J. Plant Biol. (2009) 52:411–416
DOI 10.1007/s12374-009-9052-8

ORIGINAL RESEARCH

Phylogenetic Position of *Corchoropsis* Siebold & Zucc. (Malvaceae s.l.) Inferred from Plastid DNA Sequences

Hyosig Won

Received: 25 May 2009 / Revised: 30 May 2009 / Accepted: 2 June 2009 / Published online: 26 June 2009

Abstract Recent phylogenetic research suggests that Malvaceae s.l. comprises formerly Tiliaceae, Byttneriaceae, Bombacaceae, and Sterculiaceae. *Corchoropsis* is traditionally included in Tiliaceae or Sterculiaceae and is distributed in China, Korea, and Japan. One to three species have been recognized for this genus. Phylogenetic relationships among the Malvacean taxa have been intensively studied with molecular data, and the evolution of their morphological characteristics has been reinterpreted accordingly. However, no *Corchoropsis* species have been included for their phylogenetic position. Here, three chloroplast coding regions—*rbcL*, *atpB*, and *ndhF*, from *Corchoropsis psilocarpa* and *Corchoropsis crenata*—were amplified and sequenced, then compared with other Malvacean taxa. This analysis of the three plastid gene sequences now places *Corchoropsis* species in Dombeyoideae, as previously proposed by Takeda (Bull Misc Inform Kew 365, 1912), Tang (Cathaya 4:131–150, 1992), and Bayer and Kubitzki (2003). Within Dombeyoideae, *Corchoropsis* forms a strongly supported sister relationship with the *Dombeya–Ruizia* clade.

Keywords *Corchoropsis* · Dombeyoideae · Malvaceae s.l. · Molecular systematics

Molecular phylogenetic studies and subsequent reinterpretation of morphological characteristics have changed fundamentally our understanding of many taxonomic groups and their phylogenetic relationships and evolution. In the case of Malvaceae s.l., this family now includes taxa formerly placed separately into Sterculiaceae, Bombacaceae, Tiliaceae, or Malvaceae. It is subdivided into nine subfamilies: Bombacoideae, Brownlowioideae, Byttnerioideae, Dombeyoideae, Grewioideae, Helicteroideae, Malvoideae, Sterculioideae, and Tilioideae (Alverson et al. 1998, 1999; Bayer et al. 1999; Bayer and Kubitzki 2003; Whitlock et al. 2003; Nyffeler et al. 2005). Of these, Bombacoideae and Malvoideae are often united and called Malvatheca (Baum et al. 2004). This subfamilial classification of Malvaceae s.l. has resulted from separate analyses of plastid *rbcL* (Alverson et al. 1998; Bayer et al. 1999), *atpB* (Bayer et al. 1999), *ndhF* (Alverson et al. 1999), and *matK* (Nyffeler et al. 2005), as well as their combined study (Bayer et al. 1999; Nyffeler et al. 2005).

Corchoropsis Siebold & Zucc. is distributed only in China, Korea, and Japan, and just one to three species with a few varieties have been recognized (Gilg and Loesener 1905; Nakai 1914; Chang 1986; Chiu and Zhong 1988; Tang 1994; Lee 1996; Tang et al. 2007; Kim 2007). The *Corchoropsis* taxa are annual herbs, characterized by simple leaves, solitary flowers in leaf axils, three caducous epicalyx bracts, five persistent sepals basally fused, five yellow deciduous petals, (5–)15 stamens in a staminal tube, five oblanceolate staminodes, a three-locular ovary, loculicidally dehiscent capsules, and two-lobed cotyledons (Tang 1992; Bayer and Kubitzki 2003; Kim 2007; Tang et al. 2007).

Its phylogenetic position has been traditionally treated as a member of Tiliaceae or Sterculiaceae. When the genus was first recognized by Siebold and Zuccarini in 1843, it was included in Tiliaceae. However, Takeda (1912) transferred *Corchoropsis* to Dombeyeae (Sterculiaceae) because it shared more floral characteristics with *Paradombeya* and *Pentapetes* than *Corchorus,* even though the external appearance of *Corchoropsis* was similar to

H. Won (✉)
Department of Biological Science, Daegu University,
15 Naeri-ri, Jillyang,
Gyeongsan, Gyeongbuk 712-714, Republic of Korea
e-mail: wonhs@daegu.ac.kr

Corchorus. Tang (1992) also suggested a close affinity between *Corchoropsis* and Dombeyeae (Sterculiaceae), in that they shared many morphological, anatomical, palynological, and cytological characteristics, e.g., axillary flowers, definite stamens, filaments, and staminodes fused at the base, a fruit capsule, two-partite or two-lobed cotyledons, a spiny pollen exine, and a base number of chromosome of $x = 10$. Bayer and Kubitzki (2003) placed *Corchoropsis* in Dombeyoideae (Malvaceae s.l.), suggesting a close relationship with a Malagasy/African *Dombeya* alliance. Nevertheless, no previous molecular phylogenetic study has included *Corchoropsis*.

Here, DNA sequences for three plastid regions—*rbcL*, *atpB*, and *ndhF*—were obtained from *Corchoropsis* and analyzed to resolve its phylogenetic position, thereby testing the hypothesis for this that had been proposed by Takeda (1912), Tang (1992), and Bayer and Kubitzki (2003).

Materials and Methods

Plant Materials and Herbarium Study

Corchoropsis samples for DNA extraction were mainly collected from the field. A few herbarium specimens of *Corchoropsis psilocarpa* were also used for DNA extraction because only one fresh sample of that species was available. Specimens from the Korea National Institute of Biological Resources (KB), Korea National Arboretum (KH), Seoul National University Herbarium (SNU), Kangwon National University Herbarium (KWNU), Kyungpook National University Herbarium (KNU), and Yeungnam University Herbarium (YNUH) were examined for proper identification and to determine their geographic distribution. Voucher information for these extracted DNAs is presented in Table 1.

DNA Extraction, PCR Amplification, and Sequencing

DNA was extracted from fresh or herbarium samples according to the user manual for the Qiagen Plant DNeasy mini kit (Qiagen, Valencia, CA, USA), with extra AP1 buffer added to dilute the mucilaginous material in the ground tissue extracts. Quality and quantity of the extracted DNA were verified under an UV illuminator after electrophoresis with an EtBr-containing 1% agarose gel.

To amplify and sequence the chloroplast *rbcL* and *atpB* regions, primer sets described by Fay et al. (1998; 1F, 724F, 724R, and 1460R) and Hoot et al. (1995; S2, S1494R, 611F, 766R), respectively, were applied. Some modification was made to *atpB*_S1494R (5′-TAA GTA CGC AAA GAT TTA AGG TCA T-3′, where underlined bases indicate changes). For the *ndhF* region, primer sets from Olmstead and Sweere (1994; 1F, 1318F, 1603F, 1318R, 2110R) and Alverson et al. (1999; 536F, 536R, 972F, 972R) were used. To match the sequences of Malvales, *ndhF*_1F was modified to 5′-ATG GAG CAK RCA TAT CAA TAT TC-3′, *ndhF*_1318F to 5′-GGA TTA ACA GCA TTT TAT ATG TTT CG-3′, and *ndhF*_1603F to 5′-CCT CAK GAA TCR GMY AAT ACT ATG C-3′. In addition, the *ndhF*_1651R primer (5′-TCC AAC AAA TAA AGT AAA TAG-3′) was newly designed based on the *ndhF*_1655R of Olmstead and Sweere (1994). PCR protocols followed those of Won and Renner (2005) and Park and Jansen (2007).

Amplified PCR products were purified with a QIAquick PCR product purification kit or a QIAquick gel extraction kit after electrophoresis on an EtBr-containing 1% agarose gel. These purified products were sequenced using an ABI Prism Big Dye Terminator cycle sequencing Ready Reaction kit (ver. 3.1; Perkin Elmer), and the same PCR primers were applied according to the user manual. Sequencing products were purified by following the sodium acetate–EDTA–ethanol purification method of the ABI Prism Big Dye Terminator cycle sequencing Ready Reaction kit, then sent for sequencing to the Biomedic Company, Bucheon, Kyunggi, Korea (www.ibiomedic.co.kr).

The resulting chromatograms were verified and checked under Sequencher (ver. 4.7; Genecodes), and contigs were generated. Consensus sequences of the *Corchoropsis* samples were compared with those of Bayer et al. (1999; *rbcL* and *atpB*), Alverson et al. (1999; *ndhF*), Whitlock et al. (2003; *ndhF*), and Nyffeler et al. (2005; *ndhF*). Afterwards, new data matrices of *rbcL*, *atpB*, and *ndhF* sequences were generated by adding the newly obtained *Corchoropsis* sequences to the existing data matrices. Likewise, a few extra sequences of *Malvaceae* s.l. taxa were added to the new data matrices from GenBank. To further analyze the phylogenetic relationships within Dombeyoideae, cp *rbcL*, *atpB*, and *ndhF* sequences from 29 representative taxa of Malvaceae s.l. (including most of the Dombeyoideae) were combined. This was done because *Corchoropsis* was unanimously included in the Dombeyoideae based on individual analyses of those *rbcL*, *atpB*, and *ndhF* sequences. These data matrices are available from the author upon request.

Phylogenetic Analyses

The newly generated data matrices of cp *rbcL*, *atpB*, and *ndhF* sequences and the combined matrix for Malvaceae s.l. were analyzed under parsimony optimization. The combined matrix was further analyzed under maximum likelihood optimization and Bayesian inferences. Parsimony and maximum likelihood analyses were conducted using PAUP* 4.0b10 (Swofford 2002), whereas Bayesian phylo-

genetic analyses relied on MrBayes 3.0b4 (Huelsenbeck and Ronquist 2001). Parsimony analyses employed heuristic searches with 100 random taxon addition replicates, holding 100 trees at each step, and tree bisection–reconnection (TBR) branch swapping, and with the options MulTrees on, collapse if maximum length is zero, and steepest descent on, without an upper limit for trees held in memory. Nonparametric bootstrap support was obtained by re-sampling the data 1,000 times with the same search options and model, except that the closest taxon addition was used and the maximum number of trees was set to 100.

Maximum likelihood analyses were conducted in PAUP*. Bayesian analyses employed four Markov chain Monte Carlo chains run for 300,000 generations, using random trees as the starting point, and sampling every 100th generation. Trees sampled before the saturation of the ML estimate were discarded as burn-in. Best-fitting models for maximum likelihood and Bayesian analyses were estimated via Modeltest 3.7 (Posada and Crandall 1998), with parameter values estimated simultaneously from the data, including the proportion of invariable sites and the shape parameter of the gamma distribution modeled with four rate categories.

Results

GenBank accession numbers for the newly obtained plastid DNA sequences (FJ848893–FJ848902, FJ848908–FJ848912) are presented in Table 1. Alignment of the cp *rbcL*, *atpB*, and *ndhF* sequences for *Corchoropsis* with the previously reported data matrices was straightforward. Plastid *rbcL* sequences introduced no gaps, whereas the *atpB* sequences had one 3-bp-long gap and the *ndhF* sequences showed several gaps of 3 to 21 bp. These were similar to those reported by Bayer et al. (1999), Alverson et al. (1999), and Nyffeler et al. (2005). Results of the parsimony analyses for these three cp regions are summarized in Table 2. The parsimony analysis of cp *atpB* sequences was incomplete, due to memory limit of the PAUP* program. Among the three cp regions, *ndhF* sequences had the largest portion of variable sites (43.8%), while *rbcL* had the largest portion of phylogenetically informative sites (27.4%).

Maximum parsimony analysis of the combined Malvaceae s.l. data resulted in four maximum parsimonious trees (Table 2). Modeltest selected the TVM+G+I model as the best-fit model, with a gamma distribution shape parameter of α=0.9006 and proportions of invariant sites=0.6251. This TVM+G+I model is identical to the GTR+G+I model, except that the two transition (A↔G and T↔C) rate parameter values are set identical to each other, making one parameter less. The likelihood score of the obtained maximum likelihood tree was $-\ln L$=13,218.35398. Because the TVM+G+I model is not available for MrBayes, the GTR+G+I model was employed for Bayesian analysis. The first 200 tree records from that analysis were discarded as burn-in, and the remaining 2,800 were combined into a majority rule consensus tree.

Parsimony analyses of the individual plastid data sets produced a consistent phylogenetic position for *Corchoropsis* within Dombeyoideae (data not shown, but available upon request to the author). The results of maximum parsimony, maximum likelihood, and Bayesian analyses of the combined data matrix are presented in Fig. 1. They are congruent with those of the individual analyses for the three plastid regions: (1) monophyly of Dombeyoideae is supported by a strong parsimony bootstrap value (96%) and Bayesian posterior probability (1.00); (2) *Corchoropsis* is a sister to the *Dombeya–Ruizia* clade, and this relationship is strongly supported by a 98% parsimony bootstrap value and 1.00 Bayesian posterior probability; (3) the *Pterospermum–Schoutenia* clade, *Burretiodendron*, and *Excentrodendron* form a grade or clade sister to the *Dombeya–Corchoropsis* clade; (4) *Nesogordonia* is sister to the rest of Dombeyoideae; (5) monophyly of *Corchoropsis* is supported by a 100% bootstrap value and 1.00 posterior probability; and (6) monophylies of two *Corchoropsis* species are also strongly supported (98% and 100% bootstrap values and 1.00 posterior probability).

Table 1 Voucher information and GenBank accession numbers for the plastid sequences of *Corchoropsis* taxa newly obtained in this study

Taxa	Voucher	Locality	*rbcL*	*atpB*	*ndhF*
Corchoropsis psilocarpa Harms & Loesn.	Won et al. 1880 (DGU)	Youngcheon, Gyeongbuk, South Korea	FJ848893	FJ848899	FJ848908
C. psilocarpa	JS Kim KJS070085 (KH)	Euiseong, Gyeongbuk, South Korea	FJ848894	FJ848898	FJ848909
C. crenata Siebold & Zucc.	Won et al. 1859 (DGU)	Milyang, Gyungnam, South Korea	FJ848895	FJ848900	FJ848910
C. crenata	Won et al. 1943 (DGU)	Gimhae, Gyungnam, South Korea	FJ848896	FJ848901	FJ848911
C. crenata	Won et al. 2042 (DGU)	Jinhae, Gyungnam, South Korea	FJ848897	FJ848902	FJ848912

DGU Department of Biological Science Herbarium, Daegu University, Gyeongsan, Gyeongbuk, South Korea; *KH* Korea National Arboretum Herbarium, Pocheon, Gyeonggi, South Korea

Table 2 Summary of independent and combined parsimony analyses of plastid *rbcL*, *atpB*, and *ndhF* gene sequences from Malvaceae s.l.

Data set	*rbcL*	*atpB*[a]	*ndhF*	Combined
Aligned length	1,470	1,499	2,288	5,257
Number of sequences	114	85	77	29
Number of characters analyzed	1,408	1,374	2,288	5,034
Number of variable sites				
All	532 (37.8%)	484 (35.2%)	1,003 (43.8%)	692 (13.7%)
Only among *Corchoropsis*	2	2	8	12
Number of informative sites				
All	386 (27.4%)	331 (24.1%)	579 (25.3%)	292 (5.8%)
Only among *Corchoropsis*	2	2	8	12
Number of MP trees obtained	17,578	80,900	7,873	4
Length of MP tree	2,124	1,359	2,216	1,005
CI (excl. uninformative sites)	0.3099	N/A	0.5175	0.6065
RI (excl. uninformative sites)	0.6808	N/A	0.6976	0.8181

For the *rbcL* and the *atpB* sequence data matrices, those of Bayer et al. (1999) were used. For the *ndhF* sequence data matrix, those of Alverson et al. (1999) and Nyffeler et al. (2005) were used. For the combined analysis, only representatives of Malvaceae s.l. were included

N/A not available

[a] Parsimony analysis was incomplete because the memory limit was met during optimization

Discussion

These molecular phylogenetic analyses of three plastid region sequences now place *Corchoropsis* within the Dombeyoideae of Malvaceae s.l., as had been proposed by Takeda (1912), Tang (1992), and Bayer and Kubitzki (2003). The diagnostic characteristics of Dombeyoideae members that distinguish them from other Malvaceae are flowers either in axillary cymes or solitary; presence of an epicalyx; petals often persistent and flat; cotyledons usually bifid; pollen usually spheroidal and spinose; stamens usually forming a staminal tube; and staminodes usually integrated in the staminal tube, entire, usually linear to ovate, and rarely absent.

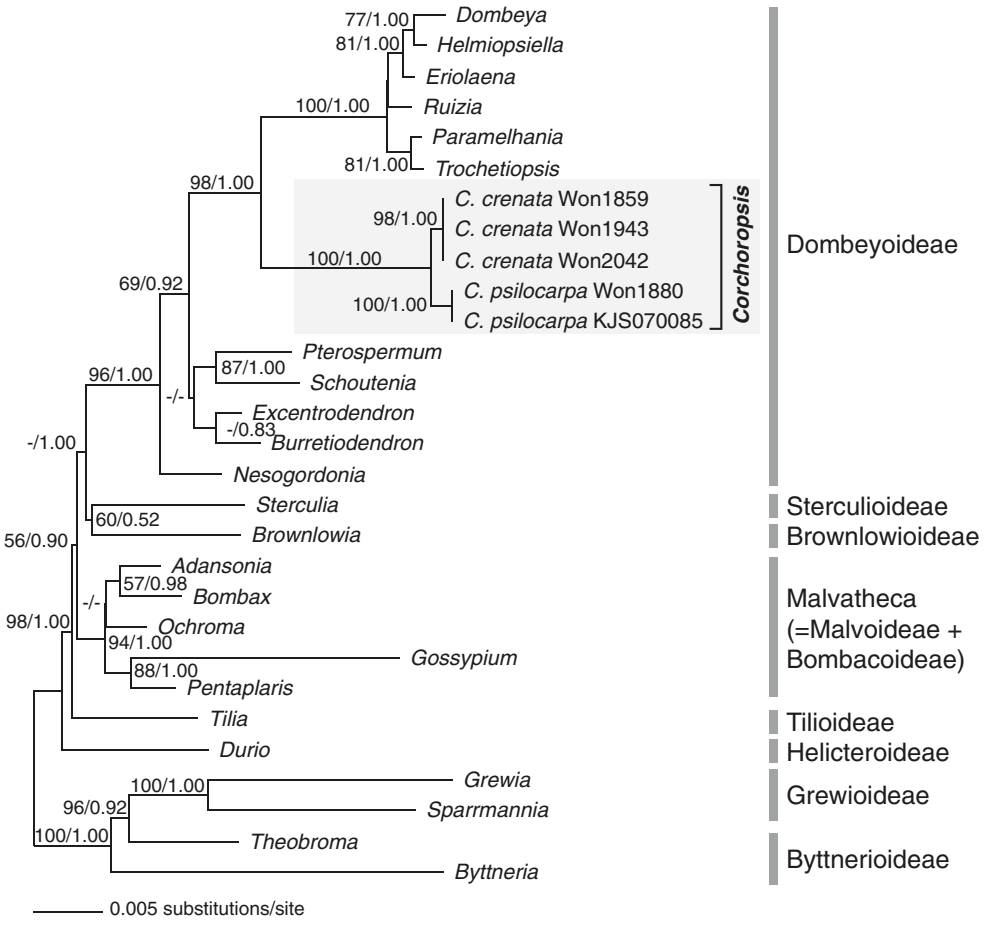

Fig. 1 Maximum likelihood tree obtained from phylogenetic analyses of combined plastid *rbcL*, *atpB*, and *ndhF* sequences from 29 Malvaceae s.l. samples. *Numbers on the branch* indicate parsimony bootstrap value, with Bayesian posterior probability after the *slash*

Corchoropsis differs from other Dombeyoideae genera in that it is an annual herb and has solitary flowers; yellow, deciduous petals; and linear capsules (Tang 1992; Bayer and Kubitzki 2003).

Current phylogenetic analyses support the phylogenetic relationships among the Dombeyoideae genera. Bayer and Kubitzki (2003) previously suggested a close relationship for Asian genera—*Corchoropsis*, *Eriolaena*, *Pentapetes*, and *Paradombeya*—with the Malagasy/African *Dombeya* alliance, whereas the other Asian genera, such as *Burretiodendron*, *Pterospermum*, *Schoutenia*, and *Sicrea*, differed from the latter. *Dombeya* is the largest genus of the Dombeyoideae, with about 200 species, mainly in Madagascar. It is included in the *Dombeya–Ruizia* clade according to both current and previous molecular phylogenetic studies (Alverson et al. 1999; Bayer et al. 1999; Nyffeler et al. 2005). *Helmiopsiella*, *Eriolaena*, *Ruizia*, *Paramelhania*, and *Trochetiopsis* comprise the remainder of the *Dombeya–Ruizia* clade, with *Eriolaena* being distributed in Asia while the others are found in Madagascar and nearby areas. *Corchoropsis* is a sister to the *Dombeya–Ruizia* clade and is distributed in NE Asia, i.e., China, Korea, and Japan. Meanwhile, the Asian genera *Pterospermum* (tropical Asia, from India to Taiwan and the Philippines, then southwards to Lesser Sunda Islands), *Schoutenia* (Indochina, Thailand, Malesia to Lesser Sunda Islands), and *Burretiodendron* (including *Excentrodendron*; SW China, North Vietnam, Myanmar, Thailand) forms a clade or grade sister to the *Corchoropsis–Dombeya* clade. The genus branching basal to the rest of Dombeyoideae is *Nesogordonia*, distributed in Madagascar and Africa. These geographic distributions and phylogenetic relationships among the Dombeyoideae genera suggest that the group dispersed independently into Asia from Malagasy/Africa several times. However, complete taxonomic sampling and a detailed biogeographic analysis are required to test this hypothesis and clarify the phylogenetic relationships among the *Dombeya–Ruizia* clade. For example, Skema (2008) has suggested that, based on preliminary parsimony analysis of chloroplast and nuclear markers, *Dombeya* is not monophyletic because *Paramelhania* and *Trochetiopsis* are nested within it, albeit with low bootstrap support.

Finally, these phylogenetic relationships should be investigated in conjunction with pollination biology. For example, some Dombeyoideae exhibit secondary pollen presentation, *Trochetiopsis* presents pollen on the petal tips (Brodie et al. 1998), and *Dombeya* species and *Pentapetes* have pollen on the staminodes (Yeo 1993; Bayer and Kubitzki 2003). *Corchoropsis* also holds its pollen on staminodes (personal observation), all of which demonstrating that four members of the *Dombeya–Corchoropsis* clade share similar characteristics for secondary pollen presentation.

Acknowledgments In addition to the gratitude for the two anonymous reviewers who commented on this manuscript, the author thanks the Korea National Institute of Biological Resources (KB), Korea National Arboretum (KH), Seoul National University Herbarium (SNU), Kangwon National University Herbarium (KWNU), Kyungpook National University Herbarium (KNU), and Yeungnam University Herbarium (YNUH) for their help, especially KH for their valuable herbarium material. This work was supported by a research grant from Daegu University.

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