral rows is variable in all clades (Table 1, Figs 71–76). It must be noted that very long straight series of tetrasporangia are typically observed only in members of the *Polysiphonia sensu stricto* clade (Fig. 71). However, straight series can also form in other clades, for example in *Neosiphonia collabens* and *Polysiphonia nuda* within the *Melanothamnus* clade, whereas tetrasporangia in *Fernandosiphonia unilateralis* form short and markedly spiral series (Fig. 18). The third tetrasporangial cover cell is exclusive to the *Polysiphonia sensu stricto* clade, but this character has not been examined in all the species.

Discussion

Phylogenetic analysis

Amongst the Polysiphoniae studied here, the early-branching clade/clades *Polysiphonia sensu stricto* 1 and 2 were resolved as separate lineages in *rbcl* analyses (Fig. 1) but together formed a robust clade in 18S analyses (Fig. 2). The marked discordance between *rbcl* and 18S trees regarding the monophyly/paraphyly of the *Polysiphonia sensu stricto* lineages requires additional research for a more accurate assessment of relationships and character evolution. Because the *Polysiphonia sensu stricto* lineages occur near the base of the tree, it is possible that the outgroups (which are relatively distant taxa compared with the ingroup) could have attached to the ingroup



Figs 25–39. Plastid arrangement in the Polysiphonieae. Scattered against all cell walls of the pericentral cells in *Polysiphonia stricta* (Figs 25–26, *Polysiphonia sensu stricto* clade 1), *Vertebrata lanosa* (Figs 27–28, *Vertebrata* clade), *P. virgata* (Figs 29–30, *Carradoriella* clade), *Polysiphonia* sp. (Fig. 31, *Strebloladia* clade) and *P. schneideri* (Figs 32–33, ‘*P.*’ *schneideri* clade). Lying exclusively on the radial walls of the pericentral cells in species of the *Melanothamnus* clade: *Neosiphonia collabens* (Figs 34–35), *N. harveyi* (Figs 36–38) and *P. forfex* (Fig. 39). Scale bars: Figs 25, 27, 29, 38 and 39, 500 μm ; Figs 26, 28 and 30, 800 μm ; Figs 31, 32, 34, 35 and 37, 100 μm ; Fig. 33, 300 μm ; Fig. 36, 50 μm .

in the wrong position in one of the analyses (Shavit *et al.*, 2007). Future work should focus on including a wider range of taxa from across the Rhodomelaceae as well as using larger, multi-gene datasets to infer the correct branching order of the two *Polysiphonia sensu stricto* lineages.

The *Vertebrata* and *Melanothamnus* clades were resolved as large, speciose clades with strong support using *rbcL*. The 18S phylogeny also resolves the *Vertebrata* clade with robust support, while *Melanothamnus* is moderately well supported. Both clades are clearly distinguished from *Polysiphonia*

sensu stricto by the rhizoid anatomy (cut off from pericentral cells). Both clades are identified by distinct morphological synapomorphies. The *Vertebrata* clade is characterized by the multinucleate cells of trichoblasts; the other key feature of *Vertebrata*, that all species have six or more pericentral cells, is shared with members of some other clades. The *Melanothamnus* clade is unequivocally distinguished from other Polysiphonieae by two synapomorphic characteristics: the plastid arrangement and the 3-celled carpogonial branches. Furthermore, branch origin is independent from trichoblasts in all the

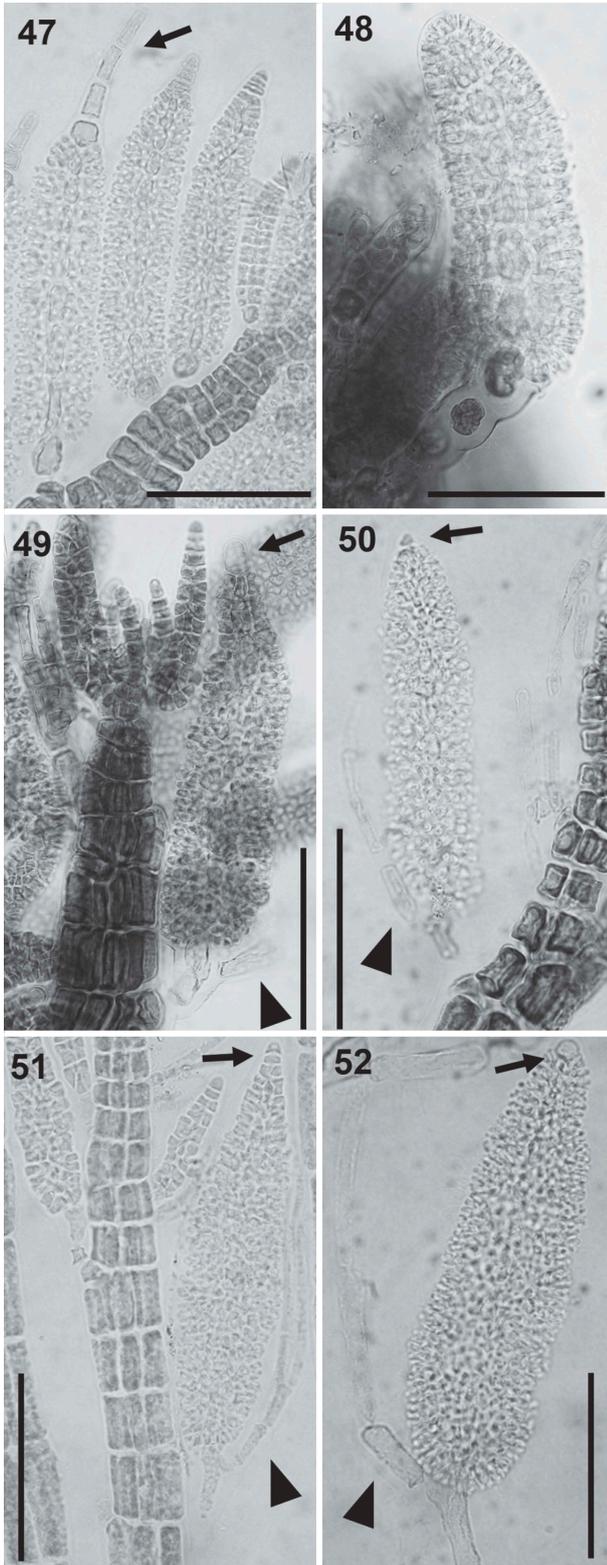


Figs 40–46. Trichoblast nuclei (arrows) in the Polysiphoniae. Uninucleate trichoblast cells in *Polysiphonia scopulorum* (Fig. 40, *Polysiphonia sensu stricto* clade 1), *P. denudata* (Fig. 44, *Carradoriella* clade), *P. schneideri* (Fig. 45, '*P.*' *schneideri* clade) and *P. blandii* (Fig. 46, *Melanothamnus* clade). Multinucleate trichoblast cells in species of the *Vertebrata* clade: *P. nigra* (Fig. 41), *Boergesenella fruticulosa* (Fig. 42) and *P. foetidissima* (Fig. 43). Scale bars: Figs 40–43, 60 μm , Fig. 44, 30 μm ; Fig. 45, 20 μm ; Fig. 46, 100 μm .

species of this clade, and the majority of species have enlarged ostiolar cells.

In addition to the above-mentioned clades, the *rbcl* phylogeny resolved six small (3–4 species) but highly supported clades, as well as indicating five species that are uncertainly positioned. The generic assignment of these lineages requires further taxon and gene sampling in order to better understand

their phylogenetic relationships and establish a natural classification – it would be premature to speculate on the outcomes of these investigations at present. One of the major shortcomings in Polysiphoniae sequence databases is the uneven geographical sampling, as the majority of sequenced taxa come from Atlantic Europe and central to north-eastern America. The generation of molecular



Figs 47–52. Spermatangial branches in the Polysiphoniae. Replacing trichoblasts and with sterile apical filaments in *Polysiphonia stricta* (Fig. 47, *Polysiphonia sensu stricto* clade 1). Replacing trichoblasts and lacking sterile apical cells in *Vertebrata lanosa* (Fig. 48, *Vertebrata* clade). On a branch of a trichoblast and with sterile apical cells in *P. fucooides* (Fig. 49, *Vertebrata* clade), *P. denudata* (Fig. 50, *Carradoriella* clade), *P. schneideri* (Fig. 51, *P. schneideri* clade) and *Neosiphonia harveyi* (Fig. 52, *Melanothamnus* clade). Scale bars: 100 μ m. Arrows show the apical sterile cells and arrowheads the sterile branch of fertile trichoblasts.

data from additional regions could contribute to acquiring a more realistic perspective of the magnitude of unplaced lineages and to delineating their corresponding genera. Also, the resolution of the commonly employed molecular markers in the Polysiphoniae is not sufficient to resolve the phylogenetic relationships among numerous lineages, which could be improved using larger gene datasets (Díaz-Tapia *et al.*, 2015).

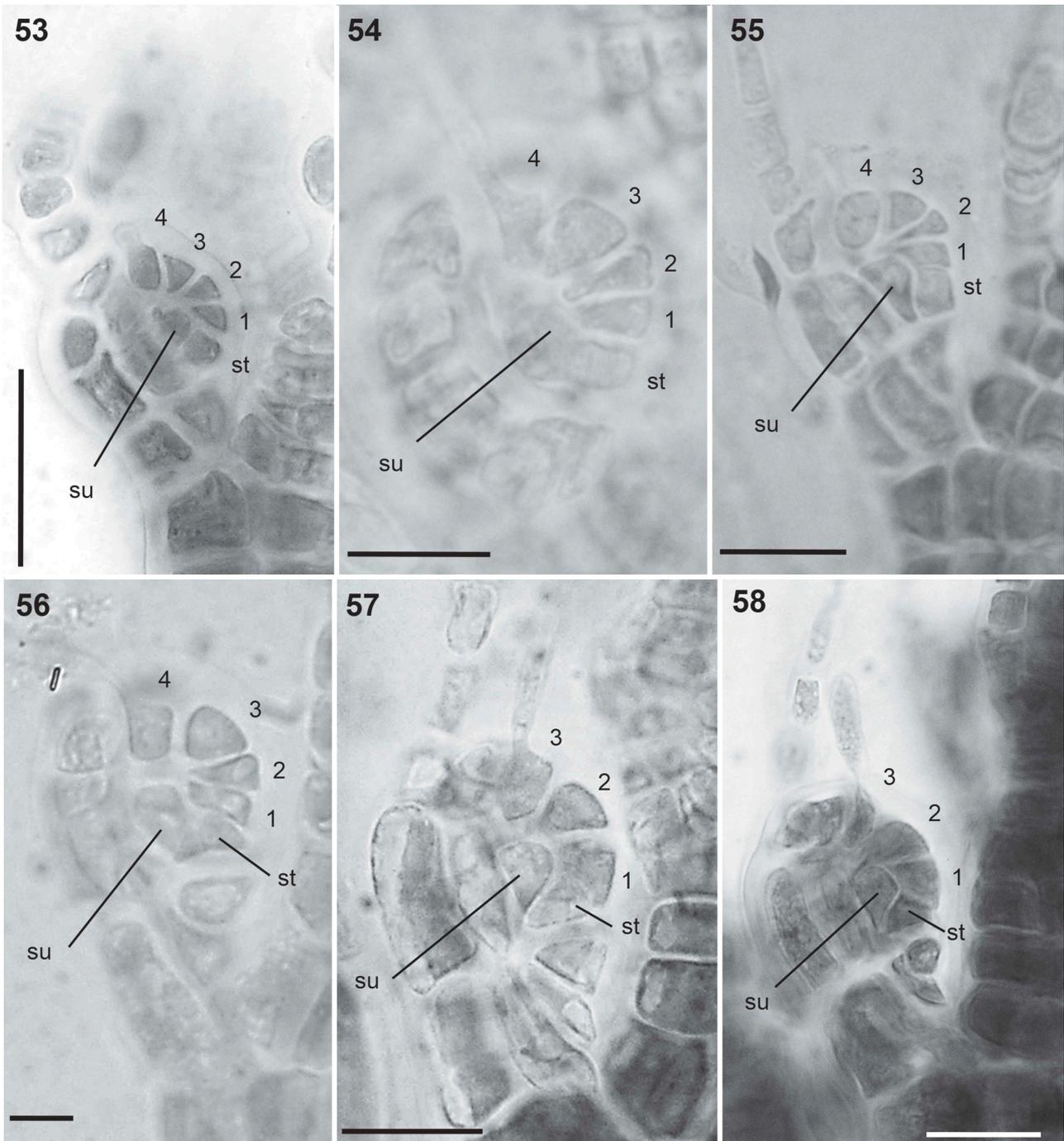
Taxonomic position of Vertebrata

Vertebrata lanosa is placed in a strongly supported clade that also includes *Brongniartella byssoides*, *Boergeseniella fruticulosa*, *Ctenosiphonia hypnoides* and *Enelittosiphonia stimpsonii*, the type species of their corresponding genera, and *Lophosiphonia reptabunda* (which is not the type species). All members of this clade have a synapomorphic characteristic that was previously overlooked in relation to systematics (but see Maggs & Hommersand, 1993): multinucleate trichoblast cells. We conclude from molecular and morphological evidence that members of this clade represent a single genus. *Vertebrata* is the oldest name among those available for this clade, as noted before (Choi *et al.*, 2001), and the new combinations proposed in Table 2 are required. Furthermore, the *Vertebrata* binomials previously established by Kuntze (1891) should be reinstated for the other 13 species included in this clade (Table 3).

Interestingly, *Brongniartella* is not monophyletic despite its distinctive persistent and pigmented trichoblasts that led to its classification in the tribes Lophothalieae (Falkenberg, 1901; Womersley, 2003) or Brongniartelleae Parsons (Parsons, 1975; Maggs & Hommersand, 1993).

Although trichoblasts are typically considered unpigmented in the Polysiphoniae, they are commonly pigmented when young before they enlarge and become colourless (Delivopoulos, 2002). The two currently recognized species of *Brongniartella*, *B. byssoides* and *B. australis*, were separated within the *Vertebrata* clade, respectively placed with *V. lanosa* and *Polysiphonia nigra*.

Ctenosiphonia is a monotypic genus segregated from *Polysiphonia* due to its very peculiar morphological characteristics, including a dorsiventral thallus and two tetrasporangia per segment (Falkenberg, 1901; Díaz-Tapia & Bárbara, 2013). This genus, together with *Lophosiphonia* Falkenberg (1897), is currently positioned within the ‘Lophosiphonia group’ (Falkenberg, 1901). *Boergeseniella* and *Enelittosiphonia* were distinguished from other Polysiphoniae by their particular branching patterns (Segi, 1949; Kylin, 1956), but our molecular evidence



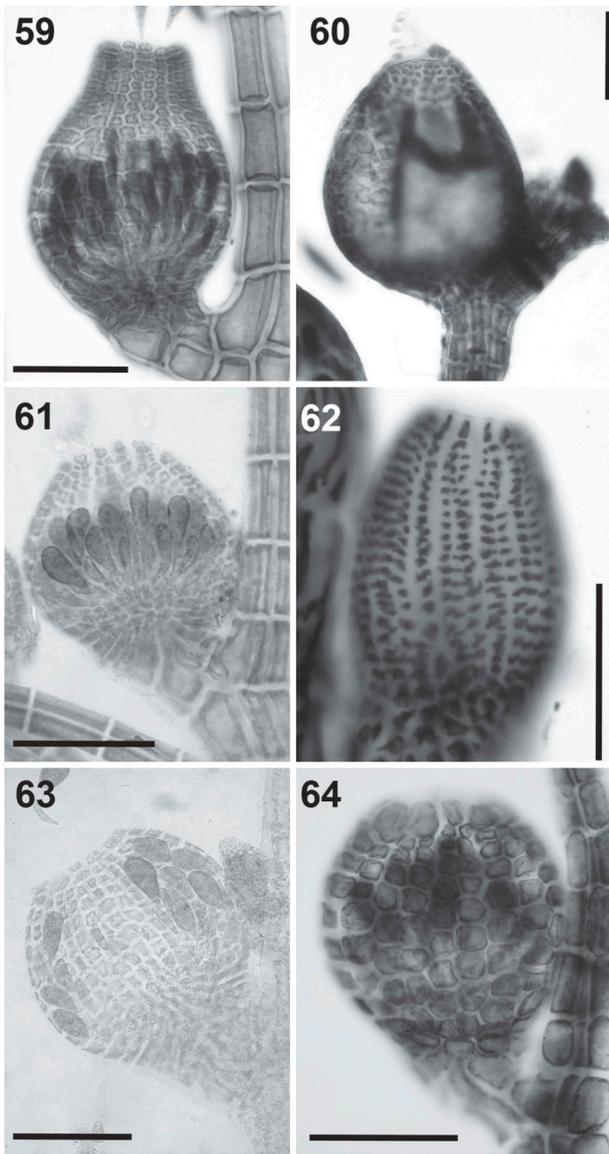
Figs 53–58. Carpopogonial branches in the Polysiphonieae. Four-celled in *Polysiphonia stricta* (Fig. 53, *Polysiphonia sensu stricto* clade 1), *P. nigra* (Fig. 54, *Vertebrata* clade), *P. denudata* (Fig. 55, *Carradoriella* clade) and *P. schneideri* (Fig. 56, '*P.*' *schneideri* clade). Three-celled in species of the *Melanothamnus* clade: *Neosiphonia harveyi* (Fig. 57) and *P. blandii* (Fig. 58). Su = supporting cell; st = sterile basal cell; 1–4 cells of carpopogonial branches. Scale bars: Fig. 53, 30 μm ; Figs 54–58, 20 μm .

(Fig. 1) does not support their recognition as independent genera.

The diversity of currently recognized genera included in this clade reflects the high variability among *Vertebrata* species in trichoblast characteristics (pigmented/unpigmented; persistent/deciduous; spirally/dorsiventrally arranged) and branching patterns (spiral/dorsiventral; presence or absence of alternating branches of determinate and indeterminate growth), which classical authors considered

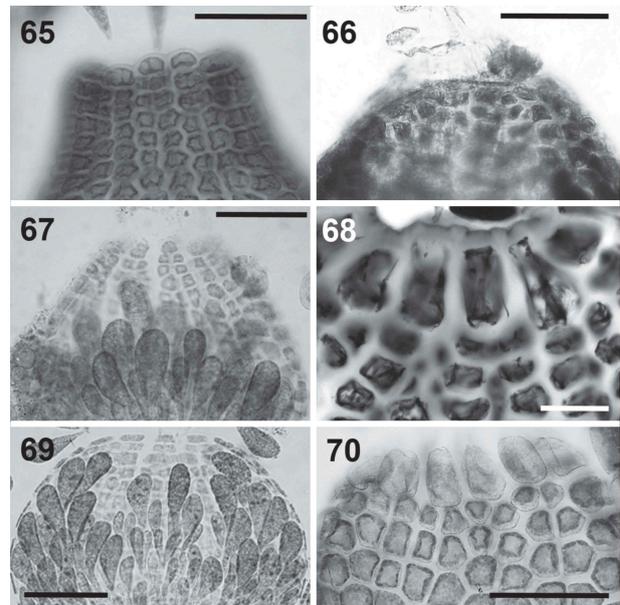
important at levels of genus and tribe (Falkenberg, 1901; Kylin, 1956; Hommersand, 1963).

The main morphological character delineating the *Vertebrata* group is that trichoblast cells are multinucleate. In the Polysiphonieae and some other Ceramiales, the apical cell is uninucleate, whereas the cells cut off from it undergo nuclear divisions during elongation, becoming multinucleate, with the number of nuclei being proportional to the volume of the cell (Goff & Coleman, 1986; McIvor *et al.*, 2002).



Figs 59–64. Cystocarps in the Polysiphoniae. Urceolate in *Polysiphonia stricta* (Fig. 59, *Polysiphonia sensu stricto* clade 1). Ovoid in *Vertebrata lanosa* (Fig. 60, *Vertebrata* clade), *P. denudata* (Fig. 61, *Carradoriella* clade), *Streblocladia glomerulata* (Fig. 62, *Streblocladia* clade). Globose in *Polysiphonia schneideri* (Fig. 63, '*P.*' *schneideri* clade) and *Neosiphonia collabens* (Fig. 64, *Melanothamnus* clade). Scale bars: Figs 59–62 and 64, 200 μm ; Fig. 63, 100 μm .

The trichoblasts of the Rhodomelaceae are usually uninucleate, whereas the polysiphonous parts of the thalli are multinucleate (Coomans & Hommersand, 1990; Garbary & Clarke, 2001; Delivopoulos, 2002). A plausible advantage of having multinucleate trichoblasts in *Vertebrata* is that their cells can reach larger sizes. In fact, trichoblasts in this genus are sometimes extremely well developed, exceeding 10 mm in length in species such as *Vertebrata* (*Lophosiphonia*) *reptabunda* and *V.* (*Ctenosiphonia*) *hypnoides*. In the red algae, cell streaming is slow compared with other algae (Pueschel, 1990), and multinuclearity of large cells may facilitate the regulation of cellular activities. Several potential functions have been attributed to

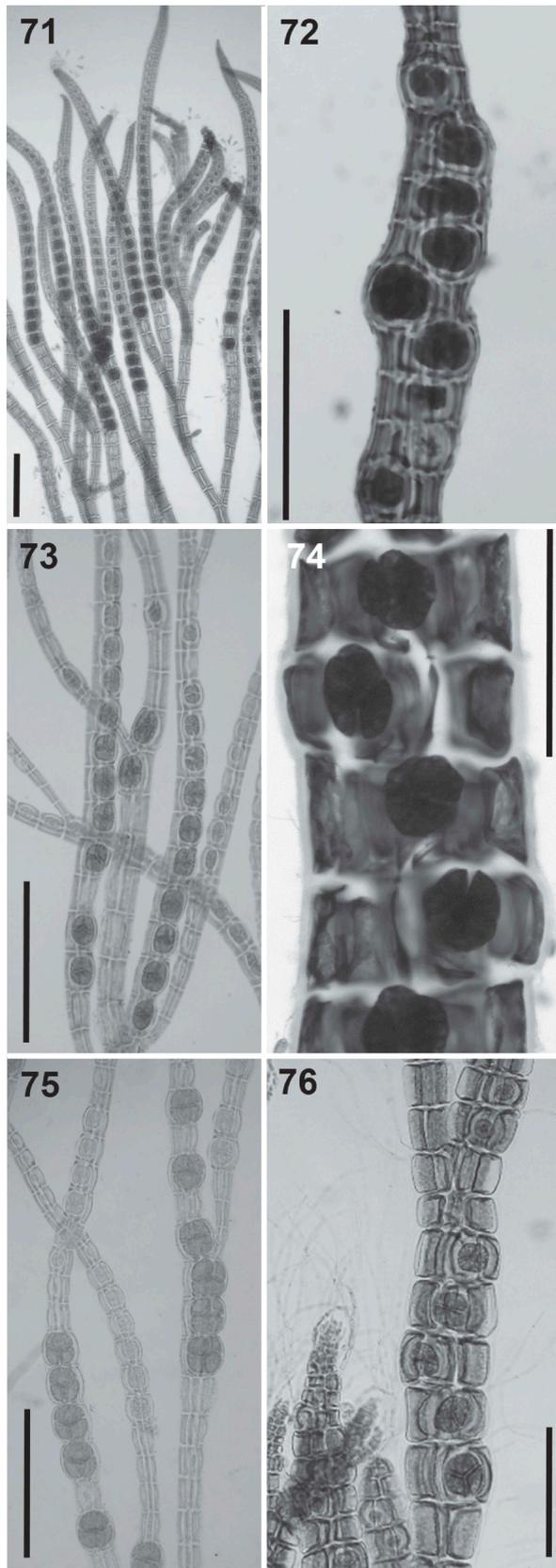


Figs 65–70. Cells surrounding the ostiole in the Polysiphoniae. Similar or slightly larger than the cells of the pericarp immediately below in *Polysiphonia stricta* (Fig. 65, *Polysiphonia sensu stricto* clade 1), *Vertebrata lanosa* (Fig. 66, *Vertebrata* clade), *P. denudata* (Fig. 67, *Carradoriella* clade), and *P. schneideri* (Fig. 69, '*P.*' *schneideri* clade). They are much larger in *Streblocladia glomerulata* (Fig. 68, *Streblocladia* clade) and *Neosiphonia collabens* (Fig. 70, *Melanothamnus* clade). Scale bars: Figs 65–68 and 70, 100 μm ; Fig. 69, 60 μm .

the vegetative hairs of the red algae or trichoblasts of the Rhodomelaceae including desiccation resistance, nutrient uptake, metabolite secretion, shading, trapping of spermatia, mucilage stabilization and monitoring of phosphorus status (Delivopoulos, 2002, and references therein). Physically, trichoblasts can form a dense network around the apices that could potentially restrict access to the cells by small grazers, such as amphipods and copepods.

Taxonomic position of *Neosiphonia*, *Fernandosiphonia* and *Melanothamnus*

The presence of the type species of the genus *Melanothamnus* (*M. somalensis*) in a strongly supported clade with the type species of the genus *Neosiphonia* (*N. flavimarina*) and *Fernandosiphonia* (*F. unilateralis*) indicates that *Neosiphonia*, *Fernandosiphonia* and *Melanothamnus* are not distinct monophyletic genera. *Neosiphonia* is a later heterotypic synonym of *Fernandosiphonia*. However, the name *Melanothamnus* is older than both *Fernandosiphonia* and *Neosiphonia*, and the new combinations proposed in Tables 4 and 5 are required. These new combinations include 31 species that were previously assigned to *Neosiphonia*; two species known to be closely related to *Neosiphonia* but that had been retained in *Polysiphonia* because



Figs 71–76. Tetrasporangia in the Polysiphonieae. Forming long straight series in *Polysiphonia stricta* (Fig. 71, *Polysiphonia sensu stricto* clade 1). Forming spiral series in *Vetrata lanosa* (Fig. 72, *Vertebrata* clade), *Polysiphonia* sp. (Fig. 74, *Strebloladia* clade) and *Neosiphonia harveyi* (Fig. 76, *Melanothamnus* clade). Forming short straight series in *P. denudata* (Fig. 73, *Carradoriella* clade), and *P. schneideri* (Fig. 75, *P. schneideri* clade). Scale bars: Figs 71, 74 and 76, 200 μm ; Figs 72, 73 and 75, 400 μm .

their morphology conflicted with Kim & Lee (1999); six species for which molecular data are presented here for the first time; and three species that are transferred to *Melanothamnus* on the basis of their morphology.

On the other hand, 10 species that are currently placed in *Neosiphonia* should be replaced in *Polysiphonia* for formal purposes pending clarification of their phylogenetic affinities and generic assignment. *Polysiphonia beaudettei*, *P. confusa*, *P. echinata*, *P. elongella*, *P. poko*, *P. rubrorhiza* and *P. profunda* were assigned to *Neosiphonia* based on morphological characteristics (Kim & Lee, 1999; Abbott *et al.*, 2002; Kim & Abbott, 2006; Mamoozadeh & Freshwater, 2011; Norris, 2014). However, they lack the plastid character, and furthermore molecular data for *P. echinata* and *P. elongella* show that they do not belong to the *Melanothamnus* clade (Fig. 1). Likewise, *Polysiphonia sertularioides* was transferred to *Neosiphonia* based on the morphology of Korean material attributed to this species (Nam & Kang, 2012). However, its type locality is in the Mediterranean, and Atlantic sequences for this species are not in the *Fernandosiphonia* clade (Fig. 1; Mamoozadeh & Freshwater, 2012). *Polysiphonia paniculata* was transferred to *Neosiphonia* (Norris, 2014), but again it is not in the *Melanothamnus* clade (Figs 1, 2). Finally, our study of the type material of *Lophosiphonia mexicana*, also transferred to *Neosiphonia* (Norris, 2014; Table S2), indicates that this species is probably not a member of the Polysiphonieae. As noted by Norris (2014), further studies are needed to clarify the generic placement of this unusual species, and meanwhile we propose to leave it in *Lophosiphonia* until more information is available.

Specimens of *Melanothamnus* collected in Oman and housed in MICH were initially assigned to *M. somalensis* (Wynne & Banaimoon, 1990), before the description of *M. afaqhusainii* from Pakistan (Shameel, 1999). Revision of the Omani materials leads us to conclude that both *M. somalensis* and *M. afaqhusainii* are represented in Oman, and their morphology agrees with the criteria proposed by Shameel (1999) and Afaq-Husain & Shameel (2000) for distinguishing them. Their *rbcL* sequences diverged by 1.4% (18 bp).

The *Melanothamnus* clade is morphologically distinguished from other members of the tribe Polysiphonieae by an unequivocal synapomorphic character: plastids lie exclusively on the radial walls of the pericentral cells and are absent from outer walls. The plastid character was previously noted by Hollenberg (1961, 1968a), who described 'hyaline cell walls' for several species (e.g. *P. pseudovillum*, *P. bajacali*), and by Maggs & Hommersand (1993). However, its significance at higher taxonomic levels

has not previously been highlighted. We observed this character in a total of 35 species, and we conclude that it is uniform in the *Melanothamnus* clade. Conversely, other Polysiphonieae and most of the Rhodomelaceae have plastids distributed within the cytoplasm, some lying against outer cell walls. In the family, the only other exception is some species of *Herposiphonia* in which the plastids form transverse bands (Hollenberg, 1968c; Womersley, 2003; Díaz-Tapia & Bárbara, 2013).

Carpogonial branches are typically 4-celled throughout the family Rhodomelaceae. Three-celled carpogonial branches were described for the first time in *Polysiphonia platycarpa* (Iyengar & Balakrishnan, 1950), and later this was one of the features proposed to delineate the genus *Neosiphonia* (Kim & Lee, 1999). Three-celled carpogonial branches have been reported in 17 species (four of them in the 'japonica-complex'), all of which are placed here in *Melanothamnus*. Alternative interpretations of the carpogonial branch configuration were found in the literature for *F. unilateralis*, as Levring (1941) described and illustrated a 4-celled structure, while Morrill (1976, plate 37, figs E and H) illustrated 3-celled carpogonial branches in the same species, also from the type locality. This character can be easily misinterpreted if the carpogonial branches are not observed at the right developmental stage. In our study of the type material, a single procarp was observed in a permanent slide (Fig. 16), most probably the same one illustrated by Levring. It is unclear how this procarp should be interpreted because it is too mature, and so it is difficult to determine with certainty which cell corresponds to the sterile basal cell and which to the basal cell of the carpogonial branch. From the evidence of the presence of both the plastid character and 3-celled carpogonial branches, *Kintarosiphonia fibrillosa* Uwai & Masuda (1999), based on *Pterosiphonia fibrillosa*, and *Polysiphonia platycarpa* are also here transferred to *Melanothamnus* (Table 1).

The other morphological characters proposed by Kim & Lee (1999) to delineate the genus *Neosiphonia* vary among closely related species, except for the rhizoid anatomy. Rhizoids are cut off from the pericentral cells in all Polysiphonieae except for *Polysiphonia sensu stricto* in which they are in open connection with the pericentral cells. After the establishment of *Neosiphonia*, numerous species were transferred to the new genus based on morphology, but commonly overlooking the number of cells in carpogonial branches. Excluding this trait, several species have all five characteristics proposed by Kim & Lee (1999) to delineate *Neosiphonia* but nevertheless are not in the *Melanothamnus* clade (e.g. *P. brodiei*, *P. echinata*, *P. elongella*), while several species are clearly in the clade (e.g. *M. collabens*, *M. nuda*, *M. pseudovillum*) but lack this combination of traits.

The key morphological feature of *Melanothamnus* is the restriction of plastids to the radial walls of the pericentral cells and their absence from the outer walls. Algae demonstrate a notable decline in photosynthesis at higher light levels possibly due to damage to the photosynthetic apparatus caused by excessive light delivery to photosystem II (Lüning, 1990; Hurd *et al.*, 2014). Many green and brown algal plastids have phototropic reactions to blue and UV light in order to protect them from irradiation damage (Lüning, 1990). Plastid movement, however, has never been demonstrated for the vast majority of red algae (Pueschel, 1990), and it appears that red algae have evolved other types of protection against UV damage. Red algae including *Polysiphonia* species have high concentrations of various mycosporine-like amino acids (MAAs) that respond rapidly to environmental change and act as defences against the photooxidative effects of sunlight (Karsten *et al.*, 1998; Navarro *et al.*, 2014). The movement of the plastids onto the radial walls of the periaxial cells, in combination with MAAs, may have given the *Melanothamnus* ancestor a selective advantage over other Polysiphonieae, allowing it to exploit new ecological niches. The prevalence of *Melanothamnus* species in habitats with exposure to high light levels, such as in Hawaii or turfs on coral reefs (Price & Scott, 1992; Kim & Abbott, 2006), supports this speculation.

Biogeography of *Vertebrata* and *Melanothamnus*

The genus *Vertebrata* is distributed worldwide, and representatives have been described from all regions where there has been a detailed study of the tribe Polysiphonieae. The majority of our sequences are from Europe, but our systematic review and unpublished data suggest that this genus is widespread. BEAST and PhyloBayes calibrations indicate radiation of the major lineages of the *Vertebrata* clade over a 20 MA period starting about 140 or 90 Ma (estimates from different methods; see Figs S1, S2). Further conclusions as to its origins and centres of diversity would be premature, pending more comprehensive sampling.

In contrast, *Melanothamnus* is predominantly Indo-Pacific (Fig. 77). Although few molecular data are available from Indian coasts, some species occur in South Africa (*M. incompta*), Oman (*M. somalensis* and *M. afaqhusainii*), India (*M. platycarpa*) and Thailand (*M. thailandica*). Among the regions for which there is a comprehensive study of the Polysiphonieae, the diversity of *Melanothamnus* is particularly high in Korea, Japan and Hawaii (14, 11 and 14 species, respectively). This genus is also well represented on North American Atlantic coasts (4–5 species), but it is almost completely absent from Atlantic and Mediterranean Europe, where only two species have been reported, which are both

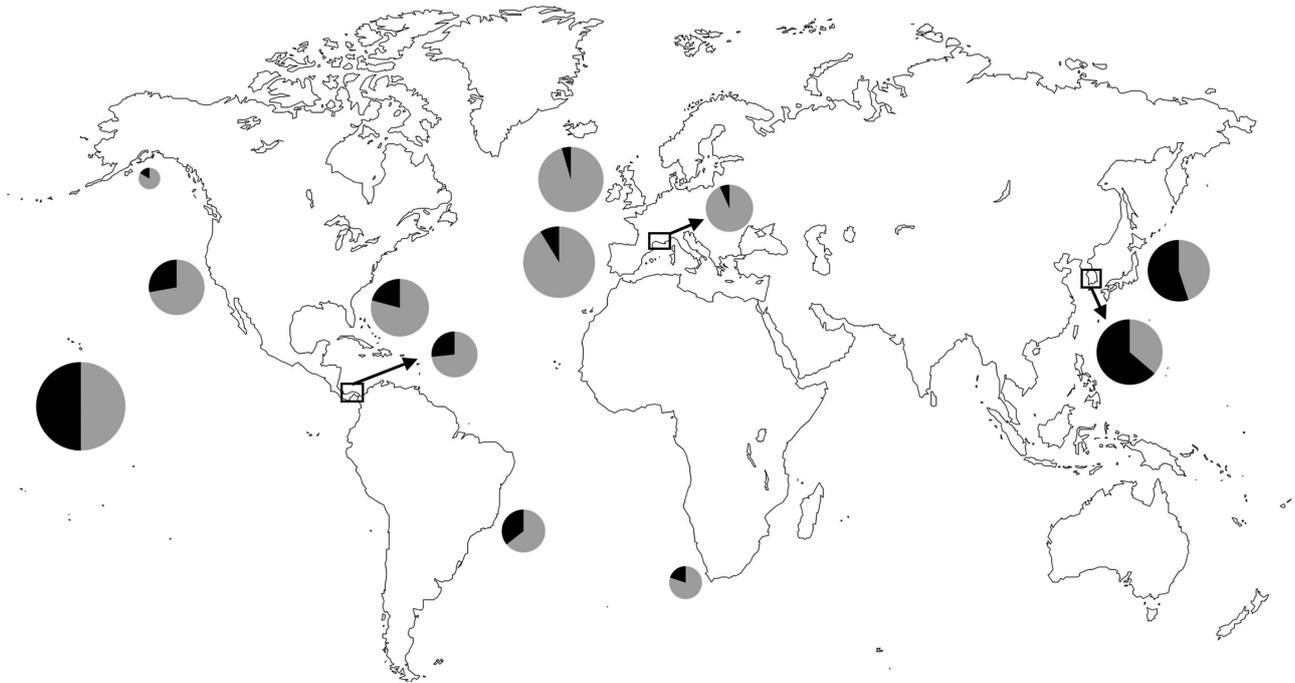


Fig. 77. World map representing the proportion of *Melanothamnus* (black) and other Polysiphoniae (grey) species in selected regions where the Polysiphoniae were studied in detail. Data were obtained from the following references after updating the species names: Alaska: Lindstrom (<http://www.seaweedssofaraska.com>); Brazil (Espírito Santo-São Paulo): Guimarães *et al.* (2004); California: Abbott & Hollenberg (1976); Florida: Schneider & Searles (1991); Hawaii: Abbott (1999); Japan: Yoshida (1998); Korea: Nam & Kang (2012); Panama: Mamoozadeh & Freshwater (2012); South Africa: Stegenga *et al.* (1997); southern France: Lauret (1967, 1970) Spain (Galicia): Bárbara *et al.* (2005); British Isles: Maggs & Hommersand (1993).

most probably examples of old human-mediated introductions. *Melanothamnus harveyi* is native to south-eastern Asia and has been introduced by multiple events onto northern Atlantic coasts (McIvor *et al.*, 2001; Savoie & Saunders, 2016). Similarly, *M. collabens* is likely to be an old introduction into the Atlantic, where it extends from the Bay of Biscay to Cape Verde, including the western Mediterranean (Díaz-Tapia & Bárbara, 2013). The finding of *M. collabens* in California (as *P. johnstonii*, see Table 4) supports this hypothesis, but although *Polysiphonia johnstonii* was first collected from the Gulf of California in 1921 (Setchell & Gardner, 1924), California was probably not the original source of the introduction. An investigation of *Polysiphonia* species from the Northern Gulf of California (Hollenberg & Norris, 1977) suggested that since its initial collection and description, *P. johnstonii* has extended its range along the Pacific coast of North America, fulfilling one of the criteria for an invasive species (Chapman & Carlton, 1991; Ribera & Boudouresque, 1995). How far this species has spread along the Pacific coast of America and along North Atlantic coastlines remains to be determined, and further sampling is needed to establish its origin.

The absence of naturally occurring *Melanothamnus* species in the Mediterranean and north-eastern Atlantic might suggest that *Melanothamnus* is of recent origin, having evolved in the Pacific Ocean after the closure of

the Tethys Seaway, between 60 and 20 million years ago, particularly as the sister '*P.*' *schneideri* clade is also primarily Pacific in distribution. In our *rbcL* phylogeny (Fig. 1), the '*P.*' *schneideri* clade includes two Korean species and two species distributed in the Pacific and North America with one of them introduced in Europe (Díaz-Tapia *et al.*, 2013a). Furthermore, our surveys in Australia revealed five other Indo-Pacific species belonging to this clade (unpublished data). However, BEAST and PhyloBayes calibrations indicate radiation of the major lineages of the *Melanothamnus* clade over a 12 Ma period starting about 95 or 75 Ma (the two methods providing different estimates), with divergence from the '*P.*' *schneideri* clade 140 or 95 Ma (Figs S1, S2). The distribution resembles a Tethyan one that originated during the Cretaceous 125–75 Ma (Lüning, 1990; Hommersand, 2007) when the Tethys Ocean formed a tropical girdle around the earth. Unlike typical Tethyan distributions, in addition to its wide occurrence throughout the tropics, *Melanothamnus* occupies more temperate regions in the North Pacific (e.g. Japan, Korea) and the South Pacific/Oceania (e.g. South Australia, New Zealand). The question of whether *Melanothamnus* failed to colonize the north-eastern Atlantic as it opened up during the Cretaceous, or whether north-eastern Atlantic lineages evolved but became extinct, perhaps during Pleistocene glaciations, cannot be answered at present.

Acknowledgements

We are pleased to recognize here that Professor Max H. Hommersand (UNC Chapel Hill) independently discovered the key morphological character of the position of plastids in the *Melanothamnus* clade. We thank Joana Costa for providing us samples of *Polysiphonia* spp. from South Africa, and Dr Erasmo Macaya for supplying material from his recent collections of *Fernandosiphonia unilateralis* from the type locality. We are grateful to Dr Cynthia Trowbridge for providing samples from Japan, and Dr Show-Mei Lin for collecting samples in Taiwan. Professor Gary Saunders is warmly thanked for generously sharing sequences for *Melanothamnus afaqhusainii* from Oman. We acknowledge the curators of the herbaria BM(NH), TFC, US and TCD; Dr Joe Zuccarello provided host support for Lynne McIvor during her Marie Curie fellowship at Leiden Herbarium. We thank two anonymous reviewers for their valuable comments on the manuscript.

Disclosure statement

No potential conflict of interest was reported by the author(s).

Funding

PDT acknowledges support by the postdoctoral programmes Axudas de apoio á etapa inicial de formación posdoutoral do Plan I2C (Xunta de Galicia). Funding for the aspects of the work carried out in the Melbourne lab was provided by the Australian Biological Resources Study (RFL213-08) and the Australian Research Council (FT110100585).

Supplementary information

The following supplementary material is accessible via the Supplementary Content tab on the article's online page at <http://dx.doi.org/10.1080/09670262.2016.1256436>

Supplementary Table S1. Sample information for the species included in the phylogenetic analysis.

Supplementary Table S2. List of herbarium specimens examined for comparison with *Melanothamnus* species.

Supplementary Table S3. Percentage of bases that differ (below) and are identical (above) for the species sequences of the *Melanothamnus* clade included in our *rbcL* alignment.

Supplementary Table S4. Percentage of bases that differ (below) and are identical (above) for the species sequences of the *Vertebrata* clade included in our *rbcL* alignment.

Supplementary Fig. S1. Chronogram resulting from the Bayesian relaxed molecular clock analysis performed with BEAST.

Supplementary Fig. S2. Chronogram resulting from the autocorrelated molecular clock analysis performed with PhyloBayes.

Author contributions

P. Díaz-Tapia: original concept, drafting and editing manuscript; determination and analysis of molecular data; L. McIvor: original concept; determination of molecular data; D.W. Freshwater: determination and analysis of molecular data; editing manuscript; H. Verbruggen: analysis of molecular data; editing manuscript; M.J. Wynne:

collection and study of *Melanothamnus somalensis* and *M. afaqhusainii*; editing manuscript; C. Maggs: original concept, drafting and editing manuscript.

ORCID

Heroen Verbruggen  <http://orcid.org/0000-0002-6305-4749>

Christine A. Maggs  <http://orcid.org/0000-0003-0495-7064>

References

- Abbott, I.A. (1999). *Marine Red Algae of the Hawaiian Islands*. Bishop Museum Press, Honolulu.
- Abbott, I.A. & Hollenberg, G.J. (1976). *Marine Algae of California*. Stanford University Press, Stanford, CA.
- Abbott, I.A., Fisher, J. & McDermid, K.J. (2002). New reported and revised marine algae from the vicinity of Nha Trang, Vietnam. In *Taxonomy of Economic Seaweeds with Reference to some Pacific Species* (Abbott, I.A. & McDermid, K. J., editors), 291–321. California Sea Grant College, La Jolla.
- Adams, N.M. (1991). The New Zealand species of *Polysiphonia* Greville (Rhodophyta). *New Zealand Journal of Botany*, **29**: 411–427.
- Adams, N.M. (1994). *Seaweeds of New Zealand. An Illustrated Guide*. Canterbury University Press, Christchurch.
- Afaq-Husain, S. & Shameel, M. (2000). Further investigations on the red alga *Melanothamnus afaqhusainii* (Ceramiales) from the coast of Pakistan. *Pakistan Journal of Botany*, **32**: 15–26.
- Agardh, C.A. (1824). *Systema algarum*. Berlinginiana, Lundae.
- Agardh, J.G. (1842). *Algae maris Mediterranei et Adriatici*, observationes in diagnosis specierum et dispositionem generum. Fortin, Masson et Cie, Paris.
- Agardh, J.G. (1863). *Species Genera et Ordines Algarum, seu descriptiones succinctae specierum, generum et ordinum, quibus algarum regnum constituitur. Volumen secundum: algas florideas complectens*. C.W.K. Gleerup, Lundae.
- Bailey, J.W. (1848). Continuation of the list of localities of algae in the United States. *American Journal of Science and Arts, series 2*, **6**: 37–42.
- Bárbara, I., Cremades, J., Calvo, S., López-Rodríguez, M.C. & Dosil, J. (2005). Checklist of the benthic marine and brackish Galician algae (NW Spain). *Anales del Jardín Botánico de Madrid*, **62**: 69–100.
- Bárbara, L., Choi, H.-G., Secilla, A., Díaz-Tapia, P., Gorostiaga, J.M., Seo, T.-K., Jung, M.Y. & Bercibar, E. (2013). *Lampisiphonia iberica* gen. et sp. nov. (Ceramiales, Rhodophyta) based on morphology and molecular evidence. *Phycologia*, **52**: 137–155.
- Børgesen, F. (1918). The marine algae of the Danish West Indies. Part 3. Rhodophyceae (4). *Dansk Botanisk Arkiv*, **3**: 241–304.
- Børgesen, F. (1934). Some Indian Rhodophyceae especially from the shores of the Presidency of Bombay-IV. *Bulletin of Miscellaneous Information, Royal Botanic Gardens, Kew*, **1934**: 1–30.
- Bornet, É. (1892). Les algues de P. K. A. Schousboe, récoltées au Maroc & dans la Méditerranée de 1815 a 1829, et déterminées par M. Édouard Bornet. *Mémoires*

- de la Société Nationale des Sciences naturelles et Mathématiques de Cherbourg, **28**: 165–376.
- Bory de Saint-Vincent, J.B.G.M. (1822). Brongniartella. Brongniartella. Bot. crypt. (Céramiales). In *Dictionnaire Classique d'Histoire Naturelle* (Audouin, I. et al., editors), 516–517. Rey et Gravier; Baudouin frères, Paris.
- Bustamante, D.E., Won, B.Y., Ramirez, M.E. & Cho, T.O. (2012). *Neosiphonia peruviana* sp. nov. (Rhodomelaceae, Rhodophyta) from the Pacific coast of South America. *Botanica Marina*, **55**: 359–366.
- Bustamante, D.E., Won, B.Y. & Cho, T.O. (2013a). *Neosiphonia ramirezii* sp. nov. (Rhodomelaceae, Rhodophyta) from Peru. *Algae*, **28**: 73–82.
- Bustamante, D.E., Won, B.Y. & Cho, T.O. (2013b). *Neosiphonia baliana* sp. nov. and *N. silvae* sp. nov. (Rhodomelaceae, Rhodophyta) from Bali, Indonesia. *Botanica Marina*, **56**: 515–524.
- Chapman, J.W. & Carlton, J.T. (1991). A test of criteria for introduced species: the global invasion by the isopod *Synidotea laevidorsalis* (Miers, 1881). *Journal of Crustacean Biology*, **11**: 386–400.
- Choi, H.G., Kim, M.S., Guiry, M.D. & Saunders, G.W. (2001). Phylogenetic relationships of *Polysiphonia* (Rhodomelaceae, Rhodophyta) and its relatives based on anatomical and nuclear small-subunit rDNA sequence data. *Canadian Journal of Botany*, **79**: 1465–1476.
- Coomans, R.J. & Hommersand, M.H. (1990). Vegetative growth and organization. In *Biology of the Red Algae* (Cole, K.M. & Sheath, R.G., editors), 275–304. Cambridge University Press, Cambridge.
- Dawes, C.J. & Mathieson, A.C. (2008). *The Seaweeds of Florida*. University Press of Florida, Gainesville, FL.
- Dawson, E.Y. (1944). The marine algae of the gulf of California. *The University of Southern California Publications*, **3**: 189–359.
- Delivopoulos, S. (2002). Ultrastructure of trichoblasts in the red alga *Osmundea spectabilis* var. *spectabilis* (Rhodomelaceae, Ceramiales). *European Journal of Phycology*, **37**: 329–338.
- Díaz-Tapia, P. & Bárbara, I. (2013). Seaweeds from sand-covered rocks of the Atlantic Iberian Peninsula. Part I. The Rhodomelaceae (Ceramiales, Rhodophyta). *Cryptogamie, Algologie*, **34**: 325–422.
- Díaz-Tapia, P., Kim, M.S., Secilla, A., Bárbara, I. & Cremades, J. (2013a). Taxonomic reassessment of *Polysiphonia foetidissima* (Rhodomelaceae, Rhodophyta) and similar species, including *P. schneideri*, a newly introduced species in Europe. *European Journal of Phycology*, **48**: 345–362.
- Díaz-Tapia, P., Bárbara, I. & Bercibar, E. (2013b). Vegetative and reproductive morphology of *Polysiphonia tripinnata* (Rhodomelaceae, Rhodophyta): a new record from the European Atlantic coast. *Botanica Marina*, **56**: 151–160.
- Díaz-Tapia, P., Maggs, C.A., West, J.A. & Verbruggen, H. (2015). Tackling rapid radiations with chloroplast phylogenomics in the Rhodomelaceae. *European Journal of Phycology*, **50**: 54–55.
- Doyle, J.J. & Doyle, J.L. (1987). A rapid DNA isolation procedure for small quantities of fresh leaf tissue. *Phytochemical Bulletin*, **1**: 11–5.
- Drummond, A.J., Ho, S.Y.W., Phillips, M.J. & Rambaut, A. (2006). Relaxed phylogenetics and dating with confidence. *PLoS Biology*, **4**: e88.
- Falkenberg, P. (1901). *Die Rhodomelaceen des Golfes von Neapel und der angrenzenden Meeres-Abschnitte. Fauna und Flora des Golfes von Neapel, Monographie 26*. Berlin.
- Freshwater, D.W. & Rueness, J. (1994). Phylogenetic relationships of some European *Gelidium* (Gelidiales, Rhodophyta) species, based on *rbcl* nucleotide sequence analysis. *Phycologia*, **33**: 187–194.
- Garbary, D.J. & Clarke, B. (2001). Apoptosis in trichoblast development in *Polysiphonia harveyi* (Rhodophyta). *Phycologia*, **40**: 324–329.
- Goff, L.J. & Coleman, A.W. (1986). A novel pattern of apical cell polyploidy, sequential polyploidy reduction and intercellular nuclear transfer in the red alga *Polysiphonia*. *American Journal of Botany*, **73**: 1109–1130.
- Goff, L.J. & Moon, D.A. (1993). PCR amplification of nuclear and plastid genes from algal herbarium specimens and algal spores. *Journal of Phycology*, **29**: 381–384.
- Gray, S.F. (1821). *A Natural Arrangement of British Plants*. ... Baldwin, Cradock & Joy, Paternoster-Row, London.
- Greville, R.K. (1824). *Flora Edinensis* ... William Blackwood, Edinburgh, & T. Cadell, London.
- Grunow, A. (1874). Algen der Fidschi-, Tonga- und Samoa-Inseln, gesammelt von Dr. E. Graeffe. *Journal des Museum Godeffroy*, **3**: 23–50.
- Guimarães, S.M.P.D.B., Fujii, M.T., Pupo, D. & Yokoya, N. S. (2004). Reavaliação das características morfológicas e suas implicações taxonômicas no gênero *Polysiphonia sensu lato* (Ceramiales, Rhodophyta) do litoral dos estados de São Paulo e Espírito Santo, Brasil. *Revista Brasileira de Botânica*, **27**: 163–183.
- Guiry, M.D. & Guiry, G.M. (2016). *AlgaeBase*. World-wide electronic publication, National University of Ireland, Galway. <http://www.algaebase.org>; searched on 7 February 2016.
- Hariot, P. (1891). Liste des algues marines rapportés de Yokoska (Japon) par M. le Dr Savatier. *Mémoires de la Société nationale des sciences naturelles de Cherbourg*, **27**: 211–230.
- Harvey, W.H. (1847). *Nereis Australis, or Algae of The Southern Ocean ... [Part I]*. Reeve Brothers, London.
- Harvey, W.H. (1853). *Nereis boreali-americana*; or, contributions towards a history of the marine algae of the Atlantic and Pacific coasts of North America. Part II. Rhodospermeae. *Smithsonian Contributions to Knowledge*, **5**: [i–ii], [1]–258, pls XIII–XXXVI.
- Harvey, W.H. (1855). Algae. In *The botany of the Antarctic voyage of H.M. discovery ships Erebus and Terror, in the years 1839–1843, under the command of Captain Sir James Clark Ross. II. Flora Novae-Zelandiae. Part II. Flowerless plants* (Hooker, J.D., editor), pp. 211–266. Reeve, London.
- Harvey, W.H. (1857 '1856'). Algae. In: *Account of the Botanical specimens* (Gray, A., editor) *Narrative of the expedition of an American squadron to the China Seas and Japan, performed in the years 1852, 1853 and 1854, under the command of Commodore M.C. Perry, United States Navy. Volume II – with illustrations*. (Anon, editors), pp. 331–332. Senate of the Thirty-third Congress, Second Session, Executive Document. House of Representatives, Washington.
- Harvey, W.H. (1859). *Phycologia Australica. Vol. 2*. Lovell Reeve & Co, London.

- Harvey, W.H. (1860). Characters of new algae, chiefly from Japan and adjacent regions, collected by Charles Wright in the North Pacific Exploring Expedition under Captain James Rodgers. *Proceedings of the American Academy of Arts and Sciences*, **4**: 327–335.
- Harvey, W.H. (1862). *Phycologia australica*. Vol. 4. Lovell Reeve & Co, London.
- Hollenberg, G.J. (1942). An account of the species of *Polysiphonia* on the Pacific coast of North America. I. Oligosiphonia. *American Journal of Botany*, **29**: 772–785.
- Hollenberg, G.J. (1944). An account of the species of *Polysiphonia* on the Pacific coast of North America. II. *Polysiphonia*. *American Journal of Botany*, **31**: 474–483.
- Hollenberg, G. J. (1961). Marine red algae of Pacific Mexico, Part 5: The genus *Polysiphonia*. *Pacific Naturalist*, **2**: 345–375.
- Hollenberg, G.J. (1968a). An account of the species of *Polysiphonia* of the central and western tropical Pacific ocean. II *Oligosiphonia*. *Pacific Science*, **22**: 56–98.
- Hollenberg, G.J. (1968b). An account of the species of *Polysiphonia* of the central and western tropical Pacific ocean. I. *Polysiphonia*. *Pacific Science*, **22**: 198–207.
- Hollenberg, G. J. (1968c). An account of the species of the red alga *Herposiphonia* occurring in the Central and Western tropical Pacific Ocean. *Pacific Science*, **22**: 536–559.
- Hollenberg, G.J. & Norris, J.N. (1977). The red alga *Polysiphonia* (Rhodomelaceae) in the Northern Gulf of California. *Smithsonian Contributions to the Marine Sciences*, **1**: 1–21.
- Hommersand, M.H. (1963). The morphology and classification of some Ceramiaceae and Rhodomelaceae. *University of California Publications in Botany*, **35**: 165–366.
- Hommersand, M.H. (2007). Global biogeography and relationships of the Australian marine macroalgae. In *Algae of Australia* (McCarthy, P.M. & Orchard, A.E., editors), 511–542. Australian Biological Resources Study, Canberra.
- Hooker, J.D. & Harvey, W.H. (1845). Algae Novae Zelandiae. *London Journal of Botany*, **4**: 521–551.
- Hudson, W. (1762). *Flora anglica; exhibens plantas per regnum angliae sponte crescentes, distributas secundum systema sexuale: cum differentiis specierum, synonymis auctorum, nominibus incolarum, solo locorum, tempore florendi, officialibus pharmacopoeorum*. J. Nourse & C. Moran, London.
- Hurd, C.L., Harrison, P.J., Bischof, K. & Lobban, C.S. (2014). *Seaweed Ecology and Physiology*. 2nd ed. Cambridge University Press, Cambridge.
- Iyengar, M. O. P., Balakrishnan, M. S. (1949). Morphology and cytology of *Polysiphonia platycarpa* Boergs. Preliminary note. *Proceedings of the Indian Academy of Sciences, Section B* **29**: 105–108.
- Iyengar, M.O.P. & Balakrishnan, M.S. (1950). Morphology and cytology of *Polysiphonia platycarpa* Boerges. *Proceedings of the Indian Academy of Science B*, **31**: 135–161.
- Karsten, U., Sawall, T., Hanelt, D., Bischof, K., Figueroa, F. L., Flores-Moya, A. & Wiencke, C. (1998). An inventory of UV-absorbing mycosporine-like amino acids in macroalgae from polar to warm-temperate regions. *Botanica Marina*, **41**: 443–454.
- Kearse, M., Moir, R., Wilson, A., Stones-Havas, S., Cheung, M., Sturrock, S., Buxton, S., Cooper, A., Markowitz, S., Duran, C., Thierer, T., Ashton, B., Mentjies, P. & Drummond, A. (2012). Geneious Basic: an integrated and extendable desktop software platform for the organization and analysis of sequence data. *Bioinformatics*, **28**: 1647–1649.
- Kim, M.S. (2003). Taxonomic reassessment of the genus *Polysiphonia* (Rhodomelaceae, Rhodophyta): morphology and reproduction of *Neosiphonia decumbens* and *N. harlandii*. *Algae*, **18**: 107–119.
- Kim, M.S. (2005). Taxonomy of a poorly documented alga, *Neosiphonia savatieri* Rhodomelaceae, Rhodophyta) from Korea. *Nova Hedwigia*, **81**: 163–176.
- Kim, M.S. & Abbott, I.A. (2006). Taxonomic notes on Hawaiian *Polysiphonia*, with transfer to *Neosiphonia* (Rhodomelaceae, Rhodophyta). *Phycological Research*, **54**: 32–39.
- Kim, M.S. & Lee, I.K. (1999). *Neosiphonia flavimarina* gen. et sp. nov. with a taxonomic reassessment of the genus *Polysiphonia* (Rhodomelaceae, Rhodophyta). *Phycological Research*, **47**: 271–281.
- Kim, B. & Kim, M.S. (2014). Three new species of *Polysiphonia sensu lato* (Rhodophyta) based on the morphology and molecular evidence. *Algae*, **29**: 183–195.
- Kim, B. & Kim, M.S. (2016). *Neosiphonia yongpili* sp. nov. (Rhodomelaceae, Rhodophyta), known as *Neosiphonia simplex* from Korea, with an emphasis on cystocarp development. *Phycologia*, **55**: 323–332.
- Kim, M.S. & Yang, E.C. (2006). Taxonomy and phylogeny of *Neosiphonia japonica* (Rhodomelaceae, Rhodophyta) based on *rbcL* and *cpeA/B* gene sequences. *Algae*, **21**: 287–294.
- Kim, M.S., Lee, I.K. & Boo, S.M. (1994). Morphological studies of the red alga *Polysiphonia morrowii* Harvey on the Korean coast. *Korean Journal of Phycology*, **9**: 185–192.
- Kim, M.-S., Maggs, C.A., McIvor, L. & Guiry, M.D. (2000). Reappraisal of the type species of *Polysiphonia* (Rhodomelaceae, Rhodophyta). *European Journal of Phycology*, **35**: 83–92.
- Kirby, R.R. & Reid, P.C. (2001). PCR from the CPR offers a historical perspective on marine population ecology. *Journal of the Marine Biological Association of the United Kingdom*, **81**: 539–540.
- Kraft, G.T. & Abbott, I.A. (2002). The anatomy of *Neotenophycus ichthyosteus* gen. et sp. nov. (Rhodomelaceae, Ceramiales), a bizarre red algal parasite from the central Pacific. *European Journal of Phycology*, **37**: 269–278.
- Kuntze, O. (1891). *Revisio generum plantarum. Pars II*. Arthur Felix, Dulau & Co., U. Hoepli, Gust. A. Schechert, Charles Klincksieck, Leipzig, London, Milan, New York, Paris.
- Kützing, F.T. (1863). *Tabulae phycologicae; oder, Abbildungen der Tange. Vol. XIII*. Gedruckt auf kosten des Verfassers (in commission bei W. Köhne), Nordhausen.
- Kützing, F.T. (1864). *Tabulae phycologicae; oder, Abbildungen der Tange. Vol. XIV*. Gedruckt auf kosten des Verfassers (in commission bei W. Köhne), Nordhausen.
- Kylin, H. (1941). Californische Rhodophyceen. *Acta Universitatis Lundensis*, **37**(2): 1–71.
- Kylin, H. (1956). *Die Gattungen der Rhodophyceen*. CWK Gleerups Förlag, Lund.
- Lam, W.D., García-Fernández, M.E., Aaboal, M. & Vis, M. L. (2013). *Polysiphonia subtilissima* (Ceramiales, Rhodophyta) from freshwater habitats in North America and Europe is confirmed as conspecific with marine collections. *Phycologia*, **52**: 156–160.
- Lartillot, N., Lepage, T. & Blanquart, S. (2009). PhyloBayes 3: a Bayesian software package for phylogenetic

- reconstruction and molecular dating. *Bioinformatics*, **25**: 2286–2288.
- Lauret, M. (1967). Morphologie, phénologie, répartition des *Polysiphonia* marins du littoral Languedocien. I. Section Oligosiphonia. *Naturalia Monspeliensia, Bot.*, **18**: 347–373.
- Lauret, M. (1970). Morphologie, phénologie, répartition des *Polysiphonia* marins du littoral Languedocien. II. Section *Polysiphonia*. *Naturalia Monspeliensia, Bot.*, **21**: 121–163.
- Lee, Y. (2008). *Marine Algae of Jeju*. Academy Publication, Seoul.
- Løvring, T. (1941). Die Meeresalgen der Juan Fernandez-Inseln. Die Corallinaceen der Juan Fernandez-Inseln. In *The Natural History of Juan Fernandez and Easter Island, Vol. 2* (Skottsberg, C., editor), 601–670, 753–757. Almqvist & Wiksells Boktryckeri, Uppsala.
- Lüning, K. (1990). *Seaweeds: Their Environment, Biogeography and Ecophysiology*. Wiley Interscience Publications, New York.
- Maggs, C.A. & Hommersand, M.H. (1993). *Seaweeds of the British Isles. Volume 1. Rhodophyta, Part 3A Ceramiales*. Natural History Museum/HMSO, London.
- Mamoozadeh, N.R. & Freshwater, D.W. (2011). Taxonomic notes on Caribbean *Neosiphonia* and *Polysiphonia* (Ceramiales, Florideophyceae): five species from Florida, USA and Mexico. *Botanica Marina*, **54**: 269–292.
- Mamoozadeh, N.R. & Freshwater, D.W. (2012). *Polysiphonia sensu lato* (Ceramiales, Florideophyceae) species of Caribbean Panama including *Polysiphonia lobophoralis* sp. nov. and *Polysiphonia nuda* sp. nov. *Botanica Marina*, **55**: 317–347.
- Masuda, M., Kudo, T., Kawaguchi, S. & Guiry, M. D. (1995). Lectotypification of some marine red algae described by W. H. Harvey from Japan. *Phycological Research*, **43**: 191–202.
- Masuda, M., Abe, T., Kawaguchi, S. & Phang, S. M. (2001). Taxonomic notes on marine algae from Malaysia. VI. Five species of Ceramiales (Rhodophyceae). *Botanica Marina*, **44**: 467–478.
- McIvor, L., Maggs, C.A., Provan, J. & Stanhope, M.J. (2001). *rbcl* sequences reveal multiple cryptic introductions of the Japanese red alga *Polysiphonia harveyi*. *Molecular Ecology*, **10**: 911–919.
- McIvor, L., Maggs, C. A., Stanhope, M. J. (2002). *Rbcl* sequences indicate a single evolutionary origin of multinucleate cells in the red algal tribe Callithamnieae. *Molecular Phylogenetics and Evolution* **23**: 433–446.
- Millar, A.J.K. (1990). Marine red algae of the Coffs Harbour region, northern New South Wales. *Australian Systematic Botany*, **3**: 293–593.
- Morrill, J.F. (1976). Comparative morphology and taxonomy of some dorsiventral and some parasitic Rhodomelaceae. Thesis, University of North Carolina, Chapel Hill.
- Muangmai, N., Yamagishi, Y., Maneekat, S. & Kaewsuralikhit, C. (2014). The new species *Neosiphonia thailandica* sp. nov. (Rhodomelaceae, Rhodophyta) from the Gulf of Thailand. *Botanica Marina*, **57**: 459–467.
- Nägeli, C. (1846). *Über Polysiphonia und Herposiphonia*. *Zeitschrift für wissenschaftliche Botanik*, **4**: 207–256.
- Nam, K.W. & Kang, P.J. (2012). *Algal flora of Korea. Volume 4, Number 4. Rhodophyta: Ceramiales: Rhodomelaceae: 18 genera including Herposiphonia*. National Institute of Biological Resources, Incheon.
- Nam, K.W., Maggs, C.A., McIvor, L. & Stanhope, M.J. (2000). Taxonomy and phylogeny of *Osmundea* (Rhodomelaceae, Rhodophyta) in Atlantic Europe. *Journal of Phycology*, **36**: 759–772.
- Navarro, N.P., Mansilla, A., Figueroa, F., Korbee, N., Jofre, J. & Plastino, E. (2014). Short-term effects of solar UV radiation and NO₃⁻ supply on the accumulation of mycosporine-like amino acids in *Pyropia columbina* (Bangiales, Rhodophyta) under spring ozone depletion in the sub-Antarctic region, Chile. *Botanica Marina*, **57**: 9–20.
- Noda, M. & Kitami, T. (1971). Some species of marine algae from Echigo Province facing the Japan Sea. *Scientific Reports Niigata University, Ser. D. (Biology)*, **8**: 35–52.
- Norris, R.E. (1994). Some cumophytic Rhodomelaceae (Rhodophyta) occurring in Hawaiian surf. *Phycologia*, **33**: 434–443.
- Norris, J.N. (2014). *Marine algae of the northern Gulf of California, II: Rhodophyta*. Smithsonian Institution Scholarly Press, Washington D.C.
- Okamura, K. (1912). *Icones of Japanese Algae. Vol. II (10), pls. 96–100*. Privately published, Tokyo.
- Parsons, M.J. (1975). Morphology and taxonomy of the Dasyaceae and the Lophothalieae (Rhodomelaceae) of the Rhodophyta. *Australian Journal of Botany*, **23**: 549–713.
- Parsons, M. J. (1980). The morphology and taxonomy of *Brongniartella* Bory sensu Kylin (Rhodomelaceae, Rhodophyta). *Phycologia*, **19**: 273–295.
- Phillips, L.E. (2001). Morphology and molecular analysis of the Australasian monotypic genera *Lembergia* and *Sonderella* (Rhodomelaceae, Rhodophyta), with a description of the tribe Sonderelleae trib. nov. *Phycologia*, **40**: 487–499.
- Phillips, L.E., Choi, H.G., Saunders, G.W. & Kraft, G.T. (2000). The morphology, taxonomy and molecular phylogeny of *Heterocladia* and *Trigenea* (Rhodomelaceae, Rhodophyta), with delineation of the little known tribe Heterocladieae. *Journal of Phycology*, **36**: 199–219.
- Price, I.R. & Scott, F.J. (1992). *The turf algal flora of the Great Barrier Reef. Part I. Rhodophyta*. Townsville, Australia: Botany Department, James Cook University.
- Provan, J., Booth, D., Todd, N.P., Beatty, G.E. & Maggs, C. A. (2008). Tracking biological invasions in space and time: elucidating the invasive history of the green alga *Codium fragile* using old DNA. *Diversity and Distributions*, **14**: 343–354.
- Pueschel, C. M. (1990). Cell structure. In *Biology of the Red Algae* (Cole, K.M. & Sheath, R.G., editors), 7–41. Cambridge University Press, Cambridge.
- Rambaut, A., Suchard, M.A., Xie, W. & Drummond, A.J. (2013). Tracer MCMC Trace Analysis Tool, Version v1.6.0. Available from: <http://tree.bio.ed.ac.uk/software/tracer/>
- Ribera, M.A. & Boudouresque, C.F. (1995). Introduced marine plants, with special reference to macroalgae: mechanisms and impact. In *Progress in Phycological Research* (Round, F.E. & Chapman, D.J., editors), 187–268. Biopress Ltd.
- Ronquist, F., Teslenko, M., Mark, P. van der, Ayres, D., Darling, A., Höhna, S., Larget, B., Liu, L., Suchard, M.A. & Huelsenbeck, J.P. (2011). MrBayes 3.2: efficient Bayesian phylogenetic inference and model choice across a large model space. *Systematic Biology*, **61**: 539–542.
- Savoie, A.M. & Saunders, G.W. (2015). Evidence for the introduction of the Asian red alga *Neosiphonia japonica*

- and its introgression with *Neosiphonia harveyi* (Ceramiales, Rhodophyta) in the Northwest Atlantic. *Molecular Ecology*, **24**: 5917–5937.
- Savoie, A.M. & Saunders, G.W. (2016). A molecular phylogenetic and DNA barcode assessment of the tribe Pterosiphoniae (Ceramiales, Rhodophyta) emphasizing the Northeast Pacific. *Botany*, **94**: 917–939.
- Saunders, G.W. & McDevit, D.C. (2012). Methods for DNA barcoding photosynthetic protists emphasizing the macroalgae and diatoms. *Methods in Molecular Biology*, **858**: 207–222.
- Saunders, G.W. & Moore, T.E. (2013). Refinements for the amplification and sequencing of red algal DNA barcode and RedToL phylogenetic markers: a summary of current primers, profiles and strategies. *Algae*, **28**: 31–43.
- Schmitz, F. & Falkenberg, P. (1897). Rhodomelaceae. In *Die natürlichen Pflanzenfamilien nebst ihren Gattungen und wichtigeren Arten insbesondere den Nutzpflanzen unter Mitwirkung zahlreicher hervorragender Fachgelehrten, Teil 1, Abteilung 2* (Engler, A. & Prantl, K. editors), 421–480. Verlag von Wilhelm Engelmann, Leipzig.
- Schneider, C. W. & Searles, R. B. (1991). *Seaweeds of the Southeastern United States: Cape Hatteras to Cape Cañaveral*. Duke University Press, Durham and London.
- Segi, T. (1949). On the systematic position of the so-called *Polysiphonia hakodatensis* Yendo from Japan. *Siebutsu*, **14**: 134–139.
- Segi, T. (1951). Systematic study of the genus *Polysiphonia* from Japan and its vicinity. *Journal of the Faculty of Fisheries, Prefectural University of Mie*, **1**: 169–272.
- Setchell, W.A. (1926). Tahitian algae collected by W.A. Setchell, C.B. Setchell and H.E. Parks. *University of California Publications in Botany*, **12**: 61–142.
- Setchell, W.A. & Gardner, N.L. (1924). XXIX Expedition of the California Academy of Sciences to the Gulf of California in 1921. The Marine Algae. *Proceeding of the California Academy of Science*, **4**: 695–949.
- Setchell, W.A. & Gardner, N.L. (1930). Marine algae of the Revillagigedo Islands expedition in 1925. *Proceedings of the California Academy of Sciences*, **4**: 109–215.
- Shameel, M. (1999). *Melanothamnus afaqhusainii*, a new red alga from the coast of Karachi. *Pakistan Journal of Botany*, **31**: 211–214.
- Shavit, L., Penny, D., Hendy, M.D. & Holland, B.R. (2007). The problem of rooting rapid radiations. *Molecular Biology and Evolution*, **24**: 2400–2411.
- Sherwood, A.R., Kurihara, A., Conklin, K.Y., Sauvage, T. & Presting, G.G. (2010). The Hawaiian Rhodophyta biodiversity survey (2006–2010): a summary of principal findings. *BMC Plant Biology*, **10**: 258.
- Silva, P.C., Basson, P.W. & Moe, R.L. (1996). Catalogue of the benthic marine algae of the Indian Ocean. *University of California Publications in Botany*, **79**: 1–1259.
- Stamatakis, A. (2014). RAxML Version 8: a tool for phylogenetic analysis and post-analysis of large phylogenies. *Bioinformatics*, **30**: 1312–1313.
- Stegenga, H., Bolton, J.J. & Anderson, R.J. (1997). *Seaweeds of the South African west coast*. Bolus Herbarium, University of Cape Town, Cape Town.
- Stuercke, B. & Freshwater, D.W. (2008). Consistency of morphological characters used to delimit *Polysiphonia sensu lato* species (Ceramiales, Florideophyceae): analyses of North Carolina, USA specimens. *Phycologia*, **47**: 541–559.
- Stuercke, B. & Freshwater, D.W. (2010). Two new species of *Polysiphonia* (Ceramiales, Florideophyceae) from the western Atlantic. *Botanica Marina*, **53**: 301–311.
- Suhr, J.N. von (1831). Beschreibung einiger neuen Algen. *Flora*, **14**: 673–687, 709–716, 725–731.
- Thiers, B. (2016) [continuously updated]. *Index Herbariorum: A global directory of public herbaria and associated staff*. New York Botanical Garden's Virtual Herbarium. <http://sweetgum.nybg.org/science/ih/>.
- Thorne, J.L. & Kishino, H. (2002). Divergence time and evolutionary rate estimation with multilocus data. *Systematic Biology*, **51**: 689–702.
- Uwai, S. & Masuda, M. (1999). *Kintarosiphonia* (Rhodomelaceae, Ceramiales), a new red algal genus based on *Pterosiphonia fibrillosa* Okamura from Japan. *Phycologia*, **38**: 225–233.
- Womersley, H.B.S. (1979). Southern Australian species of *Polysiphonia* Greville (Rhodophyta). *Australian Journal of Botany*, **27**: 459–528.
- Womersley, H.B.S. (2003). *The Marine Benthic Flora of Southern Australia. Rhodophyta. Part IIID. Ceramiales-Delesseriaceae, Sarcomeniaceae, Rhodomelaceae*. Australian Biological Resources Study & State Herbarium of South Australia, Canberra & Adelaide.
- Wulfen, F.X. (1789). *Plantae rariorae carinthiacae*. In *Collectanea ad botanicam, chemiam, et historiam naturalem, spectantia cum figuris* (Jacquin, J.N., editor). Ex Officina Wappleriana, Vienna.
- Wynne, M.J. & Banaimoon, S.A. (1990). The occurrence of *Jolya laminarioides* (Phaeophyta) in the Arabian Sea and the Indian Ocean and a new report of *Melanothamnus somalensis* (Rhodophyta). *Botanica Marina*, **33**: 213–218.
- Yang, E.U., Boo, S.M., Bhattacharya, D., Saunders, G.W., Knoll, A.H., Fredericq, S., Graf, L. & Yoon, S.H. (2016). Divergence time estimates and the evolution of major lineages in the florideophyte red algae. *Scientific Reports*, **6**: 21361.
- Yoon, H.Y. (1986). A taxonomic study of genus *Polysiphonia* (Rhodophyta) from Korea. *Korean Journal of Phycology*, **1**: 3–86.
- Yoshida, T. (1998). *Marine Algae of Japan*. Uchida Rokakuho Publishing Co., Tokyo.
- Zuccarello, G.C., West, J.A., Kamiya, M. & King, R.J. (1999). A rapid method to score plastid haplotypes in red seaweeds and its use in determining parental inheritance of plastids in the red alga *Bostrychia* (Ceramiales). *Hydrobiologia*, **401**: 207–214.