

## A Taxonomic Study of *Rosa clinophylla* var. *glabra* (Rosaceae), Newly Recorded from Myanmar, Based on Morphological and Molecular Data

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*Rosa clinophylla* Thory var. *glabra* S. C. Ghora & Panigrahi, recently described as a distinct taxon, is newly recorded from Kachin State, Myanmar as the result of the floristic research of the country. It is commonly growing along the Irrawaddy River, and is adapted to tropical seasonal climate. Molecular investigation in this study also supported that *R. bracteata* and *R. clinophylla* are recognized as distinct species in sect. *Bracteatae*, and *R. clinophylla* var. *glabra* should be classified under *R. clinophylla* as a variety rank. A key to the genus *Rosa* sect. *Bracteatae* presently recorded from Myanmar is provided.

**Key words:** Myanmar, *Rosa clinophylla* var. *glabra*, Rosaceae, section *Bracteatae*.

Field expeditions to Kachin State, northern Myanmar have been conducted by a team from the Makino Botanical Garden and the Botanical Gardens of the University of Tokyo in cooperation with the Forest Department, Union of Myanmar Ministry of Forestry since 2005. In the course of the inventory studies, the genus *Rosa* growing on the sandy river bank of the Irrawaddy River in Myitkyina was observed, however, at that time they were sterile. At a later date we visited the same place to see their flowers, and collected specimens for identification and molecular analysis.

*Rosa clinophylla* Thory, belonging to the section *Bracteatae*, consisting of two species, *R. clinophylla* and *R. bracteata* Wendl., have been considered to be distributed from India to Thailand and China (Ghora and Panigrahi 1985), however, Gu and Robertson

(2003) didn't treat this species in the Flora of China. The section *Bracteatae* is characterized by a combination of pectinate stipules with base adnate to petiole, solitary flowers with large bracts and tomentose hypanthium (Ghora and Panigrahi 1995, Gu and Robertson 2003). Ghora and Panigrahi (1995) classified three species of *Rosa*, *R. clinophylla*, *R. bracteata* and *R. lyellii* Lindl. in the section *Bracteatae*. However, sect. *Bracteatae* is presently considered to be consisting of two species, *R. bracteata* and *R. clinophylla* (Gu and Robertson 2003, Wissemann 2003). Kress et al. (2003) recorded two species, namely, *R. clinophylla* and *R. bracteata* Wendl. in this section from Myanmar.

Kingdon-Ward (1930) reported the wild rose found in Myanmar in his article, "Plant Hunting at the Edge of the World", that it

was growing in Myitkyina as a bush 10–15 feet high among rocks submerged for three months of the year. Then he identified it as *R. bracteata*. Bean (1981) mentioned that the plant Kingdon-Ward came across along the Irrawaddy River was *R. clinophylla*.

Ghora and Panigrahi (1985) recognized three infraspecific taxa under *R. clinophylla*, namely, var. *clinophylla*, var. *glabra* and var. *parvifolia*. Thus far only var. *clinophylla* has been recorded from Myanmar (Ghora and Panigrahi 1985).

In addition to morphological study, chloroplast DNA sequences were examined to reevaluate taxonomic status of the rosaceous plants collected in Myanmar from molecular biological aspects, in comparison to *R. bracteata* and *R. clinophylla*.

### Materials and Methods

Field investigations were carried out in September 2005 and February 2007, along the Irrawaddy River, Myitkyina in Kachin State, northern Myanmar. Herbarium specimens and leaf samples for DNA analysis were taken in the field. The plants of *R. bracteata* and *R. clinophylla* var. *clinophylla* were obtained from cultivated specimens. Materials and their voucher specimens used in the molecular analysis are shown in Appendix. Morphological investigations were examined based on herbarium specimens held in MBK and TI. One set of voucher specimens was retained in the Forest Department Office in Tanaing, a center of Hukaung Valley Tiger Reserve.

The DNA extraction, PCR amplification and sequence detection were performed using the procedure of Ohi-Toma et al. (2006). Nine regions of chloroplast DNA were selected, and their published primers were used for PCR amplification and sequencing: *trnL-trnF* including *trnL* intron were amplified by using primers ‘c’ and ‘f’, and primers ‘d’ and ‘e’ were also used in sequencing (Taberlet et al. 1991); *atpB-rbcL*

(Terachi 1993); *trnK* including *matK* gene were amplified by ‘3914F’ and ‘2R’ (Johnson and Soltis 1994) and sequenced by adding the two primers to ‘AF’ and ‘8R’ of Ooi et al. (1995), ‘1412F’ and ‘1470R’ of Johnson and Soltis (1994), and ‘*matK*-10’ and ‘*matK*-07’ of Kato et al. (1998); *trnS-trnG* and *rpl20-5’rps12* (Hamilton 1999); *psbB-psbH* (Xu et al. 2000); ‘F71’ of Jordan et al. (1996) and ‘R1516’ of Kelchner and Clark (1997) for *rpl16* intron; *rps16* intron (Nishizawa and Watano 2000); *trnG* intron (Kitano et al. 2005). Sequences were registered in the DNA Data Bank of Japan (DDBJ) linked with GenBank, and their accession numbers are shown in Appendix.

### Results and Discussion

After careful investigation of morphological characters of the plant, it was identified as *Rosa clinophylla* var. *glabra*, recognized by Ghora and Panigrahi (1985). Var. *glabra* is separated from var. *clinophylla* by glabrous leaflets and branches, larger flowers and subglobose hypanthium. Thus far this variety has been recorded only from India and Bangladesh, and this is the first record of this variety from Myanmar. It is considered that the plant which Kingdon-Ward reported from the Irrawaddy River as *R. bracteata* was *R. clinophylla* var. *glabra*. The sandy river bank of Irrawaddy is submerged for three months and arid for six months of the year. The variety having glabrous branches and leaflets may be considered to adapt to this kind of tropical seasonal climate.

In molecular analysis, the determined sequence of each material reached a total of ca. 9000 bp: *trnL-trnF* including *trnL* intron (1046–1047 bp), *atpB-rbcL* (777bp), *trnK* including *matK* (2484–2486), *trnS-trnG* (595–603 bp), *rpl20-5’rps12* (802 bp), *psbB-psbH* (691 bp), *rpl16* intron (950–955 bp), *rps16* intron (660 bp), and *trnG* intron (936 bp). No variations were observed between two plants of *R. clinophylla* var. *clinophylla*. The se-

Table 1. Summary of sequence variation of chloroplast DNA in *Rosa bracteata*, *R. clinophylla* var. *clinophylla* and var. *glabra*, used for DNA analysis in this study

	<i>trnL</i> intron	<i>trnK</i> 5'intron	<i>trnK</i> 3'intron	<i>trnS-trnG</i>	<i>rpl20-5'rps12</i>	<i>psbB-psbH</i>	<i>rps16</i> intron
<i>R. bracteata</i>	T (10)	A (11)/T (9)	C	TAGTTATT	G	CGAT	AAACC/A/A
<i>R. clinophylla</i>							
var. <i>clinophylla</i>	T (9)	A (8)/T (10)	C	—	A	ATCG	—/G/T
var. <i>glabra</i>	T (9)	A (8)/T (10)	A	—	A	CGAT	—/G/T

quences of *trnL-trnF*, *matK*, *atpB-rbcL*, *rps16* intron and *trnG* intron were identical for all materials. The sequence variations from other regions are summarized in Table 1. Each of *trnL* intron and *trnK* 5'intron included one or two length variations of mononucleotide repeat (poly-T or poly-A) between *R. bracteata* and *R. clinophylla*. One substitution was observed in *trnK* 3'intron between *R. clinophylla* var. *glabra* and others, one duplication of tandem repeat in *trnS-trnG* sequences of *R. bracteata*, compared with that of *R. clinophylla*, and one substitution in *rpl20-5'rps12* between the two species. In *psbB-psbH*, an inversion of 4 base pairs was observed, and *R. bracteata* and *R. clinophylla* var. *glabra* have an identical sequence. In *rpl16* intron, one insertion of 5 base pairs was included *R. bracteata*, and two substitutions were also observed between the two species.

Three previous studies, which showed the phylogenetic relationship of the genus *Rosa*, were based on sequences of *matK* gene (Matsumoto et al. 1998), *atpB-rbcL* (Wissemann and Ritz 2005), and *trnL* intron and *trnL-trnF* (Bruneau et al. 2007), respectively. The *matK* and *atpB-rbcL* sequences obtained from *R. bracteata* and *R. clinophylla* in this study were identical to the sequences of *R. bracteata* from the former two studies. The sequences of either species from the last study did not agree with our data, but the data in the last study was unreliable because the authors suggested a contamination for *R. bracteata* samples. The results that *R. bracteata* and *R. clinophylla*

have nearly identical sequences showed a close relationship between the species. Based on variation of sequences detected in this study (Table 1), *R. bracteata* and *R. clinophylla* are recognized as distinct species in sect. *Bracteatae*. For two varieties of *R. clinophylla*, it is reasonable to recognize them as variety of the same species rather than species rank, because a few differences were observed.

#### Key to the genus *Rosa* sect.

##### *Bracteatae* recorded in Myanmar

- 1a. Shrubs with long repent branches; prickles hooked; leaflets 5–9, apex truncate, rounded-obtuse; flowers 4.5–9 cm in diam. .... *R. bracteata*
- 1b. Shrubs a dense bush; prickles not hooked; leaflets 9–11, apex acute; flowers 3.5–5.5 cm in diam.
- 2a. Leaflets abaxially tomentose; branches tomentose; flowers 3.5 cm in diam. ....  
..... *R. clinophylla* var. *clinophylla*
- 2b. Leaflets glabrous on both surfaces; branches glabrous; flowers 5–5.5 cm in diam. .... *R. clinophylla* var. *glabra*

Sect. **Bracteatae** Thory, Prodr. Mongr. Gen. Rosier.: 124 (1820); Brandis, For. Fl.: 199 (1874); Hook., Fl. Brit. Ind. 2: 364 (1878); Rehder, Bibl. Cult. Trees & Shrub.: 317 (1949); Ghora & Panigrahi, Fam. *Rosac.* Ind. 2: 214 (1995); Gu & Robertson in Wu & Raven (eds.), Flora of China 9: 380 (2003). Type: *R. bracteata* Wendl. in Bot. Beobacht. 50: 7, t. 22 (1798).

***Rosa clinophylla*** Thory var. ***glabra*** S. C.



Fig. 1. A. Habitat of *Rosa clinophylla* var. *glabra* growing as bush along Irrawaddy River, Myitkyina, Kachin State, Myanmar. B. Flower enlarged.

Ghora & Panigrahi in J. Jpn. Bot. **60**: 57 (1985). [Fig. 1 A, B]

Specimens examined: MYANMAR: KACHIN STATE; on sandy soil, along the Irrawaddy River, near the industrial estate, Myitkyina, alt. 70 m., 9 February 2007, J. Murata & al. 041319 (MBK, TI).

Ecology: Growing on sandy river bank submerged for three months and arid for six months of the year. Var. *glabra* having glabrous leaflets and branches may be adapted to this kind of tropical seasonal climate.

Distribution: India, Bangladesh and northern Myanmar.

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田中伸幸<sup>a</sup>, 大井・東馬哲雄<sup>b</sup>, 邑田 仁<sup>b</sup>: ミャンマーから新しく記録された *Rosa clinophylla* var. *glabra* (バラ科) の形態および分子データに基づく分類学的検討

日華区系の西端としての南ヒマラヤの植物多様性を解析する一連の研究で、2005年の雨季終盤の9月にミャンマー北部のカチン州ミッチーナのイラワジ川河川敷で採集した際、見慣れないバラ属が生育していたが、花もなく同定は不可能であった。枝は灌木状と言うよりは横に這って、棘が比較的少なかった。半世紀以上前に当時のビルマを調査した英国のプラントハンター、Kingdon-Ward (1930) は、ミッチーナのイラワジ川の流域にカカヤンバラ *Rosa bracteata* Wendl. が灌木状になって生育していることを報告している。2007年2月の乾季に再び同地を調査に訪れたところ、河原の砂地に高さ2–3メートルの灌木状に生育し、単生花をまばらにつけている同植物を発見した。そこで、正確な同定のために開花期の標本、DNA解析用の葉の乾燥試料を採取した。

イラワジ川で採集されたバラは形態的に *Bracteatae* 節に属する *Rosa clinophylla* Thory に類似していた。一方、Bean (1981) も Kingdon-Ward が報告しているイラワジ川の野生バラは *Rosa clinophylla* にあたることを述べている。その後、Ghora and Panigrahi (1985) は、*R. clinophylla* の

種内に2つの変種を認めた。ミャンマーで採集されたイラワジ川流域の *R. clinophylla* がどちらの変種に当たるのかについては、いままで報告はなかった。そこで、採集した標本の詳細な形態比較を行ったところ、ミャンマーで採集されたものは、*R. clinophylla* var. *glabra* に当たることが明らかとなった。var. *glabra* は、これまでインドとバングラデシュからしか報告がなく、ミャンマーからの報告は初めてである。*Rosa clinophylla* var. *glabra* は、var. *clinophylla* に比べて明らかに花が大きく、枝や幹は無毛である。また、1年のうちで雨季の数ヶ月間は水流の中に埋没する特殊な環境に適応していると考えられる。変種とする Ghora and Panigrahi (1985) の分類学的取り扱い、形態的にも生態的にも妥当であると考えられる。

一方、DNA解析でも、葉緑体DNAの約9000 bpの塩基配列を決定したが、配列の類似性から *R. clinophylla* が *R. bracteata* と非常に近縁で *Bracteatae* 節であることが示された他、配列の違いから、*R. clinophylla* と *R. bracteata* はそれぞれ独立した種として、var. *clinophylla* と var. *glabra* は変種として扱うのが妥当であると考えられた。

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## APPENDIX

Accessions of *Rosa* sect. *Bracteatae* used for DNA analysis in this study. Format: species, geographical origin or source, voucher, and DDBJ accession number for *trnL-trnF* including *trnL* intron, *atpB-rbcL*, *trnK* including *matK*, *trnS-trnG*, *rpl20-5' rps12*, *psbB-psbH*, *rpl16* intron, *rps16* intron, and *trnG* intron.

*Rosa clinophylla* var. *clinophylla*—India (cult. in Gifu International Academy of Horticulture), Ohi-Toma 080610 (TI); India (cult. in Natural History Museum and Institute, Chiba), Tanaka 9911

(MBK), AB457816, AB457819, AB457822, AB457825, AB457828, AB457831, AB457834, AB457837, AB457840.

*Rosa clinophylla* var. *glabra*—Myanmar: Kachin, Myitkyina, Murata & al. 041319 (MBK, TI), AB457817, AB457820, AB457823, AB457826, AB457829, AB457832, AB457835, AB457838, AB457841.

*Rosa bracteata*—Japan: Okinawa, Iriomote Isl. (cult. in Makino Botanical Garden, Kochi), Tanaka 9908 (MBK), AB457815, AB457818, AB457821, AB457824, AB457827, AB457830, AB457833, AB457836, AB457839.