

## Article

# Reappraisal of the Identity of *Batrachium pekinense* (Ranunculaceae) and Its Implication for Biodiversity Conservation and Management

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**Abstract:** Because of human-induced habitat deterioration and climate change, a large number of species are threatened and even endangered. *Batrachium pekinense* Liang Liu, a perennial aquatic plant endemic to Beijing, was synonymized as a widely distributed species in Northern China, *B. bungei* Steudel, but it was later listed in the national key protected wild plants in China. Taxonomic uncertainty should be clarified especially when related species may be allocated with a limited conservation budget. In this study, we reappraised the identity of *B. pekinense* based on results from molecular phylogenetic analysis and 15-year field observations. Our result shows that 77% of the 65 individuals collected from various sites share identical sequences in both the nuclear ribosomal ITS and the plastid *psbA-trnH* markers, all samples of the two species consist of one monophyletic clade with strong support, and continuous morphological variations on the key distinguishing character, the leaf shape, are observed in the field. Integrative evidence from both our and the others' studies supports the same identity for *B. pekinense* and *B. bungei*. Hence, we suggest excluding *B. pekinense* from the list of national key protected wild plants of China in its next update and advocate the integrative evidence-based taxonomy and systematics for biodiversity conservation and management.



**Citation:** Mu, X.-Y.; Gu, H.-Z.; Yu, H.; Dong, W.-G.; Liang, T.-S. Reappraisal of the Identity of *Batrachium pekinense* (Ranunculaceae) and Its Implication for Biodiversity Conservation and Management. *Diversity* **2024**, *16*, 54. <https://doi.org/10.3390/d16010054>

Academic Editors: Hong-Hu Meng, Yann Hénaout and Salima Machkour-M'Rabe

Received: 8 December 2023

Revised: 1 January 2024

Accepted: 11 January 2024

Published: 15 January 2024



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**Keywords:** *Batrachium pekinense*; *Ranunculus pekinense*; endangered species; integrative evidence; taxonomic uncertainty; biodiversity conservation

## 1. Introduction

Though debates exist about the cause of the sixth mass extinction on the earth, the fact is that human-induced biodiversity loss is widely accepted [1–3]. Biodiversity conservation efforts at international and national scales are both performed, and many endangered species have been better protected and managed in China in recent years [4,5]. In 2021, the lists of national key protected wild animals and plants in China are both issued, which further develop biodiversity conservation. A fundamental question in biodiversity conservation is that what is the definition of species? It is hard to hold the same standard in species classification between animals and plants and even among various plant lineages. Another concern is which kind of species should be listed. The low percentage of species coverage and the species with disputable taxonomic identity included in the list of key protected organisms and the poor outcomes of threatened species with a high proportion of budget allocation may suffer criticism [6–8]. Considering the shortfalls in budgets for conservation, policymakers are tasked with hard decisions about how to allocate the limited resources.

Aquatic plants often exhibit a high degree of phenotypic plasticity [9], which may be induced by environmental characteristics (e.g., nutrient, light, and water) and reproductive strategies (e.g., vegetative reproduction, hybridization, and polyploidy) [10,11], and consequently, leading to controversy in species classification and endemic species'

conservation. Among them, the monophyletic group *Batrachium* (DC.) Gray (Ranunculaceae) is a representative example, the taxonomy of which is obscure and is considered a taxonomist's nightmare [11–13]. Based on results from molecular phylogeny, it has recently been classified as a section within the genus *Ranunculus* L. [14]. Several species in *Batrachium* are well known for the simultaneous occurrence of both capillary submerged leaves and laminar floating leaves, especially those distributed in Europe [11]. Because of frequent hybridization and polyploidy, species delimitation in *Batrachium* is challenging. The plastid *psbA-trnH* and the nuclear ribosomal ITS regions (nrITS) are suggested as informative in the identification of *Batrachium* [12,15].

*Batrachium pekinense* Liang Liou, a previously reported narrowly distributed species endemic to Beijing, China, was first listed as a key protected plant in Beijing in 2008, then listed as the national key protected plant in 2021 [16]. It is reported as a new species by Liang Liu in 1980 [17], with the original description that both submerged capillary and floating laminar leaves exist, and the ultimate lobules of floating laminar leaves are 0.2–0.6 mm in width (Figure 1A). However, this kind of dimorphic leaves and the filiform leaves (Figure 1B) are distinctly different from those species with typical dimorphic leaves, which grow in Europe as mentioned above. In the taxonomic revision of *Batrachium* [14], the morphology of the type specimen of *B. pekinense* designated by Liang Liu [17] is found to be the same as that of *B. hydrophilus* Bunge, which was published in 1831, and they are both treated as synonyms of a widely distributed species in Northern China whose type specimen was also collected from Beijing, *B. bungei* Steudel [14]. Furthermore, *B. bungei* (Steudel) Liang Liu is also synonymized under *B. bungei* Steudel [14]. Recently, *B. mongolicus* (Krylov) Serg. was found as a new record in China, which is documented as the only species with dimorphic leaves (i.e., both broad palmate floating laminar (Figure 1C) and filiform submerged capillary leaves) in Chinese *Batrachium* species [18]. Hence, it is necessary to reappraise the identity of *B. pekinense* from the point of view of both taxonomy and biodiversity conservation and management.



**Figure 1.** Leaf morphology of *Batrachium pekinense* (A), *B. bungei* (B), and the typical floating broad laminar leaves in *B. mongolicus* (C). (A,B) are photographed by Xian-Yun Mu, and (C) by Ce Shang. The species identification follows the classification system of *Batrachium* recorded in *Flora Reipublicae Popularis Sinicae*.

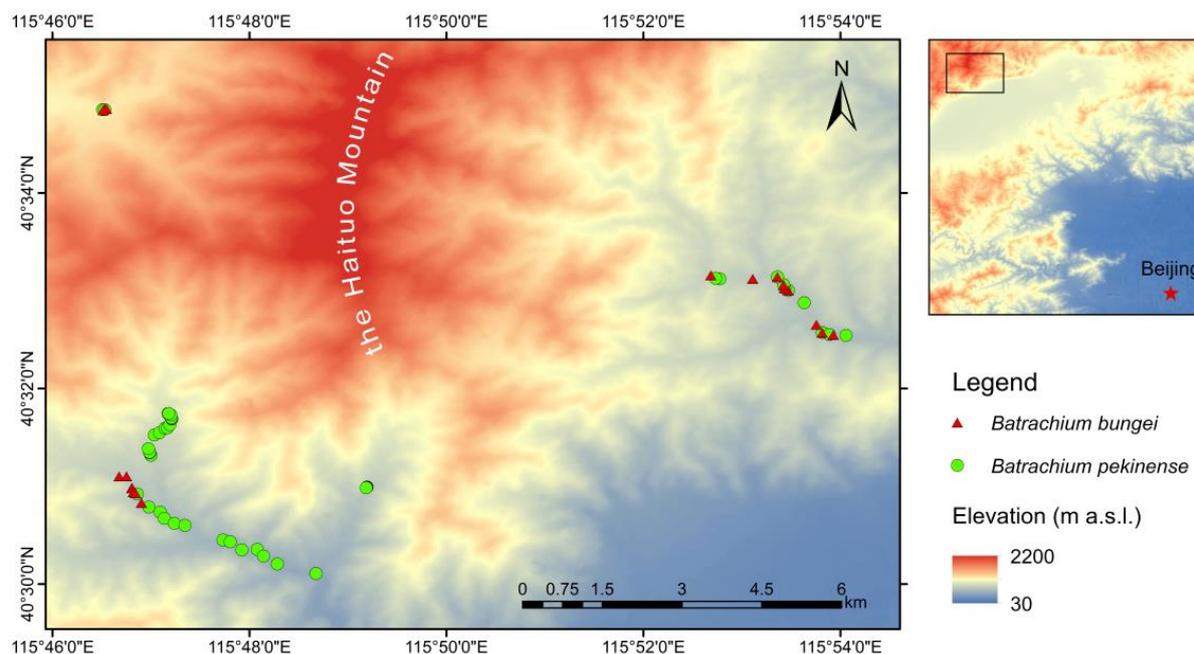
In this study, a large number of samples of *B. bungei* and *B. pekinense* from Beijing were collected and sequenced, and the variation of leaf morphology was investigated based on our 15-year field observations. We aim to elucidate the taxonomic position of *B. pekinense* and its relationships with other congeners based on integrated evidence. Our work will also be informative for policymakers during the assessment of species endangerment and the update of the lists of national key protected organisms.

## 2. Materials and Methods

### 2.1. Field Investigation and Sampling

We embarked on extensive field investigation in the type locality of *B. pekinense*, the streams within the mountains near Nankou where the Badaling Great Wall is located. Fur-

thermore, consecutive field observations including its morphological variation, distribution pattern, and habitat were performed in streams in the mountains of Beijing, especially from Haituo Mountain in the northwest to Wuling Mountain in the northeast. Samples of 46 individuals of *B. pekinense* and 19 individuals of *B. bungei* used for phylogenetic study were collected from its distribution center, the Haituo Mountain (Figure 2, detailed sample information see Appendix A) in 2014. Voucher specimens are preserved at the herbarium of Beijing Forestry University. Related sequences of *B. aquatilis* L. and *B. trichophyllus* (Chaix ex Villars) Bosch were obtained from Lumbreras et al. [15], and *R. japonicus* Thunb. was selected as the outgroup, whose sequences were obtained from Hörandl et al. [19] (Appendix B).



**Figure 2.** Distribution map of samples of 49 *Batrachium pekinense* and 16 *B. bungei* at the distribution center of the former, the Haituo Mountain in Northern Beijing.

### 2.2. DNA Extraction, Amplification, and Sequencing

Total genomic DNA was extracted from silica gel-dried leaf tissue using a plant genomic DNA kit (Tiangen Biotech Co., Ltd., Beijing, China) following the manufacturer's protocol. Two regions, nrITS and *psbA-trnH*, were selected and amplified following Lumbreras et al. [15]. The nrITS region that comprises ITS1, 5.8S gene, and ITS2 was sequenced with the universal ITS1 and ITS4 primers. Double-stranded DNA amplifications were performed on 20  $\mu$ L with 14.1  $\mu$ L of ddH<sub>2</sub>O, 2  $\mu$ L of Taqbuffer, 1.6  $\mu$ L of dNTP, 0.5  $\mu$ L of each primer (forward and reverse), 0.3  $\mu$ L of Taq-polymerase (2.5 U/ $\mu$ L), and 1  $\mu$ L of total DNA using a Eppendorf 580BR Thermal Cycler. The amplified products were sent to the Beijing Ruibo Xingke Biotechnology Co., Ltd. in Beijing, China for sequencing. The raw sequence fragments were assembled using Sequencher v.4.1.4 (Gene-Codes Corporation, Ann Arbor, MI, USA). The sequence data were aligned using MAFFT v.7.520 software [20] with default parameters.

### 2.3. Phylogenetic Inference

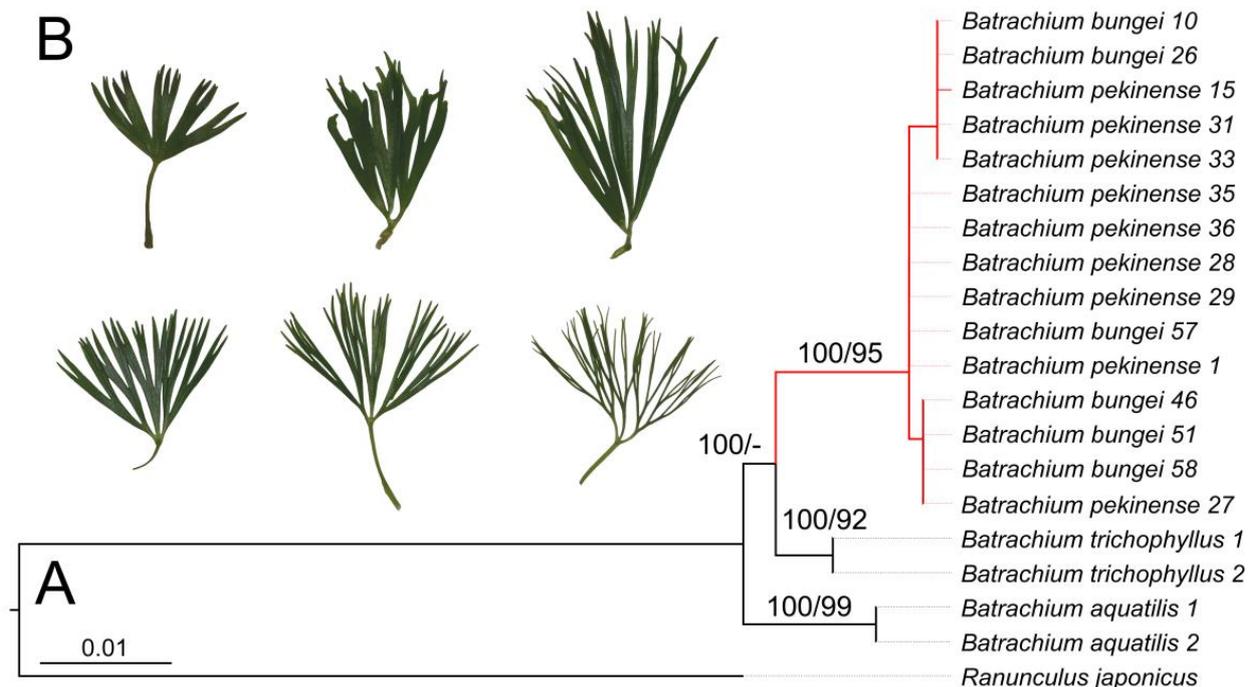
Topological incongruence between the ribosomal and the plastid partitions was tested using the incongruence length difference (ILD) test [21]. Congruence between nrITS and *psbA-trnH* was examined using PAUP\* for 100 replicates (heuristic search, simple addition, TBR branch-swapping), and all trees were saved in each step. Two regions were combined because the *p* value is 0.07 in the ILD test.

Both maximum likelihood (ML) and maximum parsimony (MP) analyses were performed for the combined data matrix. For ML analysis, the IQ-TREE 2 software (v.2.1.3) [22] was employed, with 1000 replicates for the estimation of ultrafast bootstrap values ( $BS_{ML}$ ) [23]. For MP analysis, heuristic searches with random stepwise addition by tree bisection-reconnection (TBR) branch swapping, with the MULTrees option, were performed using the PAUP\* software (v.4.0) [24]. All character states were treated as unordered and equally weighted and gaps as missing data. The bootstrap value ( $BS_{MP}$ ) was estimated from 1000 replicates using a heuristic search with simple addition with the TBR and MULTrees options implemented [25].

### 3. Results

#### 3.1. Molecular Phylogenetic Analysis

The length of nrITS and *psbA-trnH* were 659 and 390 bp, respectively (Datas S1 and S2). A low number of variable sites were identified, that is, eight sites in nrITS and two in *psbA-trnH*. After combining them and excluding the identical sequences, six individuals of *B. bungei* and nine of *B. pekinense* were kept in the IQ-TREE 2 software, and a final data matrix with a length of 982 bp was generated and used for phylogenetic analysis (Data S3). Both the ML tree and the MP tree yielded the same tree topology at the species level. Neither *B. bungei* nor *B. pekinense* was supported as monophyletic. However, all 15 samples of these two species were nested together and consisted of one strongly supported clade ( $BS_{MP} = 100\%$ ,  $BS_{ML} = 95\%$ ) (Figure 3A). The two samples of *B. trichophyllus* nested as monophyletic ( $BS_{MP} = 100\%$ ,  $BS_{ML} = 92\%$ ), and sisters to *B. pekinense*–*B. bungei* clade. Samples of *B. aquatilis* were resolved as the basal clade on the tree of the *Batrachium* group (Figure 3A).



**Figure 3.** A molecular phylogenetic tree inferred from maximum likelihood analysis shows that all individuals of *Batrachium pekinense* and *B. bungei* consist of a monophyletic clade (A), and the leaf morphology varies from relatively broad lobes to the capillary in these two species (B). The numbers above the nodes indicate bootstrap values generated from maximum parsimony ( $BS_{MP}$ ) and maximum likelihood ( $BS_{ML}$ ) analysis, respectively. The hyphen denotes  $BS_{ML}$  less than 50%. The scale bar indicates substitution per site.

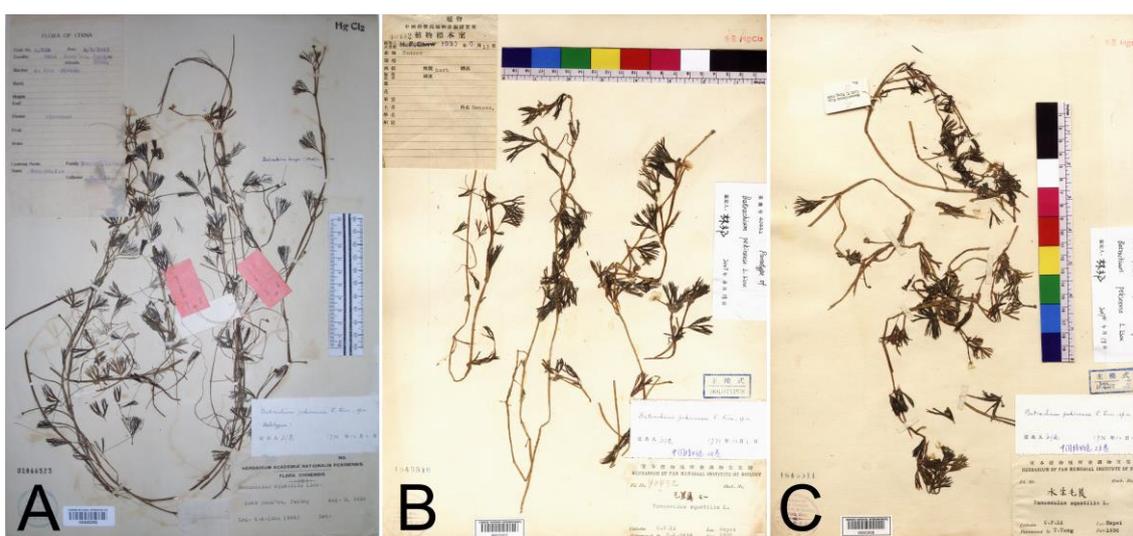
### 3.2. Morphological Comparison of Key Characters

The breadth of terminal leaf lobes is the only difference between *B. pekinense* and *B. bungei* [17]. The type specimen of *B. pekinense* is collected from the stream in the hills, where the Badaling Great Wall is located. However, our field investigation shows that Haituo Mountain, which is located north of the type locality, is the distribution center of *B. pekinense*. The giant Haituo Mountain consists of many mountains, such as the Song Mountain and the Yudu Mountain. Furthermore, *B. bungei* is observed to co-exist with *B. pekinense* in the streams of the Haituo Mountain. After careful morphological observation and comparison, one of the key differences between the two species, the leaf morphology that includes leaf lobes and the breadth of terminal lobes, is found without a clear cut (Figure 3B). Successive morphological variation was also detected in the population at the Bai River, which is located in northeast Beijing. Leaf morphology and the breadth of blades vary randomly, which also implies the obscure morphological boundary between *B. pekinense* and *B. bungei*, and this further corroborates the results of the phylogenetic study.

## 4. Discussion

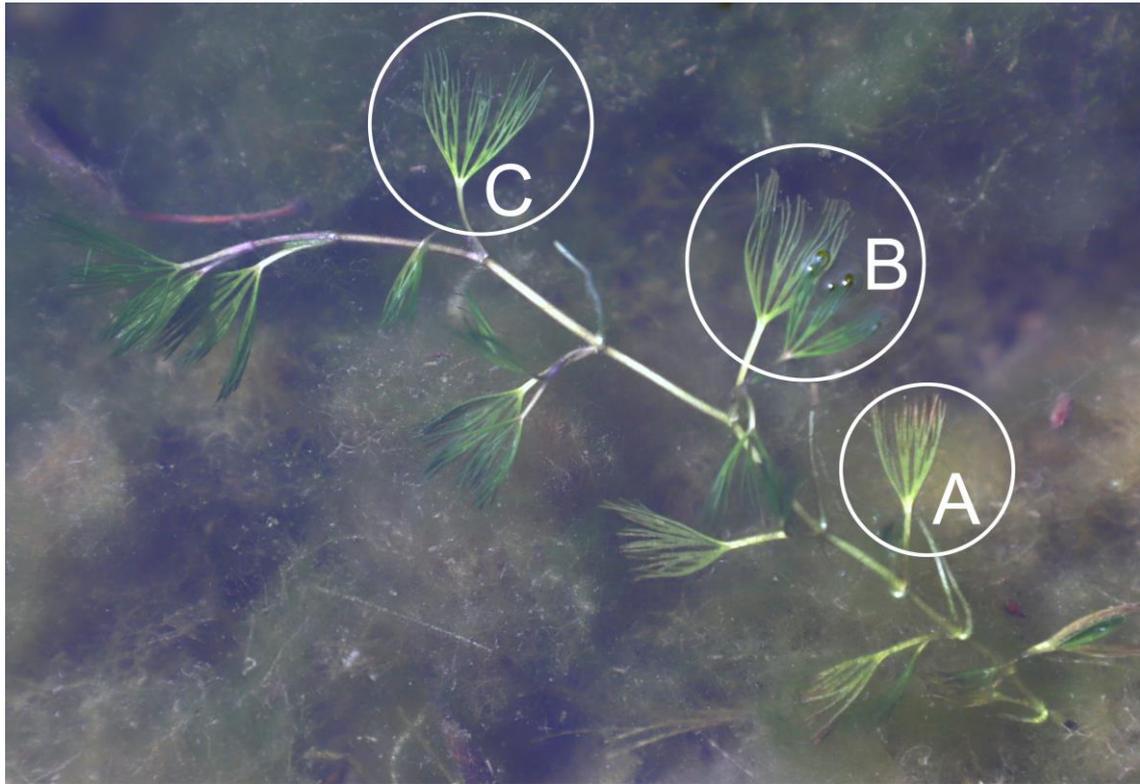
### 4.1. The Identity of *Batrachium pekinense* Inferred from Multiple Evidence

Because phenotypic plasticity, vegetative reproduction, hybridization, and polyploidy are common in aquatic plants, taxonomic studies are difficult, and a large number of taxonomic uncertainties need further clarification. As an example of aquatic plants, the genus *Batrachium* is often considered a nightmare for taxonomists [11]. *Batrachium pekinense*, a narrowly distributed species endemic to north Beijing, is reported as Liang Liu with definite diagnostic characteristics, both floating and submerged leaves, when compared with *B. bungei*, which only have submerged leaves [17,26]. On the one hand, *B. mongolicus* is the only species with real floating leaves in China [18] (Figure 1C). On the other hand, the relatively broad floating type of leaves designated by Liang Liu (Figure 4) are not floating but submerged in streams in the field based on our long-term field observation. Hence, just as Wiegleb et al. mentioned [11], leaf type (submerged vs floating) in *B. pekinense* may be misunderstood and utilized in species identification in previous literature (e.g., *Flora of Beijing* and *Flora of China*). Floating broad laminar leaves like those in Figure 1C exist in neither the type specimens (Figure 4) nor the field populations of *B. pekinense*, the leaves with relatively broad terminal lobes are submerged but not floating leaves.



**Figure 4.** The holotype (A) and paratypes (B,C) of *Batrachium pekinense* designated by Liang Liu in *Flora Reipublicae Popularis Sinicae*. These specimens are preserved in PE.

Based on our 15-year field observation, individuals with relatively broad terminal leaf lobes type (commonly identified as *B. pekinense*) and capillary type (commonly identified as *B. bungei*) are frequently discovered in the same spot (e.g., in streams in mountains with high elevation and ponds in lowland area), and leaves with intermediate type are also found (Figure 3B). Furthermore, leaves are found with the broad terminal lobes type at the basal part of the stem and then turned gradually to the capillary type at the top on the same individual (Figure 5). Hence, the definite diagnostic character employed in *B. pekinense*, leaf morphology, is less applicable when distinguished from *B. bungei* based on our long-term and extensive field observation.



**Figure 5.** Leaf lobes of *Batrachium pekinense* vary from the relatively broad capillary type (A) at the basal stem to semi-capillary type (B) at the middle and turn to capillary at the top (C) on the same individual. Photographed by Xian-Yun Mu.

Besides morphological plasticity, hybridization and polyploidy further complicate the taxonomy of *Batrachium* [11,27,28]. Based on previous phylogenetic and DNA barcoding studies focusing on *Batrachium* [12,15], plastid *psbA-trnH* and nrITS markers were employed for phylogenetic inference of *B. pekinense* in this study. A dense sampling of *B. pekinense* was performed focusing on its distribution center, which covered several origins of streams in the Haituo Mountain in northwest Beijing. The result shows that all individuals of *B. pekinense* and *B. bungei* nested in one clade with strong support (Figure 3A). Genome size, chromosome number, and ploidy are other important factors in species identification in *Batrachium* [11]. However, the same ploidy and approximately the same genome size are reported in both *B. pekinense* and *B. bungei* [29]. It is worth noting that, though Wiegleb et al. [14] questioned the chromosome number of Chinese *Batrachium* species ( $2n = 16$ ), which is reported by Dahlgren [30], these results are independently reported by both Xue-Hua Liu [29] and Qin-Er Yang [31] in *B. pekinense* and *B. bungei*. Furthermore, phylogenomic inferences based on genomic datasets of plastome and nuclear genome with a whole distributional range sampling scheme for *B. pekinense* show that samples of these two species are recovered as monophyletic with full support [29]. Hence, multiple pieces of evidence from morphological, molecular phylogenetic, genomic, and

chromosomal data support the same identity for the locally endemic one, *B. pekinense*, and the broadly distributed one, *B. bungei*. Consequently, the taxonomic treatment proposed by Wiegleb et al. [14], reducing *B. pekinense* to a synonym of *B. bungei*, is supported here.

#### 4.2. Integrative Systematics for Species Conservation and Management

Species are key components in biodiversity conservation, while the delimitation and taxonomic treatment of several species are still controversial [32]. Though challenging, accurate species identification and classification are exclusive goals of taxonomy [33]. We are approaching the natural species definition much closer than in history; more and more new species are found, while several synonyms that may make conservationists frustrated are also reported. For example, *Mussaenda anomala* Li (Rubiaceae), an endemic species endemic to Guangxi, China with only the type specimen, was listed as a national key protected wild plant in China in 1999. However, its unique petaloid calyx lobes are clarified as unstable characteristics [34]. Hence, it is finally reduced to a synonym of a widely distributed species, *M. shikokiana* Makino [35], and is consequently removed from the national list of the 2021 version. *Pyrus hopeiensis* Yü (Rosaceae), a critically endangered species endemic to North China that was funded a lot for its conservation [36], is reduced to a synonym of a widely distributed species, *P. ussuriensis* Maxim. based on integrative evidence from both phylogenomic and morphological data [32]. In this study, the same identity is again revealed between the locally endemic species, *B. pekinense* and the broadly distributed species, *B. bungei*. It is worthy of further investigating the relationship between *B. bungei* and the other species with an overlapping distribution range, e.g., *B. subrigidus* and *B. trichophyllus*.

Just as the Chinese Academician De-Yuan Hong says, we need a scientific and operative species concept in biodiversity pursuits [37]. On the one hand, all wild individuals of a genus as a whole can be included in the national key protected list, especially those with obscure taxonomic identities and complicated interspecies relationships but facing strong threats, such as *Cycas* L., *Taxus* L., and *Fritillaria* L. [7,38]. On the other hand, we also need a scientific attitude when facing the development of taxonomy and the change of species names. We suggest excluding *B. pekinense* from the list of national key protected wild plants in China in the next update. During the era of multi-omics, integrative systematics proceeded quickly, and we know much better about species and their evolution [39]. Considering the limited funds and resources, critically endangered organisms with clear taxonomic identities, including a special endangered group—taxonomists [40], should be given priority for conservation by policymakers during biodiversity conservation and management. Furthermore, the lists of national or regional key protected organisms should be updated regularly, e.g., every five years. By doing this, the precious budgets and resources may be better allocated, and the species conservation and management can be adjusted timely and appropriately.

#### 5. Conclusions

Taxonomic studies of several aquatic plants are difficult because of their morphological plasticity, hybridization, polyploidy, and so on. *Batrachium pekinense*, a previously thought threatened species endemic to Beijing, which is listed as a national key protected wild plant in China, is found to share the same identity with the widely distributed *B. bungei*, based on integrative evidence. Hence, we agree with the taxonomic treatment of Wiegleb et al. [14], which reduces *B. pekinense* to be a synonym of *B. bungei*, and suggest its deletion from the list of national key protected wild plants in China in the coming update. Considering the limited budgets and resources, conservation priority should be given to endangered but taxonomically uncontroversial species based on multiple pieces of evidence from integrative systematics.

**Supplementary Materials:** The following supporting information can be downloaded at <https://www.mdpi.com/article/10.3390/d16010054/s1>, Datas S1 and S2: The nrITS and *psbA*-

trnH data matrix of 65 samples of *B. pekinense* and *B. bungei*, respectively; Data S3: Combined data matrix of the 20 samples used in this study.

**Author Contributions:** Conceptualization, X.-Y.M.; methodology, X.-Y.M.; software, X.-Y.M. and H.-Z.G.; validation, X.-Y.M., H.-Z.G. and H.Y.; formal analysis, X.-Y.M. and H.Y.; investigation, X.-Y.M., H.Y., W.-G.D. and T.-S.L.; resources, X.-Y.M., W.-G.D. and T.-S.L.; data curation, X.-Y.M.; writing—original draft preparation, X.-Y.M. and H.Y.; writing—review and editing, X.-Y.M.; visualization, X.-Y.M., H.Y. and H.-Z.G.; supervision, X.-Y.M.; project administration, X.-Y.M.; funding acquisition, X.-Y.M. All authors have read and agreed to the published version of the manuscript.

**Funding:** This research was funded by the Natural Science Foundation of China (grant number 32070235) and the Natural Science Foundation of Beijing (grant number 5242014).

**Institutional Review Board Statement:** Not applicable.

**Data Availability Statement:** All data are available in this article.

**Acknowledgments:** We thank Wan-Jie Jiang and Yong-Xia Li during our field investigation at the Haituo Mountain and the anonymous reviewers for their constructive suggestions.

**Conflicts of Interest:** The authors declare no conflict of interest.

## Appendix A

Sample information of the 65 individuals of *Batrachium pekinense* and *B. bungei* collected and sequenced from the Haituo Mountain, Beijing.

Sample	Species	Locality	Northern Latitude	East Longitude	Altitude (m)
1	<i>B. pekinense</i>	Beijing Songshan Nature Reserve, Tangzigou	40°30'59.53"	115°49'11.60"	880
2	<i>B. pekinense</i>	Beijing Songshan Nature Reserve, Tangzigou	40°30'59.46"	115°49'11.57"	880
3	<i>B. pekinense</i>	Beijing Songshan Nature Reserve, Tangzigou	40°30'59.35"	115°49'11.53"	880
4	<i>B. pekinense</i>	Beijing Songshan Nature Reserve, Tangzigou	40°30'59.21"	115°49'11.32"	880
5	<i>B. pekinense</i>	Beijing Songshan Nature Reserve, Tangzigou	40°30'59.12"	115°49'11.21"	880
6	<i>B. pekinense</i>	Hebei Dahaituo Nature Reserve, Dahaituo	40°34'51.18"	115°46'30.58"	1255
7	<i>B. bungei</i>	Hebei Dahaituo Nature Reserve, Dahaituo	40°34'51.82"	115°46'31.77"	1255
8	<i>B. bungei</i>	Hebei Dahaituo Nature Reserve, Dahaituo	40°34'51.62"	115°46'32.92"	1255
9	<i>B. pekinense</i>	Hebei Dahaituo Nature Reserve, Dahaituo	40°34'51.15"	115°46'31.90"	1255
10	<i>B. bungei</i>	Hebei Dahaituo Nature Reserve, Dahaituo	40°34'50.85"	115°46'30.85"	1255
11	<i>B. pekinense</i>	Beijing Songshan Nature Reserve, Beigou	40°31'18.92"	115°46'59.96"	901
12	<i>B. pekinense</i>	Beijing Songshan Nature Reserve, Beigou	40°31'20.49"	115°46'59.21"	910
13	<i>B. pekinense</i>	Beijing Songshan Nature Reserve, Beigou	40°31'20.88"	115°46'59.08"	911
14	<i>B. pekinense</i>	Beijing Songshan Nature Reserve, Beigou	40°31'23.05"	115°46'58.45"	917
15	<i>B. pekinense</i>	Beijing Songshan Nature Reserve, Beigou	40°31'31.46"	115°47'02.07"	946
16	<i>B. pekinense</i>	Beijing Songshan Nature Reserve, Beigou	40°31'32.84"	115°47'05.10"	954
17	<i>B. pekinense</i>	Beijing Songshan Nature Reserve, Beigou	40°31'35.67"	115°47'08.52"	955
18	<i>B. pekinense</i>	Beijing Songshan Nature Reserve, Beigou	40°31'35.83"	115°47'10.02"	968
19	<i>B. pekinense</i>	Beijing Songshan Nature Reserve, Beigou	40°31'37.74"	115°47'11.53"	970
20	<i>B. pekinense</i>	Beijing Songshan Nature Reserve, Beigou	40°31'41.26"	115°47'12.68"	984
21	<i>B. pekinense</i>	Beijing Songshan Nature Reserve, Beigou	40°31'41.65"	115°47'12.42"	985
22	<i>B. pekinense</i>	Beijing Songshan Nature Reserve, Beigou	40°31'42.03"	115°47'12.37"	986
23	<i>B. pekinense</i>	Beijing Songshan Nature Reserve, Beigou	40°31'43.76"	115°47'12.11"	988
24	<i>B. pekinense</i>	Beijing Songshan Nature Reserve, Beigou	40°31'44.61"	115°47'10.39"	989
25	<i>B. pekinense</i>	Beijing Songshan Nature Reserve, Beigou	40°31'44.69"	115°47'10.83"	990
26	<i>B. bungei</i>	Beijing Songshan Nature Reserve, Gonglu	40°30'49.82"	115°46'54.29"	853
27	<i>B. pekinense</i>	Beijing Songshan Nature Reserve, Gonglu	40°30'47.27"	115°46'58.73"	845
28	<i>B. pekinense</i>	Beijing Songshan Nature Reserve, Gonglu	40°30'44.18"	115°47'05.61"	816
29	<i>B. pekinense</i>	Beijing Songshan Nature Reserve, Gonglu	40°30'40.31"	115°47'08.10"	815
30	<i>B. pekinense</i>	Beijing Songshan Nature Reserve, Gonglu	40°30'37.28"	115°47'14.20"	801
31	<i>B. pekinense</i>	Beijing Songshan Nature Reserve, Gonglu	40°30'36.02"	115°47'20.71"	799
32	<i>B. pekinense</i>	Beijing Songshan Nature Reserve, Gonglu	40°30'27.07"	115°47'43.95"	798
33	<i>B. pekinense</i>	Beijing Songshan Nature Reserve, Gonglu	40°30'25.95"	115°47'48.30"	790

Sample	Species	Locality	Northern Latitude	East Longitude	Altitude (m)
34	<i>B. pekinense</i>	Beijing Songshan Nature Reserve, Gonglu	40°30'21.01"	115°47'55.38"	788
35	<i>B. pekinense</i>	Beijing Songshan Nature Reserve, Gonglu	40°30'21.30"	115°48'04.74"	780
36	<i>B. pekinense</i>	Beijing Songshan Nature Reserve, Gonglu	40°30'17.18"	115°48'08.58"	711
37	<i>B. pekinense</i>	Beijing Songshan Nature Reserve, Gonglu	40°30'12.30"	115°48'16.98"	706
38	<i>B. pekinense</i>	Beijing Songshan Nature Reserve, Gonglu	40°30'06.48"	115°48'40.51"	682
39	<i>B. bungei</i>	Beijing Songshan Nature Reserve, Cunqian	40°31'06.19"	115°46'40.67"	912
40	<i>B. bungei</i>	Beijing Songshan Nature Reserve, Cunqian	40°31'06.20"	115°46'45.13"	899
41	<i>B. bungei</i>	Beijing Songshan Nature Reserve, Cunqian	40°30'59.11"	115°46'48.49"	878
42	<i>B. bungei</i>	Beijing Songshan Nature Reserve, Cunqian	40°30'58.89"	115°46'48.64"	875
43	<i>B. bungei</i>	Beijing Songshan Nature Reserve, Cunqian	40°30'56.52"	115°46'49.33"	873
44	<i>B. bungei</i>	Beijing Songshan Nature Reserve, Cunqian	40°30'55.78"	115°46'50.72"	870
45	<i>B. pekinense</i>	Beijing Songshan Nature Reserve, Cunqian	40°30'55.35"	115°46'51.64"	869
46	<i>B. bungei</i>	Beijing Songshan Nature Reserve, Yudushan	40°32'39.26"	115°53'45.44"	825
47	<i>B. bungei</i>	Beijing Songshan Nature Reserve, Yudushan	40°32'34.27"	115°53'48.97"	824
48	<i>B. pekinense</i>	Beijing Songshan Nature Reserve, Yudushan	40°32'34.27"	115°53'48.97"	824
49	<i>B. pekinense</i>	Beijing Songshan Nature Reserve, Yudushan	40°32'34.27"	115°53'48.97"	824
50	<i>B. pekinense</i>	Beijing Songshan Nature Reserve, Yudushan	40°32'33.15"	115°53'53.35"	804
51	<i>B. bungei</i>	Beijing Songshan Nature Reserve, Yudushan	40°32'32.92"	115°53'55.68"	800
52	<i>B. pekinense</i>	Beijing Songshan Nature Reserve, Yudushan	40°32'32.55"	115°54'3.40"	791
53	<i>B. pekinense</i>	Beijing Songshan Nature Reserve, Yudushan	40°32'52.64"	115°53'37.97"	839
54	<i>B. bungei</i>	Beijing Songshan Nature Reserve, Yudushan	40°33'0.15"	115°53'28.29"	845
55	<i>B. pekinense</i>	Beijing Songshan Nature Reserve, Yudushan	40°33'0.15"	115°53'28.29"	845
56	<i>B. pekinense</i>	Beijing Songshan Nature Reserve, Yudushan	40°33'1.58"	115°53'26.10"	846
57	<i>B. bungei</i>	Beijing Songshan Nature Reserve, Yudushan	40°33'1.58"	115°53'26.10"	846
58	<i>B. bungei</i>	Beijing Songshan Nature Reserve, Yudushan	40°33'3.68"	115°53'25.37"	848
59	<i>B. pekinense</i>	Beijing Songshan Nature Reserve, Yudushan	40°33'3.68"	115°53'25.37"	848
60	<i>B. pekinense</i>	Beijing Songshan Nature Reserve, Yudushan	40°33'8.49"	115°53'21.65"	852
61	<i>B. bungei</i>	Beijing Songshan Nature Reserve, Yudushan	40°33'8.49"	115°53'21.65"	852
62	<i>B. bungei</i>	Beijing Songshan Nature Reserve, Yudushan	40°33'7.38"	115°53'6.63"	868
63	<i>B. pekinense</i>	Beijing Songshan Nature Reserve, Yudushan	40°33'7.32"	115°52'46.51"	883
64	<i>B. pekinense</i>	Beijing Songshan Nature Reserve, Yudushan	40°33'7.63"	115°52'44.00"	893
65	<i>B. bungei</i>	Beijing Songshan Nature Reserve, Yudushan	40°33'9.53"	115°52'41.23"	902

## Appendix B

The sequences of nrITS and *psbA-trnH* for *B. trichophyllus* 1 and 2, *B. aquatilis* 1 and 2, and *R. japonicas* are downloaded from NCBI with the following numbers: KC620479, KC620495, KC620480, KC620496, KC620484, KC620498, KC620485, KC6204991, AY680164, and AB244028.

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