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Molecular, chromosomal and morphological characters reveal a new diploid species in the *Smilax china* complex (Smilacaceae)

ZHONG-SHUAI SUN, YI-HAN WANG, YUN-PENG ZHAO[1] & CHENG-XIN FU
Key Laboratory of Conservation Biology for Endangered Wildlife of the Ministry of Education, and Laboratory of Systematic & Evolutionary Botany and Biodiversity, College of Life Sciences, Zhejiang University, Hangzhou 310058, China.
[1]*Author for correspondence; E-mail: ypzhao@zju.edu.cn*

Abstract

The *Smilax china* complex (Smilacaceae) is a typical mixoploid species complex including five extant diploid taxa with a widespread distribution across eastern Asia. The diploid population (mHB, 2n = 32), which was originally considered to be diploid *S. china*, together with two newly discovered diploid populations (mZZ and mYXS) is supported here as a distinct species by morphological, karyotypic and molecular data. These three populations present consistent morphological characters of sub-erect stems, rudimentary tendrils and minutely serrulate leaf margins, in which they differ from *S. china*. Molecular phylogenetic analyses also confirm its monophyly with a closer relationship to the other two erect species *S. biflora* and *S. trinervula* in the complex. These three populations are thus proposed to be a clear new diploid species and described with the name of *Smilax microdontus* Z. S. Sun & C.X. Fu, sp. nov. The discovery of this new species highlights the importance of closer examinations on species complexes by integrating multiple evidence.

Key words: New diploid species; mixoploid species complex; *Smilax microdontus*; eastern Asia

Introduction

The *Smilax china* Linnaeus (1753: 1029) complex (Smilacaceae) is a typical mixoploid species complex, which included initially five extant diploid taxa, i.e., *S. china* L., *S. davidiana* A. de Candolle (1878: 104), *S. trinvervula* Miquel (1868: 87), *S. biflora* Siebold ex Miquel (1868: 87) and *S. nantoensis* Koyama (1975: 120), and an array of autopolyploid or allopolyploid lineages of *S. china* according to chromosomal and molecular data (Cameron & Fu 2006; Kong *et al*. 2007; Qi *et al*. 2013b). This species complex occurs widely in eastern Asia, and some species are common woody vines and shrubs in the evergreen and deciduous broad-leaved forests. Despite taxonomic debates, four diploid species, *S. davidiana*, *S. trinvervula*, *S. biflora* and *S. nantoensis*, are morphologically distinguishable while the mixoploid *S. china* demonstrates a remarkable morphological variation partly overlapping with the other species of the complex (Hatusima1971; Koyama 1957, 1960; Wang & Tang 1978; Chen & Koyama 2000, Chen *et al*. 2006).

Smilax china mainly comprises tetraploids and hexaploids widely distributed in mainland China and Japan (Fu *et al*. 1993, 1995; Huang *et al*. 1997; Kong *et al*. 2007), diploids being only known from Taiwan island (Nakajima 1937; Hsu 1971). However, our previous cytological investigation identified a diploid (2n=32) of *S. china* from Mt. Shennongjia, Hubei, China mainland (Huang *et al*. 1997), differing from the diploid in Taiwan (2n=30; Nakajima 1937; Hsu 1971) and other polyploids (2n=64, 96; Fu *et al.* 1993, 1995; Huang *et al*. 1997, Kong *et al*. 2007). Field observations further showed that it has nearly erect stems, which are distinct from the usually climbing ones in *S. china*. This later discovered diploid indicated that the mixoploid *S. china* might be taxonomically miscellaneous and requires a closer examination using multiple analytic approaches.

Thus, the *Smilax china* complex provides an ideal opportunity to test the hypotheses of species delineation within a widespread species complex with different ploidy levels. By integrating morphological, karyotypic and phylogenetic data, we aim to address the following specific questions, 1) if the later discovered diploid *S. china* also occurs in additional localities in mainland China, whether they statistically resemble each other in morphology and show discontinuity from other taxa of the *S. china* complex; 2) whether they share the same karyotype (2n=32); 3) whether they form a monophyly and what are the phylogenetic relationships between the diploid species of the *S. china* complex. With congruent evidence, we propose and describe the focal diploid as a new species.

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