DNA Barcoding of Scirpus mariqueter and Its Closely Related Species: A Postprint

Authors: Zhang Shilan, Wang Yucen, Liu Wenliang

Date: 2023-08-25T00:00:00+00:00

Abstract

The taxonomic status of Bolboschoenoplectus mariqueter (Tang & F. T. Wang) Tatanov remains unresolved. To clarify the taxonomic position of B. mariqueter and investigate its relationship with related species, this study amplified and sequenced DNA barcode sequences from one nuclear gene (ITS) and five chloroplast genes (matK, ndhF, rbcL, trnL, and trnL-trnF) for 21 batches of samples representing four species of B. mariqueter and its close relatives. Species identification efficiency of individual sequences and sequence combinations was evaluated using similarity search algorithms, and phylogenetic analysis was conducted by constructing trees based on Bayesian inference methods. The results demonstrated: (1) The ITS + matK sequence combination yielded the highest species identification success rate at 71.4%, enabling effective interspecific discrimination and identification of B. mariqueter and its related species; (2) Phylogenetic trees constructed from the ITS + matK sequence combination showed robust clustering of samples from the same species, with B. mariqueter grouping with species of the genus Bolboschoenus (Asch.) Palla, clearly distinct from species of the genus Schoenoplectus (Rchb.) Palla, and B. mariqueter forming a monophyletic clade with Bolboschoenus maritimus (Linnaeus) Palla. In summary, the ITS + matK sequence combination represents the optimal DNA barcode for species identification of B. mariqueter and its relatives. The findings do not support the classification of B. mariqueter as a natural hybrid; rather, it should be assigned to the genus Bolboschoenus, and B. mariqueter should be recognized as a synonym of B. maritimus. This study provides molecular evidence for the taxonomic research of B. mariqueter and its related species.

Full Text

Abstract

The taxonomic status of × Bolboschoenoplectus mariqueter (Tang & F.T.Wang) Tatanov remains controversial. To clarify its taxonomic position and explore its

relationships with closely related species, we amplified and sequenced DNA barcode sequences from one nuclear gene (ITS) and five chloroplast genes (matK, ndhF, rbcL, trnL, and trnL-trnF) across 21 samples representing four species, including × Bolboschoenoplectus mariqueter and its relatives. We then evaluated the identification efficiency of individual and combined sequences using similarity search algorithms, and finally conducted phylogenetic analysis using Bayesian inference. The results showed: (1) The ITS + matK sequence combination achieved the highest species identification success rate of 71.4%, enabling interspecific differentiation and identification of × Bolboschoenoplectus mariqueter and its close relatives; (2) The phylogenetic tree based on ITS + matK sequences revealed good clustering of samples within species, with × Bolboschoenoplectus mariqueter grouping with all Bolboschoenus (Asch.) Palla species, clearly separate from Schoenoplectus (Rchb.) Palla species, and forming a monophyletic clade with Bolboschoenus maritimus (Linnaeus) Palla. In conclusion, the ITS + matK sequence combination represents the optimal barcode for identifying × Bolboschoenoplectus mariqueter and its relatives. Our findings do not support the hypothesis that × Bolboschoenoplectus mariqueter is a natural hybrid; rather, it should be classified within Bolboschoenus, likely as a synonym of B. maritimus. This study provides molecular evidence for the taxonomic research of × Bolboschoenoplectus mariqueter and its closely related species.

Keywords: × Bolboschoenoplectus mariqueter, related species, DNA barcoding, species identification, genetic relationship

Introduction

× Bolboschoenoplectus mariqueter (Tang & F. T. Wang) Tatanov belongs to the family Cyperaceae and is primarily distributed in the tidal flats of the Yangtze River estuary and Hangzhou Bay. It was once reported as a pioneer species endemic to China's coastal wetlands (Tang & Wang, 1965) and provides multiple ecological functions, including maintaining biodiversity, wave attenuation, bank stabilization, sediment promotion, and habitat provision for migratory waterbirds (Zhu et al., 2007; Chen & Sun, 2011). However, its taxonomic status and phylogenetic position remain unresolved.

Initially described as a natural hybrid between Scirpus triqueter L. and S. planiculmis Fr. Schmidt (Tang & Wang, 1965), the species was named Scirpus × mariqueter Tang et F. T. Wang. As systematic studies of Scirpus L. s.l. progressed, the broad genus was divided into several genera, with the putative parental species reassigned to different genera: Schoenoplectus (Rchb.) Palla (S. triqueter) and Bolboschoenus (Asch.) Palla (S. planiculmis). Consequently, the species names were updated to Schoenoplectus triqueter (L.) Palla and Bolboschoenus planiculmis (F. Schmidt) T. V. Egorova. Tatanov (2007) recognized × Bolboschoenoplectus mariqueter as an intergeneric hybrid and established the hybrid genus × Bolboschoenoplectus Tatanov, resulting in the current name (Dai et al., 2010). In contrast, Koyama (1980) treated Scirpus × mariqueter as

a synonym of Bolboschoenus planiculmis, considering it merely a single-spikelet variant of that species.

With advances in sequencing technology, molecular markers have been developed to analyze the genetic structure and phylogenetic relationships of × Bolboschoenoplectus mariqueter and its relatives. Yang et al. (2009, 2013) used AFLP markers and interspecific hybridization experiments to examine relationships with potential parents B. planiculmis and S. triqueter, concluding that × B. mariqueter is not of hybrid origin and is more closely related to B. planiculmis, though without clearly defining species boundaries. Other studies using AFLP and microsatellite markers revealed genetic structural similarities between B. planiculmis and B. maritimus (Linnaeus) Palla, with numerous hybrid offspring in overlapping regions that could not be definitively classified (Zhou et al., 2009; Píšová et al., 2017). Research has shown that achene cross-section shape and pericarp thickness are important diagnostic characters for interspecific differentiation in Bolboschoenus (Hroudová et al., 2007; Píšová et al., 2017). Song et al. (2019) compared nutlet morphology and pericarp microstructure, finding that × B. mariqueter differed significantly from B. planiculmis but resembled B. maritimus, suggesting it should be synonymized under B. maritimus subsp. paludosus (A. Nelson) T. Koyama. Overall, the taxonomic status of × B. mariqueter and its relationship with close relatives requires further investigation.

In 2003, Hebert first proposed DNA barcoding technology, which uses a standardized, easily amplified short DNA fragment with sufficient variation for rapid species identification (Hebert et al., 2003a, 2003b). Over the past two decades, DNA barcoding has become one of the fastest-developing fields in life sciences, demonstrating broad application prospects in plant species identification and molecular systematics (Costion et al., 2011; Li et al., 2015; Hu et al., 2022). The technology has been widely applied to study identification, phylogenetic relationships, and evolutionary history of Cyperaceae, yielding significant results (Yen & Olmstead, 2000; Starr et al., 2009; Clerc-Blain et al., 2010; Glon et al., 2017; Léveillé-Bourret et al., 2020). However, molecular phylogenetic studies based on gene fragments for × B. mariqueter and its relatives remain limited. Accurate species identification is fundamental to biodiversity conservation (Vane-Wright et al., 1991), and achieving scientific identification of × B. mariqueter holds significant theoretical and practical value for phylogenetic reconstruction and taxonomic research in Bolboschoenus.

This study employed six candidate sequences (ITS, matK, ndhF, rbcL, trnL, and trnL-trnF) to investigate DNA barcoding of × B. mariqueter and three related species across 21 samples. By comparing sequence characteristics, intra- and interspecific variation rates, and identification success rates, we evaluated the species resolution capabilities of different candidate sequences and their combinations to identify the optimal DNA barcode for × B. mariqueter and its relatives. Additionally, we conducted phylogenetic analysis using tree-building methods to further explore relationships between × B. mariqueter and its close

relatives, providing a scientific basis for taxonomic research.

Materials and Methods

1.1 Experimental Materials

We selected 21 samples of × Bolboschoenoplectus mariqueter and three related species: Bolboschoenus planiculmis (F. Schmidt) T. V. Egorova, B. maritimus (Linnaeus) Palla, and B. yagara (Ohwi) Y. C. Yang & M. Zhan (Table 1). Detailed morphological characteristics and geographic distributions are provided in Tables 2 and 3 .

1.2 DNA Extraction, PCR Amplification, and Sequencing

Total genomic DNA was extracted using a plant genomic DNA kit (Tiangen Biotech Co., LTD). Primer information and PCR amplification protocols are listed in Table 4 . PCR reactions were performed in 100 L volumes containing: 69.5 L sterile ddH_2O, 10 L TaqBuffer, 8 L dNTPs, 4 L each of forward and reverse primers, 4 L DNA template, and 0.5 L EX Taq polymerase. Amplified products were checked on 1% agarose gels, and samples with clear bands were sent to BGI for bidirectional sequencing.

1.3 Data Processing

Bidirectional chromatograms were edited and assembled using SeqMan software (DNASTAR LaserGene package, DNASTAR Inc., Madison, WI, USA), with low-quality sequences at both ends removed. Multiple sequence alignments were performed using MEGA7.0, and sequence characteristics and variable sites were analyzed using DnaSP 6.0. Intra- and interspecific genetic distances were calculated based on the Kimura 2-parameter (K2P) model to evaluate variation patterns. Identification success rates were assessed using BLAST (Basic Local Alignment Search Tool). Query sequences were batch-analyzed against GenBank using NCBI-BLAST. Identification was considered successful when the top hit (Identity > 95% and E-value < 1$×10^{-5}$) matched the query species with no other species included. For combined fragments, identification was deemed successful if any single fragment correctly identified the species (Liu et al., 2015). Identification efficiency was calculated as: (number of successfully identified individuals) / (total number of individuals). Phylogenetic trees were constructed using Bayesian inference for genetic clustering analysis.

Results

2.1 Sequence Characteristics of Candidate DNA Barcodes

Statistical analysis of the six candidate DNA barcode sequences revealed that rbcL was the longest at 1,374 bp, while trnL-trnF was the shortest at 597 bp (Table 5). ITS showed the highest GC content, whereas trnL had the lowest.

The number of variable sites ranked as: ITS > ndhF > matK > rbcL > trnL > trnL-trnF. The nuclear ITS gene exhibited substantially more variable sites than the five chloroplast genes.

2.2 Intra- and Interspecific Genetic Variation

An ideal DNA barcode should show significantly lower intraspecific than interspecific variation (Ning et al., 2008). Our K2P distance analysis (Table 6) revealed that intraspecific variation was 0 for all six barcodes. Except for ITS, the other five chloroplast barcodes showed varying degrees of overlap between intra- and interspecific variation. Interspecific variation ranked as: ITS > ndhF > matK > trnL > rbcL > trnL-trnF.

2.3 Identification Efficiency Evaluation

BLAST analysis showed that no single barcode achieved 100% identification success (Table 7). matK performed best at 52.4%, followed by ndhF and rbcL (both 28.6%), ITS (23.8%), while trnL-trnF and trnL showed zero resolution. Among combined barcodes, ITS + matK achieved the highest efficiency (71.4%), followed by matK + ndhF and matK + rbcL (both 61.9%). Other combinations (ITS + ndhF, ITS + rbcL, ITS + trnL-trnF, ITS + trnL) showed lower efficiencies of 52.4%, 52.4%, 23.8%, and 23.8%, respectively.

2.4 Phylogenetic Analysis

To investigate relationships among × B. mariqueter and its relatives, we constructed a phylogenetic tree based on ITS + matK sequences. Outgroup sequences downloaded from GenBank are listed in Table 8 . We performed an Incongruence Length Difference (ILD) test in PAUP*4.0b10 (Dolphin et al., 2000) to assess data congruence between nuclear and chloroplast sequences. The P-value of 1.0 indicated no significant conflict ($P > 0.05$) between ITS and matK datasets, justifying combined analysis. Bayesian inference was used for multigene tree construction, as it offers greater flexibility and reliability (Heled & Drummond, 2009). The combined ITS + matK matrix comprised 1,839 bp with 39 variable sites. The phylogenetic tree (Figure 1 [Figure 1: see original paper]) showed well-supported clades, mostly monophyletic. Outgroup species from two genera formed the basal lineage. Schoenoplectus (Rchb.) Palla species formed a distinct clade, clearly separated from Bolboschoenus (Asch.) Palla species with posterior probability of 1. The Bolboschoenus clade, comprising × B. mariqueter and its relatives, divided into three main branches: the largest branch included all × B. mariqueter and B. maritimus samples; the second branch contained six B. planiculmis samples; and the third branch comprised all B. yagara individuals, all with high support values. In the ITS + matK tree, × B. mariqueter clustered with B. maritimus, forming a sister clade to B. planiculmis. One B. maritimus individual grouped with × B. mariqueter, albeit with low support. Bolboschoenus yagara formed a separate clade, most distantly related to × B. mariqueter.

Discussion and Conclusion

Due to the complex evolutionary history of plants, no single plastid region equivalent to the animal COI gene has been identified as a universal barcode. Selecting efficient and universal DNA barcode candidates from chloroplast and nuclear genomes remains a major challenge for botanists (Li et al., 2015). This study evaluated six candidate sequences (one nuclear: ITS; five chloroplast: matK, ndhF, rbcL, trnL, and trnL-trnF) for species identification capability and phylogenetic relationships among × B. mariqueter and three related species.

3.1 Identification Efficiency of Different DNA Barcodes in × B. mariqueter and Relatives

The ITS region of nuclear ribosomal DNA is widely used in Cyperaceae phylogenetic studies due to its highly conserved flanking regions, high copy number, rapid concerted evolution, and ease of amplification, sequencing, and alignment (Baldwin et al., 1995). Our ITS sequences, amplified with universal primers ITS4/ITS5, included ITS1, 5.8S rDNA, and ITS2, totaling 706 bp. This differs from reports of 636 bp in Schoenoplectus and Schoenoplectiella (Shiels et al., 2014) and 712 bp in Scirpus (Jung & Choi, 2010). The variation rate in our study (4.96%) was substantially lower than the 47.3% reported for Scirpus and 32.4% for Schoenoplectus/Schoenoplectiella, likely due to differences in sample sizes, indicating that ITS sequence length and variation differ among Cyperaceae genera.

The matK gene is one of the most rapidly evolving protein-coding regions in the chloroplast genome (Chase et al., 2007), though primer universality remains debated, often requiring multiple primer pairs across taxonomic groups (Fazekas et al., 2008). We used two universal primer sets to obtain a 1,188 bp matK fragment, consistent with Gilmour et al. (2013). While matK showed 52.4% identification efficiency in our study, Clerc-Blain et al. (2010) reported 95% resolution for Carex and Kobresia in the Canadian Arctic Archipelago, possibly due to fewer congeneric species and insufficient sampling of close relatives in that region (Fazekas et al., 2008).

The ndhF gene, located in the chloroplast small single-copy region, shows conserved 5 ends but more variable 3 ends, evolving at twice the rate of rbcL (Kim & Jansent, 1995). Our 1,038 bp ndhF sequences matched previous reports but showed far fewer variable sites than in Schoenoplectus and Schoenoplectiella (Gilmour et al., 2013), with only 28.6% identification efficiency.

The relatively conserved rbcL gene, widely used in plant phylogenetics due to its strong universality, showed low variation (0.15%) in our study, far below levels reported for Korean Scirpus (Jung & Choi, 2010), making it ineffective for distinguishing × B. mariqueter from its relatives. Starr et al. (2009) previously noted low success rates (18%) for rbcL amplification in Carex using specific primers.

The chloroplast tRNA gene trnL (UAA) intron, with catalytic function and secondary structure (Kuhsel et al., 1990), shows low variation, while the trnL-trnF spacer, under weaker selective pressure, evolves faster and suits lower-level phylogenetic studies (Taberlet et al., 1991). However, both sequences were highly conserved in our study group, with minimal or zero interspecific variation, making them unsuitable as candidate barcodes.

Starr et al. (2009) evaluated five barcodes (matK, rbcL, rpoC1, rpoB, trnH-psbA) in three Carex sections, finding single-barcode resolution never exceeded 60%. Similarly, our best single-barcode efficiency was 52.4% (matK), identifying only 11 of 21 individuals, confirming that single barcodes cannot effectively differentiate these species. Combined chloroplast barcodes improved resolution: matK + rbcL and matK + ndhF both reached 61.9%, demonstrating that multilocus analysis provides more informative sites and enhances accuracy (Fazekas et al., 2008).

Angiosperms have complex genetic backgrounds, with relatively conserved, slowly evolving chloroplast genes providing limited information. Nuclear markers like ITS may more closely track speciation with faster divergence rates (Wang et al., 2011), prompting recommendations to combine chloroplast and nuclear barcodes for closely related species (Liu, 2015). Our ITS + matK combination achieved the highest efficiency (71.4%), enabling reliable identification of × B. mariqueter and its relatives. Considering cost-effectiveness, universality, and resolution, we recommend ITS + matK as the optimal barcode for this species complex.

3.2 Taxonomic Status of × Bolboschoenoplectus mariqueter

Initially considered a hybrid origin (Tang & Wang, 1965; Tatanov, 2007), × B. mariqueter's morphological features (Table 2)—including "tubers present; 2–3 leaflike involucral bracts, erect or scattered"—differ from Schoenoplectus, and its achene surface reticulation contrasts with the smooth type in Schoenoplectus, supporting its placement in Bolboschoenus (Dai et al., 2010). Our ITS + matK phylogeny robustly placed × B. mariqueter with other Bolboschoenus species, separate from Schoenoplectus (posterior probability = 1), rejecting its status as an intergeneric hybrid and supporting its classification in Bolboschoenus, consistent with Yang (2010).

Furthermore, × B. mariqueter formed a monophyletic group with B. maritimus, providing molecular evidence that they may represent the same species. However, one B. maritimus individual grouped with × B. mariqueter with low support (PP = 0.09), suggesting this node may be questionable, though reciprocal monophyly cannot be excluded. Morphological and geographic analyses (Tables 2 and 3) revealed near-identical morphology, with only minor differences in glume length and perianth bristle length. Both species share identical habitats (coastal marshes, inland lake/river banks) and similar floristic composition in their distributions (Wu et al., 2010), though specific localities differ, particu-

larly the lack of international distribution records for × B. mariqueter—likely resulting from long-standing taxonomic confusion.

In summary, our results reject the hybrid origin hypothesis from S. triqueter × B. planiculmis (Yang et al., 2009) and Koyama's (1980) synonymization under B. planiculmis. Instead, we support the conclusion that × B. mariqueter should be synonymized under B. maritimus (Song et al., 2019).

Conclusion

This DNA barcoding study of × Bolboschoenoplectus mariqueter and its relatives identified ITS + matK as the most effective barcode combination and, through integration of molecular, morphological, and phytogeographic evidence, clarified that × B. mariqueter should be classified within Bolboschoenus as a synonym of B. maritimus. This establishes a theoretical foundation for future taxonomic research. However, with only six barcode candidates examined, further DNA barcoding studies of Bolboschoenus are warranted.

References

BALDWIN BG, SANDERSON MJ, PORTER JM, et al., 1995. The ITS region of nuclear ribosomal DNA: a valuable source of evidence on angiosperm phylogeny[J]. Ann Mo Bot Gard, 82(2): 247-277.

CHASE MW, COWAN RS, HOLLINGSWORTH PM, et al., 2007. A proposal for a standardised protocol to barcode all land plants[J]. Taxon, 56(2): 295-299.

CHEN XZ, SUN Y, 2011. Chinese endemic salt marsh plant — Bulrush (Scirpus mariqueter), current status of its conservation and utilization[J]. Wetland Sci & Manage, 7(1): 60-63.

CLERC-BLAIN JL, STARR JR, BULL RD, et al., 2010. A regional approach to plant DNA barcoding provides high species resolution of sedges (Carex and Kobresia, Cyperaceae) in the Canadian Arctic Archipelago[J]. Mol Ecol Resourc, 10(1): 69-91.

COSTION C, FORD A, CROSS H, et al., 2011. Plant DNA barcodes can accurately estimate species richness in poorly known floras[J]. PLoS ONE, 6(11): e26841.

DAI L, LIANG S, ZHANG S, et al., 2010. Cyperaceae[M]// Wu Z, Raven PH eds. Flora of China. Beijing: Science Press: 179-181.

DOLPHIN K, BELSHAW R, ORME CDL, et al., 2000. Noise and incongruence: interpreting results of the Incongruence Length Difference Test[J]. Mol Phylogen Evol, 17(3): 401-406.

FAZEKAS AJ, BURGESS KS, KESANAKURTI PR, et al., 2008. Multiple multilocus DNA barcodes from the plastid genome discriminate plant species equally well[J]. PLoS ONE, 3(7): e2802.

GILMOUR CN, STARR JR, NACZI RFC, 2013. Calliscirpus, a newgenus for two narrow endemics of the California Floristic Province, C. criniger and C. brachythrix sp. nov. (Cyperaceae)[J]. Kew Bull, 68: 85-105.

GLON HE, SHIELS DR, LINTON E, et al., 2017. A five gene phylogenetic study of Fuireneae (Cyperaceae) with a revision of Isolepis humillima[J]. Syst Bot, 42(1): 29-36.

HEBERT PDN, CYWINSKA A, BALL SL, et al., 2003. Biological identifications through DNA barcodes[J]. Proc R Soc Lond B, 270(1512): 313-321.

HEBERT PDN, RATNASINGHAM S, DEWAARD JR, 2003. Barcoding animal life: cytochrome coxidase subunit 1 divergences among closely related species[J]. Proc R Soc Lond B, 270(Supply 1): S96-S99.

HELED J, DRUMMOND AJ, 2009. Bayesian inference of species trees from multilocus data [J]. Mol Biol Evol, 27(3): 570-580.

HROUDOVÁ Z, ZáKRAVSKý P, DUCHáČEK M, et al., 2007. Taxonomy, distribution and ecology of Bolboschoenus in Europe[J]. Ann Bot Fenn, 44(2): 81-102.

HROUDOVÁ Z, ZÁKRAVSKÝ P, WÓJCICKI JJ, et al., 2005. The genus Bolboschoenus palla (Cypereceae) in Poland[J]. Polish Bot J, 50(2): 117-137.

HU JL, CI XQ, LIU ZF, et al., 2022. Assessing candidate DNA barcodes for Chinese and internationally traded timber species[J]. Mol Ecol Resour, 22: 1478-1492.

JUNG J, CHOI H-K, 2010. Systematic rearrangement of Korean Scirpus L. s.l. (Cyperaceae) as inferred from nuclear ITS and chloroplast rbcL sequences[J]. J Plant Biol, 53: 222-232.

KIM K-J, JANSENT RK, 1995. ndhF sequence evolution and the major classes in the sunflower family[J]. Proc Natl Acad Sci USA, 92(22): 10379-10383.

KOYAMA T, 1980. The genus Bolbosahoenus Palla in Japan[J]. Acta Phytotaxon Geobot, 31(4-6): 99-103.

KUHSEL MG, STRICKLAND R, PALMER JD, 1990. An ancient group I Iintron shared by eubacteria and chloroplasts[J]. Science, 250(4987): 1570-1573.

LÉVEILLÉ-BOURRETÉ, CHEN BH, GARON-LABRECQUE M-È, et al., 2020. RAD sequencing resolves the phylogeny, taxonomy and biogeography of Trichophoreae despite a recent rapid radiation (Cyperaceae)[J]. Mol Phylogen Evol, 145: 106727.

LI DZ, ZENG CX, 2015. Prospects for plant DNA barcoding[J]. Biodivers Sci, 23(3): 297-298.

LI X, YANG Y, HENRY RJ, et al., 2015. Plant DNA barcoding: from gene to genome[J]. Biological Reviews, 90(1): 157-166.

LIANG SC, 2022. List of wetland vascular plants in China[M]. Beijing: Science Press: 168-169.

LIANG SY, TUCKER GC, KOYAMA T, 2010. Cyperaceae[M]// WU Z, RAVEN PH. Flora of China. Beijing: Science Press: 181-188.

LIU J, YAN HF, NEWMASTER SG, et al., 2015. The use of DNA barcoding as a tool for the conservation biogeography of subtropical forests in China[J]. Divers Distrib, 21: 188-199.

LIU JQ, 2015. DNA barcodes, speciation and taxonomy in plants[J]. Biodivers Sci, 23(3): 283-285.

NING SP, YAN HF, HAO G, et al., 2008. Current advances of DNA barcoding study in plants[J]. Biodivers Sci, 16(5): 417-425.

OKIHITO Y, TAKUJI. H, 2005. Molecular phylogeny and chromosomal evolution of Japanese Schoenoplectus (Cyperaceae), based on ITS and ETS 1f sequences[J]. Acta Phytotaxon Geobot, 56: 177-189.

OLMSTEAD RG, MICHAELS HJ, SCOTT KM, et al., 1992. Monophyly of the Asteridae and identification of their major lineages inferred from DNA sequences of rbcL[J]. Ann Mo Bot Gard, 79(2): 249-265.

PÍŠOVÁ S, HROUDVÁ Z, CHUMOVÁ Z, et al., 2017. Ecological hybrid speciation in central-European species of Bolboschoenus: genetic and morphological evaluation[J]. Preslia, 89: 277-305.

SHIELS DR, HURLBUT DL, LICHTENWALD SK, et al., 2014. Monophyly and phylogeny of Schoenoplectus and Schoenoplectiella (Cyperaceae): evidence from chloroplast and nuclear DNA sequences[J]. Syst Bot, 39(1): 132-144.

SHINOZAKI K, SUGIURA M, 1982. The nucleotide sequence of the tobacco chloroplast gene for the large subunit of ribulose-l, 5-bisphosphate carboxylase/oxygenase[J]. Gene, 20: 91-102.

SONG CW, TANG Q, ZhAO ZP, et al., 2019. Nutlet shape and pericarp micromorphology of × Bolboschoenoplectus mariqueter and related species[J]. Plant Sci J, 37(3): 290-294.

STARR JR, NACZI RFC, CHOUINARD BN, 2009. Plant DNA barcodes and species resolution in sedges (Carex, Cyperaceae)[J]. Mol Ecol Resour, 9(Supply 1): 151-163.

TABERLET P, GIELLY L, PAUTOU G, et al., 1991. Universal primers for amplification of three non-coding regions of chloroplast DNA[J]. Plant Mol Biol, 17: 1105-1109.

TANG J, WANG FZ. 1965. Flora of China: Vol. 23[M]. Beijing: Science Press: 72-108.

TATANOV I, 2007. Hybrida intergenricua nouvus ×Bolboschoenoplectus Tatanov(Cypereceae)[J]. Novit SystemPlant Vascul, 39: 154.

VANE-WRIGHT RI, HUMPHRIES CJ, WILLIAMS PH, 1991. What to protect? systematics and the agony of choice[J]. Biol Conserv, 55(3): 235-254.

WANG Q, YU QS, LIU JQ, 2011. Are nuclear loci ideal for barcoding plants? A case study of genetic delimitation of two sister species using multiple loci and multiple intraspecific individuals[J]. J Syst Evol, 49(3): 182-188.

WARD E, ADAMS MJ, 1998. Analysis of ribosomal DNA sequences of Polymyxa species and related fungi and the development of genus- and species-specific PCR primer[J]. Mycol Res, 102(8): 965-974.

WHITE TJ, BRUNS T, LEE S, et al., 1990. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics[M]. San Diego: Academic Press: 315-322.

WU ZY, SUN H, ZHOU ZK, et al., 2010. Floristics of seed plants from China[M]. Beijing: Science Press: 52-83.

YANG M, 2010. The study on species biology of Scirpus × mariqueter and its genetic structure in China[D]. Shanghai: Fudan University: 26-37.

YANG M, LV X, FAN W, et al., 2013. Interspecific hybridization between Scirpus mariqueter Ts. Tang & F.T. Wang and S. planiculmis F. Schmidt and their selfing[J]. Aquat Bot, 110: 92-96.

YANG M, ZHOU Y, ZHU Q, et al., 2009. AFLP markers in the detection of Scirpus × mariqueter (Cyperaceae) hybrid in China[J]. Aquat Bot, 91: 298-302.

YEN AC, OLMSTEAD RG, 2000. Molecular systematics of cyperaceae tribe Cariceae based on two chloroplast DNA regions: ndhF and trnL intron-intergenic spacer[J]. Syst Bot, 25 (3): 389-401.

ZHOU Y, WANG H, YANG M, et al., 2009. Development of microsatellites for Scirpus mariqueter Wang et Tang (Cyperaceae) and cross-species amplification in Scirpus planiculmis F. Schmidt[J]. Mol Ecol Resour, 9(1): 370-372.

ZHU J, JING K, GAN XJ, et al., 2007. Food supply in intertidal area for shorebirds during stopover at Chongming Dongtan, China[J]. Acta Ecol Sin, 27(6): 2149-2159.

Note: Figure translations are in progress. See original paper for figures.