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Descriptions of two new species of *Nepenthes* (Nepenthaceae) from Thailand and their phylogenetic analysis based on AFLP technique species confirmation

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Summary. Two new species of *Nepenthes* (Nepenthaceae) from Southern Thailand, *N. bracteosa* sp. nov. and *N. hirtella* sp. nov. are described and illustrated. Descriptions of the species are given compared to their related species, *N. krabiensis* and *N. kerrii*, collected in Thailand. They are separated from *N. krabiensis* and *N. kerrii* by characteristics of leaf shape, pitcher shape, indumentum, and bracteole. Confirmation of these two new species was determined by phylogenetic relationships using the Amplified Fragment Length Polymorphism (AFLP) technique.

Key Words. monkey cup, phylogenetic relationship, pitcher plant, taxonomy.

Introduction

The genus *Nepenthes*, known as the tropical pitcher plant or monkey cup, is in the family Nepenthaceae (order Caryophyllales). The first encounter with *Nepenthes* in the mid-17th century focused on its taxonomy, diversity and distribution. The *Nepenthes* flora of Indochina and the Philippines, in particular, have attracted much recent attention (McPherson 2009; McPherson 2012). *Nepenthes* comprises approximately 160 species, with the greatest diversity with many endemic species, in the Philippines and Borneo. Several taxa have been recently described from Indochina: *N. bokorensis* Mey (Mey 2009), *N. thai* Cheek (Cheek & Jebb 2009), *N. holdenii* Mey (Mey *et al.* 2010), *N. mirabilis* var. *globosa* M.Catal. (Catalano 2010a), *N. suratensis* M.Catal. (Catalano 2010b), *N. andamana* M.Catal. (Catalano 2010c), *N. kerrii* M.Catal. & Kruetr. (Catalano 2010d), *N. chang* M.Catal. (Catalano 2010e), *N. rosea* M.Catal. & Kruetr. (Catalano 2014), *N. kongkandana* M.Catal. & Kruetr. (Catalano 2015), *N. krabiensis* Nuanlaong, Onsanit, Chusangr. & Sura. (Nuanlaong *et al.* 2016), *N. malayensis* A.Amin, M.N.Faizal & Dome (Tamizi *et al.* 2020), *N. latiffiana* M.N.Faizal, A.Amin & Dome and *N. domei* M.N.Faizal, A.Amin & A.Latif (Ghazalli *et al.* 2020). In Thailand, 14 species and one variety of *Nepenthes* are currently recognised. Of these, nine species including our new species are considered to be closely related and they have been attributed to section *Pyrophytae* Cheek & Jebb (Cheek & Jebb 2016). Seven species in section *Pyrophytae* are found on the summit areas of mountains, namely, *N. chang* (300 m a.s.l.), *N. kerrii* (500 m a.s.l.), *N. krabiensis* (500 – 600 m a.s.l.), *N. rosea* (450 – 520 m a.s.l.), *N. smilesii* Hemsl. (16 – 1,500 m a.s.l.), *N. bracteosa* sp. nov. (710 – 760 m a.s.l.) and *N. hirtella* sp. nov. (348 – 355 m a.s.l.). While four species, *N. andamana*, *N. kampotiana* Lecomte, *N. kongkandana* and *N. suratensis* are found at sea level. Generally, plants in section *Pyrophytae* have tuberous rootstocks, which is an adaptation for fire, and are found in seasonal habitats. Members of section *Pyrophytae* lack a climbing stem and the upper pitchers (which arise after the inflorescence is first produced) are borne on stems with short internodes. The tendril of the upper pitchers lacks a coil, and the partial-peduncles are 1-flowered, with short pedicels/partial-peduncles (Cheek & Jebb 2016). During the dry season, *Nepenthes* decrease leaf, flower, and pitcher production. The tuberous rootstock is likely to act as a water and nutrient store for survival during periods of unsuitable environmental conditions (Mey *et al.* 2010).

However, the pyrophytic species are found to be closely related to section *Montanae* Danser (Danser 1928), which share most of their characteristics. Plants from section *Montanae* lack fleshy tubers, have climbing stems, coiled tendrils, and longer pedicels (Cheek & Jebb 2009). In southern Thailand near the Malaysian border, two species namely, *N. sanguinea* Lindl. and *N. thai* Cheek, have been found, which are relatives of Penisular *Montanae*, such as *N. benstonei* C.Clarke, *N. gracillima* Ridl., *N. macfarlanei* Hemsl. and *N. ramispina* Ridl. in highland Malaysia (Cheek & Jebb 2009). However, the two new species are similar in overall appearance and share many characteristics

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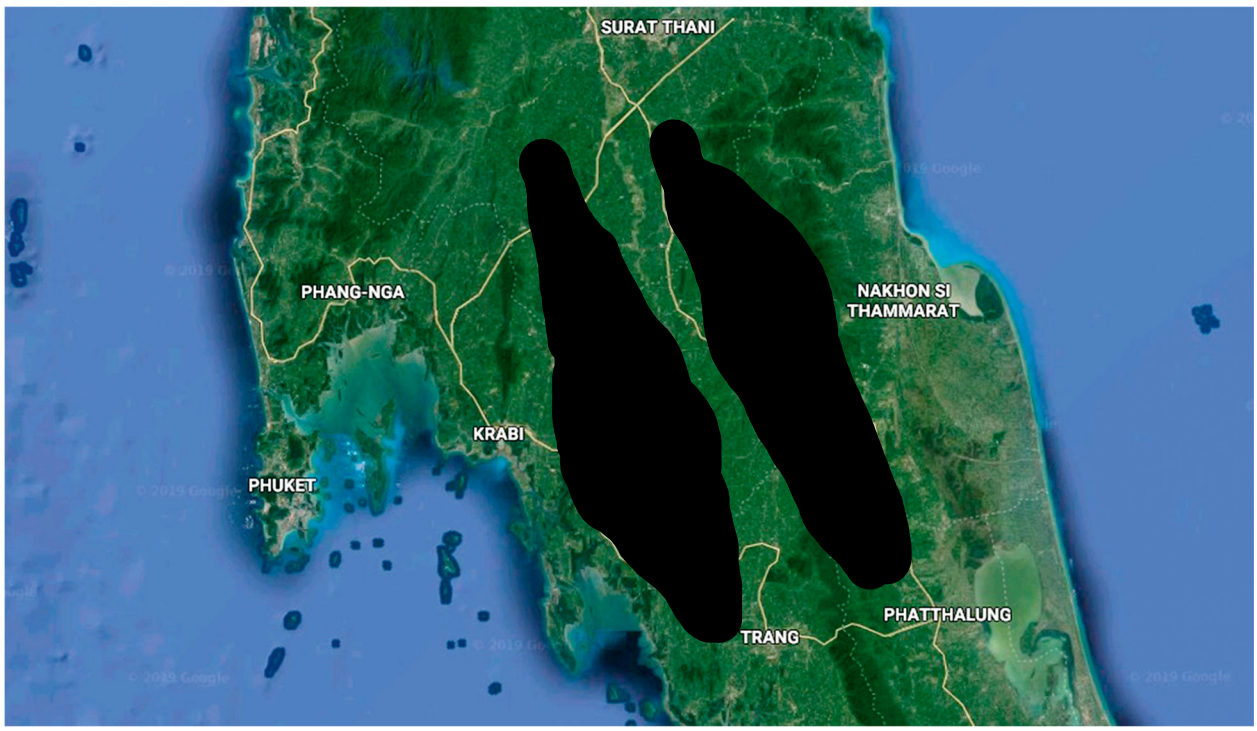
with pyrophytic species such as: long racemose inflorescence, flowering at the rosette stage, seed with reduced filiform appendages, coriaceous narrow leaves, decurrent leaf attachment, tuberous rootstock, and pyrophytic habitat. As a result, the two new species could not be classified as members of section *Montanae*.

Despite previous research, evolution within the genus *Nepenthes* is still poorly understood. Currently, research in *Nepenthes* has been focused on their actual diversity. These include biogeographical and environmental processes, and animal-plant interactions that are facilitated mostly by characteristics of pitchers and flowers (Ellison & Adamec 2018). *Nepenthes* is a cross-pollinated plant, in which the wide differential architectures of the phenotypic traits differ resulting from both genetic and environmental factors found in their habitat (Rowe & Speck 2005). Genetic analyses have revolutionised systematics and provided important insights into the understanding plant evolution. The complete genome of *Nepenthes* has not yet been published, resulting in a limited number of molecular markers available for characterisation of *Nephethes* taxa. AFLPs, a PCR-based method that selectively amplifies a subset of digested DNA fragments to produce distinctive fingerprints, is the common molecular marker used for comparing genetic differences (Paun & Schönswetter 2012). The effectiveness of AFLP analysis is derived from its capacity to rapidly produce large numbers of marker fragments for any organism without the requirement of understanding any genomic sequence. The AFLP technique has been used for various applications, including genetic mapping, genealogical studies among closely related individuals, quantification of genetic diversity within and among species, and phylogenetic studies of closely related species (Savelkoul *et al.* 1999). Thus, in this study, the two new *Nepenthes* found on the mountain tops of [redacted] (Nakhon Si Thammarat Province) and [redacted] (Krabi Province) are described and classified through their phylogenetic relationships using AFLP techniques.

Materials and Methods

Morphological analysis

Study samples of *Nepenthes* were collected during a field investigation from [redacted] Nakhon Si Thammarat Province, [redacted], and [redacted] Krabi Province, [redacted] Southern Thailand in 2017 – 2018 (Map 1). The extent of occurrence (EOO) and area of occurrence (AOO) of the *Nepenthes* populations was calculated to determine the conservation status using Geocat (Bachman *et al.* 2011). All data and dimensions were either collected in the field from live plant specimens, or by a more thorough examination using a Nikon SMZ-U Stereo Zoom Microscope with a Nikon DXM1200F digital camera and ACT-1 software (ver-



Map 1. Sample collecting sites for the *Nepenthes* species in this study in Southern Thailand. Locality **1** *N. bracteosa* and locality **2** *N. hirtella*. Map was modified from Google Maps.

sion 2.70). Morphological terminology applied for the descriptive studies follows Cheek & Jebb (2009) and Nuanlaong *et al.* (2016).

AFLP analysis and phylogenetic relationships

To determine the putative phylogenetic relationships of the new *Nepenthes* species studied, they were compared with five *Nepenthes* species distributed on mountain tops, specifically *N. chang*, *N. kerrii*, *N. krabiensis*, *N. rosea*, and *N. smilesii*. In addition, *N. thorelii* and *N. mirabilis* were used as an ingroup and an outgroup, respectively (Table 1). Each species included five replications (random plants and sex).

Total genomic DNA was isolated from fresh pitcher tissues using the methods of Suraninpong *et al.* (2015). Before analysis, DNA from five replicates was pooled in equal quantities. The AFLP analysis was conducted as described by Vos *et al.* (1995) with some modifications. Initial/first genomic DNA (300 ng) was digested with 10 units of *Eco*RI (Roche, USA) and 5 units of *Tru*9I (Roche, USA) in 1X SuRE/Cut™ Buffer A (Roche, USA) to a final volume of 30 µl for 24 h at 37°C. The mixture was added to 10 µl of ligation mix containing 50 pmol adapters of *Eco*RI and *Tru*9I, 1 unit of T4-DNA ligase (Roche, USA), and 1x ligation buffer. The ligation reaction was performed at 37°C for 12 h. Then, 5X DNA template (digestion-ligation mixture) dilution was prepared for pre-amplification (PCR I) by adding 2 µl 5X DNA template dilution with 0.25 µM of primer (1 selective base of *Eco*RI and *Tru*9I), 1X TopTaq Master Mix Kit (QIAGEN, USA) and dH_2O, in a final volume of 10 µl. The thermal conditions for PCR I were 20 cycles of 30 s at 95°C, 1 min at 56°C, 1 min at 72°C with a final extension of 7 min at 72°C (T100 Thermal Cycler Bio Red, USA). Then, the PCR I product was diluted to 10X (1 µl PCR I plus with 9 µl dH_2O) and was mixed with 0.25 µM of primer (2 or 3 selective bases of *Eco*RI and *Tru*9I), 1X TopTaq Master Mix Kit and dH_2O to a final volume of 10 µl. The PCR II was conducted with the annealing temperature reduced by 1°C every cycle. It started with 10 cycles of 30 s at 95°C, then 30 s at 65°C, and 1 min at 72°C. Continuously, 30 cycles of 30 s at 95°C, 30 s at 56°C, 1 min at 72°C, 7 min at 72°C and 4°C until the reaction was complete. Then, the reaction was stopped by adding 10 µl of stop-loading dye (10 mM EDTA pH 8.0, 98% formaldehyde, Bromophenol Blue & Xylenecyanol). The PCR product was separated on 5% (w/v) polyacrylamide gel electrophoresis. The DNA bands were visualised via silver staining and were scored manually.

The NTSYSpc (version 2.20j N) program was used for cluster analysis based on a similarity matrix. Genetic distances were obtained using the NTSYS-pc 2.20j statistic package program according to Jaccard's similarity coefficient. The matrix was analysed by the unweighted pair-group method with arithmetic mean (UPGMA) (Rohlf 1998). The relationships between species are illustrated as a dendrogram. The presence and absence of bands were scored as 1 and 0, respectively. Bands of similar size and intensity were assumed to be homologous. The polymorphism (*P*) was calculated as $P = (K/N) \times 100\%$; where *K* is the number of polymorphic bands and *N* is the total number of amplified bands. The polymorphism information content (PIC) value for each AFLP primer combination was calculated based on Roldàn-Ruiz *et al.* (2000) as $PIC_i = 2f_i(1 - f_i)$; where PIC_i is the PIC of marker *i*, f_i is the frequency of the marker bands which were present, and $1 - f_i$ is the frequency of marker bands which were absent. The final PIC value was the average of the PIC values of each marker over the bands for each primer combination. The Jaccard's similarity coefficient was computed for each pair of species (Jaccard 1908). The FreeTree program (Pavlicek *et al.* 1999) was used for the bootstrap analysis with 1,000 resampled datasets. We applied the same levels of support to the Bootstrap values as Richardson *et al.* (2000), which indicates that a value in the range of 85 – 100% means high reliability, 71 – 84% means medium reliability, and 50 – 70% means low reliability.

Table 1. Plant material examined in this study.

Species	Source
Nepenthes mirabilis (Lour.) Druce	Nakhon Si Thammarat Province, Thailand
Nepenthes thorelii Lecomte	Tay Ninh Province, Vietnam
Nepenthes chang M.Catal.	cult. Krabi Carnivores Nursery, Thailand
Nepenthes kerrii M.Catal. & Kruetr.	cult. Krabi Carnivores Nursery, Thailand
Nepenthes smilesii Hemsl.	Phu Kradueng National Park, Loei province, Thailand
Nepenthes krabiensis Nuanlaong, Onsanit, Chusangr. & Suran.	[redacted] Krabi Province, Thailand
Nepenthes rosea M.Catal. & Kruetr.	[redacted] Krabi Province, Thailand
Nepenthes bracteosa Suran. & Nuanlaong **sp. nov.**	[redacted] Nakhon Si Thammarat Province, Thailand
Nepenthes hirtella Nuanlaong & Suran. **sp. nov.**	[redacted] Krabi Province, Thailand Krabi Province, Thailand

Results and Discussion

Morphological evidence

Several morphological characters allow the new species, namely *Nepenthes bracteosa* and *N. hirtella*, to be distinguished from presently recognised species. The morphology of the two new species differs distinctly from *N. krabiensis* and *N. kerrii* as shown in Table 2.

AFLP and phylogenetic relationship

In this study, more than 50 primer combinations from *Eco*RI and *Tru*9I were examined, but only eleven primer combinations were clearly classified for polymorphic profiling on nine *Nepenthes* species (Table 3). A total of 1,415 DNA fragments scored across all primer sets were observed, out of which 1,323 (93.30%) were polymorphic, which enabled differentiation of the accessions analysed. The size of AFLP fragments generated by different primer combinations ranged from 200 to 800 bp. The highest number of polymorphisms were for ER-AC/Tru-CGT (98.64%) followed by ER-AC/Tru-CAG (94.96%) and ER-AGC/Tru- CAG (94.44%). Conversely, the lowest polymorphism at 90.65% was found on ER-AT/Tru -CAG primer combinations. Monomorphism was lower than polymorphism. Low monomorphism indicates that there is a high degree of variation between the species. The PIC value ranged from 0.71 to 0.86 with an average of 0.76 per fragment. The highest value 0.86 was obtained for the ER-AC/Tru-CGT primer combination, followed by 0.78 for ER-ATG/Tru-GAG. A strong correlation between polymorphism and PIC (r^2=0.86) was revealed.

Jaccard's similarity coefficient was found to vary from 0.22 to 0.65, with an average of 0.42, suggesting a high genetic diversity among the nine *Nepenthes* species (Table 4). The dendrogram from the cluster analysis, constructed from 11 AFLP markers, indicates that the *Nepenthes* species can be divided into two groups and two separate individual species at 0.46 of similarity coefficient with a bootstrap value of 100% (Fig. 1). This grouping was consistent with the existing morphological and geographical classification of *Nepenthes* species and was similar to previous reports of the relationships from geographical and genetic distribution by Suraninpong *et al.* (2015).

From the results, the first group contains two subgroups, the first of which is *Nepenthes rosea*, and the second subgroup is *N. krabiensis*, *N. bracteosa*, *N. kerrii* and *N. hirtella*. All species of this group were distributed throughout southern Thailand but confined to different places. *Nepenthes bracteosa* clearly showed separation from *N. krabiensis* with a similarity coefficient of 0.65 with a bootstrap value of 88%. *Nepenthes hirtella* was identified in the same related subgroup with *N. krabiensis* and *N. bracteosa*. From the result of the low similarity coefficient (0.45 – 0.65), high genetic variation among these species was discovered. In contrast, the species from North-East Thailand namely, *N. chang* and *N. smilesii* were separated into the second subgroup and were shown to have a similarity coefficient of 0.50 with a bootstrap value of 96%. The results published here suggest that all *Nepenthes* species found in Thailand are derived from a common ancestor, of which *N. thorelii* Lecomte was the first species separated from the aggregate group. Likewise, the phylogenetic relationship infor-

Table 2. Comparisons of geography and selected plant characteristics of *Nepenthes bracteosa* and *N. hirtella* with *N. krabiensis* and *N. kerrii*.

	N. krabiensis	*N. bracteosa*	*N. kerrii*	*N. hirtella*
Geography	SW Thailand, 500 – 600 m a.s.l.	SE Thailand, 710 – 760 m a.s.l.	Island, SW Thailand, 500 m a.s.l.	SW Thailand, 348 – 355 m a.s.l.
Leaf shape	lanceolate	linear to lanceolate	obovate	oblanceolate
Leaf apex	acute	acute to acuminate	acuminate	acuminate
Lid of lower pitcher	broadly ovate	broadly ovate to round	round	ovate
Spur	filiform	filiform with branches	simple or branches	filiform with branches
Male inflorescence	2-flowered, partial peduncles	1-flowered	1-flowered	1-flowered or rarely 2-flowered, partial peduncles
Androphore length (mm)	4	2.0 – 2.5	1.5	1.0 – 2.0
Bracts	male inflorescence: present at base or lower half of the pedicel female inflorescence: absent	present all flowers of both male and female inflorescences	absent	present at base of rachis (1 – 2 flowers) both male and female inflorescences
Indumentum	lacking on stem, lamina, and leaf margin	lacking on stem, lamina, leaf margin, and shoot	covering leaf axil and inflorescence	covering all vegetative parts

Table 3. Description of AFLP results from seven *Nepenthes* species and two new species, *N. bracteosa* and *N. hirtella* in Thailand: **PB** polymorphic bands; **MB** monomorphic bands; ***P*** percentage of polymorphism; ***M*** percentage of monomorphism; **PIC** polymorphism information content.

Primer pairs	Total no. of bands	PB	MB	*P*	*M*	PIC
ER-AG/Tru-CAG	100	92	8	92.00	8.00	0.74
ER-AG/Tru-CGT	101	93	8	92.08	7.92	0.71
ER-AT/Tru-CAG	107	97	10	90.65	9.35	0.71
ER-AC/Tru-CAG	139	132	7	94.96	5.04	0.74
ER-AC/Tru-CGT	147	145	2	98.64	1.36	0.86
ER-AGA/Tru-CGA	113	106	7	93.81	6.19	0.76
ER-AGA/Tru-CCA	136	127	9	93.38	6.62	0.76
ER-AGA/Tru-CAG	119	108	11	90.76	9.24	0.72
ER-AAG/Tru-CAG	149	139	10	93.29	6.71	0.75
ER-AGC/Tru-CAG	162	153	9	94.44	5.56	0.79
ER-ATG/Tru-GAG	142	131	11	92.25	7.75	0.78
Total	1415	1323	92	1026.26	73.74	8.32
Mean	128.64	120.27	8.36	93.30	6.70	0.76

mation explains the genetic link between the two newly discovered *Nepenthes* species, *N. bracteosa* and *N. hirtella*. *Nepenthes bracteosa* and *N. hirtella* are separated from *N. krabiensis* by their genetic background, even though they are found in close proximity.

Taxonomic Treatment

1. Nepenthes bracteosa *Suran. & Nuanlaong* **sp. nov.** Type: Thailand, Nakhon Si Thammarat Province, [redacted] ♂ fl. lower and upper pitchers, 29 August 2019, *Nuanlaong* BKF197454 (holotype BKF!; isotype PSU!).

[redacted]

Scandent, glossy climbing herb. *Root* branched, fleshy tubers. *Stem* terete, 0.5 – 1.0 m long, 0.3 – 0.5 cm diam.; internodes 1.3 – 2.7 cm long; axillary bud present in 2 – 3 axils in the upper part of the plant when flowering; upper part green to red and lower part brown. *Leaves* pseudo-petiolate, coriaceous, linear to lanceolate; 14.0 – 24.0 × 2.0 – 3.9 cm, longitudinally folded to form a V-shape; blade apex acute to acuminate, base attenuate, entire, dilating at the node, projecting along the stem as a wing, clasping the stem by three-quarters of its circumference, decurrent for 0.8 – 2.8 cm of its length, obscure longitudinal nerves on each side of the midrib at the edge of lamina, as leaves are thickened, inconspicuous reticulated pinnate venation; midrib conspicuous; tendrils straight, terete, 15.0 – 26.0 cm long in lower pitchers, upper pitchers 10.0 – 15.0 cm long, coiling; glossy light green when young, green when mature, emerging from an orange–amber, triangular lateral bud at the base of the midrib near the leaf attachment. *Lower pitcher* c. 10.5 – 19.0 × 3.4 – 5.6 cm; ovate in the lower part with digestive glands on the inner surface, cylindrical to narrowing in the upper part; conspicuous midsection hip; with two fringed wings, 0.6 – 1.3 cm wide, along the length of the pitcher, 6.5 – 12.0 cm from pitcher mouth to tendril, fringed elements 0.3 – 1.2 cm long, 4 – 8 fringed elements per cm; mouth ovate, concave; peristome cylindrical, undulate, revolute when pitcher mature, 0.7 – 1.9 cm wide, inner edge with teeth 0.6 – 1.7 mm long, ridges approximately 0.4 – 0.5 mm apart;

Table 4. Percentage similarity matrix for nine *Nepenthes* accessions, generated from Jaccard's similarity coefficient (Jaccard 1908). **S1** *N. rosea*; **S2** *N. krabiensis*; **S3** *N. bracteosa*; **S4** *N. hirtella*; **S5** *N. smilesii*; **S6** *N. kerrii*; **S7** *N. chang*; **S8** *N. thorelii*; **S9** *N. mirabilis*.

	S1	**S2**	**S3**	**S4**	**S5**	**S6**	**S7**	**S8**	**S9**
S1	**1.00**								
S2	0.62	**1.00**							
S3	0.49	0.65	**1.00**						
S4	0.45	0.56	0.53	**1.00**					
S5	0.42	0.48	0.45	0.43	**1.00**				
S6	0.48	0.60	0.54	0.52	0.46	**1.00**			
S7	0.44	0.51	0.47	0.45	0.50	0.50	**1.00**		
S8	0.36	0.39	0.38	0.34	0.35	0.37	0.39	**1.00**	
S9	0.22	0.24	0.24	0.23	0.24	0.24	0.25	0.22	**1.00**

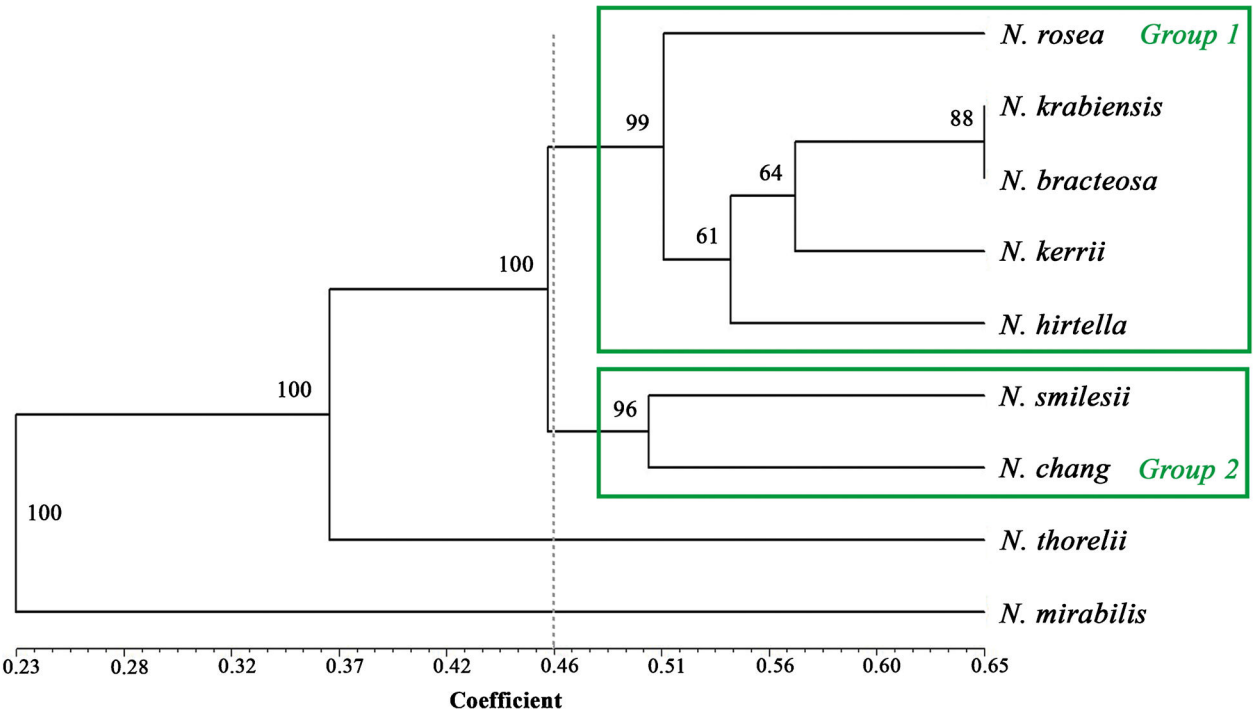


Fig. 1. UPGMA-based dendrogram showing genetic relationships among the nine *Nepenthes* species used in this study. The dendrogram is based on the genetic distance calculated according to Jaccard's similarity coefficient with 1000 bootstrap.

lid broadly ovate to round, 2.8 – 4.4 × 3.3 – 5.0 cm, larger than mouth, sometimes vaulted or bent towards the mouth, base cordate 1.0 – 4.0 mm, apex occasionally emarginate, indurate, like appendage on the lower surface, nectar glands numerous, elliptic, 0.16 – 0.26 mm diam. along the midrib, and with crateriform glands 0.12 – 0.18 mm diam. dispersed over the lower surface of the lid; spur filiform, 0.2 – 0.8 cm long, with branches; green to red on lower part and red on upper part with red speckled outside, light green to red with red blotches over the inside, absent in digestive zone, peristome green to dark red, lid green to red. *Upper pitcher* tubulose, with a conspicuous hip one third from the base; 10.0 – 19.0 cm long, 2.0 – 3.4 cm wide; two fringed wings, 0.1 – 0.3 cm wide, along the length of the pitcher, 9.0 – 16.0 cm long, fringe elements 0.3 – 0.7 cm long, 3 – 6 per cm; pitcher mouth similar to lower pitcher; peristome cylindrical, revolute when pitcher mature, 0.4 – 1.0 cm wide, inner edge with teeth 0.29 – 0.43 mm long, ridges approximately 0.4 – 0.5 mm apart; lid ovate to round, 2.1 – 3.4 × 2.1 – 4.4 cm, base cordate 1.0 – 3.0 mm, apex emarginate similar to lower pitcher, elliptic nectar gland along the midrib on the lower surface of lid, numerous near base, 0.1 – 0.2 mm diam. and with crateriform glands 0.05 – 0.1 mm diam. scattered over the lower surface of the lid; spur filiform, 3.0 – 7.0 mm long, with branches; pitchers green or green to red, sometimes red-speckled outside, light green with red blotches over the inner surface, absent in digestive zone, peristome and lid light green. *Male inflorescence* a raceme, 70.0 – 85.0 cm long, peduncle 47.0 – 55.0 cm long, 0.2 – 0.5 cm diam., rachis 13.0 – 32.0 cm long, flowers 84 – 135, solitary, pedicels 3.0 – 5.0 mm long, with bracts at the base or lower half of pedicel 0.8 – 1.0 mm long, present on all flowers; tepals 4, ovate, apex obtuse, 4.0 – 5.0 × 3.0 – 4.0 mm, reflexed in mature flower, densely covered with orbicular to elliptic nectar glands 0.20 – 0.36 mm diam.; androphore 2.0 – 2.5 mm long, anther head globular 2.1 – 2.5 mm diam., anther basifixed, synandrium, 8 – 13 anther sacs, longitudinal dehiscence; tepals light green when young, red when old. *Female inflorescence* a raceme, 50.0 – 86.0 cm long, 0.2 – 0.4 cm diam., peduncle 42.0 – 74.0 cm long, rachis 9.0 – 16.0 cm long, flowers 20 – 42, solitary, pedicels 0.5 – 1.7 cm long, with bracts 1.0 – 1.5 mm long, inserted at one third of pedicel height, conspicuous on upper part of rachis, present on all flowers; tepals 4, ovate to elliptic, 3.8 – 4.0 × 2.0 – 2.2 mm, nectar glands similar to male inflorescence, 0.20 – 0.28 mm diam., green when young, green to brown when old;. ovary superior, with four syncarpous carpels, oblong, 5.0 – 6.0 mm long, 2.3 – 2.5 mm diam., stigma head 1.0 – 1.5 mm diam. *Infructescence* similar to female inflorescence; tepals persistent; four valves, 1.0 – 1.2 cm long with septicidal capsule; seeds linear, 6.0 – 6.6 mm long c. 98 – 125 seeds per pod. *Indumentum* conspicuous, inflorescence and outer pitcher pubescent with simple brown hairs; tendril with simple and branched hairs; wing fringed

elements puberulent, with branched brown hairs; leaf midrib puberulent with inconspicuous, simple, dark green hairs; absent on stem, leaf axils, lamina, leaf margin, and shoot. Figs 2 – 4.

RECOGNITION. *Nepenthes bracteosa* belongs to section *Pyrophytae*, a section distributed in the strongly seasonal region of Indochina. It is closely related to *N. krabiensis*, but differs in its shorter stem 0.5 – 1.0 m (vs 1.5 – 2.5 m), spur filiform with branches (vs filiform with no branches), axillary bud present in 2 – 3 axils in the upper part of plant when flowering (vs a single axillary bud), fringed wing present in the upper pitcher (vs absent), occasionally emarginate and indurate lid apex (vs a blunt apex and not indurate), solitary flowers both male and female inflorescences (vs 2-flowered partial peduncles in male inflorescence and solitary flower in female inflorescence), bracts present on all flowers of both male and female inflorescences (vs present at the base or lower half of the pedicel in male inflorescence but absent in female inflorescence), short androphore (2.0 – 2.5 mm vs 4.0 mm), and 8 – 13 anther sacs (vs 15 – 22 anther sacs).

DISTRIBUTION. Nakhon Si Thammarat Province, Thailand (Map 1).

SPECIMENS EXAMINED. THAILAND. [redacted] ♂ fl. upper and lower pitchers, 29 August 2019, *Nuanlaong* BKF197454 (holotype BKF; isotype PSU).

HABITAT. On summit areas, in sandy soil, generally growing in open forest and limestone rock crevices, 710 – 760 m a.s.l.

CONSERVATION STATUS. This species is distributed at a single site on [redacted]. A decline in the quality of the habitat will lead to the disturbance of the long-term survival of the remaining population and may thus increase the extinction risk of this species. The calculation of EOO as 0.034 km^2 and AOO as 4.00 km^2 together with the information above suggests that this species is Critically Endangered (CR) [B1a,b (iii) + B2a,b (iii)] (IUCN 2019).

PHENOLOGY. Flowering June to December.

ETYMOLOGY. From the Latin "*bracteosa*" derived from "*bracteosus*": bracts are present on the pedicels of all flowers of both male and female inflorescences.

VERNACULAR NAME. From the Thai language "Seua–Nakhonsi": "Seua" means the red-speckled tiger-like pattern on the pitcher surface and "Nakhonsi" means *Nepenthes* of Nakhon Si Thammarat Province.

NOTES. *Nepenthes bracteosa* was first discovered from a photograph taken by a tourist. It is only found at a single location in Southern Thailand. It belongs to section *Pyrophytae* on account of sharing several characteristics such as long racemose inflorescence, climbing stem, flower from the rosette stage, tuberous rootstocks, and pyrophytic habitat. *Nepenthes bracteosa* is similar to *N. krabiensis*, with which it shares being glossy, climbing, its leaf shape and an inconspicuous indumentum, but differs in many other morphological characteristics. However, the results of the phylogenetic relationship revealed a similarity coefficient as 0.65 between *N. bacteosa* and *N. krabiensis*, which is also the highest of all species pairs recorded (Table 3).

2. Nepenthes hirtella *Nuanlaong & Suran.* **sp. nov.** Type: Thailand, Krabi Province, [redacted] ♂fl. lower pitchers, 29 August 2019, *Nuanlaong* BKF197453 (holotype BKF!; isotype PSU!).

[redacted]

Scandent, climbing herb. *Root* branched, fleshy tubers. *Stem* terete, 0.8 – 1.2 m long, 0.35 – 0.65 cm diam.; internode 1.5 – 5.5 cm long; upper part of the stem green, lower part of the stem brown. *Leaves* pseudopetioles, coriaceous, oblanceolate; 15.8 – 29.5 × 3.5 – 5.4 cm, longitudinally folded to form a V-shape, blade apex acuminate, base attenuate, entire, dilating at the node, projecting along the stem as wing, clasping the stem by three quarters of its circumference, decurrent for 1.7 – 4.3 cm of its length, leaf thickness 0.5 – 1.0 mm, inconspicuous longitudinal nerves 3 – 4 on each side of the midrib in outer third of lamina, inconspicuous lamina reticulated pinnate venation; midrib conspicuous; light green in a rosette when young, green when mature, emerging from a red triangular lateral bud at the base of the midrib near the leaf attachment. *Lower pitcher* c. 14.0 – 19.7 × 4.4 – 5.9 cm; ovate at the lower part with digestive zone at the inner surface, narrowing at the upper part; hip conspicuous above digestive zone; with two fringed wings, 0.3 – 0.9 cm wide, along the length of the pitcher, 9.0 – 14.3 cm from pitcher mouth to tendril, serrate to double serrate, fringed elements 0.4 – 0.9 cm long, 4 – 11 fringed elements per cm; mouth oblique ovate, concave; peristome cylindrical, entire to repand, revolute when pitcher mature, 0.9 – 1.5 cm wide, inner edge with teeth 0.64 – 1.48 mm long, ridges approximately 0.72 – 1.2 mm apart; lid ovate, 3.9 – 4.9 × 3.3 – 4.9 cm, repand, base cordate 0.5 – 3.0 mm; absent appendage, nectar glands numerous, elliptic 0.18 – 0.28 mm diam. along the midrib, with crateriform gland 0.09 – 0.28 mm diam. dispersed over the lower surface of the lid; spur filiform, 0.1 – 0.9 cm long, with branches; tendril straight, 12.0 – 20.0 cm long, 2.0 – 3.5 mm diam., approximately 10 – 30% longer than pitcher, mostly on the side of the pitcher; light green to yellow or green to red on the lower and green to red or red on the upper part with

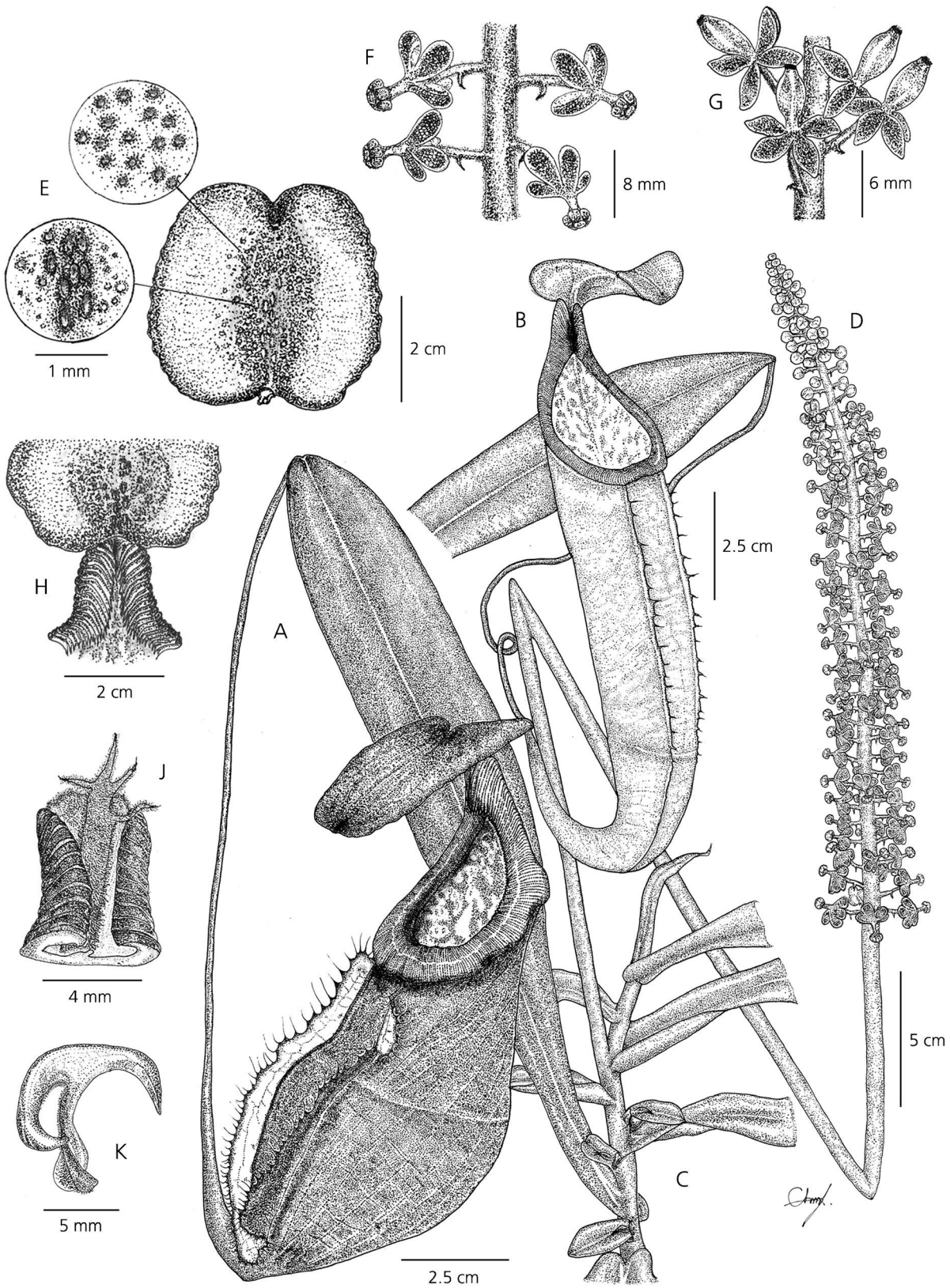


Fig. 2. *Nepenthes bracteosa*. **A** leaf shape and lower pitcher; **B** upper pitcher; **C** stem; **D** male inflorescence; **E** lower surface of lid with two types of nectar glands and a ridge-like appendage at the lid apex; **F** male flower; **G** female flower after pollination; **H** peristome; **J** filiform spur with branches; **K** peristome in transverse section. Drawn from live specimens. DRAWN BY CHAYAN YODTHAMMARAT.

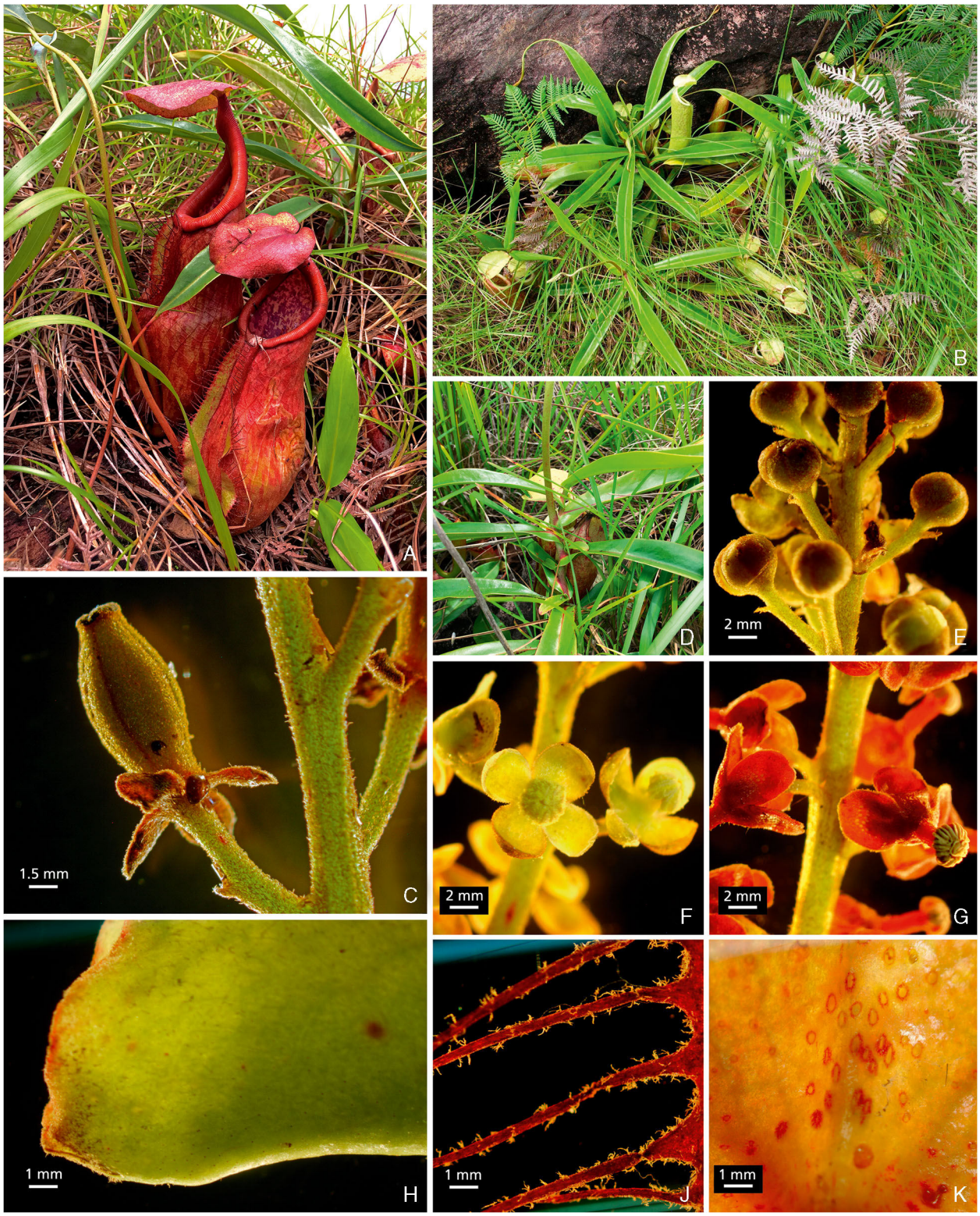


Fig. 3. *Nepenthes bracteosa*. **A** lower pitcher; **B** upper pitcher and habitat; **C** infructescence; **D** axillary bud present in 2 – 3 axils when flowering; **E** – **G** male inflorescence (immature, young, and old flowers); **H** ridge-like appendage under lid apex; **J** branched hairs on fringe elements; **K** nectar glands on lower surface of lid. PHOTOS: POTJAMARN SURANINPONG & SUNYA NUANLAONG.

Fig. 4. *Nepenthes bracteosa*. **A** lower pitcher; **B** upper pitcher; **C – D** habit and habitat. PHOTOS: POTJAMARN SURANINPONG & SUNYA NUANLAONG.

red blotches on the outside surface, light green present or absent red blotches on inside surface, absent in digestive zone, peristome light green, green to red or red peristome, lid green or green to red. *Upper pitcher* tubulose, with a conspicuous hip one third from the base or middle of pitcher; 13.0 – 21.0 cm long, 0.9 – 2.8 cm wide; two fringed wings, 1.5 – 8.0 × 0.1 – 0.2 cm, which run down the ventral exterior surface from pitcher mouth to the middle or tendril of the pitcher, serrate or double serrate, fringed elements 0.5 – 2.0 mm long, 4 – 8 per cm; mouth oblique ovate; peristome cylindrical, revolute when pitcher mature, 0.3 – 1.0 cm wide, inner edge with teeth 0.48 – 0.84 mm long, ridges approximately 0.92 – 1.40 mm apart; lid ovate, 2.1 – 4.3 × 2.0 – 4.3 cm, base cordate 0.5 – 1.0 mm, nectar gland similar to lower pitcher; spur filiform, 2.0 – 7.0 mm long; tendril straight, 7.0 – 15.0 cm long, 1.0 – 3.0 mm diam., coiling, shorter than pitcher; green or green to orange outside surface with or without red blotches, green with red blotches over inner surface, absent in digestive zone, peristome light green sometimes red stripes present, lid green. *Male inflorescence* a raceme, 65.0 – 105.0 cm long, 0.3 – 0.4 cm diam., peduncle 44.0 – 56.0 cm long, rachis 22.5 – 47.0 cm long, flowers 68 – 194, solitary flowers or rarely two-flowered partial peduncle, pedicels 0.5 – 0.8 cm long, bracts present at base of peduncle 0.5 – 3.0 mm long, absent on upper half; tepals 4, elliptic to obovate, 2.0 – 4.0 × 1.0 – 2.0 mm, explanate and not reflexed, cruciferous, densely covered with orbicular to elliptic nectar glands 0.35 – 0.70 mm diam.; androphore 1.0 – 2.0 mm long, anther head globular, 1.0 – 2.0 mm diam., anther basifixed, synandrium, 8 – 12 anther sacs, longitudinal dehiscence; tepals light green when young, green to red when old. *Female inflorescence* a raceme, 65.0 – 75.0 cm long, 0.30 – 0.35 mm diam., peduncle 40.0 – 58.5 cm long, rachis 12.0 – 20.0 cm long, bracts similar to male inflorescence; flowers 26 – 57, solitary, pedicels 0.2 – 1.0 mm long; tepals 4, elliptic to ovate, 2.0 – 3.0 × 1.0 – 2.0 mm, nectar glands similar to male inflorescence, 0.35 – 0.60 mm diam.; tepals green when young, green to red when old; ovary superior, with four syncarpous carpels, oblong, 3.0 – 5.0 mm long, 2.0 – 2.5 mm diam., stigma head 1.5 – 2.0 mm diam. *Infructescence* similar to female inflorescence; tepals persistent; four valves, 1.5 – 1.6 cm long with septicidal capsule; seed linear, 4.0 – 8.0 mm long c. 73 – 109 seeds per pod. *Indumentum* conspicuous, simple brown hairs, pubescent, 0.17 – 0.30 mm long, present on all vegetative parts; fringed elements puberulent. Figs 5 – 7.

RECOGNITION. *Nepenthes hirtella* belongs to section *Pyrophytae*, a section distributed in strongly seasonal Indochina. It is closely related to *N. kerrii* which grows in Tarutao Marine Park, Satun Province, Thailand. *Nepenthes hirtella* differs from *N. kerrii* in having an oblanceolate leaf shape (vs obovate), tendrils longer than pitcher approximately 10 – 30% (vs longer than pitcher approximately 40 – 50%), tendrils displaced to the side of lower pitcher (vs tendril centrally placed at base of lower pitcher), longer lower pitcher (14.0 – 19.7 cm vs 6.0 – 14.0 cm), lid ovate (vs round), spur filiform (vs simple or branched), solitary flowers or rarely two-flowered partial peduncle (vs solitary flowers), and indumentum covering all the vegetative parts (vs covering leaf axil and inflorescence).

DISTRIBUTION. Krabi Province, Thailand (Map 1).

SPECIMENS EXAMINED. THAILAND. [redacted] ♂ fl. lower pitchers, 29 August 2019, *Nuanlaong* BKF197453 (holotype BKF!; isotype PSU!).

HABITAT. On summit areas, in sandy soil, generally growing in open forest, savannas and scrubland, at 348 – 355 m a.s.l.

CONSERVATION STATUS. *Nepenthes hirtella* is found only on a single site on th[redacted] A decline in the quality of the habitat will lead to the disturbance of the long-term survival of the remaining population. It is easy to get to the habitat, which might put this species at risk of extinction. The calculation of EOO as 0.053 km^2, AOO as 8.00 km^2 together with the information above suggests that this species is Critically Endangered (CR) [B1a,b (iii) + B2a,b (iii)] (IUCN 2019).

PHENOLOGY. Flowering June to December.

ETYMOLOGY. From the Latin "*hirtella*" derived from "*hirtellus*": short hairs are present on all the vegetative parts of the plant and rather hairy.

VERNACULAR NAME. From the Thai language "Seua-Amphon": "Seua" means the red-speckled tiger-like pattern on the pitcher surface and "Amphon" is the name of the first collector, Mr Amphon Dumkliang who found them in 2014 – 2015.

NOTES. *Nepenthes hirtella* was first discovered in 2014 – 2015 by a Nepenthes collector/seller and was known as "Seua-Amphon". *Nepenthes hirtella* is a member of section *Pyrophytae* like *N. bracteosa* and is only found in [redacted] Krabi Province, Thailand. *Nepenthes hirtella* is closely related to *N. kerrii* but differs in several morphological details, in particular in tendril and indumentum. *Nepenthes hirtella* is covered by brown hairs throughout, while in *N. kerrii* the indumentum is confined specifically to the leaf axil and inflorescence. It is distributed on the mainland while *N. kerrii* is distributed on an island. Moreover, the phylogenetic relationship results show a low similarity index between them (Table 4).

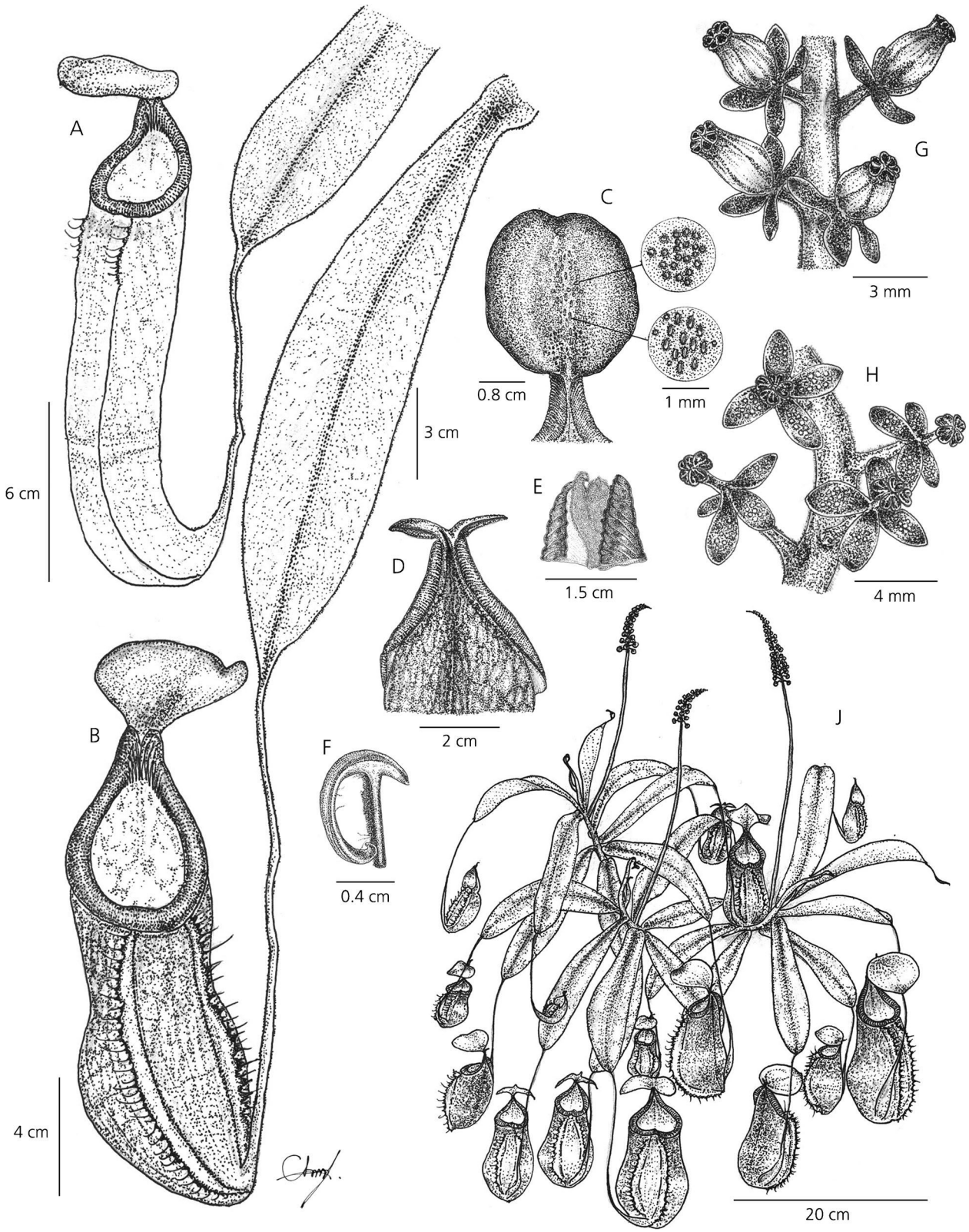


Fig. 5. *Nepenthes hirtella*. **A** upper pitcher; **B** lower pitcher with leaf shape; **C** lower surface of lid with two types of nectar glands; **D** rear view of pitcher mouth of **B**; **E** spur of lower pitcher; **F** peristome of lower pitcher in transverse section; **G** female inflorescence after pollination; **H** male inflorescence; **J** habit. Drawn from live specimens. DRAWN BY CHAYAN YODTHAMMARAT.

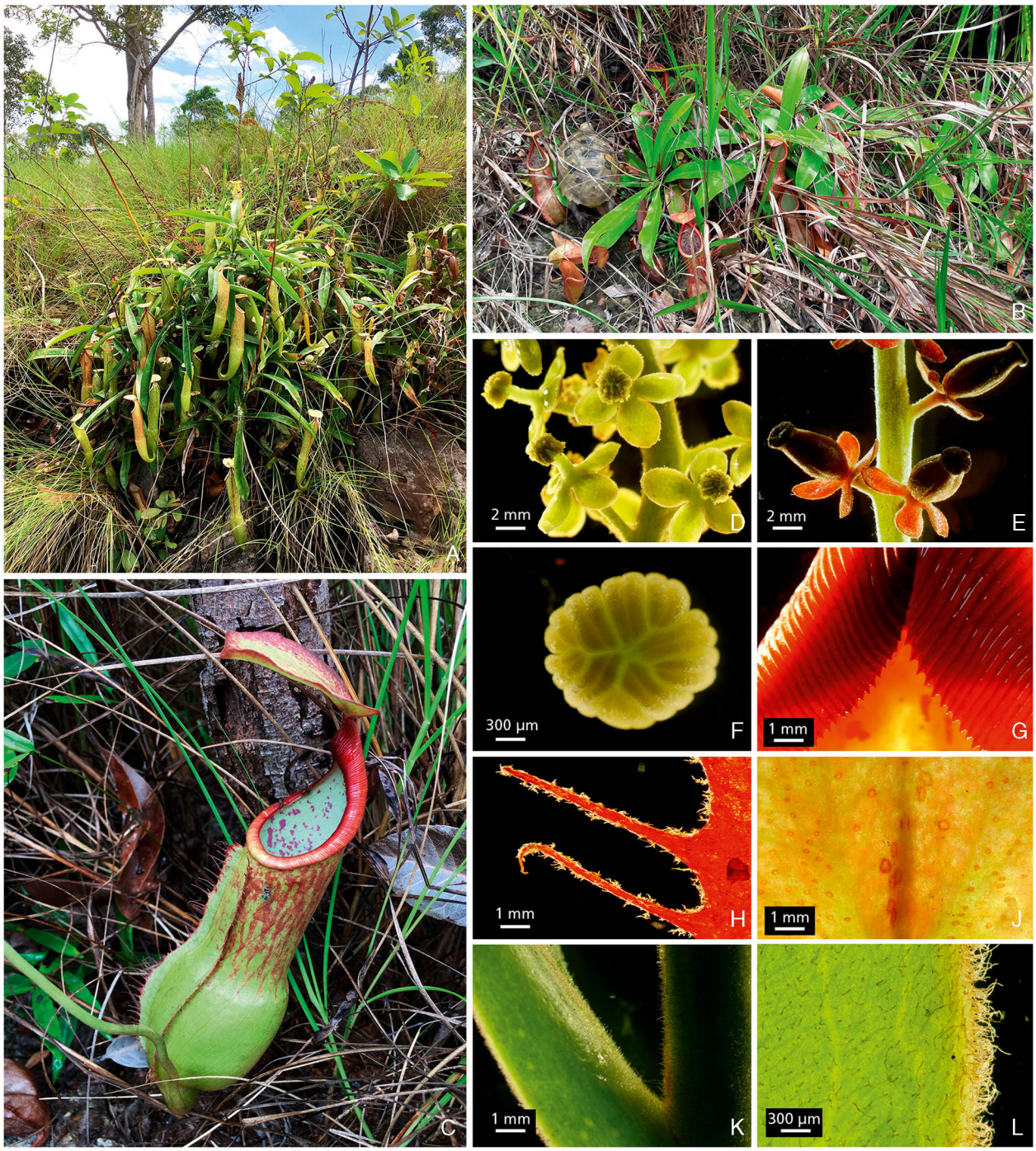


Fig. 6. *Nepenthes hirtella*. A upper pitcher and habitat; B leaves and lower pitcher; C lower pitcher; D male inflorescence; E female inflorescence; F anther head; G peristome with teeth; H branched hairs on fringed element; J nectar glands on lower surface of lid; K stem and leaf axil; L brown hairs on lamina and leaf margin. PHOTOS: POTJAMARN SURANINPONG & SUNYA NUANLAONG.

Key to *Nepenthes* species in Thailand

1. Tuberous rootstock **2**
1. Non–tuberous rootstock **12**
2. Inflorescence racemose with flowers all arranged singly (a 1-flowered) or rarely 2-flowered partial peduncle **3**
2. Inflorescence racemose with 2-flowered partial peduncles **10**

Fig. 7. *Nepenthes hirtella*. **A** lower pitcher; **B** upper pitcher; **C – D** habit and habitat. PHOTOS: POTJAMARN SURANINPONG & SUNYA NUANLAONG.

3. Leaves obovate or oblanceolate **4**
3. Leaves linear-lanceolate **5**
4. Leaves obovate, tendril longer than pitcher approximately 40 – 50%; indumentum covering leaf axil and inflorescence . . . . . ***N. kerrii***
4. Leaves oblanceolate, tendril longer than pitcher approximately 10 – 30%; indumentum covering all vegetative parts . . . . . ***N. hirtella***
5. Short hairs present on all parts or some part of plant **6**
5. Stem and leaves glabrous (pitcher, inflorescence, tendril excluded) **9**
6. Indumentum on all vegetative parts; tendril as long as or shorter than pitcher . . . . . ***N. smilesii***
6. Indumentum present on some parts of the plant; tendril longer than pitcher. **7**
7. Lower pitcher narrowly ovate in the lower third or lower half and tubular above……..…***N. kongkandana***
7. Lower pitcher ovate in lower half and narrowing above or completely ovate **8**
8. Lid smaller than mouth, triangular mouth as large as $^{1}/_{3}$ to ½ of the lower pitcher length; flower green with red margins. . . . . ***N. suratensis***
8. Lid larger than mouth, ovate mouth as large as ¼ of the lower pitcher length; flower red . . . . ***N. andamana***
9. Pseudo-petiolate leaves; lid broadly ovate to round; bract present in all flowers in both male and female inflorescences. . . . . ***N. bracteosa***
9. Sub-petiolate leaves; lid orbicular; bract present on partial peduncles . . . . . ***N. kampotiana***
10. Hip present at the mid-section or absent in lower pitcher; indumentum covering all aerial parts except upper surface of leaves . . . . . ***N. chang***
10. Hip present at the lower $^{1}/_{3}$ in both lower and upper pitchers; indumentum present on some parts of the plant **11**
11. Indumentum often covering leaf margin and midrib; lower pitcher green to light pink, with dark speckles outside and uniformly green to dark pink within . . . . . ***N. rosea***
11. Indumentum lacking on stem, lamina, leaf margin and shoot; lower pitcher green to red, with red speckles outside and light green or green with blotches over the inside. . . . . ***N. krabiensis***
12. Leaves petiolate; fimbriate leaf margin **13**
12. Leaves sessile or with a short, poorly defined petiole; entire leaf margin. **14**
13. Lower pitcher ovoid in the basal third, narrowing slightly to form a hip and cylindric above. . . . ***N. mirabilis***
13. Lower pitchers round to oval . . . . . ***N. mirabilis*** var. ***globosa***
14. Producing ground pitchers **15**
14. Not producing ground pitchers. **16**
15. Lower pitcher urceolate, peristome incurved; inflorescence paniculate. . . . . ***N. ampullaria***
15. Lower pitcher elongated and smaller, very thin peristome; inflorescence racemose. . . . . ***N. gracilis***
16. Leaf base amplexicaul, clasping the stem transversely; lid ovate, truncate at base . . . . . ***N. sanguinea***
16. Leaf base decurrent, running longitudinally down the stem as wings; lid elliptic, round at base . . . . ***N. thai***

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