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# *Batrachospermum arcuatum* Kylin (Batrachospermales, Rhodophyta), a Freshwater Red Alga Newly Recorded in Taiwan

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**ABSTRACT:** A freshwater red alga, *Batrachospermum arcuatum* Kylin, is recorded for the first time to the freshwater algal flora of Taiwan. It was found attaching on submerged stones in a clean river in Yuanshan Village, Yilan County. In addition to describe the morphological and reproductive structures of *B. arcuatum* in detail, we also used *rbc*L sequences to confirm its phylognetic relationship to other species of *Batrachospermum*.

**KEY WORDS: *Batrachospermum arcuatum*, Freshwater red algae, *rbc*L gene, Rhodophyta, Taiwan.**

## INTRODUCTION

More than 200 taxa of Rhodophyceae are found in freshwater habitats (Kumano, 2002). These red algae have been divided into two classes and seven orders (Kumano, 2002) and were reported to appear in clear, aquatic environments with low nutrient and high oxygen concentrations (Sheath and Hambrook, 1990; Liu and Wang, 2004).

Few studies have been conducted on the macroscopic freshwater red algae in Taiwan (Wu, 1999, 2001; Liu and Wang, 2004). The Order Batrachospermales can be distinguished from other rhodophytes by its heterotrichous life history, lacking of tetraspore production, containing a two-layered pit plug (the outer layer being dome-shaped), and strictly freshwater habitation (Sheath, 1984; Vis et al., 1998). Only three species of *Batrachospermum* have been previously reported from Taiwan, i.e. *B. anatinum* Sirodot (= *B. ectocarpum* Sirod), *B. gelatinosum* (Linnaeus) De Candolle (= *B. moniliforme* Roth), and *B. tapirense* Kumano *et* Phang (Wu, 1999, 2001). In this study, specimens of *Batrachospermum* were collected from a clean river in Yuanshan Village, Yilan County, northern Taiwan. We described its morphological characters and also used the Rubisco large subunit (*rbc*L) gene to infer its taxonomic status.

## MATERIALS AND METHODS

The materials were collected from a clean river in Yuanshan Village (E121°41' 43.2'', N24°44' 44.7''; Fig. 1), Yilan County, northern Taiwan on 5 February, 2005. Specimens used for the morphological study were preserved in freshwater containing 5-10% formalin or over dried as herbarium specimens, and those used for the molecular study were preserved in silica gel or 95% ethanol. The vegetative and reproductive structures were examined by light microscope under 200 × 400 (Zeiss Axioskop 2) and by a dissecting microscope (Zeiss Stemi SV11). Photographs were taken with a camera (Zeiss MC80) or with a cool CCD system (Pixera Penguin 600CL with Automontage Software, CA, USA) attached to the microscope. Specimens used in this study were deposited in the Department of Biology, National Changhua University of Education, Taiwan.

Genomic DNA extraction was aonducted following the manufactures procedure (DNeasy Plant Mini Kit) (Qiagen, Valencia, CA, USA). The *rbc*L gene was selected for the molecular analysis. The primers and protocols for gene amplification and automated sequencing used in this study were as outlined by Wang et al. (2005). The maximum parsimonious tree was generated by using a heuristic search under the constraints of random (100 replicates) sequence addition, steepest descent, and tree bisection－reconnection (TBR) branch swapping implanted in the programe PAUP (Phylogenetic Analysis Using Parsimony, Swofford, 2002). Each character in the DNA sequence analysis was performed with equal weight. The *rbc*L sequences of *Audouinella arcuata*, *Palmaria palmata*, *Rhodogorgon carriebowensis*, and *Thorea violacea* obtained from previous studies

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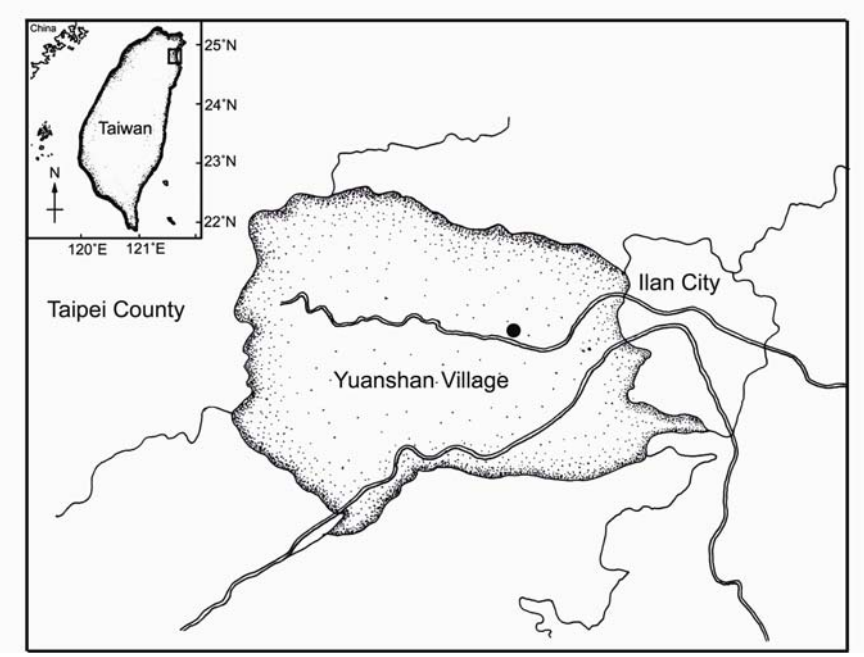


Fig. 1. Locality of collection of B. *arcuatum* (●) from Yuanshan Village, Yilan County, northern Taiwan.

(Freshwater et al., 1994; Vis et al., 1998; Müller et al., 2002) were used as outgroups, and 13 species of *Batrachospermum* were included as ingroups (Table 1). The robustness of the tree topogloy was estimated by 1000 replicates of bootstrap resampling using the Maximum Parsimony and Neighbor-Joining methods (Felsenstein, 1985).

## RESULTS

***Batrachospermum arcuatum*** Kylin 1912

Figs. 2A-F

Basionym: *Batrachospermum arcuatum* Kylin 1912: 22, Figs. 7A-E.

Descriptions: Plants are dioecious, consisting of confluent barrel-shaped whorls, and secondary branches are not curled at the tip (Fig. 2A). Whorls are 370-800 μm in diameter with 1-3 peripheral carposporophytes (Fig. 2A). The main axes with cortication consist of cylindrical cells only (Fig. 2B). Spermantagia are terminal on fascicle (Fig. 2C). Carposporophytes are spherical, pedicellate, 90-120 μm in diameter (Fig. 2D). Gonimoblast filaments consist of 2-4 cylindrical cells. Carposporangia are obovoidal, 15-18 μm in length and 10-12 μm in width (Fig. 2E). Carpogonia are 15-20 μm long with lanceolate trichogynes (Fig. 2F).

Specimens examined: Yilan County, Taiwan, collected by Chou J.-Y, 5.ii.05, NCUE-JYC-940205.

Type locality: Hör, Skane, Sweden in Europe.

World distribution: North Island and South Island, New Zealand, Sweden, North America, and Northern Taiwan.

Habitat: Plants are epilithic on submerged stones in a well-aerated, moderately agitated, and clear river at a mean depth of 50 cm in Yuanshan Village, Yilan County, northern Taiwan.

Remarks: Both *B. arcuatum* and *B. anatinum* are included in *B. arcuatum* group by Entwisle and Foard (1997) for having the carpogonia subtended by relatively short filaments of unmodified cells. The two species belong to the section *Batrachospermum*, which have small, numerous carposporophytes distributed in whorls (Kumano, 2002). *B. arcuatum* can be separated from *B. anatinum* by having regular cortication and dioecious plants.

### Molecular analyses

A set of 17 *rbc*L sequences of selected species (Table 1), including 1201 aligned sites with 344 potentially parsimony-informative characters was analyzed. Only one maximum parsimonious tree was revealed from the MP analysis (Fig. 3), which was based on a heuristic search using the stepwise addition of 100 random replications. Bootstrap proportion values (1000 replicates) derived from both MP and NJ analyses are shown on the nodes (only bootstrap values $\geqq$ 50% were shown). The tree length is 1204 steps, with consistency index (CI) = 0.5615 and retention index(RI) = 0.5397. The

Table 1. List of species used in *rbc*L analysis and accession numbers in GenBank.

| Taxon | Collection information | Accession number |
|---|---|---|
| Ingroup | | |
| *Batrachospermum arcuatum* Kylin | Yuanshan Village, Yilan County, northern Taiwan | DQ141320 |
| *Batrachospermum atrum* (Hudson) Harvey | Wairarapa Stream, New Zealand | AY297055 |
| | Blue Pool, west of Bradfield, UK | AF029139 |
| | North Dnadalup, South Dnadalup River, Australia | AF209979 |
| *Batrachospermum antipodites* Entwisle | clone Ptilo_West4182 coll. by Vis, Entwisle, West, Ott | AY423421 |
| *Batrachospermum boryanum* Sirodot | Rt. 4, 3km north of Grafton, New Hampshire, USA | AF029140 |
| *Batrachospermum campyloclonum* Skuja ex Entwisle *et* Foard | Mill Creek, Stewart I., New Zealand | AY297053 |
| *Batrachospermum gelatinosum* (Linnaeus) De Candolle *emend*. Vis, Sheath *et* Entwisle | Rainbow Creek, Rt. 33, north of Edmonton, Alberta, Canada | AF029141 |
| *Batrachospermum involutum* Vis *et* Sheath | San Marcos River, San Marcos, Texas, USA | AF029143 |
| *Batrachospermum pseudogelatinosum* Entwisle *et* Vis | Yarra River, Victoria, Australia | AF209983 |
| | Little Wheeney Creek, New South Wales, Australia | AY297052 |
| *Batrachospermum* sp. (Christchurch) | Wairarapa Stream, New Zealand | AY297054 |
| Outgroup | | |
| *Audouinella arcuata* (Drew) Garbary, Hansen *et* Scagel | San Juan Island, Washington, USA | AF029138 |
| *Palmaria palmata* (Linnaeus) Kuntze | | U04186 |
| *Rhodogorgon ramosissima* Norris *et* Bucher | St. Ann's Bay, Jamaica | U04183 |
| *Rhodogorgon carriebowensis* Norris *et* Bucher | | |
| *Thorea violacea* Bory *emend*. Sheath, Vis *et* Cole | New Braunfels, Texas, USA | AF506271 |

pairwise distance, based on the Kimura 2-parameter method, between the populations of *B. arcuatum* from Taiwan and New Zealand is 4.50%.

## DISCUSSION

*B. arcuatum* is similar to *B. gelatinosum* in all morphometric characteristics, but the latter differs from the former by having a monoecious thallus, and is recognized as a distinct species by Vis et al. (1995). Entwisle et al. (2004) regarded that *Batrachospermu* sp. Christchurch (collection no. TJE3094) from New Zealand should be identified as *B. arcuatum*. The molecular data in their study also supported the notion that they are different species (Entwisle and Vis, personal communications). Previously, *B. arcuatum* has been synonymized with *B. anatinum* Sirodot (= *B. ectocarpum* Sirodot) on the basis of morphological similarities such as thick mucilage, spermatangia, and carposporophytes in the whorl periphery and lack of terminal hairs (Israelson, 1942). Vis et al. (1995) maintained *B. arcuatum* different from *B. anatinum* based on a regular cortication and dioecious plants in the former species and irregular cortication and monoecious plants in the latter species. Based on both molecular and morphological data, we identified the species that we collected as *B. arcuatum*. The species has not been reported in Taiwan, hence it is new to the freshwater algal flora of Taiwan.

The *rbc*L sequence divergence between the populations of *B. arcuatum* found in Taiwan and that of New Zealand is 4.50%. The *rbc*L genetic distance between the populations of *B. arcuatum* from the Hawaiian Islands is less than 1% based on the uncorrected-p method (Vis et al., personal communications). The sequence divergence values fall within previously published intraspecific values of the Rhodophyta, e.g., *rbc*L gene of *B. pseudogelatinosum* Entwisle et Vis (1.0-4.8%, Entwisle et al., 2004), *Bangia* (among congeners 0-16%, Müller et al., 1998), *Tricleocarpa cylindrica* (J. Ellis *et* Solander) Huisman et Borowitzka (3.3% to 5.7%, Wang et al., 2005), and *Galaxaura rugosa* (Ellis et Solander) Lamouroux (0.6% to 6.4%, Wang et al., 2005). The divergence between our samples and those of New Zealand may result from the biogeographical isolation. Vis et al. (2001) pointed out that long-distance dispersal most likely played a role in the distribution of freshwater red algae. The mechanisms of dispersal (possibly waterfowl flyways) and potential resistant propagules (perhaps thallus fragments) have been highlighted by Hall and Vis's study (2002).

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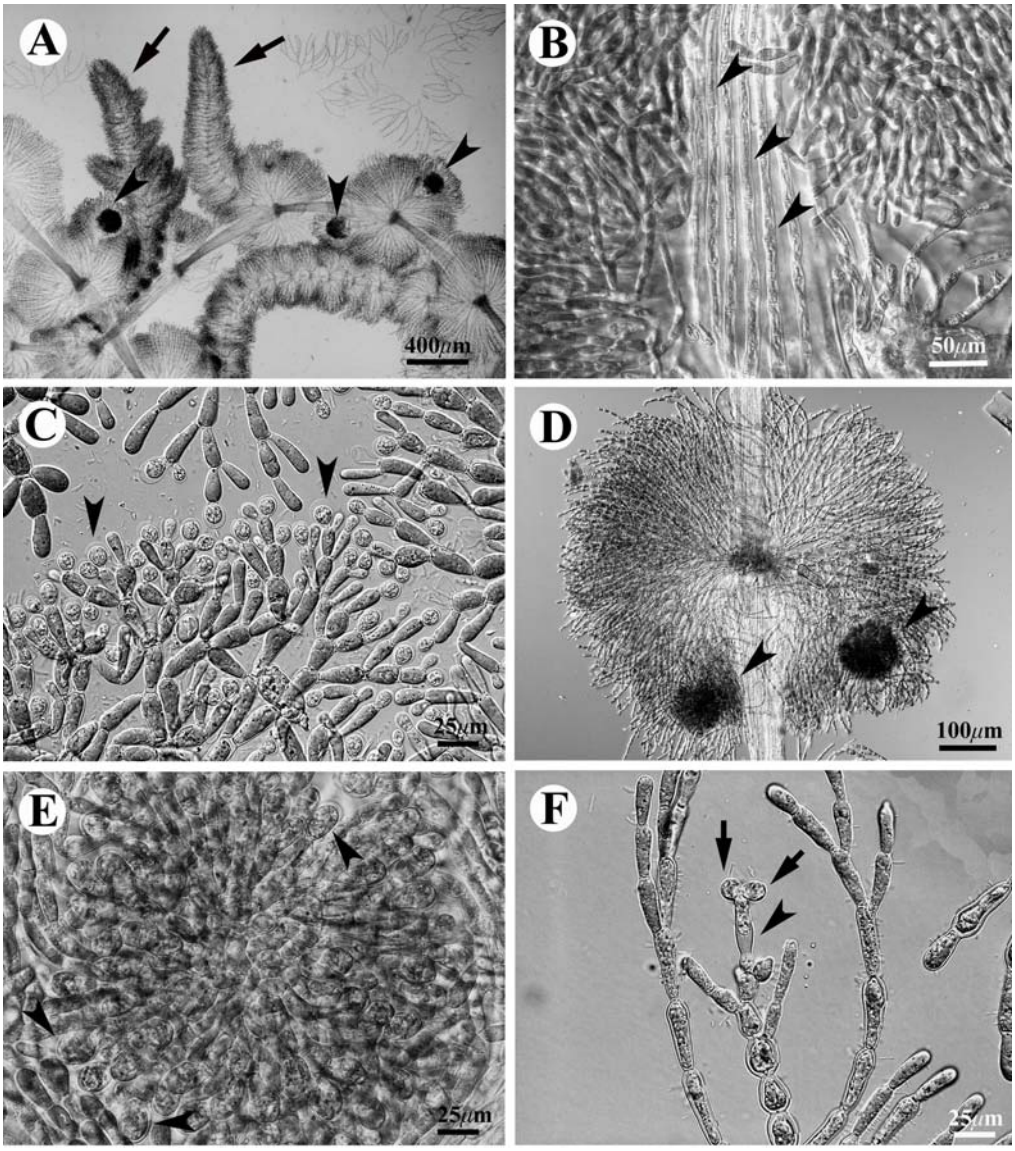


Fig. 2. *Batrachospermum arcuatum* Kylin. A: Female plant main axis with confluent, barrel-shaped whorls with secondary branches (arrows) containing spherical carposporophytes (arrowheads). B: Cortication of main axis with cylindrical cells (arrowheads) only. C: Spermatangia (arrowheads) terminal on fascicle. D: Spherical carposporophytes on branch (arrowheads). E: Compact carposporophyte with obovoidal carposporangia (arrowheads). F: Fertilized carpogonium attached by spermatangia (arrows) with lanceolate trichogyne (arrowhead).

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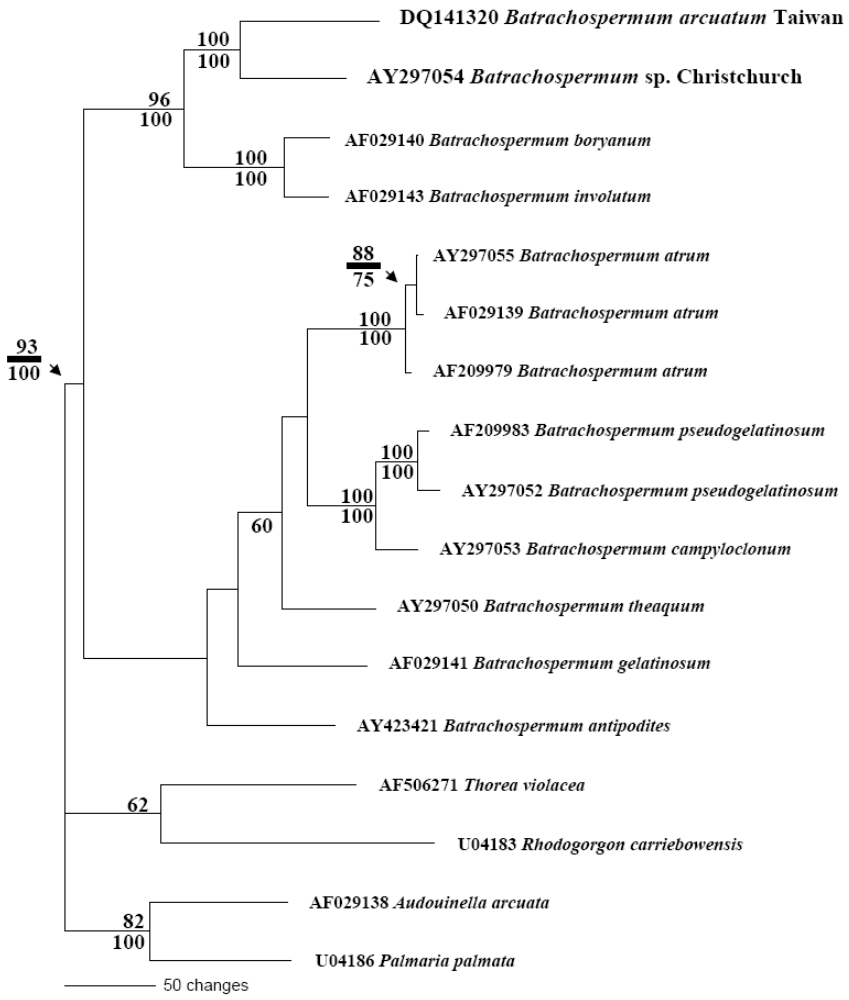


Fig. 3. The maximum parsimonious (MP) tree inferred from a partial *rbc*L sequence data set of 1201 aligned characters using 13 OTUs of the Batrachospermalean taxa as ingroup and *Audouinella arcuata*, *Palmaria palmata*, *Rhodogorgon carriebowensis*, and *Thorea violacea* as outgroup (tree length = 1204 steps, CI = 0.5615, RI = 0.5397). The numbers on the branches are bootstrap values (≧50%) in MP (above the branch) or NJ (below the branch) analysis.

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# 臺灣新紀錄淡水紅藻弧形串珠藻 (串珠藻目，紅藻門)

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## 摘　　要

本文報導一種臺灣新紀錄淡水紅藻弧形串珠藻，此種藻類生長於臺灣宜蘭縣員山鄉清澈溪流的石頭上。文中除對於此種藻類的形態作詳細描述外，也以葉綠體二磷酸核酮糖羧化酵素大次單位基因(*rbc*L gene)序列確定其親緣關係的分類地位。

關鍵詞：弧形串珠藻、淡水紅藻、葉綠體二磷酸核酮糖羧化酵素大次單位基因、紅藻、臺灣。

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