Morphological and molecular characteristics of *Homoeostrichus formosana* sp. nov. (Dictyotaceae, Phaeophyceae) from Taiwan

Wei-Lung Wang¹*, Ching-Su Lin¹, Wook-Jae Lee² and Shao-Lun Liu³

**Abstract**

**Background:** In the marine brown macroalgae, the morphological characters are highly similar between two widely distributed genera, *Homoeostrichus* and *Zonaria* (Dictyotaceae), thereby resulting in the difficulty of exploring their hidden biodiversity. Owing to the help of the molecular tools, it is now easy for scientists to objectively describe a new species in nature. In this study, we make a description on the *Homoeostrichus formosana* sp. nov. from Taiwan, Indo-Pacific Ocean based on the morphological evidence and molecular data.

**Results:** Our morphological observations revealed that this species has marginal row of apical cells responsible for thallus growth and the thallus with four layers of cells except the marginal regions. The cortical cell lies upon each medullary cell in transverse section, and two cortical cells upon each medullary cell in longitudinal section. Tetrasporangium is developed from cortical cell with stalk cell and singly scattered over the thallus surface, and has no indusia and paraphyses. Molecularly, the phylogenetic trees based on *SSU*, *psaA*, *psbA*, and *rbcL* gene sequences supported that *Homoeostrichus* species are closely related to *Exallosorus* species and clearly separated from each others in addition to *Zonaria* species.

**Conclusions:** *Homoeostrichus formosana* sp. nov. can now be clearly distinguished from *E. harveyanus* and Japanese *H. flabellatus*.

**Keywords:** Dictyotaceae; *Homoeostrichus formosana*; Phaeophyceae; Taiwan; Zonarieae

**Background**

The three genera, *Exallosorus* Phillips 1997, *Homoeostrichus* J. Agardh 1894 and *Zonaria* C. Agardh 1817 were established based on the characteristics of their reproductive structures, which used as key characters in the taxonomy of Dictyotaceae (Papenfuss 1944; Womersley 1987; Phillips and Clayton 1993, 1994, 1997; Phillips et al. 1994; Phillips 1997). Genus *Homoeostrichus* was established to include *Zonaria canaliculata* J. Agardh, *Z. multifida* Harvey ex J. Agardh, *Z. sinclairii* Hooker et Harvey and *Z. stuposa* R. Brown ex J. Agardh (J. Agardh 1894). Genus *Zonaria* had included five sections with ca. 50 species (C. Agardh 1817, see Silva 1952), of which several species were transferred to *Dictyota* Lamouroux and *Padina* Adanson. Ten species of *Zonaria* are currently recognized (Phillips 1997; Phillips and Nelson 1998), and most of them are endemic to Australia (Womersley 1987; Phillips 1997; Phillips and Nelson 1998), whereas *Z. diesingiana* J. Agardh and *Z. tournefortii* (Lamouroux) Montagne are widely distributed from subtropical to temperate waters (Børgeresn 1926; Taylor 1960; Gayral 1966; Allender and Kraft 1983; Seagarief 1984; Yoshida et al. 1985; Silva et al. 1987, 1996; Womersley 1987; Farrant and King 1989; Ribera et al. 1992; Phillips et al. 1994; Phillips 1997; Phillips and Clayton 1997; Yoshida 1998).

Papenfuss (1944) suggested that *Homoeostrichus* and *Zonaria* shared characteristics in vegetative morphology and subsumed *Homoeostrichus* in *Zonaria*. However, Womersley (1987) argued that species of *Zonaria* had octosporangia and paraphyses whereas species of *Homoeostrichus* had only tetrasporangia and no paraphyses. He kept distinguishing *Homoeostrichus* from *Zonaria* and recognized three species of *Homoeostrichus* (*H. canaliculatus* J. Agardh, *H. olsenii* Womersley and *H. sinclairii* (Hooker et Harvey) J. Agardh)
Phillips (1997) established *Exallosorus* based on two Australian species, *Zonaria harveyana* (Pappe ex Kützing) Areschoug (as *Homoeostrichus multifidus* J. Agardh) and *Homoeostrichus olsenii* Womersley [as *E. harveyanus* (Pappe ex Kützing) Phillips and *E. olsenii* (Womersley) Phillips]. She suggested that these species of *Exallosorus* have tetrasporangia with a stalk cell and within the indusiate sori which lack paraphyses and mucilage. The plants of genus *Homoeostrichus* commonly distributed in southeastern Australia and currently are recognized as two species: *H. canaliculatus* and *H. sinclairii* (Womersley 1987; Phillips 1997).

A species of brown alga with external morphology similar to *Exallosorus* and *Zonaria* was collected from several collecting sites (Figure 1) in southern Taiwan. The plants of *Homoeostrichus formosana* Wang, Lin, Lee et Liu sp. nov. have been identified as *Z. diesingiana* or *Z. harveyana* in Taiwan, due to short information of their reproductive structures and morphological characteristics, especially no gametangia. It is the first time to describe the characteristics of sporangia of *H. formosana* sp. nov. in this study. We also described the morphological and phenological characteristics of this species, and determined its phylogeny among the related species based on nuclear-encoded SSU rRNA and plastid encoded *rbcL, psaA*, and *psbA* gene sequences.

**Methods**

**Survey on morphological characteristics**

Collections were made by SCUBA or snorkeling in southern Taiwan (Figure 1) from 1999 to 2002. Voucher specimens were fixed with 10% formalin/sea water or pressed on herbarium sheets and deposited in the Herbarium of the Department of Biology, National Chunghua University of Education, Taiwan. Microscopic sections were made using a freezing microtome (Leica CM1850), then stained with 0.1% Toluidine Blue O (TBO) and mounted in 50% Karo syrup. Microphotographs were taken on a Pixera digital camera attached to a Carl Zeiss Axioskop 2 microscope with differential interference contrast (DIC) optics.

Other specimens deposited in the following institutions were also examined: the Institute of Oceanography, National Taiwan University, Taipei (IONTU), the National Museum of Natural Science, Taichung, Taiwan (NMNS) and the National Museum of Marine Biology and Aquarium, Hengchun, Taiwan (NMMBA).

**Gene sequence analyses**

Collections for gene sequencing were made by SCUBA or snorkeling at Kenting, in southern Taiwan on 23 April 2004. Nuclear-encoded SSU rRNA and plastid encoded *rbcL* gene were selected for elucidating the phylogenetic relationship of *Homoeostrichus formosana* sp. nov. with other Dictyotaceae. Genomic DNA was extracted from 0.01 g of powder ground in liquid nitrogen using Dneasy Plant Mini Kit™ (Qiagen, Hilden in Germany), according to the manufacturer’s instructions. The partial *rbcL* gene and *rbcS*, except for short 3′-terminal of *rbcL* and 5′-terminal region of the *rbcS*, were amplified and sequenced as two fragments using the primers sets, DRL1F-DRL2R and DRL3F-DRL4R.
DRL2F-RU2 (Hwang et al. 2005). The psaA gene sequences were also amplified and sequenced by two 130 F-970R and 870 F-1760R primers sets, psbA gene by one fragment with psbA F- psbA R primers set (Yoon et al. 2002). The partial 18S rRNA gene (SSU) was amplified and sequenced using primers set, SRI-SR7 and SR4-SR12. The amplified DNA was purified using High Pure PCR Product Purification Kit™ (Roche, Indianapolis, USA), in accordance with the manufacturer’s instructions. The forward and the reverse sequences were determined for all samples using an ABI PRISM 377 DNA sequencer. The sequences were aligned using PHYDIT (Chun 1995) with final visual confirmation and then submitted to GenBank under the accession numbers (Table 1). The alignment of each coding gene sequence was based on the alignment of inferred amino acid sequences, and reconfirmed by eye. The Padina species were selected as the outgroup species in the phylogenetic analyses.

Phylogenetic analysis was conducted using the software MEGA with a maximum likelihood method (Tamura et al., 2011). Prior to the phylogenetic analysis, the best fit of nucleotide evolutionary model for each gene was selected based on maximum-likelihood model fitting in the software MEGA. The chosen model is TN93+G model for SSU [lnL = −4717.63, rates of nucleotide changes (AT: 0.05, AC: 0.04, AG: 0.08, TA: 0.05, TC: 0.20, TG: 0.06, CA: 0.05, CT: 0.25, CG: 0.06, GA: 0.07, GT: 0.05, GC: 0.04), G = 0.08, and nucleotide frequencies (A: 0.24, T: 0.26, C: 0.22, G: 0.28)], GTR+G model for rbcL [lnL = −8507.61, rates of nucleotide changes (AT: 0.12, AC: 0.02, AG: 0.09, TA: 0.11, TC: 0.13, TG: 0.03, CA: 0.04, CT: 0.27, CG: 0.02, GA: 0.12, GT: 0.04, GC: 0.02), G = 0.22, and nucleotide frequencies (A: 0.30, T: 0.32, C: 0.16, G: 0.22)], TN93+G+I model for psaA [lnL = −10500.23, rates of nucleotide changes (AT: 0.06, AC: 0.03, AG: 0.07, TA: 0.05, TC: 0.15, TG: 0.03, CA: 0.05, CT: 0.33, CG: 0.03, GA: 0.12, GT: 0.06, GC: 0.03), I = 0.47, G = 0.53, and nucleotide frequencies (A: 0.30, T: 0.36, C: 0.16, G: 0.19)], and GTR+G model for psbA [lnL = −4454.15, rates of nucleotide changes (AT: 0.15, AC: 0.01, AG: 0.06, TA: 0.11, TC: 0.17, TG: 0.02, CA: 0.01, CT: 0.37, CG: 0.004, GA: 0.07, GT: 0.03, GC: 0.003), G = 0.17, and nucleotide frequencies (A: 0.26, T: 0.36, C: 0.17, G: 0.21)]. The ML bootstrap analyses were conducted with 500 replicates because of high computational demands.

Results
Species description
*Homoeostrichus formosana*

W.-L. Wang, C.-S. Lin, W.-J. Lee & S.-L. Liu sp. nov. (Figures 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12 and 13)

*Huius plantae thallus, qui mensuratur 5–23 cm altitudine ac (1-)3-7(−10) cm latitudine, est fuscus, planus ac flabellatus; in ramos dividitur quorum axes inferiors angusti, superiors vero segmentati atque flabellate sunt. Folia in basi sunt erecta aut plana atque exsurgunt e stipite manifeste rhizoidali. Thallus autem componitur ex duo aut quattuor cellularum ordinibus, crassitudine 88–100 μm. In transversali sectione, medullares cellae, 80–157 μm altitudine ac 15–25 μm latitudine, conteguntur a singulari cellula corticali, cui mensuratio est 25–50 μm altitudine ac 15–25 μm crassitudine. In sectione autem longitudinali, duo tresve corticales cellae contegunt singuliam cellulam medularem. Tetrasporangia sphaerica, dispersa supra superficies, marginibus exceptis, mensuram habent 80–100 μm altitudine ac 85–95 μm in diametro, cum singularia cellula basilaris quae se protrudit ultra thalli superficiem, sed sine uillis excrecentiis sori, indussii aut paraphysis.

Thalli are 5–23 cm in height and (1-)3-7(−10) cm in width, dark brown in color, complanate, flabellate, split to form branches with narrow lower axes and upper flabellate segment, and prostrate at the base arising from a conspicuously rhizoid holdfast to upright blades. Thallus composed of two to four layers of cells throughout, 88–100 μm in thickness. In transverse section medullary cells, 80–157 μm in height, 15–25 μm in width, are overlain by a single cortical cell, 25–50 μm in height, 15–25 μm in width, and then in longitudinal section, two to three cortical cells over lay each medullary cell. Tetrasporangia are spherical, scattered over the both sides of thallus except the margins, 80–100 μm in height by 85–95 μm in diameter, with one basal stalk cell projecting out from the thallus surface, without forming a sorus, indussii and paraphyses absent.

Holotype

The holotype is deposited at Department of Biology, National Changhua University of Education, Changhua (NCUE-CAF91072101) (Figure 2).

Type locality

Chuan-Fan-Shih, Southern Taiwan (21°56’01″N, 120°49’21″E).

Etymology

“formosana” refers to Taiwan, where the alga was collected.

Distribution

Known only from southern Taiwan (Figure 1).

Habitat and phenology

Absence of perennial stipes indicates that this species may be annual. Plants were found all year round, mainly at 2–5 m depth, where they were abundant on coral reefs or on reef rocks.
| Species name                      | Collection site & reference                                      | GenBank accession number |
|----------------------------------|------------------------------------------------------------------|--------------------------|
| **Dictyopteris divaricata**      | Atsumi, Yamagata, Japan; 28.iv.2002 (Hoshina et al. 2004)         | AB087112                 |
| (Okamura) Okamura                | Jindo, Jeolnamdo, Korea; 20.vi.1998, coll. W.J. Lee & I.-K. Hwang (2004) | -                        |
|                                  | Anin, Gangwondo, Korea; 19.vi.2000, coll. I.K. Hwang (Hwang et al. 2004) | -                        |
|                                  | Haegumgang, Gyoungsangnamdo, Korea; 14.vii.2000, coll. I. K. Hwang (Hwang et al. 2004) | -                        |
| **Dic. pacifica** (Yendo)         | Gampo, Gyoungsangnamdo, Korea; 20.xi.2002, coll. W.J. Lee (Hwang et al. 2004) | -                        |
| H.S. Kim & W.J. Lee              |                                                                    |                          |
| **Dic. polypodioides** (A.P. De Candolle) J. V. Lamouroux |                                                                    |                          |
|                                  |                          |                          |
| **Dic. prolifera** (Okamura) Okamura | Tsumekizaki, Shizuoka, Japan; 19.iii.2002 (Hoshina et al. 2004)          | AB095294                 |
|                                  |                                                                    |                          |
| **Distromium decumbens**         |                                                                    |                          |
| (Okamura) Levring                |                                                                    |                          |
|                                  |                                                                    |                          |
| **Exallosorus harveyanus** (Pappe ex Kützing) J.A. Phillips |                                                                    |                          |
|                                  |                                                                    |                          |
| **E. olsenii** (Womersley) J.A. Phillips |                          |                          |
|                                  |                                                                    |                          |
| **Homoeostrichus canaliculatus** (J. Agardh) J. Agardh |                                                                    |                          |
|                                  |                                                                    |                          |
| **H. flabellatus**                |                                                                    |                          |
| Okamura                          |                                                                    |                          |
|                                  |                                                                    |                          |
| **H. formosana** W.L. Wang, C.S. Lin, W.J. Lee & S.L. Liu sp. nov. |                                                                    |                          |
|                                  |                                                                    |                          |
| **Lobophora pachyventera** Z. Sun, P.-E. Lim, J. Tanaka & H. Kawai |                                                                    |                          |
|                                  |                                                                    |                          |
| **Lobophora vaniegata** (Lamouroux) Womersley |                                                                    |                          |
|                                  |                                                                    |                          |
| **Lobophora australis** Z. Sun, F. C. Gurgel & H. Kawai |                                                                    |                          |
|                                  |                                                                    |                          |
| **Padina arborescens** Holmes    |                                                                    |                          |
|                                  |                                                                    |                          |
| **P. australis** Hauck            |                                                                    |                          |
|                                  |                                                                    |                          |
| **P. crassa** Yamada             |                                                                    |                          |
Table 1 The list of materials and accession number of nucleotide sequences determined and used in these analyses (Continued)

| Species                           | Localities                                                                 | Accession Numbers                  |
|-----------------------------------|-----------------------------------------------------------------------------|------------------------------------|
| *P. japonica* (Yamada)            | Gyokpo, Jeolabukdo, Korea; 11.Aug.1998, coll. I.-K. Hwang (This study)      | AB096905 AY430319 AY430360          |
| *Spatoglossum crassum* (Tanaka)   | Anin, Gangwondo, Korea; 23.xii.1998, coll. I.K. Hwang (Lee and Bae 2002; Hwang et al. 2004) | AF350222 AY430336 AY430314 AY430355 |
|                                   | Yumigahama, Shizuoka, Japan; 18.iii.2002 (Hoshina et al. 2004)              | AB087129 AB096909 - -              |
| *Stypopodium flabelliforme* (Weber-van Bosse) | Alona Beach, Panglao Is., Bohol, the Philippines; 17.xii.2003, coll. W.J. Lee (This study) | - DQ866928 DQ866959 DQ866947 |
|                                   | Pratas Is., South China Sea; 22.iv.2004, coll. S.M. Lin (This study)        | - DQ866927 DQ866960 DQ866949 |
| *St. schimperi* (Kützing) M.Verlaque | Lebanon, France; 1.v.2005, coll. G. Bitra (This study)                      | - DQ866926 DQ866961 DQ866948 |
| *St. zonale* (U.V. Lamouroux) (Papenfuss) | Okakime Is., Kahoshima, Japan; 4.ix.1999 (Hoshina et al. 2004)               | AB087133 - - -                    |
| *Zonaria angustata* (C. Agardh)   | Emubay, Kanggaroo Is., Australia; 15.iv.2003, coll. W.J. Lee & E.C. Yang (This study) | - DQ866932 DQ866966 DQ866946 |
| *Z. crenata* (J. Agardh)          | Emubay, Kanggaroo Is., Australia; 15.iv.2003, coll. W.J. Lee & E.C. Yang (This study) | DQ866937 - DQ866965 DQ866945 |
|                                   | Nora Creina Bay, New South Wales, Australia; 17.iv.2003, coll. W.J. Lee & E.C. Yang (This study) | - DQ866933 DQ866967 DQ866950 |
|                                   | Nora Creina Bay, New South Wales, Australia; 17.iv.2003, coll. W.J. Lee & E.C. Yang (This study) | - DQ866936 DQ866970 DQ866955 |
| *Z. desigiana* (J. Agardh)        | Ishigaki Is., Japan; 21.i.1998, coll. W.J. Lee & J.H. Oak (Lee and Bae 2002) | AF350234 AF353377 - - |
| *Z. desigiana* (J. Agardh)        | Seongsan, Korea; 15.viii.2001, coll. W.J. Lee (Hwang et al. 2005)            | - AY422682 AY422606 AY422644 |

Abbreviation: CCMP Provasoli-Guillard National Center for Culture of Marine Phytoplankton.

Specimens examined and localities

Pingtung County, southern Taiwan: Chu-Shui-Kou, 5–7 m, coll. C-S Lin, CAF91041401, 14 April 2002; Chuan-Fan-Shih, 1–4 m, coll. C-C Peng, 840013 (NTU), 25 Oct.1995; coll. W-L Wang, CAF85053101, 31 Mar.1996; coll. C-S Lin, CAF90030301, sporophyte, 03 Mar.2001; coll. C-S Lin, CAF90050501, 05 May 2001; coll. S-M Lin, CAF90102601, 26 Oct. 2001; coll. S-M Lin, CAF90128801, sporophyte, 28 Nov. 2001; coll. C-S Lin, CAF91011301, sporophyte, 13 Jan. 2002;

Figure 2 Homoeostrichus formosana sp. nov. Mature thallus, Holotype (Scale bar: 10 cm).

Figure 3 Homoeostrichus formosana sp. nov. Young thallus, with an enlarged holdfast (arrow) at the base (Scale bar: 5 cm).
coll. C-S Lin, CAF91020601, 06 Feb. 2002; coll. C-S Lin, CAF91030101, 01 Mar. 2002; coll. S-M Lin, CAF91031401, 14 Mar. 2002; coll. C-S Lin, CAF91041301, 13 April 2002; coll. C-S Lin, CAF91061501, 15 June 2002; coll. C-S Lin, CAF91072101, sporophyte, 21 July 2002; coll. C-S Lin (Holotype), CAF91100201, 02 Oct. 2002; coll. S-L Lau, CAF91103001, sporophyte, 30 Oct. 2002; Hsiao-Wan, 1–3 m, coll. S-M Lin, CAF91032901, 29 May 2002; Hsiang-Chiao-Wan, 1–3 m coll. S-M Lin, CAF91051101, 11.v.2002; coll. C-S Lin, CAF91072001, sporophyte, 20 July 2002; Chiu-Peng, 1–5 m, coll. S-M Lin, CAF91051102, 11 May 2002; Feng-Chui-Sha, 1–5 m, coll. C-S Lin, CAF91051102, 11 May 2002; Chiu-Peng, 2–3 m, coll. G-L Lin, CAF82071101, 11 July 1993; coll. G-L Lin, CAF82102901, 29 Oct. 1993.

**Habitat and anatomical structures**

Thalli are yellow or dark brown in color, composed of upright blades (Figures 2 and 3), and which basal portions are creeping with a conspicuously rhizoid holdfast. They are 5–23 cm in height and (1-)3-7(–10) cm in width (Figures 2 and 3). Thalli are fan-shaped when young and splitting into numerous bladelets when old. The surfaces of thallus are covered with hyaline hairs that are arranged in interrupted concentric bands (Figure 4), and with the blanketing brown rhizoidal filament at the base (Figure 5). Thallus growth is by a row of marginal meristem cells, which are dark in color (Figure 6). The apical cell is 120–240 μm in length and 70–78 μm in width (Figure 12). The blades are polystromatic, two or four cell layers, with 88–100 μm in...
thickness. Cortical cells are 25–50 μm in height and 15–25 μm in width. Those cells occurred on either side of two-cell layers of medullary cells, which measure 80–157 μm in height and 15–25 μm in width (Figures 7 and 8). In longitudinal section of thallus, two or three cortical cells overlay a single medullary cell (Figure 8), whereas a single cortical cell overlays each medullary cell in transverse section (Figures 11 and 13).

Reproductive structures
Sporangia are scattered over the surface on both sides of the blade (Figures 9 and 10). Tetrasporangia are roughly spherical and projected above the surface of the thallus, 80–100 μm in height and 85–95 μm in diameter, with a basal stalk cell which measured 12–26 μm in height by 17–25 μm in diameter, and lacked indusium and paraphyses (Figures 11 and 13). Gametophytes were not observed.

Characteristics of gene sequences
The SSU sequences determined and aligned in this study were 1,814 nucleotides long. The 20 aligned SSU sequences had 106 (5.8%) variable bases and 176 (9.7%) parsimoniously informative sites and 49.4% G+C contents. Transitions occurred more than transversions (Ts/Tv=1.16). The average of uncorrected pairwise distances (p-distances) was 0.059 from the aligned data set (Figure 14). The uncorrected pair wise distance (p-distances) between Zonaria species and Homoeostrichus species ranged from 0.057 to 0.077, and between Exallosorus species and Homoeostrichus species from 0.009 to 0.014. We could find 5 nucleotide base pairs differences in the
aligned 1,723 nucleotide base pairs sequences between *H. formosana* sp. nov. and *H. flabellatus* from Japan (~0.3%), and 11 nucleotide base pairs differences between *H. formosana* sp. nov. and *H. sinclairii*. We determined and aligned 1,351 nucleotides long *rbcL* sequences in this study. The 28 aligned *rbcL* sequences had 82 (6.07%) variable bases and 420 (31.08%) parsimoniously informative sites. The G+C content was 38.2% in the aligned sequence data set. Transitions were almost less than transversions (Ts/Tv=0.89). The average of *p*-distances was 0.122 from the aligned data set (Figure 14). The "*p*-distances" between *Zonaria* species and *Homoeostrichus* species ranged from 0.143 to 0.152, and between *Exallosorus* species and *Homoeostrichus* species from 0.132 to 0.137. We found 182 nucleotide differences between *H. formosana* sp. nov. and *H. sinclairii* in aligned sequences of 1,394 base pairs.

The total 845 base pairs of *psbA* sequences were determined and aligned in this study. The aligned 25 *psbA* sequences had 45 (5.33%) variable bases and 213 (25.21%) parsimoniously informative sites with 37.8% G+C content. Transitions occurred more frequently than transversions (Ts/Tv=1.22) and *p*-distance ranged from 0.030 to 0.134 with average of 0.089 in aligned *psbA* sequences data set (Figure 14). The "*p*-distances" between *Zonaria* species and *Homoeostrichus* species ranged from 0.072 to 0.102, and between *Exallosorus* species and *Homoeostrichus* species from 0.084 to 0.098.

Overall, the sequence divergence is smallest in *SSU*, followed by *psbA* (Figure 14). In contrast, the sequence divergence is much larger in *rbcL* and *psaA* (Figure 14). Our observations suggest that *SSU* is more suitable to resolve the phylogenetic relationship of higher taxonomic level and other plastid genes used in this study are more suitable to tackle the phylogenetic relationship for the lower taxonomic level.

The phylogeny based on gene sequences

The phylogenetic tree based on *SSU* sequences showed that genera of tribe Zonarieae made four clades with no phylogenetic resolution among them in the ML analyses (Figure 15). The clade of *Homoeostrichus* and *Exallosorus* species is separated from that of *Zonaria* and *Lobophora* species. Three *Homoeostrichus* species made a subclade distinguished from *Exallosorus* species except for *H. canaliculatus*. Especially *H. formosana* sp. nov. made a clade with *H. flabellatus* with very low supporting value in three analyses.

The topology of phylogenetic tree based on *rbcL* sequences also show that four clades are distinguished (Figure 15). The clade comprising *Homoeostrichus* and *Exallosorus* species figured out as basal sister group in this phylogeny although the results showed pale phylogenetic resolution. *Homoeostrichus formosana* sp. nov. made a clade with *H. flabellatus* with very high supporting value, and a sister group of *H. sinclairii* with low supporting value in three analyses. *Exallosorum olsenii* also made a sister group to three *Homoeostrichus* species clade and closely related to *H. canaliculatus*. The clade of *Zonaria* and *Lobophora* species made a
concrete clade with high supporting value distinguished from others.

The aligned \textit{psaA} gene sequences data set made the phylogenetic tree with five clades, which have a basal clade of \textit{Stypopodium} species although with pale phylogenetic resolution (Figure 15). As in the former trees, the clade of \textit{Homeostrichus} and \textit{Exallosorus} species is distinguished as basal sister group in this phylogeny although having pale phylogenetic resolution. \textit{Homoeostrichus formosana} sp. nov. made a clade with \textit{H. flabellatus} with very high supporting value, with a sister group of \textit{H. sinclairii} with low supporting value in three analyses. \textit{Exallosorum} \textit{olenii} also made a sister calde with three \textit{Homoeostrichus} species and closely related to \textit{H. canaliculatus} as in the phylogeny of \textit{rbcL}. The clade of \textit{Zonaria} and \textit{Lobophora} species made a concrete clade with high supporting value distinguished from \textit{Homoeostrichus} and \textit{Exallosorus} species.

The phylogenic tree based on \textit{psbA} gene also show that \textit{H. formosana} sp. nov. is involved in a clade with \textit{H. sinclairii} and \textit{H. canaliculatus}. This phylogenic tree is composed of five clades with very pale phylogenic resolution (Figure 15). \textit{Exallosorus} species are closely related to \textit{Homoeostrichus} species as in the other phylogenic trees.

\textbf{Discussion}

The taxonomy of the Dictyotales is largely based on the comparison of vegetative and reproductive growth and organization (Phillips 1997). \textit{Homoeostrichus formosana} sp. nov. is mainly characterized by blades composing of two to four layers of cells, single tetrasporangia scattered over both thallus surfaces, sporangia borne on a stalk cell, and lacking indusium and paraphyses. In the erect to recumbent fan-like fronds of \textit{Lobophora}, unusual large medullary cell and indusiate sporangial sorus, and \textit{Padina}, rolling margin and concentric arrangement of reproductive structures, which both are conspicuously differed from \textit{Homoeostrichus}, whatever the habit, texture, anatomical and reproductive structures. \textit{Homoeostrichus} has been very easily confused with \textit{Exallosorus} and \textit{Zonaria}, based on the vegetative and reproductive characters used for separating among them summarized in Table 2. The phylogenic trees based on \textit{SSU}, \textit{psaA}, \textit{psbA}, and \textit{rbcL} gene sequences supported that \textit{Homoeostrichus} species are closely related to \textit{Exallosorus} species but clearly separated from each others in addition to \textit{Zonaria} species.

The genus \textit{Exallosorus} is separated from \textit{Zonaria} and \textit{Homoeostrichus} in having regularly arranged cells in transverse section, densely placed basally stalked sporangia within sori that possess brown paraphyses and indusium (Phillips 1997) (Table 2). Sporangia of \textit{Zonaria} lacked basal stalk cells, are surrounded by whitish paraphyses (except in \textit{Z. angustata}) in the indusiate sori, and released eight spores (Womersley 1987; Phillips et al. 1994; Phillips 1997) (Table 2). Sporangia of \textit{Homoeostrichus} are distributed among brown paraphyses in non-indusiate sori, and released four spores (Womersley 1987; Phillips et al. 1994;
In this study, we also found the sporangia in *H. formosana* sp. nov. are singly scattered over the surfaces of the thallus without forming a sorus and lacking indusium and paraphyses (Table 3). Classifying the genera of tribe Zonariae based on these morphological and anatomical characteristics is basically agreed to five clades in phylogenetic analyses based on gene sequences.

*Homoeostrichus formosana* sp. nov. is superficially similar to *Zonaria diesingiana* found from Taiwan in external morphology. However, *H. formosana* sp. nov. can be distinguished vegetatively and reproductively from *Z. diesingiana*, especially it makes four cell layers. The thallus of *Z. diesingiana* is composed of 4–8 layers of cells, in which the one medullary cell is flanked by 2 cortical cells in transverse section, the octosporangia are borne on no stalk cell, and white paraphyses are present in indusiate sori. However, the tetrasporangium of *H. formosana* sp. nov. is borne on a basal stalk cell and lacks paraphyses and indusium.
Homoeostrichus formosana sp. nov. was previously misidentified as E. harveyanus (as Z. harveyana, H. multifidus) in Taiwan (Yamada 1925; Okamura 1936; Shen and Fan 1950; Chiang 1960; Lewis and Norris 1987). The thallus of E. harveyanus is composed of 6 layers of cells, which measured 120–170 μm in thickness, and the sporangia are formed in a dark brown band of an indusiate sorus, whereas the sporangia in H. formosana are singly scattered over the surfaces of the thallus without forming a sorus (see Table 3). Although Yamada (1925) and Okamura (1936) had documented the thallus of "Homoeostrichus multifidus" (as H. formosana sp. nov. in this study) as being composed of four layers of cells, they did not observe reproductive structures, moreover, it is now known that E. harveyanus (as H. multifidus) is only distributed in southern Africa, the type locality (Silva et al. 1996; Phillips 1997). All molecular data also supported that H. formosana sp. nov. is clearly distinguished from E. harveyanus in the psbA sequences molecular analyses in this study. Another Exallosorus species, E. olsenii, comprised of six cell layers, has sporangia assembled in indusiate sori that are connected with hairs and paraphyses, and with the reproductive structures only occurring on one thallus surface (Womersley 1987, as H. olsenii; Phillips et al. 1994; Phillips 1997), which is not agreed with H. formosana sp. nov. (Table 3).

### Table 2 Comparative features of the genera Exallosorus, Homoeostrichus, and Zonaria

|                | Exallosorus | Homoeostrichus | Zonaria |
|----------------|-------------|----------------|---------|
| No. of cell layers | 4–6        | 2–4–7          | 4–12    |
| No. of cortex / medulla in transverse section | 1          | 1              | 2       |
| Sporangia       | Sorus       | Single, Sorus  | Sorus   |
| No. of stalk cells | 1          | 1 or more     | 0       |
| No. of spores   | 4           | 4              | 8       |
| Indusium        | +           | -              | +       |
| Paraphyses      | + or -      | + or -         | +       |
| Color           | Brown       | Brown          | White   |
| Structure       | Spherical cells | Spherical cells | Elliptic cells in upper, spherical cells in middle and base |
| Distribution    | Australia and South Africa | Australia and Taiwan | widespread |
| References      | b, c        | a, b, c, this study | a, b, c |

*a: Womersley, 1987; b: Phillips et al., 1994; c: Phillips, 1997; -: Absent.*

### Table 3 Comparisons of vegetative and sporangial structures among the species of Exallosorus, Homoeostrichus and Zonaria

| Characters                      | H. formosana | H. canaliculatus | H. sinclairii | E. harveyanus | E. olsenii | Zonaria spp. |
|--------------------------------|--------------|------------------|--------------|---------------|-------------|--------------|
| Thallus thickness              | 88–100 μm    | 150–200 μm       | 105–180 μm   | 120–170 μm    | 90–115 μm   | 66–300 μm    |
| No. of cell layers             | 2–4          | 6–7              | 4–7          | 6             | 4–6         | 2–1          |
| Cortical / Medullary cells     | 1            | (2)              | 1(2)         | (2)           | (2)         | (2)          |
| Sporangia                      | Tetra-, Single | unknown       | Tetra-, Sorus | Tetra-, Sorus | Tetra-, Sorus | Octo-, Sorus |
| Indusium                       | -            | x                | -            | +             | +           | +            |
| Paraphyses                     | -            | x                | Brown, in sori | -             | Brown, near sori | Whitish, in sori |
| Stalk cell                     | 1            | x                | Multicellular | 1             | 1           | -            |
| Oogonia                        | unknown      | unknown          | Sorus        | unknown       | Sorus       | Sorus        |
| Indusium                       | x            | x                | -            | x             | +           | +            |
| Paraphyses                     | x            | x                | Brown, among oogonia | x           | Brown, near sori | -            |
| Stalk cell                     | x            | x                | -            | x             | 1           | 1            |
| Antheridial sorus              | unknown      | unknown          | sorus        | unknown       | sorus       | sorus        |
| Sorus border                   | x            | x                | Brown paraphyses and sterile filaments | x           | Cortical cells | Slightly elongate sterile cells |
| Stalk cell                     | x            | x                | 1            | x             | 1           | 1            |
| References                     | f            | b                | b, c, d, e   | a, e          | b, d, e     | b, c, d, e   |

*a: Simons, 1964; b: Womersley, 1987; c: Phillips et al., 1994; d: Phillips and Clayton, 1997; e: Phillips; 1997; f: This study. +: Present, -: Absent, x: not found.*
Homoeostrichus formosana sp. nov. can possibly be confused with other species of Homoeostrichus: H. canaliculatus and H. sinclairii (Womersley 1987; Phillips 1997). However, H. formosana sp. nov. can be distinguished from the other species of Homoeostrichus by its 2–4 layers of cells thallus and sporangial stalk cells opposed to a 6–7 cell layer thallus and by multilayered stalk cells which are found in Homoeostrichus (see Table 3). The phylogenetic tree especially based on psbA gene sequences showed that H. canaliculatus is distinguished from other Homoeostrichus species and from Exallosorus species. Moreover, H. flabellatus Okamura, another Dictyotaceae species from Taiwan, might also be confused with H. formosana sp. nov. (Taniguti 1976; Lewis and Norris 1987; Wang and Chiang 2001). Okamura (1936) reported the thallus of H. flabellatus was composed of three layers of cells but he did not observe reproductive structures. Womersley (1987) speculated that Japanese H. flabellatus did not belong to the genus Homoeostrichus, and Papenfuss (1944) transferred H. flabellatus to Zonaria flabellata (Okamura) C. However, this combination is not recognized by some phycologists (see Phillips 1997; Phillips and Nelson 1998). The molecular characteristics of SSU show Japanese H. flabellatus is more related to H. formosana sp. nov. in this study. These show that the status of this taxon should be required further study especially examining voucher specimens of H. flabellatus. Furthermore, it is noted that an undescribed Zonaria sp. was recently reported from Chaojing, Keelung, northern Taiwan by Kitayama and Lin (2012). Though they only showed single photo of the thallus of this alga without any anatomical observations, this alga is highly similar to H. formosana in appearance. Considering that H. flabellatus (as Zonaria flabellatus) in Okinawa is biogeographically close to northern Taiwan (Figure 15), it will be interesting to examine the phylogenetic affinity of this undescribed Zonaria sp. from the northern Taiwan to test whether this alga is phylogenetically close to H. flabellatus or H. formosana.

Conclusions
We describes a new species, Homoeostrichus formosana Wang, Lin, Lee et Liu, collected from Taiwan. This species has marginal row of apical cells responsible for thallus growth and the thallus with four layers of cells except the marginal regions. The cortical cell lies upon each medullary cell in transverse section, and two cortical cells upon each medullary cell in longitudinal section. Tetrasporangium is observed for the first time, which is developed from cortical cell with stalk cell and singly scattered over the thallus surface, and has no indusia and paraphyses. The phylogenetic trees based on SSU, psaA, psbA, and rbcL gene sequences supported that Homoeostrichus species are closely related to Exallosorus species but distinctly different from Zonaria species.

Competing interests
The authors declare that they have no competing interests.

Authors’ contributions
WL Wang and CS Lin carried out the morphological characteristics of this species and drafted the manuscript, while WJ Lee and SL Liu participated in the molecular genetic studies. All authors read and approved the final manuscript.

Acknowledgments
We are very grateful to Dr. S.-M. Lin (National Taiwan Ocean University, Taiwan) for collecting the materials from the Pratas Island used in this study, to Mr. C.-K. Lin (National Museum of Natural Science, Taiwan) for loaning some Dictyotaceae specimens, and to Dr. Lawrence Liao (Hiroshima University, Japan) for providing critical Dictyotaceae references. We also thank to Mr. L.-C. Wang at Department of Biology, National Chunchua University of Education for the assistance of the field work and the map drawing. We also thank to Prof. Emerit. S. J. Fernando Mateos (Tien Educational Center, Taiwan) for translating the Latin of genus and species descriptions. This study was partly supported by a Taiwan National Science Council (NSC) grant (NSC89-2611-M-018-001) to W.-L. Wang, by a Korea Research Foundation grant (KRF2002-070-C00983) to W.J. Lee, and by Taiwan NSC grant (NSC-101-2621-B-029-004) to S.-L. Liu.

Author details
1Department of Biology, National Chunghua University of Education, Chunghua 500, Taiwan. 2Research Group for Cosmetic Materials, Jeju Biodiversity Research Institute (JBRI) & Jeju Hi-Tech Industry Development Institute (HiDI), Jeju 697-943, Korea. 3Department of Life Science, Tunghai University, Taichung 40704, Taiwan.

Received: 2 May 2012 Accepted: 19 March 2013
Published: 21 August 2013

References
Agardh CA (1817) Synopsis algarum Scandinaviae. Berling, Lund, pp 1–135, I-xl + Agardh JG (1894) Anadecta algologica. Continuato I. Lund, universitetets Ar-skiften and afdelningen, konigl. Fysiografiska salskapets I. Lund Handlingar 299(1):1–144
Allender BA, Kraft GT (1983) The marine algae of Lord Howe island (New south Wales): the Dicrnatolae and Cutetolae (Phaeophyta). Brunonia 673–130
Bergesen F (1926) Marine algae from the canary islands, especially from Tenerife and Gran Canaria, II: Phaeophyceae: Biol Meddelelses 621–112
Chiang YM (1960) Marine algae of northern Taiwan (Cyanophyta, Chlorophyta, Phaeophyta): Taiwania 751–76
Chun J (1995) Computer-assisted classification and identification of actinomycetes, Ph. D. Thesis, University of Newcastle, UK
Farrant PA, King RJ (1989) The Dicrnatolae (algae: phaeophyta) of New South Wales. Proc Linnean Soc New South Wales 110:369–406
Gayral P (1965) Les algues de côte françaises, Dion, Manche et Atlantique, Paris
Hoshina R, Hasegawa K, Tanaka J, Hara Y (2004) Molecular phylogeny of the dictyotaceae (Phaeophyceae) with emphasis on their morphology and its taxonomic implication. Jpn J Phycol 52(suppl)189–194
Hwang K, Kim HS, Lee WJ (2004) Confirmation on taxonomic status of Spataglossum pacificum yendo (Dicrnatolae, Phaeophyceae) based on morphology and plastid protein coding rbcL, rbcS, psaA, and psbA gene sequences. Algae 19:161–174
Hwang K, Kim HS, Lee WJ (2005) Polymorphism in the brown algaDictyota dichotoma(Dictyotaceae, Dictyotales) from Korea. Mar Biol 147:999–1015
Kitayama T, Lin S-M (2012) Brown alga from Chaojing, Keelung City, Taiwan. Mem Natl Mus Sci, Tokyo 48:149–157
Lee WJ, Bai GS (2002) Phylogenetic relationship among several genera of Dicrnatolae (Dicrnatae, Phaeophyceae) based on 18S rDNA and rbcL gene sequences. Mar Biol 140:1107–1115
Lewis JE, Norris JN (1987) A history and annotated account of the benthic marine alga of Taiwan. Smithsonian Contr Mar Sci 217:38
Okamura K (1936) Nippon Kaiso-si. Uchi-da-ro-kaku-ho, Tokyo, pp 185–185, pp. (In Japanese)
Papenfuss GF (1944) Notes on algal nomenclature. II: Miscellaneous species of Chlorophyceae, Phaeophyceae and Rhaeophyta. Farlowia 1:337–346
Phillips JA (1997) Genus and species concepts in Zonaria and Homoeostrichus (Dictyotales, Phaeophyceae), including the description of Exallosorus gen. nov. Eur J Phycol 32:303–311

Phillips JA, Clayton MN (1993) Comparative flagellar morphology of spermatozoids of the Dictyotales (Phaeophyceae). Eur J Phycol 28(2):123–127

Phillips JA, Clayton MN (1994) Flagellate spores in Homoeostrichus olsenii Womersley (Dictyotales, Phaeophyceae); the largest know motile reproductive cells of marine macroalgae. Phycologia 33(6):415–419

Phillips JA, Clayton MN (1997) Comparative studies on gametangial distribution and structure in species of Zonaria and Homoeostrichus (Dictyotales, Phaeophyceae) from Australia. Eur J Phycol 32:25–34

Phillips JA, Nelson WA (1998) Typification of the Australasian brown alga Zonaria turneriana J. Agardh (Dictyotales) and description of the endemic New Zealand species, Zonaria aureomarginata sp. nov. Bot Mar 41:77–86

Phillips JA, Clayton MN, Harvey AS (1994) Comparative studies on sporangial distribution and structure in species of Zonaria, Lobophora and Homoeostrichus (Dictyotales, Phaeophyceae) from Australia. Eur J Phycol 29:93–101

Ribera MA, Gómez-Garreta A, Gallardo T, Comez-Garreta M, Cormaci M, Furnari G, Giaccone G (1992) Check-list of Mediterranean seaweeds. I. Fucophyceae (Warming 1884). Bot Mar 35:109–130

Seagarief SC (1984) A catalogue of south African green, brown and red marine algae. Mem Bot Surv South Africa 47:72

Shen YF, Fan KC (1950) Marine algae of Formosa. Taiwania 1(2–4):317–345

Silva PC (1952) A review of nomenclatural conservation in the algae from the point of view of the type method. Univ Calif Pub Bot 25:241–324

Silva PC, Menez G, Moe RL (1987) Catalog of benthic marine algae of the Philippines. Smithsonian Contr Mar Sci 27:1–179

Silva PC, Basson PW, Moe RL (1996) Catalogue of the benthic marine algae of the Indian ocean. Univ California Publ Bot 79:1–1259

Simons RH (1964) Notes on the species of Zonaria in south Africa. Bothalia 8:195–197

Tamura K, Peterson D, Peterson N, Stecher G, Nei M, Kumar S (2011) MEGA5: molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. Mol Biol Evol 28:2731–2739

Taylor WR (1960) Marine algae of the eastern tropical and subtropical coasts of the Americas. University of Michigan Press, America, pp 214–238, 718–812 pp

Wang WL, Chiang YM (2001) The marine macroalgae of Lu Tao (green island), Taiwan. Taiwania 46:49–61

Yamada Y (1925) Studien über die meeresalgen von der insel Formosa 2. Phaeophyceae Bot Mag (Tokyo) 39:239–254

Yoon HS, Hackett JD, Bhattacharya D (2002) A single origin of the peridinin- and fucoxanthin-containing plastids in dinoflagellates through tertiary endosymbiosis. Proc Natl Acad Sci USA 99:11724–11729

Yoshida T (1998) Marine algae of Japan. Uchida Rokakuho Publishing, Tokyo, Japan, pp 205–234

Yoshida T, Nakajima Y, Nakata Y (1985) Preliminary checklist of marine benthic algae of Japan. I. Chlorophyceae and Phaeophyceae. Jap. J Phycol 33:57–74

doi:10.1186/1999-3110-54-13

Cite this article as: Wang et al.: Morphological and molecular characteristics of Homoeostrichus formosana sp. nov. (Dictyotaceae, Phaeophyceae) from Taiwan. Botanical Studies 2013 54:13.