Taxonomic and molecular phylogenetic studies in the Scytosiphonaceae (Ectocarpales, Phaeophyceae)

【カヤモノリ科（褐藻綱シオミドロ目）の分類学的および分子系統学的研究】

Wilfred John Eria Santiañez

Department of Natural History Sciences
Graduate School of Science
Hokkaido University

March 2018

"*There is the sea, vast and spacious, teeming with creatures beyond number—living things both large and small.*" ~Psalm 104:25

「かしこに大いなる広い海がある。その中に無数のもの、大小の生き物が満ちている。」 ~詩篇 104:25

ABSTRACT

The systematics of the brown algal family Scytosiphonaceae poses an interesting question due to the inconsistencies between the taxonomies and molecular phylogenies of its members. The complexity of the Scytosiphonaceae is also highlighted in the discovery of several new species possessing morphological characters that were intermediate to at least two genera, consequently blurring generic boundaries. As such, it has been widely accepted that traditional characters used to define genera in the family (e.g., thallus morphology, thallus construction, and shape and nature of plurangial sori) were unreliable.

In this study, I attempted to resolve some of the glaring problems in the taxonomy and molecular phylogeny of several genera in the Scytosiphonaceae by integrating information on their morphologies, molecular phylogenies, and life histories. I focused my studies on the relatively under-examined representatives from tropical to subtropical regions of the Indo-Pacific as most studies have been conducted on the subtropical to temperate members of the family.

I assessed the taxonomy, molecular phylogeny, and distribution of the circumtropical genus *Hydroclathrus* using samples collected from its known distribution range. I confirmed the independence of all known *Hydroclathrus* species especially that of *Hydroclathrus stephanosorus* as well as described two new species, *Hydroclathrus minutus sp. nov.* and *Hydroclathrus rapanuii sp. nov.*, based on morphological and molecular phylogenetic criteria. A putative new species from Western Australia, which closely resembles the Hawaiian species *Hydroclathrus tumulis*, is also described. Despite sampling bias in the western Pacific, the distribution limits of *H. stephanosorus* and *H. tenuis* were expanded in this study, the former being the most

widely distributed throughout my study areas. Species distributions also showed some distinct biogeographic patterns between species despite some regions of overlap.

Based on multi-gene phylogenies and by comparing their morpho-anatomies and life histories, I also attempted to resolve the taxonomy and classification of several genera in the Scytosiphonaceae. Aside from the discovery and description of *Tronoella ryukyuana gen. et sp. nov.* from southern Japan, I have also introduced several taxonomic revisions including the proposal to recognize two new genera, *Pseudochnoospora gen. nov.* and *Dactylosiphon gen. nov.* The former was erected to accommodate the species previously known as '*Chnoospora implexa*' [= *Pseudochnoospora implexa comb. nov.*], while the latter was established for species with finger-like thalli that were previously circumscribed under the genus *Colpomenia* [= *Dactylosiphon bullosus comb. nov.*, *D. durvillei comb. nov.*, *D. wynnei comb. nov.*]. I also suggested the transfer of *Scytosiphon tenellus* to the genus *Petalonia* [as *Petalonia tenella comb. nov.*] due to its molecular and life history similarities with other *Petalonia* species as well as proposed the transfer of *Petalonia filiformis* to the newly established genus *Planosiphon* [as *Planosiphon filiformis comb. nov.*] based on morphological and life history criteria. I have also expanded the descriptions of *Petalonia* and *Planosiphon* to account for the characters of the newly transferred species. Two tribes, Hydroclathreae *trib. nov.* and Scytosiphoneae *trib. nov.*, were also newly proposed for the two phyletic groups that are unified primarily by their similarities in the type of reproductive structures that are borne by their sporophytic thalli.

Finally, I provided a synthesis of the current classification of the Scytosiphonaceae in light of the taxonomic revisions I have proposed. As the relationships among the different taxa in the tribe Hydroclathreae is yet to be resolved, I also underscored the need to examine further several genera in the tribe that were not covered in this study (i.e., *Colpomenia*, *Rosenvingea*, and *Iyengaria*).

ACKNOWLEDGEMENTS

Rallying past all the difficulties and hardships in this PhD journey would have not been possible without the support from different people and institutions. Herein, I take the opportunity to thank all of them.

I would like to thank the Japanese Ministry of Education, Culture, Sports, Science and Technology (MEXT) for the PhD scholarship grant that has enabled me to study for four years (as both a Research and PhD student) here in Hokkaido University.

I am deeply indebted to my supervisor, Prof. Kazuhiro Kogame, for his understanding, wise counsels, and guidance throughout my PhD. I sincerely commend and appreciate his unwavering support and patience especially of my whims about my ever dynamic research scope as well as my occasional stubbornness. I am thankful to my advisor, Prof. Takeo Horiguchi, for his sound advices and encouragements to be bold in pushing boundaries. I am grateful to Prof. Hiroshi Kajihara for critically reading through my PhD manuscript and his insightful feedback for its improvement. I also appreciate the help of several phycologists who have contributed to the samples and/or sequences that I used in this study: Dr Kyung Min Lee and Dr Sung Min Boo (Chungnam University), Dr Erasmo Macaya (Universidad de Concepción), Dr John Huisman (Western Australian Herbarium), Dr Ana Isabel Neto (University of the Azores), Dr John A. West (University of Melbourne), Dr Shinya Uwai (Niigata University), Dr Akira Kurihara (Kyushu University), Dr Hiroshi Kawai (Kobe University), Dr Yukimasa Yamagishi (Fukuyama University), Dr Paul Geraldino (University of San Carlos), Dr Edna T. Ganzon-Fortes (University of the Philippines), Mr Masakazu Hoshino (Hokkaido University), Dr Le Nu Hau (Vietnam Academy of Science and Technology), Dr Stefano Draisma (Prince of Songkla University), and Dr Emilia Croce (Consejo Nacional de

Investigaciones Científicas y Técnicas). I also wish to thank Dr Michael Wynne (University of Michigan) for his correspondence that encouraged me to dabble in the world of algal nomenclature and Dr Michael Guiry (National University of Ireland) for initiating and maintaining *Algaebase*, which has made the lives of students of algal taxonomy and nomenclature easier. I also thank the curators and staff of the Herbarium Hamburgense, University of Hamburg, Germany (HBG), Herbarium of the Swedish Natural History Museum (S), and the Trustees of the Natural History Museum, London (BM) for the scans of the type specimens of several scytosiphonacean species I examined in this study.

My sanity has been kept in check through my friends here in Sapporo and abroad. Particularly, I would like to extend my sincerest gratitude to the *Titas of Sapporo*: Mr Delburg Mitchao for being a dependable wingman; Dr West Paraiso for the candid conversations about life and living through PhD; Ms Kaye Kristine Vergel for the feeding programs; Mr Benjamin Magallon for the funny antics; Ms Fatima Cruz for being a bubble of fun energy; Dr Loida Casalme for giving us fits of laughter every time we get together; and, Dr Rachael Uson-Lopez and Dr Julius Lopez, thank you for sharing your lives with me, especially for bringing upon the whole gang our little bundle of joy, my adorable godson Aiden. To the *Kitas of Sapporo*, especially Mr Kevin Garas and Mr Randulph Morales, thank you for spicing up HAFS. My thanks also goes to Mr Joemark Narsico for being that one *wa-is bisdak* friend that you can rely on. To the *Humans of GenSan* (HoGS), Mrs Giff Paraba-Dayondon, Mr Apolo Novicio, Mr Jopy Cañeda, and Mrs Beverly Besmanos, thank you all for keeping me happy despite our long distance. I am also thankful for these two godly ladies, Dr Karen Bondoc and Ms Joyce Nieva, for keeping me reminded and grounded to the word of God. To my brothers from different mothers, Mr Lloyd Yales, Mr Iris Orizar, and Mr Francis Sune, thank you for being always there and for walking together with me in faith.

I am grateful for the support and camaraderie afforded to me by the past and present members of the Algal and Protist Systematics laboratory, Department of Natural History Sciences, Hokkaido University, particularly Ms Yukiko Mizuo, Dr Ryo Onuma, Davis Iritani, and Dr Kevin Wakeman. Special thanks go to my best buddies in the lab, Ms Suttikarn Sutti and Mr Mahmutjan Dawut—both of you are such great additions to my life and I will forever treasure our friendship.

I also thank the members of the Seaweed Biodiversity and Culture lab, the Seaweed Chemistry lab, and the Marine Genomics and Molecular Genetics lab of The Marine Science Institute, University of the Philippines (UP-MSI) for all their emotional and "technical" support. More importantly, I am indebted to my mentor Dr Gavino C. Trono, Jr. of UP-MSI for cultivating my passion for seaweed biology (diversity and ecology) and mariculture.

To my mother Selphonie and my father Wilfredo, words are not enough to express my gratitude for your love, patience, and understanding of my passion for advanced learning and, well, my life choices in general. It is my hope that I will be able to honor and make both of you proud throughout my lifetime. To my brothers, Francis Ryan, Jay Wilbert and Christopher, thank you all for your encouragements and for taking care of our parents in my absence.

And last but definitely not the least, I thank God, my Almighty Father, for the gift of love and life as well as the grace to finish. To You, I bring all this glory and honor!

WILFRED JOHN E. SANTIAÑEZ

11 December 2017

CONTENTS

CHAPTER 1

General Introduction

Systematics of brown algae (Class Phaeophyceae) has greatly benefited from the advances in molecular biology, particularly as PCR technologies become more accessible (Draisma *et al.* 2003). The growing body of molecular-based phylogenetic studies on the Phaeophyceae has dramatically changed our current understanding of the phylogeny of the group and, consequently, has allowed the updating of brown algal systematics towards a natural classification (Rousseau & Reviers 1999, Draisma *et al.* 2003, Silberfeld *et al.* 2010, Silberfeld *et al.* 2014, Kawai *et al.* 2015, Kawai & Henry 2017). In the most recent updating of the classification of brown algae, Silberfeld *et al.* (2014) recognized 19 orders (Phaesiphoniellales Silberfeld, F.Rousseau *et* Reviers was newly proposed therein); a year later, Kawai *et al.* (2015) proposed the recognition of the Stschapoviales H.Kawai. More recently, Kawai and Henry (2017) provided a summary of the Phaeophyceae and they did not recognize Phaeosiphoniellales as a distinct order.

Among the most interesting orders in the Phaeophyceae is the Ectocarpales Bessey 1907. The long and confused circumscription of the Ectocarpales has been reviewed in detail by Rousseau and Reviers (1999). By re-examining the concept of the Ectocarpales with the aid of multigene phylogenies, Rousseau and Reviers (1999) made significant progress and had since broadened the ordinal concept to include all brown

algae that possess plastids with stalked pyriform pyrenoids. By doing so, the Chordariales Setchell *et* Gardner 1925, Dictyosiphonales Setchell *et* Gardner 1925, and Scytosiphonales J. Feldmann 1949 were merged with the Ectocarpales *sensu stricto* based on priority. Silberfeld *et al.* (2014) noted that Silva and Reviers (2000) suggested the priority of use of Mesogloiales Nägeli 1847 as the earlier available name for the group. However, to stabilize the nomenclature in view of the widely used name Ectocarpales, the latter is being conserved based on the proposal of Wynne (2005).

The ectocarpalean family Scytosiphonaceae Ardissone *et* Straforello 1877, often erroneously attributed to Farlow (1881), was first established to accommodate the genus *Scytosiphon* C. Agardh. The family now includes 10 other genera: *Chnoospora* J. Agardh (previously under the monotypic family Chnoosporaceae Setchell *et* Gardner 1925), *Colpomenia* (Endlicher) Derbès *et* Solier in Castagne, *Hydroclathrus* Bory de Saint-Vincent, *Iyengaria* Børgesen, *Jolyna* S.M. Guimarães, *Melanosiphon* (D.A. Saunders) Wynne, *Myelophycus* Kjellman in Engler *et* Prantl, *Petalonia* Derbès *et* Solier, *Planosiphon* McDevit *et* G.W. Saunders, and *Rosenvingea* Børgesen. The assignment of the genus *Symphyocarpus* Rosenvinge to the family Scytosiphonaceae is yet to be confirmed; as such, it is presently not considered herein. The pioneering molecular phylogenetic work by Kogame *et al.* (1999) has provided support for the proposal of Rousseau and Reviers (1999) that the Scytosiphonales be reduced to familial rank (Scytosiphonaceae) and be classified in the Ectocarpales *sensu lato*. Scytosiphonacean species are distinguished based on possessing a large pyrenoid in a single plastid and heteromorphic life histories where a large, erect gametophytic and plurangia-bearing thalli alternate with small, prostrate sporophytic and unangia- and/or plurangia-bearing thalli. Genus-level classification, however, was traditionally based on the gross morphologies and structure of erect thalli (Kogame *et al.* 1999). In their phylogenetic trees, Kogame *et al.* (1999) showed unresolved relationships among scytosiphonacean

genera and highlighted the incongruences in their classification based on erect gametophytic thallus morphologies. Conversely, the morphologies of the prostrate sporophytes corresponded well with their molecular trees; thus, they suggested the use of sporophyte morphologies in delineating genera (Kogame *et al.* 1999). By including *Myelophycus* into the Scytosiphonaceae based on molecular data, G.Y. Cho and S.M. Boo in Cho *et al.* (2003) amended the description of the family to include isomorphic life history. Before this transfer, the familial position of *Myelophycus* had been debated between Punctariaceae and Chordariaceae (Tanaka & Chihara 1984). Lee *et al.* (2014b) also provided the first molecular evidence of the inclusion to the Scytosiphonaceae of the monotypic genus *Melanosiphon*, which also exhibit an isomorphic life history. Despite several taxonomic, molecular phylogenetic, and life history studies on the Scytosiphonaceae (Kogame 2001, Kogame & Masuda 2001, Cho *et al.* 2003, 2006, Kraft & Abbott 2003, West *et al.* 2010, 2015, Boo *et al.* 2011, Kogame *et al.* 2011, Lee *et al.* 2012, 2014a, 2014b, Matsumoto *et al.* 2014), the classification of the different genera included in the family remained confused. This is perhaps because recent studies were focused at the genus and species level while the latest study at the family level was done a decade ago (Cho *et al.* 2006). The problem is also confounded by the conservative approaches of previous workers, which is understandable considering the lack and/or patchy studies on scytosiphonacean taxa.

CLASSIFICATION OF GENERA IN THE FAMILY SCYTOSIPHONACEAE: MORPHOLOGY-BASED TAXONOMIES IN THE AGE OF MOLECULAR PHYLOGENETICS

The prevailing problem in the generic classification in the Scytosiphonaceae can be implicated in their broad and ambiguous delineations (Kogame *et al.* 1999). This is

further complicated by the wide morphological plasticity among the different species such that generic demarcations are blurred. Below, I briefly surveyed the current morphological circumscription of the known genera in the Scytosiphonaceae (Figure 1) and outlined how these broad generic delineations conflicted with their known molecular phylogenies.

Colpomenia (Figure 1, A) species are among the most variable: they possess saccate, globular, to irregularly convoluted, some branched or with numerous irregular protrusions, while others are erect and may have adventitious laterals. Possessing such highly polymorphic forms, several *Colpomenia* species have intermediate morphologies that widely overlap with those of *Iyengaria* (Wynne 1972), *Rosenvingea*, and *Scytosiphon* (Wynne & Norris 1976). As such, generic assignments of some species have been repeatedly questioned or some were erroneously described or transferred to other genus. Kogame *et al.* (1999) were first to show the unresolved relationships of *Colpomenia*, which were reflected in their distinct morphological, life history, and molecular phylogenies. Subsequent studies (e.g., Cho *et al.* 2003, 2006) on the family with increased taxon sampling were not able to resolve this problem. More recent molecular phylogenetic studies conducted on *Colpomenia* were focused to either the saccate (Boo *et al.* 2011a) or on the elongate species (Lee *et al.* 2012, Lee *et al.* 2014a). Studies on the generitype *Colpomenia sinuosa* (Mertens *ex* Roth) Derbès *et* Solier suggested the presence of three cryptic species with widely variable morphologies (Lee *et al.* 2013). The long discussion on how to define morpho-species boundaries among saccate *Colpomenia* species (e.g., Clayton 1975, Wynne & Norris 1976, Parsons 1982) is thus unsurprising. But, this also complicates species identification based solely on morphologies and other saccate species must be treated with caution. Nonetheless, there is a need to consider *Colpomenia* in a broader context to finally address the problem on their ambiguous relationships.

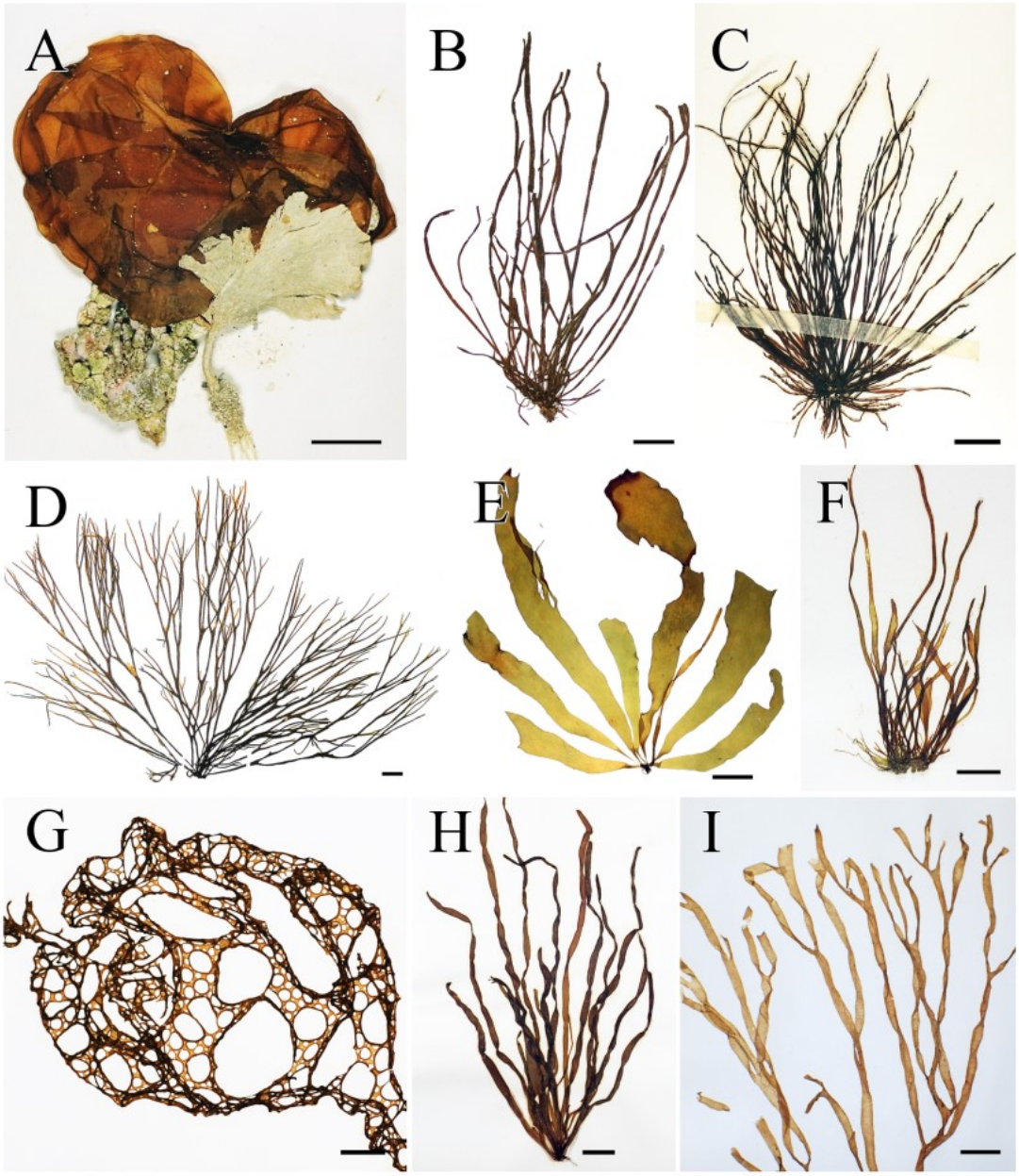

Figure 1. Representatives of the known genera in the Scytosiphonaceae. **A**, Saccate and epiphytic *Colpomenia sinuosa* (SAP059275). **B**, Erect and simple *Melanosiphon simplex*. **C**, Erect and simple thalli of *Myelophycus simplex* (SAP091221) showing its twisted apical portions. **D**, Erect and branched *Chnoospora minima* (SGO168260). **E**, Erect and leaf-like *Petalonia fascia* (SAP050352). **F**, Erect and compressed *Planosiphon zosterifolius*. **G**, Net-like *Hydroclathrus stephanosorus* (SAP115308). **H**, Erect and constricted thalli of *Scytosiphon lomentaria* (SAP059358). **I**, Erect and branching *Rosenvingea orientalis* (SAP115374). Scale bars: A–E, G–I = 1 cm; F = 0.5 cm.

The caespitose, upright and simple, solid (when young) to hollow (when old) and isomorphic *Myelophycus* (Tanaka & Chihara 1984) (Figure 1, C) is morphologically similar to the monotypic genus *Melanosiphon* (Figure 1, B). Tanaka and Chihara (1984)

reviewed in detail the taxonomy of both genera and suggested these are congeneric based on their widely overlapping characteristics. Kawai *et al.* (1994) assessed the reproduction and sexual pheromones in *Myelophycus simplex* (Harvey) Papenfuss and suggested that *Myelophycus* is best assigned in the Scytosiphonaceae (as Scytosiphonales) rather than in Dictyosiphonaceae (as Dictyosiphonales) as suggested by Tanaka and Chihara (1984). Cho *et al.* (2003) confirmed the placement of *Myelophycus* in the Scytosiphonaceae using molecular phylogenetics; consequently, the description of the family was also emended therein to accommodate the isomorphic life history of the genus. Concerning *Melanosiphon*, it is primarily distinguished from *Myelophycus* in having paraphyses with longitudinal septa (H. Kawai in Guiry & Guiry 2017). Lee *et al.* (2014b) provided the first molecular phylogenetic information on the distinction of *Melanosiphon*, which was corroborated in the multigene tree of McDevit and Saunders (2017). However, on both occasions, no observations on the morphology of the species were reported.

The erect to decumbent and branching or inter-adhesive *Chnoospora* (Figure 1, D) are distinguished from *Rosenvingea* in possessing solid thalli. *Chnoospora* species previously constituted the monogeneric family Chnoosporaceae based on its subapical growth pattern (Setchell & Gardner 1925). Kogame *et al.* (1999), based on *Chnoospora implexa* J. Agardh, provided molecular evidence to consider *Chnoospora* under Scytosiphonaceae. By including a specimen of *Chnoospora minima* (K. Hering) Papenfuss, Cho *et al.* (2006) showed that *Chnoospora* was not monophyletic.

Scytosiphon (Figure 1, H) species were traditionally distinguished from the similarly erect and unbranched *Petalonia* based on its subterete to compressed, hollow thalli and the presence of ascocysts among their plurangia. Conversely, *Petalonia* has leaf-like and flattened thalli (Figure 1, E) that are solid and has entangled rhizoidal filaments in its medulla (Kogame *et al.* 1999). Several species in both genera have

intermediate characters such as the compressed to flattened and hollow to partially hollow *Scytosiphon complanatus* (Rosenvinge) Doty and *Petalonia zosterifolia* (Reinke) Kuntze. Both species are now considered as members of the newly established genus *Planosiphon* McDevit *et* Saunders (McDevit & Saunders 2017) (Figure 1, F) and the aforementioned morphological characters, as well as the absence of paraphyses (ascocysts) among their plurangia, are considered their diagnostic characters. *Scytosiphon* is the most widely-studied genus in the Scytosiphonaceae. In the latest survey by McDevit and Saunders (2017), *Scytosiphon* was not recovered as monophyletic. These results suggest that, similar to other genera in the family, traditional morphological characters used to define the genus were not reliable or were loosely applied.

The net-like and spreading genus *Hydroclathrus* (Figure 1, G) possess the most distinct morphology in the Scytosiphonaceae, making it readily identifiable even in the field. However, the young saccate thalli of *Hydroclathrus* can be mistaken with the saccate and sometimes perforated *Colpomenia*, particularly *Colpomenia sinuosa* (Mertens *ex* Roth) Derbès *et* Solier. Before *C. sinuosa* was assigned as the generitype of *Colpomenia* by Derbès *et* Solier (1851), Zanardini (1843) considered it as a species of *Hydroclathrus* (i.e., *Hydroclathrus sinuosus* (Mertens *ex* Roth) Zanardini). Wynne (1972) noted the debate on segregating *Colpomenia* and *Hydroclathrus* as a distinct genus. Kraft and Abbott (2003) provided the first detailed taxonomic assessment of the genus based on global samples. While the generic delineation is distinct, species boundaries are unclear and interpretation of the generitype *Hydroclathrus clathratus* (C. Agardh) Howe and *Hydroclathrus tenuis* C.K. Tseng *et* Lu were widely varied. To resolve this problem, Kraft and Abbott (2003) encouraged the use of DNA data.

Rosenvingea (Figure 1, I) species are distinguished by their erect, dichotomous or alternately branched, cylindrical to somewhat compressed, hollow thalli (Børgesen

1914, Norris 2010, West *et al.* 2010, Lee *et al.* 2014b). In most species, branches are free, co-adhering in some. Norris (2010) noted that the taxonomy of the erect *Rosenvingea* was problematic as species were segregated primarily on their branching pattern. He further suggested the use of molecular data to define each species. In their life history and molecular phylogenetic studies on *Rosenvingea orientalis* (J. Agardh) Børgesen, West *et al.* (2010) noted two *Rosenvingea intricata* (J. Agardh) Børgesen entities: one from Japan, the other is from New Caledonia. The *psa*A tree of Lee *et al.* (2014b) recovered four *Rosenvingea* lineages, three of which were identified as *R. intricata*. Aside from the two entities mentioned previously, the newly well-supported clade consisted of specimens collected from Vietnam and (Atlantic) Panama. Lee *et al.* (2014b), however, did not provide detailed morphological descriptions for their specimens from Vietnam and Panama. *Rosenvingea* thus needs further reassessment especially in delineating species boundaries.

Iyengaria was established based on specimens previously assigned to *Rosenvingea* [*Rosenvingea stellata* Børgesen (Børgesen 1928)] and *Colpomenia* [*Colpomenia stellata* (Børgesen) Børgesen (Børgesen 1930)] (Børgesen 1939). Then, the monotypic *Iyengaria* [*viz. Iyengaria stellata* Børgesen (Børgesen)] was distinguished by Børgesen (1939) from *Colpomenia* based on "its semiglobular thallus provided with more or less solid, conical projections giving the plant a semi-stellate appearance." According to his examinations, the South African species *Colpomenia capensis* Levring is also conspecific with *I. stellata*. Several authors have pointed to the blurred generic boundaries between *Iyengaria* and *Colpomenia* (Wynne 1972, Wynne & Norris 1976). Due to its peculiar distribution, it has been repeatedly suggested that the status of *Iyengaria* must be reassessed and that resurrection of *C. capensis* for the South African specimens must be considered (Stegenga *et al.* 1997, Anderson *et al.* 2016). Recently, West *et al.* (2015) provided a brief review of the taxonomy of the genus, including the

molecular data on their newly described species *Iyengaria quadris*eriata J.A.West, Zuccarello, E.K.Ganesan *et* Loiseaux-de Goër.

AIMS AND OUTLINE OF THE THESIS

My study intends to clarify the taxonomy and phylogeny of the members of the family Scytosiphonaceae in the Indo-Pacific through increased taxon sampling and by integrating single- (*cox*1, *cox*3, *psa*A, and *rbc*L) and multi-gene phylogenies with information on their morphologies, anatomies, and life histories.

In **Chapter 2**, I investigated the validity of the known *Hydroclathrus* species based on global samples. Three putative new species were considered as distinct, two of which were formally described based on morphological and phylogenetic criteria. My study also showed indications of biogeographic break among the different species, including putative endemism. Due to the high morphological plasticity that I have observed, I also discussed therein the need to correctly identify species in light of bioprospecting and biodiversity conservation and management, among others.

In **Chapter 3**, I attempted to resolve the taxonomy and classification of several genera in the Scytosiphonaceae based on the multi-gene-based phylogenetic reconstruction and comparative morpho-anatomical and life history analyses. I described the morphology and phylogeny of the newly discovered monotypic genus *Tronoella* from southern Japan. To provide a more natural classification in the family and address the phylogenetic problem in the genus *Chnoospora* and *Colpomenia*, two new genera were established for species that were genetically segregated and morphologically distinct from their respective generitypes. I proposed the monotypic genus *Pseudochnoospora* to accommodate '*Chnoospora implexa*' and *Dactylosiphon* for

the three known *Colpomenia* species with finger-like hollow thalli that arise from a saccate base.

Finally, in **Chapter 4**, in light of the taxonomic proposals done in this work, I provided a synthesis on the current classification of the Scytosiphonaceae. Considering the limitations of this study and the apparent need to clarify the relationships among several genera, I also point herein key areas that need to be addressed.

CHAPTER 2

Casting the net wider: Uncovering the diversity and molecular phylogeny of the clathrate algal genus *Hydroclathrus* (Scytosiphonaceae, Phaeophyceae)[1]

INTRODUCTION

Hydroclathrus species (Scytosiphonacae, Phaeophyceae) are brown marine macrobenthic algae (seaweeds) that are widely distributed in the tropical to warm temperate waters of the Pacific, Indian, and Atlantic Oceans (Guiry & Guiry 2017; Figure 2). Members of this genus are distinct in possessing spreading and clathrate (net-like) thalli, the latter a result of numerous irregular holes found on their membranes. *Hydroclathrus* occurs seasonally with their macrothalli being observed in late winter to early summer in subtropical to warm temperate waters (Kogame 1994) and may form blooms during early summer in the tropics (Trono 1997). The observed distinct

[1] Portions of this chapter appeared in:

Santiañez, W.J.E., Lee, K.M., Uwai, S., Kurihara, A., Geraldino, P.J.L., Ganzon-Fortes, E.T., Boo, S.M. & Kogame, K. (2018). Untangling nets: Elucidating the diversity and phylogeny of the clathrate brown algal genus *Hydroclathrus*, with the description of a new genus *Tronoella* (Scytosiphonaceae, Phaeophyceae). *Phycologia* 57: 61–78.

Santiañez, W.J.E., Macaya, E.C., Lee, K.M., Cho, G.Y., Boo, S.M., & Kogame, K. (2018). Taxonomic reassessment of the Indo-Pacific Scytosiphonaceae (Phaeophyceae): *Hydroclathrus rapanuii* sp. nov. and *Chnoospora minima* from Easter Island, with proposal of *Dactylosiphon* gen. nov. and *Pseudochnoospora* gen. nov. *Botanica Marina* 61: 47–64.

seasonality in their occurrence may be due to their heteromorphic life history where a macroscopic plurangia-bearing thalli alternate with a microscopic unangia- and plangia-bearing microscopic thalli (Hurtado-Ponce & Umezaki 1987, Kogame 1994, 1997, Toste *et al.* 2003a, b). Although, it has also been reported that *Hydroclathrus* in culture has a monophasic (direct type) life history wherein macrothalli plurispores directly develop into new macrothalli (Clayton 1982, Hurtado-Ponce & Umezaki 1987, Toste *et al.* 2003a).

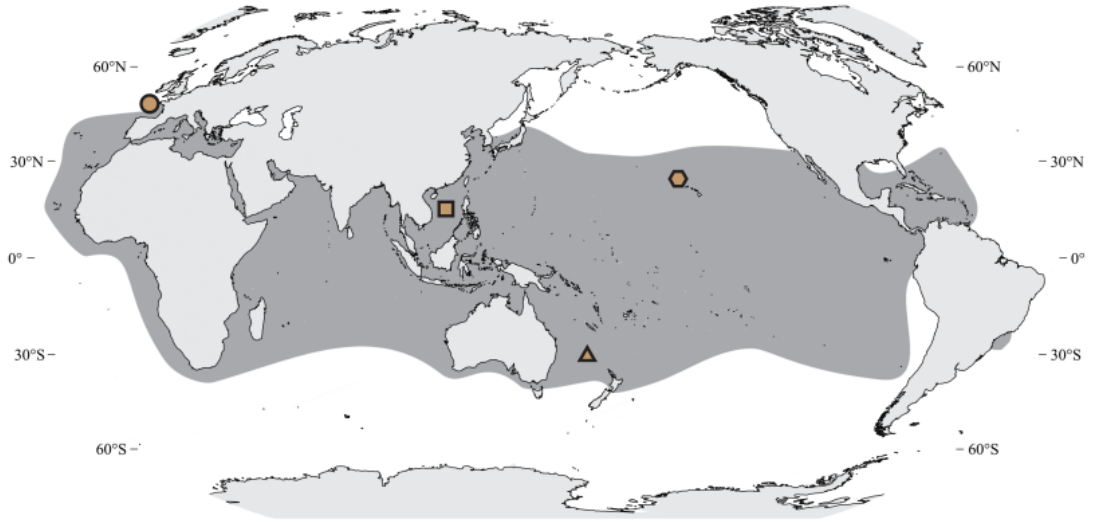

Figure 2. Generalized global distribution of *Hydroclathrus* (Guiry & Guiry 2017) showing type localities of known species: *H. clathratus* (Belle-Île, France (?); circle), *H. stephanosorus* (Lord Howe Is., Australia; triangle), *H. tenuis* (Paracel Is.; square), and *H. tumulis* (Maro Reef, Hawaii; hexagon).

Hydroclathrus species are used as food (seaweed salad), animal fodder, and fertilizer (Trono 1997) as well as hold potential as a feedstock for biogas production (Marquez *et al.* 2014). As with other brown algae, they also produce economically important metabolites such as alginates and fucoidan. *Hydroclathrus* has been subjected for bioprospecting in search of natural products with medicinal, pharmaceutical, and industrial applications (Wang *et al.* 2007, 2010a, b, Kamal & Sethuraman 2013).

The taxonomy of *Hydroclathrus* has been long and challenging. Several problems have been raised since it was established based on *Hydroclathrus cancellatus* Bory de Saint-Vincent from Belle-Île, Brittany, France (Bory de Saint-Vincent 1825). Bornet (1892) noted that the shells found in Bory's specimen were not found in Brittany's coastal fauna and suggested that the provenance may be erroneous (Hamel 1937). Consequently, the type locality is now considered as uncertain (Silva *et al.* 1987). More than nine decades after its description, the generitype *H. cancellatus* was synonymized to *H. clathratus* (C. Agardh) Howe to prioritize the earlier described taxon based on the same type collection *Encoelium clathratum* C. Agardh (Howe 1920; Silva *et al.* 1987).

Currently, there are four taxonomically accepted *Hydroclathrus* species: *H. clathratus* (type), *H. tenuis* Tseng *et* Lu (Tseng & Lu 1983), *H. stephanosorus* Kraft, and *H. tumulis* Kraft *et* Abbott (Kraft & Abbott 2003). Despite being species poor, the genus remains taxonomically difficult due to the high morphological plasticity of its members. This problem is further complicated by the differences in the interpretations of each species such that when observations are consolidated, descriptions were inconsistent and widely overlapping (Kraft & Abbott 2003). As such, uncertainties on the taxonomic validity of the known species have been growing (Kraft & Abbott 2003, Kraft 2009). Particularly, the generitype *H. clathratus* has been widely known to exhibit various forms and large size ranges that overlap with *H. tenuis* and *H. stephanosorus* (Kraft & Abbott 2003, Kraft 2009). *Hydroclathrus tenuis* is traditionally distinguished from *H. clathratus* based on its thinner thalli, smaller cells, and longer plurangia. However, vegetative and reproductive characters of these two species have been known to overlap (Kraft & Abbott 2003). *Hydroclathrus stephanosorus* is distinct in possessing membranous thalli with subcircular, punctate sori surrounding hair tufts, broadly rounded cortical cells, and moniliform hair primordia (Kraft & Abbott 2003). However,

Kraft (2009) suggested that most of the characters used to segregate *H. stephanosorus* from *H. clathratus* were unreliable and had since doubted the durability of the former as an independent taxon. To settle issues on delineating species within the genus, Kraft & Abbott (2003), as reiterated by Kraft (2009), advocated the use of DNA fingerprinting.

Hydroclathrus clathratus and *H. tenuis* have been shown to be distinct from each other based on several molecular phylogenetic studies on the family Scytosiphonaceae (Cho *et al.* 2006, West *et al.* 2010, Kogame *et al.* 2011). The *rbc*L tree of Matsumoto *et al.* (2014) hinted at the existence of putative new and/or cryptic species. These findings are unsurprising as the molecular-assisted taxonomic studies has repeatedly suggested seaweed biodiversity is underestimated and cryptic species may be apparent especially among widespread taxa (e.g., Lee *et al.* 2013, Payo *et al.* 2013, Belton *et al.* 2014, Hind *et al.* 2014, 2015, Kogame *et al.* 2015, Yaegashi *et al.* 2015, Gabriel *et al.* 2016). In line with this, I hypothesized that the genus may be more diverse than currently known and species distributions may be wider than previously reported. Herein, I present the findings of my taxonomic and molecular phylogenetic assessment of *Hydroclathrus* species based on samples collected from their known distribution ranges, including the description of the two new species, *Hydroclathrus minutus* Santiañez *et* Kogame from Okinawa Island and *Hydroclathrus rapanuii* Santiañez, Macaya *et* Kogame from Easter Island, Chile. Based on a sample from Western Australia, a putative new species is also described. I also discussed species delineation and distribution within the genus based on integrated morpho-anatomical and single and multiple gene-based phylogenies.

MATERIALS AND METHODS

Hydroclathrus samples were collected from several localities, primarily from within the Pacific Ocean (Table S1). A subsample of each specimen was dried in silica gel for use in

DNA extraction and molecular analyses. The remainder of each specimen was air-dried on herbarium sheets as voucher specimen, or, whenever possible, a subsample was also soaked in salt or 10% seawater-formalin solution for subsequent morphological analyses. Voucher specimens are deposited in the following herbaria: Chungnam National University, Daejeon, Korea (CNUK); GT Velasquez Phycological Herbarium of the Marine Science Institute, University of the Philippines, Diliman, Quezon City, Philippines (MSI); Western Australian Herbarium, Perth, Western Australia, Australia (PERTH); Faculty of Science, Hokkaido University, Sapporo, Japan (SAP); and Herbarium, Botany Section, Museo Nacional de Historia Natural, Santiago, Chile (SGO) (Table S1).

For molecular analyses, total genomic DNA was extracted from silica gel-dried samples using QuickExtract™ FFPE DNA Extraction Kit (Epicentre Technologies Corp., Chicago, Illinois, USA) following the manufacturer's instructions. After which, extracts were used as template to amplify the targeted four genetic regions: mitochondrial cytochrome oxidase subunit 1 (*cox*1) and cytochrome oxidase subunit 3 (*cox*3) and plastidial photosystem I subunit A (*psa*A) and RuBisCo large subunit (*rbc*L) genes. DNA extraction, PCR amplification, and sequencing of *cox*3 genes of several samples from Korea, the Philippines, Indonesia, Panama, Mexico, and South Africa followed the methods described in Boo *et al.* (2011b). The primers used for PCR and sequencing used in this study are listed in Table S2. PCR was done using TaKaRa Ex Taq DNA Polymerase (TAKARA Bio Inc., Otsu, Japan) or Q5® High-Fidelity DNA Polymerase (New England Biolabs Inc., Massachusetts, USA) added with dimethylsulfoxide (5% in reaction volume) under the following conditions: 1 min at 96˚C for denaturation, followed by 50 cycles of 30 s at 94˚C, 30 s at 50˚C, 30 s at 72˚C, with a final extension of 5 min at 72˚C. The PCR was performed with a GeneAmp PCR System 9600 or 9700 (PE Applied Biosystems). To remove residual primers and dNTP, polyethylene glycol (PEG

#6000, Nakalai Tesque, Kyoto, Japan) was used. The precipitated PCR products were directly sequenced in ABI Prism 310 or 3130 Genetic Analyzer (PE Applied Biosystems) using an ABI Prism BigDye Terminator Cycle Sequencing Ready Reaction Kit v. 1.1 (PE Applied Biosystems, Texas, USA) following the manufacturer's protocols. Both forward and reverse sequences were determined; these were subsequently edited, aligned, and multiple sequence alignments were generated using MEGA v.6 (Tamura *et al.* 2013).

To infer phylogenies based on individual (*cox*1, *cox*3, *psa*A, and *rbc*L) sequence alignments (including sequences downloaded from GenBank; see Table S1), Maximum likelihood (ML) and Bayesian Inference (BI) were used. The suitable model of sequence evolution for single gene analyses was assessed using MrModeltest 2.3 (Nylander 2004). Using the Akaike Information Criterion, GTR + I + Γ model was selected for *cox*3, *psa*A, and *rbc*L, while the GTR + Γ model was chosen for *cox*1. ML analyses were conducted in CIPRES Phylogenetic Portal (Miller *et al.* 2010) using RAxML v.8 (Stamatakis 2014) with 1000 bootstrap pseudoreplicates. BI was conducted in MrBayes v.3.2.1 (Huelsenbeck & Ronquist 2001). Markov-chain Monte Carlo iterations were run for 25 million generations until the average standard deviations of split frequencies fell below 0.01, indicating a convergence of the iterations. Trees were calculated after 25% burn-in was removed. In addition, pairwise sequence differences (p-distances) within *Hydroclathrus* were also calculated using MEGA v.6.

Sections were made by hand primarily on salt- or Formalin-preserved specimens; when both were unavailable, sections were made on the rehydrated tissue. These were subsequently stained using 0.5% aniline blue with phenol and mounted in 50% glycerol- or 30% Karo-distilled water solution. Observations were made on Nikon Optiphot-2 microscope and photomicrographs were taken using the microscope-mounted Nikon Digital Sight DS-L1 camera (Nikon, Tokyo, Japan). Measurements were done using ImageJ 1.50i (Schneider *et al.* 2012).

RESULTS

Phylogenetic results

One hundred and seventy-nine (179) new *Hydroclathrus* sequences generated from 117 samples were used in this study (Table S1). These were primarily mitochondrial *cox*3 sequences (107), followed by *cox*1 (57). Fewer plastidial *psa*A (8) and *rbc*L (6) genes were amplified as they were only used to represent each genetic species identified in mitochondrial gene data.

Based on *cox*3 sequence data, I have identified six species lineages (Lineages 1–6) within *Hydroclathrus*. Five species lineages (Lineages 1–3, 5, 6) were recovered with long branches and highly supported nodes, while one (Lineage 4) was unresolved (Figure 3). The *cox*1 gene-based analyses resulted in a similar tree topology, albeit clades were arranged differently, particularly those of Lineages 1–3 (Figure 4). In both *cox*1 and *cox*3 trees, Lineage 4 was not recovered as monophyletic. In the former, Lineage 4 was clearly segregated in two phyletic groups, with the latter having . Intraspecific differences (based on *cox*3 gene data) among the species lineages was 0–2.6%, with the largest found among those in Lineage 4. Meanwhile, interspecific differences ranged from 3.2% to 11.8%.

Regarding distribution, three of these species lineages were found to have limited distribution. Particularly, Lineage 2 was only recovered in Easter Is., Lineage 3 was found in White Is., Western Australia, and Lineage 6 in several islands in the Ryukyu arc. The other three lineages were more widely distributed. Lineage 1 was found in tropical to subtropical waters of the Philippines, Japan, Korea, South Africa, and Australia (based on a single GenBank sequence data). Lineage 4, the most widely distributed lineage, occurred in tropical (Panama, Hawaii, Taiwan), subtropical (Korea

and Australia [including type locality, Lord Howe Is. (based on GenBank sequence data)] to warm temperate (Japan) waters. Lastly, Lineage 5 was recovered in the warmer regions of the Indo-Pacific (Philippines, Indonesia, China (Hainan), Hawaii, Mexico, and southern Japan).

Phylogenetic reconstruction of the family Scytosiphonaceae based on plastidial *rbc*L and *psa*A genes resulted in a similar tree topology but showed some slight differences in the arrangement of the different lineages (Figure 5,Figure 6). In both *psa*A and *rbc*L trees, all *Hydroclathrus* species lineages, except Lineage 6, were recovered as a highly supported clade that was closely related to *Ch. implexa* and *R. intricata*. In addition, relationships among *Chnoospora*, *Rosenvingea*, *Colpomenia*, and *Scytosiphon* species were unresolved.

Taxonomic observations

Based on morphological and anatomical observations, I have assigned names of currently known *Hydroclathrus* species to three of the species lineages: *H. clathratus* (Lineage 1), *H. stephanosorus* (Lineage 4), and *H. tenuis* (Lineage 5). The two other species lineages are herein described as new species: *H. rapanuii* Santiañez, Maya *et* Kogame *sp. nov.* (Lineage 2) and *H. minutus* Santiañez *et* Kogame *sp. nov.* (Lineage 6). Meanwhile, Lineage 3 was similar to *H. tumulis* but I have not assigned it to the species due to some morphological and ecological differences.

Hydroclathrus **Bory de Saint-Vincent 1825: 419**

Type species: *Hydroclathrus cancellatus* Bory de Saint-Vincent 1825: 419, *nom. illeg*. (= *Hydroclathrus clathratus* (C. Agardh) Howe 1920: 590).

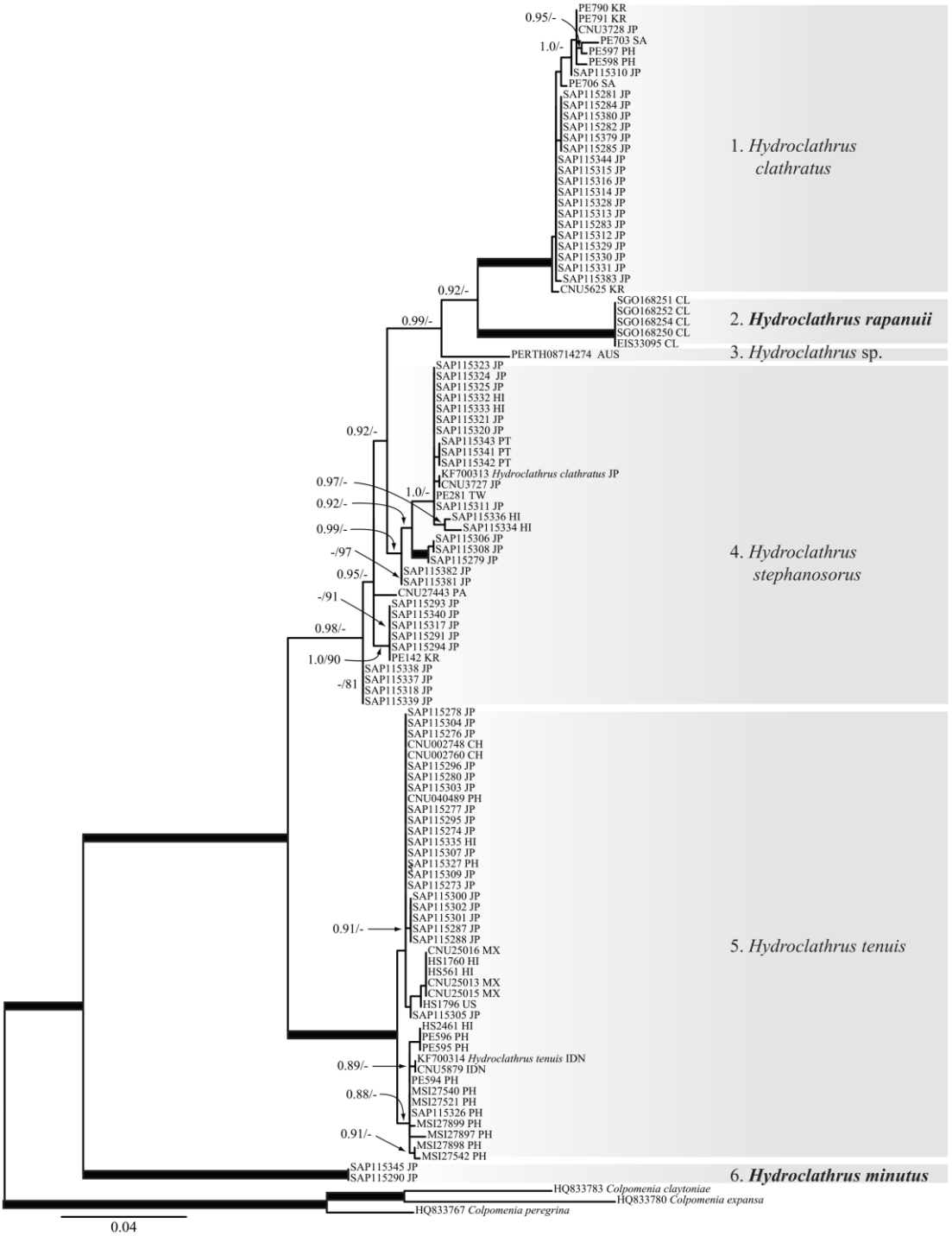

Figure 3. Relationships of *Hydroclathrus* species based on *cox*3 sequence data. Support values appended at each node are Bayesian posterior probabilities (PP) and maximum likelihood bootstrap percentages (BP), respectively. Thickened lines indicate highly supported nodes (PP: ≥ 0.98 and BP: ≥ 95%). Values <80% BP and <0.80 PP are removed. Collection localities of each specimen are indicated as: Japan: JP, Philippines: PH, Korea: KOR, China: CH, Taiwan: TW, Indonesia: IDN, Hawaii (USA): HI, Panama: PA, South Africa: SA, Portugal: PT, Australia: AUS, Chile: CL, Mexico: MX. Numbers preceding taxon names indicate lineage number. Bold names = newly proposed taxa.

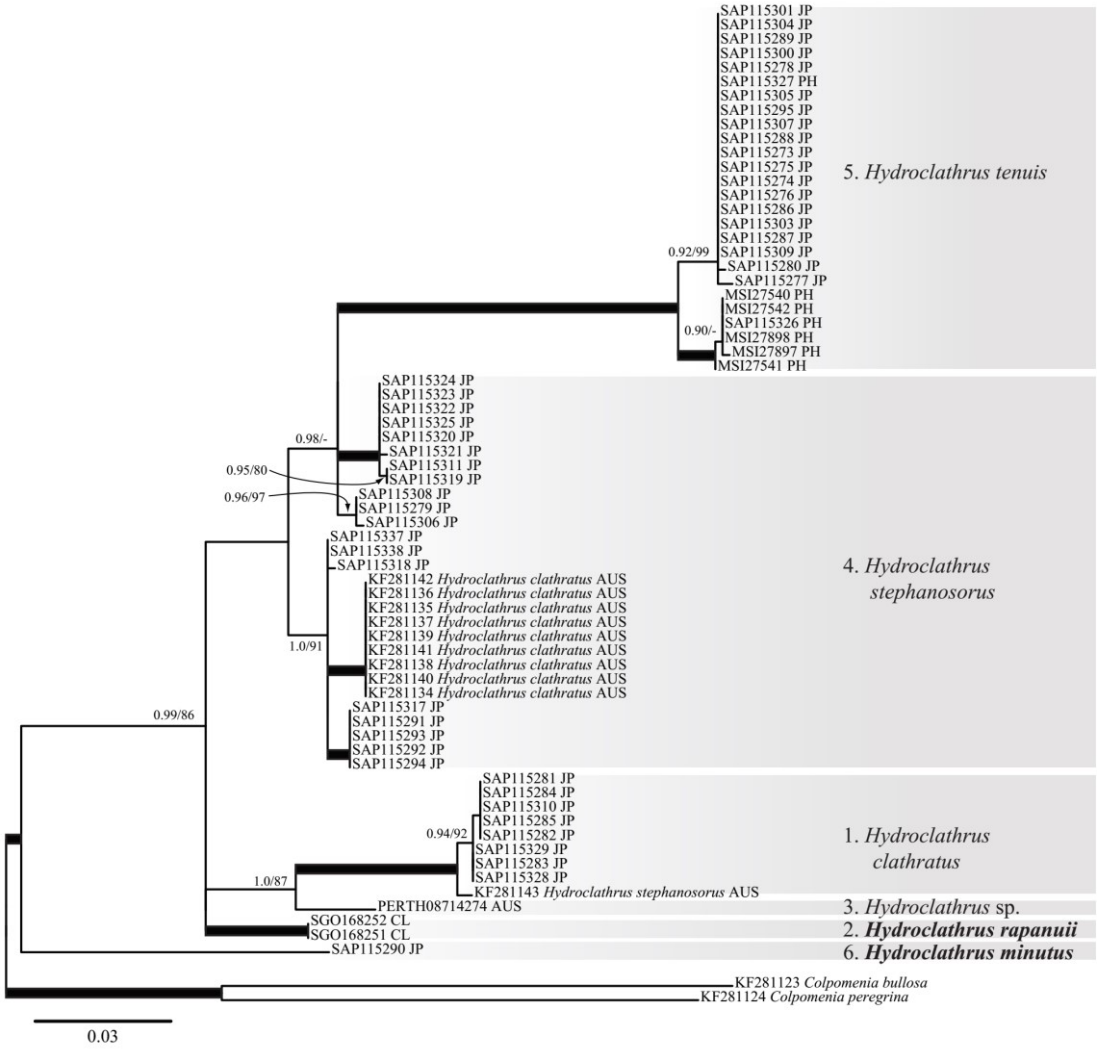

Figure 4. Relationships of *Hydroclathrus* species based on *cox*1 sequence data. Support values appended at each node are Bayesian posterior probabilities (PP) and maximum likelihood bootstrap percentages (BP), respectively. Thickened lines indicate highly supported nodes (PP: ≥ 0.98 and BP: ≥ 95%). Values <80% BP and <0.80 PP are removed. Collection localities of each specimen are indicated as: Japan: JP, Philippines: PH, Australia: AUS, Chile: CL. Numbers preceding taxon names indicate lineage number. Bold names = newly proposed taxa.

Description: Thalli perforated with numerous round to irregularly shaped holes; hollow, saccate when young, later torn into membranous sheets or irregularly spreading and/or strap-shaped. Thalli epiphytic or epilithic through rhizoidal holdfasts, sometimes unattached and drifting on the sea floor. Cells possess a single plastid with a pyrenoid. Cortex composed of 1–2 (rarely 3) layers of small, pigmented cells. Medulla composed of up to 9 layers of clear cells that were progressively larger toward the dorsal hollow thalli portions. Hairs epidermal and often occurring as scattered tufts in

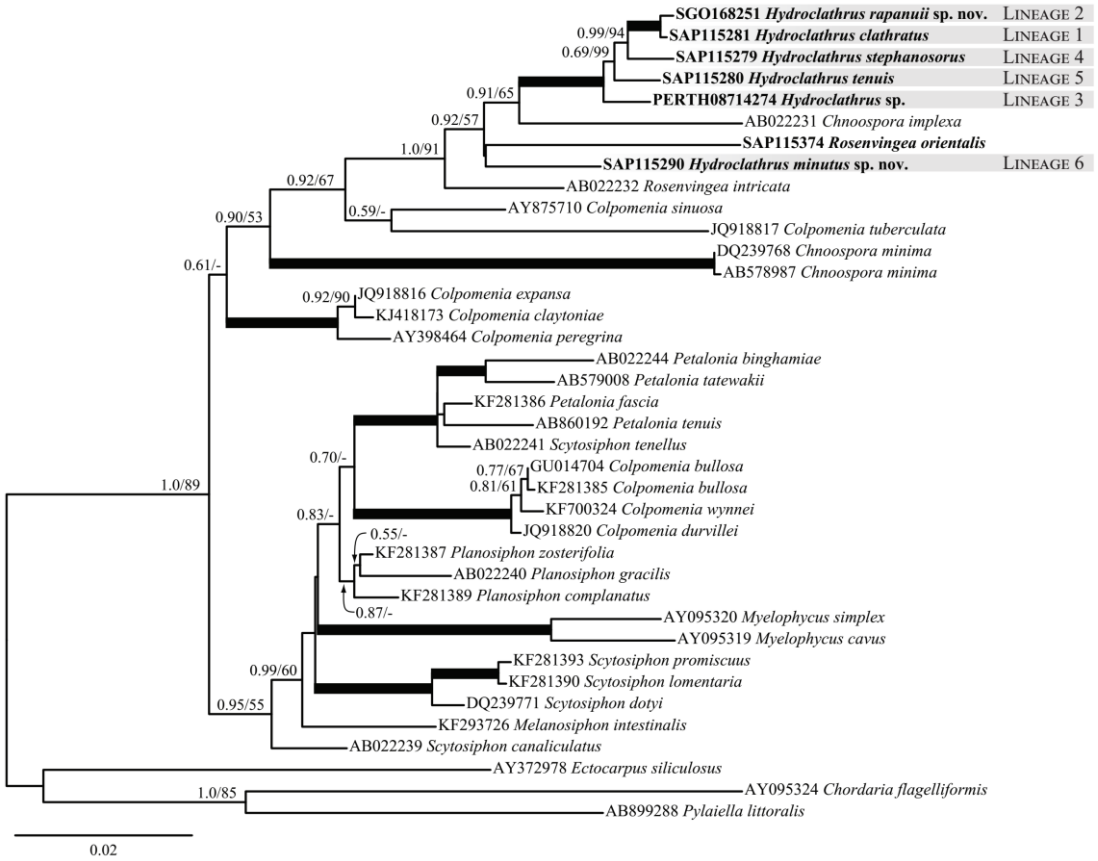

Figure 5. Phylogeny of the family Scytosiphonaceae inferred from *rbc*L sequence data. Support values appended at each node are Bayesian posterior probabilities (PP) and maximum likelihood bootstrap percentages (BP), respectively. Thickened lines indicate highly supported nodes (PP: ≥ 0.98 and BP: ≥ 95%). Values <50% BP and <0.50 PP are removed. Bold names = newly generated sequences.

pits or depressions. Reproduction through erect quadriseriate plurangia arranged in discrete to confluent angular to subcircular sori. Life history monophasic ("direct") or heteromorphic; in the latter, a macrothalli bearing plurilocular sporangia alternate with a discoid or pseudodiscoid microscopic thalli bearing unangia and plurangia. Paraphyses (ascocysts) absent.

LINEAGE 1

***Hydroclathrus clathratus* (C. Agardh) Howe 1920: 590**

Figure 7

Basionym: *Encoelium clathratum* C. Agardh 1823.

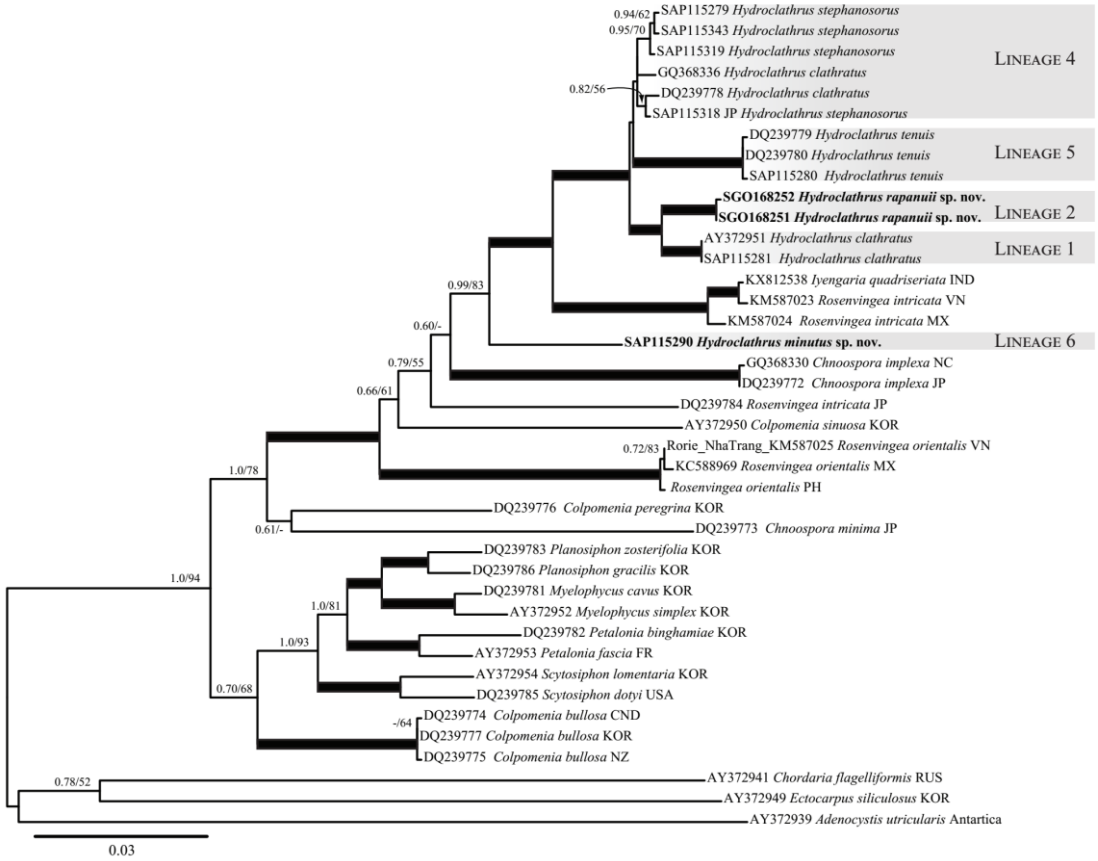

Figure 6. Phylogeny of the family Scytosiphonaceae inferred from *psa*A sequence data. Support values appended at each node are Bayesian posterior probabilities (PP) and maximum likelihood bootstrap percentages (BP), respectively. Thickened lines indicate highly supported nodes (PP: ≥ 0.98 and BP: ≥ 95%). Values <50% BP and <0.50 PP are removed. Bold names = newly generated sequences.

Synonym: *Hydroclathrus cancellatus* Bory de Saint-Vincent 1825: 419, *nom. illeg.*; *Asperococcus cancellatus* (Bory de Saint-Vincent) Sonder *fide* J. Agardh 1848: 76; *Asperococcus clathratus* (C. Agardh) J. Agardh.

Type locality and specimen: Uncertain (Silva *et al.* 1987).

Distribution: Pantropical to warm temperate.

Local names: Kagomenori (Japanese); Balbalolang, Lukot-lukot, Poko-poko (Filipino).

Specimens examined: JAPAN: SAP115281, SAP115283, SAP115284, 29 March 2006, Sakurajima Is., Kagoshima, *K. Kogame*; SAP115312, SAP115313, SAP115315, 23 March 2015, Tsuyazaki, Fukuoka, *W.J.E. Santiañez & K. Kogame*; SAP115328, SAP115330, 17 August 2015, Tassya, Sado Is., Niigata, *S. Uwai*.

Observations: Thalli were light to dark brown in color, with numerous irregular and variedly sized holes resulting to its characteristic net-like appearance (Figure 7, A–D). Thalli initially saccate becoming irregularly spreading, strap-shaped, and sometimes entangled, 100–690 μm thick. These are epiphytic to other seaweeds or epilithic through rhizoids, or occur as drifts on the sea floor. Membrane surfaces were smooth, sometimes punctuated by small holes and/or shallow pits with peripheral creases that rarely merge with others. These pits are sites of hair primordia growth (Figure 7, E), hair primordia in groups of five to more than 25 and were often extended to long hyaline distal cells.

Membranes were composed of 1–2 (rarely 3) layers of pigmented cortical cells and 5–9 layers of clear medullary cells (Figure 7, F). Cortical cells in surface view were oblong to rectilinear to polygonal (Figure 7, E, G), 4–9 × 5–11 (–14) μm; in cross-section, cells were thin-walled, broadly ovate, ovate to papillate, 5–11 (–12) μm wide by 6–14 (–13) μm in height (Figure 7, H). Medullary cells were clear and thin-walled, subcircular to broadly oblong, periclinal and progressively becoming larger towards the hollow basal thalli portions (Figure 7, F), up to 260 μm at its widest.

Plurangial sori associated with hair tufts, with irregular but often angular margins (Figure 7, G). Plurangial initials were derived from cortical cells; mature plurangia were laterally biseriate, each tier divided into four locules.

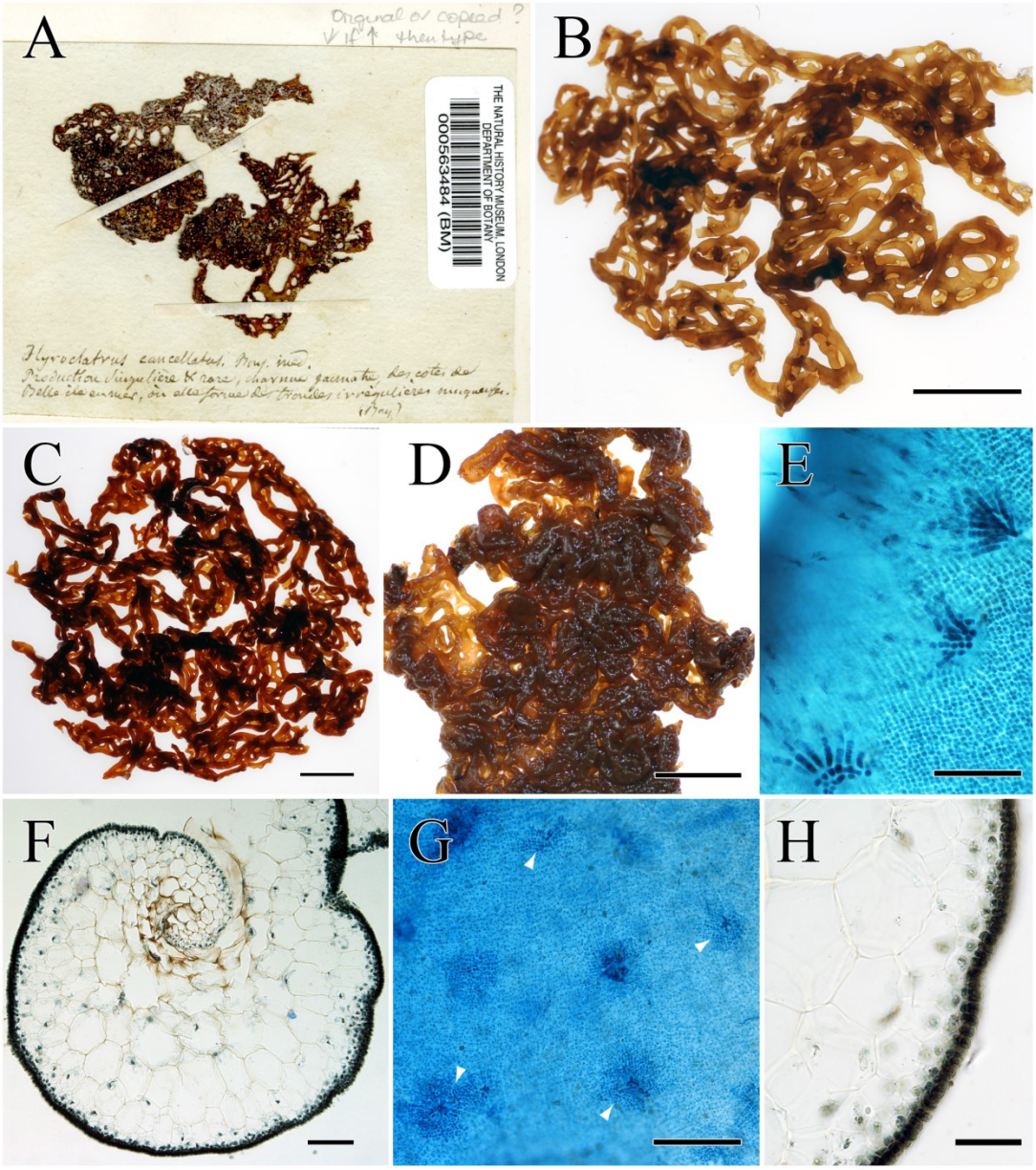

Figure 7. *Hydroclathrus clathratus* (C. Agardh) Howe habit and morphological characters. **A**, Scanned image of *H. clathratus* (= *H. cancellatus*) collected by Bory de Saint-Vincent in Belle-Île, France, including his handwritten notes. **B–C**, Wet habit of *H. clathratus* collected from Kagoshima, Japan (SAP115284 and SAP115283, respectively) showing the typical robust, perforated, and strap-shaped membranes. **D**, Wet habit of a membranous and convoluted *H. clathratus* collected from Tsuyazaki, Fukuoka, Japan (SAP115313). **E**, Group of hair primordia with long hyaline hair extensions and growing on shallow pits (SAP115313). **F**, Section through a revolute membrane edge showing pigmented cortical cells bounding several layers of clear medullary cells. **G**, Discrete patches of plurangial sori with irregular margins (arrowheads) surrounding hair tufts (SAP115285). **H**, Ovoid to broadly ovoid cortical cells with somewhat domed apices (SAP115312). Scale bars: B–D= 1 cm; E–G= 100 µm; H = 50 µm.

LINEAGE 2

Hydroclathrus rapanuii* Santiañez, Macaya *et* Kogame *sp. nov.

Figure 8

Description: Thalli membranous, up to 10 cm in diameter; membranes up to 350 μm thick, perforated with numerous holes. Cortical cells thin-walled, oblong to broadly oblong, 4.5–12.5 μm wide by 7–14 μm long; medullary cells thin-walled, up to 190 μm wide. Hair primordia in groups, mostly extended into hyaline hairs. Plurangial sori angular, block-like, may be confluent with adjacent sori. Plurangia erect, quadriseriate, cylindrical to slightly clavate, 15–23 μm long.

Holotype: SGO168251 (Figure 8, A), Vaihu, Easter Is., Chile, 1.5–2 m depth, 20 March 2016, *E.C. Macaya*; deposited in SGO.

Paratypes: SGO168250, SGO168252, Vaihu, Easter Is., 20 March 2016, *E.C. Macaya*; deposited in SGO.

Type locality: Vaihu, Easter Is., Chile (27° 9′ 57.22″ S, 109° 21′ 48.20″ W).

Etymology: Named in honor of the Rapanui people, the aboriginal people group of Easter Island ("Rapa Nui" in the indigenous language).

Distribution: Currently only known from the type locality.

Representative sequences: Genbank accession numbers: *cox*3 = MG450663, *psa*A = MG450661, *rbc*L = MG251837 (sequenced from SGO168251).

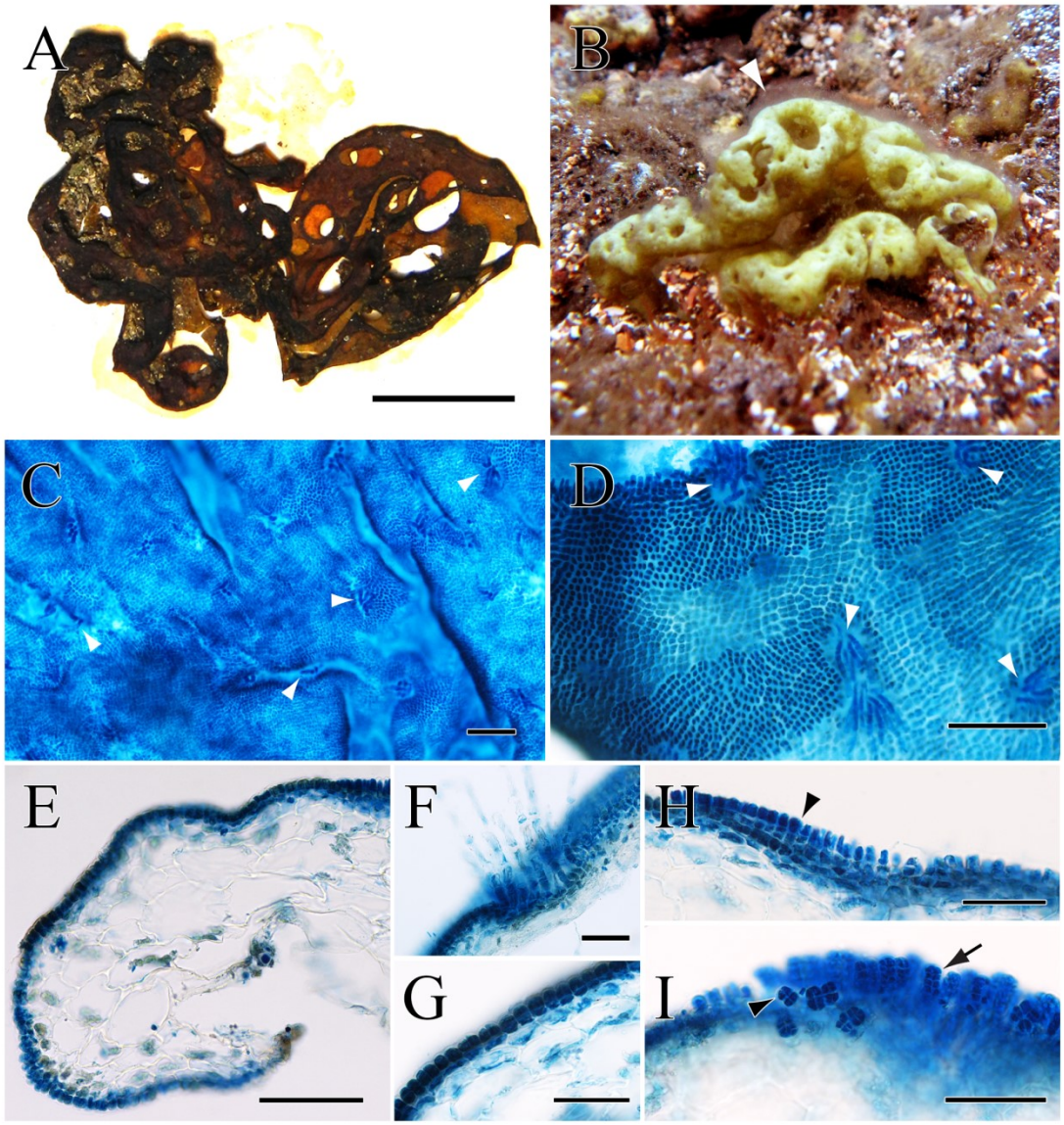

Figure 8. *Hydroclathrus rapanuii* Santiañez, Macaya *et* Kogame *sp. nov.* habit and morphological characters. **A**, Holotype specimen (SGO168251) showing membranous thallus perforated with numerous, variously sized holes; large holes have thickened margins resulting from its folded to revolute membrane edges. **B**, Underwater habit of *H. rapanuii* showing its olive green color as well as the long hyaline hair extensions surrounding its thallus (arrowhead). **C**, Portion of the membranous thalli showing hair tufts (arrowheads) growing on depressions and/or channels that are sometimes interconnected (SGO168251). **D**, Block-like, angularly margined, and confluent plurangial sori of *H. rapanuii* that are closely associated with hair tufts (arrowheads) (SGO168250). **E**, Section through the thallus showing the undulate profile of the membranes and its folded edge. Membranes composed of a layer of small and pigmented cortical cells and several layers of large and clear medullary cells (SGO168253). **F**, Group of hair primordia with hyaline hair extensions growing on shallow depressions (SGO168252). **G**, Oblong to broadly oblong cortical cells with somewhat domed apices. **H**, Transverse section showing plurangial initials (arrowhead) that are differentiated from cortical cells. **I**, A group of mature quadriseriate plurangia (arrowhead)—each series are divided in four layers (arrow)—arranged in vertical palisades. Scale bars: A= 1 cm; C–E= 100 μm; F–I = 50 μm.

Specimens examined: EASTER ISLAND: SGO168250–SGO168254, Vaihu, Easter Is., 20 March 2016, *E.C. Macaya*; EIS3-3095, Vaihu, Easter Is., 18 November 2016, *E.C. Macaya*.

Observations: Living thalli were yellowish to light brown in color, dark brown when dried on herbarium sheets (Figure 8, A, B). Thalli were membranous, saccate (when young) to irregularly lobed, 60–350 μm thick, perforated by holes of various sizes (Figure 8, A, B) rimmed by slightly thickened and mildly revolute margins; attached to the substrata by rhizoids. Membrane surfaces were dimpled and furrowed (Figure 8, C–E), with hair tufts formed in depressions and creases (Figure 8, D, F).

Membranes were composed of a layer of small pigmented cortical cells and 3–6 (rarely 7) layers of clear medullary cells (Figure 8, E, G). Cortical cells in surface view were square or rectilinear to polygonal, (4–) 5–9 × (5–) 6–12 (–14) μm (Figure 8, D); in cross-section, cells were thin-walled, mostly oblong to broadly oblong, and were 4.5–12.5 μm wide by 7–14 μm in height (Figure 8, E, G). Cortical cell apices were smooth, sometimes domed to obtuse; those adjacent to hair primordia often becoming papillate. Medullary cells were also thin-walled, progressively larger towards the hollow dorsal portion, up to 190 μm wide (Figure 8, E).

Hair primordia slightly clavate and often tapered to an abruptly narrow base; these grew in clusters of 4–20 and usually extended into hyaline hairs (Figure 8, D, F).

Plurangial sori progressively becoming angular and block-like around or adjacent to hair pits (Figure 8, D); these are often confluent with nearby sori, becoming irregularly outlined (Figure 8, D). Erect plurangial primordia were differentiated from cortical cells (Figure 8, H). Mature plurangia were quadriseriate, cylindrical and almost similar in size as those of the basal cortical cells but sometimes becoming slightly clavate, occurring as densely aggregated palisades, 15–23 μm long, each tier divided into four locules (Figure 8, I).

LINEAGE 3

***Hydroclathrus* sp.**

Figure 9

Distribution: AUSTRALIA: White Is. Kimberley, Western Australia.

Specimen examined: AUSTRALIA: PERTH08714274, 16 October 2011, White Is. (15° 2′ 45.6″ S, 124° 19′ 16.8″ E), Kimberley, Western Australia, *J.M. Huisman*; deposited in PERTH.

Observations: Thallus was light to dark-brown, somewhat membranous and partially saccate, net-like (Figure 9, A). Membranes 120–400 μm thick, were perforated with large and small subcircular to irregularly shaped holes rimmed by thickened inwardly folded or inrolled membranes. Membrane surfaces were wrinkled, with many deep depressions and interconnected channels that were sites of hair development (Figure 9, B–C). Membranes were composed of 1 to sporadically 2 layers of small pigmented cortical cells and 4–7 layers of large, clear medullary cells (Figure 9, D–E). Cortical cells in surface view were rectilinear to pentagonal, 4–6 × 6–10 μm (Figure 9, C). Cortical cells were thin-walled and distinctly subacutely papillate, some were ovate with domed apices, 7–11 μm wide by 9–14 μm long (Figure 9, E). Medullary cells were also thin-walled, periclinal, and progressively larger towards the hollow dorsal portion (Figure 9, D), 210 μm at its widest.

Hair primordia subclavate, narrowed sharply near the base, borne on papillate cells; these grew as tufts with elongated hyaline hairs (Figure 9, F).

Plurangial initials derived from surface cortical cells; young plurangia arranged in cylindrical to clavate palisades, quadriseriate, 9–11 μm long (Figure 9, G).

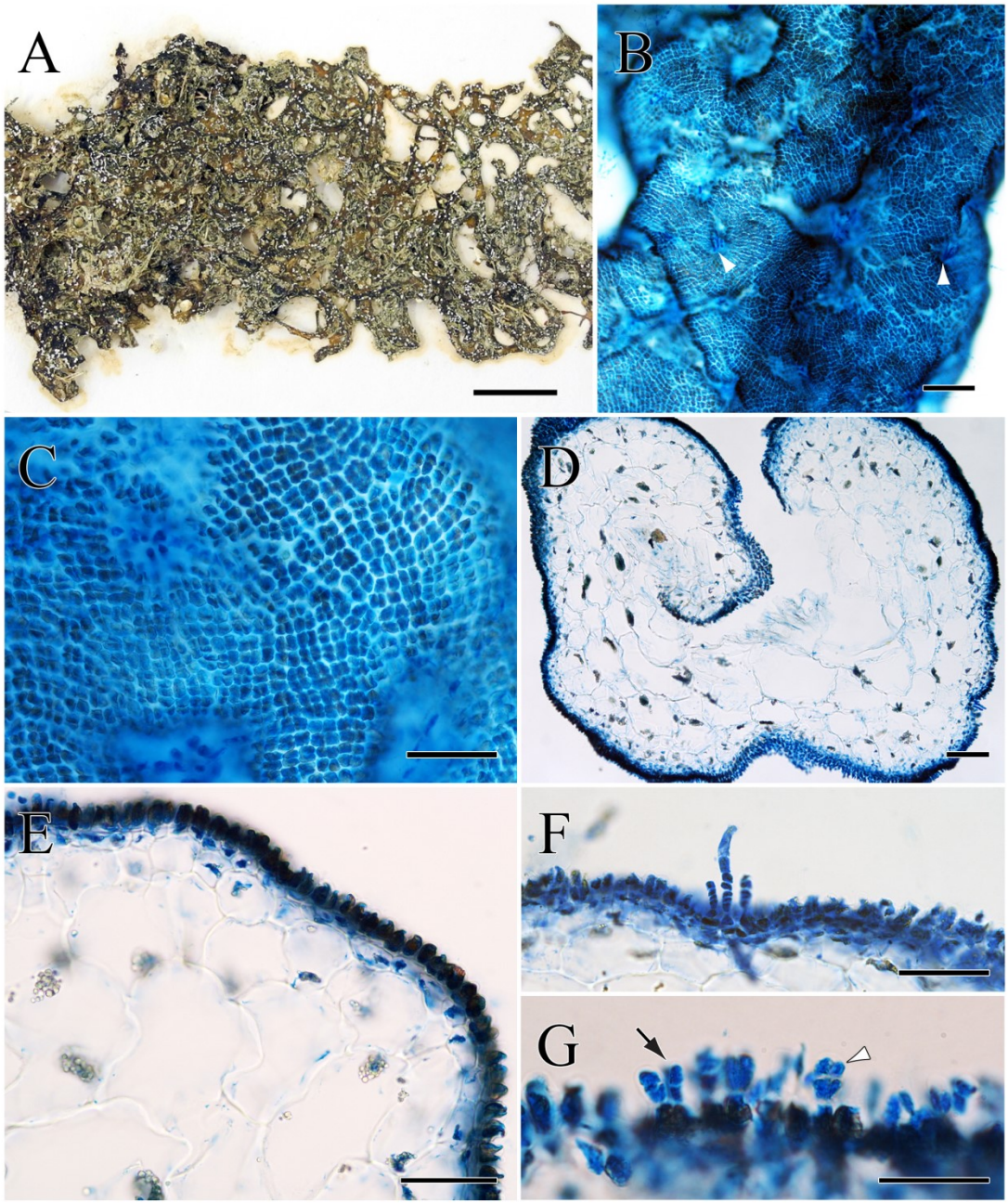

Figure 9. *Hydroclathrus* sp. habit and morphological characters. **A**, Pressed herbarium specimen of *H*. sp. (PERTH08714274) showing torn and somewhat membranous portions, which are punctuated by holes. **B**, Uneven thallus surface resulting from numerous deep depressions and channels that are often connected and are sites of hair growth and development (arrowheads). **C**, Close-up view of thallus showing rectangular to polygonal surface cortical cells. **D**, Transverse section showing undulate profile of the thallus membrane. Membrane consisted by several layers of large and clear medullary cells bounded by small and pigmented surface cortical cells. **E**, Closer view of a layer of cortical cells, each of these cells are in varying degrees of being subacutely papillate. **F**, A group of hair primordia, each tapering and borne on distinctly apiculate cells. **G**, Young and short, extremely thin-walled plurangia borne on subapiculate cortical cells. Plurangial initials appear to be transversely uniseriate and at first horizontally cut-off (arrow); each locule subsequently cut vertically, consequently becoming transversely biseriate (arrowhead). Scale bars: A= 1 cm; B, D = 100 µm; C, E, F = 50 µm; G = 25 µm.

LINEAGE 4

***Hydroclathrus stephanosorus* Kraft in Kraft *et* Abbott 2003: 252**

Figure 10

Type locality and specimen: Comets Hole, Lord Howe Is., New South Wales, Australia; MELU LHI-9967 [as corrected in Kraft (2009)] 30 September 1976, *G.T. Kraft & C.E. O'Brien*.

Distribution: TROPICAL TO WARM TEMPERATE INDO-PACIFIC: Lord Howe Is., Victoria, and Western Australia (Kraft 2009); New Zealand [as *H. clathratus* in Johnson & Dromgoole (1977) and Nelson (2013)]; French Polynesia [as *H. clathratus* in N'Yeurt & Payri (2006)]; Japan, Korea, Taiwan, Hawaii, Panama. ATLANTIC ISLANDS: Azores, Portugal.

Specimens examined: JAPAN: SAP115279, 28 April 2013, Sumuide, Nago, Okinawa, *K. Kogame*; SAP115294, 24 March 2008, Moroiso, Kanagawa, *K. Kogame*; SAP115306,12 March 2008, Haemita, Iriomote Is., Okinawa, *K. Kogame*; SAP115317, 20 April 2015, Innoshima Is., Hiroshima, *Y. Yamagishi*; SAP115320, SAP115323, Sado Is., Niigata, *S. Uwai*; SAP059218, 8 June 1989, Aburatsubo, Kanagawa, *M. Matsumoto*; SAP043620, 25 May 1983, *T. Yoshida*; SAP115337, SAP115338, 21 March 2016, Tateyama, Chiba, *W.J.E. Santiañez & K. Kogame*. SAP115381, 26 March 2017, Inoshiri, Kochi, *M. Hoshino*. HAWAIIAN ISLANDS: SAP115332, 12 June 2007, Pupukea, Oahu; SAP115333, 14 June 2007, Kahala, Oahu, *A. Kurihara*. ATLANTIC ISLANDS: SAP115341, SAP115342, May 2016, Azores, Portugal, *A.I. Neto*.

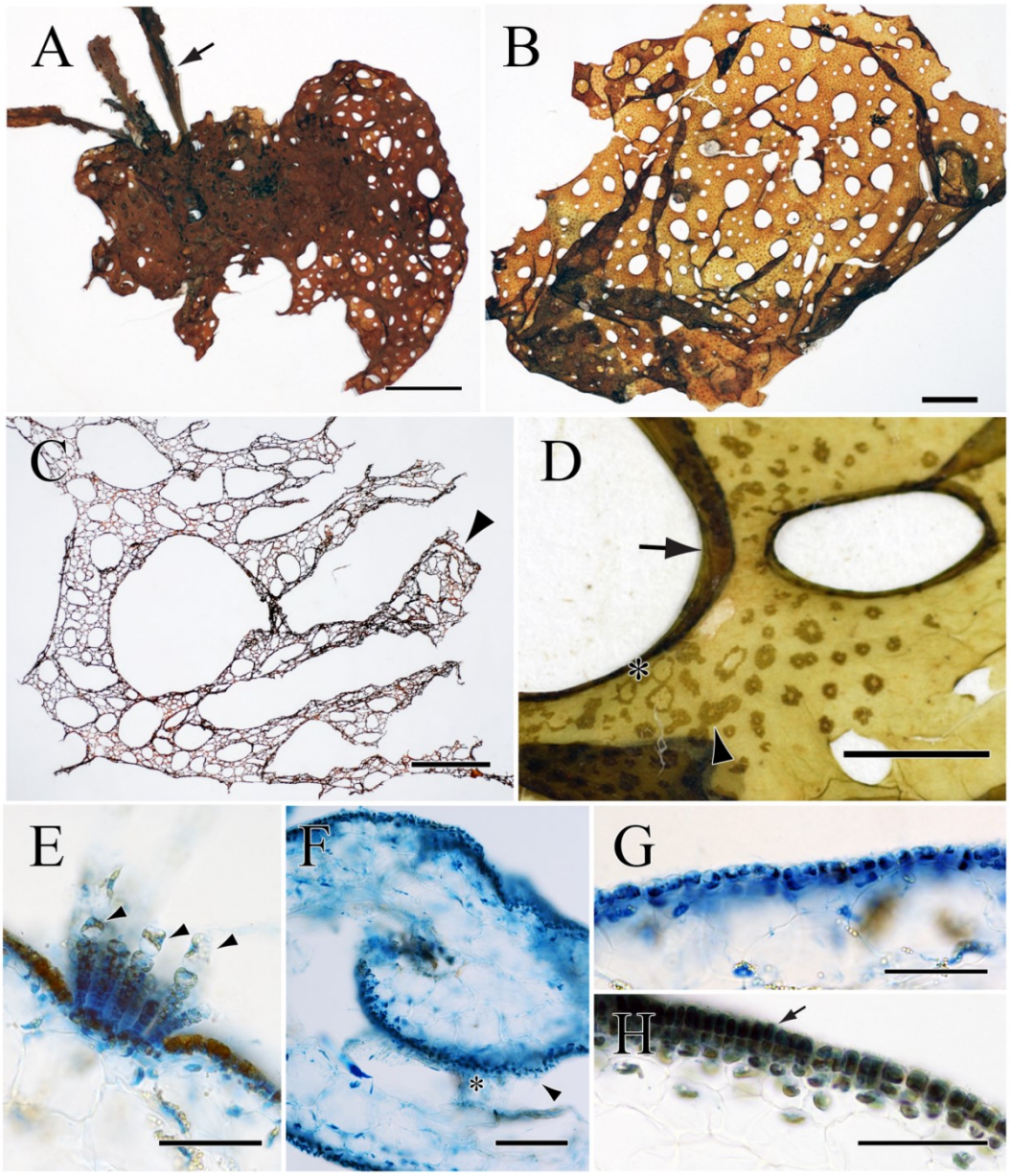

Figure 10. *Hydroclathrus stephanosorus* Kraft habit and morphological characters. **A**, Young and saccate dark-brown *H. stephanosorus* thallus (SAP115324) epiphytic to *Sargassum* sp. (arrow). **B**, Torn, sheet-like, light-brown thallus (SAP043620) covered with subcircular plurangial sori, which can be seen here as dark spots on the membrane. **C**, Stringy, torn, strap-shaped thalli collected from Inoshiri, Kochi (SAP115381) shown here with the typical large and evenly bounded subcircular holes. Periphery of the thallus shows remnants of saccate portions (arrowhead). **D**, Closer view of fertile portion of the thallus (SAP059218) showing thickened margins of large holes due to inrolled membrane edges (arrow), fused plurangial sori wherein each sorus maintains their respective circular margins (arrowhead), and a subcircular band of plurangia surrounding remnants of sori after the release zooids (asterisk). **E**, Section through a sample from Tassya, Niigata (SAP115320) showing a cluster of moniliform hair primordia some bearing the basal cell of hyaline hair extensions (arrowheads). **F**, Inrolled portion of the membrane of a specimen collected from Azores, Portugal (SAP115341) showing 3–4 layers of large, clear medullary cells bounded by one (to sporadically two) layer of small, pigmented cortical cells. Also shown here are some cortical cells developing into rhizoids (arrowhead), some of which are found attached to the basal medullary cell (asterisk). **G**, Rounded to broadly rounded and pigmented surface cortical cells bounding larger, clear, and thin-walled medullary cells (SAP115323). **H**, Transverse section through a fertile portion of the branch showing plurangial initials (arrow) that are differentiated from ovoid to papillate cortical cells. These cortical cells initially rounded to broadly rounded as seen on profiles of adjacent cells (SAP115338). Scale bars: A–B = 1 cm; C = 5 cm; D = 2.5 mm; E, G = 50 µm; F = 100 µm.

Observations: Thalli were olive green to dark brown in color, membranes 50–520 μm thick, expansive, with numerous large subcircular to elliptic holes with thickened margins, membranous parts were interspersed with smaller subcircular to irregularly shaped perforation. Thalli were saccate when young and torn into sheets that were peripherally partially saccate when old (Figure 10, A–D); these may also be stringy and convoluted here and there, but maintain the numerous subcircular holes and saccate form at the periphery (Figure 10, C).

Membranes composed of 1–2 layers of oblong to rectilinear to pentagonal cells (surface view), and (2–) 3–6 layers of clear, thin-walled medullary cells (Figure 10, F). Cortical cells in cross-section were thin-walled, broadly rounded to ovoid, sometimes highly domed and becoming subacutely papillate especially near hair primordia, (4–) 5–11 (–13) μm wide by (5–) 6–10 (–15) μm long (Figure 10, E–G). Medullary cells were round to elliptic and/or angular, larger towards the dorsal portion, up to 220 μm wide.

Moniliform hair primordia grow in tufts of 5 or more in shallow depressions or pits; primordia clavate, with large and domed uppermost cell, these gradually tapers to an attenuate base and sometimes extended into hyaline hairs (Figure 10, E).

Plurangial sori were conspicuous, often occurring at the periphery of the thalli, always surrounding hair tufts with a distinct circular margin (Figure 10, B, D). Sori are often discrete but may merge with adjacent sori, retaining its even when merged (Figure 10, D). Plurangial initials differentiated from surface cortical cells (Figure 10, G), each developing into an 18–28 μm long quadriseriate plurangium.

LINEAGE 5

***Hydroclathrus tenuis* C.K. Tseng *et* Lu 1983: 185**

Figure 11

Type locality and specimen: Paracel Is., South China Sea; AST 76-1276, 24 March 1976.

Distribution: TROPICAL TO SUBTROPICAL INDO-PACIFIC.

Local name: Hosokagomenori (Japanese).

Specimens examined: JAPAN: SAP115280, 28 April 2013, Sumuide, Okinawa, *K. Kogame*; SAP115301, SAP115302, 30 March 2009, Senaga, Naha Is., Okinawa, *K. Kogame*. PHILIPPINES: MSI27540, 10 April 2015, Calatagan, Batangas, *E.T. Ganzon-Fortes*; MSI27541, 22 May 2015 in Bolinao, Pangasinan, *E.T. Ganzon-Fortes*; SAP115326, SAP115327, 6 June 2015, Panglao Is., Bohol, *P.J.L. Geraldino*. CHINA: CNU002747–CNU002748, 12 March 2009, Daedonghae Bay, Hainan Is.

Observations: Thalli were light to dark brown in color, with numerous holes; spreading, often highly convoluted. Strap-shaped membranes generally fine, often widely separated from each other, resulting to a seemingly loose appearance (Figure 11, A–C). In the field, these were epiphytic or epilithic through rhizoidal holdfasts derived from surface cortical cells, or unattached, sometimes forming mats on seagrass beds or on corals.

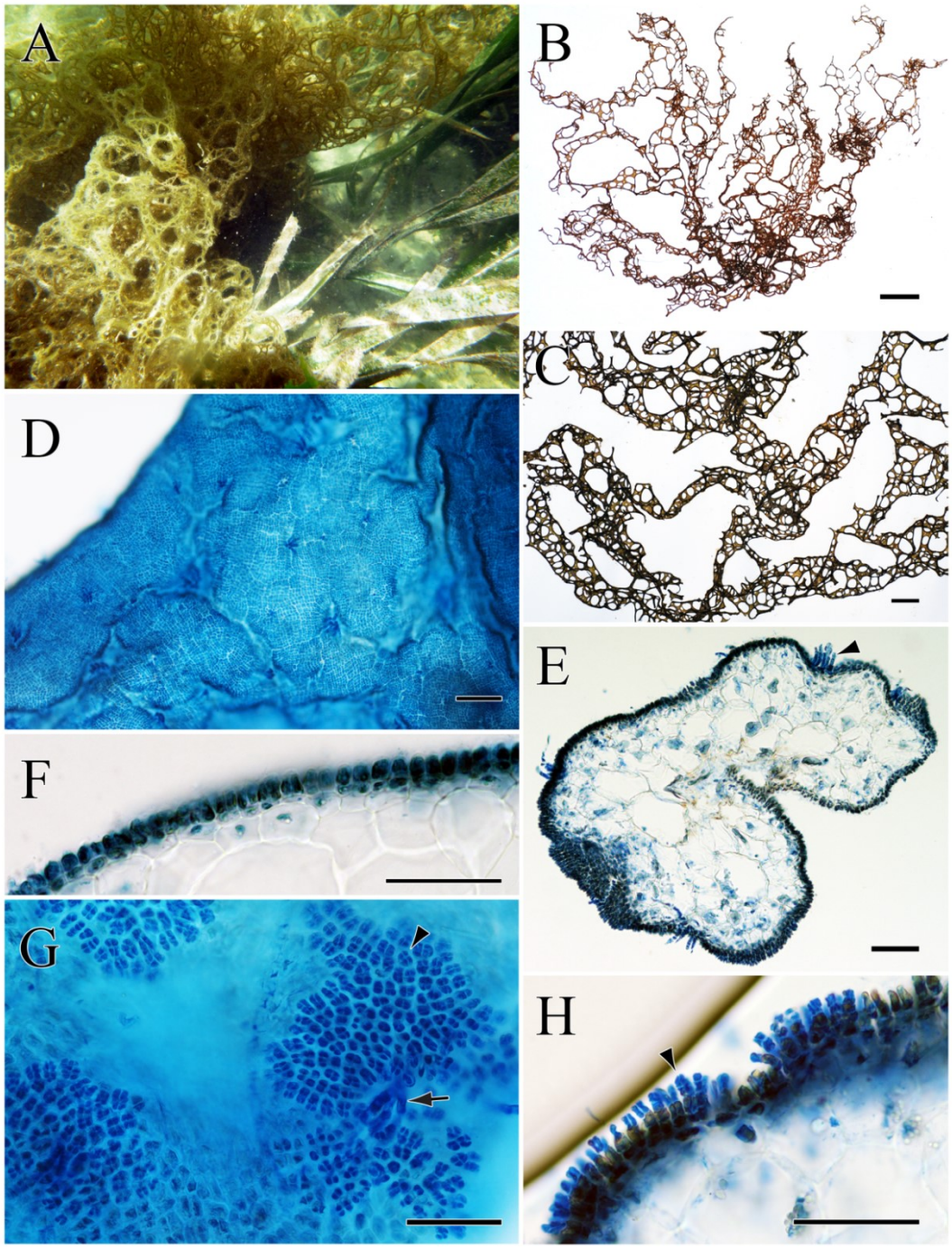

Figure 11. *Hydroclathrus tenuis* Tseng *et* Lu habit and morphological characters. **A**, Spreading habit of *H. tenuis* found growing in a shallow *Enhalus acoroides* bed in Calatagan, Batangas, Philippines (photo by Edna T. Ganzon-Fortes, 10 April 2015). **B**, Typical thin and fibrous *H. tenuis* specimen collected from Bolinao, Pangasinan, Philippines mounted on herbarium sheet (MSI27542). **C**, Strap-shaped and relatively robust morphotype of *H. tenuis* from Calatagan, Batangas, Philippines (MSI27540). **D**, Closer view of the uneven thallus surface showing a network of channels and deep depressions, which are sites of hair development (SAP115277). **E**, Transverse section of the thallus (MSI27541) showing the rugged outline of the membrane whose edges are folded, incurved, or inrolled and groups of hairs growing in depressions (arrowhead). **F**, Pigmented and disctinctly papillate cortical cells bounding clear, larger medullary cells (SAP115301). **G**, Sori of mature quadriseriate plurangia (arrowhead) surrounding a tuft of hairs (arrow) that are extended into hyaline hairs (MSI27541). **H**, Series of young plurangia derived from cortical cells (CNU002748). Shown herein is a transversely biseriate plurangium with four locules (arrowhead). Scale bars: B–C = 1 cm; D–E = 100 µm; F–H = 50 µm.

Membrane surfaces were uneven, creased, with numerous interconnected depressions and channels; these depressions and channels being site of hair development (Figure 11, D). Membranes were highly variable in form and thickness, usually thin and fibrous but may also be thick and robust, 40–590 μm thick. Membranes composed of 1–2 layers of small, pigmented cortical cells that were square to rectilinear, 4–8 × 6–9 (–11) μm in size (surface view), and 3–6 (–8) layers of larger, clear medullary cells (Figure 11, E–F). Cortical cells were thin-walled, generally papillate but may range from being broadly ovate to ovate (Figure 11, F), 5–9 (–10) wide by 7–11 (–12) long. Medullary cells were also thin-walled, round to elliptic, up to 250 μm in size (Figure 11, E).

Hair primordia were composed of similar-sized cells that gradually narrow into an abrupt constriction near the base. Primordia occurred in groups of 5 or more and were often extended into long hyaline hairs (Figure 11, E, G).

Plurangia differentiated from surface cortical cells (Figure 11, H) often developing adjacent to hair tufts; these were arranged in sori with irregular and angular margins, spreading extensively on the thalli surface (Figure 11, G–H). Plurangia were loosely arranged in erect, quadriseriate palisades.

LINEAGE 6

Hydroclathrus minutus* Santiañez *et* Kogame *sp. nov.

Figure 12

Description: Thalli fine and fibrous; membranes extremely narrow and thin, generally 250–750 μm wide and less than 130 μm thick, sometimes inter-adhesive. Cortical cells in cross-section 4–7 μm × 7–9 μm, broadly obovate to ovate, domed; medullary cells

clear, thick-walled, up to 200 μm wide. Hair primordia in groups of 4–9, extended to hyaline hairs. Plurangial sori subcircular, surrounding hair tufts. Plurangia erect, arranged in vertical palisades, up to 18 μm long.

Holotype: SAP115290 (Figure 12, B), 30 March 2009, Senaga, Naha, Okinawa, Japan, *K. Kogame*; deposited in SAP.

Type locality: Senaga, Naha, Okinawa, Japan (127° 38′ 23″ N, 26° 10′ 30″ E).

Etymology: This species is named after its minute thallus.

Distribution: TROPICAL TO SUBTROPICAL NORTH-WESTERN PACIFIC: Okinawa Is., Japan; Ly Son Is. and My Hoa, Vietnam.

Representative sequences: GenBank accession numbers: *cox*1 = MF432078, *cox*3 = MF432005, *psa*A = MF431956, *rbc*L = MF431948.

Additional specimens examined: JAPAN: SAP113344, 13 April 1935, Ishigaki Is., Okinawa, *Y. Yamada*; SAP113767, May 1961, Chinenumino, Naha, Okinawa, *Y. Yamada*. VIETNAM: NITRA1024, 24 April 2007, My Hoa; NITRA2018, 24 March 2010, Ly Son Is.; NITRA1020, 9 April 2014, An Hai, Ly Son Is.

Observations: Thalli were light to dark brown in color, forming delicate skeins with extremely fine membranes that are 250–750 (–1100) μm wide and less than 130 μm at the thickest portion (Figure 12, A–C). Membrane surfaces had numerous depressions, some were interconnected and were often sites of hair development (Figure 12, D).

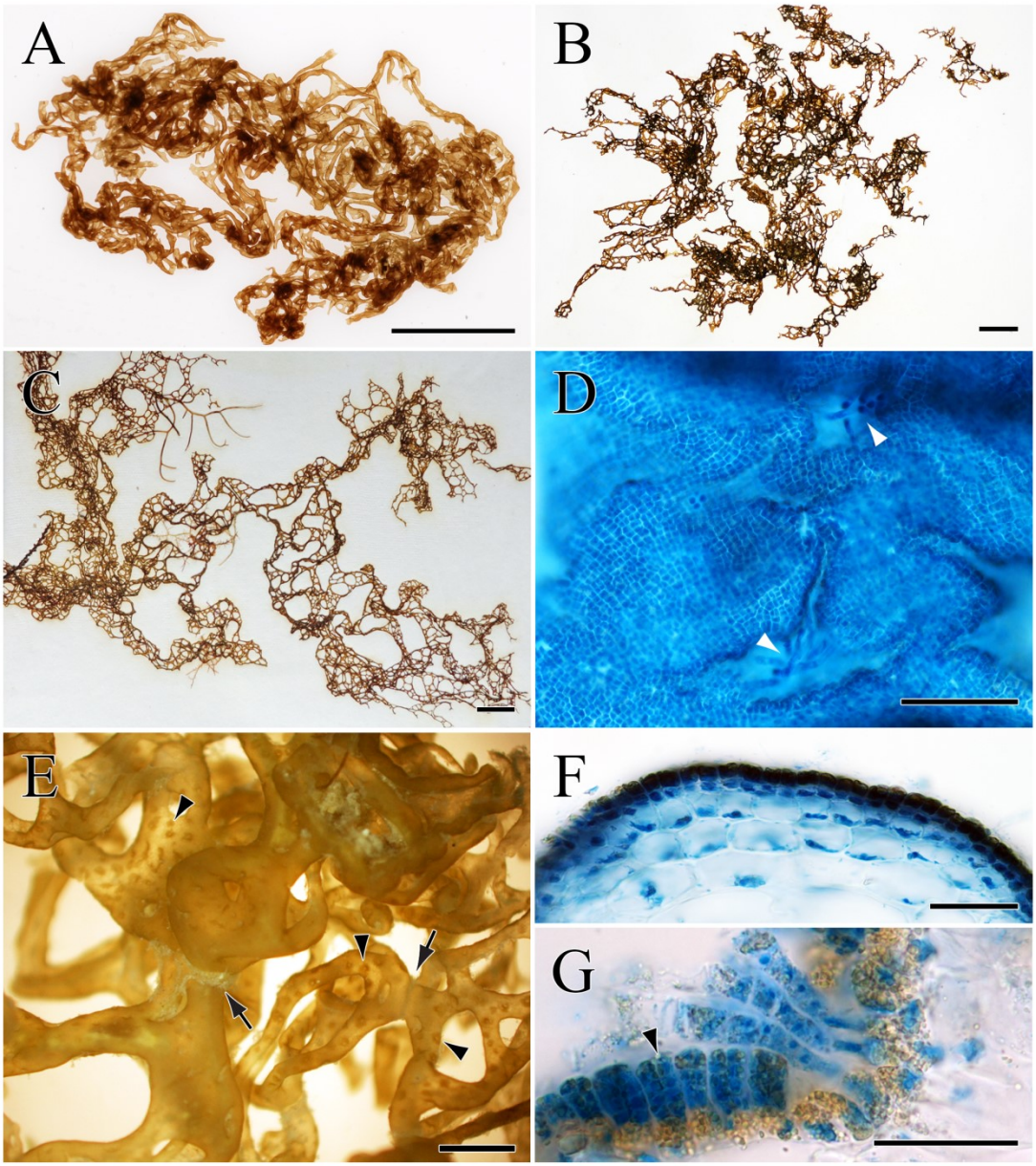

Figure 12. *Hydroclathrus minutus* Santiañez *et* Kogame habit and morphological characters. **A**, Wet habit of the fine and convoluted thallus of the holotype (SAP115290) prior to mounting on herbarium sheet. **B**, Dried herbarium specimen of the holotype. **C**, Herbariums specimen of *H. minutus* from Ly Son Is., Vietnam (NITRA2018) showing fine, fibrous, and strap-shaped portions reminiscent of *H. tenuis* (Photo by Le Nhu Hau). **D**, Thallus surface showing interconnected pits which are sites of hair primordia development; these primordia often extended into long hyaline hairs (arrowheads). **E**, Portion of the holotype thallus showing inter-adhesive membranes (arrows) with numerous subcircular plurangial sori with a sterile center (arrowheads). **F**, Transverse section of the thin thallus of the holotype showing a layer of small broadly obovate to ovate to sometimes papillate and pigmented cortical cells bounding several layers of larger, clear, and thick-walled medullary cells. **G**, Section through a fertile portion of a specimen from Chinenumino, Okinawa, Japan (SAP113767) showing a row of transversely biseriate plurangia (arrowhead), each column is divided into four locules. Scale bars: A–C = 1 cm; D = 100 µm; E = 1 mm; F = 50 µm; G = 25 µm.

Membranes were sometimes inter-adhesive, making those portions highly convoluted (Figure 12, A–D). Plurangial initials developed radially with reference to hair tufts, distinctly marking the thalli with nearly circular sori with sterile center (Figure 12, E).

Membranes composed of one layer of small pigmented cortical cells and one to five layers of larger clear medullary cells (Figure 12, F). Cortical cells in surface view were square to rectilinear to polygonal (Figure 12, D), 3–6 × (4–) 5–8 (–9) μm, each with one pyrenoid that is 1–2 μm in diameter. Transversely, cortical cells were thin-walled, broadly obovate to ovate, with domed apices, sometimes papillate, 4–7 (–9) μm wide by 7–9 (–11.5) μm high (Figure 12, F). Thick-walled and clear medullary cells were periclinal, broadly ovate to elliptical, becoming larger towards the dorsal portion (Figure 12, F) , up to 200 μm wide.

Hair primordia were in groups of 4–9, but can occur singly. They were subclavate to clavate, progressively narrowing towards the attenuate base, often extending into hyaline hairs (Figure 12, D, G).

Plurangial sori discrete, often nearly circular, surrounding hair tufts (Figure 12, E); expansive sori merged with adjacent sori but maintained their rounded margin. Plurangia arranged in vertical palisades, quadriseriate, short, up to 18 μm high (Figure 12, G).

Hydroclathrus tumulis Kraft *et* Abbott 2003: 254

Figure 13

Type locality and specimen: Necker Is., northwestern Hawaiian Is.; BISH697080.

Distribution: SUBTROPICAL NORTH-CENTRAL PACIFIC: Necker Is. and Maro Reef, northwestern Hawaiian Is.

Specimens examined: NORTHWESTERN HAWAIIAN ISLANDS: TC96-07, 22 June 1996, Necker Is.; TC96-01, 26 June 1996, Necker Is.; IA26078a, 30 June 2001, Necker Is., *R. Moffitt.*

Observations: Thalli olive to dark brown, membranous, perforated with various subcircular to oblong holes, saccate and/or torn into thin sheets; these were adherent on herbarium paper, up to 12.5 cm in diameter at its widest (Figure 13, A–B). Thalli epiphytic, epilithic, and can be interadhesive through rhizoids derived from cortical cells.

Membranes were up to 190 μm thick, smooth, somewhat creased here and there, with shallow pits or depressions (Figure 13, C), which were sites of hair development (Figure 13, D). Membranes composed of 1 (rarely 2) layer of small, pigmented cortical cells and 2–4 layers of periclinal, oblong to widely elongate, clear medullary cells (Figure 13, E–G). Cortical cells in surface view were squarish to rectilinear to polygonal, 7–12 μm wide by 11–16 μm long; in cross-section, cells were thin-walled, broadly ovate to oblong, often subacutely papillate (Figure 13, F–G), 8–13 (–15) μm wide by 9–12.5 μm long. Medullary cells were thin-walled, (Figure 13, AE, G), periclinally ovoid, up to 300 μm at its widest.

Hair primordia in groups of two to four, clavate, apical cell large, narrowed steeply towards the base, borne on domed apical cell (Figure 13, D, F).

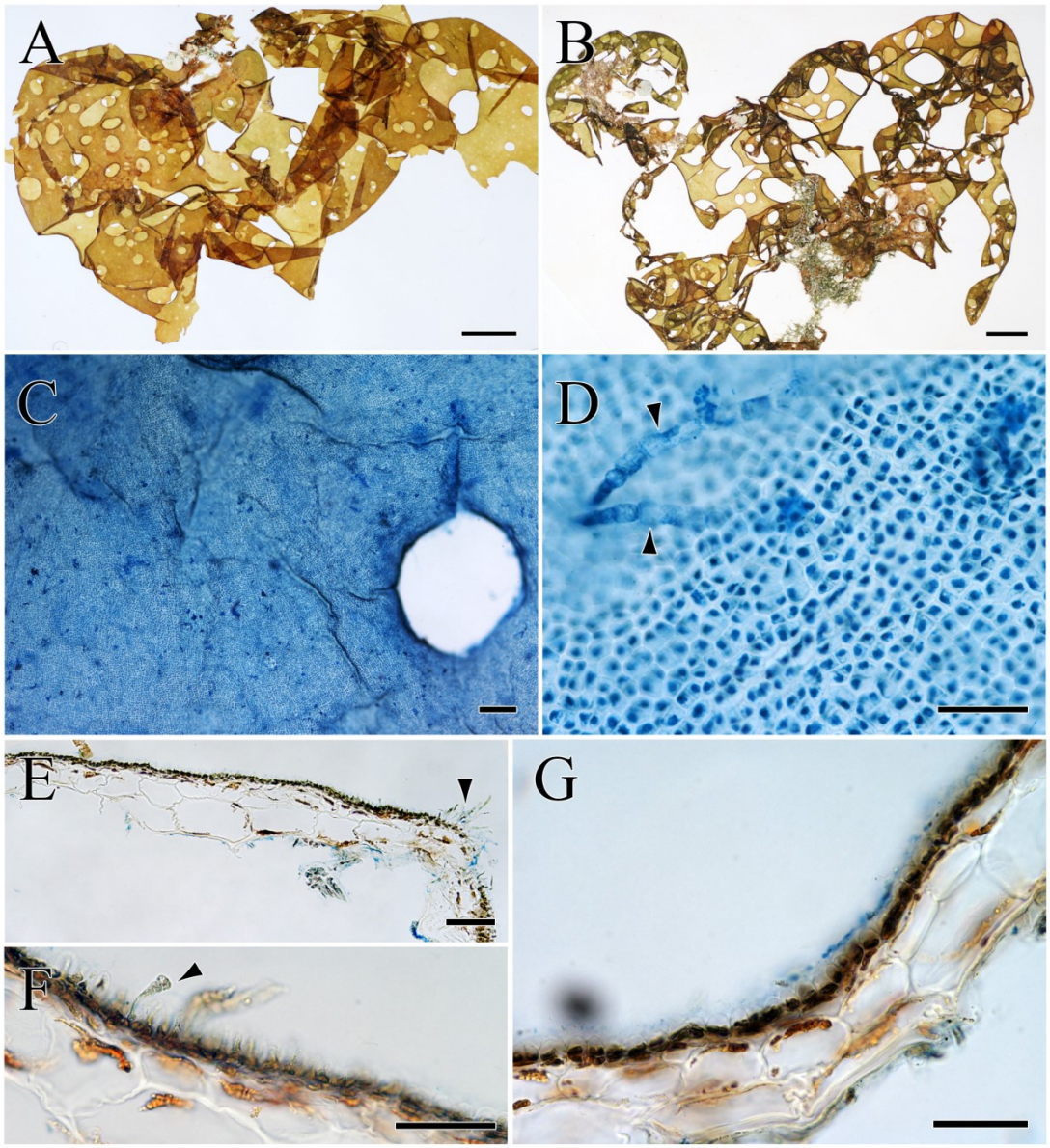

Figure 13. *Hydroclathrus tumulis* Kraft *et* Abbott habit and morphological characters. **A**, Saccate thallus with expansive membranes having numerous, variedly sized perforations. **B**, Partially saccate and torn thalli collected from within the type region. **C**, Portion of the thallus surface having several channels and depressions. **D**, Closer view of the membrane surface showing rectangular to polygonal profile of cortical cells and hair primordia with hyaline hair extensions (arrowheads). **E**, Transverse section through the thalli showing a thin membrane composed of a layer of pigmented cortical cells and up to four layers of clear medullary cells. Rhizoids derived from cortical cells were also apparent near the membrane edges (arrowhead). **F**, Cuneate hair primordia (arrowhead) associated with (sub-) apiculate cells. **G**, Transverse section of the membrane showing the highly domed to papillate to variedly subapiculate cortical cells bounding two to three layers of clear medullary cells. Scale bars: A–B = 1 cm; C = 200 µm; D, F, G = 50 µm; E = 100 µm.

DISCUSSION

The advent of DNA phylogenies has resulted in considerable taxonomic revisions in many seaweed groups and has greatly impacted our understanding of global seaweed biodiversity. Using molecular phylogenetic tools, recent studies reported high cryptic and pseudo-cryptic diversity among supposed widely distributed taxa, suggesting that seaweed diversity has been grossly underestimated. For example, in the pantropical red alga *Portieria hornemanii* (Lyngbye) P.C. Silva , Payo *et al.* (2013) recognized 21 cryptic species within the Philippine archipelago, and in the brown algal species *Colpomenia sinuosa* and *Scytosiphon lomentaria* (Lyngbye) Link, at least three (Lee *et al.* 2013) and four cryptic species (Kogame *et al.* 2015), respectively, were reported. The brown algal genus *Lobophora* J. Agardh from New Caledonia was reported to be consisted of 31–39 (pseudo-) cryptic species, 10 of which were formally described as new species (Vieira *et al.* 2014) while in the red algal genus *Bossiella* P.C. Silva , Hind *et al.* (2014, 2015) revealed the presence of 17 genetic species, five of which were described as new to science. Together with species discovery, molecular phylogenetic analyses have also been used to revise morpho-species boundaries such as in the taxonomically problematic *Caulerpa* Lamouroux (Belton *et al.* 2014) and *Sargassum* C. Agardh (Mattio *et al.* 2008, 2009, 2010) as well as in the supposedly morphologically variable and cosmopolitan species *Lobophora variegata* (J.V. Lamouroux) Womersley *ex* E.C. Oliveira (Schultz *et al.* 2015), *Gibsmithia hawaiiensis* Doty (Gabriel *et al.* 2016), *Centroceras clavatum* (C. Agardh) Montagne (Schneider *et al.* 2015), and *Corallina officinalis* Linnaeus (Brodie *et al.* 2013).

Similarly, my studies on *Hydroclathrus* have led to the discovery of two pseudo-cryptic species, which I described as *H. minutus* and *H. rapanuii*. I also confirmed herein that *H. stephanosorus* is an independent species that is both morphologically and

genetically distinct from other *Hydroclathrus*. Similar to Kraft and Abbott (2003), I also observed high morphological overlap between *H. tenuis* and *H. clathratus*. Distinguishing them from each other was difficult especially in localities where both species were known to be sympatric. As a consequence, the traditional way of distinguishing the latter two species based on membrane size and thickness is deemed unreliable and may have resulted in misidentifications.

Hydroclathrus clathratus was assigned to Lineage 1 based on the similarities of the specimens to the gross morphology of Bory's *H. cancellatus* specimen collected from Belle-Île, France (Figure 7, A). Assigning this lineage to the generitype by comparing gross morphologies despite the known phenotypic plasticity in the genus is admittedly crude. However, I did not attempt to morphologically and genetically examine Bory's specimen due to several technical difficulties. Old herbarium specimens of *Hydroclathrus* rehydrate poorly such that characters are often muddled. DNA extraction on decades-old brown algal herbarium specimens also remains challenging due to DNA degradation. In addition, Bory's specimen has doubtful provenance; thus, barcoding for typification studies would be counterproductive. For practicality, Kraft and Abbott (2003) suggested to relectotypify the genus through topotype studies on the relatively narrow geographic areas of syntype localities, Shark Bay, Australia and/or from Rauki, Waigeo Is., Moluccas, Indonesia (Agardh 1823, Silva *et al.* 1996).

Lineage 2 consisted of specimens from the geographically isolated Easter Is., all of which was initially considered as the cosmopolitan *H. clathratus*. Based on its distinct morphological and genetic characters, I described this lineage as *H. rapanuii*. The young thalli of *H. rapanuii* were saccate and membranous similar to *H. stephanosorus* and *H. tumulis*. *Hydroclathrus stephanosorus* can be distinguished based on its broadly rounded cortical cells (vs oblong to broadly oblong cells of the latter), thicker thallus, and discrete and subcircular plurangial sori (vs often confluent and angular, block-like

plurangial sori of the latter). Meanwhile, *H. tumulis* is differentiated from *H. rapanuii* in having broadly membranous thalli, subapiculate cortical cells, stalked plurangia, and its restriction to subtidal habitats. When compared with *H. clathratus*, *H. rapanuii* can be distinguished through its confluent, angular and block-like plurangial sori and its thinner membranes due to fewer cortical and medullary cell layers (Table 1).

Lineage 3 was a putative new species represented by a single sample from the intertidal of White Is., Kimberley, Australia. The species was morphologically distinct when compared with other *Hydroclathrus* species in having 120–400 μm thick membrane composed of subapiculate cortical cells and 4–7 layers of medullary cells, as well as in its thin-walled, clavate plurangia borne on apiculate cells. However, the shape and nature of the cortical cells and plurangia of this species are similar to those of *H. tumulis* (Table 1). The latter was known to be restricted to the subtidal (*H*. sp. was collected in the intertidal) and possessed thinner, saccate to torn broadly membranous thalli. Considering the high morphological plasticity in *Hydroclathrus*, it was also likely that the broader membranes of *H. tumulis* were environmentally-induced variations. That is, subtidal algal species tend to have expansive membranes as an adaptation to maximize light absorption. The absence of waves in the subtidal may also promote the formation of wider membranes; conversely, wave action in shallower waters can induce morphological change such as those reported in other brown seaweeds (Charrier *et al.* 2012). Considering the morphological plasticity in *Hydroclathrus*, the absence of reference sequence for *H. tumulis* collected from within the type region, the geographic distance between White Is., Western Australia, and the difference in their habitat, I refrained from assigning this lineage to *H. tumulis*.

Hydroclathrus stephanosorus was assigned to Lineage 4 based on the morphological and genetic similarities of the specimens with those collected and described from the type locality, Lord Howe Is., Australia. The specimens I examined

herein possessed the typical characters described by Kraft and Abbott (2003: figs 7–28, p. 253): 1) membranous sac-like to torn and/or sheet-like thalli, 2) large and small subcircular to elliptic holes, 2) moniliform hairs, 3) broadly rounded cortical cells, and 4) subcircular plurangial sori (Table 1). Kraft (2009) doubted the validity of *H. stephanosorus* noting the wide overlap in characters of the species with those of *H. clathratus*. As I have shown here, the former can be consistently distinguished from the latter based on the previously outlined characters. However, Kraft's doubt is understandable as *H. stephanosorus* appears to be a usually misidentified species. For example, the Japanese *Hydroclathrus* flora was once thought to be composed of only two species, *H. clathratus* and *H. tenuis*. The former was attributed to most *Hydroclathrus* species collected from Kyushu to southern Hokkaido while the latter was only found in the Okinawa archipelago. My current assessment indicated that *H. stephanosorus* was more common and widely distributed around Japan than *H. clathratus*. The '*H. clathratus*' reported by Johnson and Dromgoole (1977: fig. 1) and Nelson (2013: p. 123) were also attributable to *H. stephanosorus* based on their saccate and membranous thalli, subcircular plurangial sori, and shape and nature of hair primordia and cortical cells. Similarly, the '*H. clathratus*' from French Polynesia, as reported by N'Yeurt and Payri (2006: fig. 42), was also identifiable as *H. stephanosorus* based on its habit, subcircular membrane holes, 3–5 layers of medullary cells, and cortical cell size and shape.

Hydroclathrus tenuis was assigned to Lineage 5 based on specimens with fine and fibrous thalli that were collected from Hainan Is., China and Bolinao, Pangasinan, Philippines. The morpho-anatomical characters of these specimens fitted those of *H. tenuis* from Paracel Is. (type locality) as originally described and illustrated by Tseng and Lu (1983: pl. I:1). Hainan Is. and Bolinao, Pangasinan were within the northern part of the South China Sea and were near Paracel Is. Surprisingly, specimens included in

this lineage also possessed thick and robust thalli akin to those of *H. clathratus*. In the Philippines, for example, *H. tenuis* and *H. clathratus* have been reported, with the latter considered as more abundant and common than the former (Trono & Ganzon-Fortes 1988; Trono 1997). My current assessment on *Hydroclathrus* based on collections from the central and western Philippines, however, indicated otherwise. It appeared that *H. tenuis* was more common within the Philippine archipelago and that *H. tenuis* with robust and thick thalli may have been misidentified as *H. clathratus*. Noting the varied and overlapping interpretations of both species, Kraft and Abbott (2003) highlighted the difficulty between the c*lathratus*/*tenuis* split based only on morphologies. When compared with *H. clathratus*, *H. tenuis* has been consistently found to have 1) thalli surfaces with many deep depressions and channels that are interconnected, 2) smaller and highly papillate cortical cells, 3) smaller medullary cells and fewer layers, and 4) thinner membranes (Table 1). Because of the widely overlapping characters of *H. tenuis* and *H. clathratus*, supplementing morphological data with molecular data would prove beneficial.

Lineage 6 was composed of specimens that were, based on gross morphology, identifiable as *H. tenuis*. However, these specimens were distinct in possessing very thin membranes that were occasionally attached to each other, subcircular plurilocular sporangia that surround hair tufts, thick-walled medullary cells, and shorter (Table 1).. The nature and arrangement of the plurangia of specimens from this lineage were comparable to *H. stephanosorus*. As this lineage is morphologically and genetically distinct, this species was described as new to science and was herein named as *H. minutus*. The phylogenetic position of this species relative to other *Hydroclathrus* species is yet to be established. In the *rbc*L tree (Figure 5), the phylogenetic relationships of *Hydroclathrus* species were not resolved. In the *psa*A tree (Figure 6), *H. minutus* was segregated with high support by *Rosenvingea intricata* and *Iyengaria*

quadriseriata from the other *Hydroclathrus* species. If the *psa*A tree would be primarily considered, *H. minutus* is best segregated from *Hydroclathrus* and should represent a distinct genus. Alternatively, by following the principle of monophyly, *R. intricata* and *I. quadriseriata* can be transferred to *Hydroclathrus*. However, this would mean that hollow, tubular, decumbent, and branching scytosiphonacean algae will be circumscribed under the genus *Hydroclathrus*. Lumping all these species with highly varied morphologies would most likely lead to confusion and, as phylogenies remain unresolved, I consider such drastic taxonomic change as premature. Thus, at present and until proved otherwise, I assigned the new species to *Hydroclathrus* because of its morphological similarities to the members of the genus.

For the deep-water Hawaiian species *H. tumulis*, I was only able to conduct morphological analyses on samples collected from the type locality as my attempts to extract DNA from the available specimens have failed. Nonetheless, the specimens I have observed were similar to those described by Kraft and Abbott (2003 (Table 1). *Hydroclathrus tumulis* has morphological characters that were similar to those of *H*. sp. (Lineage 3) but, as mentioned earlier, I have not assigned this name to the latter due to morpho-anatomical, geographic, and habitat differences.

The distribution of each *Hydroclathrus* species in my current study, despite the collection bias within the tropical to warm temperate northwestern Pacific, showed interesting patterns (Figure 14). Particularly, *H. tenuis* and *H. stephanosorus* were recorded for the first time in several localities, and, their distribution ranges were considerably extended. For *H. tenuis*, aside from its known distribution in the tropical to subtropical western Pacific (Philippines, Indonesia, China, and Japan), the species was recovered in tropical eastern Pacific (Mexico), subtropical central Pacific (Hawaiian Is.) (Figure 14, A). Surprisingly, *H. stephanosorus*, a species that was once reported only from subtropical to warm temperate Australia (Kraft & Abbott 2003, Kraft 2009), was

found to be widely distributed and recorded for the first time throughout the tropical (Panama), subtropical (Hawaii, Taiwan, Korea, Japan) to warm temperate (Japan) northern Pacific as well as in the subtropical eastern Atlantic (Azores, Portugal) (Figure 14, B). Meanwhile, *H. clathratus* was mainly recovered in subtropical (South Africa, Korea, Japan) to warm temperate waters (Japan), and was not recovered in the tropics except in relatively cool tropical waters in the northwestern Philippines (Figure 14, C). The other three lineages have limited distributions: *H. minutus* was only found in several areas in Okinawa, Japan and Vietnam, *H. rapanuii* was only recorded in Easter Is., while *H*. sp. only occurred in White Is., Western Australia (Figure 14, D). The observed disjunct distribution of the different species may be sampling artifacts as this study did not include samples from the northern Indian Ocean as well as the majority of the Atlantic. Thus, to properly account for the distribution range limits of the different species, a global survey would be necessary.

The current integrative taxonomic study on *Hydroclathrus* highlights the need to reassess the identity of species previously identified as *'H. clathratus*' and '*H. tenuis*'. As I have shown, these species may be confused with the other species I have observed and described herein. Most of the *Hydroclathrus* species, such as *H. stephanosorus*, *H. rapanuii*, and *H. minutus*, can be reliably identified based on morpho-anatomical analyses; however, distinguishing between *H. clathratus* and *H. tenuis* remains difficult. *Hydroclathrus* species exhibit high morphological plasticity and multiple characters tend to overlap. Biodiversity assessment must therefore consider detailed morpho-anatomical studies. Phycologists conducting in situ rapid biodiversity assessments must resist the temptation to identify species based on gross morphologies alone (i.e., attributing thin and fibrous specimens to *H. tenuis*, and thick and robust ones to *H. clathratus*).

Assigning correct names to seaweed species has become more relevant especially as global marine biodiversity faces several challenges including species loss due to environmental degradation and the changing climate. Seaweeds remain as often neglected resources when it comes to biodiversity conservation and management. However, proper accounting of biological resources is crucial in environmental planning and management as it provides importance values especially when ecological goods and services are considered. Correct species identification is more pressing in coastal resource management, particularly in addressing the impacts of marine macroalgal blooms such as *Sargassum* spp. in the Atlantic (Sissini *et al.* 2017) and/or those that have invaded other areas, causing wide-scale ecological imbalances like *Caulerpa taxifolia* (M. Vahl) C. Agardh in the Mediterranean and *Sargassum muticum* (Yendo) Fensholt in the northern Atlantic (Global Invasive Species Database 2017a, b). Lastly, with the seas being the last frontier, bioprospecting of seaweeds in search of high-value natural products has become more common. As names announce and enhance the value, kind, and quality of a seaweed product (Abbott 1985), uncertainties or misidentifications of a seaweed crop can have significant negative consequences to the seaweed processing industry (Ganzon-Fortes *et al.* 2012), among others. All these underscore an imperative for good practices such as including several lines of evidence for species identification and/or delineation, especially among highly polymorphic taxonomic groups such as seaweeds.

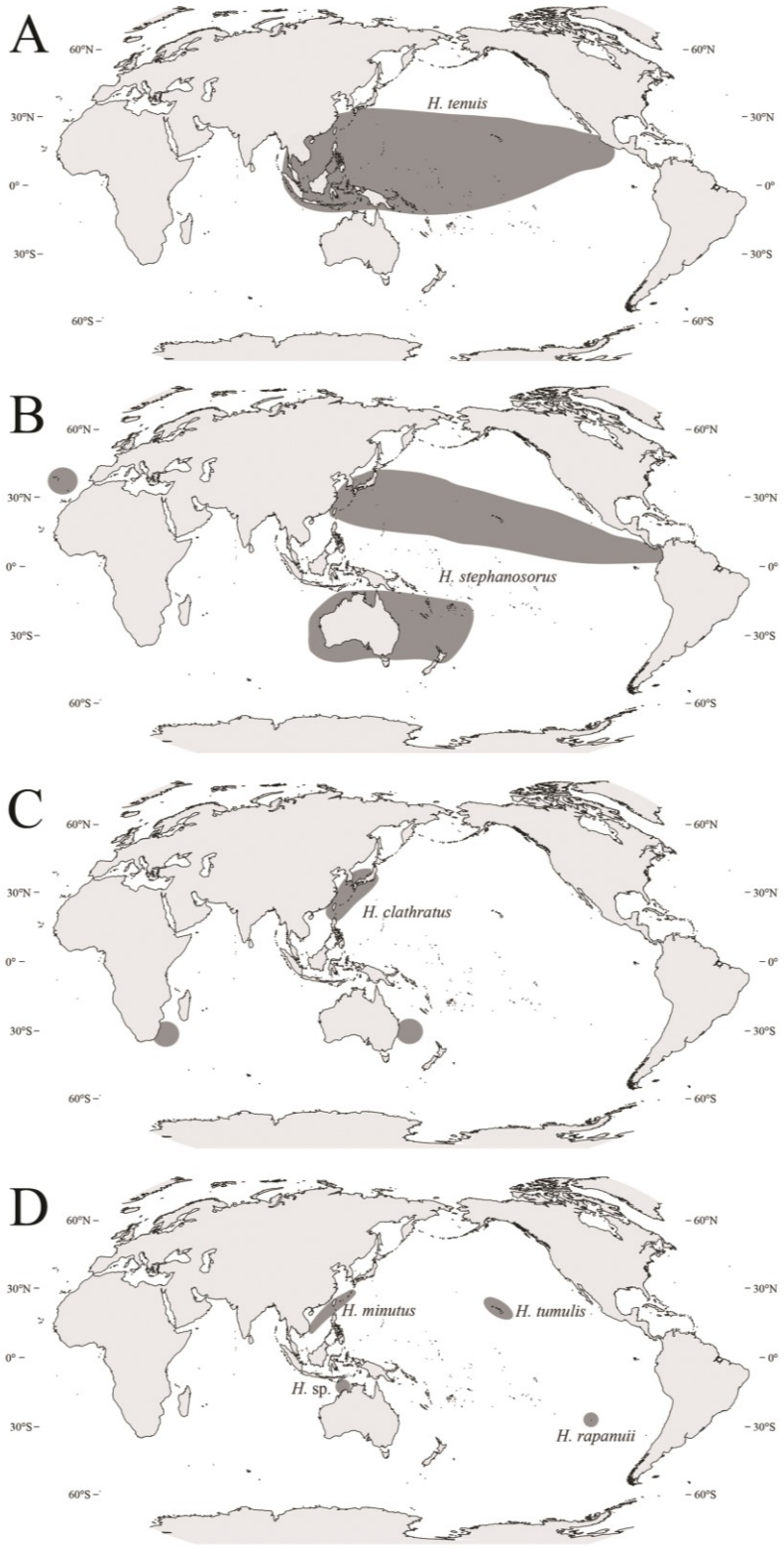

Figure 14. Distribution of *Hydroclathrus* species (gray) reported in this study. **A**, Tropical to warm subtropical distribution range of *H. tenuis* in the Pacific. **B**. Extended distribution range of *H. stephanosorus* in cold tropical to warm temperate waters of the northern Pacific and eastern Atlantic. **C**, Disjunct distribution of *H. clathratus* in cold tropical to warm temperate waters of the Indo-Pacific. **D**, Limited distribution ranges of *H. minutus sp. nov.*, *H. rapanuii sp. nov.*, *H. tumulis*, and *H.* sp. in the warmer waters of the Indo-Pacific.

Table 1. Morphological characters of *Hydroclathrus* species examined in this study*.

Character	***Hydroclathrus clathratus*** (C. Agardh) Howe	***Hydroclathrus rapanuii*** Santiañez, Macaya *et* Kogame	***Hydroclathrus stephanosorus*** Kraft	***Hydroclathrus* sp.**	***Hydroclathrus tumulis*** Kraft *et* Abbott	***Hydroclathrus tenuis*** Tseng *et* Lu	***Hydroclathrus minutus*** Santiañez *et* Kogame
Thallus form	strap-shaped to net-like, convoluted; irregularly perforated	saccate to convoluted; membranous, irregularly perforated	saccate to sheet-like; membranous, perforated by subcircular holes	net-like; somewhat membranous; irregularly perforated	saccate to torn, sheet-like; membranous, irregularly perforated	net-like, convoluted; perforated membranes thin to fibrous	net-like, convoluted, sometimes inter-adhesive; membrane strands extremely thin and delicate
Membrane thickness (µm)	100–690	60–350	60–520	120–400	120–280	40–590	Less than 130
Cortical cells							
No. of cell layer	1–2 (3)	1 (–2)	1–2	1 (–2)	1	1–2	1
Shape (surface)	oblong to rectilinear to pentagonal/hexagonal	rectilinear to polygonal	oblong to rectilinear to pentagonal	rectilinear to polygonal	square to rectilinear to pentagonal	square to rectilinear	square to rectilinear to polygonal
Size (surface; width × length, µm)	(3–) 4–9 (–11) × 5–11 (–14)	(4–) 5–9 × (5–) 6–12 (–14)	4–7 (–8) × (5–) 6–12	4–6 × 6–10	7–13 × 7–15	4–8 × 6–9 (–11)	3–6 × (4–) 5–8 (–9)
Shape (cross section)	ovate to broadly ovate to papillate	oblong to broadly oblong, apices domed to obtuse	broadly rounded to ovoid, apices sometimes domed to obtuse	ovate, subacutely papillate	subacutely papillate	ovate to broadly ovate, domed to papillate	broadly obovate to ovate, apices protuberant
Size (cross section; width × length, µm)	(4.5–) 5–11 (–12) × (5–) 6–14 (–16)	4.5–12.5 × 7–14 µm	(4–) 5–11 (–13) × (5–) 6–10 (–15)	7–11 × 9–14	8–10 × 10–13	5–9 (–10) × 7–11 (–12)	4–7 (–9) × 7–9 (–11.5)
Medullary cells	5–9 layers; thin-walled	3–6 (–7) layers; thin-walled	(2–) 3–6 layers; thin-walled	4–7 layers; thin-walled	2–4 layers; thin-walled	(3–) 5–6 (–8) layers; thin-walled	1–5 layers; thick-walled
Size (width, µm)	up to 260	up to 190	up to 220	up to 210	250	up to 250	up to 200
Hair primordia							
Nature and arrangement	occur in groups of not more than 25 in depressions, pits, and channels; often extended into long hyaline hairs	occur in groups of up to 20 in depressions, pits, and channels; each usually extended into	occur in groups of not less than five in shallow depressions, sometimes in pits; sometimes	occur in groups; elongated into hyaline hairs	occur singly or in groups of three to six	occur in groups of not less than five; often with long hyaline hair extensions	occur in groups of not more than eight, often with long hyaline hair extensions

Character	***Hydroclathrus clathratus*** (C. Agardh) Howe	***Hydroclathrus rapanuii*** Santiañez, Macaya *et* Kogame	***Hydroclathrus stephanosorus*** Kraft	***Hydroclathrus* sp.**	***Hydroclathrus tumulis*** Kraft *et* Abbott	***Hydroclathrus tenuis*** Tseng *et* Lu	***Hydroclathrus minutus*** Santiañez *et* Kogame
		long hyaline hairs	extended into hyaline hairs				
Plurangia							
Sori shape and nature	sori often associated with hair tufts; diffused with angular margins, sometimes confluent	sori associated with hair tufts, occur in angular and irregular blocks, often merged with adjacent sori	sori surrounds hair tufts, discrete, nearly circular in outline; maintains circular margins when merged with adjacent sori	—	sori angular to irregularly block-like, rarely confluent	sori confluent, irregularly shaped with margins angular, may occur extensively across the surface; may grow without reference to hairs	sori often surround hair tufts; generally discrete with subcircular margins, sometimes merged with adjacent sori
Arrangement	erect; in lateral view biseriate, each column divided into four locules	erect; in lateral view biseriate, each column divided into four locules	erect; in lateral view biseriate, each column divided into four locules	erect; borne on papillate cells	erect, laxly arranged and often borne on papillate cells; in lateral view biseriate, each column divided into four locules	erect; in lateral view biseriate, each column divided into four locules	erect; in lateral view biseriate, each column divided into four locules
Length (cross section, µm)	10–30	15–23	(12–) 18–28	9–11 (young)	17–20	22–25	up to 18

*Included information from Taylor (1960), Srinivasan (1969), Womersley (1987), Kogame (1994), Trono (1997), Littler and Littler (2000), Trono (2001b), Kraft and Abbott (2003), Abbott and Huisman (2004), Kraft (2009).

CHAPTER 3

Taxonomic reassessment in the family Scytosiphonaceae (Ectocarpales), including the description of three new genera *Tronoella*, *Pseudochnoospora*, and *Dactylosiphon*[2]

INTRODUCTION

Members of the brown algal family Scytosiphonaceae are distributed worldwide, occurring in the upper intertidal down to the subtidal (~60 m) of tropical to warm temperate waters (Kogame *et al.* 1999, Cho *et al.* 2001, 2006, Kraft & Abbott 2003, N'Yeurt & Payri 2006). Scytosiphonacean taxa are classified in the Order Ectocarpales and are characterized by thalli possessing the following: 1) apical or later intercalary growth; 2) single plate-like plastid containing one large pyrenoid; 3) exhibiting isomorphic to heteromorphic life histories; and, 4) producing hormosirene as main

[2]Portions of this chapter appeared in:

Santiañez, W.J.E., Macaya, E.C., Lee, K.M., Cho, G.Y., Boo, S.M., & Kogame, K. (2018). Taxonomic reassessment of the Indo-Pacific Scytosiphonaceae (Phaeophyceae): *Hydroclathrus rapanuii* sp. nov. and *Chnoospora minima* from Easter Island, with proposal of *Dactylosiphon* gen. nov. and *Pseudochnoospora* gen. nov. *Botanica Marina* 61: 47–64.

Santiañez, W.J.E., Lee, K.M., Uwai, S., Kurihara, A., Geraldino, P.J.L., Ganzon-Fortes, E.T., Boo, S.M. & Kogame, K. (2018). Untangling nets: Elucidating the diversity and phylogeny of the clathrate brown algal genus *Hydroclathrus*, with the description of a new genus *Tronoella* (Scytosiphonaceae, Phaeophyceae). *Phycologia* 57: 61–78.

Santiañez, W.J.E. & Kogame, K. (2017). Transfer of *Petalonia filiformis* (Batters) Kuntze to the genus *Planosiphon* McDevit & G.W.Saunders (Scytosiphonaceae, Phaeophyceae). *Notulae algarum* No. 40: 1–3.

sexual pheromone (Kogame *et al.* 1999, Cho *et al.* 2003), among others. Currently, 11 genera are included in the family: *Chnoospora*, *Colpomenia*, *Hydroclathrus*, *Iyengaria*, *Jolyna*, *Melanosiphon*, *Myelophycus*, *Petalonia*, *Planosiphon*, *Rosenvingea*, *Scytosiphon* (Wynne 1969, Kogame *et al.* 1999, Cho *et al.* 2003, Lee *et al.* 2014b, McDevit & Saunders 2017).

Scytosiphonacean taxa have complex and confused phylogenetic relationships, and previous studies indicated unresolved relationships in *Chnoospora*, *Colpomenia*, *Scytosiphon*, *Petalonia, Rosenvingea,* and *Hydroclathrus* (Kogame *et al.* 1999, Cho *et al.* 20032006, McDevit & Saunders 2017). Kogame *et al.* (1999) suggested that the morphologies of prostrate sporophytic thalli (i.e., thallus structure and presence/absence of plurilocular zoidangia) were important taxonomic criteria. However, at that time only a few species have been subjected to life history and phylogenetic studies such that formal taxonomic revisions had been stalled. Recently, several scytosiphonacean genera have attention and certain aspects of their biology including their life histories, taxonomies, and phylogenies, have been elucidated. These include those of *Chnoospora* (Kogame 2001), *Colpomenia* (Kogame & Masuda 2001, Boo *et al.* 2011a, Lee *et al.* 2012,. 2014a), *Hydroclathrus* (Kraft & Abbott 2003), *Iyengaria* (West *et al.* 2015), *Melanosiphon* (Lee *et al.* 2014b), *Myelophycus* (Cho *et al.* 2003), *Rosenvingea* (West *et al.* 2010, Lee *et al.* 2014b), and *Petalonia* (Kogame *et al.* 2011, Matsumoto *et al.* 2014). Albeit patchy, these studies not only provided collectible information that would aid in untangling the complexities of the Scytosiphonaceae but also ushered the way to delineating boundaries both at the species and genus levels.

Herein, I reassessed the taxonomy of the family Scytosiphonaceae by integrating information on their morpho-anatomies, life histories, and molecular phylogenies. Together with the discovery and description of a monotypic genus *Tronoella* Santiañez *et* Kogame *gen. nov*., I also propose herein the recognition of two new genera:

Pseudochnoospora Santiañez, G.Y. Cho *et* Kogame *gen. nov*. and *Dactylosiphon* Santiañez, K.M. Lee, S.M. Boo *et* Kogame *gen. nov*. The former is a monotypic genus represented by '*Chnoospora implexa*' while the latter accommodates the erect, hollow and elongate (finger-like) scytosiphonacean species previously assigned to *Colpomenia*.

MATERIALS AND METHODS

Scytosiphonacean taxa used in this study were listed in Table S1. DNA extraction, PCR amplification, and sequencing of targeted mitochondrial *cox*3 and plastidial *psa*A and *rbc*L genes were done according to those outlined in Chapter 2.

Phylogenetic analyses of newly generated individual genes together with sequences of other scytosiphonacean taxa downloaded from GenBank were conducted similarly to those described in Chapter 2. For this study, I used a concatenated dataset (*cox*3: 610 bp, *psa*A: 740 bp, and *rbc*L: 1,383 bp = 2,733 bp) to settle the phylogenetic problem of the family Scytosiphonaceae. ML and BI analyses of a concatenated sequence alignment of newly generated and GenBank-downloaded sequences (Table S1) were performed under GTR + I + Γ model with partitioning (by gene and by codon position) similar to those of single gene-based phylogenetic analyses.

Observations on gross morphologies and anatomies of cross-sections of specimens, photomicrographs, and measurements were also done similar to those in Chapter 2. Morpho-anatomical studies on the genus *Chnoospora* were based on the supposed generitype *Chnoospora minima* (K. Hering) Papenfuss collected from within the Pacific (Easter Is., Cook Is., Hawaiian Is., and Japan). Photographs of type specimens of both *C. minima* and its congener *C. implexa* J. Agardh—courtesies of the Herbarium Hamburgense of the University of Hamburg, Germany (HBG) and the Trustees of the

Natural History Museum, London (BM), respectively—were also examined. Concerning *Colpomenia*, photographs of type specimens were examined as published in Vandermeulen *et al.* (1984) for *C. sinuosa* (Mertens *ex* Roth) Derbès *et* Solier (neotype), *C. peregrina* Sauvageau, and *C. bullosa* (D.A. Saunders) Yamada; Ramírez and Rojas (1992) for *C. durvillei* (Bory de Saint-Vincent) M.E. Ramírez (lectotype); and Lee *et al.* (2014) for *C. wynnei* K.M. Lee, R. Riosmena-Rodriguez, Kogame *et* S.M. Boo. A scanned image of a *C. sinuosa* sample collected by Mertens in Cadiz, Spain (type locality), as solicited from the Swedish Museum of Natural History in Stockholm, Sweden (S), was also observed.

RESULTS

Phylogenetic results

Fourteen (14) sequences of scytosiphonacean algae collected from several localities in the Pacific were newly added and analyzed in this study (*cox*3: 10, *psa*A: 2, *rbc*L: 2; Table S1).

Phylogenetic reconstruction based on single- and three-gene (*cox*3+*psa*A+*rbc*L) based phylogenies suggested two major clusters within the family Scytosiphonaceae (Figures 13–15). One cluster (hereon the '*Scytosiphon* group') consisted of mainly subtropical to temperate species. Meanwhile, the other cluster (the '*Hydroclathrus* group') was comprised of tropical to warm temperate species. In all my analyses, *Colpomenia*, *Chnoospora*, *Hydroclathrus*, *Rosenvingea*, and *Scytosiphon* were not recovered as monophyletic groups. In *Chnoospora*, *C. minima* was consistently segregated from *C. implexa*. The former was found in the basal portion of the

'*Hydroclathrus* group' while the latter appeared to be a recently diverged taxon that was closely associated with *Hydroclathrus* and *Rosenvingea*. Relationships among *Colpomenia* species were not resolved as these were recovered in at least three distinct lineages. All *Colpomenia* lineages consisted mostly of saccate species, were recovered within the '*Hydroclathrus* group' while the elongate and siphonous *Colpomenia* species (i.e., *C. bullosa*, *C. durvillei*, and *C. wynnei*) clustered within the '*Scytosiphon* group'. In *Hydroclathrus*, all species were recovered in a highly supported clade except the recently described *H. minutus* whose phylogenetic position remains ambiguous. With respect to *Rosenvingea*, the relationship between *R. intricata* (J. Agardh) Børgesen and *R. orientalis* (J. Agardh) Børgesen remains to be elucidated.

In the *cox*3-based phylogenetic tree (Figure 15), several of the new sequences clustered with the known taxa such as *Rosenvingea orientalis*, *Chnoospora minima*, and *Colpomenia bullosa*. One unidentified lineage within the '*Hydroclathrus* group' was recovered as a distinct taxon and described below as *Tronoella ryukyuana gen. et sp. nov. Rosenvingea* was shown to have two segregated entities identified as '*Rosenvingea intricata*'. One of the '*R. intricata*' clade was composed of samples from subtropical Japan and Hawaii and was clustered albeit with low support with *Rosenvingea orientalis* specimens from Vietnam and the Philippines. The other '*R. intricata*' clade was more widely distributed and collected from subtropical Pacific Mexico and tropical New Caledonia. The latter clade was found to be closely related to an unidentified *Rosenvingea* species from Vietnam and *Hydroclathrus* species. The genus *Colpomenia* exhibited the most ambiguous relationships, with at least five clades. Four of these clades were found within the '*Hydroclathrus* group' while one was within '*Scytosiphon* group'. The relationship between these clades were unresolved but one that contained the generitype *Colpomenia sinuosa* clustered with those of the clade which contained the similarly globular/saccate *Colpomenia peregrina* Sauvageau, *Colpomenia expansa*

(D.A. Saunders) Y.P. Lee, and *C. claytoniae* S.M. Boo, K.M. Lee, G.Y. Cho *et* W. Nelson, while a clade composed of species with tuberculate or branched thalli consisted of *Colpomenia tuberculata* D.A. Saunders and *Colpomenia ramosa* W.R. Taylor, clustered with *Chnoospora minima*. *Colpomenia ecuticulata* Parsons was recovered as a segregated and an early-diverged taxon within the '*Hydroclathrus* group' while all elongate *Colpomenia* were nested as a highly supported clade in the '*Scytosiphon* group'. With respect to *Scytosiphon*, two lineages were apparent; one contained the generitype *Scytosiphon lomentaria* and *Scytosiphon dotyi* M.J. Wynne while the other was composed only of *Scytosiphon canaliculatus* (Setchell *et* Gardner) Kogame.

The *rbc*L gene-based phylogenetic reconstruction similarly showed unresolved relationships within the Scytosiphonaceae (Figure 16). In *Hydroclathrus*, *H. minutus* clustered with *Rosenvingea* species. *Chnoospora implexa* seemed more closely related to *Hydroclathrus* spp. and *Rosenvingea* spp. than to its congener *C. minima*. The latter was found to be in between two *Colpomenia* lineages. The elongate *Colpomenia* species were found to be more closely related to the other elongate scytosiphonacean taxa, *Petalonia* spp. and *Planosiphon* spp. The unresolved relationship in *Scytosiphon* was similarly observed; additionally, sequence data of *Scytosiphon tenellus* Kogame was effectively segregated and clustered within *Petalonia*. Moreover, *T. ryukyuana* was recovered as a moderately supported taxon (bootstrap percentage (BP) = 89%, posterior possibility (PP) = 1.0) that was basal to *C. implexa*, *Rosenvingea* spp., and *Hydroclathrus* spp. (Figure 16).

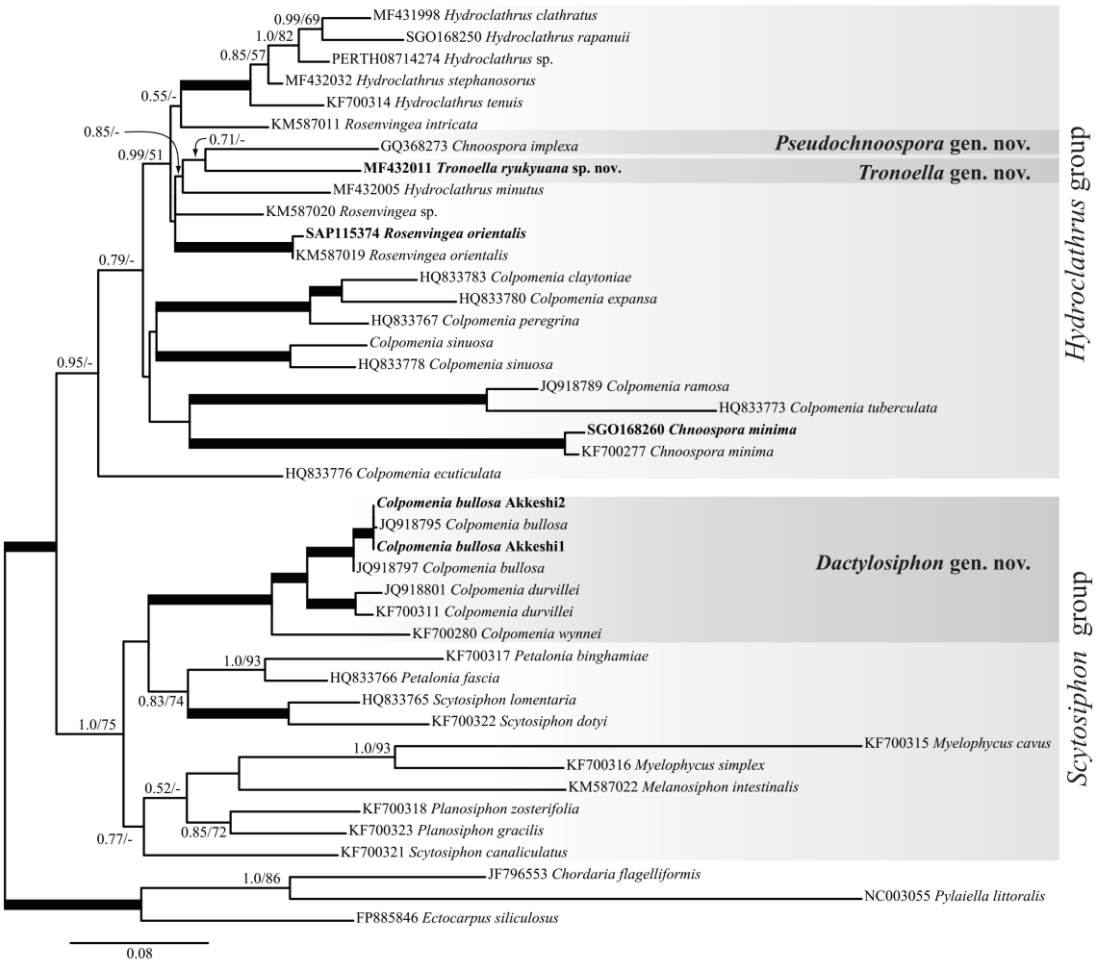

Figure 15. Phylogeny of the family Scytosiphonaceae inferred from *cox*3 sequence data. Support values appended at each node are Bayesian posterior probabilities (PP) and maximum likelihood bootstrap percentages (BP), respectively. Thickened lines indicate highly supported nodes (PP: ≥ 0.98 and BP: ≥ 95%). Values <50% BP and <0.50 PP are removed. Bold names = newly generated sequences.

In the concatenated tree (Figure 17), the two major clusters were also apparent. The relationship among species of *Rosenvingea* and *Hydroclathrus*, as well as relative to *Tronoella* remained unresolved, having nodes with low support. Similar to previous trees, *Chnoospora* species in our concatenated tree was not recovered as monophyletic and each was effectively segregated by a highly supported node wherein *Colpomenia sinuosa* was basal. Elongate *Colpomenia*, as represented by *C. bullosa*, formed a highly supported clade that was basal within the '*Scytosiphon* group'.

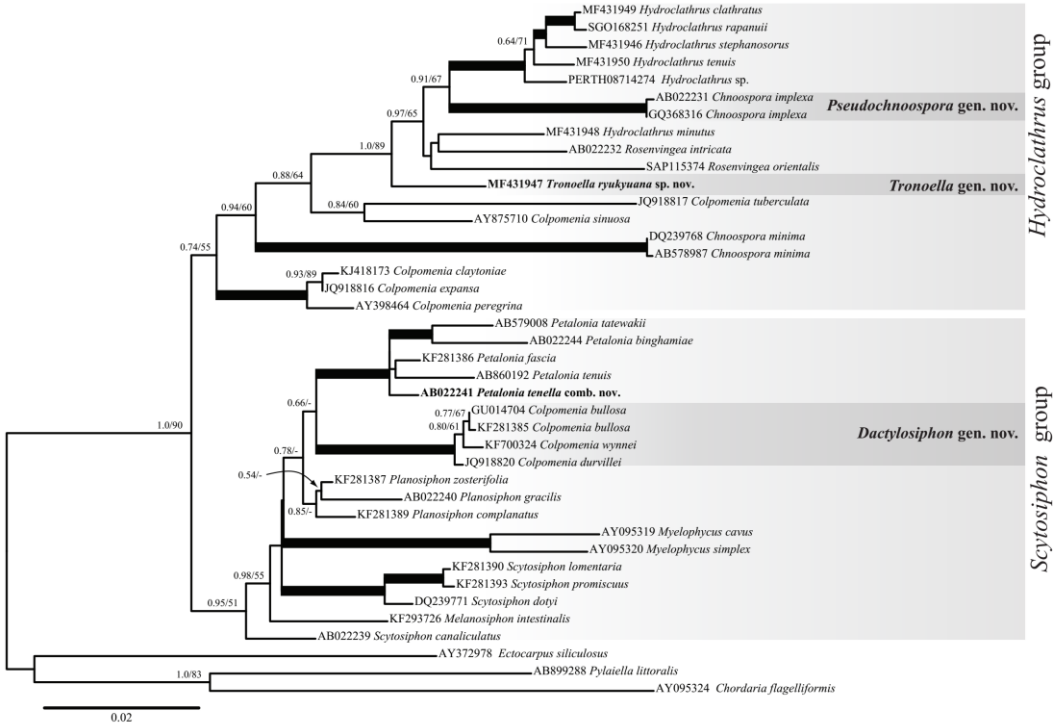

Figure 16. Phylogeny of the family Scytosiphonaceae inferred from *rbc*L sequence data. Support values appended at each node are Bayesian posterior probabilities (PP) and maximum likelihood bootstrap percentages (BP), respectively. Thickened lines indicate highly supported nodes (PP: ≥ 0.98 and BP: ≥ 95%). Values <50% BP and <0.50 PP are removed. Bold names = newly proposed taxa.

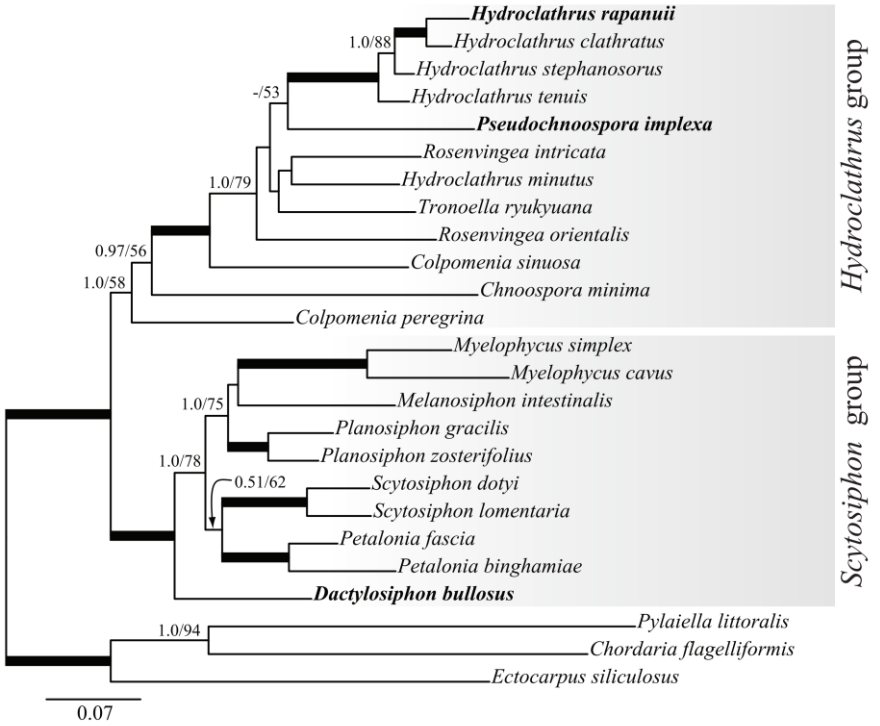

Figure 17. Phylogeny of the family Scytosiphonaceae inferred from concatenated sequence (*cox*3 + *psa*A + *rbc*L: 2,733 bp) data. Support values appended at each node are Bayesian posterior probabilities (PP) and maximum likelihood bootstrap percentages (BP), respectively. Thickened lines indicate highly supported nodes (PP: ≥ 0.98 and BP: ≥ 95%). Values <50% BP and <0.50 PP are removed. Bold names = newly proposed taxa.

Taxonomic observations

Tronoella* Santiañez *et* Kogame *gen. nov.

Figure 18

Description: Thalli membranous to branched, highly convoluted, sometimes adhering to each other, spreading. Membrane surface perforated with variedly sized holes. Revolute branches initially arise at random on the surface as siphonous protrusions. In cross-section, pigmented cortical cells round to broadly elliptic. Medullary cells clear, becoming larger towards the center. Hair primordia short and scattered. Plurangial sori conspicuous and discrete. Plurangia differentiated from cortical cells, firmly coherent, massive, and highly protruded.

Type species: *Tronoella ryukyuana* Santiañez *et* Kogame *sp. nov.*

Etymology: The genus is named after Dr. Gavino C. Trono, Jr., Emeritus Professor of Marine Science of the University of the Philippines–Marine Science Institute and National Scientist of the National Academy of Science and Technology–Philippines, to honor his contributions to the understanding of the diversity and ecology of central and western tropical Pacific seaweeds. Dr. Trono has trained and inspired phycologists within the Asia-Pacific region and was instrumental in the development and the success of *Kappaphycus* and *Eucheuma* farming, which sustainably support the supply of biomass for the carrageenan industry.

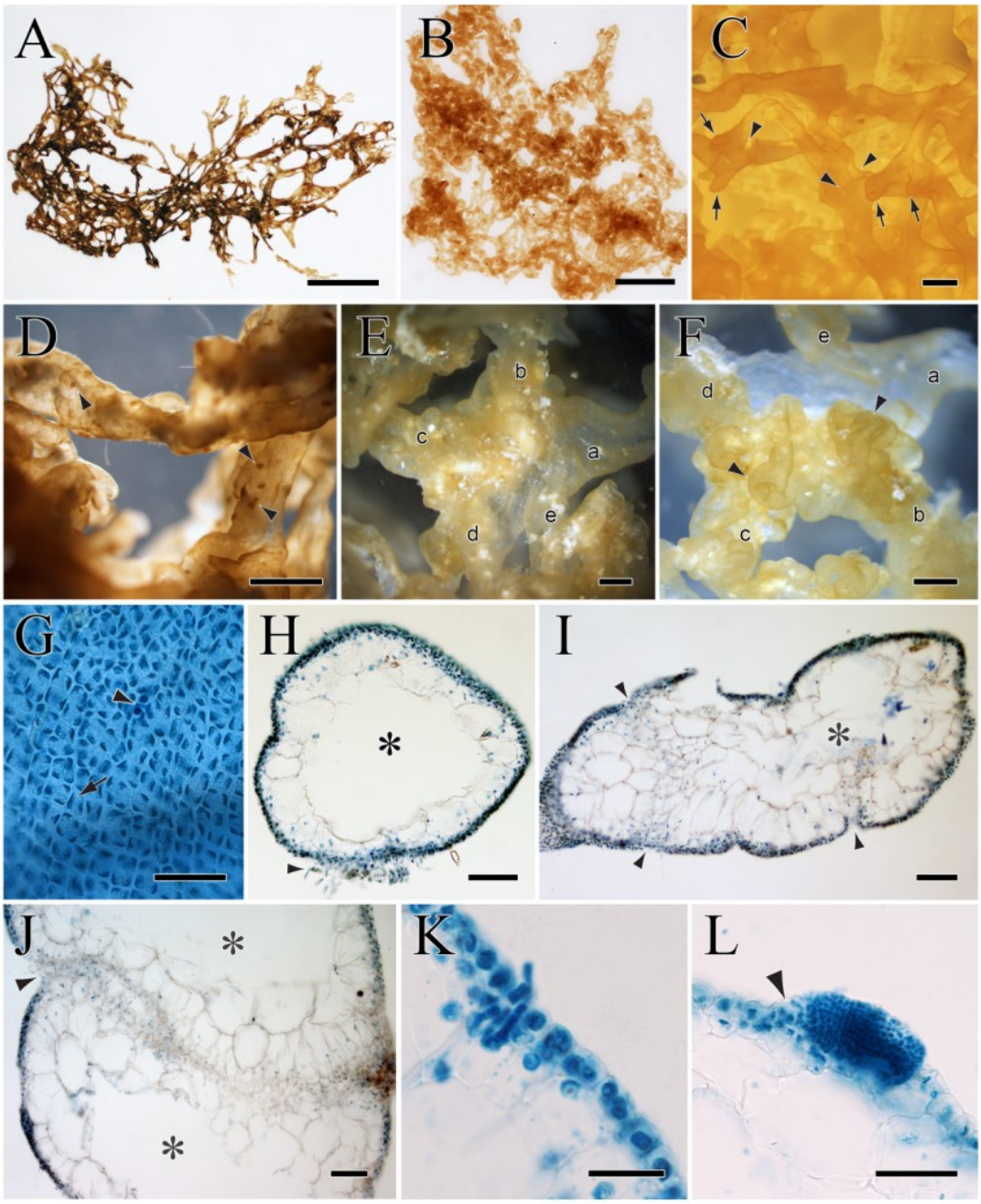

Figure 18. *Tronoella ryukyuana* Santiañez *et* Kogame *gen. et sp. nov.* habit and morphological characters. **A**, The highly convoluted, fine and fibrous thallus of the holotype specimen (SAP115297). Scale bar = 1 cm. **B**, Wet habit of SAP115299 showing the network-like and spreading morphology of the species, resembling those of *Hydroclathrus*. Scale bar = 1 cm. **C**, Convoluted portion of the thallus (SAP115299) showing numerous variedly sized holes (arrowheads) and hollow branch protrusions (arrows) arising at various points and oriented at different directions. Scale bar = 1 mm. **D**, Slightly twisted branch portions showing the discrete clumps of plurangia (arrowheads) scattered on the undulate to rugose surface (SAP115297). Scale bar = 1 mm. **E,** Dorsal part of the thallus (SAP115299) showing membranous portion with five branches (a–e). Scale bar = 2 mm. **F,** Ventral part of the thallus (SAP115299) showing hollow and sinuous protrusions (arrows) arising from the membranous portion in **E**; letters in branches correspond to those in **E**. Scale bar = 2 mm. **G,** Surface of thallus showing short hairs (arrowhead) and cortical cells each of which possessing a single conspicuous pyrenoid (arrow). Scale bar = 50 µm. **H,** Section through a subterete hollow branch made up of a layer of small, pigmented cortical cells, clear and large medullary cells bordering a hollow portion (asterisk) and derived from cortical cells (arrowhead). Scale bar = 100 µm. **I**, Section through the basal portion of the branch protrusion showing the initiation of cleavage (arrowheads) into the hollow portion (asterisk); this branch portion subsequently becoming revolute. (Continued…)⟾

Tronoella ryukyuana* Santiañez *et* Kogame *sp. nov.

Figure 18

Diagnosis: With the morphological characters of the genus *Tronoella*.

Holotype: SAP115297 (Figure 18, A), 28 March 2009, Odo, Itoman, Okinawa Is., Japan, *K. Kogame*; deposited in SAP.

Isotypes: SAP115298, SAP115299, 28 March 2009, Odo, Itoman, Okinawa Is., Japan; deposited in SAP.

Type locality: Odo, Itoman, Okinawa I., Japan (127° 42′ 36″ N, 26° 5′20″ E).

Etymology: Named after the Kingdom of Ryukyu which ruled the Okinawa Is. from the 15th to the 19th century. Incidentally, Okinawa Is. is also part of the Ryukyu Arc, a chain of volcanic islands in the southernmost region of Japan.

Distribution: Known only from its type locality.

Representative sequences: GenBank accession numbers: *cox*1 = MF432084, *cox*3 = MF432011, *psa*A = MF431952, *rbc*L = MF431947 (sequenced from SAP115297).

⇚ (Continued…) At the left side portion, the branch is also simultaneously being cleaved, with some medullary cells being divided into smaller cells, later differentiated into rhizoidal cells. Scale bar = 100 μm. **J**, Section through a branch showing revolute branches (hollow portions marked with asterisks), connected by numerous rhizoidal cells (arrowhead) at the left side, while the right side still on the process of cellular differentiation and cleavage. Scale bar = 50 μm. **K**, Short hair primordia developing on medullary cells, emerging and crowded between pigmented cortical cells Scale bar =25 μm. **L**, Section through a fertile portion of the branch showing the firmly cohesive and massive plurangia which are differentiated from cortical cells (arrowhead). Scale bar = 25 μm.

Observations: Thalli were light to dark brown, occurring as amorphous, highly convoluted clumps (Figure 18, A–C) attached to the substrata through rhizoidal holdfasts that are derived from cortical cells. The highly convoluted habit of the species a result of inter-adhesive branches attached to each other through rhizoids.

Membranes were perforated and divided into slightly twisted and hollow strands that were 500–1500 (–2100) μm wide (Figure 18, A–D). In some cases, the basal parts of the highly convoluted portions of the thallus were distinctly membranous; from these membranes, sinuous and often perforated sacs were abundantly found (Figure 18, C, E, F). Membrane surfaces were uneven, undulate to rugose, some with siphonous, sinuous protrusions (Figure 18, C–F). These sacs/protrusions were later on extended as branches growing on various planes (Figure 18, C). Siphonous branches (Figure 18, H) were later on perforated and were cleaved inwardly along its length (Figure 18, I, J), becoming revolute similar to *Hydroclathrus*. In some cases, a siphonous branch may differentiate into two separate revolute branches, which were initially attached to each other through rhizoids (Figure 18, I, J). Apparently, this complex branch development pattern has significantly contributed to the highly convoluted and amorphous habit of the species

Membranes were thin, up to 250 μm thick, and composed of 1–2 layers of small, domed pigmented cortical cells and 1–3 (rarely 4) layers of clear and relatively larger medullary cells (Figure 18, H–J). Cortical cells were square to rectilinear to polygonal in surface view, (7-) 10–12 (-15) × (13-) 14–18 (-21) μm, each with a single pyrenoid, 2–3 μm in diameter (Figure 18, G). In cross-section, cells were thin-walled, round to broadly elliptic, (8-) 9–16 (-18.5) μm wide and (5-) 9–14 (-15) μm in height, with highly domed apices (Figure 18, K, L). Medullary cells were thin-walled, ovate to broadly oblong, periclinal and progressively becoming larger towards the dorsal portion (Figure 18, H–J), up to 180 μm wide.

Hair primordia arranged in groups of 2–3, short, subepidermal, and rarely extended into hyaline distal cells. Primordia emerged from tight indentations in the membrane surface, crowded between cortical cells (Figure 18, G, K).

Plurangial sori were scattered, in discrete and conspicuous clumps, often associated with hair primordia. In cross-section, clumps were composed of firmly coherent and massive plurangia that were differentiated from sufacecortical cells, (Figure 18, L), up to 50 μm high.

Chnoospora minima (K. Hering) Papenfuss

Figure 19

Basionym: *Fucus minimus* K. Hering 1841: 92.

Synonyms: *Chnoospora pacifica* J. Agardh *fide* Papenfuss 1956: 69.

Chnoospora fastigiata J. Agardh 1848: 171, *nom. illeg.*, *Chnoospora fastigiata* var. *pacifica* (J. Agardh) J. Agardh *fide* Silva *et al.* 1996: 626.

Lectotype: HBG024509 (Figure 20, A), Port Natal (Durban), South Africa, *F. Krauss*; deposited in HBG: the lectotype was designated by Papenfuss (1956).

Distribution: TROPICAL TO SUBTROPICAL INDO-PACIFIC.

Specimens examined: EASTER ISLAND: SGO168260–SGO168262, Tahai, Easter Is., 15 March 2016, *E.C. Macaya*; EIS2-0193, Vaihu, Easter Is., 20 March 2016, *E.C. Macaya*. COOK ISLANDS: SAP115375, Ngatangiia, Rarotonga, 13 February 1993, *A.D.R. N'Yeurt*. HAWAIIAN ISLANDS: SAP115377, Wittington Beach Park, Naalehu, 24 January 2008, *A.*

Kurihara; SAP115378, Moloa'a Beach Park, Kauai, 15 February 2008, *A. Kurihara*. JAPAN: SAP115376, Uganzaki, Ishigaki, Okinawa, 10 March 2000, *S. Kawaguchi & A. Kato*.

Observations: Thalli were erect and yellow brown in the field (Figure 19, A), basally dark brown, apically lighter when dried on herbarium sheets (Figure 19, B, C). Thalli were erect, growing in dense clumps in the low to mid-intertidal (Figure 19, A), attached to substrata by a consolidated rhizoidal holdfast (Figure 19, B, C). Longer thalli lay prostrate on rocky surfaces when exposed during low tide.

Thalli were up to 16 cm long and (sub-)dichotomously branched up to seven orders. The basal portions of the thalli were subterete, progressively thinner and compressed towards the blunt tip (Figure 19, B, C).

Cortex was 11–26 μm thick, composed of 2–4 layers of thick-walled, pigmented cells, surrounding a medulla of intermixed small and large, thick-walled, clear cells (Figure 19, E, F). Surface cells narrowly to broadly oblong, slightly domed, those in subsurface layers were rounded to cuboidal (Figure 19, E, F). Medullary cells were roundish near the cortical cells, becoming larger and narrowly elliptical towards the center, up to 150 μm long and 100 μm wide (Figure 19, D–F). Variedly sized cavities occur in the medulla (Figure 19, D–F), these intercellular spaces were found in sections near the holdfast up to the terminal branches. However, these hollow portions localized and discontinuous.

Hair primordia were formed epidermally and initially covered in a cuticle (Figure 19, F) or in deep pits subtended by several layers of small, clear subcortical cells, often forming a distinct arc (Figure 19, E). The primordia were in dense, longitudinally aligned tufts along the midlines of the compressed axes especially of the apical branches; these are extended into long hyaline hairs (Figure 19, D, E), creating a whitish halo effect.

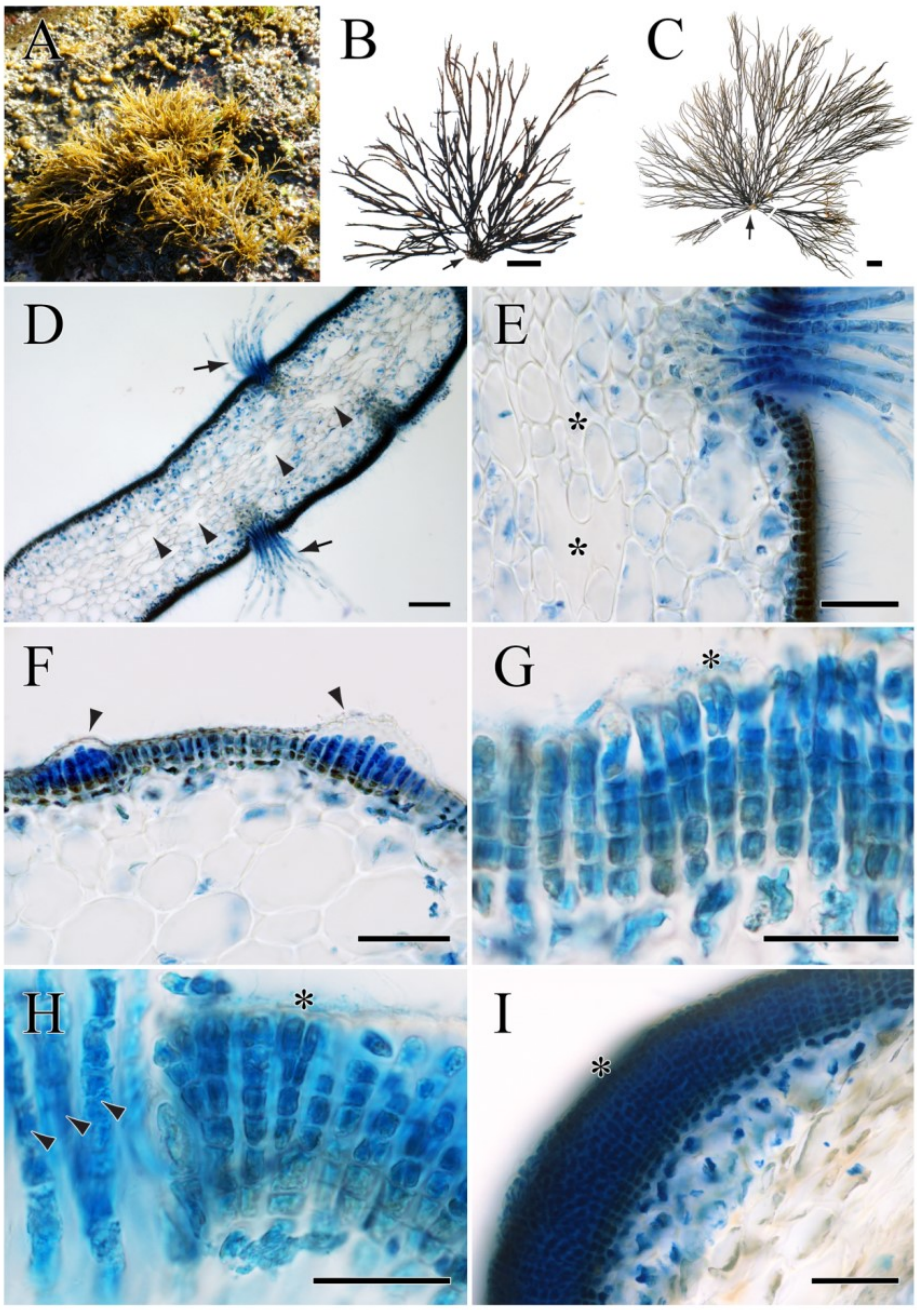

Figure 19. *Chnoospora minima* (K. Hering) Papenfuss habit and morphological characters. **A**, Erect *Chnoospora minima* thalli growing on rocks in Ahu Tahai, Easter Island, exposed during low tide. **B**, Pressed herbarium specimen of *C. minima* from Cook Is. (SAP115375) showing a consolidated holdfast (arrow) and (sub)dichotomous branching; branch portions at the base of dichotomy noticeably wider. Scale bar = 1 cm. **C**, Pressed herbarium specimen of a relatively longer *C. minima* thalli collected from Easter Is. (SGO168262) showing the typical holdfast (arrow) and several orders of branching. Scale bar = 1 cm. **D**, Section through a compressed branch (SGO168260) showing medulla with several irregularly shaped hollow (arrowheads). Scale bar = 100 µm. **E**, Closer view of the intercellular space (asterisk) bounded by thick-walled medullary cells (SGO168260). Shown here also are hairs arising from deep into the medulla; cells directly below the hair primordia distinctly smaller and were arranged into a cup-like manner. Scale bar = 50 µm. **F**, Groups of hair primordia developing on the cortical layer shown covered with loosened cuticles (arrowheads) (SGO168261). Scale bar = 50 µm. **G**, Cortical layer of a specimen from Cook Is. (SAP115375) showing uniseriate and biseriate (asterisk) plurangial initials derived from the outermost cortical cell. Scale bar = 25 µm. **H**, Young biseriate plurangia (asterisk) growing adjacent to hairs (arrowheads) (SAP115375). Scale bar = 25 µm. **I**, Section through a mature plurangia showing biseriate plurangia (asterisk) compounds of several layers of cells (SAP115377). Scale bar = 50 µm.

Plurangial sori often associated with hairs and were covered with cuticles that are loosened as plurangia matures. Plurangial initials were differentiated from cortical cells, these becoming biseriate and elongated (Figures 15 and 16), up to 50 μm long (Figure 19, G, H). Biseriate plurangia were subdivided into smaller, squarish to rectangular locules 4–5 μm by 2–5 μm in size (Figure 19, I).

DISCUSSION

The molecular phylogenetic trees of the family Scytosiphonaceae in this study suggested two major phyletic groups (i.e., the '*Scytosiphon* group' and '*Hydroclathrus* group'), which were similarly found but not specified in previous reports (e.g., Cho *et al.* 2006, Lee *et al.* 2014a, McDevit & Saunders 2017). Taxa within the '*Scytosiphon* group' are typically distributed in subtropical to temperate waters (Kogame *et al.* 1999). They have upright, elongate and terete to flattened, siphonous to partially hollow or solid thalli and their prostrate sporophytic thalli only produce unilocular zoidangia (Kogame *et al.* 1999, Kogame & Masuda 2001, Cho *et al.* 2006). Meanwhile, taxa within the '*Hydroclathrus* group' are typical flora of tropical to warm to temperate waters. They possess varied morphologies ranging from erect to spreading, or saccate and solitary or gregarious to network-like to branching, either hollow or partially hollow, or solid. Unlike those reported from the '*Scytosiphon* group', prostrate sporophytes of representatives from the '*Hydroclathrus* group' produce both unilocular and plurilocular zoidangia (Kogame *et al.* 1999, 2001, Cho *et al.* 2006). Unresolved relationships among the different species in the Scytosiphonaceae may be implicated to some factors including incomplete taxon sampling, the low resolving power of genetic markers, and the wide and ambiguous morphological circumscription of each genera

resulting to several incorrect generic assignments, among others. One of the possible actions to settle the problematic phylogenetic relationships in the family, as suggested by McDevit and Saunders (2017), is to lump several genera into a large genus. However, they cautioned that this would mean that the genus would have species with widely varied characters such as what I have previously outlined. Nonetheless, lumping can be merited if we are to consider the aforementioned reproductive structures produced by their prostrate sporophytes as well as the morphologies of the early development stage of the erect thalli. For the latter, taxa in the '*Hydroclathrus* group' were reported to develop initially from massive thalli (Hurtado-Ponce & Umezaki 1987, Kogame 1994, 1997, 2001, Kogame & Yamagishi 1997, Toste *et al.* 2003a, b) while those of the '*Scytosiphon* group' were derived earlier from filamentous thalli (Nakamura & Tatewaki 1975, Kogame 1994, 1998), except for *Colpomenia bullosa* in which erect thalli develop initially from massive thalli (Nakamura & Tatewaki 1975). Should this proposal be made, all taxa in the '*Hydroclathrus* group' and '*Scytosiphon* group' can be circumscribed under the genus *Hydroclathrus* and *Scytosiphon*, respectively, based on nomenclatural priority. This move would be problematic as the '*Scytosiphon* group' includes *Myelophycus* spp. and *Melanosiphon intestinalis* which have isomorphic life histories. As noted by Cho *et al.* (2006), such life histories are reliable taxonomic criteria at the generic level. The most pragmatic move would be to adhere to the principle of monophyly by recognizing new taxonomic groups (McDevit & Saunders 2017) along with defining more clearly the morphological boundaries especially at the generic level. I am underscoring the latter as it appears that the confused relationships in the Scytosiphonaceae were rooted in the rather broad morphological circumscription such that several taxa were lumped into a genus despite possessing several distinct characteristics. In this study, I have made several taxonomic proposals and revisions by

integrating morpho-anatomical and phylogenetic criteria, and, where possible, including life history information.

The 'Hydroclathrus *group'*

The new genus *Tronoella* was established based on its distinct molecular and morphological characters. *Tronoella* possessed a curious mix of morphological characters that are reminiscent of several related taxa: *Hydroclathrus*, for its spreading, hollow, and perforated thalli with membrane edges that are folded to revolute; *Rosenvingea*, for its (initially) siphonous and sometimes inter-adhesive branches; and, *Chnoospora*, for its coherent plurangia. The network-like and spreading thalli of *Tronoella* were most similar to *Hydroclathrus*, the genus to which the specimens were initially assigned. *Tronoella* is differentiated from the latter based on the nature and arrangement of its plurangia as well as its tendency to form sinuous sacs or subterete hollow elongations which are later on cleaved inwards into membranes with revolute margins or to form two hollow branches, each possessing the typical inrolled margins.

Among the problematic genera is *Chnoospora*, which includes scytosiphonacean species with erect to decumbent thalli that are solid in construction. However, there are several morpho-anatomical differences between these taxa that would merit segregating and establishing a new genus for '*C. implexa*'. The *C. minima* specimens I examined were similar to those reported from Hawaii (Fotos 1981, Abbott & Huisman 2004), Philippines (Trono 1997), French Polynesia (N'Yeurt & Payri 2006), Australia (Kraft 2009), and South Africa (Anderson *et al.* 2016); however, I have found here that *C. minima* thalli are partially hollow. *Chnoospora minima* reported from New Zealand (Nelson & Duffy 1991, fig. 3) and South Africa (Anderson *et al.* 2016) also appear to

have intercellular spaces but these were perhaps overlooked. In the smaller and thinner *C. minima* thalli collected from the Hawaiian Is., hollow portions were often inconspicuous and can be easily missed. Cuticles were also observed on the uni- to biseriate plurangia of the *C. minima* specimens I have examined, and these were similar to previous reports by J. Agardh (1848) and Barton (1896) in *Chnoospora fastigiata* (= *C. minima*). This character was, however, not mentioned in recent reports (Fotos 1981, Trono 1997, Abbott & Huisman 2004, Kraft 2009, Anderson *et al.* 2016). With respect to the character of plurangia, Kraft (2009) refuted that those illustrated by Fotos (1981, figs. 4, 11) and Barton (1898, pl. 28, figs 4, 5) were 'anticlinal cortical filaments that are typical of vegetative cells lining the flat faces of the fronds'. However, I confirm herein that those illustrated by Fotos and Barton were plurangia, similar to what I have observed and with those illustrated in the South African specimen (Anderson *et al.* 2016). Meanwhile, like those illustrated in Kraft (2009, fig. 38I), I have observed similar plurangial initials that are differentiated from cortical cells, later further subdivided into long biseriate strands with small locules. Among the main differences between *C. minima* and '*C. implexa*' is their habit: the former possesses an erect and tufted, (sub) dichotomously branched thalli with a consolidated discoid holdfast (Barton 1898, Fotos 1981, Trono 2001a, Abbott & Huisman 2004, Kraft 2009, Anderson *et al.* 2016) while the latter exhibits an erect to decumbent, cushion-like, entangled thalli that are laxly branched, and anchored at various points on the substrate by rhizoids (Abbott & Huisman 2004, Kraft 2009). The cortex of *C. minima* is composed of 2–5 layers of pigmented, thick-walled cells; its medulla solid to partially hollow, is intermixed large and small, clear, thick-walled to lamellate cells [those from New Zealand possess a distinct central zone of smaller cells (Nelson & Duffy 1991)] (Fotos 1981, Abbott & Huisman 2004, Kraft 2009, Anderson *et al.* 2016). Meanwhile, the cortical layer of '*C. implexa*' is composed of only 1–2 small, pigmented cells, which bounds a solid medulla

of large, clear, and thin-walled cells (Børgesen 1941, Fotos 1981, Trono 2001a, Abbott & Huisman 2004, Kraft 2009, Anderson *et al.* 2016). Both also possess differences in habitat: *C. minima* is restricted to the wave-swept, upper intertidal area, while '*C. implexa*' is subtidal (Fotos 1981, Trono 2001a, Abbott & Huisman 2004, Kraft 2009, Anderson *et al.* 2016).

When *Chnoospora* was established by J. Agardh in 1847, he described two species, *Chnoospora pacifica* J. Agardh from Pacific Mexico and *Chnoospora atlantica* J. Agardh from Venezuela (Agardh 1847). A year later, he synonymized both species under *C. fastigiata* J. Agardh and added two new species, *Chnoospora implexa* J. Agardh and *Chnoospora pannosa* J. Agardh (Agardh 1848). However, he did not designate a type species for the genus on both occasions. The conspecificity of *C. fastigiata* with *Fucus minimus* K. Hering was proposed by Papenfuss (1956: 69) and a new combination, *Chnoospora minima* (K. Hering) Papenfuss, was chosen based on taxonomic priority. In the same publication, Herings's specimen (HBG024509; Figure 20, A), collected by F. Krauss from Port Natal [Durban], South Africa was designated by Papenfuss as the lectotype. He also noted the similarity of Hering's specimen with the South African *C. minima* (as *C. fastigiata*) reported by Barton (1986). As I have mentioned earlier, the morphological characters of *C. minima* from various localities were consistent, suggesting that it is a widely distributed species. Taking all these into consideration, to fill in the absence of the type species of the genus *Choospora, C. minima* is designated herein as the generitype. Moreover, when *Sphaerococcus implexus* Hering *nom. nud*. (Figure 20, B) was described by Agardh (1848) as a new species, he assigned it to *Chnoospora* under 'S*pecies inquirenda*'. The original assignment of '*C. implexa*' has since been used and left unquestioned. Similar to *C. minima*, the morphological characters of '*C. implexa*' from several localities have been consistently described. Kraft (2009) provided the most detailed morphological work on the taxon based on Australian and

Hawaiian samples, while Kogame (2001) described the life history of the species in a culture based on Japanese materials. The morphological and genetic of the taxon cast doubt on the placement of the *C. implexa* in the genus *Chnoospora*, suggesting that Agardh's tentative generic assignment of the species has been merited. As such, I propose herein that this entity be removed from the genus *Chnoospora* and a new genus be established—that is, *Pseudochnoospora* Santiañez, G.Y. Cho *et* Kogame—to accommodate its distinct morpho-anatomical and genetic characteristics.

The genus *Colpomenia*, a widely distributed genus currently known to include 13 species (Lee *et al.* 2013, Guiry & Guiry 2017), also has a problematic taxonomic history. As with other scytosiphonacean genera, this problem is largely due to the ambiguous and wide morphological delineation of the genus. *Colpomenia* includes scytosiphonacean species with hollow, thin-walled thalli that are variously shaped (saccate to irregularly ellipsoidal and spreading on the surface, some linear-elongate, some erect and/or branched); with 1–3-celled paraphyses associated with its plurangia, the latter may or may not be covered by cuticles; and, whose prostrate sporophytic thalli produce only unilocular sporangia or both uni- and plurilocular sporangia (Womersley 1987, Kogame 1997, Boo *et al.* 2011a, Lee *et al.* 2012, 2013, Kraft 2009). In addition, morpho-anatomical features used to delineate between *Colpomenia* species has long been a source of confusion and several attempts were made to discriminate one from the other. Efforts included the reexamination and designation of type materials by Vandermeulen *et al.* (1984) for *C. sinuosa* (generitype), *C. peregrina*, and *C. bullosa*. For *C. sinuosa*, studies were made on a neotype [as selected by Vandermeulen *et al.* (1984)] collected from Tenerife, Spain, which were deposited in BM. The neotypification was made as the original collection by Mertens from Cadiz, Spain (type locality) has been presumed lost and the senior author's attempts to search for the type collection in several herbaria that possibly houses the holotype [suggested by Parsons

(1982)] were futile. Nonetheless, analyses on the neotype showed that it possesses the typical punctate plurangial sori covered by cuticle. However, the shape of the thalli was different to those illustrated by Roth (1806, pl. 12). A search on the on-line database of herbaria found in Sweden through 'Sweden's Virtual Herbarium' (http://herbarium.emg.umu.se/standard_search.html) resulted in finding a *C. sinuosa* specimen collected from Cadiz presumably by Mertens. The subspherical and epiphytic specimen A37119 currently deposited at the Sweden Museum of Natural History, Stockholm (S) resembles the epiphytic sample illustrated by Roth (1806, plate 12b). The label on the sheet contains the following text: "*Aspercoccus sinuosus Bory. / Ulva sinuosa m n. descr. / in 3 [...] Catal. Fasc. Icon [...] / prope Gades. / Mertens scripsit et misit. Herb. Swartzii*". Considering all these, it seems best to designate this specimen as the type of *C. sinuosa* rather than those assigned by Vandermeulen *et al.* However, detailed morpho-anatomical characterization must first be done on the specimen to ascertain its identity.

Recently, *Colpomenia* has been subjected to numerous taxonomic and molecular phylogenetic studies. These works resulted in the description of two new species (i.e., *C. claytoniae* and *C. wynnei*), the synonymy of *C. phaeodactyla* under *C. durvillei* based on nomenclatural priority, and the discovery of cryptic diversity in *C. sinuosa* (Boo *et al.* 2011a, Lee *et al.* 2012, 2013, 2014). However, the relationships of the elongate *Colpomenia* species relative to their globular/saccate congeners as well as to other taxa in the Scytosiphonaceae were not explored in more detail as done in this current study. Herein, in both single and multigene phylogenies, *Colpomenia* was not recovered as monophyletic. That is, *C. bullosa*, *C. durvillei*, and *C. wynnei* were segregated and found within the '*Scytosiphon* group' while the other *Colpomenia* species including the generitype *C. sinuosa* were found within the '*Hydroclathrus* group'. This polyphyly is reflected and supported by the distinct morphological and life history differences of the

bullosa-durvillei-wynnei clade from the other *Colpomenia*. The *bullosa-durvillei-wynnei* clade was composed of species that have upright, hollow, and elongate (finger-like) thalli that arise from a colpomenioid base and whose prostrate sporophytic thalli only produce unilocular zoidangia—at least for *'C. bullosa*' (Nakamura & Tatewaki 1975, Kogame *et al.* 1999). Meanwhile, the other *Colpomenia* species within the '*Hydroclathrus* group' have hollow, globular/saccate to amorphous and spreading thalli and its prostrate sporophytic thalli produce both unilocular and plurilocular zoidangia. Based on the distinct morpho-anatomy, molecular phylogeny, and life history of the elongate species of *Colpomenia*, I proposed herein that these species be transferred into a new genus *Dactylosiphon* Santiañez, K.M. Lee, S.M. Boo *et* Kogame (Table 2).

The 'Scytosiphon group'

The genus *Scytosiphon* (Table 3) is also among the problematic groups in the family despite being the relatively well-studied genus in the Scytosiphonaceae. The taxonomic problem in the genus is aggravated by high cryptic species diversity, especially in the generitype *S. lomentaria* (Kogame *et al.* 2015). The recent taxonomic treatment of *Scytosiphon* based mainly on samples from both the Atlantic and Pacific coasts of Canada has highlighted the difficulty in morphological species delineation in the genus (McDevit & Saunders 2017). Nonetheless, McDevit and Saunders (2017) have made considerable changes in the taxonomy of several species that are found within the '*Scytosiphon* group' including amending the description of *S. lomentaria*, adding a new species *Scytosiphon promiscuus* McDevit *et* G.W. Saunders, and establishing the new genus *Planosiphon* McDevit *et* G.W. Saunders. In their phylogenetic trees, this highly supported clade included taxa previously assigned to *Scytosiphon* and *Petalonia*; these

taxa were subsequently transferred to the new genus. Typically, *Planosiphon* species possess flattened thalli that are non-constricted, hollow to partially hollow, and have uniseriate plurangia that lack paraphyses (Table 4; McDevit & Saunders 2017). Comparing the morphologies and life histories of *Planosiphon* species and other closely related genera suggested that the genus can be further defined through the life histories in culture of its members. That is, *Planosiphon* species have been reported to have *Compsonema*-like prostrate sporophytic thalli that only bear unangia (Kogame & Kawai 1993, Kogame 1998). As such, the description of the genus is expanded below. Moreover, *Petalonia filiformis* (Batters) Kuntze, a minute species found in the Atlantic, seems best assigned to *Planosiphon*. The morphology and life history of *P. filiformis* has been described in detail by Fletcher (1987). Based on morphology, the species can be placed under *Planosiphon* or *Petalonia*. However, Fletcher (1987) reported that the species has *Compsonema*-like prostrate sporophytic thallus, which is different to the other *Petalonia* species which has *Stragularia*-type thalli (Brophy & Murray 1989, Kogame 1998, Kogame *et al.* 1999, Parente *et al.* 2003). All species under *Planosiphon* has prostrate sporophytic thalli that are *Compsonema*-like; as such, I believe the species in question is best assigned under the genus and propose the new combination *Planosiphon filiformis* (Batters) Santiañez *et* Kogame *comb. nov*.

In the *cox*3 and *rbc*L trees, *Scytosiphon canaliculatus* (Setchell *et* N.L. Gardner) Kogame did not cluster with the other *Scytosiphon* species. Exploratory phylogenetic analyses of the family Scytosiphonaceae based on *rbc*L gene which included the '*S. canaliculatus*' of McDevit and Saunders (2017) suggested that these entities were not similar. The question as to which of these entities should correctly bear the name *S. canaliculatus* can only be settled by studying materials from the type locality, San Pedro, California, USA. At present, I refrained from using the '*S. canaliculatus*' sequence of McDevit and Saunders (2017) because the Japanese entity, whose sequences were also

available in GenBank, was the basis of transferring *Hapterophycus canaliculatus* Setchell *et* N.L. Gardner to *Scytosiphon*. Whichever is the true '*S. canaliculatus*' between the two entities, I suggest the transfer of the Japanese entity to a different genus because of its life history (formation of hapteron-like prostrate thalli (Kogame 1996)) and genetic difference from other *Scytosiphon* species.

In the *rbc*L gene tree, *Scytosiphon tenellus* Kogame formed a highly supported clade with *Petalonia* species. The species was originally placed under *Scytosiphon* due to its hollow, cylindrical to flattened thallus that was simple/unconstricted, as well as the presence of ascocysts among its plurangia. As for *Petalonia*, its members are distinguished in having complanate, linear to lanceolate thalli that are generally solid in construction and whose medulla possesses rhizoidal filaments (Table 4). *Scytosiphon tenellus*, as also noted by Matsumoto *et al.* (2014), was similar to *Petalonia binghamiae* (J. Agardh) V.L. Vinogradova and *Petalonia fascia* (O.F. Müller) Kuntze in having a *Stragularia*-type prostrate sporophytic thallus (Kogame *et al.* 1999). The absence of ascocysts in *Petalonia* was initially perceived an important generic criterion but is now considered of little taxonomic value after the description of *Petalonia tatewakii* Kogame *et* Kurihara, a species which possesses ascocysts (Kogame *et al.* 2011). Based on the aforementioned morphological and phylogenetic similarities, I propose that *S. tenellus* be transferred to the genus *Petalonia* and from hereon be recognized as *Petalonia tenella comb. nov*. Consequently, I also expanded herein the generic diagnosis of *Petalonia* to include the characters found in *P. tatewakii* and *P. tenella*.

Moreover, considering that the Scytosiphonaceae has been consistently divided into two phyletic groups that are unified by several morphological and life history characteristics, I believe that this phylogenetic grouping can be formally designated as distinct tribes: Hydroclathreae *trib. nov.*, to accommodate genera under the

'*Hydroclathrus* group' (Table 6), and, Scytosiphoneae *trib. nov.*, for genera found within the '*Scytosiphon* group' (Table 6).

Taxonomic proposals: New taxa, new combinations, and lectotypification

Pseudochnoospora* Santiañez, G.Y. Cho *et* Kogame *gen. nov.

Description: Thalli decumbent, branched, with branches attached to the substrata and other branches at various points; branches solid, terete to compressed. Cortex up to two layers of small, pigmented cells; medullary cells clear, thin-walled, becoming larger towards the center. Plurangial sori may be covered with a cuticle; plurangia coherent, cylindrical to clavate, uni- to partially to completely biseriate, paraphysis absent. Prostrate sporophyte produces both uni- and plurilocular zoidangia.

Type species: *Pseudochnoospora implexa* (J. Agardh) Santiañez, G.Y. Cho *et* Kogame *comb. nov.*

Etymology: *Pseudochnoospora*, or the 'false *Chnoospora*', is in reference to the erroneous assignment of the taxon to the genus *Chnoospora* to which *P. implexa* previously belonged.

Pseudochnoospora implexa* (J. Agardh) Santiañez, G.Y. Cho *et* Kogame *comb. nov.

Figure 20. Scanned photographs of the type specimens of (A) *Chnoospora minima* (K. Hering) Papenfuss (HBG024509), deposited at the Herbarium Hamburgense of the University of Hamburg, Germany (HBG); (B) *Pseudochnoospora implexa* (J. Agardh) Santiañez, G.Y. Cho *et* Kogame *gen. et comb. nov.* (BM000569565); and (C) *Planosiphon filiformis* (Batters) Santiañez *et* Kogame (BM 000562830), deposited at the Natural History Museum, London. Scale bar = 1 cm.

Basionym: *Chnoospora implexa* J. Agardh 1848: *Species genera et ordines algarum, seu descriptiones succinctae specierum, generum et ordinum, quibus algarum regnum constituitur*. *Volumen primum: Algas fucoideas complectens*: 172.

Synonyms: *Dictyota obtusangula* Kützing 1859: 13, *Chnoospora obtusangula* (Kützing) Sonder 1871: 45 *fide* Grunow 1874: 25.

Chnoospora pannosa J. Agardh 1848: 172 *fide* Kraft 2009: 108.

Holotype: BM000569565 (Figure 20, B), Tor, Sinai Peninsula, *W. Schimper*; deposited in BM.

Distribution: Tropical to subtropical waters (M.D. Guiry in Guiry and Guiry 2017).

Dactylosiphon Santiañez, K.M. Lee, S.M. Boo *et* Kogame *gen. nov.*

Diagnosis: Thalli composed of elongate (finger-like) tubes, each tapering to an attenuate to cuneate basal portion; these arising from a saccate base. Hollow center bounded by several layers of large, clear, thick-walled medullary cells; outer layer composed of small pigmented cortical cells. Plurangia in sori, confluent, extensive throughout the thalli; mostly uniseriate, others partially to completely biseriate; always associated with short to slightly longer paraphyses (ascocysts).

Type species: *Dactylosiphon bullosus* (D.A. Saunders) Santiañez, K.M. Lee, S.M. Boo *et* Kogame *comb. nov.*

Etymology: Named after the finger-like (Greek: *dactylo*-) and siphonous thalli of the species belonging to this genus.

Dactylosiphon bullosus (D.A. Saunders) Santiañez, K.M. Lee, S.M. Boo *et* Kogame *comb. nov.*

Basionym: *Scytosiphon bullosus* D.A. Saunders 1898: *Proceedings of the California Academy of Science, Series 3, Botany 1*: 163, pl. XXXI: figs 1–7.

Synonym: *Colpomenia bullosa* (D.A. Saunders) Yamada in Yamada & Kinoshita 1948: 6, pl. 4.

Type locality and specimen: Pacific Grove, California, USA; FH, August 1896, *D.A. Saunders*, deposited in HUH (Parsons 1982).

Distribution: Temperate waters of the Pacific (Lee *et al.* 2012).

Remarks: The taxonomy of *D. bullosus* (as *C. bullosa*) has been confused, having been placed under *Colpomenia* or *Scytosiphon* by various authors (Parsons 1982). Prior to this transfer, the species had been classified in the genus *Colpomenia* based on the opinion of Yamada (1948), and Hollenberg and Abbott (1966) provided the rationale for segregating it from *Scytosiphon*. This confusion is understandable as both *Colpomenia* and *Scytosiphon* have been described very broadly.

Dactylosiphon durvillei (Bory de Saint-Vincent) Santiañez, K.M. Lee, S.M. Boo *et* Kogame *comb. nov.*

Basionym: *Aspercoccus durvillei* Bory de Saint-Vincent 1828: Botanique, Cryptogamie. In (Duperrey, L.I. Eds): *Voyage autour du monde, exécuté par ordre du Roi, sur la corvette de Sa Majesté, la Coquille, pendant les années 1822, 1823, 1824 et 182*: 200, pl. 11: fig. 3 (as '*durvillaei*').

Synonyms: *Colpomenia durvillei* (Bory de Saint-Vincent) M.E. Ramírez in Ramírez & Rojas 1991: 17.

Colpomenia phaeodactyla M.J. Wynne *et* J.N. Norris 1976 *fide* Lee *et al.* 2014a: 485.

Type locality and specimen: Concepcion, Chile; TA5284, *d'Urville*; deposited in PC Herb. Bornet-Thuret (Ramírez & Rojas 1991).

Distribution: Temperate waters of the Pacific (Lee *et al.* 2012).

Dactylosiphon wynnei* (K.M. Lee, R. Riosmena-Rodriguez, Kogame *et* S.M. Boo) Santiañez, K.M. Lee, S.M. Boo *et* Kogame *comb. nov.

Basionym: *Colpomenia wynnei* K.M. Lee, R. Riosmena-Rodriguez, Kogame *et* S.M. Boo 2014: *Phycologia* 53 (5): 481, figs 1–7.

Type locality and specimen: Hoedong, Jindo, Korea; CNU33055, 2 March 2013; deposited in CNUK (Lee *et al.* 2014a).

Distribution: SUBTROPICAL NORTHWESTERN PACIFIC: Central and southern Japan; southern Korea (Lee *et al.* 2014a).

Remarks: *Dactylosiphon wynnei* is a recently described species (as *Colpomenia wynnei*) from the northeast Pacific (i.e., Korea and Japan). This species is unique among its congeners in possessing adventitious branchlets along the length of its contorted, mature elongated tubes.

Petalonia Derbès *et* Solier *nom. cons.*

Expanded description: Thalli erect, gregarious, leaf-like, compressed, generally flattened, linear to lanceolate or oblanceolate; entire, generally unbranched, old thalli sometimes perforated or torn at the apical portion; hollow to partially hollow to solid. Medulla typically interspersed with rhizoidal filaments. Plurangia occurs extensively throughout the thallus, sometimes in sori; generally biseriate, closely packed, covered with cuticle; paraphysis (ascocyst) may be present. Prostrate sporophytic thalli *Stragularia*-like, only bear unangia.

Petalonia tenella (Kogame) Santiañez *et* Kogame *comb. nov.*

Basionym: *Scytosiphon tenellus* Kogame 1998: *Phycological Research* 46: 44, figs 29–51.

Type locality and specimen: Muroran, Hokkaido, Japan; SAP059746, 2 February 1988, *K. Kogame*, deposited in SAP.

Distribution: Hokkaido, Japan (Kogame 1994, 1998); Northern Chile (Camus *et al.* 2005).

Etymology: The generic name *Petalonia* is considered feminine while the specific epithet '*tenellus*' is masculine; as such, the epithet should take its feminine form and be corrected to '*tenella*'.

Planosiphon McDevit *et* Saunders

Expanded description: Thalli erect, linear, compressed to flattened; hollow to partially hollow; non-constricted. Plurangia grows extensively throughout the thallus; uniseriate, closely packed, usually covered with cuticle; paraphysis (ascocyst) absent. Prostrate sporophytic thalli *Compsonema*-like, only bear unangia.

Planosiphon filiformis (Batters) Santiañez *et* Kogame *comb. nov.*

Basionym: *Phyllitis filiformis* Batters 1888: *Journal of the Linnean Society of London, Botany* 24: 451, pl. XVIII [18]: figs 1–6.

Synonym: *Petalonia filiformis* (Batters) Kuntze 1898: 419.

Lectotype: BM000562830 (Here designated: see remarks below)

Type locality: Berwick-[up]on-Tweed, [Northumberland], England (Batters 1888).

Remarks: When Batters (1888) described *Phyllitis filiformis*, he did not assign the type specimen for the species. This is understandable considering that such designation was not required at the time to validly publish a new species. Based on the online database of the Natural History Museum, London (Natural History Museum 2014), two possible type collections were recorded: BM000562830 and BM000563650. Several specimens were found on both sheets and were labelled "121. Phyllitis filiformis, Batters. / Berwick, January, 1887, E. Batters." Thus, it appears that specimens on both sheets were part of a gathering and can be considered a collection (ICN Articles 8.2 and 8.3

(Melbourne Code; McNeill *et al.* 2012)). Herein, BM000562830 (Figure 20, C) was designated as the lectotype of *P. filiformis*, and BM000563650 is considered as its isolectotype.

Hydroclathreae *trib. nov.*

Description: Thalli erect to decumbent, branching freely or inter-adhesive, or saccate to network-like, spreading and amorphous; generally hollow (*Colpomenia, Hydroclathrus, Rosenvingea, Tronoella, Iyengaria*), partially hollow (*Chnoospora*), or solid in construction (*Pseudochnoospora*); plurangia in sori, uni- to biseriate in lateral view, mostly ecuticulate, some (initially) covered with cuticles (*Colpomenia, Chnoospora*, *Pseudochnoospora*); plurangia arranged in loose vertical palisades or as cohesive mass; paraphyses absent (except in *Colpomenia*). Life history generally heteromorphic, alternating between a macroscopic gametophyte bearing plurangia and a discoid microscopic sporophyte bearing both unangia and plurangia; some showing a direct-type life history patterns in culture; erect macrothalli derived from unispores develop initially from massive parts.

Type genus: *Hydroclathrus* Bory de Saint-Vincent 1825.

Remarks: The genus *Iyengaria* was included in this tribe based on the morphologies and phylogenetic positions of the generitype *Iyengaria stellata* (Børgesen) Børgesen (data not shown) and *Iyengaria quadriseriata*.

Scytosiphoneae *trib. nov.*

Description: Thalli erect, solitary or gregarious, rarely branched; mostly terete to compressed hollow (*Dactylosiphon, Scytosiphon, Melanosiphon, Myelophycus*), some flattened and partially hollow (*Planosiphon*), to generally solid (*Petalonia*); plurangia develop basipetally (but acropetally in *Dactylosiphon*) and grows extensively throughout the thalli; plurangia uni- to biseriate, arranged in firmly coherent columns and covered by cuticles (*Planosiphon, Petalonia, Melanosiphon*) or in somewhat loose palisades (*Dactylosiphon, Scytosiphon*); paraphyses commonly found together with reproductive structure, although absent in some (*Planosiphon, Petalonia* (some species)). Life history isomorphic (*Myelophycus, Melanosiphon*) or heteromorphic (*Dactylosiphon, Planosiphon, Petalonia, Scytosiphon*), the latter has discoid to filamentous microscopic sporophytes; sporophytic thalli only produce unangia; erect macrothalli derived from unispore develop initially from uniseriate filaments (except for *Dactylosiphon*).

Type genus: *Scytosiphon* C. Agardh 1820 *nom. cons.*

Remarks: The name 'Scytosiphoneae' was first used by Thuret in Le Jolis (1863) but was attributed to a group that corresponded to a family. Meanwhile, Cho *et al.* (2006) also suggested the name 'Scytosiphonieae' but no formal proposal was made at that time.

Table 2. Morphology, anatomy and life history characteristics of known *Dactylosiphon* species*.

Character	***Dactylosiphon durvillei* (Bory de Saint-Vincent) Santiañez, K.M. Lee, S.M. Boo *et* Kogame *comb. nov.***	***Dactylosiphon bullosus* (D.A. Saunders) Santiañez, K.M. Lee, S.M. Boo *et* Kogame *comb. nov.***	***Dactylosiphon wynnei* (K.M. Lee, R. Riosmena-Rodriguez, K. Kogame *et* S.M. Boo) Santiañez, K.M. Lee, S.M. Boo *et* Kogame *comb. nov.***
Thallus form	erect, numerous elongated (finger-like) tubes arising from saccate base	erect, numerous elongated (finger-like) tubes arising from a saccate base	erect, numerous elongated (finger-like) tubes arising from a saccate base; tubes sometimes undulate to somewhat twisted; adventitious protrusions found occasionally among older thalli
Thallus size(length × width)	15 cm × 0.8 cm	30 cm × 3 cm	23 cm × 2 cm
Hair primordia	subcortical, in tufts, extended into long hyaline hairs	subcortical, in tufts, extended into long hyaline hairs	subcortical, in tufts, extended into long hyaline hairs
Cortex	1–3 cell layers; small, pigmented, 8–15 µm in diameter	1–3 cell layers; small, pigmented,	1–2 cell layers; small, pigmented, 10–15 µm in diameter
Medulla	up to 5 layers; small, cuboidal; thick-walled	3–8 layers; round to broadly rounded; clear, thick-walled, up to 80 µm wide	3–5 layers; round to broadly rounded; clear, thick-walled, up to 80 µm wide
Paraphysis	present	present	present
Plurangia	uni- to biseriate, loosely arranged in vertical palisades; ecuticulate; up to 40 µm long	extensive, uni- to biseriate, loosely arranged in vertical palisades; ecuticulate; up to 60 µm long	extensive; biseriate, loosely arranged in vertical palisades; ecuticulate up to 75 µm long
Initiation of macrothalli	—	filamentous, branched, tufted	—
Prostrate thallus morphology	—	bilateral-type, associated with paraphysis	—

*Information from Saunders (1898), Wynne and Norris (1976), Parsons (1982), Ramírez and Rojas (1991), Kogame (1994), Kogame and Masuda (2001), Lee et al. (2012, 2014a).

Table 3. Morphology, anatomy and life history characteristics of known *Scytosiphon* species*.

Character	***Scytosiphon lomentaria* (Lyngbye) Link**	***Scytosiphon dotyi* M.J.Wynne**	***Scytosiphon canaliculatus* (Setchell *et* N.L.Gardner) Kogame**	***Scytosiphon promiscuus* McDevit *et* G.W.Saunders**
Thallus form	erect; constricted at intervals; cylindrical to compressed; hollow	erect, linear to slightly twisted; unconstricted or with few inconspicuous constrictions; terete to compressed; hollow	erect, constricted, terete to compressed; hollow	erect, constricted, compressed (?); hollow
Thallus size(length × width)	50 cm × 3–8 mm	17 cm × 1–2 mm	40 cm × 7 mm	35 cm × 4 mm
Cortex	1–3 layers	1–3 layers	1–3 layers	2 layers
Medulla	2–5 layers, clear, thick-walled, round to elongate	2–5 layers, clear, thick-walled, round to elongate	2–5 layers, clear, thick-walled, round to elongate	2–3 layers; thick-walled
Paraphysis	present (?)	absent	present	present
Plurangia	loose, uni- to biseriate, ecuticulate	extensive on thallus surface, uniseriate (?), covered with cuticle, 35 µm long, 3–5 µm broad	extensive on thallus surface, uniseriate (?), ecuticulate, 54 µm long	Uniseriate, closely packed
Initiation of macrothalli	filamentous, branched	—	filamentous, uniseriate	—
Prostrate thallus morphology	*Microspongium*-like, produce unagia only	—	hapteron-like, produce unangia only	—

*Information from Wynne (1969), Clayton (1980), Fletcher (1987), Kogame (1994, 1996, 1998), McDevit and Saunders (2017).

Table 4. Morphology, anatomy and life history characteristics of known *Planosiphon* species*.

Character	***Planosiphon complanatus*** **(Rosenvinge) McDevit *et* G.W.Saunders**	***Planosiphon gracilis*** **(Kogame) McDevit *et* G.W.Saunders**	***Planosiphon zosterifolius*** **(Reinke) McDevit *et* G.W.Saunders**	***Planosiphon filiformis*** **(Batters) Santiañez *et* Kogame *comb. nov.***
Thallus form	erect, linear; highly compressed; partially hollow	erect, linear; flattened to compressed or subterete; hollow	erect, linear; flattened; partially hollow	erect, very narrow, filiform; compressed to slightly flattened; solid to partially hollow
Thallus size(length × width)	28 cm × 5 mm	10–25 cm × 0.5–4 mm	10–20 cm × 2–5 mm	8 cm × 0.4 mm
Cortex	2 layers	1–3 layers	1–3 layers	1–3 layers
Medulla	2–4 layers; clear and thick-walled; round to collapsed	—	—	2–4 (–6) layers; clear and thick-walled; round to collapsed
Paraphysis	absent	absent	absent	absent
Plurangia	closely packed, uniseriate, covered with cuticle	develop throughout thalli; closely packed, covered with cuticle	closely packed, covered with cuticle	extensive; closely packed, uniseriate, up to 30 μm long
Initiation of macrothalli	filamentous, uniseriate	filamentous, branched	filamentous, branched	filamentous, branched
Prostrate thallus morphology	*Compsonema*-like; only bears unilocular sporangia	*Compsonema*-like; only bears unilocular sporangia	*Compsonema*-like; only bears unilocular sporangia	*Compsonema*-like; only bears unilocular sporangia

*Information from Clayton (1976, 1980), Fletcher (1987), Kogame and Kawai (1993), Kogame (1994, 1998), McDevit and Saunders (2017).

Table 5. Morphology, anatomy and life history characteristics of known *Petalonia* species*.

Character	*Petalonia fascia* (O.F. Müller) Kuntze	*Petalonia binghamiae* (J. Agardh) K.L. Vinogradova	*Petalonia tatewakii* Kogame *et* Kurihara	*Petalonia tenuis* Matsumoto *et* Shimada	*Petalonia tenella* (Kogame) Santiañez *et* Kogame *comb. nov.*
Thallus form	erect, broadly linear, lanceolate to oblanceolate; flattened; solid	erect, broadly linear, lanceolate to oblanceolate; flattened; solid	erect, linear to linear-lanceolate; compressed; solid	erect, lanceolate to oblanceolate; flattened; solid	erect, linear; flattened; hollow
Thallus size(length × width)	25 cm × 4 cm	20 cm × 3 cm	15 cm × 0.8 cm	15 cm × 4.4 cm	15 cm × 5 mm
Cortex	1–3 layers	1–3 layers			1–3 layers
Medulla	3–6 layers; clear and thick-walled; round to elongate or collapsed; central area interspersed with rhizoidal filaments	clear and thick-walled; round to ellipsoid; central area interspersed with rhizoidal filaments	clear and thick-walled; round to ellipsoid; central area interspersed with rhizoidal filaments	3–6 layers; clear and thick-walled; round to ellipsoid; central area interspersed with rhizoidal filaments	2–3 layers; clear and thick-walled; round to ellipsoid
Paraphysis	absent	absent	present	absent	present
Plurangia	closely packed, up to 40 µm long, covered with cuticle	closely packed, ~ 20 µm long, covered with cuticle	closely packed, ~ 30 µm long, covered with cuticle	extensive; closely packed, ~ 30 µm long, covered with cuticle	closely packed, up to 40 µm long, covered with cuticle
Initiation of macrothalli	filamentous, branched, discoid	filamentous, discoid	—	—	filamentous, branched, discoid
Prostrate thallus morphology	*Stragularia*-like; bears unangia only	*Stragularia*-like; bears unangia only	—	*Stragularia*-like	*Stragularia*-like; bears unangia only

*Information from Wynne (1969), Clayton (1976, 1980), Fletcher (1987), Brophy and Murray (1989), Kogame (1998), Parente et al. (2003), Kogame et al. (2011), Matsumoto et al. (2014).

Table 6. Morpho-anatomical and life history characteristics of the different genera in the Scytosiphonaceae*.

Characters	Tribe Scytosiphoneae					
	Dactylosiphon	***Scytosiphon***	***Petalonia***	***Planosiphon***	***Myelophycus***	***Melanosiphon***
Thallus form	Erect, elongated tubes; each finger-like tube arise from a common lobed base	erect, generally constricted at intervals; cylindrical to compressed, hollow	erect, leaf-like(i.e., flattened, broadly linear, lanceolate to oblanceolate); solid	erect, non-constricted; compressed to flattened; hollow or partially hollow	erect, elongate; solid when young, hollow when mature	erect, elongate; solid when young, hollow when mature; cylindrical
Cortex	1–3 cell layers, pigmented	1–3 layers, pigmented	1–3 layers, pigmented	1–3 layers	3–4 layers	2–4 layers
Medulla	2–5 cell layers; clear, thick-walled to lamellate	2–5 layers, clear, thick-walled, round to elongate	3–6 layers; clear, thick-walled; round to elongate or collapsed; interspersed with rhizoidal filaments	2–5 layers, clear, thick-walled, round to elongate	4–5 layers; clear, thick-walled; round to elongate or collapsed	2–5 layers; clear, thick-walled; round to elongate or collapsed
Paraphysis	present	present (absent in some)	absent (present in some)	absent	present	present
Plurangia	extensive on thallus surface; cuticle absent; cylindrical to subclavate, mostly uniseriate, some partially or completely biseriate (lateral view)	extensive on surface, especially at terminal portions; loose, uniseriate (some biseriate); mostly ecuticulate	extensive in terminal portions of thallus surface, closely packed, covered with cuticle, generally uniseriate (lateral view)	extensive on surface; closely packed, covered with cuticle, uniseriate (lateral view)	extensive; closely packed, covered with cuticle, uniseriate (lateral view)	extensive, in patches; closely packed, covered with cuticle, uniseriate (lateral view)
Fertilization	isogamy (?)	isogamy/anisogamy	isogamy	isogamy		
Initiation of macrothalli	-	filamentous, branched or uniseriate	filamentous, branched	filamentous, branched	filamentous, uniseriate	filamentous, branched
Prostrate thallus morphology	Bilateral type; bears unangia only	*Microspongium*/hapteron-like; bears unangia only	*Stragularia*-like; bears unangia only	*Compsonema*-like; only bears unilocular sporangia	isomorphic; bears unangia only	isomorphic; bears unangia only

*Information from Barton (1898), Saunders (1898), Wynne (1969), Clayton (1975, 1980), Nakamura and Tatewakii (1975), Wynne and Norris (1976), Fotos (1981), Parsons (1982), Tanaka and Chihara (1984), Fletcher (1987), Ramírez and Rojas (1991), Kogame and Kawai (1993), Kogame (1994, 1996,1997, 1998, 2001), Kogame and Yamagishi (1997), Trono (1997, 2001a), Yoshida (1998), Kogame and Masuda (2001), Cho et al. (2003), Kraft and Abbott (2003), Parente et al. (2003), Kraft (2009), Boo et al. (2011a), Kogame et al. (2011), Lee *et al.* (2012, 2014a, 2014b), Matsumoto et al. (2014), West *et al*. (2015).

(Continued…) Table 6. Morpho-anatomical and life history characteristics of the different genera in the Scytosiphonaceae*.

Characters	Tribe Hydroclathreae					
	Chnoospora	***Colpomenia***	***Rosenvingea***	***Tronoella***	***Pseudochnoospora***	***Hydroclathrus***
Thallus form	erect; solid to partially hollow, compressed to flattened; branched	saccate, globular to cushion-like, surface sometimes perforated or with (branched) protrusions; hollow	erect to decumbent to mat-forming; branched; terete to compressed; hollow	spreading; perforated hollow membranes initially arise as siphonous protrusions; somewhat branched	decumbent, terete to compressed; branched, entangled, mat-forming; solid	saccate to spreading; membranes perforated, network-like; margins of perforations folded to revolute; hollow
Cortex	2–5 cell layers; cells thick-walled, pigmented	1–2 (–3) cell layers, pigmented	1 cell layer, pigmented	1–2 cell layers, pigmented	1–2 cell layers; cells thin-walled, pigmented	1–3 cell layers, pigmented
Medulla	clear cells thick-walled with lamellate regions; large and small cells intermixed	3–6 cell layers; clear, thin- to thick-walled	1–5 cell layers; clear, thin-walled	1–4 cell layers; colourless, thin-walled	Cells clear and thin-walled, often becoming larger towards the center	1–9 cell layers; clear, generally thin-walled
Paraphysis	absent	present	absent	absent	absent	absent
Plurangia	linear; associated with hairs; covered with cuticle; coherent, cylindrical to clavate, uni- to biseriate (lateral view)	discrete to confluent, may be associated with hairs or covered with cuticle; cylindrical to clavate, uni- to biseriate (lateral view)	discrete to confluent, shape linear to irregular, may be associated with hairs; densely arranged, cylindrical to clavate, uni- to biseriate (lateral view)	discrete, irregularly shaped, associated with hairs; firmly coherent and massive	irregularly shaped, generally not associated with hairs; may be covered with cuticle; coherent, cylindrical to clavate, uni- to partially or completely biseriate (lateral view)	discrete to confluent, circular to irregular, angular to block-like, often associated with hairs; loosely arranged, cylindrical to clavate, biseriate (lateral view)
Fertilization	—	anisogamy				
Initiation of macrothalli	—	massive	—	—	massive	massive
Prostrate thallus morphology	—	discoid; produce both unangia and ectocarpoid plurangia	discoid; produce plurangia	—	discoid; produce both unangia and firmly coherent plurangia	discoid; produce both unangia and ectocarpoid plurangia

*Information from Barton (1898), Saunders (1898), Wynne (1969), Clayton (1975, 1980), Nakamura and Tatewakii (1975), Wynne and Norris (1976), Fotos (1981), Parsons (1982), Tanaka and Chihara (1984), Fletcher (1987), Ramírez and Rojas (1991), Kogame and Kawai (1993), Kogame (1994, 1996,1997, 1998, 2001), Kogame and Yamagishi (1997), Trono (1997, 2001a), Yoshida (1998), Kogame and Masuda (2001), Cho et al. (2003), Kraft and Abbott (2003), Parente et al. (2003), Kraft (2009), Boo et al. (2011a), Kogame et al. (2011), Lee *et al.* (2012, 2014a, 2014b), Matsumoto et al. (2014), West *et al*. (2015).

CHAPTER 4

The family Scytosiphonaceae: Current perspectives and outlook

Untangling the complex yet interesting systematic problems in the family Scytosiphonaceae has been long overdue. My current study addresses some of the prevailing inconsistencies in the taxonomy, classification, and phylogeny of the family by integrating several lines of evidences to facilitate appropriate systematic changes. Kogame (1994) was first to introduce the suite of morphological and life history characteristics that I used in delineating the different species and genera in the Scytosiphonaceae. By integrating these suite of characters into the molecular phylogeny of the Scytosiphonaceae—as first proposed in Kogame *et al.* (1999)—I provided herein a framework on which future systematic changes in the family should be conducted. That is, aside from morphological and molecular information, knowledge on their life histories is fundamental in clarifying the generic-level distinctions in the Scytosiphonaceae, particularly those in the tribe Scytosiphoneae.

ROLE OF MOLECULAR PHYLOGENIES IN THE TAXONOMY AND CLASSIFICATION OF THE SCYTOSIPHONACEAE

The use of molecular tools has recently gained traction in phycology, ushering renewed interests in the algae. Recognizing and discovering taxa have been simplified by

molecular techniques, resulting in the apparent "explosion" of described taxa across phyletic levels. As Huisman (2016) keenly observed, "the Age of Discovery is still with us." Molecular-assisted taxonomic studies have become standard practice in recent years and were instrumental in refining species boundaries especially among many algal groups with simple yet highly plastic morphologies. Concomitant with the increase of our understanding on the boundaries and phylogenetic relationships of the different taxa, more informed systematic revisions are currently being made especially at suprageneric levels (e.g., Silberfeld *et al*. 2014, Kawai *et al.* 2015, 2017).

The family Scytosiphonaceae provides an interesting case study on the utility of DNA phylogenies as tools to clarify both the taxonomy and classification of its members. In this study, the use of molecular data has facilitated delineating species- and/or genus-level boundaries for *Chnoospora*, *Dactylosiphon*, *Hydroclathrus*, *Pseudochnoospora*, and *Tronoella*. Based on the results of my molecular-assisted alpha-taxonomic studies on *Hydroclathrus*, as well as on other seaweeds [e.g., *Lobophora* (Vieira *et al*. 2014), *Acinetospora* Bornet (Yaegashi *et al.* 2015), and *Gibsmithia* Doty (Gabriel *et al*. 2016)], the existence of pseudo-cryptic species underscores the need to question the widespread assumptions on the existence of a single, cosmopolitan, and highly polymorphic species. Molecular phylogenies have been providing objective bases and opportunities to revise the taxonomy and classification of several taxa. For example, the multi-gene phylogeny of Scytosiphonaceae has allowed me to expand the descriptions of *Petalonia* and *Planosiphon* wherein I specified a particular type of sporophyte morphology to each genus: *Stragularia*-like for the former and *Compsonema*-like for the latter. Aside from these, it has also allowed me to update the classification of the group by establishing two tribes, Hydroclathreae and Scytosiphoneae. Clearly, the family is among the many algal groups that have greatly benefitted from the advent of DNA phylogenies.

ON THE TRIBES IN THE SCYTOSIPHONACEAE

The tribes Hydroclathreae and Scytosiphoneae were established in this study to accommodate the two major phylogenetic clades in the Scytosiphonaceae which share distinct life history characteristics, particularly the type of reproductive organ produced by the sporophytic thalli.

Kogame (1994) suggested that the initiation of macrothalli (filamentous vs massive) is best used at delineating taxa at the family level, rather than at the genus level as proposed by Wynne and Norris (1976) for *Colpomenia* and *Scytosiphon*. Until 1999, the Order Scytosiphonales, which was constituted by two families Chnoosporaceae and Scytosiphonaceae, are still acknowledged. As the Order Scytosiphonales was relegated to the family level, i.e., Scytosiphonaceae (Rousseau & Reviers 1999, Kogame *et al.* 1999), the recommendation by Kogame (1994) may still hold at suprageneric level. Cho *et al.* (2006) suggested classifying the two phyletic groups into two tribes, 'Scytosiphonieae' and 'Chnoosporieae', as recognized by molecular data, distributional patterns, and reproductive organs in their sporophyte stages. However, this proposal was not formally made as it was initially intended to settle the problematic relationships of the different taxa. Distributional patterns are unreliable in delineating these two groups as several taxa in both phyletic groups have overlapping distributions (e.g., *Hydroclathrus* and *Colpomenia* can be found in warm temperate waters of Japan while *Petalonia* and *Planosiphon* can be found in warm subtropical waters). Conversely, the reproductive organs among sporophyte provided a clear distinction between the two groups. As such, Hydroclathreae and Scytosiphoneae were distinguished primarily based on these characters. Secondarily, Scytosiphoneae

are united by erect and unbranched morphologies while those of Hydroclathreae are erect to decumbent to spreading and branched or network-like.

MORPHOLOGY-BASED CLASSIFICATION IN THE SCYTOSIPHONACEAE

Genus-level delineation and identification among taxa under the tribe Hydroclathreae is relatively straightforward as the different genera have distinct gross morphologies (i.e., erect, decumbent, saccate, spreading, branched, or net-like) compared to those of tribe Scytosiphoneae (i.e., erect, unbranched) (Table 6). Their phylogenetic relationships, however, remained unresolved. As mentioned previously, these unresolved relationships among taxa in the Hydroclathreae may be due to a number of factors including incomplete taxon sampling and misidentifications (e.g., erroneous designation to a genus). The latter was the case of both *Pseudochnoospora* and *Dactylosiphon* species. The taxonomic problem in the Hydroclathreae may also be due to the fact that most genera in the tribe received little attention compared to those of Scytosiphoneae. In my current study, some progress has been made on the "older" genera *Hydroclathrus* and *Chnoospora*. Attention should be shifted now to the hollow and branching *Rosenvingea*, which has never been recovered as monophyletic. This problem is confounded by the description of *Iyengaria quadriseriata*, which clustered with *Rosenvingea*. The identity of *I. quadriseriata* as well as the generitype *I. stellata*, together with all *Rosenvingea* species with known sequence data, must be verified by conducting both detailed morpho-anatomical and multi-gene based phylogenetic analyses. The problem in *Colpomenia* should also be similarly assessed. Due to their distinct morphological characters from the typical *Colpomenia*, exploring the possibility of segregating the branched *Colpomenia ramosa* and *Colpomenia tuberculata* as either

member of a distinct, new genera or each as representatives of a monotypic genus seems reasonable. Nonetheless, the genera I have assessed in this study—that is, *Chnoospora*, *Hydroclathrus*, *Pseudochnoospora*, and *Tronoella*—can be consistently segregated based on traditional criteria. That is, gross morphologies, thallus construction, and nature and arrangement of plurangia are useful characters in generic delineation within tribe Hydroclathreae.

Difficulties in genus-level segregation were pronounced within the tribe Scytosiphoneae, particularly in the *Scytosiphon-Petalonia-Planosiphon* (SPP) complex (Table 6). Largely due to the simple morphologies of taxa in the Scytosiphoneae, the characters that were used to segregate genera in the Hydroclathreae appear to be of limited use. The most distinct among the genera in the tribe is *Dactylosiphon* because of the presence of the hollow saccate base. *Scytosiphon* species can be distinguished from *Petalonia* and *Planosiphon* based on their constricted thalli, except *S. dotyi* whose constrictions were few and inconspicuous. Most *Petalonia* species—except *Petalonia tenella*—can be consistently distinguished from other genera by their leaf-like, flattened, and typically solid thalli with medullary rhizoidal filaments. Meanwhile, *Planosiphon* has linear, unconstricted, compressed to flattened, and hollow to partially hollow thalli, with uniseriate plurangia that lacks paraphysis. The morphological characters used to segregate *Planosiphon* are deemed unreliable as these are also found among *Petalonia* and *Scytosiphon* species. In fact, *Planosiphon* species were previously referred to either *Petalonia* or *Scytosiphon*. Despite the overlapping morphological characters between *Petalonia* and *Planosiphon*, both are consistently recovered as distinct monophyletic groups. Both also possessed distinct sporophytic thallus morphology: that of *Petalonia* is *Stragularia*-like while that of *Planosiphon* is *Compsonema*-like. Meanwhile, *Scytosiphon* has *Microspongium*-type or hapteron-like sporophytic thalli (Table 6). Thus, within the SPP complex, I would underscore that,

aside from the use of molecular data, information on their life histories (sporophytic thallus morphology) is taxonomically crucial. Meanwhile, the case of *Myelophycus* and *Melanosiphon* remained unsettled. Tanaka and Chihara (1984) considered *Melanosiphon intestinalis*, the only species in the genus, as a member of *Myelophycus*, citing the similarities between two species (Table 6). Kawai in Guiry and Guiry (2017) noted that only the biseriate nature of the paraphyses of *Melanosiphon* distinguishes it from *Myelophycus*. These two genera, however, were recovered as distinct in all phylogenetic trees where they were included. Nonetheless, detailed reassessment of the morphological boundaries between these genera is needed.

Despite the fuzzy boundaries among the different genera due to species that have intermediate character, several genera can be distinguished based on a combination of morphological traits that are typically found to the majority of the species attributed to them. As such, I provided herein a dichotomous key for the identification of the different genera in the Scytosiphonaceae based solely on morpho-anatomical characteristics (Table 7). The utility of this key is understandably limited in several genera in the tribe Scytosiphoneae and must, therefore, be used carefully.

FRONTIERS FOR FUTURE STUDY

The complexities in the systematics of the Scytosiphonaceae that I simultaneously unraveled and reiterated herein presented several opportunities in contributing to the understanding of the group. The revisions that I introduced herein—with the help and support of various partners from different parts of the globe—to disentangle the intricacies of numerous taxa, point to the need for wide-scale efforts and partnerships if we are to resolve the taxonomic, nomenclatural, and molecular phylogenetic issues in

the Scytosiphonaceae. Indeed, the fuzzy generic delineations in the Scytosiphoneae need attention but I would encourage a shift of focus on studying the tropical to subtropical members of the Hydroclathreae. In the latter tribe, while its generic delineation is relatively more intuitive than the former, the relationships among the different taxa are yet to be resolved. That is, integrated morphological, life historical, and molecular phylogenetic (wide taxon and genes sampling) studies are best conducted at least among seaweeds considered as putative members of the genus *Rosenvingea*, *Colpomenia*, and *Iyengaria*. However, to facilitate taxonomic revisions on these genera, studies must also include type materials or those collected from the type locality when type collections are missing or not viable. It is expected that, upon closer inspection, several new taxa will be discovered at different phyletic levels. Ultimately, it is hoped that after scrutinizing Hydroclathreae, some new taxa will be discovered. Such discoveries will, hopefully, resolve the relationships of taxa within the tribe.

Table 7. Key to the genera in the family Scytosiphonaceae.

1	Macrothalli erect, unbranched, typically hollow tubes; paraphysis generally present among extensively distributed plurangia	**2**
1	Macrothalli decumbent, or spreading, some saccate or erect and branched; paraphysis generally absent among plurangia that are in sori	**3**
2	Macrothalli arise from a colpomenioid base	***Dactylosiphon***
2	Macrothalli do not arise from a colpomenioid base	**4**
3	Macrothalli saccate and/or irregularly spreading, some with (branched) protrusions	***Colpomenia***
3	Macrothalli branched or net-like	**5**
4	Macrothalli linear to constricted at various points, terete to compressed; hollow to partially hollow	**6**
4	Macrothalli leaf-like, flattened, some compressed; solid or hollow; medulla generally with rhizoidal filaments	***Petalonia***
5	Macrothalli branched, hollow, partially hollow, or solid	**7**
5	Macrothalli net-like and spreading	**9**
6	Macrothalli hollow, often constricted; plurangia loosely packed	***Scytosiphon***
6	Macrothalli hollow to partially hollow, unconstricted; plurangia compactly arranged	**8**
7	Macrothalli solid, decumbent, entangled	***Pseudochnoospora***
7	Macrothalli hollow to partially hollow, branched freely or inter-adhesive	**11**
8	Macrothalli linear, compressed to somewhat flattened; paraphysis absent	***Planosiphon***
8	Macrothalli terete, some twisted towards the apex; paraphysis present	**10**
9	Plurangia in sori, loosely arranged, without a cuticle	***Hydroclathrus***
9	Plurangia in dense clumps, firmly coherent, covered with cuticle	***Tronoella***
10	Paraphyses uniseriate	***Myelophycus***
10	Paraphyses biseriate	***Melanosiphon***
11	Macrothalli solid to partially hollow, branched freely; plurangia compact, covered with cuticle	***Chnoospora***
11	Macrothalli hollow, branched freely, inter-adhesive in some; plurangia loose, without cuticle	***Rosenvingea***

REFERENCES

Abbott, I.A. & Huisman, J.M. (2004). *Marine green and brown algae of the Hawaiian Islands*. Honolulu: Bishop Museum Press. 259 pp.

Abbott, I.A. (1985). Introduction. In: *Taxonomy of economic seaweeds with reference to some Pacific and Caribbean species*. (Abbott, I.A. & Norris, J.N., eds). pp. xv + 167. La Jolla: California Sea Grant College Programme.

Agardh, C.A. (1823). *Species algarum rite cognitae, cum synonymis, differentiis specificis et descriptionibus succinctis*. Volumen primum pars posterior. Lundae [Lund]: ex officina Berlingiana. pp. [399]-531.

Agardh, J.G. (1847). Nya alger från Mexico. *Öfversigt af Kongl. Vetenskaps-Adademiens Förhandlingar, Stockholm* 4: 5-17.

Agardh, J.G. (1848). *Species genera et ordines algarum*, seu descriptiones succinctae specierum, generum et ordinum, quibus algarum regnum constituitur. Volumen Primum. Algas fucoideas complectens. Lundae [Lund]: C.W.K. Gleerup. 363 pp.

Anderson, R.J., Stegenga, H., & Bolton, J.J. (2016). Seaweeds of the South African South Coast. World Wide Web electronic publication, University of Cape Town, http://southafrseaweeds.uct.ac.za.

Ardissone, F. & Strafforello, J. (1877). *Enumerazione delle Alghe di Liguria*. pp. [1]-238. Milano: Tip. Editrice Lombarda.

Barton, E.S. (1896). Cape algae. *Journal of Botany, British and Foreign* 34: 193-198.

Barton, E.S. (1898). On the fruit of *Chnoospora fastigiata*. *Journal of the Linnean Society of London, Botany* 33: 507-508.

Batters, E.A.L. (1888). A description of three new marine algae. *Journal of the Linnean Society of London, Botany* 24: 450-453.

Belton, G.S., Prud'homme van Reine, W.F., Huisman, J.M., Draisma, S.G.A. & Gurgel, C.F.D. (2014). Resolving phenotypic plasticity and species designation in the morphology challenging

Caulerpa racemosa-peltata complex (Caulerpaceae, Chlorophyta). *Journal of Phycology* 50: 32-54.

Bessey, C.E. (1907). A synopsis of plant phyla. *University Studies of the University of Nebraska* 7: 275-373.

Boo, G.H., Lindstrom, S.C., Klochkova, N.G., Yotsukura, N., Yang, E.C., Kim, H.G., Waaland, J.R., Cho, G.Y., Miller, K.A. & Boo, S.M. (2011b). Taxonomy and biogeography of *Agarum* and *Thalassiophyllum* (Laminariales, Phaeophyceae) based on sequences of nuclear, mitochondrial, and plastid markers. *Taxon* 60: 831–840.

Boo, S.M., Kim, H.S., Shin, W., Boo, G.H., Cho, S.M., Jo, B. Y., Kim, J.H., Yang, E.C., Siver, P.A., Wolfe, A.P., Bhattacharya, D., Andersen, R.A. & Yoon, H.S. (2010). Complex phylogeographic patterns in the freshwater alga *Synura* provide new insights on ubiquity versus endemism in microbial eukaryotes. *Molecular Ecology* 19: 4328–4338.

Boo, S.M., Lee, K.M., Cho, G.Y. & Nelson, W. (2011a). *Colpomenia claytonii* sp. nov. (Scytosiphonaceae, Phaeophyceae) based on morphology and mitochondrial cox3 sequences. *Botanica Marina* 54: 159-167.

Børgesen, F. (1914). The marine algae of the Danish West Indies. Part 2. Phaeophyceae. *Dansk Botanisk Arkiv* 2(2): 1-68.

Børgesen, F. (1928). On *Rosenvingea stellata*, a new Indian alga, and on an interesting littoral algal vegetation in which this species is a characteristic constituent. *Dansk Botanisk Arkiv* 5(6): 11.

Børgesen, F. (1930). Some Indian green and brown algae especially from the shores of the Presidency of Bombay. *Journal of the Indian Botanical Society* 9: 151-174.

Børgesen, F. (1939). Marine algae from the Iranian Gulf especially from the innermost part near Bushire and the Island Kharg. In: *Danish Scientific Investigations in Iran, Part 1*. (Jessen, K. & Spärck, R. Eds) Copenhagen: Ejnar Munksgaard. pp. 47-141.

Bornet, E. (1892). Les algues de P.-K.-A. Schousboe. *Mémoires de la Société des Sciences Naturelles et Mathématiques de Cherbourg* 28: 165-376.

Bory de Saint-Vincent, J.B.G.M. (1825). *Hydroclathre*. *Dictionnaire d'histoire naturelle* 8: 419.

Bory de Saint-Vincent, J.B.G.M. (1828). *Botanique, Cryptogamie*. In: *Voyage autour du monde, exécuté par ordre du Roi, sur la corvette de Sa Majesté, la Coquille, pendant les années 1822, 1823, 1824 et 1825*. (Duperrey, L.I. Eds). Paris: Bertrand. pp. 97-200.

Brodie, J., Walker, R.H., Williamson, C. & Irvine, L.M. (2013). Epitypification and redescription of *Corallina officinalis* L., the type of the genus, and *C. elongata* Ellis et Solander (Corallinales, Rhodophyta). *Cryptogamie Algologie* 34: 49-56.

Brophy, T. C & Murray, S.N. (1989). Field and culture studies of a population of *Endarachne binghamiae* (Phaeophyta) from southern California. *Journal of PhycoIogy* 25: 6-15.

Calumpong, H.P. & Meñez, E.G. (1997). Field guide to the common mangroves, seagrasses and algae of the Philippines. Bookmark Inc., Makati City, Philippines. 197 pp.

Camus, C., Meynard, A.P., Faugeron, S., Kogame, K. & Correa, J.A. (2005). Differential life history phase expression in two coexisting species of *Scytosiphon* (Phaeophyceae) in northern Chile. *Journal of Phycology* 41: 931-941.

Charrier, B., Bail, A.L. & Reviers, B.D. (2012). Plant Proteus: brown algal morphological plasticity and underlying developmental mechanisms. *Trends in Plant Science* 17: 468-477.

Cho, G.Y. Kogame, K. & Boo, Y.-M. (2006). Molecular phylogeny of the family Scytosiphonaceae (Phaeophyceae). *Algae* 12(2): 175-183.

Cho, G.Y., Yoon, H.S., Choi, H.-G., Kogame, K. & Boo, S.M. (2001). Phylogeny of the family Scytosiphonaceae (Phaeophyta) from Korea based on sequences of plastid-encoded RuBisCo spacer region. *Algae* 16: 145-150.

Cho, T.O., Cho, G.Y., Yoon, H.S., Boo, S.M. & Lee, W.J. (2003). New records of *Myelophycus cavus* (Scytosiphonaceae, Phaeophyceae) in Korea and the taxonomic position of the genus on the basis of a plastid DNA phylogeny. *Nova Hedwigia* 76: 381-397.

Clayton, M.N. (1975). A study of variation in Australian species of *Colpomenia* (Phaeophyta, Scytosiphonales). *Phycologia* 14: 187-195.

Clayton, M.N. (1976). The morphology, anatomy and life history of a complanate form of *Scytosiphon lomentaria* (Scytosiphonales, Phaeophyta) from southern Australia. *Marine Biology, Berlin* 38: 201-208.

Clayton, M.N. (1980). Sexual reproduction—a rare occurrence in the life history of the complanate form of *Scytosiphon* (Scytosiphonaceae, Phaeophyta) from southern Australia. *British Phycological Journal* 15: 105-118.

Clayton, M.N. (1982). Life history studies in the Ectocarpales (Phaeophyta): contributions toward the understanding of evolutionary processes. *Botanica Marina* 25: 111-116.

Derbès, F. & Solier, A.J.J. (1851). *Algues*. In: *Supplément au catalogue des plantes qui croissent naturellement aux environs de Marseille*. (Castagne, L.). Aix: Nicot & Pardigon. pp. [1]-125.

Draisma, S.G.A., Peters, A.F., & Fletcher, R.L. (2003) Evolution and taxonomy in the Phaeophyceae: effects of the molecular age on brown algal systematics. In: *Out of the Past* (Norton, T.A., Ed). pp. 87-102.

Edelstein, T., Chen, L.C.-M. & McLachlan, J. (1971). On the life histories of some brown algae from eastern Canada. *Canadian Journal of Botany* 49: 1247-1251.

Farlow, W.G. (1881 '1882'). The marine algae of New England. In: *Report of the U.S. Commissioner of Fish and Fisheries for 1879*. (Eds), pp. 1-210. Washington.

Feldmann, J. (1949). *L'ordre des Scytosiphonales*. *Mémoires hors-série de la Société d'Histoire Naturelle de l'Afrique du Nord* 2(Travaux botaniques dédiés a René Maire): 103-115.

Fletcher, R.L. (1987). *Seaweeds of the British Isles. Vol. 3. Fucophyceae (Phaeophyceae). Part 1.* British Museum (Natural History), London. 359 pp.

Fotos, S.F. (1981). Observations on *Chnoospora minima* (Hering) Papenfuss (Phaeophyta, Scytosiphonales) in the field and in culture. *Japanese Journal of Phycology* 29: 101-108.

Gabriel, D., Draisma, S.G.A., Sauvage, T., Schmidt, W.E., Schils, T., Lim, P.-E., Harris, J. & Fredericq, S. (2016). Multilocus phylogeny reveals *Gibsmithia hawaiiensis* (Dumontiaceae, Rhodophyta) to be a species complex from the Indo-Pacific, with the proposal of *G. eilatensis* sp. nov. *Phytotaxa* 277: 1-20.

Ganzon-Fortes, E.T., Trono, G.C., Jr, Villanueva, R.D., Romero, J.B., Montaño, M.N.E. (2012). 'Endong', a rare variety of the farmed carrageenophyte *Eucheuma denticulatum* (Burman) Collins & Hervey from the Philippines. *Journal of Applied Phycology* 24: 1107-1111.

Global Invasive Species Database. (2017a). Species profile: *Caulerpa taxifolia*. Downloaded from http://www.iucngisd.org/gisd/speciesname/Caulerpa+taxifolia.

Global Invasive Species Database. (2017b). Species profile: *Sargassum muticum*. Downloaded from http://www.iucngisd.org/gisd/speciesname/Sargassum+muticum.

Grunow, A. (1873/1874). Algen der Fidschi-, Tonga- und Samoa-Inseln, gesammelt von Dr. E. Graeffe. *Journal des Museum Godeffroy* 3: 23-50.

Guiry, M.D. & Guiry, G.M. (2017). *AlgaeBase*. World-wide electronic publication, National University of Ireland, Galway. http://www.algaebase.org.

Hamel, G. (1937). Phaeophycées de France, fasc. III. Paris. pp. 177-240.

Hering, [K.] (1841). Diagnoses algarum novarum a cl. Dre. Ferdinand Krauss in Africa Australi lectarum. *Annals and Magazine of Natural History [Series 1]* 8: 90-92.

Hind, K. R., Gabrielson, P. W. & Saunders, G. W. (2014). Molecular-assisted alpha taxonomy reveals pseudocryptic diversity among species of *Bossiella* (Corallinales, Rhodophyta) in the eastern Pacific Ocean. *Phycologia* 53: 443-456.

Hind, K.R., Miller, K. A., Young, M., Jensen, C., Gabrielson, P. W. & Martone, P. (2015). Resolving cryptic species of *Bossiella* (Corallinales, Rhodophyta) using contemporary and historical DNA. *American Journal of Botany* 102: 1-19.

Hollenberg, G.J. & Abbott, I.A. (1966). *Supplement to Smith's marine algae of the Monterey Peninsula*. Stanford: Stanford University Press. 130 pp.

Howe M.A. (1920). Algae. In: *The Bahama Flora*. (Britton, N. L. & Millspaugh, C. F., Eds) New York, pp. 553-618.

Huelsenbeck, J.P. & Ronquist, F.R. (2001). MrBayes: Bayesian inference of phylogeny. *Biometrics* 17: 754-755.

Huisman, J. (2016). The age of discovery is still with us (Algae Highlights). *Journal of Phycology* 52(1): 37-39.

Hurtado-Ponce, A.Q. & Umezaki, I. (1987). Life history of *Hydroclathrus* (Bory) Howe (Scytosiphonales, Phyeophyceae) in laboratory culture. *The Philippine Journal of Science* 116: 227-244.

Johnson K.A. & Dromgoole F.I. (1977). Occurrence of *Hydroclathrus clathratus* in northern New Zealand waters. *New Zealand Journal of Marine and Freshwater Research* 11: 269-272.

Kamal, C. & Sethuraman, M.G. (2013). *Hydroclathrus clathratus* marine alga as a green inhibitor of acid corrosion of mild steel. *Research on Chemical Intermediates* 39: 3813-3828.

Kawai, H. & Henry, E.C. (2017). Phaeophyta. In: *Handbook of the Protists* (Archibald *et al.*, Eds). Springer International Publishing Switzerland. pp. 267-304.

Kawai, H., Hanyuda, T., Draisma, S.G.A., Wilce, R.T. & Andersen, R.A. (2015). Molecular phylogeny of two unusual brown algae, *Phaeostrophion irregular* and *Platysiphon glacialis*, proposal of the Stschapoviales ord. nov. and Platysiphonaceae fam. nov., and a re-examination of divergence times for brown algal orders. *Journal of Phycology* 51: 918-928.

Kawai, H., Hanyuda, T., Gao, X., Terauchi, M., Miyata, M., Lindstrom, S.C., Klochkova, N.G. & Miller, K.A. (2017). Taxonomic revision of the Agaraceae with a description of *Neoagarum* gen. nov. and reinstatement of *Thalassiophyllum*. *Journal of Phycology* 53(2): 261-270.

Kawai, H., Holand, W. & Müller, D.G. (1994). Sexual reproduction and sexual pheromones in *Myelophycus simplex* (Harvey) Papenfuss (Phaeophyceae). *Japanese Journal of Phycology* 42: 227-231.

Kogame, K & Masuda, M. (2001). Crustose sporophytes of *Colpomenia bullosa* (Scytosiphonaceae, Phaeophyceae) in nature. *Cryptogamie, Algologie* 22: 201–208.

Kogame, K. & Kawai, H. (1993) Morphology and life history of *Petalonia zosterifolia* (Reinke) O. Kuntze (Scytosiphonales, Phaeophyceae) from Japan. *Japanese Journal of Phycology* 41: 29-37.

Kogame, K. & Yamagishi, Y. (1997). The life history and phenology of *Colpomenia peregrina* (Scytosiphonales, Phaeophyceae) from Japan. *Phycologia* 36: 337-344.

Kogame, K. (1994). A taxonomic study of the family Scytosiphonaceae (Scytosiphonales, Phaeophyceae) in Japan. Ph.D. dissertation, Hokkaido University, Sapporo. 158 pp.

Kogame, K. (1996). Morphology and life history of *Scytosiphon canaliculatus* comb. nov. (Scytosiphonales, Phaeophyceae) from Japan. *Phycological Research* 44: 85-94.

Kogame, K. (1997). Life history of *Colpomenia sinuosa* and *Hydroclathtus clathratus* (Scytosiphonales, Phaeophyceae) in culture. *Phycological Research* 45: 277-231.

Kogame, K. (1998). A taxonomic study of Japanese *Scytosiphon* (Scytosiphonales, Phaeophyceae), including two new species. *Phycological Research* 46: 39-51.

Kogame, K. (2001). Life history of *Chnoospora implexa* (Chnoosporaceae, Phaeophyceae) in culture. *Phycological Research* 49: 123-128.

Kogame, K., Horiguchi, T. & Masuda, M. (1999). Phylogeny of the order Scytosiphonales (Phaeophyceae) based on DNA sequences of *rbc*L, partial *rbc*S, and partial LSU nr DNA. *Phycologia* 38: 496-502.

Kogame, K., Kurihara, A., Cho, G.Y, Lee, K.M., Sherwood, A.R. & Boo, S.M. (2011). *Petalonia tatewakii* sp. nov. (Scytosiphonaceae, Phaeophyceae) from the Hawaiian Islands. *Phycologia* 50: 563-573.

Kogame, K., Rindi, F., Peters, A.F. & Guiry, M.D. (2015). Genetic diversity and mitochondrial introgression in *Scytosiphon lomentaria* (Ectocarpales, Phaeophyceae) in the north-eastern Atlantic Ocean. *Phycologia* 54: 367-374.

Kogame, K., Uwai, S., Shimada, S. & Masuda, M. (2005). A study of sexual and asexual populations of *Scytosiphon lomentaria* (Scytosiphonaceae, Phaeophyceae) in Hokkaido, northern Japan, using molecular markers. *European Journal of Phycology* 40: 313-322.

Kraft, G.T. & Abbott, I.A. (2003). *Hydroclathrus* (Scytosiphonaceae, Phaeophyceae): conspectus of the genus and proposal of new species from Australia and Hawaii. *Phycological Research* 51(4): 244-258.

Kraft, G.T. (2009). *Algae of Australia. Marine benthic algae of Lord Howe Island and the southern Great Barrier Reef, 2. Brown algae*. Canberra & Melbourne: Australian Biological Resources Study and CSIRO Publishing. 364 pp.

Kuntze, O. (1898). *Revisio generum plantarum. Pars III (3)*. Leipzig, London, Milano, New York, Paris: Arthur Felix, Dulau & Co., U. Hoepli, Gust. A. Schechert, Charles Klincksierck. 576 pp.

Lane, C.E., Mayes, C., Druehl, L.D. & Saunders, G.W. (2006). A multi-gene molecular investigation of the kelp (Laminariales, Phaeophyceae) supports substantial taxonomic re-organization. *Journal of Phycology* 42: 493-512.

Le Jolis, A. (1863). Liste des algues marines de Cherbourg. *Mémoires de la Société Impériale des Sciences Naturelles de Cherbourg* 10: 5-168.

Lee, K.M., Boo, S.M., Kain (Jones), J.M. & Sherwood, A.R. (2013). Cryptic diversity and biogeography of the widespread brown alga *Colpomenia sinuosa* (Ectocarpales, Phaeophyceae). *Botanica Marina* 56: 15-25.

Lee, K.M., Hong, D.D. & Boo, S.M. (2014b). Phylogenetic relationships of *Rosenvingea* (Scytosiphonaceae, Phaeophyceae) from Vietnam based on *cox*3 and *psa*A sequences. *Algae* 29: 289-297.

Lee, K.M., Mansilla, A, Nelson, W.A. & Boo, S.M. (2012). *Colpomenia durvillei* (Scytosiphonaceae, Phaeophyceae): its distribution and relationships with other elongate species of the genus. *Botanica Marina* 55: 367-375.

Lee, K.M., Riosmena-Rodriguez, R., Kogame, K. & Boo, S.M. (2014a). *Colpomenia wynnei* sp. nov. (Scytosiphoniaceae, Phaeophyceae): a new species of marine algae from Northeast Asia. *Phycologia* 53: 480-487.

Littler, D.S. & Littler, M.M. (2000). *Caribbean reef plants*. An identification guide to the reef plants of the Caribbean, Bahamas, Florida and Gulf of Mexico.

Marquez G.P.B., Santiañez W.J.E., Trono G.C. Jr., Montaño M.N.E, Araki H., Takeuchi H. & Hasegawa T. (2014). Seaweed biomass of the Philippines: sustainable feedstock for biogas production. *Renewable and Sustainable Energy Reviews* 38: 1056-1068.

Matsumoto, K., Ichihara, K. & Shimada, S. (2014). Taxonomic reinvestigation of *Petalonia* (Phaeophyceae, Ectocarpales) in southeast of Honshu, Japan, with a description of *Petalonia tenuis sp. nov. Phycologia* 53: 127-136.

Mattio, L., Payri, C.E. & Stiger-Pouvreau, V. (2008). Taxonomic revision of *Sargassum* (Fucales, Phaeophceae) from French Polynesia based on morphological and molecular analyses. *Journal of Phycology* 44: 1541-1555.

Mattio, L., Payri, C.E. & Verlaque, M. (2009). Taxonomic revision and geographic distribution of the subgenus *Sargassum* (Fucales, Phaeophyceae) in the western and central Pacific islands based on morphological and molecular analyses. *Journal of Phycology* 45: 1213-1227.

Mattio, L., Payri, C.E., Verlaque, M. & De Reviers, B.D. (2010). Taxonomic revision of *Sargassum* sect. *Acanthocarpicae* (Fucales, Phaeophyceae). *Taxon* 59: 896-904.

McDevit, D.C. & Saunders, G.W. (2017). A molecular investigation of Canadian Scytosiphonaceae (Phaeophyceae) including descriptions of *Planosiphon* gen. nov. and *Scytosiphon promiscuus* sp. nov. *Botany* 95: 653-671.

McNeill, J., Barrie, F.R., Buck, W.R., Demoulin, V., Greuter, W., Hawksworth, D.L., Herendeen, P.S., Knapp, S., Prado, J., Prud'homme van Reine, W.F., Smith, G.F., Wiersema, J.H. & Turland, N.J. (2012). *International Code of Nomenclature for algae, fungi and plants (Melbourne Code) adopted by the Eighteenth International Botanical Congress Melbourne, Australia, July 2011. Regnum Vegetabile*, Vol. 154. Königstein: Koeltz Scientific Books. 208 pp.

Miller, M.A., Pfeiffer, W. & Schwartz, T. (2010). Creating the CIPRES Science Gateway for inference of large phylogenetic trees. In: Proceedings of the Gateway Computing Environments Workshop (GCE). Orleans, LA, pp. 1-8.

Misra, J.N. (1966). Phaeophyceae in India. *Indian Council of Agricultural Research Monographs on Algae* 7. Indian Council of Agricultural Research, New Delhi. 203 pp.

Nakamura, Y. & Tatewaki, M. (1975). The life history of some species of Scytosiphonales. *Scientific Papers of the Institute of Algological Research, Faculty of Science, Hokkaido University* 6: 57-93.

Natural History Museum (2014). Dataset: Collection specimens. Resource: Specimens. Natural History Museum Data Portal (data.nhm.ac.uk). http://dx.doi.org/10.5519/0002965. Retrieved 22 October 2017.

Nelson, W. (2013). *New Zealand seaweeds: An illustrated guide*. Te Papa Press, Wellington, New Zealand. 328 pp.

Nelson, W.A. & Duffy, C.A.J. (1991). *Chnoospora minima* (Phaeophyta) in Port Underwood, Marlborough—a curious new algal record for New Zealand. *New Zealand Journal of Botany* 29: 341-344.

Norris, J.N. (2010). Marine algae of the Northern Gulf of California: Chlorophyta and Phaeophyceae. *Smithsonian Contributions to Botany* 94: 1-276 .

N'Yeurt, A.D.R. & Payri, C.E. (2006). Marine algal flora of French Polynesia I. Phaeophyceae (Ochrophyta, brown algae). *Cryptogamie, Algologie* 27: 111-152.

Nylander, J.A.A. (2004). *MrModeltest v2*. Program distributed by the author. Evolutionary Biology Centre, Uppsala University.

Papenfuss, G.F. (1956). Notes on South African marine algae. IV. *Journal of South African Botany* 22: 65-77.

Parente, M.I., Neto, A.I. & Fletcher, R.L. (2003). Morphology and life history studies of *Endarachne binghamiae* (Scytosiphonaceae, Phaeophycota) from the Azores. *Aquatic Botany* 76: 109-116.

Parsons, M.J. (1982). *Colpomenia* (Endlicher) Derbès *et* Solier (Phaeophyta) in New Zealand. *New Zealand Journal of Botany* 20: 289-301.

Payo, D.A., Leliaert, F., Verbruggen, H., D'hondt, S., Calumpong, H.P. & De Clerck, O. (2013). Extensive cryptic species diversity and fine-scale endemism in the marine red alga *Portieria* in the Philippines. *Proceedings of the Royal Society of London B Biological Sciences* 280: 20122660.

Ramírez, M.E. & Rojas, V.G. (1991). El genero *Colpomenia* (F.C. Mertens ex Roth) Derbes et Solier (Phaeophyceae), en Chile. *Boletin del Museo Nacional de Historia Natural [Santiago de Chile]* 42: 11-24.

Roth, A.G. (1806). *Catalecta botanica quibus plantae novae et minus cognitae describuntur atque illustrantur. Fasciculus tertius cum tabulis aenaeis XII*. Lipsiae [Leipzig]: in Bibliopolio Io. Fr. Gleditschiano. 350 pp.

Rousseau, F. & Reviers, B.D. (1999). Circumscription of the order Ectocarpales (Phaeophyceae): bibliographical synthesis and molecular evidence. *Cryptogamie, Algologie* 20: 5-18.

San Diego-McGlone, M.L., Villanoy, C.L. & Aliño, P.M. (1995). Nutrient mediated stress on the marine communities of a coastal lagoon (Puerto Galera, Philippines). *Marine Pollution Bulletin* 31: 355-366.

Saunders, D.A. (1898). Phycological memoirs. *Proceedings of the California Academy of Sciences. Series 3, Botany* 1: 147-168.

Schneider, C.A., Rasband, W.S., & Eliceiri, K.W. (2012). NIH Image to ImageJ: 25 years of image analysis. *Nature Methods* 9: 671-675.

Schneider, C.W., Cianciola, E.N., Popolizio, T.R., Sagnuolo, D.S., & Lane, C. (2015). A molecular assisted alpha taxonomic study of the genus *Centroceras* (Ceramiaceae, Rhodophyta) in Bermuda reveals two novel species. *Algae* 30: 15-33.

Schultz, N.E., Lane, C.E., Le Gall, L., Gey, D., Bigney, A.R., De Reviers, B.D., Rousseau, F. & Schneider, C.W. (2015). A barcode analysis of the genus *Lobophora* (Dictyotales, Phaeophyceae) in the western Atlantic Ocean with four novel species and the epitypification of *L. variegata* (J.V. Lamouroux) E.C. Oliveira. *European Journal of Phycology* 50: 481-500.

Setchell, W.A. & Gardner, N.L. (1925). The marine algae of the Pacific coast of North America. Part III. Melanophyceae. *University of California Publications in Botany* 8: 383-898.

Silberfeld, T., Leigh, J.W., Verbruggen, H., Cruaud, C., Reviers, B.D. &Rousseau, F. (2010). A multi-locus time-calibrated phylogeny of the brown algae (Heterokonta, Ochrophyta, Phaeophyceae): Investigating the evolutionary nature of the "brown algal crown radiation". *Molecular Phylogenetics and Evolution* 56: 659-674.

Silberfeld, T., Rousseau, F. & Reviers, B.D. (2014). An updated classification of brown algae (Ochrophyta, Phaeophyceae). *Cryptogamie Algologie* 35: 117-156.

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.

Silva, P.C. & Reviers, B.D. (2000). Ordinal names in the Phaeophyceae. *Cryptogamie, Algologie* 21: 49-58.

Silva, P.C., Meñez, E.G. & Moe, R.L. (1987). *Catalog of the benthic marine algae of the Philippines*. *Smithsonian Contributions to Marine Sciences* 27, Smithsonian Institution Press, Washington. 179 pp.

Sissini, M.N., Barbosa de Barros Barretao, M.B., Széchy, M.T.M., Bouças de Lucena, M., Oliveira M.C., Gower, J., Liu, G., de Oliveira Bastos, E., Milstein, D., Gusmão, F., Martinelli-Filho, J.E., Alves-Lima, C., Colepicolo, P., Ameka, G., de Graft-Johnson, K., Gouveia, L., Torrano-Silva, B., Nauer, F., de Castros Nunes, J.M., Barufi, J.B., Rörig, L., Riosmena-Rodríguez, R., Mello, T.J., Lotufo, L.V.C. & Horta, P.A. (2017). The floating *Sargassum*(Phaeophyceae) of the South Atlantic Ocean- likely scenarios. *Phycologia* 56(3): 321-328.

Srinivasan, K.S. (1969). *Phycologia Indica: (Icones of Indian marine algae) vol. 1*. Botanical Survey of India, Calcutta. 52 pp.

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*. pp. [2] + 1-655. Cape Town: Bolus Herbarium, University of Cape Town.

Tamura, K., Stecher, G., Peterson, D., Filipski, A. & Kumar, S. (2013). MEGA6: Molecular Evolutionary Genetics Analysis version 6.0. *Molecular Biology and Evolution* 30: 2725-2729.

Tanaka, J. & Chihara, M. (1984). A new species of *Myelophycus* (*M. cavum* sp. nov.) with special reference to the systematic position of the genus (Dictyosiphonales, Phaeophyceae). *Phykos* 23: 12-162.

Taylor, W.R. (1960). *Marine algae of the eastern tropical and subtropical coasts of the Americas*. The University of Michigan Press, Ann Arbor. 870 pp.

Toste, M.F., Parente, M.I., Neto, A.I. & Fletcher, R.L. (2003a). Life history and phenology of *Hydroclathrus clathratus* (Scytosiphonaceae, Phaeophyceae) in the Azores. *Cryptogamie, Algologie* 24: 209-218.

Toste, M.F., Parente, M.I., Neto, A.I. & Fletcher, R.L. (2003b). Life history of *Colpomenia sinuosa* (Scytosiphonaceae, Phaeophyceae) in the Azores. *Journal of Phycology* 39: 1268-1274.

Trono G.C. Jr. (2001a). *Chnoospora* J. Agardh. In: *Plant resources of South-East Asia 15(1). Cryptogams: Algae* (Prud'homme van Reine, W. F. & Trono, G.C. Jr., Eds). Backhuys Publishers, Leiden, The Netherlands. pp. 131-132.

Trono G.C. Jr. (2001b). *Hydroclathrus* Bory. In: *Plant Resources of South-East Asia 15(1). Cryptogams: Algae* (Prud'homme van Reine, W. F. & Trono, G.C. Jr., Eds). Backhuys Publishers, Leiden, The Netherlands. pp. 207-208.

Trono, G.C. Jr. (1997). *Field guide and atlas of the seaweed resources of the Philippines***.** Bookmark, Makati City. 306 pp.

Trono, G.C., Jr & Ganzon-Fortes, E.T. (1988). *Philippine Seaweeds*. Manila, Philippines: National Book Store Inc. 330 pp.

Tseng, C.K. & Lu, B.R. (1983). Two new brown algae from the Xisha Islands, south China Sea. *Chinese Journal of Oceanology and Limnology* 1: 185-189.

Vandermeulen, H., DeWreede, R.E. & Cole, K.M. (1984). Nomenclatural recommendations for three species of *Colpomenia* (Scytosiphonales, Phaeophyta). *Taxon* 33: 324-329.

Vieira, C., D'Hondt, S., De Clerck, O. & Payri, C.E. (2014). Toward an inordinate fondness for stars, beetles and *Lobophora*? Species diversity of the genus *Lobophora* (Dictyotales, Phaeophyceae) in New Caledonia. *Journal of Phycology* 50: 1101-1119.

Wang H., Ooi, V.E.C. & Ang, P.O. Jr. (2007). Antiviral polysaccharides isolated from Hong Kong brown seaweed *Hydroclathrus clathratus*. *Science in China Series C* 50: 611-618.

Wang, H., Chiu, L.C.M., Ooi, V.E.C. & Ang, P.O. Jr. (2010b). A potent antitumor polysaccharide from the edible brown seaweed *Hydroclathrus clathratus*. *Botanica Marina* 53: 265-274.

Wang, H., Ooi, V.E.C. & Ang, P.O. Jr. (2010a). Anti-herpesviral property and mode of action of a polysaccharide from brown seaweed (*Hydroclathrus clathratus*). *World Journal of Microbiology and Biotechnology* 26: 1703-1713.

West, J.A., Zuccarello, G.C., Ganesan, E.K. & Loiseaux de Goër, S. (2015). Investigations into *Iyengaria*, a poorly known genus of the Scytosiphonaceae (Phaeophyceae) and description of a new species *Iyengaria quadriseriata*. *Phykos* 45: 43-50.

West, J.A., Zuccarello, G.C., Pedroche, F.F. & Loiseaux de Goër, S. (2010). *Rosenvingea orientalis* (Scytosiphonaceae, Phaeophyceae) from Chiapas, Mexico: life history in culture and molecular phylogeny. *Algae* 25: 187-195.

Womersley, H.B.S. (1987). *The marine benthic flora of southern Australia. Part II*. Adelaide: South Australian Government Printing Division. 481 pp.

Wynne, M.J. & Norris, J.N. (1976). The genus *Colpomenia* Derbès et Solier (Phaeophyta) in the Gulf of California. *Smithsonian Contributions to Botany* 35: 1-18.

Wynne, M.J. (1969). Life history and systematic studies of some Pacific North American Phaeophyceae (brown algae). *University of California Publications in Botany* 50: 1-88.

Wynne, M.J. (1972). Studies on the life forms in nature and in culture of selected brown algae. In: *Contributions to the systematics of benthic marine algae of the North Pacific*. (Abbott, I.A. & Kurogi, M. Eds). Kobe, Japan: Japanese Society of Phycology. pp. 133-143.

Wynne, M.J. (2005). A check-list of benthic marine algae of the tropical and subtropical western Atlantic: second revision. *Beihefte zur Nova Hedwigia* 129: 1-152.

Yaegashi, K., Yamagishi, Y., Uwai, S., Abe, T., Santiañez, W.J.E. & Kogame, K. (2015). Two species of the genus *Acinetospora* (Ectocarpales, Phaeophyceae) from Japan: *A. filamentosa* comb. nov. and *A. asiatica* sp. nov. *Botanica Marina* 58(5): 331-343.

Yamada, Y. (1948). Marine algae. In: *Icones of the marine animals and plants of Hokkaido. No. 1*. (Yamada, Y. & Kinoshita, S. Eds) Yoichi, Hokkaido: Hokkaido Fisheries Scientifc Institution.

Yoon, H.S., Hackett, J.D., Pinto, G. & Bhattacharya, D. (2002). The single ancient origin of chromist plastids. *Proceedings of the National Academy of Sciences of the United States of America* 99: 15507-15512.

Zanardini, G. (1843). *Saggio di classificazione naturale delle Ficee del Dottore Giovanni Zanardini aggiunti nuovi studii sopra l'androsace degli antichi con tavola miniata ed enumerazione di tutte le specie scoperte e raccolte dall'autore in Dalmazia*. pp. [1]-64. Venezia [Venice]: Dallo Stabilimento tipographico enciclopedico di Girolamo Tasso.

SUPPLEMENTARY TABLES

Table S1. List of species and their respective collection details, herbarium voucher codes, and GenBank accession numbers.

Species and collection details	Voucher	GenBank accession number			
		*cox*3	*cox*1	*psa*A	*rbc*L
***Chnoospora minima* (K. Hering) Papenfuss**					
Yonagunijima, Okinawa, Japan		KF700277		DQ239773	DQ239768
Polihua Beach, Lanai, Hawaii, U.S.A.; 27 Mar. 2008					AB578987
Tahai, Easter Is., Chile; 15 Mar. 2016	SGO168260	MG570399			
Tahai, Easter Is., Chile; 15 Mar. 2016	SGO168261	MG570400			
Tahai, Easter Is., Chile; 15 Mar. 2016	SGO168262	MG570401			
Vaihu, Easter Is., Chile; 18 Nov. 2016	EIS3-3095	MG584830			
***Colpomenia claytoniae* S.M. Boo, K.M. Lee, G.Y. Cho *et* W. Nelson**					
Sangkojam, Goseong, Korea; 12 Jan. 2005		HQ833785			
Island Bay, Wellington, New Zealand; 30 Jul. 2004					KJ418173
***Colpomenia expansa* (D.A. Saunders) Y.P. Lee**					
Damuraemi, Chujado, Korea; 26 May 2009		HQ833780			
Mukri, Chujado, Korea; 24 May 2009					JQ918816
***Colpomenia peregrina* Sauvageau**					
Sancheon, Gangreung, Korea; 23 Feb. 1999		HQ833767			
Anin, Gangreung, Korea; date unknown				DQ239776	
Monterey Bay, California, USA; 11 Dec. 1999					AY398464
***Colpomenia ramosa* W.R. Taylor**					
Punta La Esmeralda, Baja California, Mexico; 2 Dec. 2006		JQ918789			
***Colpomenia sinuosa* (Mertens *ex* Roth) Derbès *et* Solier**					
Black Bock, South Africa; 10 Aug. 2005		HQ833778			
Castillo san Cristobal, Gran Canaria, Spain; 25 Apr. 2004					AY785710
Guryongpo, Pohang, Korea; 16 Nov. 2002				AY372950	
***Colpomenia tuberculata* D.A. Saunders**					
El Sargento, Baja California, Mexico; 11 May 2009		HQ833773			JQ918817
Dactylosiphon bullosus* (D.A. Saunders) Santiañez, K.M. Lee, S.M. Boo *et* Kogame *comb. nov.					
Cattage, Melbourne, Australia; 18 Dec. 2010		JQ918797			
San Juan Island, Seattle, U.S.A.; 10 Feb. 2010		JQ918795			
Weller's Rock, Dunedin, New Zealand; date unknown				DQ239775	
Kainga Reef, Burrewarra Point, Australia; 1 Jan. 2009					GU014704
Pachena Beach, Bamfield, British Columbia, Canada; 12 Jun. 20016					KF281385
Akkeshi, Hokkaido, Japan; 9 Jul. 2017					
Akkeshi, Hokkaido, Japan; 9 Jul. 2017					
Dactylosiphon durvillei* (Bory de Saint-Vincent) Santiañez, K.M. Lee, S.M. Boo *et* Kogame *comb. nov.					
Los Molles, Coquimbo, Chile; 28 Oct. 2011		JQ918806			JQ918820
Puerto Peñasco, Sonora, Mexico; 8 Feb. 2013		KF700311			
Dactylosiphon wynnei* (K.M. Lee, R. Riosmena-Rodriguez, Kogame *et* S.M. Boo) Santiañez, K.M. Lee, S.M. Boo *et* Kogame *comb. nov.					
Hoedong, Jindo, Korea; 2 Mar. 2013		KF700280			KF700324

Species and collection details	Voucher	GenBank accession number			
		*cox*3	*cox*1	*psa*A	*rbc*L
***Hydroclathrus clathratus* (C.Agardh) Howe**					
Sakurajima I., Kagoshima, Japan; 29 Mar 2006	SAP115281	MF431998	MF432069	MF431955	MF431949
Sakurajima I., Kagoshima, Japan; 29 Mar 2006	SAP115282	MF431999	MF432070		
Sakurajima I., Kagoshima, Japan; 29 Mar 2006	SAP115283	MF432000	MF432071		
Sakurajima I., Kagoshima, Japan; 29 Mar 2006	SAP115284	MF432001	MF432072		
Sakurajima I., Kagoshima, Japan; 29 Mar 2006	SAP115285	MF432002	MF432073		
Tsuyazaki, Fukuoka, Japan; 23 Mar 2015	SAP115310	MF432024	MF432096		
Tsuyazaki, Fukuoka, Japan; 23 Mar 2015	SAP115312	MF432026			
Tsuyazaki, Fukuoka, Japan; 23 Mar 2015	SAP115313	MF432027			
Tsuyazaki, Fukuoka, Japan; 23 Mar 2015	SAP115314	MF432028			
Tsuyazaki, Fukuoka, Japan; 23 Mar 2015	SAP115315	MF432029			
Tassya, Sado I., Niigata, Japan; 17 Aug 2015	SAP115328	MF432040	MF432109		
Tassya, Sado I., Niigata, Japan; 17 Aug 2015	SAP115329	MF432041	MF432110		
Tassya, Sado I., Niigata, Japan; 17 Aug 2015	SAP115330	MF432042			
Tassya, Sado I., Niigata, Japan; 17 Aug 2015	SAP115331	MF432043			
Banshozaki, Wakayama, Japan; 8 Mar 2015	SAP115316	MF432030			
Sado I., Niigata, Japan; 8 Jul 1990	SAP115344	MF432113			
Tonggumi, Ulleungdo, Korea; 27 Aug 2003	PE790	MF431989			
Tonggumi, Ulleungdo, Korea; 27 Aug 2003	PE791	MF431990			
Shinyang, Jeju, Korea; 6 Jun 2009	CNU5625	MF431968			
Nishiura, Tsushima I., Nagasaki, Japan; 26 Mar 2009	CNU3728	MF431967			
Pangil South, Ilocos Norte, Philippines; 11 Mar 2005	PE597	MF431985			
Pangil South, Ilocos Norte, Philippines; 11 Mar 2005	PE598	MF431986			
Rocktail Beach, South Africa; 11 Aug 2005	PE703	MF431987			
Black Bock, South Africa; 10 Aug 2005	PE706	MF431988			
***Hydroclathrus stephanosorus* Kraft**					
Sumuide, Nago, Okinawa, Japan; 28 Apr 2013	SAP115279	MF431996	MF432067	MF431957	
Tsuyazaki, Fukuoka, Japan; 23 Mar 2015	SAP115311	MF432025	MF432097		
Moroiso, Misaki, Kanagawa, Japan; 24 Mar 2008	SAP115291	MF432006	MF432079		
Moroiso, Misaki, Kanagawa, Japan; 24 Mar 2008	SAP115292		MF432080		
Moroiso, Misaki, Kanagawa, Japan; 24 Mar 2008	SAP115293	MF432007	MF432081		
Moroiso, Misaki, Kanagawa, Japan; 24 Mar 2008	SAP115294	MF432008	MF432082		
Haemita, Iriomote I., Okinawa, Japan; 12 Mar 2008	SAP115306	MF432020	MF432092		
Haemita, Iriomote I., Okinawa, Japan; 12 Mar 2008	SAP115308	MF432022	MF432094		
Innoshima I., Hiroshima, Japan; 20 Apr 2015	SAP115317	MF432031	MF432098		
Innoshima I., Hiroshima, Japan; 20 Apr 2015	SAP115318	MF432032	MF432099	MF431951	MF431946
Innoshima I., Hiroshima, Japan; 20 Apr 2015	SAP115319		MF432100		
Tassya, Sado I., Niigata, Japan; May 2015	SAP115320	MF432033	MF432101		
Tassya, Sado I., Niigata, Japan; May 2015	SAP115321	MF432034	MF432102		
Tassya, Sado I., Niigata, Japan; May 2015	SAP115322		MF432103		
Tassya, Sado I., Niigata, Japan; May 2015	SAP115323	MF432035	MF432104		
Tassya, Sado I., Niigata, Japan; May 2015	SAP115324	MF432036	MF432105		
Tassya, Sado I., Niigata, Japan; May 2015	SAP115325	MF432037	MF432106		
Pupukea, Oahu, Hawaii; 12 Jun 2007	SAP115332	MF432044			
Kahala, Oahu, Hawaii; 14 Jun 2007	SAP115333	MF432045			
Site 3, Hawaii; 1 Mar 2008	SAP115334	MF432046			
Ulehawa, Hawaii; 1 Feb 2009	SAP115336	MF432048			
Banda, Tateyama, Chiba, Japan; 21 Mar 2016	SAP115337	MF432049	MF432111		
Banda, Tateyama, Chiba, Japan; 21 Mar 2016	SAP115338	MF432050	MF432112		
Banda, Tateyama, Chiba, Japan; 21 Mar 2016	SAP115339	MF432051			
Yura, Awaji I., Hyogo, Japan; 23 Mar 2016	SAP115340	MF432052			
Azores, Portugal; May 2016	SAP115341	MF432053			
Azores, Portugal; May 2016	SAP115342	MF432054			

Species and collection details	Voucher	GenBank accession number			
		*cox*3	*cox*1	*psa*A	*rbc*L
Azores, Portugal; May 2016	SAP115343	MF432055		MF431954	
Chagwido, Jeju, Korea; 17 Apr 2003	PE142	MF431980			
Sail Rock, Kaosiung, Taiwan; 27 Mar 2004	PE281	MF431981			
Hitakatsu, Tsushima, Japan; 25 Mar 2009	CNU3727	MF431966			
Isla Canal de Afuera, Veraguas, Panama; 14 Jan 2011	CNU27443	MF431965			
***Hydroclathrus tenuis* Tseng *et* Lu**					
Awase, Okinawa, Japan; 22 Apr 2013	SAP115273	MF431991	MF432061		
Awase, Okinawa, Japan; 22 Apr 2013	SAP115274	MF431992	MF432062		
Awase, Okinawa, Japan; 22 Apr 2013	SAP115275		MF432063		
Ohura, Okinawa, Japan; 27 Apr 2013	SAP115276	MF431993	MF432064		
Ohura, Okinawa, Japan; 27 Apr 2013	SAP115277	MF431994	MF432065		
Ohura, Okinawa, Japan; 27 Apr 2013	SAP115278	MF431995	MF432066		
Sumuide, Nago, Okinawa, Japan; 28 Apr 2013	SAP115280	MF431997	MF432068	MF431958	MF431950
Shiiragawa, Iriomote I., Okinawa, Japan; 11 Mar 2008	SAP115286		MF432074		
Senaga, Naha, Okinawa, Japan; 30 Mar 2009	SAP115287	MF432003	MF432075		
Senaga, Naha, Okinawa, Japan; 30 Mar 2009	SAP115288	MF432004	MF432076		
Senaga, Naha, Okinawa, Japan; 30 Mar 2009	SAP115289		MF432077		
Hoshisuna, Iriomote I., Japan; 10 Mar 2008	SAP115295	MF432009	MF432083		
Hoshisuna, Iriomote I., Japan; 10 Mar 2008	SAP115296	MF432010			
Senaga, Naha, Okinawa, Japan; 30 Mar 2009	SAP115300	MF432014	MF432087		
Senaga, Naha, Okinawa, Japan; 30 Mar 2009	SAP115301	MF432015	MF432088		
Senaga, Naha, Okinawa, Japan; 30 Mar 2009	SAP115302	MF432016			
Maeragawa, Iriomote I., Okinawa, Japan; 11 Mar 2008	SAP115303	MF432017	MF432089		
Maeragawa, Iriomote I., Okinawa, Japan; 11 Mar 2008	SAP115304	MF432018	MF432090		
Maeragawa, Iriomote I., Okinawa, Japan; 11 Mar 2008	SAP115305	MF432019	MF432091		
Haemita, Iriomote I., Okinawa, Japan; 12 Mar 2008	SAP115307	MF432021	MF432093		
Haemita, Iriomote I., Okinawa, Japan; 12 Mar 2008	SAP115309	MF432023	MF432095		
Barangay II, Calatagan, Batangas, Philippines; 10 Apr 2015	MSI27540	MF431975	MF432056		
Patar, Bolinao, Pangasinan, Philippines; 22 May 2015	MSI27541	MF431974	MF432057		
Patar, Bolinao, Pangasinan, Philippines; 22 May 2015	MSI27542	MF431976	MF432058		
Binabalian, Bolinao, Pangasinan, Philippines; 27 May 2015	MSI27897	MF431977	MF432059		
Silaki I., Bolinao, Pangasinan, Philippines; 27 May 2015	MSI27898	MF431978	MF432060		
Panglao I., Bohol, Philippines; 6 Jun 2015	SAP115326	MF432038	MF432107		
Panglao I., Bohol, Philippines; 6 Jun 2015	SAP115327	MF432039	MF432108		
Daedonghae Bay, Hainan, China; 12 Mar 2009	CNU002748	MF431959			
Daedonghae Bay, Hainan, China; 12 Mar 2009	CNU002760	MF431960			
Polihua, Lanai, Hawaii; 27 Mar 2008	SAP115335	MF432047			
Santiago Beach, Camotes I., Cebu, Philippines; 17 Jan 2017	MSI27899	MF431979			
Dapdap, Bulusan, Sorsogon, Philippines; 22 Apr 2013	CNU040489	MF431961			
Pangil South, Ilocos Norte, Philippines; 11 Mar 2005	PE594	MF431982			
Pangil South, Ilocos Norte, Philippines; 11 Mar 2005	PE595	MF431983			
Pangil South, Ilocos Norte, Philippines; 11 Mar 2005	PE596	MF431984			
Tembowong, Sekotong, Lombok, Indonesia; 25 Aug 2008	CNU5879	MF431969			
Mexican Pacific; date unknown	CNU25013	MF431962			
Mexican Pacific; date unknown	CNU25015	MF431963			

Species and collection details	Voucher	GenBank accession number			
		*cox*3	*cox*1	*psa*A	*rbc*L
Mexican Pacific; date unknown	CNU25016	MF431964			
Oahu Portlock, Hawaii; 17 Jul 2005	HS561	MF431973			
Oahu Paiko, Hawaii; 22 Dec 2006	HS1760	MF431970			
Oahu Portlock, Hawaii; 17 Jul 2005	HS1796	MF431971			
Hookena, Hawaii; 17 Mar 2007	HS2461	MF431972			
Hydroclathrus minutus* Santiañez *et* Kogame *sp. nov.					
Senaga, Naha, Okinawa, Japan; 30 Mar 2009	SAP115290	MF432005	MF432078	MF431956	MF431948
Taketomi I., Okinawa, Japan; 24 Mar 2001	SAP115345	MF432114			
Hydroclathrus rapanuii* Santiañez, Macaya *et* Kogame *sp. nov.					
Vaihu, Easter Is., Chile; 20 Mar. 2016	SGO168251	MG450663		MG450664	MG251837
Vaihu, Easter Is., Chile; 20 Mar. 2016	SGO168252	MG450662			
Vaihu, Easter Is., Chile; 20 Mar. 2016	SGO168250	MG450661			
Vaihu, Easter Is., Chile; 20 Mar. 2016	SGO168254	MG450660			
***Hydroclathrus* sp.**					
White Is., Kimberley, Australia; 11 Oct. 2016	PERTH 08714274				
***Melanosiphon intestinalis* (D.A. Saunders) M.J. Wynne**					
Kruz of Island, Sitka, Alaska; 13 Jul. 2006		KM587022			
Pachena Beach, Bamfield, British Columbia, Canada; 14 Sep. 2005				KF293725	KF293726
***Myelophycus cavus* Tanaka *et* Chihara**					
Woongdo, Taean, Korea; 15 Jun. 2000		KF700315		AY095319	DQ239781
***Myelophycus simplex* (Harvey) Papenfuss**					
Kurohae Beach, Chiba, Japan; 31 Jul. 2004		KF700316			
Daesado, Wando, Korea				AY372952	AY095320
Pseudochnoospora implexa* (J. Agardh) Santiañez, G.Y. Cho *et* Kogame *gen. et comb. nov.					
Port-Boisé, New Caledonia; date unknown		GQ368273			GQ368316
Sesoko, Okinawa, Japan; 8 Mar. 1990				DQ239772	AB022231
***Petalonia binghamiae* (J.Agardh) K.L. Vinogradova**					
Puraengi, Chujado, Korea; 23 May 2004		KF700317			
Munseom, Jeju, Korea; date unknown				DQ239782	
Kasumi, Hyogo, Japan; 3 Jun. 1991					AB022244
***Petalonia fascia* (O.F. Müller) Kuntze**					
St. Andrew's, New Brunswick, Canada; 5 Jun. 2006				KF281541	KF281386
Munseom, Jeju, Korea		HQ833766			
***Petalonia tatewakii* Kogame *et* A. Kurihara**					
Halawa, Molokai, Hawaii, USA; 12 Dec. 2009					AB579008
***Petalonia tenuis* Matsumoto *et* Shimada**					
Isshiki, Hayama, Kanagawa, Japan; 30 Dec. 2010					AB860192
***Petalonia tenella* (Kogame) Santiañez**					
Caleta, Palito, Chile; Jul. 2007		GU252556			
Muroran, Hokkaido, Japan; 22 Jan. 1989					AB022241
***Planosiphon complanatus* (Rosenvinge) McDevit *et* G.W. Saunders**					
Bay of Fundy, NB, Canada; 22 Mar. 2007				KF281561	KF281389
***Planosiphon gracilis* (Kogame) McDevit *et* G.W. Saunders**					
Hado, Jeju, Korea; 22 Mar. 2000		KF700323		DQ239786	
Ohma, Aomori, Japan; 2 Feb. 1990					AB022240
***Planosiphon zosterifolia* (Reinke) McDevit *et* G.W. Saunders**					
Onyangri, Uljin, Korea; 12 Jan. 2002		KF700318			
Bay of Fundy, NB, Canada; 31 Jan. 2007				KF281542	KF281387
***Rosenvingea intricata* (J. Agardh) Børgesen**					
Playa La Concha, La Paz, Mexico; 31 Mar. 2009		KM587011			
Isla Canal de Afuera, Veraguas, Panama; 14 Jan. 2011				DQ239784	
Gushikawa, Okinawa, Japan; 8 Mar. 1990					AB022260
***Rosenvingea* sp.**					
Canal de San Lorenzo, Baja California Sur, Mexico; 5 Dec. 2013		KM587021			

Species and collection details	Voucher	GenBank accession number			
		*cox*3	*cox*1	*psa*A	*rbc*L
***Rosenvingea orientalis* (J. Agardh) Børgesen**					
Manjagaw, Surigao, Philippines; date unknown	SAP115374	MG450659		MG450665	MG251836
Cam Ranh Bay, Nha Trang, Vietnam; 9 Apr. 2011		KM587019			
***Scytosiphon canaliculatus* (Setchell *et* N.L. Gardner) Kogame**					
San Clemente Island, CA, U.S.A., 17 Oct. 2009		KF700321			
Oshoro, Hokkaido, Japan; 16 May 1990					AB022239
***Scytosiphon dotyi* M.J. Wynne**					
Monterey Bay, CA, U.S.A., 11 Dec. 1999		KF700322		DQ239771	DQ239785
***Scytosiphon lomentaria* (Lyngbye) Link**					
Sormsangi, Chujado, Korea; 23 May 2005		HQ833765			
Narragansett, Rhode Is., USA; 23 Apr. 2007				KF281563	KF281390
***Scytosiphon promiscuus* McDevit *et* G.W. Saunders**					
Blacks Harbour, New Brunswick, Canada; 30 Mar. 2007				KF281594	KF281393
Tronoella ryukyuana* Santiañez *et* Kogame *gen. et sp. nov.					
Odo, Itoman, Okinawa, Japan; 28 Mar 2009	SAP115297	MF432011	MF432084	MF431952	MF431947
Odo, Itoman, Okinawa, Japan; 28 Mar 2009	SAP115298	MF432012	MF432085	MF431953	
Odo, Itoman, Okinawa, Japan; 28 Mar 2009	SAP115299	MF432013	MF432086		
***Ectocarpus siliculosus* (Dillwyn) Lyngbye**					
San Juan de Marcona, Peru; 1988		FP885846			
Hoedong, Jindo, Korea; 9 Mar. 2011					AY372978
Hanrim, Jeju, Korea; 15 May 2006				FN564540	
***Chordaria flagelliformis* (O.F. Müller) C. Agardh**					
Schleimünde, Germany; date unknown		JF796553			
Avacha Bay, Kamchatka, Russia; 24 Jul. 1998				AY372941	AY095324
***Pylaiella littoralis* (Linnaeus) Kjellmann**					
Roscoff, France; 1970		NC003055			
Friday Harbor, Washington, U.S.A.; Jul. 1992				AB899222	AB899288

Table S2. Sequencing primers used in this study.

Genetic region	Forward	Reverse
***cox*3**[1,2]	CAF4A	CAR4A
	F49	
***cox*1**[3]	GazF2	GazR2
***rbc*L**[4]	PRB-F0	PRB-R1A
	PRB-F2	PRB-R2
	PRB-F3	PRB-R3A
	rbcL3F	RSPR
***psa*A**[5]	psaA130F	psaA970R
	psaA870F	psaA1760R

[1]Kogame *et al.* (2005), [2]Boo *et al.* 2010, [3]Lane *et al.* (2006), [4]Kogame *et al.* (1999), [5]Yoon *et al.* (2002).

CURRICULUM VITAE

Wilfred John E. Santiañez was born on the 27th of September 1987 in General Santos City, Philippines. He obtained his high school diploma from the Mindanao State University–College of Education Training Department in 2004. After graduating from high school, Fred began studying marine biology as a scholar of the Department of Science and Technology of the Government of the Philippines at the College of Fisheries, Mindanao State University, General Santos City campus where he graduated *cum laude* in 2008. Shortly after graduating, he was employed as one of the in-house marine biologists at the Environmental Conservation and Protection Center (ECPC) of the Provincial Government of Sarangani. Therein, he worked as a technical team leader in coastal resources assessments and management as well as led several community-based environmental information and education campaigns. While working at ECPC, he realized the apparent need for coastal resource managers (CRM) and in May 2010 enrolled in the professional master's program for CRM at the Faculty of Management and Development Studies of the University of the Philippines Open University (UPOU). In August 2010, he moved to Diliman, Quezon City to work as a research associate at the Seaweed Biodiversity and Culture laboratory of Dr Gavino C. Trono Jr. in The Marine Science Institute, University of the Philippines. Therein, he began his training as a phycologist where he conducted basic and applied phycological research, especially on the taxonomy and ecology of tropical seaweeds as well as the mariculture of carrageenan-producing red seaweeds *Kappaphycus* and *Eucheuma*. Four years later, Fred obtained his Master in Environment and Natural Resources Management degree (with distinction) from UPOU in 2014. In that same year, he moved to Sapporo, Hokkaido, Japan to start his one-year research studentship at Hokkaido University through a Monbukagakusho scholarship grant from the Government of Japan (MEXT). Under the same scholarship program, Fred started his PhD in 2015 under the guidance of Dr Kazuhiro Kogame. Originally, his PhD research was focused on the taxonomy, molecular phylogeny, and distribution of the clathrate brown algal genus *Hydroclathrus*. Upon the discovery of the new genus *Tronoella* in early 2017, the scope of his PhD project was changed to cover the systematics (taxonomy, nomenclature, classification and molecular phylogeny) of the brown algal family Scytosiphonaceae.

LIST OF PUBLICATIONS

Santiañez, W.J.E., E.C. Macaya, K.M. Lee, G.Y. Cho, S.M. Boo and K. Kogame. (2018). Taxonomic reassessment of the Indo-Pacific Scytosiphonaceae (Phaeophyceae): *Hydroclathrus rapanuii sp. nov.* and *Chnoospora minima* from Easter Island, with proposal of *Dactylosiphon gen. nov.* and *Pseudochnoospora gen. nov*. *Botanica Marina* 61: 47–64. **[part of Chapters 2 and 3]**

Santianez, W.J.E., K.M. Lee, S. Uwai, A. Kurihara, P.J.L. Geraldino, E.T. Ganzon-Fortes, S.M. Boo and K. Kogame. 2018. Untangling nets: Elucidating the diversity and phylogeny of the clathrate brown algal genus *Hydroclathrus*, with the description of a new genus *Tronoella* (Scytosiphonaceae, Phaeophyceae). *Phycologia* 57: 61–78. **[part of Chapters 2 and 3]**

Santiañez, W.J.E. and K. Kogame. 2017. Transfer of *Petalonia filiformis* (Batters) Kuntze to the genus *Planosiphon* McDevit & G.W.Saunders (Scytosiphonaceae, Phaeophyceae). *Notulae Algarum* 40: 1–3. **[part of Chapter 3]**

Santiañez, W.J.E., H.J. Suan-Flandez and G.C. Trono, Jr. 2016. White rot disease and epiphytism on *Halymenia durvillei* Bory de Saint-Vincent (Halymeniaceae, Rhodophyta) in culture. *Science Diliman* 28: 54–60.

Marquez, G.P.B., **W.J.E. Santiañez**, G.C. Trono, Jr., S.R.B. de la Rama, H. Takeuchi and T. Hasegawa. 2015. Seaweeds: a sustainable fuel source*. In* Tiwari B.K. and D.J. Troy (eds.). *Seaweed sustainability–Food and Non-food applications*. Academic Press, Elsevier Inc. pp. 421–458.

Santiañez, W.J.E., R.S. Sariego and G.C. Trono, Jr. 2015. The seaweed flora of the Balabac Marine Biodiversity Conservation Corridor (BMBCC), Southern Palawan, Western Philippines. *Plant Ecology and Evolution* 148: 267–282.

Yaegashi, K., Y. Yamagishi, S. Uwai, T. Abe, **W.J.E. Santiañez** and K. Kogame. 2015. Two species of the genus *Acinetospora* (Ectocarpales, Phaeophyceae) from Japan: *A. filamentosa* comb. nov. and *A. asiatica* sp. nov. *Botanica Marina* 58: 331–343.

Marquez, G.P.B., **W.J.E. Santiañez,** G. C. Trono, Jr., M.N.E. Montaño, H. Araki, H. Takeuchi and T. Hasegawa. 2014. Seaweed biomass of the Philippines: Sustainable feedstock for biogas production. *Renewable and Sustainable Energy Reviews* 38: 1056–1068.

Santiañez, W.J.E. and G. C. Trono, Jr. 2013. Taxonomy of the genus *Sargassum* (Fucales, Phaeophyceae) in Alabat Island, Quezon, Northeastern Philippines. *Science Diliman* 25: 29–50.