

## Research Article

Algae 2013, 28(1): 73-82

<http://dx.doi.org/10.4490/algae.2013.28.1.073>

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# *Neosiphonia ramirezii* sp. nov. (Rhodomelaceae, Rhodophyta) from Peru

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The genus *Neosiphonia* Kim and Lee 1999 contains approximately 30 species worldwide. Unidentified samples from Peru are here described as a new species, *Neosiphonia ramirezii* sp. nov., on the basis of morphological and molecular data. *N. ramirezii* sp. nov. is characterized by the presence of a limited prostrate system, well-developed erect filaments, rhizoids cut off from pericentral cells by cross walls, four pericentral cells that are completely ecorticate, scarce trichoblasts, inconspicuous scar cells, procarps with three-celled carpogonial branches, spermatangial branches developed from basal cells of forked trichoblasts, and spirally arranged tetrasporangia. Our new species is distinct from *N. flaccidissima* (Hollenberg) Kim et Lee, *N. sphaerocarpa* (Børgesen) Kim et Lee, and *N. savatieri* (Harriot) Kim et Lee from the Pacific temperate coast of South America and from 14 *Neosiphonia* species reported worldwide by having limited prostrate filaments attached by numerous rhizoids, dichotomous (“Y” shaped) branches in the main axes, and scarce trichoblasts. Phylogenetic *rbcl* analyses confirm the placement of the new taxon as a distinct species in the genus *Neosiphonia*.

**Key Words:** morphology; *Neosiphonia ramirezii*; Peru; *rbcl*; Rhodophyta; taxonomy

## INTRODUCTION

The segregation of the genus *Neosiphonia* Kim et Lee from the large genus *Polysiphonia* Greville was based on *Neosiphonia flavimarina* Kim et Lee from Korea (Kim and Lee 1999, Kim 2005). *Neosiphonia* is characterized by having lateral branch initials and trichoblasts produced on successive segments, rhizoids cut off from pericentral cells by a cross wall, procarps bearing a three-celled carpogonial branch, spermatangial branches arising as a furcation of the trichoblasts, and spirally arranged tetrasporangia (Kim and Lee 1999, Kim 2003).

Recently, new combinations were made for *Neosiphonia upolensis* (Grunow) Kim et Boo from Malaysia (Kim et al. 2008), *N. bajacali* (Hollenberg) Mamoozadeh et Freshwater from Caribbean Mexico and *N. echinata* (Harvey) Mamoozadeh et Freshwater from Florida, USA (Mamoozadeh and Freshwater 2011), and *N. sertularioides* (Grate-

loup) Nam et Kang from Geojedo, Korea (Nam and Kang 2012). In the most recent study, Bustamante et al. (2012) reported a new species, *N. peruviansis* Bustamante, Won, Ramírez et T. O. Cho from Pisco, Ica, Peru.

Four *Neosiphonia* species are currently recognized from the Pacific temperate coast of South America: *N. flaccidissima* (Hollenberg) Kim et Lee; *N. peruviansis* Bustamante, Won, Ramírez et Cho; *N. savatieri* (Harriot) Kim et Lee; and *N. sphaerocarpa* (Børgesen) Kim et Lee (Howe 1914, Dawson et al. 1964, Ramírez and Santelices 1991, Bustamante et al. 2012). These species are characterized by rhizoids cutting off pericentral cells by a cross wall, ecorticate axes, and spirally arranged tetrasporangia.

We collected unidentified samples from Peru in 2008 and 2012 which we here describe as new species, *Neo-*

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Received January 8, 2013, Accepted February 19, 2013

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*siphonia ramirezii* sp. nov., on the basis of their morphology. We also provide evidence of their phylogenetic relationship with other similar species, using comparative *rbcL* sequence analyses.

## MATERIALS AND METHODS

### Morphology

Samples were collected at Lagunillas, Pisco, Peru on August 2008 and July 2012, preserved in 4-5% formalin / seawater for the morphological study and dried in silica gel for the molecular study. Microscope observations were made on material stained with 1% aqueous aniline blue acidified with 0.1% diluted HCl. Photomicrographs were taken on an Olympus microscope (BX51TRF; Olympus, Tokyo, Japan) with an Olympus DP71 camera. A total of 25 individuals from 5 tufts were selected for measuring quantitative characters. Voucher specimens were deposited in the herbarium of Chosun University Korea (CUK).

### Molecular study

Genomic DNA was extracted from silica gel-dried samples using the G-spin Iip genomic DNA extraction kit (iNtRON Biotechnology, Inc., Seongnam, Korea). The *rbcL* gene was amplified using the primer combinations F7-R753 and F645-Rrbcst, as listed in Lin et al. (2001), and was sequenced with the primers F7, F645, F993, R376, R753, R1150, and RrbcStart (Freshwater and Rueness 1994, Lin et al. 2001, Gavio and Fredericq 2002). PCR and sequencing protocols were as described in Cho et al. (2003). Sequences were determined for both forward and reverse strands using an ABI Prism 3100 Genetic Analyzer (PE Applied Biosystems, Foster City, CA, USA). New *rbcL* sequences were obtained from *N. ramirezii* and have been deposited in EMBL / GenBank under the accession numbers KC493351 and KC493352. All *rbcL* sequence data were compiled and the sequences aligned in the Genetic Data Environment (GDE 2.2) program (Smith et al. 1994). Phylogenetic analyses were conducted using MEGA version 5 (Tamura et al. 2011). Support for nodes in the maximum parsimony tree was determined by calculating 500 bootstrap proportion replicates (Felsenstein 1985). For maximum likelihood, we performed a likelihood ratio test using Modeltest 3.06 (Posada and Crandall 1998) to determine the best available model for the *rbcL* data. Maximum likelihood analyses were conducted using the GTR + I + G model, with 500 bootstrap replicates.

## RESULTS

### *Neosiphonia ramirezii* D. E. Bustamante, B. Y. Won et T. O. Cho sp. nov.

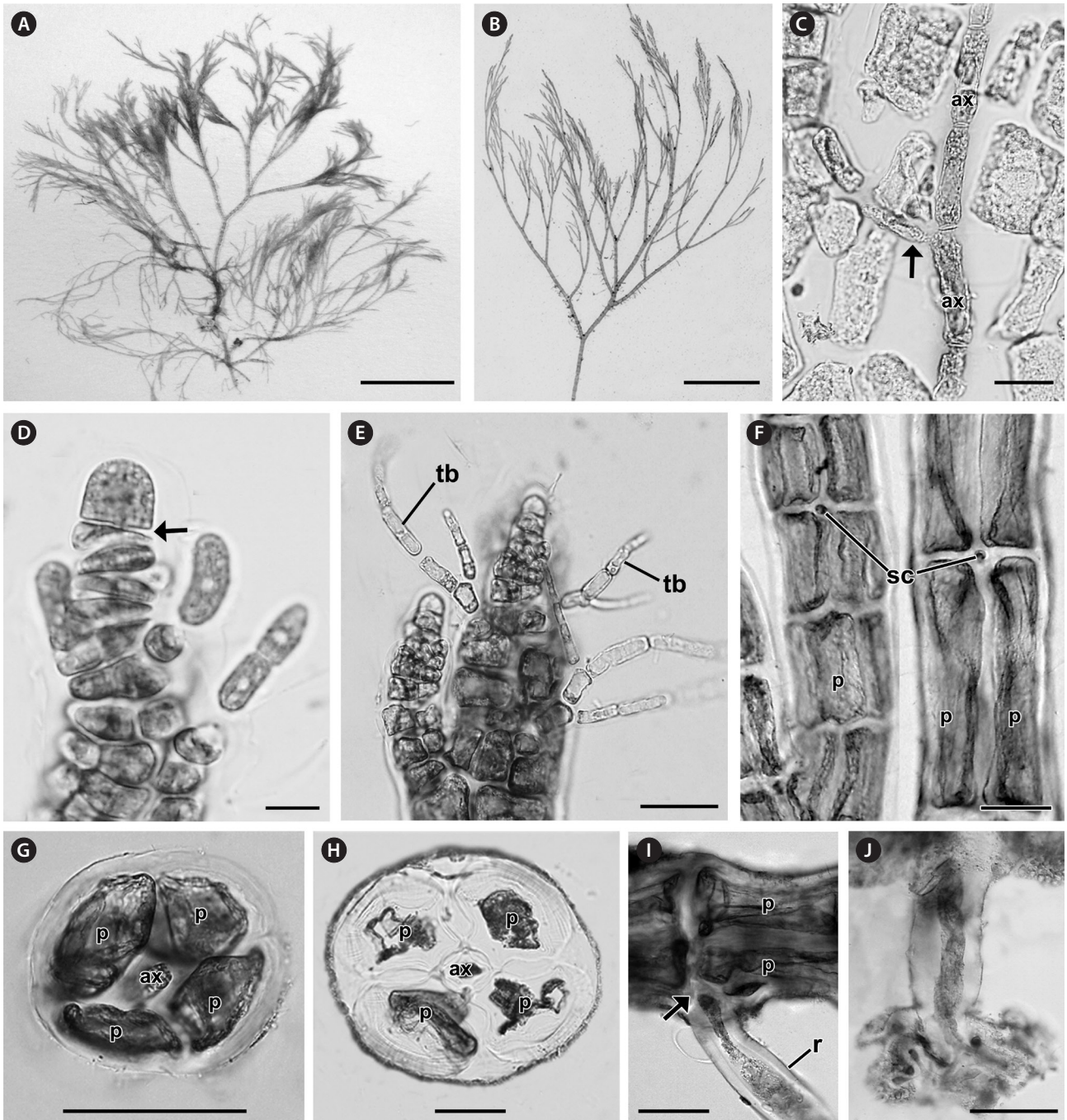
**Diagnosis.** Plants 2.4-5 cm high, saxicolous, consisting of limited prostrate and well-developed erect systems; branches dichotomous, attached by abundant unicellular rhizoids. Pericentral cells four, ecorticated along the entire thallus. Trichoblasts scarce, simple or forked once. Rhizoids cut off from pericentral cells by cross walls along the thallus. Procarps with three-celled carpogonial branches. Spermatangial branches arising from basal cells of forked trichoblasts. Tetrasporangia tetrahedral, spirally arranged.

**Holotype.** Lagunillas, Pisco, Ica, Peru, collected by T. O. Cho, Aug 21, 2008, CUK 6511 (Fig. 1A).

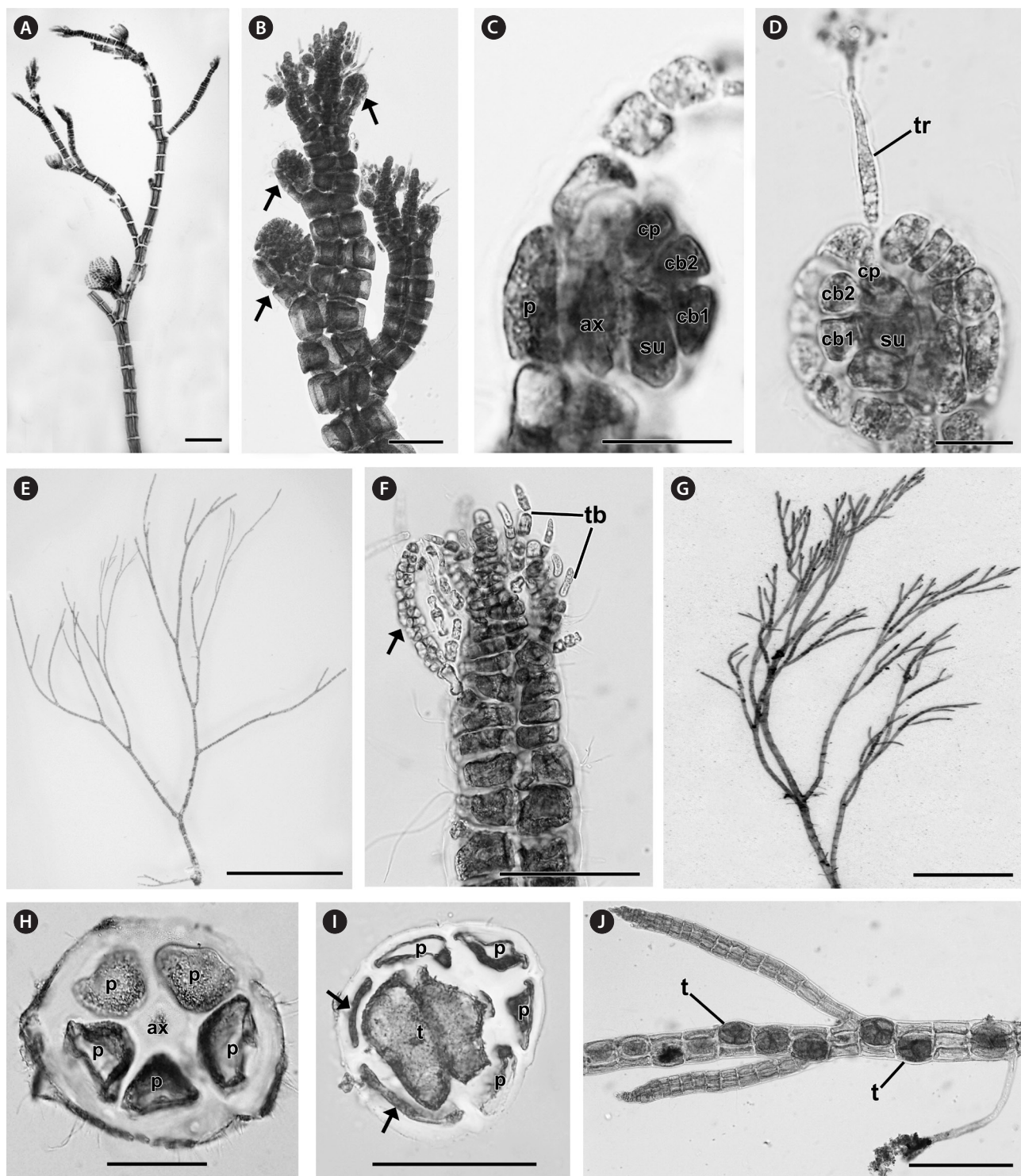
**Other specimens examined.** Lagunillas, Pisco, Ica, Peru, collected by T. O. Cho, Aug 21, 2008, CUK 6520; Lagunillas, Pisco, Ica, Peru, collected by T. O. Cho and D. E. Bustamante, Jul 5, 2012, CUK 8360.

**Etymology of specific epithet.** The new species honors María Eliana Ramírez for her pioneering and valuable contributions to the understanding of marine algae from the Pacific temperate coast of South America.

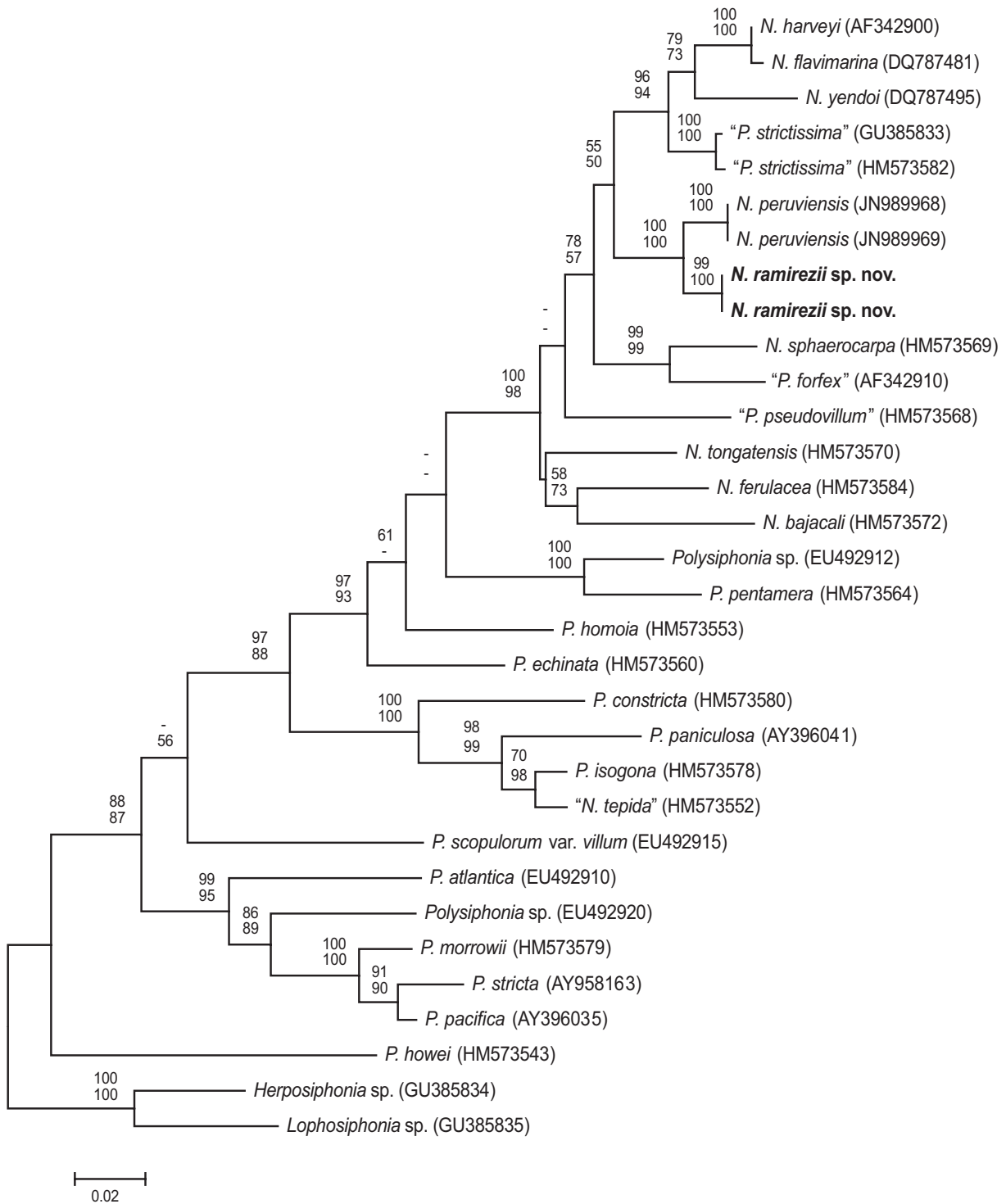
**Description.** Plants grow in the intertidal zone on rocks in association with other filamentous species. Thalli are 2.4 to 5 cm high (Fig. 1A), red-brown in color and occur in dense tufts (Fig. 1B) consisting of a limited prostrate system and well-developed erect filaments. Erect axes are  $95.3 \pm 9.8 \mu\text{m}$  in diameter (Fig. 1B) and arise endogenously from prostrate axes (Fig. 1C). Apices have a prominent apical cell,  $8.2 \pm 1.4 \mu\text{m} \times 6.9 \pm 1 \mu\text{m}$  in size (Fig. 1D). Trichoblasts are  $22.7 \pm 8.2 \mu\text{m}$  in length, deciduous, delicate, and scarce, produced from each segment near the apex, and once or twice forked (Fig. 1E). Inconspicuous scar cells are  $11.8 \pm 3.9 \mu\text{m} \times 11.9 \pm 3.9 \mu\text{m}$ , visible on the surface after shedding of trichoblasts (Fig. 1F). Adventitious branchlets arise on basal and older segments of the main axes as well as on its dichotomies. Four pericentral cells remain completely ecorticated throughout the thallus (Fig. 1G & H). Segments of erect branches measure  $136.4 \pm 43.6 \mu\text{m}$  in length and are 0.7 broader than long ( $1.4 \pm 0.4$  in length/breadth). Prostrate axes are  $168.5 \pm 11.1 \mu\text{m}$  in diameter and are attached on the surface of rocky substrata by numerous unicellular rhizoids located in the proximal and distal parts of the pericentral cells. Segments of prostrate axes are  $279.4 \pm 24.8 \mu\text{m}$  in length and 0.6 broader than long ( $1.7 \pm 0.2$  in length/breadth). Branching pattern is dichotomous in the main axes (Fig.



**Fig. 1.** Vegetative structures of *Neosiphonia ramirezii* sp. nov. (A) Holotype specimen from Lagunillas, Pisco, Peru, CUK 6511. (B) Habit of vegetative plant showing dichotomous branching pattern and pseudofastigiata appearance, CUK 6511. (C) Axial filament (arrow) endogenously developed from main axial cells (ax), CUK 6511. (D) Apical region of thallus showing a prominent apical cell with transverse division (arrow) of subapical cell, CUK 8360. (E) Apical region with trichoblasts (tb), CUK 8360. (F) Middle part of thallus with scar cells (sc), CUK 8360. (G & H) Cross section views of upper (G) and basal (H) parts of thallus (ax, axial cell; p, pericentral cell), CUK 8360. (I) Rhizoid (r) cut off from pericentral cell (p) by a wall (arrow), CUK 6511. (J) Unicellular rhizoid with digitate tips, CUK 8630. Scale bars represent: A, 1 cm; B, 5 mm; C & E, 20 µm; D, 5 µm; F-J, 50 µm.



**Fig. 2.** Reproductive structures of *Neosiphonia ramirezii* sp. nov. (A) Female gametophyte bearing cystocarps, CUK 8630. (B) Apical part of thallus with cystocarps (arrows) at various stages of development, CUK 8630. (C & D) Young (C) and mature procarp (D) with a three-celled carpogonial branch and trichogyne (tr), CUK 8630. ax, axial cell; cb1-cb2, sequence of carpogonial branch cells; cp, carpogonium; p, pericentral cell; su, supporting cell. (E) Male gametophyte, CUK 8630. (F) Apex showing spermatangial branch (arrow) developed on the basal cell of trichoblast (tb), CUK 8630. (G) Tetrasporangial plant, CUK 6511. (H & I) Cross section view of fertile segment (H) showing 5 pericentral cells, and mature stage (I) showing one tetrasporangium (t) surrounded by cover cells (arrows), CUK 6511. (J) Upper part of tetrasporangial thallus showing interrupted and spiral series of mature tetrasporangia (t), CUK 6511. Scale bars represent: A, 200  $\mu$ m; B, F, H & I, 50  $\mu$ m; C & D, 10  $\mu$ m; E & G, 5 mm; J, 100  $\mu$ m.



**Fig. 3.** Phylogenetic tree based on *rbcL* sequences inferred from maximum-likelihood (ML) analysis using the general time reversible model (GTR model) + invariable sites (I) + Gamma distribution (G). Bootstrap proportion values (>50%) for ML (500 replicates, upper) and maximum parsimony (MP) (500 replicates, lower) are shown at the nodes.

1B) but alternate near the apex, showing a pseudofastigiate appearance (Fig. 1B). Branching points occur at intervals of 5-14 ( $8.4 \pm 2.6$ ) axial cells in main axes and 6-11 ( $7.3 \pm 1.5$ ) cells in lateral axes replacing trichoblasts. Rhizoids are  $362.1 \pm 139.9 \mu\text{m}$  in length, cut off as separate cells from any position by pericentral cells (Fig. 1I) usually more than one per segment, and terminating in digitate tips (Fig. 1J).

In female plants (Fig. 2A), cystocarps are narrowly globose, ovate,  $152 \pm 50 \mu\text{m} \times 175 \pm 57 \mu\text{m}$  in size, scattered on branchlets (Fig. 2B), and produced on short pedicels. Procarps consist of three-celled carpogonial branches (Fig. 2C & D). In male plants (Fig. 2E), spermatangial branches each arise from the basal cell of a forked trichoblast (Fig. 2F). In tetrasporangial plants (Fig. 2G), the fertile segments comprise 5 pericentral cells (Fig. 2H). Tetrasporangia are tetrahedral, swollen, distorted, and  $59.8 \pm 13 \mu\text{m} \times 87.8 \pm 18.4 \mu\text{m}$  in size. A single tetrasporangium is produced per single segment (Fig. 2I). Tetrasporangia are spirally arranged (Fig. 2J) with successively maturing sporangia.

**Phylogenetic analyses.** The 1,244-bp portion of the 1,467-bp *rbcL* gene (84.8% sequenced) was analyzed, includes 347 parsimony informative sites. The phylogenetic trees were obtained from the alignment of the *rbcL* sequences which were newly generated and downloaded from GenBank. *Herposiphonia* sp. and *Lophosiphonia* sp. were selected as an outgroup taxa. Phylogenetic analyses reveal a strongly supported clade of predominantly *Neosiphonia* species in the *rbcL* maximum likelihood phylogeny (Fig. 3), with *N. ramirezii* located within this strongly supported clade, sister to *N. peruviansis*. The *rbcL* sequence divergence (%) for *N. ramirezii* specimens differed from *N. peruviansis* by 2.4%, from "*Polysiphonia strictissima*" by 5.2%, from *N. yendoii* by 7.0%, from *N. flavimarina* by 6.3 %, and from *N. harveyi* by 6.2% sequence divergence.

## DISCUSSION

*Neosiphonia ramirezii* sp. nov. is newly described from Peru based on morphological and molecular evidence. It is recognized by the presence of erect filaments arising from limited prostrate filaments, numerous rhizoids cut off from irregular positions of pericentral cells by a cross wall, 4 pericentral cells throughout, ecorticate axes, scarce trichoblasts, inconspicuous scar cells, procarps with three-celled carpogonial branches, spermatangia arising from the basal cell of trichoblasts, and a spiral

arrangement of tetrasporangia. *RbcL* molecular analysis corroborates the taxonomic placement of *N. ramirezii* in *Neosiphonia*.

Four *Neosiphonia* species have been reported from the Pacific temperate coast of South America: *N. flaccidissima*, *N. peruviansis*, *N. savatieri*, and *N. sphaerocarpa*. Although these *Neosiphonia* species co-occur with our new species in the Pacific temperate coast of South America, they are morphologically distinct from *N. ramirezii* sp. nov. *N. flaccidissima* reported from Lima, Peru, is distinguished by having branches arising in connection with trichoblasts, indistinct main axes, and abundant trichoblasts (Hollenberg 1942, 1968, Dawson et al. 1964). *N. peruviansis* described from Ica, Peru, has 6 pericentral cells and 1 or 2 tetrasporangia per segment (Bustamante et al. 2012). *N. savatieri* reported from Isla de Pascua, has a rhizoidal cluster and abundant trichoblasts (Hariot 1891, Santelices and Abbott 1987, Kim 2005). *N. sphaerocarpa*, reported from Lima and Talara, Peru, is distinguished by having creeping prostrate filaments and abundant trichoblasts with 3-4 dichotomies (Børgesen 1918, Dawson et al. 1964, Hollenberg 1968, Hollenberg and Norris 1977, Guimarães et al. 2004, Mamoozadeh and Freshwater 2011).

*N. ramirezii* sp. nov. resembles the other 14 worldwide *Neosiphonia* species (Harvey 1853, Kützing 1863, Hollenberg 1942, 1961, 1968, Kapraun 1977, Maggs and Hommersand 1993, Womersley 2003, Guiry and Guiry 2013) by having 4 pericentral cells, ecorticate axes, exogenous lateral branches, procarps with three-celled carpogonial branches, spermatangia developed from the basal cell of a forked trichoblast, and spirally arranged tetrasporangia (Table 1). However, the species (symbol a) labeled in Table 1 are distinguished from *N. ramirezii* by the presence of a discoidal base or rhizoidal cluster (Harvey 1853, Segi 1951, Hollenberg 1968, Kapraun 1979, Yoon 1986, Kim and Lee 1999, Guimarães et al. 2004, Kim and Abbott 2006, Kim et al. 2008, Mamoozadeh and Freshwater 2012). Furthermore, the species (symbol b) labeled in Table 1 are distinct from *N. ramirezii* by having creeping filaments or extended prostrate filaments (Agardh 1863, Hollenberg 1942, 1968, Segi 1951, Kim and Lee 1996, 1999, Abbott et al. 2002, Womersley 2003, Kim and Abbott 2006, Kim et al. 2008, Lee 2008, Creed et al. 2010, Nam and Kang 2012). The remaining species in Table 1, *N. apiculata* (Hollenberg) Masuda et Kogame, is characterized by abruptly tapered apices and pseudodichotomous branches (Hollenberg 1968, Tani et al. 2005, Kim and Abbott 2006).

Our molecular phylogenetic analyses using *rbcL* sequences revealed sequence divergence between *N. rami-*

**Table 1.** Morphological comparisons of species similar in appearance to *Neosiphonia ramirezii*

Species	Height (cm)	Rhizoidal position on pericentral cells	Rhizoid nature	Attachment system	Apex shape	Branch relationship to trichoblasts	Trichoblast nature	Branching pattern	Type locality	References
<i>N. ramirezii</i> sp. nov.	2.4-5	Proximal, distal, or middle	Unicellular	Limited prostrate filaments	Narrowed gradually	Replacing trichoblast	Scarce or absent, short, simple or once forked	Distinct main axes, dichotomous ("Y" shape)	Pisco, Peru	This study
<i>N. apiculata</i>	2	Proximal or middle	Unicellular	Basal attachment by a number of rhizoids	Apiculate	Replacing trichoblast	Short, rudimentary	Pseudodichotomous	O'ahu, Hawaii	Hollenberg 1968, Tani et al. 2005, Kim and Abbott 2006
<i>N. ferulacea</i> <sup>a</sup>	4 (15)	Proximal	-	Discoid base	Acute	Replacing trichoblast	Short, abundant, several dichotomies	Pseudodichotomous, alternate to irregular	Guadeloupe, Mexico; Hawaii; Australia	Segi 1951, Guimarães et al. 2004, Mamoozadeh and Freshwater 2012
<i>N. flaccidissima</i>	1-2	Proximal	Unicellular	Creeping prostrate filaments	Narrowed gradually	Arising in connection with trichoblast	Abundant, simple or forked once	Indistinct main axes, irregular	California, USA	Hollenberg 1942, 1968, Kim and Lee 1999
<i>N. gorgoniae</i> <sup>a</sup>	3	Distal	-	Rhizoidal cluster and prostrate filaments	Narrowed gradually	Replacing trichoblast	Scarce	Sparsely dichotomously with few spine-like branchlets	Florida, USA	Harvey 1853, Kapraun 1979, Guimarães et al. 2004
<i>N. hawaiiensis</i> <sup>a</sup>	4.5-5	Proximal	Unicellular	Discoid base and prostrate filaments	Not tapered	Replacing trichoblast	Short, abundant, 4 dichotomies	Dense branching, subdichotomously	O'ahu, Hawaii	Hollenberg 1968, Kim and Abbott 2006
<i>N. peruviana</i> <sup>b</sup>	1-5	Proximal	Unicellular	Prostrate filament	Narrowed gradually	Replacing trichoblast	Abundant, 1-2 dichotomies	Alternate to pseudodichotomous	Pisco, Peru	Bustamante et al. 2012
<i>N. pokob</i> <sup>b</sup>	0.4	Proximal	Unicellular	Prostrate filaments	-	-	Short, or long with four dichotomies	Unbranched erect filaments	North Island, Johnston Atoll	Hollenberg 1968, Abbott et al. 2002
<i>N. profunda</i> <sup>b</sup>	0.4-0.6	Proximal	Unicellular	Prostrate filaments	-	Arising in connection with trichoblast	Slender, abundant, long with 4 dichotomies	-	O'ahu, Hawaii	Hollenberg 1968, Kim and Abbott 2006
<i>N. rubrorhiza</i> <sup>a</sup>	0.8	-	Saccate unicellular	Rhizoidal cluster and prostrate filaments	-	Arising independent of trichoblasts	Long with 2-4 dichotomies	Pseudodichotomous	Midway Atoll, Hawaii	Hollenberg 1968, Kim and Abbott 2006

**Table 1.** Continued

Species	Height (cm)	Rhizoidal position on pericentral cells	Rhizoid nature	Attachment system	Apex shape	Branch relationship to trichoblasts	Trichoblast nature	Branching pattern	Type locality	References
<i>N. saccorhiza</i> <sup>b</sup>	Minute	Middle	Saccate unicellular	Well-developed prostrate filaments	-	-	Short with 2 dichotomies	Always prostrate		Hollenberg 1968, Creed et al. 2010
<i>N. savatieri</i>	0.5-1	Proximal	Unicellular	Rhizoidal cluster	-	Arising not associated with trichoblasts	Abundant	Dichotomous ("Y" shaped)	Yokosuka, Japan	Kim and Lee 1999, Kim 2005
<i>N. sertularioides</i> <sup>b</sup>	2-4	Proximal	Unicellular	Prostrate filaments	Dome shape	Arising in connection with trichoblast	Abundant	Dichotomous to alternate	Cette, France	Agardh 1863, Kim and Lee 1996, Womersley 2003, Nam and Kang 2012
<i>N. simplex</i> <sup>b</sup>	1-3(7)	Proximal	Unicellular	Creeping prostrate filaments	-	Replacing trichoblast	1-3 dichotomies	Sparingly branched	California, USA	Hollenberg 1942, Lee 2008
<i>N. sparsa</i> <sup>b</sup>	1	-	Unicellular	Prostrate filaments	-	Arising independent of trichoblasts	Long with 3-5 dichotomies	Not dichotomous	Arue Reef, Tahiti	Hollenberg 1968, Abbott et al. 2002
<i>N. sphaerocarpa</i>	1-1.5	-	Unicellular	Creeping prostrate filaments	-	Arising independent of trichoblasts	Abundant with 3-4 dichotomies	Subdichotomous	St. Thomas, Virgin Islands	Kim and Lee 1999
<i>N. tongatensis</i> <sup>a</sup>	5	Proximal	-	Rhizoidal cluster	-	Arising independent of trichoblasts	Abundant with several dichotomies	Pseudodichotomous	Tonga, Friendly Islands	Yoon 1986, Kim and Lee 1999, Kim et al. 2008
<i>N. upolensis</i> <sup>b</sup>	1-2	Proximal	-	Well-developed prostrate filaments	-	Arising independent of trichoblasts	Abundant and long with 3 dichotomies	Pseudodichotomous branched	Upolu, Samoa	Hollenberg 1968, Kim et al. 2008
<i>N. yendo</i> <sup>b</sup>	3.3-5	Corner or middle	Unicellular	Creeping prostrate filaments	Acute	Arising independent of trichoblasts	Short with 1 dichotomy	Subalternate and irregular	Muroran, Japan	Segi 1951, Kim and Lee 1999

<sup>a</sup>Species having discoidal base or rhizoidal cluster.<sup>b</sup>Species having creeping filaments or extended prostrate filaments.



*rezii* sp. nov. and other previously reported *Neosiphonia* species, sufficient to classify our new species among *Neosiphonia*. *Neosiphonia ramirezii* is nested in a monophyletic, well-supported clade.

## ACKNOWLEDGEMENTS

This research was supported by Basic Science Research Program through a grant from Marine Biotechnology Program Funded by Ministry of Land, Transport and Maritime Affairs of the Korean Government and the National Research Foundation of Korea (NRF) funded by the Ministry of Education, Science and Technology (2010-0003405) to Tae Oh Cho.

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