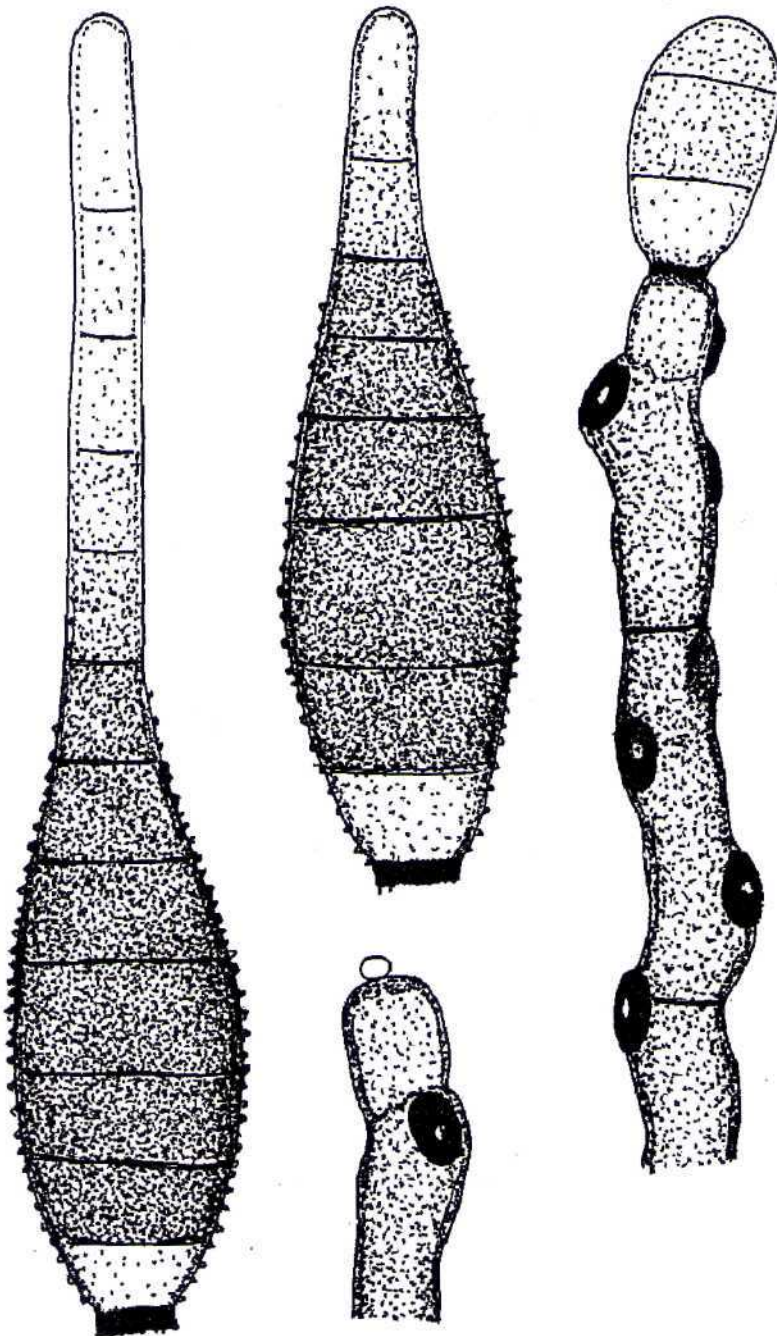




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THE PHYLOGENETIC POSITION OF THE PAPUASIAN GENUS SARCOCHILUS R.BR. (ORCHIDACEAE: AERIDINAE): EVIDENCE FROM MOLECULAR DATA

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ABSTRACT

HIDAYAT, T.; ITO, M.; YUKAWA, T. 2008. The phylogenetic position of the Papuan genus *Sarcochilus* R.Br. (*Orchidaceae: Aeridinae*): evidence from molecular data. *Reinwardtia* 12(4). 281 – 284. — The taxonomic status of the Papuan orchid genus *Sarcochilus* R.Br. remains unresolved. Represented by *Sarcochilus chrysanthus* Schltr., a phylogenetic analysis to evaluate relationships between the Papuan species and those from Australia was conducted using molecular characters. Parsimony analysis using DNA sequences of the internal transcribed spacer (ITS) region showed that this Papuan species, is in a distant position from the so-called the true *Sarcochilus* sensu stricto. These results provide additional evidence for the establishment of a new genus *Monantochilus*.

Keywords: ITS region, *Orchidaceae*, Papua New Guinea, phylogenetic relationships, *Sarcochilus chrysanthus*

ABSTRAK

HIDAYAT, T.; ITO, M.; YUKAWA, T. 2008. Posisi filogenetika anggrek Papua marga *Sarcochilus* (*Orchidaceae: Aeridinae*): bukti dari data molekuler. *Reinwardtia* 12(4): 281 – 284. — Status taksonomi marga anggrek *Sarcochilus* yang berasal dari Papua masih belum terselesaikan. Dengan menggunakan *Sarcochilus chrysanthus* Schltr., analisis filogenetik untuk mengevaluasi hubungan antara jenis yang berasal dari Papua. dengan jenis yang berasal dari Australia telah dilakukan menggunakan ciri molekuler. Analisis parsimoni dengan menggunakan urutan DNA dari daerah *internal transcribed spacer* (ITS) menunjukkan bahwa jenis yang berasal dari Papua ini sangat berbeda dengan jenis *Sarcochilus* yang sebenarnya. Hasil ini menyediakan bukti tambahan bagi pembentukan marga baru, yaitu *Monantochilus*.

Kata kunci: Daerah ITS, hubungan filogenetik, *Orchidaceae*, Papua, *Sarcochilus chrysanthus*

INTRODUCTION

The orchid genus *Sarcochilus* R. Br., belongs to the vandaceous subtribe *Aeridinae* Pfitzer, which contains numerous poorly delimited genera providing many taxonomic and phylogenetic problems. *Sarcochilus* is primarily distributed in Australia, with only a few species occurring in neighbouring Papua New Guinea. The relationship between the Papuan species of *Sarcochilus* and those of *Sarcochilus* sensu stricto from mainland Australia has long remained unanswered. Rice

(2004) separated the Papuan members of *Sarcochilus* from the *Sarcochilus* sensu stricto, placing them into a new genus *Monantochilus* (Schltr.) R. Rice. This separation was based upon habit, flower number, labellum mobility, and the existence of a callus in the spur. However, these characters vary greatly throughout the species. Given the shortcomings of these characters, data obtained from nucleotide substitutions of appropriate molecules are preferable in clarifying the phylogenetic relationships of this group (e.g., Moritz and Hillis 1996).

This study aims to evaluate the phylogenetic relationships between Papuanian *Sarcochilus* and *Sarcochilus* sensu stricto at molecular level using DNA sequences of the internal transcribed spacer (ITS) region. The ITS region has been widely used by plant systematists because of its small size, highly conserved flanks, high copy number, and rapid concerted evolution (Baldwin et al. 1995). Recently, the ITS region has been used in the phylogenetic analysis in many groups of *Orchidaceae* and has provided resolutions for previously obscure relationships.

MATERIALS AND METHODS

Six species of *Sarcochilus*, viz. the Papuanian *S. chrysanthus* and five from Australia, were examined in this study. *Rhinerrhiza* was used as an outgroup, since this genus is recognized as the sister group to *Sarcochilus* based upon morphological and macromolecular characters (Topik et al. 2005). Specimens were primarily collected from various regions of Australia, and another obtained from Tsukuba Botanical Garden, Japan. Detailed information about plant materials is provided in Table 1.

Table 1. Plant materials examined in this study

Taxon	Source	Voucher
<i>Rhinerrhiza divitiflora</i> (Benth.) Rupp	AU	NA
<i>Sarcochilus hartmannii</i> F. Mueller	TBG	TBG145793
<i>Sarcochilus hirticalcar</i> (Dockrill) MA Clem & B.J. Wallace	AU	NA
<i>Sarcochilus moorei</i> Schltr.	AU	NA
<i>Sarcochilus spathulatus</i> R.S. Rogers	AU	NA
<i>Sarcochilus chrysanthus</i> Schltr.	TBG	TBG145831
<i>Sarcochilus weinthalii</i> F.M Bailey	AU	NA

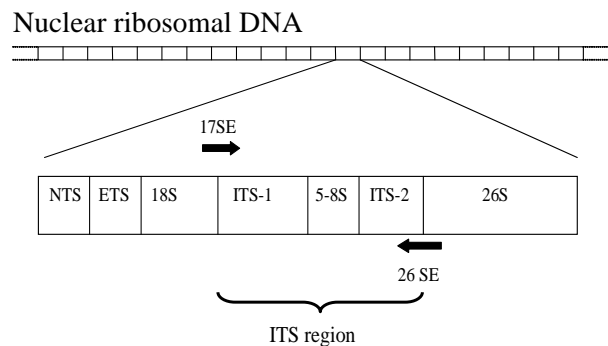
Notes:

AU : Specimens were collected in Australia

TBG : Specimens were collected Tsukuba Botanical Garden-Japan (TBG).

NA : Not Available

The total DNA was extracted from fresh material or silica-gel dried plant tissue with a QIAGEN DNeasy Mini Plant Kit following the manufacturer's instructions. Amplification, purification, and sequencing of ITS region were carried out as described in Topik et al. (2005) using a primer pair, 17SE and 26SE (Fig.1; Sun et al. 1994).



17SE= ACGAATTCATGGTCCGGTGAAGTGTTTCG

26SE= GAATTCCCCGGTTCGCTCGCCGTAC

Fig. 1. Sequence of ITS region with location of primers used in this study. Information on the primers is also provided.

The DNA sequence obtained from the ITS region was aligned with Clustal X and was then adjusted manually following the guidelines described in Kelchner (2000). Phylogenetic analysis based on the maximum parsimony criterion was performed using PAUP* version 4.0b10 (Swofford 1998). Insertions and deletions were treated as missing data. All characters were equally weighted and unordered (Fitch 1971). Data were analyzed by the heuristic search method with tree bisection-reconnection (TBR) branch swapping and the MULTREES option on, ten replications of random addition sequences with the stepwise addition option, and all most parsimonious trees (MPTs) were saved. Evaluation of internal support of clades was conducted by the bootstrap analysis (Felsenstein 1985) utilizing 1,000 replicates with TBR branch swapping and the MULTREES option off. Number of steps, consistency indices (CI) and retention indices (RI) were calculated on one of the MPTs in each analysis with the TREE SCORES command in PAUP*.

RESULTS

The aligned ITS region comprised 662 characters. Of these, 547 (83%) were constant and 32 (4.8%) were potentially informative. The analysis resulted in one MPT with the length of 153 steps, CI (excluding autapomorphies) of 0.876 and RI of 0.548. The strict consensus tree (Fig. 2) showed that Papuanian *Sarcochilus*, in this study represented by *S. chrysanthus*, is separated from *Sarcochilus* sensu stricto. Moreover, monophyly of *Sarcochilus* from Australia is clearly defined, although bootstrap

percentages are relatively low (only 66).

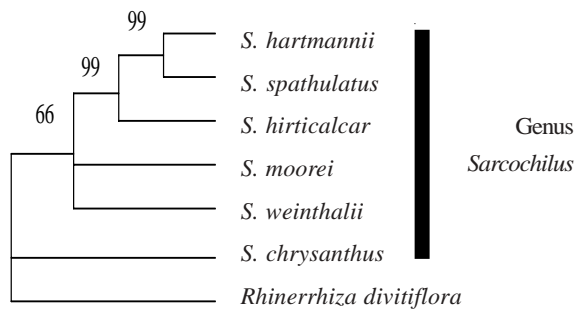


Fig. 2. Strict consensus tree derived from the parsimony analysis of the ITS region of nrDNA. Bootstrap percentages of > 50 are shown above each branch.

DISCUSSION

From global phylogenetic analyses on *Aeridinae*

Regarding the phylogenetic position of the genus *Sarcochilus*, a recent global phylogenetic analysis of subtribe *Aeridinae* based on DNA (ITS and *matK* sequences) contributes some information (Topik *et al.* 2005). In that analysis, *Sarcochilus* is represented by *S. chrysanthus* and *S. hartmannii*. On this limited basis, *Sarcochilus* appears to be non-monophyletic. The former makes up *Thrixspermum* alliance with *Thrixspermum*, *Dimorphorchis*, *Abdominea*, *Microsaccus*, and *Cleisomeria*, whereas the latter makes monophyletic with *Rhinerrhiza* and *Bogoria*. Using much greater taxon sampling of *Sarcochilus* more robust phylogenetic hypotheses for the genus at molecular level could be established, as can be seen in this study

Generic circumscription

As mentioned above that, at the molecular level, Papuan *Sarcochilus* is different from *Sarcochilus* *sensu stricto*. In this case, the consequences were whether to align the Papuan species into the Australian ones, or to split the former into a new genus. The latter option seemed to be the most practical choice, to avoid *Sarcochilus* in becoming non-monophyletic.

Schlechter (1913) defined *Sarcochilus* rather broadly subdividing it into three sections based mainly upon stem length, the number of flowers on the sessile inflorescences, and lip lobe number; Schlechter's sections are *Monantochilus*, *Eu-Sarcochilus*, and *Ascochilus*. Rice (2004), however,

circumscribed *Sarcochilus* in a narrower sense, including only species with a short leafy stem and many-flowered inflorescences. These two morphological characters are synapomorphic for the genus (Fig. 2). The result of this analysis demonstrates wide phylogenetic separation between *S. chrysanthus* and the Australian species of *Sarcochilus*.

In comparison with Australian *Sarcochilus* (section *Eu-Sarcochilus*), the Papuan *S. chrysanthus* has more elongated stems and spurs, and a single-flowered inflorescence. In addition, the presence of a rectangular, flattened callus in a distinct spur, and the absence of a column-foot are distinctive. The raising of section *Monantochilus* to a generic rank by Rice (2004) is supported by this study at the molecular level.

Two additional Papuan species, *S. iboensis* and *S. unifloris*, have been transferred to *Monantochilus* by Rice (2004).

Biogeographic overview

In the past, Papua was connected with Australia before finally it was separated as today (White 1990). Due to its strategic position, Papua since long time has been acting as a corridor for many groups of plant to migrate from Asia to Australia, and vice versa. Such pattern of migration has been suggested for *Nothofagus* (Setoguchi *et al.* 1997), *Araucaria* (Setoguchi *et al.* 1999), *Amaryllidaceae* (Ito *et al.* 1999), *Dendrobium* (Yukawa *et al.* 2000), and *Aeridinae* (Topik, 2005).

We assumed that several members of *Sarcochilus* have separated at the time of the separation of Papua from Australia. Consequently, those Papuan species gradually have to shift their morphological features to fit with new conditions. It is natural that migration to a new region may be accompanied by several morphological changes to adapt to new environmental conditions before subsequently they diverge (White 1990).

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