

Supplementary tables, figures and alignment data:

Morphological and phylogenetic analyses reveal the taxonomic distinctiveness between *Nymphaea pygmaea* and *N. tetragona* (Nymphaeaceae)

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Table S1 Primers used for amplification and sequencing in the present study

Designation	Sequence (5' to 3')	Tm (°C) /extension (s)	References
ITS			
ITS5 ¹	GGAAGTAAAAGTCGTAACA	53/30	White et al. (1990)
ITS4 ²	TCCTCCGCTTATTGATATGC		White et al. (1990)
<i>rps4-trnF</i> region			
trnL(UAA)5 exonC ¹	CGAAATCGGTAGACGCTACG	52/60	Taberlet et al. (1991)
trnF F ²	ATTTGAACTGGTGACACGAG		Taberlet et al. (1991)
rps4-5R ¹	AGGCCCTCGGTAACGSG	52/60	Sauquet et al. (2003)
trnL110R ²	GATTTGGCTCAGGATTGCCC		Borsch et al. (2003)
Nt_rps4-trnF_F1 ¹	CTTCTTCTTATAACTGGCAAGG	52/60	Newly designed
Nt_rps4-trnF_R1 ²	ATTGAGAACACAAGACTTAGCT		Newly designed
Nt_rps4-trnF_F2 ¹	CTTAACGCCTATGTAGTTACTA		Newly designed

¹ Forward primer² Reverse primer

Table S2 Accession numbers of nrDNA [internal transcribed spacer (ITS) region] and cpDNA (*rps4-trnF* spacer) sequences from GenBank

Taxon	Country	Accession no.		Ribotype and haplotype		Reference
		ITS	<i>rps4-trnF</i>	ITS	<i>rps4-trnF</i>	
Subgenus						
<i>Nymphaea</i>						
<i>Nymphaea alba</i>	Germany	HG518071	AM422066	nrN5	cpN5	Borsch et al. 2014
<i>N. candida</i>	Russia	HG518075	AM422070	nrN5	cpN6	Borsch et al. 2014
<i>N. leibergii</i>	Canada	HG518084	LN590015	nrN6	cpN7	Borsch et al. 2014
<i>N. mexicana</i>	USA	AY771813	AM422071	nrN7	cpN8	Woods et al. 2007
	USA	AY771814	AM422072	nrN8	cpN9	Woods et al. 2007
<i>N. odorata</i> subsp. <i>odorata</i>	USA	AY771821	LN590036	nrN9	cpN10	Woods et al. 2007
<i>N. odorata</i> subsp. <i>tuberosa</i>	USA	AY771848	AM422073	nrN10	cpN11	Woods et al. 2007
	USA	AY771853	LN590031	nrN11	cpN12	Woods et al. 2007
<i>N. pygmaea</i>	Unknown ¹	HG518076	LN590038	nrN12	cpN1	Borsch et al. 2014
	India	FJ597745	-	nrN13	-	Dakhr et al. 2010
<i>N. tetragona</i>	Canada	HG518081	LN590041	nrN3	cpN3	Borsch et al. 2014
Subgenus						
<i>Hydrocallis</i>						
<i>N. amazonum</i>	Mexico	FR717598	AM422026	-	cpN13	Borsch et al. 2011
<i>N. jamesoniana</i>	Ecuador	FR717597	AM422033	nrN14		Borsch et al. 2011
<i>N. lingulata</i>	Guyana	HG518070	AM422031	nrN15		Borsch et al. 2014
<i>N. novogranatensis</i>	Mexico	FM242154	AM422034	-	cpN14	Borsch et al. 2009

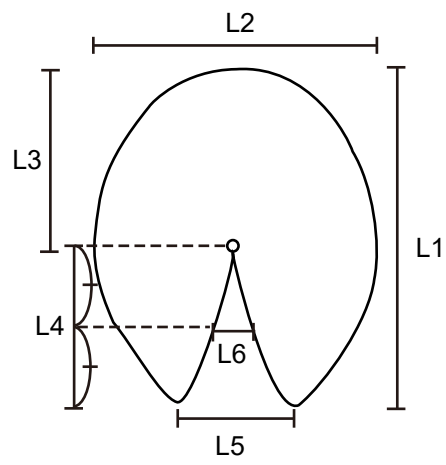
¹ From cultivation

Table S3 Loadings of 13 morphological characters for the first three factors from principal component analysis of 40 populations of *Nymphaea*. Arcsine transformations were performed for L9 and F13

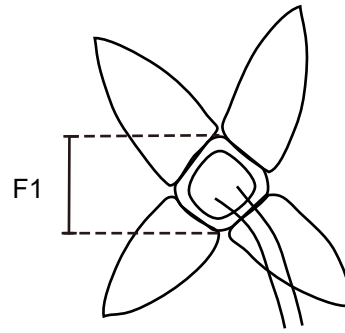
Characteristics	PCA1	PCA2	PCA3
L3	0.9155	-0.0411	-0.0217
L5	0.7304	0.4777	-0.1923
L7	0.5873	0.2647	0.6526
L8	0.7900	-0.0254	0.4813
L9	0.2083	0.7085	0.3863
L10	0.1806	0.6749	-0.3078
L11	0.7728	-0.0434	0.0786
F2	0.7512	0.3071	-0.2962
F3	0.8553	-0.2972	-0.2184
F4	0.2983	-0.8225	0.0219
F7	0.4374	-0.6244	-0.0304
F9	0.7035	0.3180	-0.3763
F13	0.4832	-0.7541	-0.0167
Eigenvalue	5.3184	3.1785	1.2237
Variance (%)	40.911	24.45	9.413
Cumulative % of variance	40.911	65.361	74.774

Table S4 Variable sites of four haplotypes of *rps4-trnF* region in sect. *Chamaenymphaea* (*Nymphaea pygmaea*, *N. tetragona*, and *N. leibergii*). Numbers in parentheses after the sequence represent the number of samples

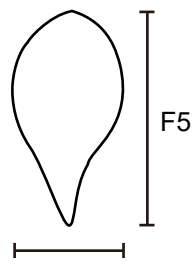
Species	Haplotype	1	2	3	1	1
		6	0	8	2	4
		4	5	8	3	7
<i>Nymphaea pygmaea</i>	cpN1 (34)	-	G	G	A	A
<i>N. pygmaea</i>	cpN2 (1)	C	T	G	-	A
<i>N. tetragona</i>	cpN3 (4)	C	T	G	A	A
<i>N. tetragona</i>	cpN4 (3)	C	T	G	A	C
<i>N. leibergii</i>	cpN6 (1)	C	T	A	A	A



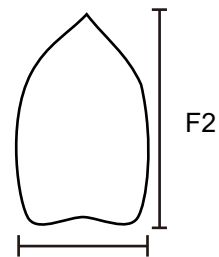
leaf



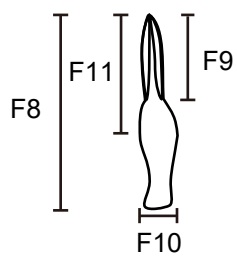
receptacle



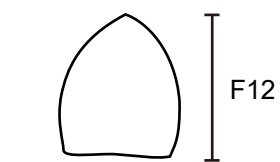
petal



sepal



stamen



carpellary appendage

Fig. S1 Morphological characteristics measured for comparison.

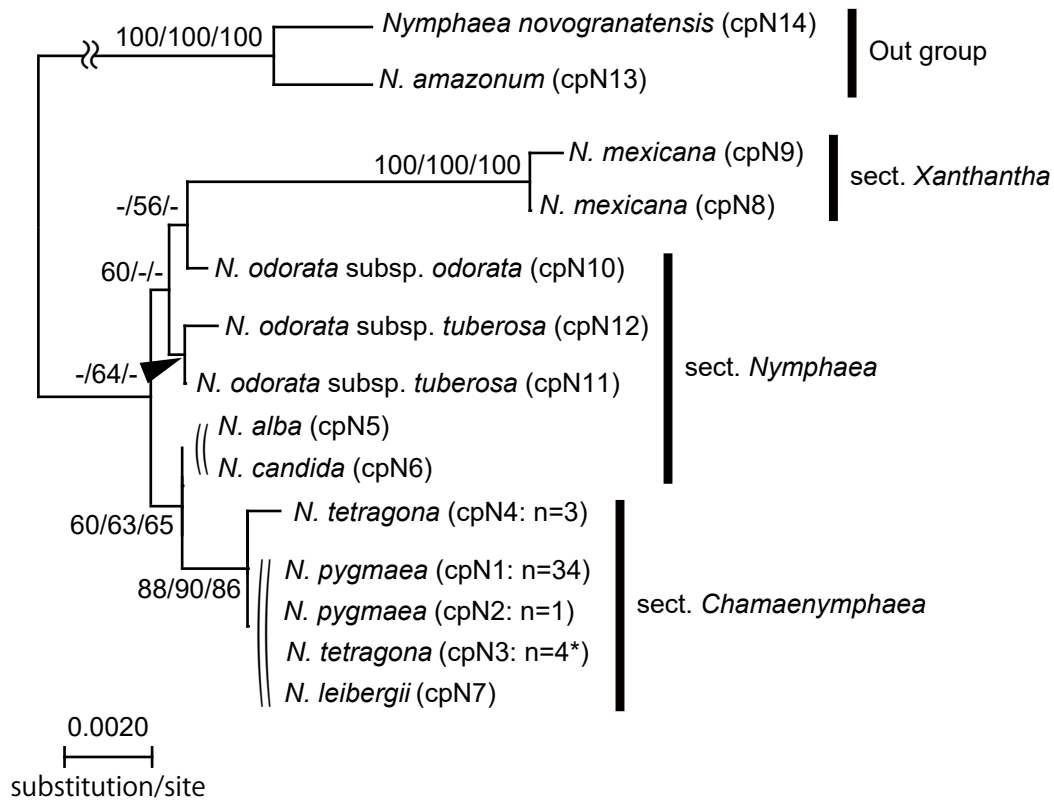


Fig. S2 Neighbor-joining (NJ) tree based on 1,401 aligned characters of chloroplast DNA (*rps4-trnF* region) of 13 haplotypes representing 10 *Nymphaea* taxa. Bootstrap values (>50 %) of each tree (maximum likelihood [ML], NJ, maximum parsimony [MP]) are shown above or under the branches. Samples representing identical sequences in 1,401 characters of alignment matrix were treated as a single operational taxonomic unit. The classification of sections given by Wiersema (1996) are indicated on the right side of each clade. *: Including three samples from this study and one sequence from GenBank

Supplementary Information 1 Alignment data of internal transcribed spacers (ITS 1 and 2) and 5.8S rRNA regions

>FR717597_N_jamesoniana

C-----CCTCATGCGGAGGGGAGCATCCGCGCCTTCACACGGTGTGGTGCTTTCCTTTGCTACT-GGCTTTTGCCTTTGC
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CGCCAAGGAACTTTT--TGATTGAAAGGAGAGGGCATCCCCCACACGAAAGTGTGGGTGGAGATATGCC-ATTTCCCCTTCC
ATT-ATAACGACTCTCGGCAACGGATATCTTGGCTCCCGCCACGATGAAGAACGTAGCGAAATGCGATACTTGGTGTGAATTG
CAGAATCCCGTGAATCATCGAGTTTTTGAACGCAAGTTGCGTCCGAGGCCATTTGGCTAAGGGCACGCCTGCCTGGGCGTCAA
GCTTCGCATCGCCCTTCCCATGTCTTCTGCTTGCGAGC-AAAGGCTGGGGTAGAGCGGAGGACTGGCCTTCGGCGTCGGCCT
TTGAGGCGCGCCGTTGGCTGAAACATTCCGGGCTTACGACGATCAGTTGATGGGCGCAACAAGCGGTGGATTTCCCACTGA--
-GTTGTGCCTCAGCTGATCGAGAAGGCCTACGGGACTCTTGAGGCAAG--CGGCTTAGGTT

>HG518070_N_lingulata

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CGCCAAGGAACTTTT--TGATTGAAAGGAGAGGGCATCCCGCTCACGAAAGTGTGGGTGGAGACATCCC-TTTTCCCCTTCC
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GCTTCGCATCGCCCTTCCCATGTCTTTTGTTCGGGCTTAAGGCTGAGGTAGAGCGGAGGACTGGCCTTCGGCGT-GAGCC
TTCAGGCGCGCCGTTGGTTGAAACATTTT-GGCTTACGACGATTGGTTGTTGGGCACGCTAAGCGGTGGATTTGCCAGTGA--
-GTTGTGCCTCAGCTGATCGAGAAGGCCCATGGGACTCTTGAGGCAAG--CGGCTTAGGTT

>AY771813_N_mexicana

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TTTTGCCATCC-CCTTTACTGTGGT--CGATGGTTTGAGC-GGCGTATTGCCAA---AACATAAAACCGGCGCTTTTAAG
CGTCAAGGATCATTGATTGAATGAAAG--GGGGACATCCTGCCAC--AAATGAGTGGGGGGAGAAGTGCC-CCTTTGCCTTCT
AATAAGAACGACTCTCGGCAACGGATATCTTGGCTCCCGTCACGATGAAGAACGTAGCGAAATGCGATAGTTGGTGTGAATTG
CAGAATCCCGTGAATCATCGAGTTTTTGAACGCAAGTTGCGCCCGAGGCCATTCGGCCAAGGGCACGTCTGCCTGGGCGTCAC
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----TCGGCGTCGTCGGCTGAAACTTTT--GGCTC---GCGATCTGTTGTGCGGCACAACAAGCGGTGGATTT-CCAGTGAGT
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GCTTAGCGTCGCCCTCCCCAGGTCTCTGTGTTTTCGAAC-CGAGGCCGAGGGAGAGCGGAGGACTGGCCTTCGGTGTCTG--CT
TTCRTCGGCGTCGTGGCTGAAACTTTC--GGCTC---ACGATCTGTTGTGCRGCACAACAAGCGGTGGATTT-CCAGTGAGT
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>AY771821_N_odorata_subsp_odorata

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AACAAAGAACGACTCTCGGCAACGGATATCTTGGCTCCTGTACGATGAAGAACGTAGCGAAATGCGATAGTTGGTGTGAATTG
CAGAATCCCGTGAATCATCGAGTTTTTTGAACGCAAGTTGCGCCCGAGGCCATTTCGGCCAAGGGGCACGTCTGCCTGGGCGTCAC
GCTTAGCGTCGCCCTCCCCAGGTCTCTGTGTTTTCGAAC-CGAGGCCGAGGGAGAGCGGAGGACTGGCCTTCGGTGTCTG--CT
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>FJ597745_N_pygmaea

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>LC775176_N_pygmaea

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>HG518084_N_leibergii

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>LC775185_N_tetragona

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Supplementary Information 2 Alignment data of *rps4-trnF* region

>AM422034_N_novogranatensis

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>AM422026_N_amazonu

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GGCACTTTTTTCCGATATCGGATCGGTATTTAATGAATTGAATGGTTCTAAC---AGGAAGGAAAGAGGGGAATGGAATCACAATAGGA
TCCCGGCCTCAAAAGAAAGGGGGATATGGCGAAATTGGTAGACGCTACGGACTTGATTGAATTGAGCCTTGGTATGGAAACCTACTAAGT
GGTAACTTCCAAATTCAGAGAAACCCTGGAATTAATAATGGGCAATCCTGAGCCAAATCCTGTTTACAGAAAAACAAGGGTTCCTTTCCTA
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ATAATTAGTTCATAACTCTTGTGTTCTGAATCGATCCCAATTTGAAAGAAAAATAAAATATTTCAGTGAGAAAAATCATTCACTCCAGAGTA
TAAGGCTGGGAGAGAAATGACTGATCGAACGAGAATAAAGATAGAGTCCATTCTACCTGTCAATGCTGACAACAATGCAATTTGTTTGT
AGTAGGAGGAAAAATCCGTCGACTTTAGAAATCGTGAGGGTTCGAGTCCCTCTATCCCCAATAAATGCCTAGTTGACGACCTAAGCATTTA
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