

# Character-based DNA barcoding for authentication and conservation of IUCN Red listed threatened species of genus *Decalepis* (Apocynaceae)

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CIMAP Communication Number: CIMAP/PUB/2016/85

## Supplementary information

**Figure S1** Morphological view of: (a - b) *Decalepis arayalpathra* fresh roots in wild (c) Raw drug samples of *D. arayalpathra* roots in markets (d) *D. hamiltonii* fresh roots in wild (e) *D. hamiltonii* dried roots in wild (f) Raw drug samples of *D. hamiltonii* roots in markets (g) *D. salicifolia* fresh roots in wild (h) *Hemidesmus indicus* fresh roots in wild (i) Raw drug samples of *H. indicus* roots in markets (j) Raw drug samples of *H. indicus* stems in markets.

**Figure S2** Relative distribution of intra-specific and inter-specific distances for all the single and combination of favourable barcodes in *Decalepis*; x-axes relate to K2P distances arranged in intervals, and the y-axes correspond to the percentage of occurrences.

**Figure S3** Maximum parsimony trees constructed using candidate barcode markers showing species resolution rates in terms of single locus. Bootstrap support values below 70% are not shown.

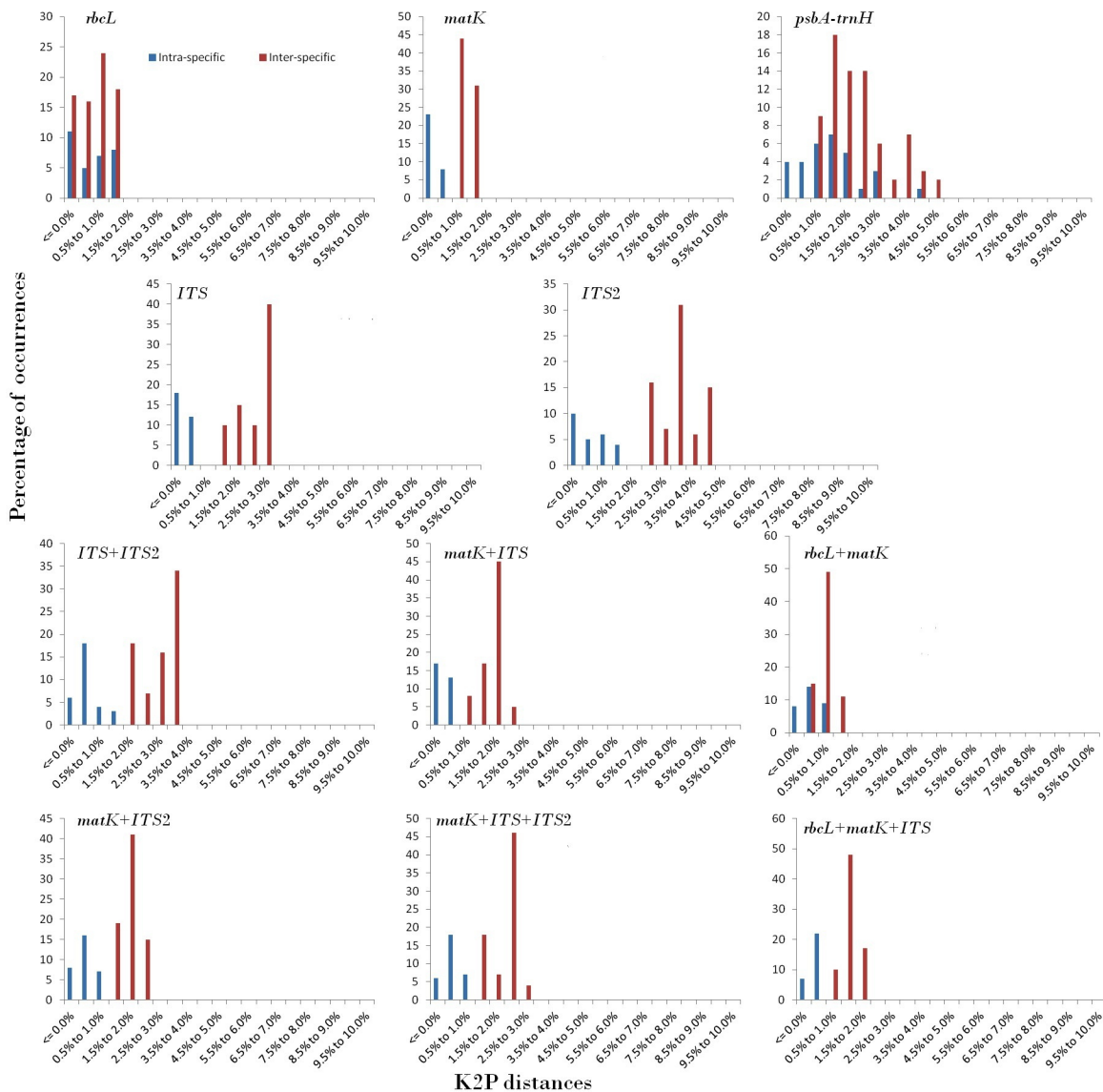
**Table S1** Primers used for amplification and sequencing for DNA barcoding of *Decalepis* species.

**Table S2** Mean and maximum intra-specific and nearest neighbor (NN) distance for all the species using candidate barcodes.

**Table S3** Genetic distance (%) measured using Kimura 2-parameter model for the candidate barcode loci and their combinations.

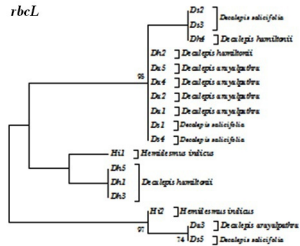
**Table S4** Identification success rates based on analysis of the 'Best Match', 'Best Close Match' and 'All Species Barcodes' function of TaxonDNA software for each DNA barcoding marker and its combinations.





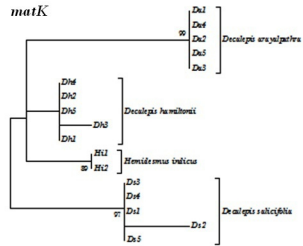
**K2P distances**

*rbcL*



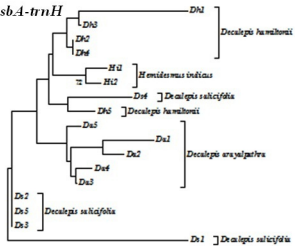
0.001

*matK*



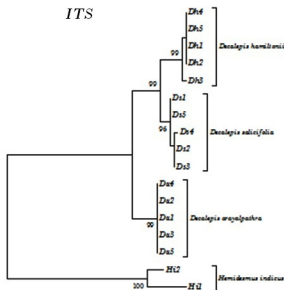
0.001

*psbA-trnH*



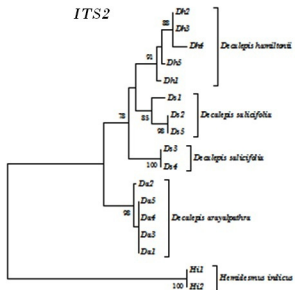
0.015

*ITS*



0.001

*ITS2*



0.001

**Table S1. Primers used for amplification and sequencing for DNA barcoding of *Decalepis* species.**

Region	Primer name	Sequence (5'-3')	Thermal cycling conditions	Mean size	Reference
<i>rbcL</i>	1F	5'-ATGTCACCACAAACAGAAAC-3'	95°C 2 min; [35 cycles: 94°C 1 min; 55°C 30 s; 72°C 1 min]; 72°C 7 min	715 bp	25
	724R	5'-TCGCATGTACCTGCAGTAGC-3'			
<i>matK</i>	390F	5'-CGATCTATTCATTCAATATTTC-3'	95°C 2 min; [30 cycles: 94°C 1 min; 48°C 30 s; 72°C 1 min]; 72°C 7 min	856 bp	70
	1326R	5'-TCTAGCACACGAAAGTCGAAAGT-3'			
<i>psbA-trnH</i>	<i>psbAF</i>	5'-GTTATGCATGAACGTAATGCTC-3'	94°C 5 min; [35 cycles: 94°C 1 min; 55°C 30 s; 72°C 1.5 min]; 72°C 7 min	400 bp	71
	<i>trnHR</i>	5'-CGCGCATGGTGGATTACAATCC-3'			
<i>ITS</i>	<i>ITS5a</i>	5'-CCTTATCATTAGAGGAAGGAG-3'	94°C 5 min; [30 cycles: 94°C 1 min; 50°C 1 min; 72°C 1.5 min]; 72°C 7 min	704 bp	25
	<i>ITS4</i>	5'- ATGCGATACTGGTGTGAAT -3'			
<i>ITS2</i>	<i>S2F</i>	5'-TCCTCCGCTTATTGATATGC-3'	94°C 5 min; [40 cycles: 94°C 30 sec; 56°C 30 sec; 72°C 45 sec]; 72°C 10 min	450 bp	53
	<i>S3R</i>	5'- GACGCTTCTCCAGACTACAAT-3'			

**Table S2. Mean and maximum intra-specific and nearest neighbor (NN) distance for all the species using candidate barcodes.**

Barcode	Species	Mean intra-specific distance	Max intra-specific distance	Nearest Neighbour	Distance to NN
<i>rbcL</i>	<i>Decalepis arayalpathra</i>	0.48	1.19	<i>D. salicifolia</i>	0
	<i>Decalepis hamiltonii</i>	0.51	0.89	<i>D. salicifolia</i>	0
	<i>Decalepis salicifolia</i>	0.57	1.34	<i>D. arayalpathra</i>	0
	<i>Hemidesmus indicus</i>	0.89	0.89	<i>D. salicifolia</i>	0.15
<i>matK</i>	<i>Decalepis arayalpathra</i>	0	0	<i>D. hamiltonii</i>	0.8
	<i>Decalepis hamiltonii</i>	0.05	0.13	<i>H. indicus</i>	0.4
	<i>Decalepis salicifolia</i>	0.11	0.27	<i>D. hamiltonii</i>	0.67
	<i>Hemidesmus indicus</i>	0	0	<i>D. hamiltonii</i>	0.4
<i>psbA-trnH</i>	<i>Decalepis arayalpathra</i>	0.97	1.62	<i>D. salicifolia</i>	0.8
	<i>Decalepis hamiltonii</i>	1.02	1.16	<i>D. salicifolia</i>	0.8
	<i>Decalepis salicifolia</i>	1.63	4.11	<i>D. hamiltonii</i>	0.8
	<i>Hemidesmus indicus</i>	0.53	0.53	<i>D. hamiltonii</i>	0.8
<i>ITS</i>	<i>Decalepis arayalpathra</i>	0	0	<i>D. salicifolia</i>	2.47
	<i>Decalepis hamiltonii</i>	0.12	0.3	<i>D. salicifolia</i>	1.37
	<i>Decalepis salicifolia</i>	0.15	0.3	<i>D. hamiltonii</i>	1.37
	<i>Hemidesmus indicus</i>	2.15	2.15	<i>D. arayalpathra</i>	11.67
<i>ITS2</i>	<i>Decalepis arayalpathra</i>	0.1	0.25	<i>D. hamiltonii</i>	3.08
	<i>Decalepis hamiltonii</i>	0.6	1.27	<i>D. salicifolia</i>	2.03
	<i>Decalepis salicifolia</i>	1.57	2.29	<i>D. hamiltonii</i>	2.03
	<i>Hemidesmus indicus</i>	0	0	<i>D. arayalpathra</i>	10.69

**Table S3. Genetic distance (%) measured using Kimura 2-parameter model for the candidate barcode loci and their combinations.**

<b>Barcode locus/loci</b>	<b>Range of Intra-specific distances (<math>\pm</math>S.D.)</b>	<b>Range of Inter-specific distances (<math>\pm</math>S.D.)</b>
<i>rbcL</i>	0.004 ( $\pm$ 0.001) – 0.008 ( $\pm$ 0.003)	0.004 ( $\pm$ 0.001) – 0.009 ( $\pm$ 0.003)
<i>matK</i>	0.000 ( $\pm$ 0.000) – 0.001 ( $\pm$ 0.000)	0.004 ( $\pm$ 0.002) – 0.013 ( $\pm$ 0.004)
<i>psbA-trnH</i>	0.005 ( $\pm$ 0.003) – 0.016 ( $\pm$ 0.004)	0.015 ( $\pm$ 0.005) – 0.021 ( $\pm$ 0.006)
<i>ITS</i>	0.000 ( $\pm$ 0.000) – 0.215 ( $\pm$ 0.005)	0.015 ( $\pm$ 0.004) – 0.132 ( $\pm$ 0.015)
<i>ITS2</i>	0.000 ( $\pm$ 0.000) – 0.015 ( $\pm$ 0.004)	0.025 ( $\pm$ 0.006) – 0.122 ( $\pm$ 0.019)
<i>rbcL+ matK</i>	0.002 ( $\pm$ 0.000) – 0.004 ( $\pm$ 0.001)	0.006 ( $\pm$ 0.002) – 0.009 ( $\pm$ 0.002)
<i>rbcL+ psbA-trnH</i>	0.006 ( $\pm$ 0.001) – 0.009 ( $\pm$ 0.001)	0.010 ( $\pm$ 0.002) – 0.013 ( $\pm$ 0.003)
<i>rbcL+ITS</i>	0.002 ( $\pm$ 0.000) – 0.015 ( $\pm$ 0.003)	0.011 ( $\pm$ 0.002) – 0.066 ( $\pm$ 0.007)
<i>rbcL+ITS2</i>	0.003 ( $\pm$ 0.001) – 0.009 ( $\pm$ 0.002)	0.013 ( $\pm$ 0.003) – 0.047 ( $\pm$ 0.006)
<i>matK+ psbA-trnH</i>	0.001 ( $\pm$ 0.001) – 0.006 ( $\pm$ 0.001)	0.008 ( $\pm$ 0.002) – 0.015 ( $\pm$ 0.003)
<i>matK+ITS</i>	0.000 ( $\pm$ 0.000) – 0.009 ( $\pm$ 0.002)	0.011 ( $\pm$ 0.003) – 0.061 ( $\pm$ 0.006)
<i>matK+ITS2</i>	0.000 ( $\pm$ 0.000) – 0.006 ( $\pm$ 0.001)	0.013 ( $\pm$ 0.003) – 0.043 ( $\pm$ 0.006)
<i>psbA-trnH+ITS</i>	0.003 ( $\pm$ 0.001) – 0.015 ( $\pm$ 0.004)	0.017 ( $\pm$ 0.003) – 0.087 ( $\pm$ 0.009)
<i>psbA-trnH+ITS2</i>	0.002 ( $\pm$ 0.001) – 0.015 ( $\pm$ 0.003)	0.023 ( $\pm$ 0.004) – 0.067 ( $\pm$ 0.009)
<i>ITS+ITS2</i>	0.000 ( $\pm$ 0.000) – 0.013 ( $\pm$ 0.003)	0.019 ( $\pm$ 0.004) – 0.128 ( $\pm$ 0.011)
<i>rbcL+ matK+ psbA-trnH</i>	0.003 ( $\pm$ 0.000) – 0.005 (0.001)	0.008 ( $\pm$ 0.002) – 0.011 ( $\pm$ 0.002)
<i>rbcL+ matK+ITS</i>	0.001 ( $\pm$ 0.000) – 0.009 ( $\pm$ 0.002)	0.010 ( $\pm$ 0.002) – 0.044 ( $\pm$ 0.004)
<i>rbcL+ matK+ITS2</i>	0.001 ( $\pm$ 0.000) – 0.005 ( $\pm$ 0.001)	0.011 ( $\pm$ 0.002) – 0.030 ( $\pm$ 0.004)
<i>rbcL+ psbA-trnH+ITS</i>	0.003 ( $\pm$ 0.000) – 0.012 ( $\pm$ 0.002)	0.013 ( $\pm$ 0.002) – 0.054 ( $\pm$ 0.005)
<i>rbcL+ psbA-trnH+ITS2</i>	0.004 ( $\pm$ 0.001) – 0.011 ( $\pm$ 0.001)	0.015 ( $\pm$ 0.003) – 0.039 ( $\pm$ 0.005)
<i>rbcL+ITS+ITS2</i>	0.002 ( $\pm$ 0.000) – 0.011 ( $\pm$ 0.002)	0.014 ( $\pm$ 0.002) – 0.078 ( $\pm$ 0.006)
<i>matK+ psbA-trnH+ITS</i>	0.002 ( $\pm$ 0.000) – 0.009 ( $\pm$ 0.002)	0.013 ( $\pm$ 0.002) – 0.052 ( $\pm$ 0.005)
<i>matK+ psbA-trnH+ITS2</i>	0.001 ( $\pm$ 0.000) – 0.008 ( $\pm$ 0.001)	0.015 ( $\pm$ 0.002) – 0.038 ( $\pm$ 0.005)
<i>matK+ITS+ITS2</i>	0.000 ( $\pm$ 0.000) – 0.007 ( $\pm$ 0.001)	0.014 ( $\pm$ 0.003) – 0.074 ( $\pm$ 0.007)
<i>psbA-trnH+ITS+ITS2</i>	0.002 ( $\pm$ 0.000) – 0.011 ( $\pm$ 0.003)	0.019 ( $\pm$ 0.003) – 0.096 ( $\pm$ 0.008)
<i>rbcL+ matK+ psbA-trnH+ITS</i>	0.002 ( $\pm$ 0.000) – 0.008 ( $\pm$ 0.002)	0.011 ( $\pm$ 0.002) – 0.040 ( $\pm$ 0.004)
<i>rbcL+ matK+ psbA-trnH+ITS2</i>	0.003 ( $\pm$ 0.000) – 0.007 ( $\pm$ 0.001)	0.013 ( $\pm$ 0.002) – 0.029 ( $\pm$ 0.003)
<i>rbcL+ psbA-trnH+ITS+ITS2</i>	0.003 ( $\pm$ 0.000) – 0.010 ( $\pm$ 0.002)	0.015 ( $\pm$ 0.002) – 0.066 ( $\pm$ 0.006)
<i>rbcL+ matK+ITS+ITS2</i>	0.001 ( $\pm$ 0.000) – 0.008 ( $\pm$ 0.001)	0.012 ( $\pm$ 0.002) – 0.055 ( $\pm$ 0.004)
<i>matK+ psbA-trnH+ITS+ITS2</i>	0.001 ( $\pm$ 0.000) – 0.007 ( $\pm$ 0.001)	0.015 ( $\pm$ 0.002) – 0.063 ( $\pm$ 0.005)
<i>rbcL+ matK+ psbA-trnH+ITS+ITS2</i>	0.002 ( $\pm$ 0.000) – 0.007 ( $\pm$ 0.001)	0.013 ( $\pm$ 0.002) – 0.050 ( $\pm$ 0.004)



**Table S4. Identification success rates based on analysis of the ‘Best match’, ‘Best close match’ and ‘All species barcodes’ function of TaxonDNA software for each DNA barcoding marker and its combinations.**

Barcode locus/loci	Threshold (%)	Best match (%)			Best close match (%)				All species barcode (%)
		Successfully identified (%)	Ambiguous (%)	Misidentified (%)	Successfully identified (%)	Ambiguous (%)	Misidentified (%)	No match (%)	
<i>rbcL</i>	1.34	3 (17.64)	10 (58.52)	4 (23.52)	3 (17.64)	10 (58.52)	4 (23.52)	0 (0.00)	0.00
<i>matK</i>	0.25	17 (100)	0 (0.00)	0 (0.00)	15 (88.23)	0 (0.00)	0 (0.00)	2 (11.76)	76.47
<i>psbA-trnH</i>	4.09	16 (94.11)	0 (0.00)	1(5.88)	16 (94.11)	0 (0.00)	1 (5.88)	0 (0.00)	17.64
<i>ITS</i>	2.15	17 (100)	0 (0.00)	0 (0.00)	15 (88.23)	0 (0.00)	0 (0.00)	2 (11.76)	88.23
<i>ITS2</i>	2.27	17 (100)	0 (0.00)	0 (0.00)	17 (100)	0 (0.00)	0 (0.00)	0 (0.00)	58.82
<i>rbcL+ matK</i>	0.76	14 (82.35)	1 (5.88)	2 (11.76)	14 (82.35)	1 (5.88)	2 (11.76)	0 (0.00)	5.88
<i>rbcL+ psbA-trnH</i>	1.73	13 (76.47)	0 (0.00)	4 (23.52)	13 (76.47)	0 (0.00)	4 (23.52)	0 (0.00)	11.76
<i>rbcL+ITS</i>	1.50	17 (100)	0 (0.00)	0 (0.00)	15 (88.23)	0 (0.00)	0 (0.00)	2 (11.76)	88.23
<i>rbcL+ITS2</i>	1.69	17 (100)	0 (0.00)	0 (0.00)	17 (100)	0 (0.00)	0 (0.00)	0 (0.00)	64.7
<i>matK+ psbA-trnH</i>	1.34	17 (100)	0 (0.00)	0 (0.00)	13 (76.47)	0 (0.00)	4 (23.52)	0 (0.00)	64.7
<i>matK+ITS</i>	0.99	17 (100)	0 (0.00)	0 (0.00)	15 (88.23)	0 (0.00)	0 (0.00)	2 (11.76)	88.23
<i>matK+ITS2</i>	0.95	17 (100)	0 (0.00)	0 (0.00)	17 (100)	0 (0.00)	0 (0.00)	0 (0.00)	88.23
<i>psbA-trnH+ITS</i>	1.64	14 (82.35)	1 (5.88)	2 (11.76)	14 (82.35)	1 (5.88)	2 (11.76)	0 (0.00)	82.35
<i>psbA-trnH+ITS2</i>	3.16	13 (76.47)	0 (0.00)	4 (23.52)	13 (76.47)	0 (0.00)	4 (23.52)	0 (0.00)	58.82
<i>ITS+ITS2</i>	1.33	17 (100)	0 (0.00)	0(0.00)	15 (88.23)	0(0.00)	0 (0.00)	2 (11.76)	88.23
<i>rbcL+ matK+ psbA-trnH</i>	1.00	17 (100)	0 (0.00)	0 (0.00)	15 (88.23)	0 (0.00)	0 (0.00)	2 (11.76)	47.05
<i>rbcL+ matK+ITS</i>	0.95	17 (100)	0 (0.00)	0 (0.00)	17 (100)	0 (0.00)	0 (0.00)	0 (0.00)	88.23
<i>rbcL+ matK+ITS2</i>	0.99	17 (100)	0 (0.00)	0 (0.00)	17 (100)	0 (0.00)	0 (0.00)	0 (0.00)	76.47
<i>rbcL+ psbA-trnH+ITS</i>	1.28	17 (100)	0 (0.00)	0 (0.00)	15 (88.23)	0 (0.00)	0 (0.00)	2 (11.76)	88.23
<i>rbcL+ psbA-trnH+ITS2</i>	1.66	17 (100)	0 (0.00)	0 (0.00)	17 (100)	0 (0.00)	0 (0.00)	0 (0.00)	47.05
<i>rbcL+ITS+ITS2</i>	1.15	17 (100)	0 (0.00)	0 (0.00)	15 (88.23)	0 (0.00)	0 (0.00)	2 (11.76)	88.23
<i>matK+ psbA-trnH+ITS</i>	0.94	17 (100)	0 (0.00)	0 (0.00)	17 (100)	0 (0.00)	0 (0.00)	0 (0.00)	88.23
<i>matK+ psbA-trnH+ITS2</i>	1.58	17 (100)	0 (0.00)	0 (0.00)	17 (100)	0 (0.00)	0 (0.00)	0 (0.00)	82.35
<i>matK+ITS+ITS2</i>	0.76	17 (100)	0 (0.00)	0 (0.00)	15 (88.23)	0 (0.00)	0 (0.00)	2 (11.76)	88.23
<i>psbA-trnH+ITS+ITS2</i>	1.83	17 (100)	0 (0.00)	0 (0.00)	17 (100)	0(0.00)	0(0.00)	0(0.00)	82.35
<i>rbcL+ matK+ psbA-trnH+ITS</i>	0.88	17 (100)	0 (0.00)	0 (0.00)	15 (88.23)	0(0.00)	0(0.00)	2(11.76)	88.23

<i>rbcL+ matK+ psbA-trnH+ITS2</i>	1.09	17 (100)	0 (0.00)	0 (0.00)	17 (100)	0 (0.00)	0 (0.00)	0 (0.00)	88.23
<i>rbcL+ psbA-trnH+ITS+ITS2</i>	1.24	17 (100)	0 (0.00)	0 (0.00)	17 (100)	0 (0.00)	0 (0.00)	0 (0.00)	88.23
<i>rbcL+ matK+ITS+ITS2</i>	0.80	17 (100)	0 (0.00)	0 (0.00)	15 (88.23)	0 (0.00)	0 (0.00)	2 (11.76)	88.23
<i>matK+ psbA-trnH+ITS+ITS2</i>	1.19	17 (100)	0 (0.00)	0 (0.00)	17 (100)	0 (0.00)	0 (0.00)	0 (0.00)	88.23
<i>rbcL+ matK+ psbA-trnH+ITS+ITS2</i>	0.91	17 (100)	0 (0.00)	0 (0.00)	17 (100)	0 (0.00)	0 (0.00)	0 (0.00)	88.23

Identification analysis for each barcoding marker and its combinations was performed at their respective threshold calculated from pairwise distances.