

See discussions, stats, and author profiles for this publication at: <https://www.researchgate.net/publication/319694631>

# Molecular analysis of Sargassum from the northern China seas

Article in *Phytotaxa* · August 2017

DOI: 10.11646/phytotaxa.319.1.3

CITATIONS

9

READS

2,409

9 authors, including:



**Zhongmin Sun**

526 PUBLICATIONS 5,559 CITATIONS

[SEE PROFILE](#)



**Dahai Gao**

Shanghai Ocean University

32 PUBLICATIONS 456 CITATIONS

[SEE PROFILE](#)



**Zi-Min Hu**

Yantai University

80 PUBLICATIONS 776 CITATIONS

[SEE PROFILE](#)



**Yuhang Li**

Institute of Oceanology, Chinese Academy of Sciences

23 PUBLICATIONS 112 CITATIONS

[SEE PROFILE](#)

Some of the authors of this publication are also working on these related projects:



Benthic diversity in shallow and deep-sea seas [View project](#)



Adaptation and evolution of seaweeds under climate change [View project](#)



## Molecular analysis of *Sargassum* from the northern China seas

CHAOHUA HUANG<sup>1,2</sup>, ZHONGMIN SUN<sup>1,2\*</sup>, DAHAI GAO<sup>1</sup>, JIANTING YAO<sup>1</sup>, ZIMIN HU<sup>1</sup>, YUHANG LI<sup>1</sup>, YONGQIANG WANG<sup>1</sup>, KUIDONG XU<sup>1</sup> & WANDONG CHEN<sup>3</sup>

<sup>1</sup>Institute of Oceanology, Chinese Academy of Sciences, 7 Nanhai Road, Qingdao 266071, China

<sup>2</sup>College of Earth Science, University of Chinese Academy of Sciences, Beijing 100049, China

<sup>3</sup>Nanji Islands National Marine Nature Reserve Administration, Pingyang, Wenzhou, 325401, China

\*Corresponding author: zhmsun@hotmail.com

### Abstract

The species-level taxonomy of the marine brown algal genus *Sargassum* is problematic. To resolve some of these systematic issues in the northern China seas, a molecular analysis of *Sargassum* species was carried out using ITS-2, *cox3*, and *rbcL* DNA sequences. Phylogenetic analyses confirmed eight species, including: *S. confusum*, *S. fusiforme*, *S. hemiphyllum* var. *chinense*, *S. horneri*, *S. muticum*, *S. siliquastrum*, *S. thunbergii*, and *S. vachellianum*. The previously described *S. shandongense* and *S. qingdaoense*, endemic to the Shandong Peninsula, are shown to be synonymous with *S. vachellianum*. Seven species previously described from the East China Sea were not observed, and require further investigation.

**Keywords:** *Sargassum*, diversity, molecular assessment, northern China seas

### Introduction

*Sargassum* C. Agardh (1820: 1) is the most diverse genus in the Phaeophyceae, with 945 species, varieties and forms listed in Algaebase, and 353 flagged as currently accepted (Guiry & Guiry 2017). The genus is distributed worldwide, but particularly abundant in tropical and subtropical regions and the Indo-Pacific (Yoshida 1989, Phillips 1995). *Sargassum* beds are an important element of marine ecosystems providing substrate, food, and habitat for other marine organisms (Godoy & Coutinho 2002). Some species of *Sargassum* can be used as food, medicine for human beings, and fertilizer for crops (Tseng & Lu 2000, Liu *et al.* 2012, Van Tu 2015).

The traditional classification system of *Sargassum* mainly depends on morphological characteristics of the holdfast, stem, branches, blades, vesicles, and receptacles (C. Agardh 1820, J. Agardh 1849, 1889). Unfortunately, these criteria can be highly variable due to environmental conditions or developmental stages of the thallus. As a result, significant morphological variation can occur between different populations, within a single population, or within an individual at different seasons and growth stages (Kilar & Hanisak 1989). This variability led early authors to describe many ecotypes as new species, which further confused the taxonomy (Mattio & Payri 2011). It is therefore likely that *Sargassum* diversity is overestimated (Mattio & Payri 2011).

DNA-based molecular phylogenetic analyses have shown great promise to resolve taxonomic ambiguity and algal diversity (Leliaert *et al.* 2014). For *Sargassum*, the nuclear internal transcribed spacer 2 (ITS-2), mitochondrial cytochrome oxidase subunit 3 (*cox3*), and chloroplast genes (*rbcL*, *rbcLS*) have been applied to investigate *Sargassum* taxonomy and phylogenetic relationships (e.g. Stiger *et al.* 2003, Phillips *et al.* 2005, Mattio *et al.* 2008), and population-level genetic differentiation and phylogeographic patterns (e.g. Uwai *et al.* 2009, Hu *et al.* 2011). Recently, the classification of *Sargassum* has been significantly revised using multiple genetic markers (Mattio & Payri 2009, 2011, Dixon *et al.* 2012, 2014, Mattio *et al.* 2013, 2015, Andrade-Sorcia *et al.* 2014, Camacho *et al.* 2015, Kantachumpoo *et al.* 2015). On the basis of these work, many taxonomic problems were resolved and a number of new taxonomic synonymies were proposed.

In the China seas, *Sargassum* systematics is particularly challenging. A total of 130 species were recorded, 17 of which were reported from the northern Chinese coasts (Yellow Sea and East China Sea) (Tseng & Lu 2000). The population genetics and evolutionary biogeography of *Sargassum* species in the China Seas have received considerable

research interest (Cheng *et al.* 2010, Hu *et al.* 2011, 2013, Bi *et al.* 2014, Li *et al.* 2016), however, species diversity has not yet been reevaluated using a multigene molecular approach. In this study we used three DNA markers (ITS-2, *cox3*, and *rbcL*) to reassess the species diversity of *Sargassum* from the northern China seas, and show that morphology alone was unable to accurately classify the many *Sargassum* taxa.

## Materials and methods

### Specimen collection and species identification

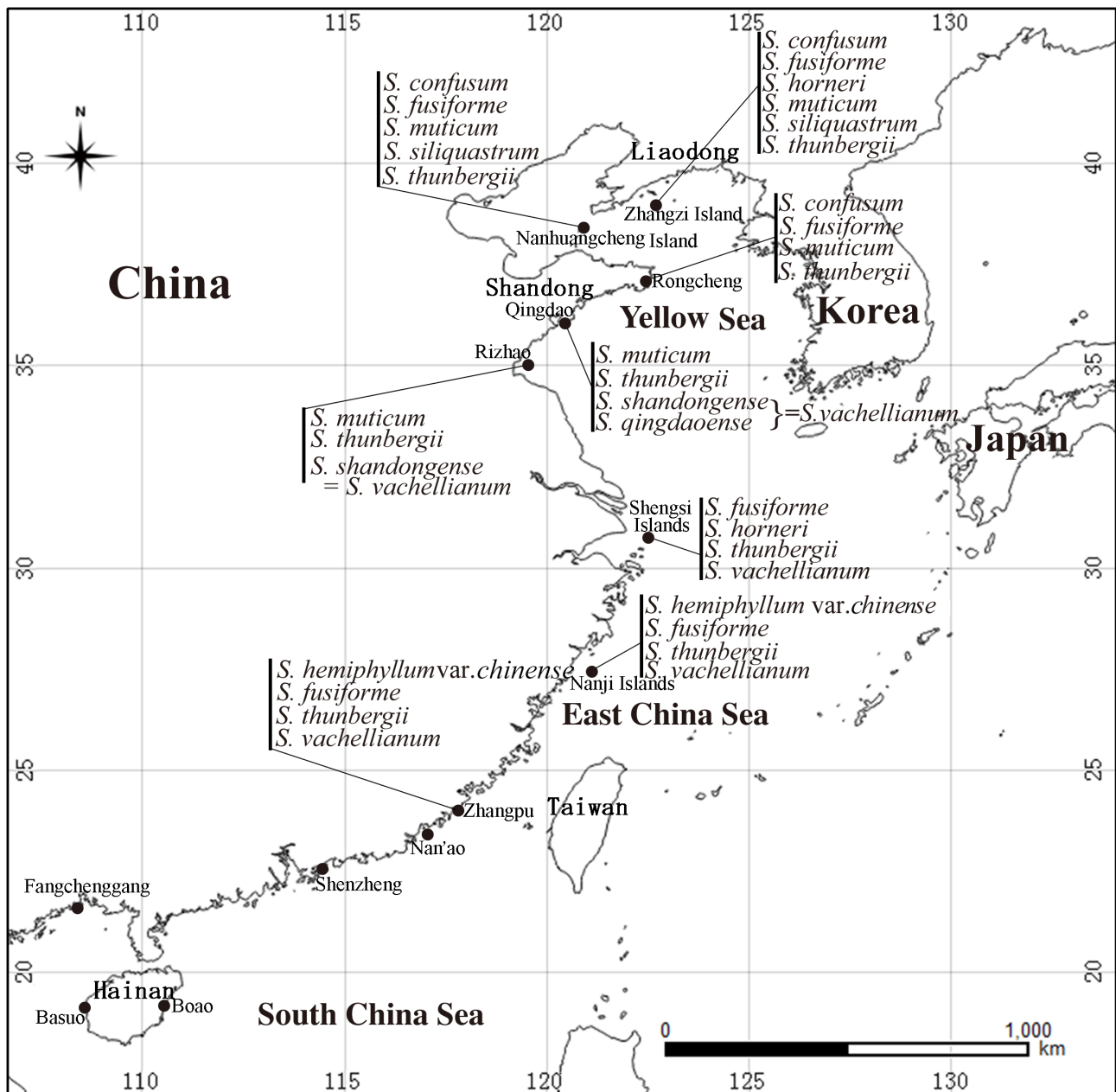


FIGURE 1. Map of collection sites showing the species of *Sargassum* collected from the China Seas.

*Sargassum* samples were mainly collected from eight localities in the Yellow Sea and East China Sea in spring and summer from 2014 to 2016 (Fig. 1). More than 20 individuals were collected from more than three sites in each locality by snorkeling or SCUBA diving. *Sargassum vachellianum* samples were also obtained from several sites in the South China Sea close to the type locality Macau, China (Greville 1848). Apical fragments from each specimen were preserved in silica gel for DNA extraction and a portion of the rest specimen was pressed as herbarium vouchers. The mature individuals were preserved in 10% formaldehyde-seawater solution for morphological examinations. Photographs

were taken with a digital camera (Canon, EOS700D). The morphological characteristics of new specimens were compared to herbaria housed at the Marine Biological Museum Chinese Academy of Sciences (MBMCAS), including the type specimens of *S. shandongense* and *S. qingdaoense*. All vouchers were deposited at MBMCAS. Preliminary identifications were accomplished using published literatures (Cotton 1915, Collins 1919, Grubb 1932, Zheng *et al.* 1960, Hang & Sun 1983, Yoshida 1983, Tseng & Lu 2000, 2004) and historical diagnoses (C. Agardh 1820, 1824, Greville 1848, Kuntze 1880, Setchell 1931, Fensholt 1955).

#### **DNA-extraction, amplification and sequencing**

Genomic DNA was extracted from silica gel-dried tissue after grinding in liquid nitrogen using a DNeasy Plant kit (Tiangen Biotech. Co. Ltd., Beijing) following the manufacturer's instructions. The PCR reactions were performed on the TakaRa TP600 thermocycler, using 50 µL reactions containing 1 µL of genomic DNA, 5 µL 10x PCR Buffer (Mg<sup>2+</sup> included), 4 µL High pure dNTPs (2.5 mM), 0.5 µL Taq DNA polymerase, 1 µL of each forward and reverse primer (10 µM), and ultrapure water. The parameters included an initial denaturation at 94°C for 5 min, followed by denaturation at 94°C for 45s, annealing at 55°C (for ITS-2), 48°C (for *cox3*), or 56°C (for *rbcL*) for 1 min, extension at 72°C for 1 min for 36 cycles, and a final extension at 72°C for 7 min. The primers used for PCR and sequencing were as follows: ITS2FC2 (Cheang *et al.* 2010) and 25BR2 (Yoshida *et al.* 2000) for ITS-2; trnY-P1 and *cox3*-P2 (Ni-Ni-Win *et al.* 2008) for *cox3*; *rbcL*-68F (Draisma *et al.* 2001) and *rbcS*-P1 (Kawai *et al.* 2007) for *rbcL*. All PCR products were examined by electrophoresis on a 1% agarose gel. PCR products were directly sequenced using the ABI 3700 autosequencer according to manufacturer's instructions (ABI, BigDye® Terminator v3.1 Cycle Sequencing Kit).

#### **Phylogenetic analyses**

The newly generated sequences were assembled and edited using DNASTAR LASERGENE 7.1 (DNASTAR Inc., Madison, USA). They were then aligned using MUSCLE 3.6 (Edgar 2004) and manually examined for accuracy and trimmed to minimize missing characters in GeneDoc 2.7. Phylogenetic analyses were first run on individual markers, and then the ITS-2 and *cox3* concatenated data sets were analyzed. The number of *rbcL* sequences published in GenBank was limited, and therefore they were not included in the concatenated analysis. The ITS-2 and *cox3* concatenated data set included 52 ingroup sequences and the *rbcL* data set included 47 sequences. The GenBank accessions are shown in Table 1.

**TABLE 1.** Taxa, collection, publication information, and GenBank accession numbers of the ITS-2, *cox3*, and *rbcL* sequences.

| Taxon   | Voucher and collection or publication      | GenBank accession number |             |             |
|---|--|--------------------------|-------------|-------------|
|   |  | ITS-2                    | <i>cox3</i> | <i>rbcL</i> |
| <b><i>S.</i> subgen. <i>Bactrophycus</i></b>                              |  |                          |             |             |
| <i>Sargassum autumnale</i> Yoshida  | PF582; Cho <i>et al.</i> 2012              | JF931831                 | JF931729    | JF931677    |
| <i>Sargassum confusum</i> C. Agardh                                       | MBMD03832; Rongcheng, May 2016             | KY411080                 | KY411106    | KY432485    |
|   | PF616; Cho <i>et al.</i> 2012              | JF931851                 | JF931733    | JF931681    |
| <i>Sargassum filicinum</i> Harvey   | PF958; Cho <i>et al.</i> 2012              | JF931860                 | JF931740    | JF931688    |
| <i>Sargassum fulvellum</i> (Turner)<br>C. Agardh                          | PF634; Cho <i>et al.</i> 2012              | JF931853                 | JF931741    | JF931689    |
|   | MBMD03488; Zhangpu, April 2016             | KY411081                 | KY411107    | KY432486    |
| <i>Sargassum fusiforme</i> (Harvey)<br>Setchell                           | PF589; Cho <i>et al.</i> 2012              | JF931859                 | JF931743    | JF931691    |
|   | PF584; Cho <i>et al.</i> 2012              | JF931854                 | JF931744    | JF931692    |
| <i>Sargassum hemiphylum</i><br>(Turner) C. Agardh                         | PF584; Cho <i>et al.</i> 2012              | JF931854                 | JF931744    | JF931692    |
| <i>Sargassum hemiphylum</i><br>(Turner) var. <i>chinense</i> C.<br>Agardh | MBMD03466; Zhangpu, April 2016             | KY411082                 | KY411108    | KY432487    |
|   | MBMD03540; Dongshan, April 2016            | KY411083                 | KY411109    | KY432488    |
|   | MBMD03688; Nanji Islands, June 2016        | KY411084                 | KY411110    | KY432489    |
| <i>Sargassum horneri</i> (Turner) C.<br>Agardh                            | MBMD04265; Nanhuangcheng Island, June 2016 | KY411085                 | KY411111    | KY432490    |
|   | MBMD04300; Gouqi Island, July 2015         | KY411086                 | KY411112    | KY432491    |
|   | PF1005; Cho <i>et al.</i> 2012             | JF931861                 | JF931747    | JF931695    |
|   | IRD5219; Dixon <i>et al.</i> 2014          | KF281925                 | KF281979    | -           |
|   | GENT-HV2037; Dixon <i>et al.</i> 2014      | KF281941                 | KF281989    | -           |

...continued on the next page

TABLE 1. (continued)

| Taxon   | Voucher and collection or publication      | GenBank accession number |          |          |
|---|--|--------------------------|----------|----------|
|   |  | ITS-2                    | cox3     | rbcL     |
| <i>Sargassum macrocarpum</i> C. Agardh                      | PF588; Cho <i>et al.</i> 2012              | JF931834                 | JF931748 | JF931696 |
| <i>Sargassum micracanthum</i> (Kützinger) Endlicher         | PF1420; Cho <i>et al.</i> 2012             | JF931838                 | JF931752 | JF931700 |
| <i>Sargassum miyabei</i> Yendo                              | PF1452; Cho <i>et al.</i> 2012             | JF931857                 | JF931755 | JF931703 |
| <i>Sargassum muticum</i> (Yendo) Fensholt                   | MBMD04284; Nanhuangcheng Island, June 2016 | KY411087                 | KY411113 | KY432492 |
|   | PF1010; Cho <i>et al.</i> 2012             | JF931857                 | JF931755 | JF931703 |
|   | GENT-ODC1702; Dixon <i>et al.</i> 2014     | KF281781                 | KF281977 | -        |
| <i>Sargassum nigrifolium</i> Yendo                          | PF1359; Cho <i>et al.</i> 2012             | JF931846                 | JF931756 | JF931704 |
| <i>Sargassum ringgoldianum</i> Harvey                       | GENT-HV2003; Dixon <i>et al.</i> 2014      | KF281937                 | KF281988 | KF281795 |
| <i>Sargassum serratifolium</i> (C. Agardh) C. Agardh        | PF1343; Cho <i>et al.</i> 2012             | JF931840                 | JF931762 | JF931710 |
| <i>Sargassum siliquastrum</i> (Mertens ex Turner) C. Agardh | MBMD04273; Nanhuangcheng Island, June 2016 | KY411088                 | KY411114 | KY432493 |
|   | MBMD01281; Zhangzi Island, June 2014       | KY411089                 | KY411115 | KY432494 |
|   | PF1423; Cho <i>et al.</i> 2012             | JF931843                 | JF931765 | JF931713 |
| <i>Sargassum thunbergii</i> (Mertens ex Roth) Kuntze        | MBMD03177; Qingdao, March 2016             | KY411090                 | KY411116 | KY432495 |
|   | PF531; Cho <i>et al.</i> 2012              | JF931858                 | JF931766 | JF931714 |
|   | IRD5241; Dixon <i>et al.</i> 2014          | KF281923                 | KF281977 | -        |
|   | SGAD0909017; Dixon <i>et al.</i> 2014      | KF281936                 | KF281987 | -        |
| <i>Sargassum yamadae</i> Yoshida et Konno                   | PF1356; Cho <i>et al.</i> 2012             | JF931847                 | JF931767 | JF931715 |
| <i>Sargassum yezoense</i> (Yamada) Yoshida et Konno         | PF1350; Cho <i>et al.</i> 2012             | JF931849                 | JF931772 | JF931720 |
| <b>S. subgen. <i>Sargassum</i></b>                          |  |                          |          |          |
| <i>Sargassum piluliferum</i> (Turner) C. Agardh             | MBMD02867; Fukui, Japan, October 2015      | KY411091                 | KY411117 | KY432496 |
|   | PF949; Cho <i>et al.</i> 2012              | JF931863                 | JF931760 | JF931708 |
| <i>Sargassum qingdaoense</i> Tseng et Lu*                   | MBMD04328; Qingdao, July 2016              | KY411092                 | KY411118 | KY432497 |
| <i>Sargassum shandongense</i> Tseng, C. F. Zhang et Lu*     | MBMD02308; Rizhao (Pingdao), July 2015     | KY411093                 | KY411119 | KY432498 |
|   | MBMD04356; Qingdao, July 2016              | KY411094                 | KY411120 | KY432499 |
| <i>Sargassum yendoii</i> Okamura et Yamada                  | MBMD02875; Chiba, Japan, March 2016        | KY411095                 | KY411121 | KY432500 |
|   | PF1032; Cho <i>et al.</i> 2012             | JF931864                 | JF931771 | JF931719 |
| <i>Sargassum vachellianum</i> Greville                      | MBMD01652; Basuo, Hainan, March 2015       | KY411096                 | KY411122 | KY432501 |
|   | MBMD01806; Boao, Haian, March 2015         | KY411097                 | KY411123 | KY432502 |
|   | MBMD01810; Boao, Hainan, March 2015        | KY411098                 | KY411124 | KY432503 |
|   | MBMD01998; Shengsi Islands, June 2015      | KY411099                 | KY411125 | KY432504 |
|   | MBMD03610; Nan'ao Island, April 2016       | KY411100                 | KY411126 | KY432505 |
|   | MBMD03684; Fangchenggang, April 2016       | KY411101                 | KY411127 | KY432506 |
|   | MBMD03708; Shenzhen, April 2016            | KY411102                 | KY411128 | KY432507 |
|   | MBMD03728; Shenzhen, April 2016            | KY411103                 | KY411129 | KY432508 |
|   | MBMD03941; Nanji Islands, June 2016        | KY411104                 | KY411130 | KY432509 |
| Other Sargassaceae  |  |                          |          |          |
| <i>Coccophora langsdorfii</i> (Turner) Greville             | MBMD02725; Aomori, Japan, December 2015    | KY411105                 | KY411131 | KY432510 |
| <i>Turbinaria rnat</i> (Turner) J. Agardh                   | IRD1536; Mattio <i>et al.</i> 2009         | EU100772                 | EU833384 | -        |
|   | MBMD02892, Sanya, Hiannan, April 2016      | -                        | -        | KY432511 |

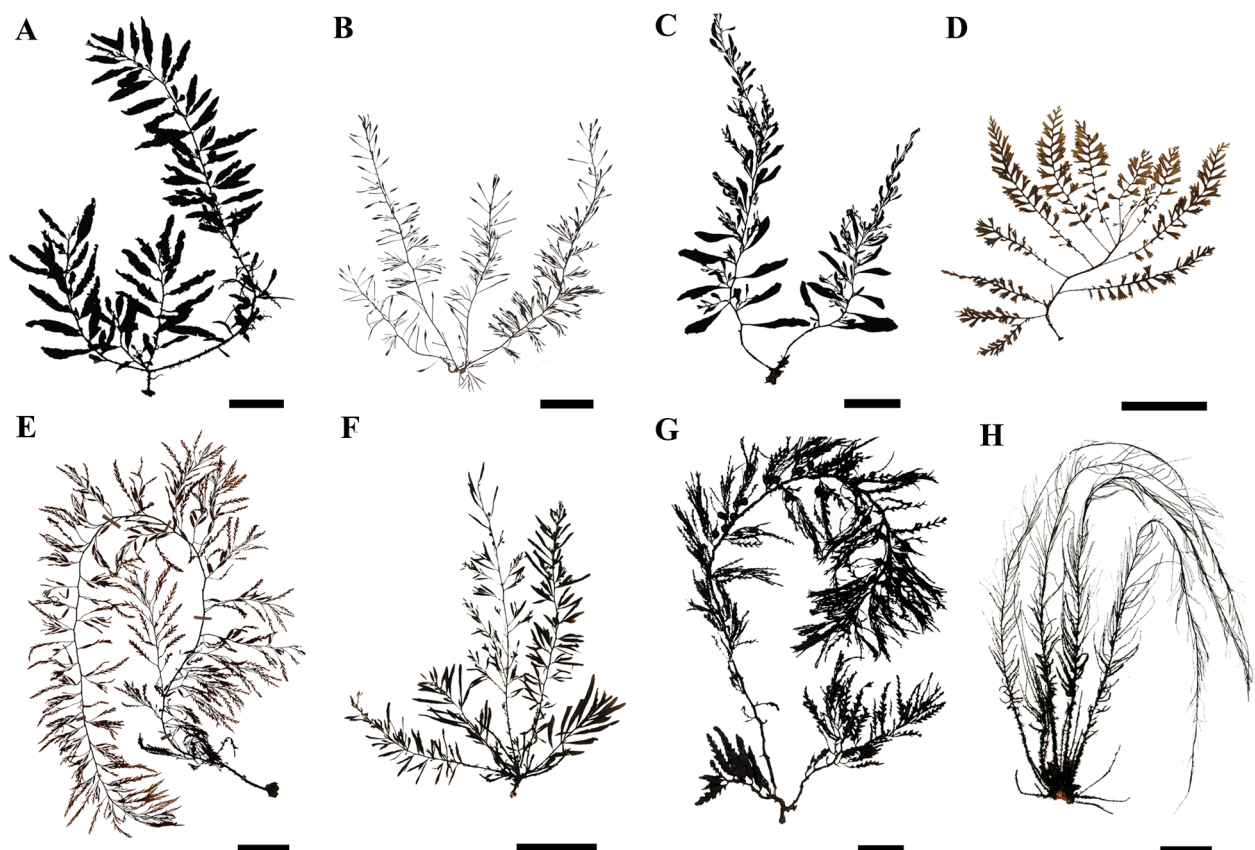
\* synonyms of *Sargassum vachellianum* Greville.

Phylogenetic relationships were inferred using Bayesian inferences (BI) and Maximum likelihood (ML) analyses. The BI was assessed with MrBayes 3.2 (Ronquist *et al.* 2012). For this analysis the best fit partitioning schemes and substitution model were determined by Partitionfinder 2.0 (Lanfear *et al.* 2016) under the Bayesian Information Criterion. For the analysis using concatenated sequences, the partition was defined by genes and codon positions (for *cox3*). The BI analysis was conducted with a random starting tree and Bayesian search included four chains, each chain running for 1 million generations with a tree sampling frequency of every 100 generations. The convergence was judged based on the average standard deviation of split frequencies (all less than 0.01) and the ESS values (more than 200) in TRACER 1.6.0 (Rambault *et al.* 2014), and 25% of the resulting trees discarded as burn-in. The final tree was visualized in FigTree v1.4.3. The ML tree was performed with RAxML8.0 (Stamatakis 2014) using the GTR+ gamma models with 1000 bootstrap replicates. The tree was rooted with *Coccophora langsdorfii* (Turner) Greville and *Turbinaria ornata* (Turner) J. Agardh as suggested by Cho *et al.* (2012). Nodes in the trees were considered well supported when Bayesian posterior probabilities (PP) were  $\geq 0.95$  (Leaché *et al.* 2002) and ML bootstrap support (BS) was  $\geq 70\%$  (Huelsenbeck *et al.* 1993).

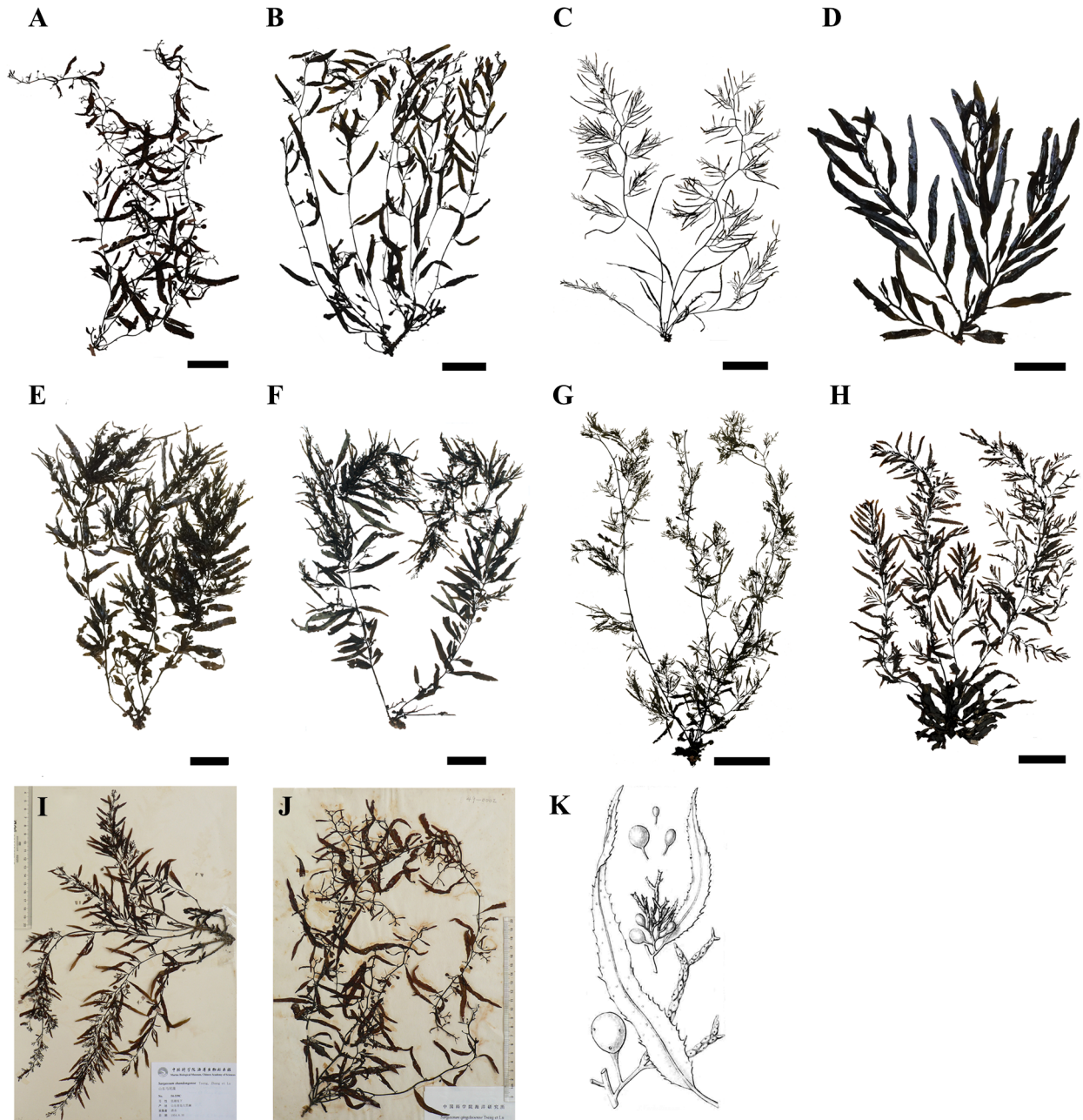
## Results

### *Morphological identification*

Among the newly collected specimens, seven common species of *Sargassum* subgen. *Bactrophyucus* were identified based on morphological characters displayed in Fig. 2. For *Sargassum* subgen. *Sargassum*, one taxon spanning the morphological continuum of three species (*S. shandongense* Tseng, Zhang et Lu, *S. qingdaoense* Tseng et Lu, and *S. vachellianum*) was collected (Figs. 3, 4).



**FIGURE 2.** *Sargassum* species collected from the Yellow Sea and East China Sea. All scale bars = 5 cm. A. *S. confusum*. B. *S. fusiforme*. C. *S. hemiphyllum* var. *chinense*. D. *S. horneri*, young individual. E. *S. horneri*, mature individual. F. *S. muticum*. G. *S. siliquastrum*. H. *S. thunbergii*.



**FIGURE 3.** *Sargassum vachellianum* collected from different regions. All scale bars = 5 cm. A. Qingdao, Shandong, floating individual. B. Qingdao, Shandong. C. Rizhao, Shandong. D. Nanji Islands, Zhejiang. E. Nan'ao Island, Guangdong. F. Shenzhen, Guangdong. G. Fangchenggang, Guangxi. H. Boao, Hainan. I. Type specimen of *S. shandongense*. J. Type specimen of *S. qingdaoense*. K. Sketch of type specimen of *S. vachellianum* (in Greville 1848).

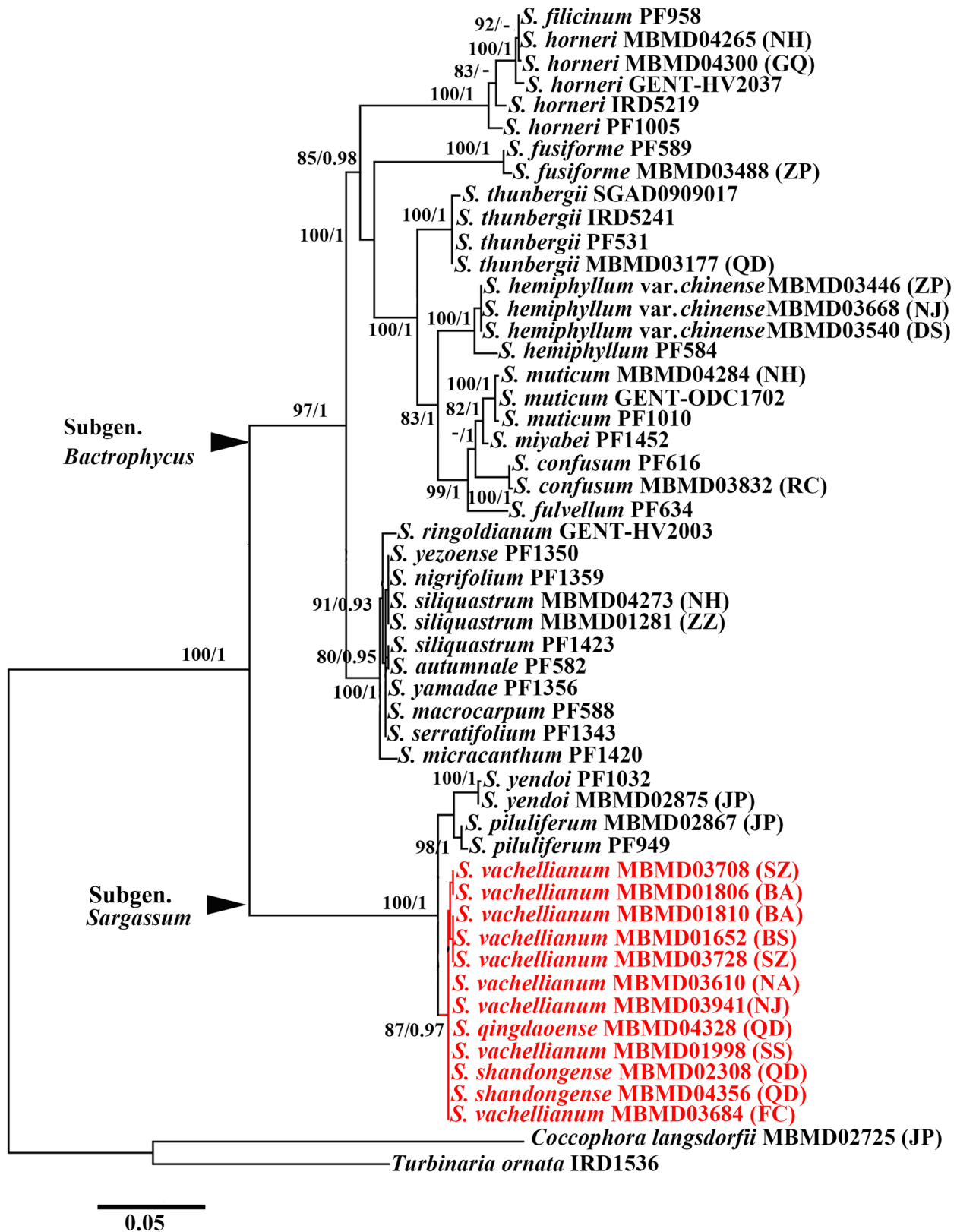
### **Phylogenetic analyses**

A total of 26 ITS-2, 26 *cox3*, and 27 *rbcL* sequences were generated for the analyses. Based on the concatenated dataset (ITS-2+*cox3*), the two main strongly supported clades corresponded to *Sargassum* subgen. *Bactrophyucus* and *Sargassum* subgen. *Sargassum*. In the *Sargassum* subgen. *Bactrophyucus* clade, all specimens from the northern China seas were grouped with previously published Korean and Japanese specimens. In the *Sargassum* subgen. *Sargassum*, all specimens referred to *S. shandongense*, *S. qingdaoense* and *S. vachellianum* clustered within one highly supported monophyletic clade, with sequence identity > 99.5%. This clade was sister to *S. piluliferum* (Turner) C. Agardh and *S. yendoii* Okamura et Yamada from Japan. The *rbcL*-based phylogenetic tree (Fig. S1) showed similar topological structure to the ITS-2+*cox3* dataset, but the relationships of some related taxa were not resolved due to the limited base substitution.



**FIGURE 4.** *Sargassum vachellianum* collected from different regions, showing morphologies of holdfast and main stem, blades, vesicles, receptacles. A. Qingdao, Shandong. B. Rizhao, Shandong. C. Qingdao, Shandong, floating individual. D. Nanji Islands, Zhejiang. E. Nan'ao Island, Guangdong. F. Shenzhen, Guangdong.





**FIGURE 5.** Phylogenetic tree using Maximum likelihood (ML) and Bayesian inferences (BI) based on concatenated ITS-2 and *cox3* sequences. Node numbers indicate bootstrap support (ML/BI). The well-supported clade in red consists of specimens previously identified as *S. vachellianum*, *S. shandongense* (specimens referring to Fig. 4. A–B) and *S. qingdaoense* (specimen referring to Fig. 4. C). BA = Boao, Hainan; BS = Basuo, Hainan; DS = Dongshan, Fujian; QD = Qingdao, Shandong; FC = Fangchenggang, Guangxi; GQ = Gouqi Island, Zhejiang; JP = Japan; NA = Nan’ao Island, Guangdong; NH = Nanhuangcheng Island, Shandong; NJ = Nanji Islands, Zhejiang; RC = Rongcheng, Shandong; RZ = Rizhao, Shandong; SZ = Shenzhen, Guangdong; SS = Shengsi Islands, Zhejiang; ZP = Zhangpu, Fujian; ZZ = Zhangzi Island, Liaoning.

## Discussion

The main goal of this study was to re-examine the diversity of *Sargassum* along the Chinese coast in the Yellow Sea and East China Sea based on molecular analysis. Among 130 species previously recorded in China, we confirmed the presence of at least eight *Sargassum* species in the northern China seas, with seven belonging to the *Sargassum* subgen. *Bactrophyucus* and one belonging to the *Sargassum* subgen. *Sargassum*. Our phylogenetic data indicated that none of the *Sargassum* species are endemic to the Yellow Sea and East China Sea, instead they are also in the South China Sea and adjacent seas.

Following our findings, we propose *Sargassum shandongense* and *S. qingdaoense* as taxonomic synonyms of *S. vachellianum*. This taxonomic treatment is supported by two lines of evidence. First, the morphology of type specimens and the original diagnoses (Greville 1848: 204, Tseng & Lu 2004: 195) revealed only one *Sargassum* subgenus *Sargassum* species with morphological characteristics compatible with the description of the three taxa. Second, our phylogenetic tree showed that the specimens from Qingdao, China (type locality of *S. shandongense* and *S. qingdaoense*) were not significantly distinct from those near Macao, China (the type locality of *S. vachellianum*). Rather, in the phylogenetic tree, these specimens clustered in a well-supported clade with *S. vachellianum* from the East China Sea (Shengsi Island, Nanji Island) and South China Sea (Nan'ao Island, Fangchenggang, Hainan Island). There were some subtle differences observed among the specimens from different localities, for example, the blades of specimens from Rizhao were narrow and almost linear, whereas the specimens from southern China had oval vesicles and spiny margins (Fig. 4). The name *S. vachellianum* (Greville 1848) has priority over *S. shandongense* and *S. qingdaoense* (Tseng & Lu 2004).

*Sargassum* in the northern China seas displayed a low genetic diversity with only one haplotype detected in each species. The Yellow-Bohai Sea and most of the East China Sea were entirely exposed during the glacial period, which probably results in a low diversity of marine flora (Oba *et al.* 1991, Ota 1998). The ancestral population of some *Sargassum* species may have survived in the southern warm sea during the Quaternary ice age, and the currently distributed northern populations might be transported from the south after the glacial period (Hu *et al.* 2011, 2013). Seawater temperature is thought to be an important factor influencing the distribution of the Chinese algal flora (Tseng & Zhang 1960). Recent study also indicated that the number of *Sargassum* species decreases northward in China, in correlation with decreasing seawater temperature (Huang *et al.* 2013). Based on our survey, *Sargassum hemiphyllyum* var. *chinense* was not observed in the Yellow Sea, and *S. vachellianum* is absent in the north of Shandong Peninsula. However *Sargassum horneri*, *S. fusiforme*, and *S. thunbergii* are naturally distributed on the Chinese coasts, therefore it seems seawater temperature may not be an major factor restricting the distribution of some species. It is interesting to note that *S. horneri* is present in Liaodong Peninsula, but absent in Shandong Peninsula, though seawater temperature in Shandong Peninsula is higher (Hua *et al.* 2016). The high nutrient concentrations throughout the year driven by ocean currents may be another important oceanographic force contributing to the distribution and diversity of *Sargassum* species in the northwest Pacific (Li *et al.* 2016).

Several previously recorded species in the East China Sea were not collected in the present study, possibly due to historical misidentification, seasonality of thallus occurrence, or scope of this work. We collected samples every month in some localities, but some previously recorded species were never present. For example, *Sargassum horneri* has disappeared from the Nanji Islands, where a huge seaweed bed of *S. horneri* was well known for decades (Sun *et al.* 2008). In the same area, *S. nigrifolioides* Tseng et Lu had been described as a new species (Tseng & Lu 1985), but we failed to find it at the type locality. The frequent human activities in the area might be the major reason of the dramatic reduction of biomass and diversity of *Sargassum* (Sun *et al.* 2009). The historical sampling localities have been destroyed or become reclaimed land, and the low light transparency in the seawater inhibits the growth of seaweeds.

About 130 *Sargassum* species were previously recorded in China, of which 49 were described as new species, however, most species have never been confirmed using molecular phylogenetics. Consequently, further studies on the diversity of *Sargassum* from the South China Sea and adjacent seas using DNA datasets are required. Ecological monitoring has also been proposed as a necessary approach to document the ontogenic and phenotypic variability at the intra and inter-population levels (Mattio & Payri 2011, Mattio *et al.* 2015). Integrative investigations using morphological, ecological, and molecular analyses are essential to resolve *Sargassum* taxonomy.

## Acknowledgements

We thank Mr. Wei Tan for collection of specimens in Hainnan Island, and Mr. Weizhou Chen for collection of specimens in Fangchenggang and Nan'ao Island. This research was supported by the Strategic Priority Research Program of Chinese Academy of Sciences (No. XDA11020303), the National Natural Science Foundation of China (No. 31200163), and the S & T Basic Project from Ministry of Science and Technology of China (No. 2014FY110500).

## References

- Agardh, C.A. (1820) *Species algarum rite cognitae, cum synonymis, differentiis specificis et descriptionibus succinctis. vol. 1.* Lund, Berling. 168 pp.
- Agardh, C.A. (1824) *Systema algarum.* Lund, Berling, 312 pp.
- Agardh, J.G. (1848) *Species Cenera et Ordines Algarum, seu Descriptiones Succinctae Specierum, Generum et Ordinum, Quibus Algarum Regnum Constituitur Algas Fucoideas Complectens vol. 1.* Gleerup CWK, Lund, Sweden, 363 pp.  
<https://doi.org/10.5962/bhl.title.1576>
- Agardh, J.G. (1889) *Species Sargassorum Australiae Descriptae et Dispositae vol. 23. Öfversigt af Kongl. Vetenskaps-Academiens Forhandlingar,* Stockholm, Sweden, 333 pp.
- Andrade-Sorcía G., Riosmena-Rodríguez, R., Muñoz-Salazar, R., López-Vivas, J.M., Boo, G.H., Lee, K.M. & Boo, S.M. (2014) Morphological reassessment and molecular assessment of *Sargassum* (Fucales: Phaeophyceae) species from the Gulf of California, México. *Phytotaxa* 183: 201–223.  
<https://doi.org/10.11646/phytotaxa.183.4.1>
- Bi, Y.H., Yang, X. & Zhou, Z.G. (2014) Characterization and phylogenetic analysis of ITS sequences in three geographic populations of *Sargassum vachellianum* (Fucales, Phaeophyceae). *Journal of Fisheries of China* 38: 1335–1342.
- Camacho O., Mattio L., Draisma, S., Fredericq, S. & Diaz-Pulido, G. (2015) Morphological and molecular assessment of *Sargassum* (Fucales, Phaeophyceae) from Caribbean Colombia, including the proposal of *S. giganteum* sp. nov., *Sargassum schnetteri* comb. nov., and *Sargassum* section *Cladophyllum* sect. nov. *Systematics and Biodiversity* 13: 105–130.  
<https://doi.org/10.1080/14772000.2014.972478>
- Cheang, C.C., Chu, K.H. & Ang, P.O. (2010) Phylogeography of the marine macroalga *Sargassum hemiphyllum* (Phaeophyceae, Heterokontophyta) in northwestern Pacific. *Molecular Ecology* 19: 2933–2948.  
<https://doi.org/10.1111/j.1365-294X.2010.04685.x>
- Cho, S.M., Lee, S.M., Ko, Y.D., Mattio, L. & Boo, S.M. (2012) Molecular systematic reassessment of *Sargassum* (Fucales, Phaeophyceae) in Korea using four gene regions. *Botanica Marina* 55: 473–484.  
<https://doi.org/10.1515/bot-2012-0109>
- Collins, F.S. (1919) Chinese marine algae. *Rhodora* 21: 203–207.
- Cotton, A.D. (1915) Some Chinese marine algae. *Bulletin of Miscellaneous Information (Royal Botanic Gardens, Kew)* 1915: 107–113.  
<https://doi.org/10.2307/4107515>
- Dixon, R.R., Huisman, J.M., Buchanan, J., Gurgel, C.F.D. & Spencer, P. (2012) A morphological and molecular study of Austral *Sargassum* (Fucales, Phaeophyceae) supports the recognition of *Phyllotricha* at genus level, with further additions to the genus *Sargassopsis*. *Journal of Phycology* 48: 1119–1129.  
<https://doi.org/10.1111/j.1529-8817.2012.01187.x>
- Dixon, R.R., Mattio, L., Huisman, J.M., Payri, C.E., Bolton, J.J. & Gurgel, C.F.D. (2014) North meets South-Taxonomic and biogeographic implications of a phylogenetic assessment of *Sargassum* subgenera *Arthrophyucus* and *Bactrophyucus* (Fucales, Phaeophyceae). *Phycologia* 53: 15–22.  
<https://doi.org/10.2216/13-173.1>
- Draisma, S.G., Prud'Homme van Reine, W.F., Stam, W.T. & Olsen, J.L. (2001) A reassessment of phylogenetic relationships within the Phaeophyceae based on Rubisco large subunit and ribosomal DNA sequences. *Journal of Phycology* 37: 586–603.  
<https://doi.org/10.1046/j.1529-8817.2001.037004586.x>
- Edgar, R.C. (2004) MUSCLE: multiple sequence alignment with high accuracy and high throughput. *Nucleic Acids Research* 32: 1792–1797.  
<https://doi.org/10.1093/nar/gkh340>
- Fensholt, D.E. (1955) An emendation of the genus *Cystophyllum* (Fucales). *American Journal of Botany* 42: 305–322.  
<https://doi.org/10.2307/2438568>

- Godoy, E.A. & Coutinho, R. (2002) Can artificial beds of plastic mimics compensate for seasonal absence of natural beds of *Sargassum furcatum*, ICES Journal of Marine Science: *Journal du Conseil* 59: S111–S115.  
<https://doi.org/10.1006/jmsc.2002.1220>
- Greville, R.K. (1848) Algae orientales XXIII: Descriptions of new species belonging to the genus *Sargassum*. *Journal of Natural History* 2: 203–206.  
<https://doi.org/10.1080/03745485809494687>
- Grubb, V. (1932) Marine algae of Korea and China, with notes on the distribution of Chinese marine algae. *Jour. Bot* 70: 245–251.
- Guiry, M.D. & Guiry, G.M. (2017) AlgaeBase. World-wide electronic publication, National University of Ireland, Galway. Available from: <http://www.algaebase.org> (accessed 20 March 2016)
- Hang, J.X. & Sun, J.Z. (1983) *Colored Illustrations of Seaweeds of Zhejiang Province*. Zhejiang Science and Technology Press, Hangzhou, 119 pp.
- Hu, Z.M., Uwai, S., Yu, S.H., Komatsu, T., Ajisaka, T. & Duan, D.L. (2011) Phylogeographic heterogeneity of the brown macroalga *Sargassum horneri* (Fucaceae) in the northwestern Pacific in relation to late Pleistocene glaciation and tectonic configurations. *Molecular Ecology* 20: 3894–3909.  
<https://doi.org/10.1111/j.1365-294X.2011.05220.x>
- Hu, Z.M., Zhang, J., Lopez-Bautista, J. & Duan, D.L. (2013) Asymmetric genetic exchange in the brown seaweed *Sargassum fusiforme* (Phaeophyceae) driven by oceanic currents. *Marine Biology* 160: 1407–1414.  
<https://doi.org/10.1007/s00227-013-2192-x>
- Hua, C.J., Wu, Y.M., Zhang, H., Cui, X.S. & Yang, S.L. (2016) Analysis on the seasonal variability of sea surface temperature in the East the Yellow Sea in 2014. *Fisheries Information & Strategy* 31: 193–205.
- Huang, B.X., Ding, L.P., Tan, H.Q. & Sun, G.D. (2013) Species diversity and distribution of genus *Sargassum* in China seas. *Oceanologia Et Limnologia Sinica* 44: 69–76.
- Huelsenbeck, J.P. & Hillis, D.M. (1993) Success of phylogenetic methods in the four-taxon case. *Systematic Biology* 42: 247–264.  
<https://doi.org/10.1093/sysbio/42.3.247>
- Kantachumpoo, A., Uwai, S., Noiraksar, T. & Komatsu, T. (2015) Systematics of marine brown alga *Sargassum*. *Ocean Science Journal* 50: 251–262.  
<https://doi.org/10.1007/s12601-015-0022-4>
- Kawai, H., Hanyuda, T., Draisma, S.G. & Müller, D.G. (2007) Molecular phylogeny of *Discosporangium Mesarthrocarpum* (Phaeophyceae) with a reinstatement of the order *Discosporangiales*. *Journal of phycology* 43: 186–194.  
<https://doi.org/10.1111/j.1529-8817.2006.00308.x>
- Kilar, J.A. & Hanisak, M.D. (1989) Phenotypic variability in *Sargassum polyceratum* (Fucales, Phaeophyta). *Phycologia* 28: 491–500.  
<https://doi.org/10.2216/i0031-8884-28-4-491.1>
- Kuntze, O. (1880) Revision von *Sargassum* und das sogenannte Sargasso-Meer. *Botanische Jahrbücher für Systematik, Pflanzengeschichte und Pflanzengeographie* 1: 191–239.
- Komatsu, T., Fukuda, M., Mikami, A., Mizuno, S., Kantachumpoo, A., Tanoue, H. & Kawamiya, M. (2014) Possible change in distribution of seaweed, *Sargassum horneri*, in northeast Asia under A2 scenario of global warming and consequent effect on some fish. *Marine Pollution Bulletin* 85: 317–324.  
<https://doi.org/10.1016/j.marpolbul.2014.04.032>
- Lanfear, R., Frandsen, P.B., Wright, A.M., Senfeld, T. & Calcott, B. (2016) PartitionFinder 2: new methods for selecting partitioned models of evolution for molecular and morphological phylogenetic analyses. *Molecular Biology and Evolution* 34 (3): 772–773.  
<https://doi.org/10.1093/molbev/msw260>
- Leaché, A.D. & Reeder, T.W. (2002) Molecular systematics of the eastern fence lizard (*Sceloporus undulatus*): a comparison of parsimony, likelihood, and Bayesian approaches. *Systematic biology* 51: 44–68.  
<https://doi.org/10.1080/106351502753475871>
- Leliaert, F., Verbruggen, H., Vanormelingen, P., Steen, F., López-Bautista, J.M., Zuccarello, G.C. & De Clerck, O. (2014) DNA-based species delimitation in algae. *European Journal of Phycology* 49: 179–196.  
<https://doi.org/10.1080/09670262.2014.904524>
- Li, J.J., Hu, Z.M., Gao, X., Sun, Z.M., Choi, H.G., Duan, D.L. & Endo, H. (2016) Oceanic currents drove population genetic connectivity of the brown alga *Sargassum thunbergii* in the north-west Pacific. *Journal of Biogeography* 44: 230–242.  
<https://doi.org/10.1111/jbi.12856>
- Liu, L., Heinrich, M., Myers, S. & Dworjanyn, S.A. (2012) Towards a better understanding of medicinal uses of the brown seaweed *Sargassum* in Traditional Chinese Medicine: A phytochemical and pharmacological review. *Journal of ethnopharmacology* 142: 591–619.  
<https://doi.org/10.1016/j.jep.2012.05.046>

- Mattio, L., Anderson, R.J. & Bolton, J.J. (2015) A revision of the genus *Sargassum* (Fucales, Phaeophyceae) in South Africa. *South African Journal of Botany* 98: 95–107.  
<https://doi.org/10.1016/j.sajb.2015.02.008>
- Mattio, L. & Payri, C.E. (2009) Taxonomic revision of *Sargassum* species (Fucales, Phaeophyceae) from New Caledonia based on morphological and molecular analyses. *Journal of Phycology* 45: 1374–1388.  
<https://doi.org/10.1111/j.1529-8817.2009.00760.x>
- Mattio, L. & Payri, C.E. (2011) 190 years of *Sargassum* taxonomy, facing the advent of DNA phylogenies. *The Botanical Review* 77: 31–70.  
<https://doi.org/10.1007/s12229-010-9060-x>
- Mattio, L., Payri, C.E. & Stiger-Pouvreau, V. (2008) Taxonomic revision of *Sargassum* (Fucales, Phaeophyceae) from French Polynesia based on morphological and molecular analyses1. *Journal of Phycology* 44: 1541–1555.  
<https://doi.org/10.1111/j.1529-8817.2008.00597.x>
- 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 analyses1. *Journal of Phycology* 45: 1213–1227.  
<https://doi.org/10.1111/j.1529-8817.2009.00737.x>
- Mattio, L., Zubia, M., Loveday, B., Crochelet, E., Duong, N., Payri, C.E., Bhagooli, R. & Bolton, J.J. (2013) *Sargassum* (Fucales, Phaeophyceae) in Mauritius and Réunion, western Indian Ocean: taxonomic revision and biogeography using hydrodynamic dispersal models. *Phycologia* 52: 578–594.  
<https://doi.org/10.2216/13-150.1>
- Ni-Ni-Win, Hanyuda, T., Arai, S., Uchimura, M., Abbott, I.A. & Kawai, H. (2008) Three new records of *Padina*, in Japan based on morphological and molecular markers. *Phycological Research* 56: 288–300.  
<https://doi.org/10.1111/j.1440-1835.2008.00510.x>
- Oba, T., Kato, M., Kitazato, H., Koizumi, I., Omura, A., Sakai, T. & Takayama, T. (1991) Paleoenvironmental changes in the Japan Sea during the last 85,000 years. *Paleoceanography* 6: 499–518.  
<https://doi.org/10.1029/91PA00560>
- Ota, H. (1998) Geographic patterns of endemism and speciation in amphibians and reptiles of the Ryukyu Archipelago, Japan, with special reference to their paleogeographical implications. *Researches on Population Ecology* 40: 189–204.  
<https://doi.org/10.1007/BF02763404>
- Phillips, N. (1995) Biogeography of *Sargassum* (Phaeophyta) in the Pacific basin. In: Abbott, I.A. (Ed.) *Taxonomy of Economic Seaweeds with Reference to Some Pacific Species Vol. V*. California Sea Grant College, La Jolla, pp. 107–144.
- Phillips, N.E., Smith, C.M. & Morden, C.W. (2005) Testing systematic concepts of *Sargassum* (Fucales, Phaeophyceae) using portions of the *rbcL-S* operon. *Phycological research* 53: 1–10.  
<https://doi.org/10.1111/j.1440-1835.2005.tb00353.x>
- Rambault, A., Suchard, M.A., Xie, D. & Drummond, A.J. (2014) Tracer v1.6 [WWW Document]. Tracer. Available from: <http://beast.bio.ed.ac.uk/Tracer> (accessed 1 August 2017)
- Ronquist, F., Teslenko, M., Van Der Mark, P., Ayres, D.L., Darling, A., Höhna, S., Larget, B., Liu, L., Suchard, M.A. & Huelsenbeck, J.P. (2012) MrBayes 3.2: efficient Bayesian phylogenetic inference and model choice across a large model space. *Systematic Biology* 61: 539–542.  
<https://doi.org/10.1093/sysbio/sys029>
- Setchell, W.A. (1931) Hong Kong Seaweeds, II. *Hong Kong Naturalist* 11: 237–253.
- Stamatakis, A. (2014) RAxML version 8: a tool for phylogenetic analysis and post-analysis of large phylogenies. *Bioinformatics* 30: 1312–1313.  
<https://doi.org/10.1093/bioinformatics/btu033>
- Stiger, V., Horiguchi, T., Yoshida, T., Coleman, A.W. & Masuda, M. (2003) Phylogenetic relationships within the genus *Sargassum* (Fucales, Phaeophyceae), inferred from ITS-2 nrDNA, with an emphasis on the taxonomic subdivision of the genus. *Phycological research* 51: 1–10.  
<https://doi.org/10.1111/j.1440-1835.2003.tb00164.x>
- Sun, J.Z., Chen, W.D., Zhuang, D.G., Zheng, H.Y., Lin, L. & Pang, S.J. (2008) In situ ecological studies of the subtidal brown alga *Sargassum horneri* at Nanji Island of China. *South China Fisheries Science* 4: 58–63.
- Sun, J.Z., Zhuang, D.G., Wang, T.G., Chen, W.D. & Yang, J.B. (2009) Study on of *Sargassum horneri* (Turn.) Agardh around Nanji Islands. *Modern Fisheries Information* 24: 19–21.
- Tseng, C.K. & Chang, C.F. (1960) An analysis of the nature of marine algal flora. *Oceanologia Et Limnologia Sinica* 3: 177–187.
- Tseng, C.K. & Lu, B.R. (1985) On a new *Sargassum* from the East China Sea *S. nigrifolioides* sp. nov. *Oceanologia Et Limnologia Sinica*

16: 169–174.

- Tseng, C.K. & Lu, B.R. (2000) *Flora Algarum Marinarum Sinicarum, Tomus 3. Phaeophyta (No.2) Fucales*, Science Press, Beijing, 238 pp.
- Tseng, C.K. & Lu, B.R. (2004) Studies on four new species of the malacocarpic *Sargassum* (Sargassaceae, Heterokontophyta) in China. *Hydrobiologia* 512: 193–199.  
<https://doi.org/10.1023/B:HYDR.0000020327.28056.fa>
- Uwai, S., Kogame, K., Yoshida, G., Kawai, H. & Ajisaka, T. (2009) Geographical genetic structure and phylogeography of the *Sargassum horneri/filicinum* complex in Japan, based on the mitochondrial *cox3* haplotype. *Marine Biology* 156: 901–911.  
<https://doi.org/10.1007/s00227-009-1136-y>
- Van Tu, N. (2015) Seaweed diversity in Vietnam, with an emphasis on the brown algal genus *Sargassum*, Doctoral dissertation, Ghent University, 191 pp.
- Yoshida, T. (1983) Japanese species of *Sargassum* subgenus *Bactrophycus* (Phaeophyta, Fucales). *Journal of the Faculty of Science, Hokkaido University, Series 5, Botany* 13: 99–246.
- Yoshida, T. (1989) Taxonomy of *Sargassum*. *The Korean Journal of Phycology* 4: 107–110.
- Yoshida, T., Stiger, V. & Horiguchi, T. (2000) *Sargassum boreale* sp. nov. (Fucales, Phaeophyceae) from Hokkaido, Japan. *Phycological Research* 48: 125–131.  
<https://doi.org/10.1111/j.1440-1835.2000.tb00207.x>
- Zheng, B.L., Yao, G.D., Lv, Y.K., Li, C.P., Li, X.Y. & Wang, G.H. (1960) Yellow Sea and Bohai Sea Economic Seaweed, *Journal of Shandong Ocean University* 1: 120–210.