A revised taxonomy of *Cirrospilus ambiguus* based on molecular systematics with notes on notauli evolution in Eulophinae

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Abstract: Eulophids provide a valuable experimental model system to investigate a wide variety of questions in the evolution of parasitic wasps. Notauli, an important taxonomic character in the family Eulophidae, is used to differentiate subfamilies, tribes, genera and species. However, abundant morphological homoplasy in this family has made it difficult to confidently identify many eulophid taxa at specific level. Both *Cirrospilus ambiguus* and *Diglyphus bimaculatus* are considered to have notauli patterns that are intermediate between their respective genera. Determining the phylogenetic position of both species may facilitate studying the evolution of notauli patterns. We analyzed COⅠ, ITS1 and 28S sequences using Bayesian method. *C. ambiguus* was transferred into the monophyletic *Diglyphus*, and the monophyly of *Cirrospilus*, however, was not supported. The types of notauli were examined in the context of the phylogeny based on 28S gene sequences. The complete notauli in the Eulophidae extending to the hind margin of the mesoscutum was inferred to represent the ancestral form. The complete notauli that curves to meet the apex of the axillae occurs independently in five clades. Each occurrence represents a unique independent evolution. The incomplete form of notauli occurred in four clades, indicating that the shape independently evolved four times.

Key words: Eulophidae; molecular systematics; *Cirrospilus ambiguus*; *Diglyphus bimaculatus*; notauli; evolution

1 INTRODUCTION

The Eulophidae (Hymenoptera: Chalcidoidea) is one of the most speciose families of parasitoid wasps. It currently contains 294 genera and 4 288 species (Noyes, 2002). Besides attacking eggs and larvae, eulophids can also attack pupae and adults of their hosts. These species occur in almost all known parasitoid forms: endoparasitoids and ectoparasitoids, idiobionts and koinobionts, solitary and gregarious, and specialists and generalists. With such a diverse biology, eulophids form excellent model systems to study the evolution of diverse life styles and numerous other questions (Godfray, 1994).

Leafminers, the hosts of eulophid parasites, cause great damage to crops and flowers (Minkenberg and van Lenteren, 1986). One of the important, effective and environmentally friendly methods to control leafminers is to use natural enemies. However, the incorrect identifications of parasitoids and their hosts will hinder their use in biological control. No matter how similar morphologically, the parasitoid species can differ in behavior, physiology, ecological preference and reproductive modes (Rossler and DeBach, 1973; Gordh, 1982). Many species of both genera *Cirrospilus* and *Diglyphus* of Eulophidae are important parasitoids of leafminers (Zhu et al., 2000; Noyes, 2002), including *Cirrospilus ambiguus* (Hansson and LaSalle, 1996), which attacks *Liriomyza trifolii* throughout the Tropics (eg, China, India, Vietnam and Tanzania) (Noyes, 2002) and in South Africa.

Three notauli shapes occur in the Eulophidae: incomplete (Fig. 1), complete and curving to meet the apex of the axilla (Fig. 2), and complete and extending to the hind margin of the mesoscutum (Fig. 3). These patterns are used to differentiate eulophid subfamilies, tribes and genera (Bouček, 1988; Schauff *et al.*, 1997). Using this character, Peck *et al.* (1964) divided the Eulophinae into two subfamilies: Eulophinae and Elachertinae. The tribe Anselmellini, established by Bouček (1988), is easily recognized with the combination of three characters, one of which is that the mesoscutum has clear-cut, deep notauli. Tribe Ophelimini, defined by Ashmead (1904), has complete notauli and two short hind tibial spurs.

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Bouček (1988) proposed the Ophelimini should accommodate most genera with deep, straight notauli.

Both *C. ambiguus* and *D. bimaculatus* have similar notauli (Fig. 2, 4), which are considered to be examples of intermediate morphological forms between *Cirrospilus* and *Diglyphus*. The complete and curved shapes of notauli are one of the key features distinguishing both species from all members of the tribe Cirrospilini (Hansson and LaSalle, 1996; Zhu *et al.*, 1999; Zhu and Huang, 2002). Most members of *Diglyphus* can be distinguished from other genera by having incomplete notauli (Zhu *et al.*, 2000).

Notauli patterns are also used to distinguish *Cirrospilus* from *Zagrammosoma* (Gordh, 1978; Bouček, 1988; LaSalle, 1989). In *Cirrospilus*, except for *C. ambiguus*, notauli extend to hind margin of mesoscutum (Fig. 3). In *Zagrammosoma*, once treated as a subgenus of *Cirrospilus* (Peck *et al.*, 1963), notauli are curved and extend to the anterior margin of the axillae. Recently, many authors recognize its generic status (Gordh, 1978; Bouček, 1988; LaSalle, 1989). Although *Cirrospilus ambiguus* is placed in its genus because of its metallic body color and non-vaulted vertex, in making this decision Hansson and LaSalle (1996) acknowledged that the inclusion of *C. ambiguus* might leave *Cirrospilus* a paraphyletic genus.

It is difficult to identify eulophids based only on morphology because of the relatively small number of informative characters and high levels of homoplasy (Ubaidillah *et al.*, 2003). Fortunately, modern molecular methods offer important alternatives to species identification and they can yield insights into the evolution of these parasitic wasps. Relatively conservative nuclear gene sequences can distinguish species and biotypes of parasitoids that are difficult to separate morphologically (Hoy *et al.*, 2000). Nuclear ribosomal ITS1 can resolve the relationships among populations and species (Avise, 2000). The second expansion segment (D2) of 28S rDNA is useful for resolving relationships among families in the Chalcidoidea (Campbell *et al.*, 2000). Gauthier *et al.* (2000) found that this region was particularly informative at the levels of subfamily and tribe. The rapid evolution and selective constraints make mitochondrial protein-encoding genes useful molecular markers for separating species (Hebert *et al.*, 2003; Hebert *et al.*, 2004).

The main purposes of this study are: (1) to investigate the generic placement of the two species, *C. ambiguus* and *D. bimaculatus* based on a phylogenetic evaluation of sequences from three genes, and (2) to investigate the evolution of notauli patterns in the Eulophinae based on 28S sequences.

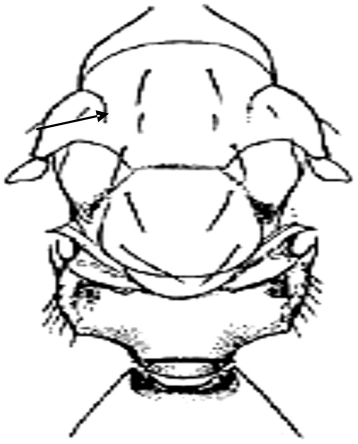

Fig. 1 Dorsal view of the thorax of *Sympiesis* (incomplete)

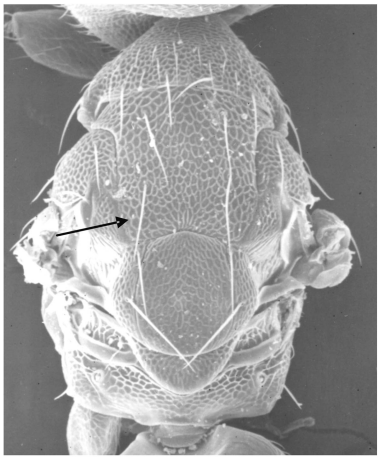

Fig. 2 Dorsal view of the thorax of *Cirrospilus ambiguus* (complete and curving to meet the apexof the axilla)

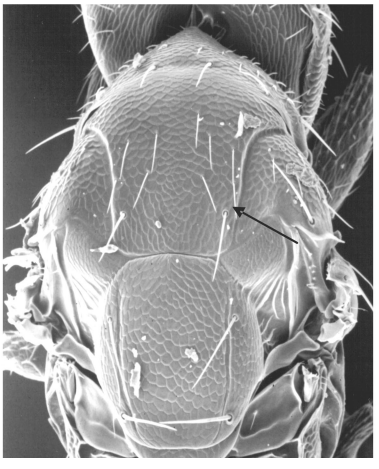

Fig. 3 Dorsal view of the thorax of *Cirrospilus brevis* (complete and extending to the hind margin of the mesoscutum)

2 MATERIALS AND METHODS

2.1 Taxa sampling

All sequenced specimens (Tables 1, 2) were identified by the second author based on morphological

features. All voucher specimens were deposited in the Institute of Zoology, Chinese Academy of Sciences, Beijing (IZCAS). *Mymar* sp. (Mymaridae) was used as the initial outgroup to re-root 50% majority rule consensus tree constructed from 28S D2. Morphologically, members of Mymaridae have closer relationships with other families than Eulophidae in Chalcidoidea (Schauff, 1984; Gibson, 1986). The genus *Euderus* was used to root the network constructed from CO Ⅰ and ITS1 sequence data. Euderinae are considered as the most primitive members of the family because they possess eight gastral segments and always four or even five funicular segments (Bouček, 1988; Gauthier *et al*., 2000).

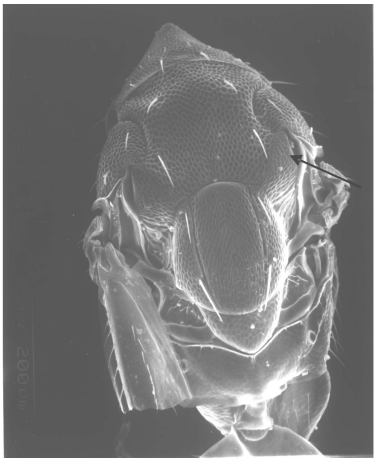

Fig. 4 Dorsal view of the thorax of *Diglyphus bimaculatus* (complete and curving to meet the apex of the axilla)

2.2 DNA extraction, PCR, and sequencing

Because most specimens were critical point dried before DNA extraction, they were submerged in tri-distilled water for 24 h. Genomic DNA was extracted using standard phenol-chloroform extraction from a single individual (Sambrook *et al*., 1989). After extraction, the DNA was dissolved in 450 μL TE buffer and stored at −20℃.

A fragment of CO Ⅰ was amplified with standard polymerase chain reaction (PCR) protocol and directly sequenced after purifications. Primers used for CO Ⅰ PCR and sequencing were as the following: CO Ⅰ SF 5′-TAAGATTTTGATTATT (AG)CC(TA)CC-3′, CO Ⅰ 2191, 5′-CCCGGTAAAATTAAAATATAAACTTC-3′, CO Ⅰ 2613, 5′-ATTGCAAATACTGCACCTAT-3′ and CO Ⅰ 2193, 5′-TTTTTTGGTCATCCAGAAGT-3′. The first primer was designed by ourselves, while others were taken from Simon *et al*. (1994) and Chen *et al*. (2004). Primers used for amplifications and sequencing of ITS1 were taken from Ji *et al*. (2003) (18sF1 and 5p8sB1d). For amplifying the D2 region, primers were taken from Campbell *et al*. (1993: 28S D2F and D2R). All PCRs were carried out in standard 25 μL reactions. For mitochondrial CO Ⅰ, 35 cycles were performed. In each cycle, melting temperature was 94℃ for 30 s, annealing at 48℃ for 45 s, and extension at 72℃ for 1 min. For nuclear ribosomal 28S and ITS1, PCR used 35 cycles of 94℃ denaturation for 30 s, 50℃ for annealing 30 s and 72℃ extension for 1 min, with an initial denaturation of 95℃ for 5 min and a final extension of 72℃ for 7 min. PCR products were purified with Minipore protocols (Qiagen) and sequenced with ABI Big Dye protocols.

CO Ⅰ and ITS1 were not always sequenced from the same samples (Table 1). The DNA of some individuals was exhausted after multiple attempts to amplify one of these gene fragments. Thus, additional samples were used to sequence other fragments.

2.3 Sequence alignment and phylogenetic analyses

Sequences were initially aligned by Clustal W (Thompson *et al*., 1994) with default parameters (gap opening penalty = 10, gap extension penalty = 5, delay divergent sequences = 40%) and then manually checked in BioEdit (Hall, 1999). Missing data were coded as "?".

To select an evolutionary model that best fits the data, hierarchical likelihood ratio tests (hLRTs) (Goldman, 1993) were implemented by comparing log-likelihood scores of 56 models of base substitution using Modeltest (Posada and Crandall, 1998). Bayesian inference was used to estimate genealogical relationships (Huelsenbeck and Ronquist, 2001; Buckley *et al*., 2002; Nylander *et al*., 2004; Ronquist, 2004). Analyses were conducted using MrBayes 2.0 (Huelsenbeck and Ronquist, 2001). All Bayesian analyses were initiated with random starting trees. Four Markov chains were used and the dataset was run for 2×10^6 generations to allow for adequate time of convergence. Trees were sampled every 100 generations. To avoid being trapped on local optima, we ran two independent analyses with different starting trees and monitored the fluctuating value of the likelihood graphically (Huelsenbeck and Bollback, 2001). We plotted the log-likelihood scores of sample points against generation time and determined that stationarity was achieved when the log-likelihood values of the sample points reached stationarity (Huelsenbeck and Ronquist, 2001). After discarding burn-in samples, the remaining samples were retained for generating a 50% majority rule consensus tree. These frequencies of resolving nodes were assumed to reflect the true probabilities of the clades under the assumed models. Consequently, Bayesian posterior probabilities (BPP) of 95% or greater were considered to be significantly supported.

3 RESULTS

Nineteen CO Ⅰ sequences from 19 species and 18 ITS1 sequences from 14 species were obtained (Table

1). Thirteen 28S D2 sequences of 12 species were also obtained (Table 2). All 28S D2 sequences of Eulophidae, except for those species transferred out of the family by Gauthier *et al*. (2000), were directly downloaded from GenBank for analyses. One sequence of *Cirrospilus* (AF345598) was discarded for its little homology with other eulophids. The aligned sequences are available upon request.

3.1 Sequence composition

The totals of 745 bp of CO Ⅰ were easily aligned because no indels were detected. Base frequencies averaged A = 0.297, C = 0.120, G = 0.152, T = 0.432. There was a strong adenine and thymine (A-T) bias. Compared with the first and second positions, the average A-T frequency in the third position was 0.922, showing very strong A/T bias, as was typical of insect mitochondrial genomes (Simon *et al*., 1994; Arias and Sheppard, 1996).

Table 1 Samples and localities for parasitoids for which sequences of CO Ⅰ and ITS1 were obtained

Species name	Voucher number	Locality	Elevation (m)	Sex	GenBank accession no.	
					CO Ⅰ	ITS1
Aulogmnus						
A. sp1 TBbsbd 101 f	Aulo101	Bangda, Basu, Xizang	4 390	Female	–	DQ390436
A. sp GXjxsts 2 f	Aulo2	Shengtangshan, Jinxiu, Guangxi	1 095	Female	DQ149154	–
Cirrospilus						
C. *ambiguus* VIETNAMhn 105 f	Cirr105	Hanoi, VIETNAM		Female	DQ390432	DQ390437
C. *diallus* TBcd 1 f	Cirr1	Changdu, Xizang	3 400	Female	DQ390434	–
C. *diallus* TBcd 102 f	Cirr102	Changdu, Xizang	3 400	Female	DQ390433	–
C. *diallus* QHgem 103 f	Cirr103	Geermu, Qinghai	2 880	Female	–	DQ390438
C. *pictus* BJmtg 101 1 f	Cirr101 1	Mentougou, Beijing	1 300	Female	–	DQ390439
C. *pictus* BJmtg 101 2 f	Cirr101 2	Mentougou, Beijing	1 300	Female	–	DQ390440
C. *variagatus* SXhyhs 4 m	Cirr4	Hengshan, Hengyuan, Shanxi	1 475	Male	DQ149157	–
C. *vittatus* QHgem 104 f	Cirr104	Geermu, Qinghai	2 880	Female	DQ149158	DQ390441
Diglyphus						
D. *begini* TBrkz 147 m	Dig147	Rekaze, Xizang	3 890	Male	–	AY948107
D. *begini* TBls 187 m	Dig187	Lasa, Xizang	3 650	Male	DQ149159	–
D. *bimaculatus* TBjz 128 f	Dig128	Relong, Jiangzi, Xizang	4 700	Female	DQ149160	AY948108
D. *bimaculatus* TBls 130 f	Dig130	Lasa, Xizang	3 650	Female	DQ149161	AY948109
D. *crassinervis* QHdl 119 f	Dig119	Deling, Qinghai	2 900	Female	DQ149162	AY948110
D. *isaea* QHql 108 m	Dig108	Qilian, Qinghai	2 790	Male	DQ149174	AY948092
D. *isaea* QHql 126 f	Dig126	Qilian, Qinghai	2 790	Female	DQ149176	AY948094
D. *minoeus* TBjzrl 101 1 m	Dig101	Relong, Jiangzi, Xizang	4 700	Male	DQ149192	AY948111
D. *minoeus* TBjzrl 102 f	Dig102	Relong, Jiangzi, Xizang	4 700	Female	–	AY948112
D. *pachyneurus* QHdl 121 m	Dig121	Delingha, Qinghai	2 900	Male	DQ149193	AY948113
D. *pulchripes* QHgem 146 f	Dig146	Geermu, Qinghai	2 880	Female	DQ390435	DQ390444
Eulophus						
E. sp2 TBcd 102 f	Eulo102	Changdu, Xizang	3 400	Female	DQ149197	DQ390443
Entedon						
E. sp2 BJmtg 1280	Ent2	Mengtougou, Beijing	1 280	Female	DQ149194	–
E. sp3 SCbx 2200	Ent3	Baoxing, Sichuan	2 200	Female	DQ149195	–
Euderus						
E. sp1 BJzhwy 101 f	Eud101	Beijing Botanical Garden, Beijing	100	Female	DQ149196	DQ390442

After manual alignment, the ITS1 sequences consisted of 1 005 bp positions, which included partial 18S sequences in 3′ and 5.8S sequences in 5′ end. Base frequencies averaged A = 0.242, C = 0.25, G = 0.276, T = 0.231. The average frequency of A/T was 47.3%, being roughly equal to that of G/C. Aligned 28S D2 sequence yielded 486 bp. Base frequencies averaged A = 0.221, C = 0.305, G = 0.139, T = 0.336.

3.2 Phylogenetic analyses

The TVM + I + G model was chosen as best-fit model for CO Ⅰ dataset by hierarchical likelihood ratio tests (– lnL = 4 644.5327). The model allowed for equal transition rates and nucleotide substitution with invariable sites plus a G distribution of variable-rate site.

After approximately 300 000 generations, the log-likelihood values of each sampled tree stabilized. The last 17 001 sample trees were used to estimate the 50% majority rule consensus tree and the BPPs (Fig. 5). The tree was moderately well resolved. Unresolved relationships occurred within the genus *Diglyphus* only (Clade D). Both *Cirrospilus ambiguus* and *D*. *bimaculatus* were resolved in the clade containing other

species of *Diglyphus*. However, the support for this group, Clade D, was low (BPP = 54). The other species of *Cirrospilus* fall into three clades: A, B and C (Fig. 5).

Table 2 Samples and localities for parasitic wasps for which sequences of 28S were obtained

Species name	Specimen no.	Locality	Elevation (m)	Sex	GenBank accession no.
Aulogmnus					
A. sp1 TBbsbd 101 f	Aulo101	Bangda, Basu, Xizang	4 390	Female	DQ390414
Cirrospilus					
C. ambiguus VIETNAMhn 105 f	Cirr105	Hanoi, VIETNAM		Female	DQ390415
C. pictus BJmtg 101 f	Cirr101	Mentougou, Beijing	1 300	Female	DQ390416
C. variagatus SXhyhs 4 m	Cirr4	Hengshan, Hengyuan, Shanxi	1 475	Male	DQ390417
Diglyphus					
D. albinervis QHdllhb 132 f	Dig132	Delingha, Qinghai	2 900	Female	DQ390418
D. bimaculatus TBjz 128 f 4700	Dig128	Relong, Jiangzi, Xizang	4 700	Female	DQ390419
D. bimaculatus TBls 130 f 3650	Dig130	Lasa, Xizang	3 650	Female	DQ390420
D. inflatus GSwx 131 m	Dig131	Wenxian, Gansu	960	Male	DQ390421
D. isaea SCgzkd 174 f	Dig174	Kangding, Ganzi, Sichuan	2 400	Female	DQ390422
D. minoeus h6	DigH6	London, U.K.		Female	DQ390423
D. pachyneurus ZJtms 116 f	Dig116	Tianmushan, Zhejiang	350	Female	DQ390424
Eulophus					
E. sp2 TBcd 102 f	Eulo102	Changdu, Xizang	3 400	Female	DQ390428
E. sp3 GSzq 103 f	Eulo103	Zhouqiu, Gansu	3 400	Female	DQ390429
Elachertus					
E. fenestratus SC 1 m	Elach1	Manigange, Dege, Sichuan	3 965	Male	DQ390425
Elasmus					
E. sp BJzwy 5 f	Elas1	Beijing Botanical Garden, Beijing	100	Female	DQ390427
E. sp1 LNdl 102 m	Elas102	Dalian, Liaoning	300	Male	DQ390426
Pnigalio					
P. sp2 SCgzkd 6 f	Pnig6	Kangding, Ganzi, Sichuan	2 400	Female	DQ390430
P. sp3 TBcd 3 f	Pnig3	Changdu, Xizang	3 400	Female	DQ390431

For the nuclear gene ITS1, K80 + I + G model was selected as the best-model by hLRTs (– lnL = 7 457.8960). This model of substitution allowed for a bias in the rate of transitions and a substitution rate that varies across sites with G distribution of variable-rate site. Following the Bayesian analysis, all species of *Diglyphus* clustered into one strongly supported clade with a high BPP (BPP > 95; Fig. 6). As with the analysis of CO Ⅰ data, the clade included both *D. bimaculatus* and *C. ambiguus*. Other species of *Cirrospilus* fell into clades A and B (Fig. 6). The monophyly of clades A and B were strongly supported (BPP > 99).

For 28S D2 sequence data, the hLRTs selected the GTR + I + G as the best-fit model (– lnL = 2 874.0681). Both *D. bimaculatus* and *C. ambiguus* clustered together with other species of *Diglyphus* (Fig. 7). *Cirrospilus* formed 2 different clades. *Diglyphus* + *C. ambiguus* was monophyletic, but the monophyly was not strongly supported (BPP = 62).

4 DISCUSSION

4.1 Phylogenetic postion of *C. ambiguus* and monophyly of the genera *Cirrospilus*

Phylogenetic analyses of three gene sequences all supported the cluster of both *D. bimaculatus* and *C. ambiguus* with the other species of *Diglyphus*. Therefore, *C. ambiguus* should be transferred from *Cirrospilus* to *Diglyphus* and referred to as *Diglyphus ambiguus* (new combination). This association is strongly supported by the analyses of ITS1 with a high value (BPP = 96). Hansson and LaSalle (1996) placed *C. ambiguus* in *Cirrospilus* because the species had the non-vaulted vertex and the metallic body coloration. However, both characters were also commonly found among species of *Diglyphus*. An anterior pair of scutellar setae were located very close to the posterior margin of mesoscutum in *C. ambiguus* (Zhu *et al*., 2002), which was more typical of *Diglyphus* (Fig. 4) than other species of *Cirrospilus* (Fig. 3). Furthermore, the body color of species in *Cirrospilus* ranges from metallic to non-metallic. Body coloration did not indicate generic membership.

Our results supported the monophyly of *Diglyphus*, but not *Cirrospilus*. In the Cirrospilini, *Cirrospilus* was the most speciose genus (Noyes, 2002). The monophyly of *Zagrammosoma* was

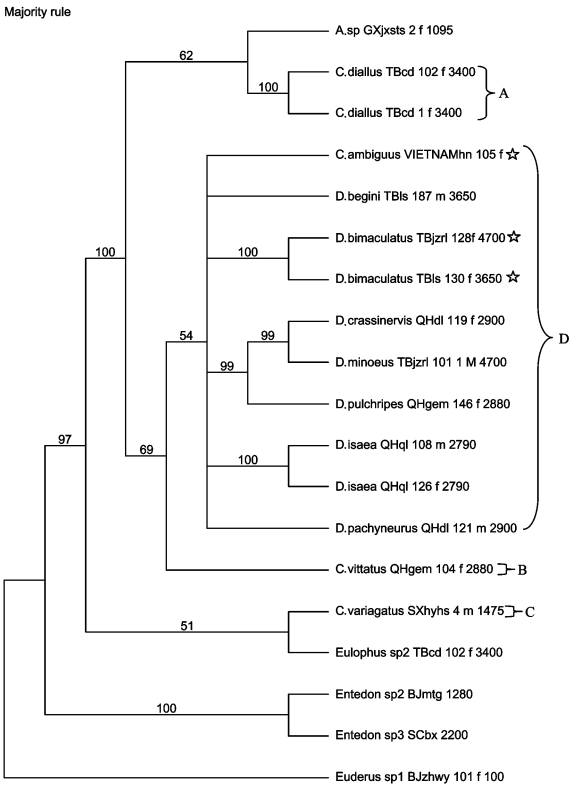

Fig. 5 A 50% majority rule consensus tree based on CO Ⅰ sequence data with Bayesian analysis for parasitic wasps

Posterior probability values are shown above the branches. Letters indicate nodes discussed in text. Stars indicate the position of *C. ambiguus* and *D. bimaculatus*. The same in Figs. 6 – 7.

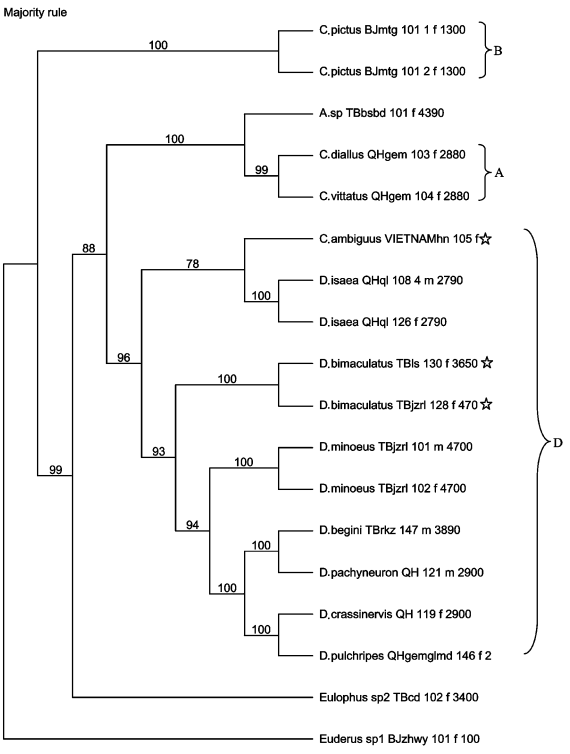

Fig. 6 A 50% majority rule consensus tree based on ITS1 sequences and Bayesian analysis for parasitic wasps

supported by two distinctive characters: non-metallic body color and a vaulted vertex. Two species, *C. variegatus* and *C. coachellae* (Gates, 2000), supported the grouping of *Cirrospilus* and *Zagrammosoma*, as both genera share a moderately vaulted vertex. However, a vaulted vertex also raises the possibility of a paraphyletic *Cirrospilus*. Ubaidillah *et al*. (2003) analyzed 56 morphological characters of 53 cirrospilines and showed that a monophyletic *Cirrospilus* could not be morphologically diagnosed. Identically, the molecular analysis of Gauthier *et al*. (2000) failed to diagnose *Cirrospilus*.

4.2 Phylogenetic postion of genera *Aulogymnus*

The higher taxonomic placement of *Aulogymnus* has been uncertain. When Zhu *et al*. (1999) recorded the genus *Aulogymnus* from China, they were uncertain whether or not Chinese species of *Aulogymnus* should be included in the Cirrospilini because their specimens did not have a transverse sulcus. The genus *Aulogymnus* was placed in Ophelimini by Bouček (1988). Moreover, this genus also differed from the other Cirrospilini in mostly having 2-4 funicular segments (Zhu *et al*., 1999; Zhu and Huang, 2000). Gauthier *et al*. (2000) revised the Eulophidae based on their molecular phylogeny but they did not include any species of *Aulogymnus*. We found that *Aulogymnus* should be included in the Cirrospilini.

4.3 Evolution of notauli

The Eulophidae is one of the most speciose families of parasitic wasps. With their diverse biology, eulophids offer very good experimental model systems to study a variety of evolutionary questions (Godfray, 1994). A sound phylogenetic tree facilitates an understanding of evolutionary history. However, the high levels of homoplasy and small number of informative morphological characters make it difficult to form a hypothesis of relationships. Fortunately, molecular data cast light on the phylogeny of wasps. Gauthier *et al*. (2000) proposed a phylogenetic framework for Eulophidae based on nucleotide sequence data from D2 region of 28S rDNA.

Phylogenetic positions of *D. bimaculatus* and *C. ambiguus* offer an excellent opportunity to study the evolution of notauli patterns. The shape of notauli is not only an important character in eulophid taxonomy, it is also associated with the ability to fly. The notauli are external indicators of the internal phragmata that separate the dorsolongitudinal and dorsoventral flight muscles (Michener, 1944). The phragmata form an internal ridge for the attachment of flight muscles (Wong, 1963). Complete notauli indicate relatively more flight muscles and, thus, a stronger capability of flight. In contrast, incomplete notauli indicate fewer

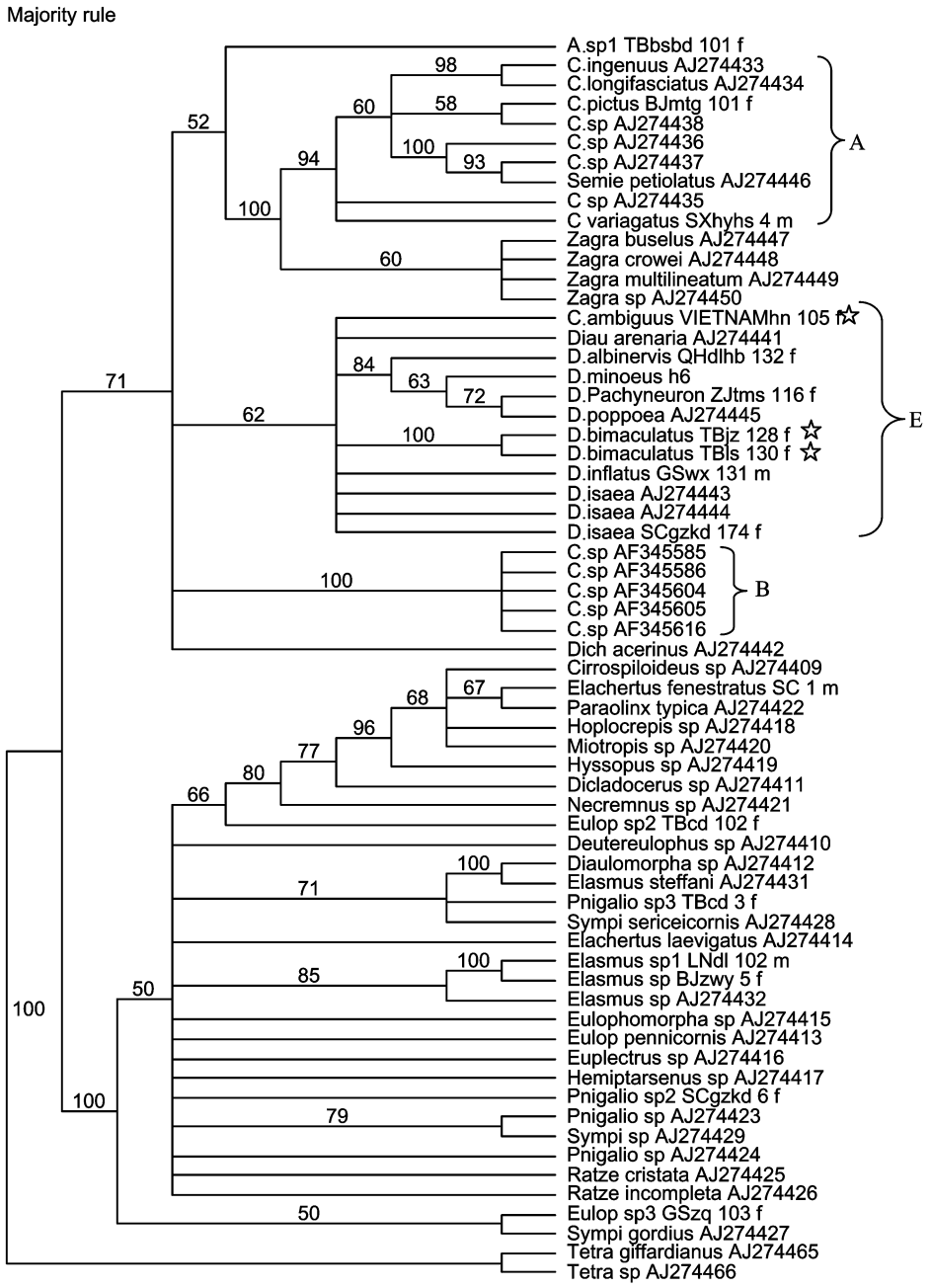

Fig. 7 A 50% majority rule consensus tree based on 28S sequences analyzed by Bayesian analysis

flight muscles and a relatively weaker ability to fly.

In the Tetrastichinae, the notauli of all species are complete and extend to the hind margin of the mesoscutum. In contrast, the notauli of most entedonines are incomplete. The notauli of euderines are complete and extend to the hind margin of the mesoscutum or sometimes disappear anteriorly. The phylogeny inferred from the 28S sequence shows that the notauli evolved from being complete to incomplete in the Eulophidae. The plesiomorphic condition is that of having complete notauli. This condition also implies that the capability of flight became gradually weaker as species of free-living eulophid ancestors specialized to their respective hosts.

The complete notauli that curve to meet the apex of the axillae occur in five clades (Fig. 8). The same type of notauli is also found in a few other eulophines, including species of *Oxycantha* and *Naumanniola ramose*. Although the phylogenetic relationships among these species are unknown due to lack of specimens, our results imply that this type of notauli evolved at least five times. Similarly, incomplete notauli are found in four clades (Fig. 8) and, thus, must have evolved independently at least four times. The multiple, independent origins of derived notauli shapes suggests that shape of this character is phylogenetically

misinformative. Notauli patterns may continue to serve as diagnostic characteristics only when taken in concert with other morphological characters.

Fig. 8 Evolutionary cladogram of notauli shape in the Eulophinae
Circled notes indicate the possible formation of incomplete notauli.

The Eulophinae is an excellent taxon for the study of the evolution of parasitic wasps. The phylogeny inferred from both molecular and morphological data can provide a historical context for study of evolution of various characters and biological features. Furthermore, work in progress on the phylogeny of the Eulophinae, and species-level phylogenies on related genera with similar interspecific variation in notauli, will greatly improve our understanding of the evolution of notauli, particularly when more detailed biological observations are available for a number of species.

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基于分子系统学的可疑瑟姬小蜂分类修订及姬小蜂亚科盾纵沟的演化分析

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摘要：姬小蜂为寄生蜂的演化研究提供了很好的材料。在姬小蜂分类中，盾纵沟是一个重要性状，曾被用来区分亚科、族、属及种。非同源性相似形态特征的存在使得物种准确鉴定存在困难。从盾纵沟形状上，可疑瑟姬小蜂 *Cirrospilus ambiguus* 和双斑潜蝇姬小蜂 *Diglyphus bimaculatus* 应介于瑟姬小蜂属 *Cirrospilus* 和潜蝇姬小蜂属 *Diglyphus* 之间。确定这两种姬小蜂的系统发育位置将有助于分析盾纵沟的演化模式。本文用贝叶斯方法分析了线粒体 CO Ⅰ 部分序列、核糖体 ITS1 序列及核糖体 28S D2 区部分序列等 3 个基因序列，结果显示可疑瑟姬小蜂应被移到潜蝇姬小蜂属中；研究结果支持潜蝇姬小蜂属是单系，而不支持瑟姬小蜂属是单系。结合 28S D2 区部分序列的贝叶斯分析结果，分析了在姬小蜂亚科中盾纵沟的演化模式。结果显示，完整且延伸到中胸背板后缘的盾纵沟代表其原始类型；完整且延伸到三角片的盾纵沟类型分别出现在 5 个独立的枝上，代表了该特征 5 次独立的演化；不完整的盾纵沟类型出现在 4 个独立的枝上，表明该类型独立演化了 4 次。

关键词：姬小蜂科；分子系统学；可疑瑟姬小蜂；双斑潜蝇姬小蜂；盾纵沟；演化

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