



Phylogenetic biogeography of *Euphrasia* section *Malesianae* (*Orobanchaceae*) in Taiwan and Malesia

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Key words

biogeography
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phylogeny
rps2 gene sequence
trnL intron sequence
trnL-trnF intergenic spacer sequence

Abstract Species of *Euphrasia* are distributed in both hemispheres with a series of connecting localities on the mountain peaks of Taiwan and the Malesian region including Luzon, Borneo, Sulawesi, Seram and New Guinea. Two hypotheses are proposed to explain this distribution pattern. The Northern Hemisphere might have been the centre of origin or the Southern Hemisphere. This study aims to reconstruct the core phylogeny of *Euphrasia* in the connecting areas and tries to identify the migratory direction of *Euphrasia* in Taiwan and Malesia. The phylogeny of *Euphrasia*, including sections *Euphrasia*, *Malesianae* and *Pauciflorae*, is reconstructed with the chloroplast molecular markers *rps2* gene, *trnL* intron and *trnL-trnF* intergenic spacer. The results suggest that the migratory direction between Taiwan and the Philippines is possibly from the north to the south. However, the migratory direction within section *Malesianae* and the centre of origin of *Euphrasia* remain unanswered from our data. More data is needed to clarify this issue.

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INTRODUCTION

Euphrasia contains about 170 species and 14 sections, each with a typical distributional area (Table 1; Barker 1982: f. 19). The section *Euphrasia* comprises about 90 species widely distributed in the northern temperate areas. Section *Atlanticae* has two species restricted to the Azores. Section *Malesianae* contains about 13 species distributed in Taiwan, the Philippines, Borneo and Seram (Ceram). Section *Pauciflorae* holds about 14 species in the mountain zone of Sulawesi (Celebes), New Guinea and New Zealand. Section *Cuneatae* has two disjunct species in New Zealand and eastern Australia. The sections *Australes*, *Lasiantherae*, *Phragmostomae*, *Scabrae* and *Striatae*, comprising together about 18 species, are endemic to Australia. Sections *Anagosperrae* and *Novaezeelandiae*, eight species, are restricted to New Zealand. The monotypic section *Paradoxae* is endemic to the Juan Fernandez Islands. Section *Trifidae* comprises 18 species restricted to the Andes. Hence the sectional diversity centre is in the Southern Hemisphere and the distribution pattern of the genus is bi-hemispherical with a series of intermediate species on the mountain peaks of Taiwan and Malesian region.

Such a distribution pattern has inspired two theories to explain the possible dispersal mechanism. One theory is that the genus is old and the pattern could have been caused by migration from various places of origin through ancient land bridges (Von Wettstein 1896, Du Rietz 1932, 1948, Van Steenis 1971, Barker 1982). The other theory is that the genus could be young and the pattern could have been the result of long distance dispersal (Raven & Axelrod 1972, Raven 1973). However, recent studies on most plants in Malesia support the first theory (Morley 1998, Van Welzen et al. 2005). As to the possible centre of origin of *Euphrasia*, the first theory considers the Southern Hemisphere

as the centre of origin (Von Wettstein 1896, Van Steenis 1962, Barker 1982), while the other theory considers it to be in the Northern Hemisphere (Raven & Axelrod 1972, Raven 1973).

The Malesian region, or the Malay Archipelago, is a natural phytogeographic area with endemic species up to 70 % (Van Steenis 1950, Van Welzen et al. 2005). In the Malesian region the genus *Euphrasia* is distributed on a series of mountain peaks in Taiwan, Luzon, Borneo, Sulawesi, Seram, New Guinea and New Zealand (Fig. 1). These species belong to sections *Malesianae* and *Pauciflorae*. Seven species and one variety in section *Malesianae*, from the isolated alpine areas of Taiwan, Luzon, Borneo and Seram, were recently studied (Barker 1982, Wu & Huang 1998, 2004). Several geographical boundaries were drawn between these islands. The first boundary is between Taiwan and Luzon and forms the northern limit of the Malesian region (Van Steenis 1950). The second one is the Merrill-Dickerson/Huxley line (a variant of Wallace's line) between Luzon and Borneo. The third one is Wallace's line and separates Seram from the Philippines and Borneo. These geographical lines may be tested through phylogenetic studies.

In Taiwan, three species and one variety in section *Malesianae* were recognized based on morphology (Wu & Huang 1998).

Table 1 Distribution of *Euphrasia* which is separated into 14 sections (modified from Barker 1982: f. 19).

Section	Number of species	Distribution
<i>Anagosperrae</i>	4	New Zealand
<i>Atlanticae</i>	2	Azores
<i>Australes</i>	5	Australia
<i>Cuneatae</i>	2	New Zealand, Australia
<i>Euphrasia</i>	± 90	northern temperate areas
<i>Lasiantherae</i>	3	Australia
<i>Malesianae</i>	± 13	Taiwan, Philippines, Borneo, Seram
<i>Novaezeelandiae</i>	4	New Zealand
<i>Paradoxae</i>	1	Juan Fernandez Islands
<i>Pauciflorae</i>	± 14	Sulawesi, New Guinea, New Zealand
<i>Phragmostomae</i>	1	Australia
<i>Scabrae</i>	5	Australia
<i>Striatae</i>	4	Australia
<i>Trifidae</i>	18	the Andes

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Fig. 1 Distribution of *Euphrasia* sections *Malesianae* (★) and *Pauciflorae* (●) in Taiwan and Malesia.

However, it was impossible to distinguish these morphological taxa with the aid of molecular markers of the chloroplast DNA (cpDNA) such as the intron of *trnL* gene and intergenic spacer of *trnL-trnF*, and the nuclear DNA (nrDNA) intergenic spacer (ITS) (Wu & Huang 2004, Wu et al. 2005). The species were also indistinguishable by pollen and seed morphology. They share tricolpate and spheroidal pollen grains and seed coats with several longitudinal ridges with numerous perpendicular small scalariform ridges (Wu 2004). The basic chromosome number is always $n = 11$ (Wu 2004), which also occurs within the sections *Euphrasia*, *Pauciflorae* (Barker 1982) and *Australes* (Barker et al. 1988). Morphologically, section *Malesianae* closely resembles sections *Euphrasia* and *Atlanticae* of the Northern Hemisphere, and they may be derived from the same ancestor as section *Pauciflorae*, which is distributed from Malesia to New Zealand (Barker 1982: f. 22). This suggests that the Taiwanese species could have been derived from the south, probably from the Philippines. A recent study on *Oreomyrrhis* (*Apiaceae*), which has a similar distribution pattern as *Euphrasia*, suggests a

long distance dispersal mechanism, because the genus seems young, and the migratory direction was from Australia to the north in the Old World based on a nrITS haplotype network (Chung et al. 2005). However, the larger part of the temperate vascular flora in Taiwan is derived from continental Asia (Hsieh 2003), thus apparently *Oreomyrrhis* with its Malesian derivation is atypical. Therefore, it is interesting to know from which region *Euphrasia* species migrated to Taiwan.

In this paper we investigate whether the *Euphrasia* species in Taiwan are derived from Malesian species (like *Oreomyrrhis*), or migrated from continental Asia as most temperate species in Taiwan. The two scenarios will be tested with the phylogeny of sections *Euphrasia*, *Malesianae* and *Pauciflorae*. The haplotypes should either be basal to the ones found in section *Euphrasia* (southern origin) or the haplotypes of sections *Malesianae* and *Pauciflorae* are derived from those of section *Euphrasia* (northern origin).

Table 2 *Euphrasia* species used for sequencing and their haplotypes (*rps2*, *trnL-trnF*, *rps2* & *trnL-trnF*) recognized in this study.

Taxa	Distribution	<i>rps2</i>	<i>trnL-trnF</i>	<i>rps2</i> & <i>trnL-trnF</i>	GenBank accession number
<i>Bartsia alpina</i>	Northern hemisphere	Ba	–	–	U48751
<i>Parentucellia viscosa</i>	Europe	Pv	–	–	U48753
<i>Tozzia alpina</i>	Europe	Ta	–	–	U48754
Section <i>Malesianae</i>					
<i>E. borneensis</i>	Borneo	A	M, N	AM, AN	FJ600675, 676, 682
<i>E. nankotaizanensis</i>	Taiwan	C	C, I	CC, CI	AY512697, 698, 711, 712, 720–723, 733, 734, 751, 752, FJ600687
<i>E. philippinensis</i>	Philippines	C	L	CL	FJ600674, 684
<i>E. tarokoana</i>	Taiwan	C	G	CG	AY512702–705, 775–778, FJ600688
<i>E. transmorisonensis</i> var. <i>transmorisonensis</i>	Taiwan	C	A, B, C, D, E, F	CA, CB, CC, CD, CE, CF	AY512684–687, 689–691, 693, 695, 696, 699–701, 706–710, 713–719, 727–730, 738, 739, 741–750, 753–769, 772–774
<i>E. transmorisonensis</i> var. <i>duritziana</i>	Taiwan	B, C	K A, C, H, J,	BK, CA, CC, CH, CJ	AY512681–683, 688, 692, 694, 724, 726, 731, 732, 735–737, 740, 770, 771, FJ600673
Section <i>Pauciflorae</i>					
<i>E. mirabilis</i>	Papua New Guinea	D	O	DO	FJ600672, 690
Section <i>Euphrasia</i>					
<i>E. hirtella</i>	Georgia	E	Y	EY	AY831432, FJ600689
<i>E. maximowiczii</i>	Japan	E	R	ER	FJ600677, 691
<i>E. officinalis</i>	UK	G	–	–	FJ600676
<i>E. petiolaris</i>	Georgia	H	X	EX	AY831431, FJ600697
<i>E. picta</i>	Austria	E	P	EP	FJ600679, 680
<i>E. spectabilis</i>	Austria	F	Q	FQ	FJ600678, 695
<i>E. stricta</i>	Denmark	E	P	EP	FJ600681, 693
<i>E. subarctica</i>	Canada	E	–	–	FJ600694

MATERIAL AND METHODS

Plant material and DNA sequencing

The sampled species cover sections *Malesianae*, *Euphrasia* and *Pauciflorae* (Table 2). Based on the *rps2* gene, 15 taxa of *Euphrasia* and 10 outgroups from *Orobanchaceae* and *Scrophulariaceae* were used to reconstruct the phylogeny. DePamphilis et al. (1997) found that *Bartsia alpina*, *Parentucellia viscosa* and *Tozzia alpina* were the most closely related taxa to *Euphrasia*. Therefore, *Bartsia*, *Parentucellia* and *Tozzia* were used as outgroups in our analysis (Table 2). Based on *trnL-trnF* sequences, which includes the partial *trnL* gene, its intron and the spacer between the genes *trnL* and *trnF*, a total of 110 samples from Taiwan (part of them based on the data of Wu et al. 2005), 8 samples from Sabah, Borneo, 6 samples from Papua New Guinea, 5 samples from the Philippines, 1–3 samples of 7 species that belong to the section *Euphrasia*, were analyzed.

Fresh leaves of the Taiwanese species and silica-gel-dried leaves were obtained of *E. borneensis*, *E. maximowicziana*, *E. mirabilis*, *E. philippinensis*, *E. picta* and *E. stricta*. The other leaf material was collected from herbarium specimens. Voucher specimens are listed in Table 2.

The protocol of Doyle & Doyle (1990) was applied to extract DNA using DNeasy plant mini kit (QIAGEN). The extracted DNA was then amplified by the polymerase chain reaction (PCR). The three cpDNA markers were used in this study, i.e., gene of *rps2*, intron of *trnL* gene, and intergenic spacer of *trnL-trnF*. The primers for *trnL-trnL* intron were 5'-CGA AAT CGG TAG ACG CTA CG-3' in forward and 5'-GGG GAT AGA GGG ACT TGA AC-3' in reverse; and the primers for *trnL-trnF* intergenic spacer were 5'-GGT TCAAGT CCC TCTATC CC-3' in forward and 5'-ATT TGA ACT GGT GAC ACG AG-3' in reverse. The

primers for *rps2* were 5'-ACC CTC ACA AAT AGC GAA TAC CAA in forward (*rps2*-47F) and 5, CTC GTT TTT TAT CTG AAG CCT G in reverse (*rps2*-661R) (DePamphilis et al. 1997).

Thirty-five thermal cycles were used during amplification, with an annealing temperature of 54 °C for 30 seconds and an extension temperature of 72 °C for 60 seconds. The PCR products were then purified with a QIAGEN PCR purification kit and sequenced with a model 373A automatic sequencer (Applied Biosystems) using the Big Dye terminator.

Phylogenetic tree analysis

DNA sequences were examined by using Sequencher 4.1 (Gene Codes Corporation, Ann Arbor, Michigan, USA) and they were aligned by using MacClade 4 software (Maddison & Maddison 2001). The phylogeny of each gene marker was reconstructed with PAUP 4.10 (Swofford 2000) either using the criterion of maximum Fitch parsimony with heuristic search and TBR swapping algorithm, or using criterion of maximum likelihood with the HKY model. Measures of support for each clade were generated using bootstrap analysis (Felsenstein 1985) with a 1 000 replicates.

RESULTS

Based on the *rps2* gene, the strict consensus cladogram (Fig. 2) has a length 487 bps (base pairs) after alignment. No indels (insertion-deletions) were invoked. Thirty-five variation sites were found of which 20 were uninformative and 15 sites were informative (Table 3). Twenty four most parsimonious trees were found with a CI index of 0.9118. The *Euphrasia* clade was well supported by a bootstrap value (BS) of 100 %. The ingroup shows a polytomy with three species, *E. borneensis*, *E. mirabilis* and *E. petiolaris*, and two clades, the larger parts of

Fig. 2 Strict consensus cladogram of 24 most parsimonious cladograms of *Euphrasia* based on the *rps2* dataset. Numbers above the branches are the number of substitutions, and numbers below the branches are the bootstrap values equal or higher than 50 %. The haplotypes (Table 3) are shown in parentheses.

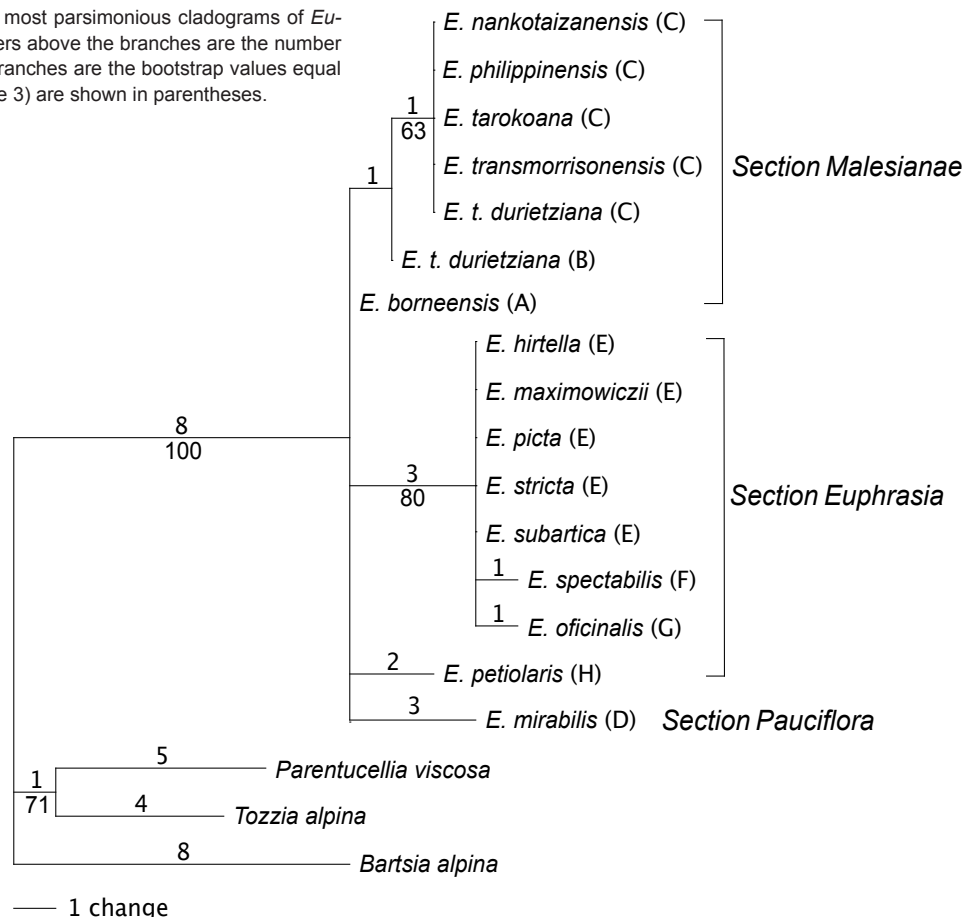


Table 3 Haplotypes (H*) of *Euphrasia* and three related species recognized in the *rps2* gene tree based on polymorphic sites. Ba = *Bartsia alpina*; Pv = *Paren-tucellia viscosa*; Ta = *Tozzia alpina*.

H*	Polymorphic site																																			
	0	0	0	0	0	1	1	1	1	1	1	1	1	2	2	2	2	2	2	2	2	2	2	2	2	3	3	3	3	3	3	3	4	4	4	4
Ba	G	G	A	A	C	C	T	A	C	T	G	A	T	C	G	G	A	C	G	G	C	G	A	G	A	C	T	G	G	C	T	C	A	A	C	
Pv	A	G	A	A	T	C	C	G	C	T	G	C	C	C	C	G	C	G	G	G	A	G	G	A	C	G	G	A	C	T	C	A	A	C		
Ta	A	G	C	A	T	C	C	G	C	T	G	C	T	C	G	A	C	G	G	A	A	C	G	A	A	C	G	G	G	C	T	A	A	C		
A	A	G	A	C	T	G	C	G	T	C	G	C	T	C	G	G	A	A	G	G	G	A	A	G	C	C	G	C	G	T	T	C	C	A	C	
B	A	G	A	C	T	G	C	G	T	C	G	C	T	T	G	G	A	A	G	G	G	A	A	G	A	C	G	C	G	T	T	C	C	A	C	
C	A	G	A	C	T	G	C	G	T	C	G	C	T	T	G	G	A	A	G	G	G	A	A	G	A	C	G	C	G	T	T	C	C	A	C	
D	A	G	A	C	T	G	C	G	T	T	C	C	T	C	G	G	A	A	G	G	G	A	A	G	A	T	G	C	G	T	T	C	C	A	C	
E	A	A	A	C	T	G	C	G	T	C	G	C	T	C	G	G	A	A	G	G	G	A	A	G	A	T	G	C	G	T	T	C	C	A	T	
F	A	A	A	C	T	G	C	G	T	C	G	C	T	C	G	G	A	A	G	G	G	A	A	G	A	T	G	C	G	T	T	C	C	C	T	
G	A	A	A	C	T	G	C	G	T	C	G	C	T	C	G	G	A	A	A	G	G	A	A	G	A	T	G	C	G	T	T	C	C	A	T	
H	A	G	A	C	T	G	T	G	T	C	G	C	T	C	G	G	A	A	G	G	G	A	A	G	C	C	G	C	G	T	T	C	C	A	C	

Table 4 *Euphrasia* haplotypes (H*) recognized in the *trnL-trnF* gene tree based on polymorphic sites.

H*	Polymorphic site																																			
	0	0	0	0	1	2	2	3	3	3	3	3	3	4	4	4	4	5	5	5	5	5	6	6	6	6	6	6	6	6	7	7	7	7	8	8
A	1	1	5	9	8	7	8	0	5	8	8	9	2	2	7	3	3	3	4	5	0	0	0	3	3	4	6	7	4	8	9	3	6			
B	3	5	7	6	4	5	5	0	1	3	4	2	2	5	5	4	6	7	4	6	0	4	5	5	7	5	1	2	5	6	5	9	8			
C	A	A	A	A	A	C	A	G	T	A	A	C	C	G	T	C	A	A	A	T	A	T	T	T	C	A	C	T	T	G	C	C	C			
D	A	A	A	A	A	C	A	G	T	A	A	C	C	G	T	C	A	A	A	T	A	T	T	T	C	A	C	T	T	G	C	C	C			
E	A	A	A	A	A	C	A	G	T	A	A	C	C	G	T	C	A	A	A	T	A	T	T	T	C	A	C	T	T	A	C	C	C			
F	A	A	A	A	A	C	A	G	T	A	A	C	C	G	T	C	A	A	A	C	A	T	T	T	C	A	C	T	T	G	C	C	C			
G	A	A	A	A	A	A	A	G	T	A	A	C	C	G	T	C	A	A	A	T	C	T	T	T	C	A	C	T	T	G	C	C	C			
H	A	A	A	A	A	C	A	G	T	A	A	C	C	G	T	C	A	A	C	T	C	T	T	T	C	A	C	T	T	G	C	C	C			
I	A	A	A	A	A	C	A	G	C	A	A	C	C	G	G	C	A	A	A	T	C	T	T	T	C	A	C	T	T	G	C	C	C			
J	A	A	A	A	A	C	A	G	T	A	A	C	C	G	T	C	A	A	A	T	C	T	T	T	C	A	C	T	T	G	C	C	G			
K	A	A	A	A	A	C	G	G	T	A	A	C	C	G	T	C	A	A	A	T	A	T	T	T	C	A	C	T	T	G	C	C	C			
L	A	A	C	A	A	C	G	G	T	A	A	C	C	G	T	C	A	A	A	T	A	T	T	G	C	A	C	T	T	G	C	C	C			
M	A	A	A	A	A	C	G	G	T	A	A	A	C	G	T	C	A	A	A	T	A	T	T	T	C	C	C	T	T	G	C	T	C			
N	A	A	A	A	A	C	G	G	T	A	A	A	C	G	T	C	A	A	A	T	A	T	T	T	C	C	C	T	T	G	C	C	C			
O	C	C	A	A	A	C	G	G	T	G	A	A	C	G	T	C	A	A	A	T	A	T	T	T	C	A	T	T	T	G	C	C	C			
P	A	A	A	C	C	C	G	G	T	G	G	A	C	A	T	C	A	A	A	T	A	T	T	T	C	A	C	T	T	G	C	C	C			
Q	A	A	A	C	C	C	G	T	T	G	G	A	C	A	T	C	C	A	A	T	A	T	T	T	C	A	C	T	T	G	C	C	C			
R	A	A	A	C	C	C	G	G	T	G	A	A	C	A	T	C	A	A	A	T	A	T	T	T	C	A	C	T	C	G	C	C	C			
S	A	A	A	C	C	C	G	G	T	G	A	C	A	T	C	A	T	A	T	A	T	T	T	T	A	C	T	T	G	C	C	C				
X	A	A	A	A	A	C	G	G	T	G	A	A	T	G	T	T	A	A	A	T	A	G	T	T	T	C	A	C	A	T	G	T	C	C		
Y	A	A	A	C	C	C	G	G	T	G	A	C	A	T	C	A	T	A	T	A	T	T	T	T	C	A	C	T	T	G	C	C	C			

section *Malesianae* and section *Euphrasia*. There were only two substitutions within the clade of section *Euphrasia*, with a further substitution in *E. officinalis* and another one in *E. spectabilis*. Two substitutions occurred in section *Malesianae*.

Based on the *trnL-trnF* dataset, the total length of the alignment was 903 bps when indels were excluded from analysis. Thirty three variation sites were found of which 21 were uninformative and 12 informative (Table 4). Fifteen most parsimonious trees with a CI = 0.9697 were found. *Euphrasia mirabilis* was selected as outgroup. The mark # is given in Fig. 3 when *E. transmorrisonensis* var. *transmorrisonensis* and *E. transmorrisonensis* var. *duritziana* share the same haplotype. The strict consensus cladogram (Fig. 3) shows a basal trichotomy of *E. petiolaris* and two clades, section *Malesianae* (BS 63) and the majority of section *Euphrasia* (BS 96). The topology of the tree is similar to the *rps2* gene tree (Fig. 2), except for *E. borneensis*, which is grouped in section *Malesianae* (Fig. 3) as sister species to the remaining taxa. While the Philippine species with haplotype L is nested inside the Taiwanese haplotypes.

The analysis of the combined *rps2* and *trnL-trnF* datasets (insufficiently known taxa were excluded, *E. mirabilis* as outgroup) showed a total alignment length of 1 400 bps (indels were excluded) and 36 variation sites (19 sites uninformative,

17 informative). The mark # is given in Fig. 4 when *E. transmorrisonensis* var. *transmorrisonensis* and *E. transmorrisonensis* var. *duritziana* share the same haplotype. Twelve most parsimonious trees were obtained (strict consensus cladogram in Fig. 4). Section *Euphrasia* formed a clade (BS 99), just like section *Malesianae* (BS 86). The maximum likelihood analysis resulted in a similar topology (data not shown) with both sections monophyletic. The Philippine haplotype was again clearly nested inside the Taiwanese species, although the relationship was not well resolved. In the section *Malesianae* clade *E. borneensis* is sister species to the remaining species of the clade, the next sister group relation is between *E. transmorrisonensis* var. *duritziana* (haplotype BK) and the others.

DISCUSSION

Migratory direction of *Euphrasia* between Taiwan and Malesia

Section *Malesianae* is monophyletic in the *trnL-trnF* gene tree (Fig. 3) and the combined tree (Fig. 4). This is not contradicted by the *rps2* gene tree (Fig. 2) that is partly unresolved (*E. borneensis* in a basal polytomy). *Euphrasia borneensis* from Borneo contains the most primitive haplotype of section *Malesianae*

Fig. 3 Strict consensus cladogram of 15 most parsimonious cladograms of *Euphrasia* based on the *trnL-trnF* dataset. Numbers above the branches are the number of substitutions, and numbers below the branches are the bootstrap values equal or higher than 50 %. The mark # indicates that *E. transmorrisonensis* var. *duritziana* shares the same haplotype. The haplotypes (Table 4) are shown in parentheses.

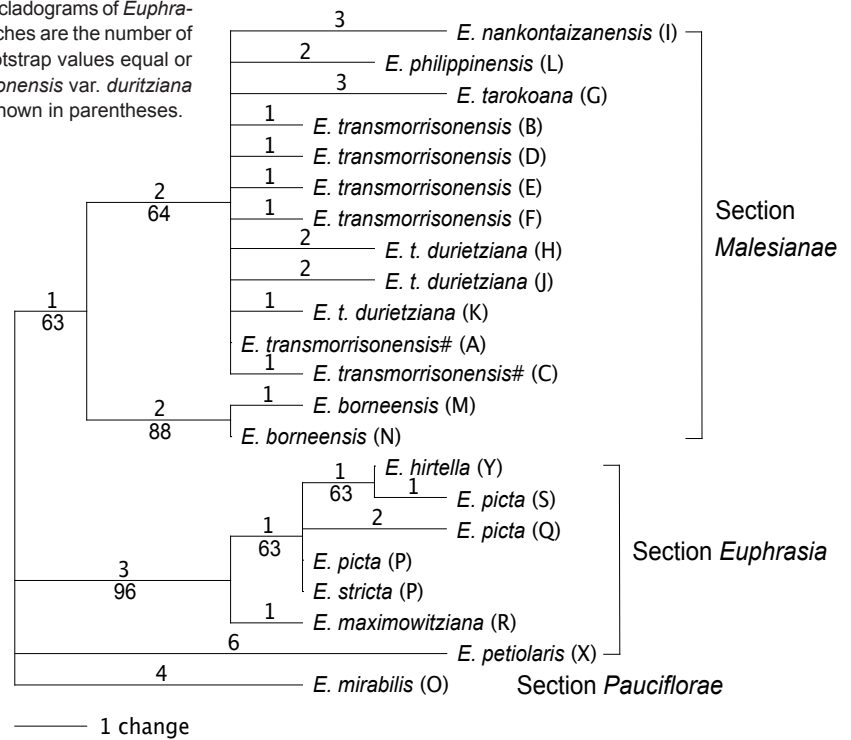
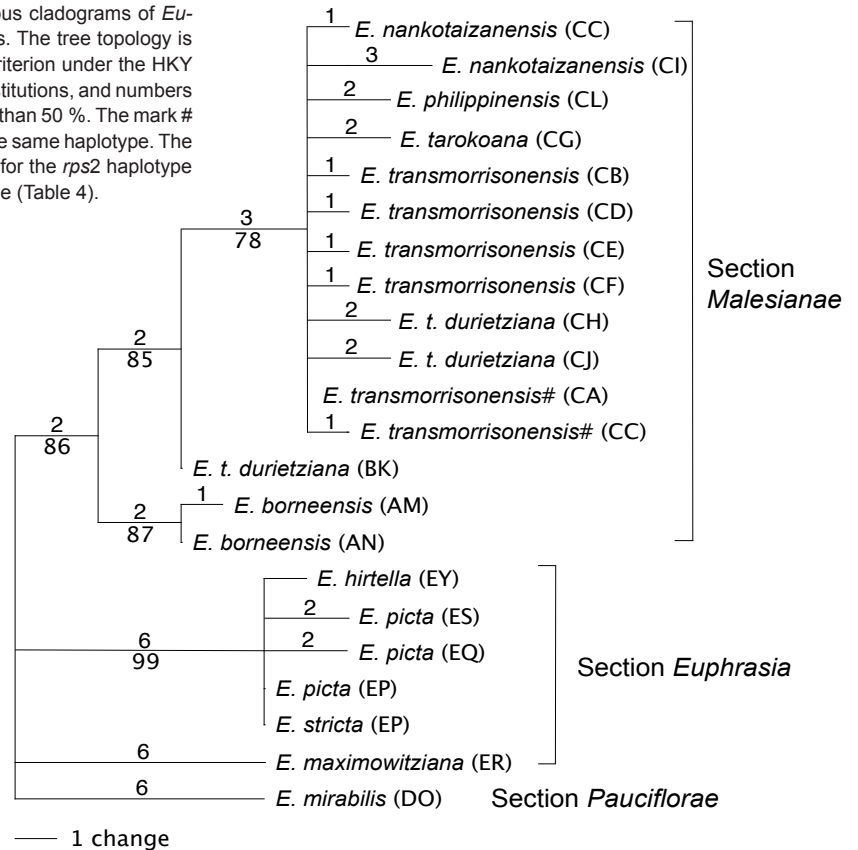


Fig. 4 Strict consensus cladogram of 12 most parsimonious cladograms of *Euphrasia* based on the combined *rps2* and *trnL-trnF* datasets. The tree topology is the same as those generated by the maximum likelihood criterion under the HKY model. Numbers above the branches are the number of substitutions, and numbers below the branches are the bootstrap values equal or higher than 50 %. The mark # indicates that *E. transmorrisonensis* var. *duritziana* shares the same haplotype. The haplotypes are shown in parentheses with the first position for the *rps2* haplotype (Table 3), and the second position for the *trnL-trnF* haplotype (Table 4).



in the *rps2* gene tree (Fig. 2) and the *trnL-trnF* and combined data gene trees. *Euphrasia borneensis* is sister species to the remaining species of section *Malesianae* (Fig. 3, 4). If we regard the relationships among sections *Euphrasia*, *Malesianae* and *Pauciflorae*, then section *Malesianae* has the same genetic distance both with *Euphrasia* and *Pauciflorae*. Because *Euphrasia transmorrisonensis* var. *duritziana* from Taiwan and *E. borneensis* from Borneo (both section *Malesianae*) have the same genetic distance in common with sections *Pauciflorae* and *Euphrasia*, the combined gene tree (Fig. 4) shows 10 substitu-

tions between sections *Malesianae* and *Pauciflorae*, and also between sections *Malesianae* and *Euphrasia*. Therefore, the migratory direction of the section *Malesianae* cannot be inferred from this result. However, the Philippine species contains haplotypes (C from *rps2* tree, CL from combined tree) that could be derived from *E. transmorrisonensis* var. *duritziana* (B from *rps2* tree, BK from combined tree), or from *E. transmorrisonensis* (C from *rps2* tree, CA from combined tree). Thus the migratory direction between Taiwan and the Philippines was possibly from the north to the south.

How the Taiwanese species connect with the Philippine species

Based on the combined data gene tree (Fig. 4), the haplotype of the Philippines (CL) differs from the closest Taiwanese haplotype (CA) in only two substitutions, while there are at least four substitutions between the haplotypes of the Borneo (AN) and Taiwan (BK). Therefore, isolation between Taiwanese and the Philippine species was probably for a relatively short period of time.

The Bashi Strait separates Taiwan from the Philippines. No land bridge ever occurred between both areas, because a trench is running through the strait. Thus the similarity in flora between these two areas should be due either to 1) long distance dispersal between Taiwan and the Philippines; or 2) the same ancestral population in continental Asia migrated to each area independently. The populations in continental Asia belong to section *Euphrasia* while the populations in Taiwan and the Philippines belong to section *Malesianae*. This does not support the idea of a shared ancestral population having migrated to each area separately. Our own data also suggests a migratory direction from Taiwan to the Philippines. Hence, the connection between Taiwan and the Philippines should be due to long distance dispersal.

The seeds of Taiwanese *Euphrasia* species are small but not minute, 1–1.5 mm long (Wu 2004). They might be transported over a long distance by strong winds such as typhoons or monsoon winds. Typhoons before landing on the Philippines and Taiwan usually bring a strong north wind that could facilitate dispersal. The prevailing northeast winter monsoons also generate optimum winds for seed transport from Taiwan to the Philippines. Another possibility is transport by migrating birds. The plants of *Euphrasia* in Taiwan inhabit rock slits on mountain slopes, or occur in wet flat, sometimes marshy places. When migrating birds take a rest in the mountains of Taiwan during their flight to the south to avoid the winter cold, the seeds of *Euphrasia* species in marshy habitats may be imbedded in the mud, which may get stuck to the legs of the birds and is thus carried by them to the south (Raven 1973). Plants of *Euphrasia* are hemiparasitic and they can establish on a wide range of host plants. Therefore, establishments of new populations is likely even without the normally associated plants (Wilkins 1963).

Our data indicates direct dispersal from Taiwan to the Philippines based on the phylogeny of section *Malesianae*, and the long distance dispersal is likely due to strong winds or avian migration.

Possible center of origin of *Euphrasia*

Our data are insufficient to explain the migratory direction of section *Malesianae* in the Malesian region. Therefore, it is also still too early to propose a centre of origin.

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