NOTE

Exacum paucisquamum (C.B. Clarke) Klack. - a New Record of Mycoheterotroph (Gentianaceae) to the Flora of Hong Kong♥

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♥ *Dedicated to Prof Shiu-Ying Hu (1910-2012)*
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ABSTRACT: A mycoheterotroph was found growing on Tai Mo Shan. Morphological characters and DNA sequence of the ITS region confirmed that it is *Exacum paucisquamum* (Gentianaceae). It is a new record to Hong Kong and also a new low altitude and the southern-most range of its distribution in China.

KEY WORDS: *Cotylanthera paucisquama*, *Exacum paucisquamum*, Gentianaceae, Hong Kong, mycoheterotroph, taxonomy.

INTRODUCTION

Gentianaceae is a big dicotyledonous family of 87 genera and about 1,650 species, which are distributed mainly in temperate regions and high altitudes (Struwe et al., 2002). The Flora of Hong Kong (Xia, 2009) recorded three species belonging to three genera, namely *Exacum tetragonum* Roxb., *Gentiana loureiroi* Griseb. and *Tripterospermum nienkui* (C. Marq.) C. J. Wu. The former two species are small herbs, and the third one is a twining herb. All of them are chlorophyllous.

Recently, a small achlorophyllous plant was discovered in Hong Kong. Both the Flora Reipublicae Popularis Sinicae (Ho, 1988) and Flora of China (Ho and Pringle, 1995) placed this herb in the genus *Cotylanthera* as *C. paucisquama* C.B. Clarke. Recent cladistic studies combined with morphological analyses, however, concluded that *Cotylanthera* is nested within the genus *Exacum* (Yuan et al., 2003, 2005). So the updated name of the plant is *Exacum paucisquamum* (C.B. Clarke) Klack. (Klackenberg, 2006). It is a new record to the Flora of Hong Kong.

This specimen was further subjected to DNA analyses. Total DNA was extracted as described previously (Li et al., 2010). Internal transcribed spacer (ITS) were amplified by polymerase chain reaction using universal primers ITS5 (forward, 5′- GGA AGT AAA AGT CGT AAC AAG G -3′) and ITS4 (reverse, 5′- TCC TCC GCT TAT TGA TAT GC -3′) through 35 cycles of 95°C for 30 s, 55°C for 20 s, and 72°C for 1 min (White et al., 1990). The amplicons were purified using Gel-M™ Gel extraction system (Viogene) before bidirectional DNA sequencing performed in BGI-Hong Kong. The DNA sequence was deposited in GenBank. A total of 115 ITS sequences of species in the tribe Exaceae were downloaded from GenBank and were aligned with our ITS sequence using BioEdit7 (Hall, 1999). A phylogenetic tree was constructed by maximum parsimony with 100 replications using MEGA5 (Tamura et al., 2011), and the results found this specimen clustering with *Cotylanthera paucisquama* (AJ489874) and nesting among *Exacum* species (Fig. 2). More refined comparison of the ITS sequences (Fig. 3), however, revealed intra-specific variation of 17.2% between the Hong Kong specimen and the *Cotylanthera paucisquama* specimen reported by Yuan et al. (2003), indicating genetic polymorphism in this species.

The mode of nutrition of this species was frequently described as saprophytic (Ho, 1988; Ho and Pringle, 1995). More refined analyses suggested that it is a mycoheterotroph that parasitizes on and obtain nutrients from mycorrhizal fungi, which in turn obtain nutrients from external sources (Furman and Trappe, 1971; Leak, 1994; Merckx and Freudenstein, 2010). Nothing is known about its reproductive biology, except that yellow anthers are open by an apical pore, through which pollen would be released, and thus may also involve buzz-pollination as the genus *Exacum* is known to be buzz-pollinated (Hardy et al., 2000).

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TAXONOMIC TREATMENT

Exacum paucisquamum (C.B. Clarke) Klack., Bot. Jahrb. Syst. 126: 477-481. 2006; *Cotylanthera paucisquama* C.B. Clarke, in Hooker, Fl. Brit. India 4: 94. 1883; Ho & Pringle, in Wu & Raven, Fl.China 16: 3. 1995; *Cotylanthera yunnanensis* W. W. Smith., Notes Roy. Bot. Gard. Edinburgh 13(63-64): 158. 1921. (Fig. 1).

Achlorophyllous herb, about 7 cm tall, with terete stem and opposite leaves which are reduced to scales of 1.5-2.5 × 0.7-1 mm in size. The flowers are terminal and tetrameric. The calyx tube is short with four membranous lobes, 4-6 mm long. The corolla is bluish, with a short tube and four petal lobes that are oblong and spreading, 1-1.2 cm long. Stamens are inserted at the throat of the corolla tube just below the sinus between corolla lobes. Anthers are yellow, 2.5-4 mm long, sagittate, with a single apical pore. The filaments are as long as the anthers. The ovary is superior, bearing a single style, 5.5-8 mm long, with a 2-lobed stigma.

Specimen examined: HONG KONG: New territories, Tai Mo Shan, ca. 600 m elev., on debris formed by decaying *Lithocarpus* and bamboo leaves nearby a small stream, 21 May 2010, *P. But and Y.M. Chan 2010502* (CUHK).

Distribution: Previous collections of this species came from forests at 1700-2400 m in Sichuan, Xizang and Yunnan provinces, China and in Bhutan, Darjeeling and Sikkim (Hara, 1975; Ho and Pringle, 1995). In 2008, a collection was made at elevation of 1040 m in Napo, Guangxi Province, China (Xu et al., 2010). The discovery of this unusual herb in Hong Kong has further expanded its distribution territory to subtropical area and lower altitude.

Habitat and Ecology: This species is found in slightly exposed areas on the floor of broadleaf or mixed forests and grows on debris formed by decaying broadleaf leaves.

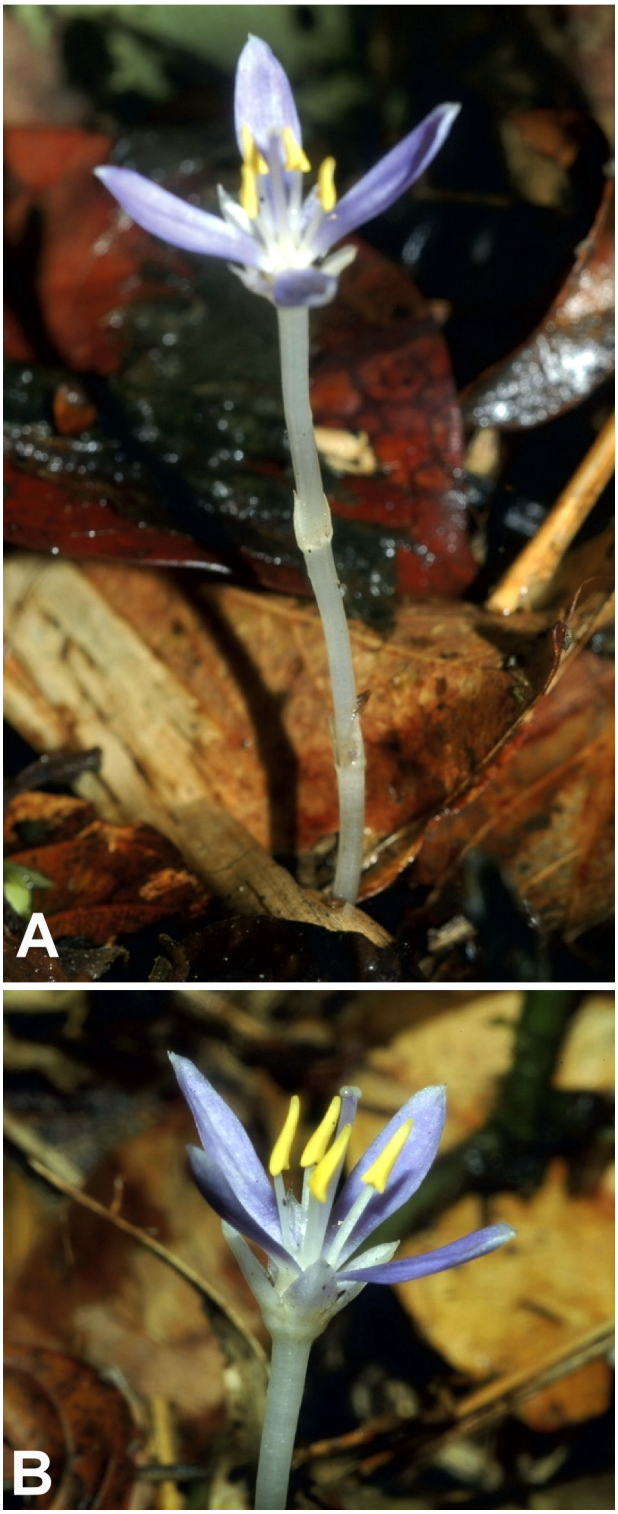


Fig. 1. Morphology of *Exacum paucisquamum*. A: Whole plant of *Exacum paucisquamum* found on Tai Mo Shan, Hong Kong. B: a flower of *Exacum paucisquamum*.

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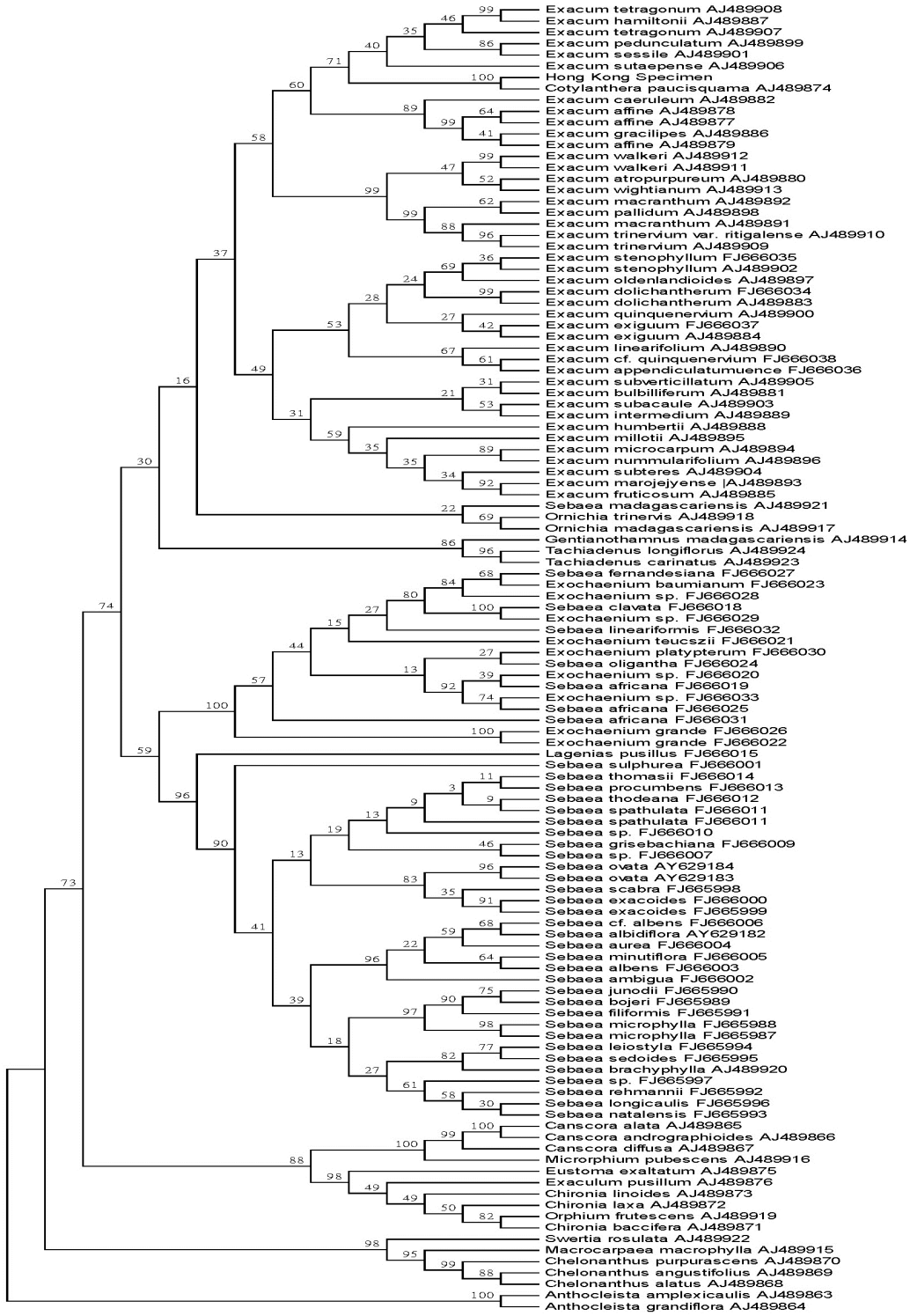


Fig. 2. Maximum parsimony analysis of the tribe Exaceae based on 116 ITS sequences. The evolutionary history was inferred using the maximum parsimony method. The bootstrap consensus tree inferred from 100 replicates is taken to represent the evolutionary history of the taxa analyzed. Branches corresponding to partitions reproduced in less than 50% bootstrap replicates are collapsed. The percentages of replicate trees in which the associated taxa clustered together in the bootstrap test (100 replicates) are shown above the branches. The maximum parsimony tree was obtained using the Close-Neighbor-Interchange algorithm with search level 1 in which the initial trees were obtained with the random addition of sequences (10 replicates). All positions containing gaps and missing data were eliminated. There were a total of 449 positions in the final dataset.

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Fig. 3. Sequence alignment of ITS sequences of the Hong Kong Specimen and *Cotylanthera paucisquama* (AJ489874) in GenBank performed using ClustalW2 alignment program. The intra-specific variation is 17.2%. '-' indicates gaps and '*' indicates conserved site. The boxes in the 5'-end and 3'-end represent the sequences of PCR primers ITS5 and ITS4, respectively.

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香港龍膽科菌生異營性植物新記錄 — 杯藥草♥

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摘要：在香港大霧山發現一種菌生異營性植物，形態及 DNA 基因間隔序列分析鑒定為龍膽科的杯藥草，屬香港新紀錄植物，而其地理分佈亦首次向南擴展到香港及更低的海拔。

關鍵詞：杯藥草、龍膽科、香港、菌生異營性植物、分類學。

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