Manzaea minuta gen. & comb. nov. (Scytosiphonaceae, Phaeophyceae) from the tropical Northwestern Pacific Ocean

Wilfred John E. Santiañez* and Kazuhiro Kogame2

Abstract

Recent molecular-assisted taxonomic studies on the brown algal genus Hydroclathrus has resulted in discoveries of new taxa in the family Scytosiphonaceae, both at the genus and species level. However, phylogenetic studies on Hydroclathrus based on wide geographical sampling also suggested that the genus is not monophyletic. That is, one of the recently described species Hydroclathrus minutus is consistently segregated from the Hydroclathrus main clade. We propose here to segregate H. minutus from the brown algal genus Hydroclathrus and establish the new monotypic genus Manzaea (i.e., Manzaea minuta gen. & comb. nov.) based on information on molecular phylogenetics and morpho-anatomy. Morphologically, M. minutus is similar to Hydroclathrus and Tronoella in having clathrate (net-like) and spreading thalli but is differentiated from the latter two genera in having membranous thalli that are sometimes interadhesive resulting in portions of the thallus forming amorphous clumps. Additionally, Manzaea is distinguished from both clathrate genera in having thick-walled medullary cells and short closely arranged quadratiseriate plurangia. Phylogenetic analyses (Maximum Likelihood and Bayesian Inference) based on single (plastidial psaA and rbcL genes) and concatenated (cox3 + psaA + rbcL) genes showed that M. minutus is consistently segregated from the highly supported clade of Hydroclathrus species and often clustering with Tronoella and/or Rosenvingea. Our proposal further increases the diversity of monotypic genera in the Scytosiphonaceae and underscores the need to conduct further studies on tropical seaweed biodiversity.

Keywords: brown seaweed, Hydroclathrus, Japan, phylogeny, taxonomy

Introduction

The net-like and spreading brown algal genus Hydroclathrus Bory de Saint-Vincent (1825) possesses the most distinct morphologies in the family Scytosiphonaceae Ardisone & Straforello (1877), making it readily identifiable in the field. Hydroclathrus species occur in tropical to warm temperate waters, inhabiting the shallow intertidal to depths of 70 m (Kraft & Abbott 2003, Santiañez et al. 2018a). Similar to other genera in the Scytosiphonaceae, Hydroclathrus is species-poor with only six known species. Of these, the type species of the genus, H. clathratus (C.Agardh) M.Howe (1920) (as H. cancellatus Bory, nom. illeg.), along with H. tenuis C.K.Tseng & Lu Baroen, H. tilesii (Endlicher) Santiañez & M.J.Wynne (as H. stephanosorus Kraft; Kraft & Abbott 2003, Santiañez & Wynne 2019), and H. tumulis Kraft & Abbott (Kraft & Abbott 2003) were described based solely on morpho-anatomy and some aspects of their ecology. Recent molecular-assisted taxonomic studies have resulted in two new additions to the genus: H. minutus Santiañez & Kogame based on specimens from Okinawa, Japan (Santiañez et al. 2018a) and H. rapanuii Santiañez, Macaya & Kogame from the geographically isolated Easter Island (Santiañez et al. 2018b). Molecular phylogenetic relationships of the species within Hydroclathrus, however, remain unresolved both in single gene- (i.e., mitochondrial cox3 and plastidial psaA and rbcL) and concatenated gene-based (i.e., cox3 + psaA + rbcL) phylogenetic analyses (Santiañez et al. 2018a, 2018b, Santiañez & Kogame 2019, Santiañez & West 2019, Santiañez et al. 2020). That is, all Hydroclathrus species with available sequences are resolved in a highly supported clade except H. minutus. In describing H. minutus, Santiañez et al. (2018a) already noted the phylogenetic segregation of the species from other Hydroclathrus as well as the apparent paraphyly in the genus. Although not explicitly mentioned in their report, the species was classified under Hydroclathrus primarily because of its net-like thallus.

The family Scytosiphonaceae has been known to exhibit a high degree of paraphyly and polyphyly in several genera based on earlier phylogenetic studies (Kogame et al. 1999, Cho et al. 2001, 2006). Recent studies on the diversity and systematics of the Scytosiphonaceae, however, underscored the weakness of using gross morphologies in delineating taxa at the genus level (McDevit & Saunders 2017, Santiañez et al. 2018a, 2018b, Santiañez & Kogame 2019). Subsequent detailed reassessment of the taxonomies of the different scytosiphonacean taxa guided by molecular phylogenies thus resulted in the segregation and transfer of several taxa to new genera such as Planosiphon McDevit & Saunders (2017), Dactylosiphon Santiañez et al. (2018b), and Pseudechnoospora Santiañez et al. (2018b). Considering that H. minutus is always excluded from other Hydroclathrus in single gene and concatenated gene-based molecular phylogenies and with the combination of distinct morpho-anatomical characteristics, we believe that the species is a representative of a distinct genus. As such, we propose here the new monotypic genus Manzaea Santiañez & Kogame to accommodate the species.

1G.T. Velasquez Phycological Herbarium and The Marine Science Institute, College of Science, University of the Philippines, Diliman, Quezon City, Philippines
2Department of Biological Sciences, Faculty of Science, Hokkaido University, Sapporo 06-0810, Japan
*Corresponding author, email: wjsantianez@msi.upd.edu.ph

Date Submitted: 16 December 2021
Date Accepted: 18 June 2022
Materials and Methods

Samples of *Hydroclathrus* collected from Japan, Hawaii, and Easter Island, along with samples used in our previous studies (Santiañez et al., 2018a, 2018b, 2020, Santiañez & West 2019), were used in this study. Morpho-anatomical observations and analyses on *Hydroclathrus minutus* samples were based on Santiañez et al. (2018a). DNA extraction to sequencing of newly generated *psaA* sequences from *Hydroclathrus* species (n=3; Table S1) followed those described in Santiañez et al. (2018a). For molecular phylogenetic analyses based on plastidial *psaA* and *rbcL* DNA sequence data, sequences of members of the family Scytosiphonaceae were downloaded from GenBank (*psaA*: 32 sequences; *rbcL*: 38 sequences). For molecular phylogenetic analyses based on *psaA* genes (n=35 sequences), *Ectocarpus siliculosus*, *Chordaria flagelliformis*, and *Adenocystis utricularis* were used as outgroups, while *E. siliculosus* and *Pylaella littoralis* were the outgroups for *rbcL* gene-based (n=38 sequences) phylogenetic analyses. These were aligned and subsequently subjected to Maximum Likelihood and Bayesian Inference analyses as described in Santiañez & Kogame (2019).

Results and Discussion

Three new *psaA* sequences from *Hydroclathrus* collected within the Pacific Ocean (Table S1) were generated in this study. Interspecific divergence (*P*-distance) among *Hydroclathrus* species based on *psaA* sequences ranged from 0.011 to 0.048. Of these, the highest divergence was observed between *H. minutus* and other *Hydroclathrus* species, which ranged from 0.033 to 0.048. Similarly, high sequence divergence between *H. minutus* and other *Hydroclathrus* species was found in *rbcL* sequences (0.023–0.026). In other brown seaweeds, *rbcL* sequence divergence among different genera ranges from 0.010 to 0.038 (Kawai & Hanyuda 2021).

![Figure 1](image-url)

*Figure 1.* Maximum likelihood (ML) phylogenetic tree for members of the family Scytosiphonaceae based on *psaA* gene sequences. Newly generated sequences in **bold**. ML bootstrap percentages (BP) and Bayesian posterior probabilities (PP) and are shown at each node. Thickened lines indicate highly supported nodes (PP: ≥0.98 and BP: ≥95%). Values <50% BP and <0.50 PP are removed.

---

**Manzaea minuta**
In both our psa and rbcL trees that included recently described and sequenced scytosiphonacean taxa (Figs. 1–2), *H. minutus* is recovered outside of the highly supported *Hydroclathrus* main clade (BP: 100%, PP: 1.00). In the psa gene tree (Fig. 1), *H. minutus* was recovered as a lineage that is basal to the highly supported *Rosenvingea* (*pro parte*)-*Hydroclathrus* clade (BP: 97%, PP: 1.00). This relationship is recovered in the rbcL tree (Fig. 2), yet *H. minutus* sits at the end of a long branch segregated from the *Hydroclathrus* main clade. In our most recent concatenated (*cox3 + psaA + rbcL*) gene-based phylogeny (Santiañez & Kogame 2019, fig. 2), *H. minutus* was shown to be more closely related to *Rosenvingea endiviifolia* (Martius) M.J.Wynne in Wynne & Nunes (2021) (*as Rosenvingea intricata* (J.Agardh) Børgesen (1914)) and *Tronoella ryukyuana* Santiañez & Kogame (Santiañez et al. 2018a), albeit with low support. Previous studies have indicated that unresolved relationships among scytosiphonacean taxa are likely artefacts of incomplete taxon sampling (i.e., tropical members of the Scytosiphonaceae remain understudied and their diversity poorly understood), the low resolving power of genetic markers currently being used, and incorrect generic assignments due to the wide and ambiguous morphological circumscriptions traditionally used among scytosiphonacean genera (Santiañez et al. 2018a, 2018b, Santiañez and Kogame 2019), among others.

*Hydroclathrus minutus* is similar in gross morphology to other *Hydroclathrus* species (Figs. 3A–B). In particular, the fine and fibrous thalli of *H. minutus* (Figs. 3A–B) are most similar to *H. tenuis*. However, *H. minutus* is distinct from other *Hydroclathrus* in having membranes that are occasionally interadhesive (Fig. 3C–D)—consequently resulting in an amorphous mass in those parts of the thallus—as well as in having very delicate, fine, and thin thalli. Unlike other *Hydroclathrus* with involute margins, those of *H. minutus* are only arched to folded inwardly. Additionally, the thalli of *H. minutus* also possess distinct thick-walled medullary cells (Fig. 3A–B).
Figure 3. *Manzaea minuta* Santíañez & Kogame gen. & comb. nov. habit and morpho-anatomical characters. A. Wet habit of the fine and convoluted thallus of the holotype (SAP15290) prior to mounting on herbarium sheet. B. Dried herbarium specimen of the holotype. C. Portion of the holotype thallus showing interadhesive membranes (arrows) with numerous subcircular plurangial sori with a sterile center (arrowheads). D. Closer view of portion of the thallus showing interadhesive membranes (arrow) and sterile center surrounded by plurangial sorus (arrowhead). E. Transverse section of the holotype showing arched membrane margins and pigmented cortical layer (stained darkly) bounding several layers of clear medullary cells with thickened cell walls. F. Closer view of a transverse section a layer of small broadly obovate to ovate to sometimes papillate and pigmented cortical cells (c) bounding several layers of larger, clear, and thick-walled medullary cells (m). Scale bars: A–B = 1 cm; C = 1 mm; D = 0.5 mm; E = 100 µm; F = 50 µm.
Morphological characters of clathrate genera in the family Scytosiphonaceae

<table>
<thead>
<tr>
<th>Characters</th>
<th>Manzaea gen. nov.</th>
<th>Hydroclathrus</th>
<th>Tronoella</th>
</tr>
</thead>
<tbody>
<tr>
<td>Thallus form</td>
<td>Clathrate, spreading; sometimes interadhesive; margins arched to folded inwardly</td>
<td>Clathrate, spreading; membranes perforated, margins involute</td>
<td>Clathrate, spreading; sometimes interadhesive; membranes perforated, margins arched to involute, with siphonous protrusions</td>
</tr>
<tr>
<td>Holdfast</td>
<td>Rhizoidal</td>
<td>Rhizoidal</td>
<td>Rhizoidal</td>
</tr>
<tr>
<td>Cortical cell layer</td>
<td>1 layer</td>
<td>1–3 layers</td>
<td>1–2 layers</td>
</tr>
<tr>
<td>Medullary cells</td>
<td>1–5 layers; thick-walled</td>
<td>1–9 layers, thin-walled</td>
<td>1–4 layers, thin-walled</td>
</tr>
<tr>
<td>Hair primordia</td>
<td>Occur in groups in pits or depressions, often extended into hyaline hairs</td>
<td>Occur in groups in pits or depressions, often extended into hyaline hairs</td>
<td>Occur singly to groups of 3 in shallow pits, not extended into hyaline hairs</td>
</tr>
<tr>
<td>Plurangular sori</td>
<td>Circular, discrete to confluent, surrounds hair tufts</td>
<td>Circular to block-like, discrete to confluent, often surrounding hair tufts</td>
<td>Irregularly shaped, discrete, often associated with hairs</td>
</tr>
<tr>
<td>Plurilocular sporangia</td>
<td>Somewhat compactly arranged, cylindrical to clavate, quadriseriate</td>
<td>Loosely arranged, cylindrical to clavate, quadriseriate</td>
<td>Firmly coherent and massive</td>
</tr>
</tbody>
</table>

Table 1. Morphological characters of clathrate genera in the family Scytosiphonaceae.

3E–F), which contrast with the thin-walled cells typical in other Hydroclathrus species.

In previous studies in the Scytosiphonaceae that resulted in descriptions of several new taxa at both genus and species levels (McDevit & Saunders 2017, Santiañez et al. 2018a, 2018b, Santiañez & Kogame 2019), taxonomists have worked under the framework that 1) using gross morphologies in the genus-level assignment in the Scytosiphonaceae is unreliable and 2) molecular phylogenies are objective guides to re-assess and clarify the problematic taxonomy and classification of scytosiphonacean taxa. McDevit & Saunders (2017) suggested two possible actions to resolve the problematic phylogenetic relationships in the Scytosiphonaceae: 1) lump several taxa into a large genus, and 2) 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 genus level (Santiañez 2018). Succeeding studies on the systematics of the Scytosiphonaceae have since favored the latter as the former tends to be very problematic when morphological, anatomical, and life history characteristics of scytosiphonacean taxa are accounted (McDevit & Saunders 2017, Santiañez et al. 2018a, 2018b, Santiañez 2018). With the above framework in mind, and considering the combination of the suite of distinct morphological and anatomical characteristics along with the persistent exclusion of H. minutus from its supposed congeners, we believe that we need to reconsider the current assignment to the genus Hydroclathrus. As such, we propose here to transfer the species to the new genus Manzaea gen. nov. Santiañez & Kogame.

Manzaea Santiañez & Kogame, gen. nov. (Fig. 3)

Description. Thallus net-like, spreading; extremely fine strands sometimes interadhesive. Membrane surface perforated with holes; margins arched to folded inwardly. Membranes composed of a single layer of pigmented thin-walled surface cells and one to several layers of thick-walled clear medullary cells. Hair primordia in tufts, extended to hyaline hairs. Plurangular sori conspicuous, discrete to confluent. Plurangia short, erect, quadriseriate, arranged in closely arranged palisades.

Etymology. Manzaea is dedicated to the late Dr. Artemio V. Manza (1896–1964), Professor of Botany and Dendrology at the University of the Philippines Los Baños, to honor his contributions to phycology, especially his work on the taxonomy of coralline red algae of the Pacific Ocean.

Type species. Manzaea minuta (Santiañez & Kogame) Santiañez & Kogame comb. nov.


Holotype. SAP115290, deposited in SAP (Santiañez et al. 2018a); with fragment (isotype) deposited at MSI [MSI29248 (Ganzon-Fortes & Santiañez 2021)].

Type locality. Senaga, Naha, Okinawa, Japan (Santiañez et al. 2018a).

Distribution. Northwestern tropical Pacific Ocean [Ly Son I. and My Hoa, Vietnam; Okinawa, Japan (Santiañez et al. 2018a)].

Remarks. In Japan, Manzaea is typically found in shallow intertidal areas. The nature and arrangement of plurangia of Manzaea as seen in surface view and transverse section is illustrated in Santiañez et al. (2018a, figs. 31, 34). The interadhesive nature of the membranes of Manzaea is reminiscent of the newly described Tronoella, Pseudochnoospora, and R. endiviifolia. This similarity is also reflected in their clustering and close relationships in single- and concatenated gene-based trees (fig. 2, Santiañez & Kogame 2019), although with low support. Manzaea is distinguished from Tronoella based on several morpho-anatomical
characteristics. Tronoella possesses siphonous protrusions that are later cleaved to form arched to somewhat involute margins (Santíañez et al. 2018a; Table 1). Regarding Rosenwingea species, they are characterized by erect, cylindrical to somewhat compressed, hollow, and freely branched or interadhesive thalli (Børgesen 1914, Norris 2010, West et al. 2010, Lee et al. 2014, Santíañez & West 2019). Pseudochnoospora has interadhesive thalli but these are decumbent, branching, solid and terete to compressed and is not membranous and clathrate as those of Manzaea (Santíañez et al. 2018b). Meanwhile, thickened medullary cells are not a common feature among the genera and species in the ‘Hydroclathrus group’ sensu Santíañez et al. (2018b) but these are commonly found in the ‘Scytosiphon group.’ Of those found in the former, only Chnoospora species are known to possess thickened medullary cells. Thickened cell walls and interadhesive branching patterns are among the main characteristics that were used to distinguish and segregate ‘Chnoospora implexa’ to a newly established genus Pseudochnoospora (Santíañez et al. 2018b).

Our proposal to erect the genus Manzaea based on ‘H. minutus’ is part of our ongoing effort to clarify the systematics of the family Scytosiphonaceae, especially to provide a more natural classification in the group. We have been attempting to resolve the problems in the taxonomy of scytosiphonaceous taxa and we have noted that the confused phylogenetic relationships in the family were rooted in the rather broad morphological circumscription of scytosiphonaceous genera (Santíañez et al. 2018b). Considering the insights that we have had in the last few years (Santíañez 2018, Santíañez et al. 2018a, 2018b, 2020, Santíañez & Kogame 2017, 2019), we believe that in continuing to classify ‘H. minutus’ under Hydroclathrus, we are only perpetuating the problems that we are trying to solve. Despite its unresolved phylogenetic position even in concatenated gene tree, ‘H. minutus’ has always been excluded from other Hydroclathrus. Taken together with its gross morphological and anatomical features, we believe that it merits segregation as a species in the new genus Manzaea.

In establishing Manzaea, we further increase the genus-level diversity in the Sctyosiphonaceae, especially the tropical to warm temperate taxa in the ‘Hydroclathrus group.’ Manzaea is the fifth monotypic genus in the Sctyosiphonaceae, the others being Iyengaria Borgesen (1939, Santíañez et al. 2020), Melanosiphon M.J.Wyne (1969), Tronoella and Pseudochnoospora (Santíañez et al. 2018a, 2018b).

Conclusion

Our studies on the family Sctyosiphonaceae—which resulted in discoveries and descriptions of new genera and species—highlight our poor knowledge and understanding of tropical seaweed diversity and underscore the need to conduct detailed taxonomic and molecular phylogenetic studies among warm-water taxa. We anticipate that with continued efforts, steady support, and active collaborations, more taxa will be sequenced and biodiversity resources will be uncovered in tropical regions. It is our best hope that these will consequently provide some insight on resolving the problematic taxonomies and molecular phylogenetic relationships of many algal groups.

Acknowledgements

WJES thanks Dr. Gavino C. Trono, Jr. and Dr. Edna T. Ganzon-Fortes for the inspiration and encouragement to conduct seaweed biodiversity and systematics research. WJES is funded by the University of the Philippines through the Balik PhD Program of the Office of the Vice President for Academic Affairs (OVPPA-BPhD-2018-05), the University of the Philippines Diliman through the In-house research grant of the Marine Science Institute, and the Ministry of Education, Culture, Sports, Science and Technology (MEXT) of the Government of Japan under the Monbukagakusho Scholarship Grant. WJES also acknowledges the support of the Department of Science and Technology (DOST)-Philippine Council for Agriculture, Aquatic and Natural Resources Research and Development (PCAARRD) of the Government of the Philippines through the DOST Balik Scientist Program.

Literature Cited


