Zoological Studies **48**(2): 270-279 (2009)

Zoological Studies

Systematic Status of *Agehana elwesi* f. *cavaleriei* Based on Morphological and Molecular Evidence

Chih-Chien Lu[1], Li-Wei Wu[1], Guo-Fang Jiang[2], He-Li Deng[3], Li-Hao Wang[1], Ping-Shih Yang[4], and Yu-Feng Hsu[1,*]

[1]*Department of Life Science, National Taiwan Normal University, Taipei 116, Taiwan*
[2]*Jiangsu Key Laboratory for Biodiversity and Biotechnology, College of Life Science, Nanjing Normal University, Nanjing, Jiangsu 210046, China*
[3]*Chongqing Parnassian Researches Company, Beibei, Chongqing, Sichuan 400700, China*
[4]*Department of Entomology, National Taiwan University, Taipei 106, Taiwan*

(Accepted June 16, 2008)

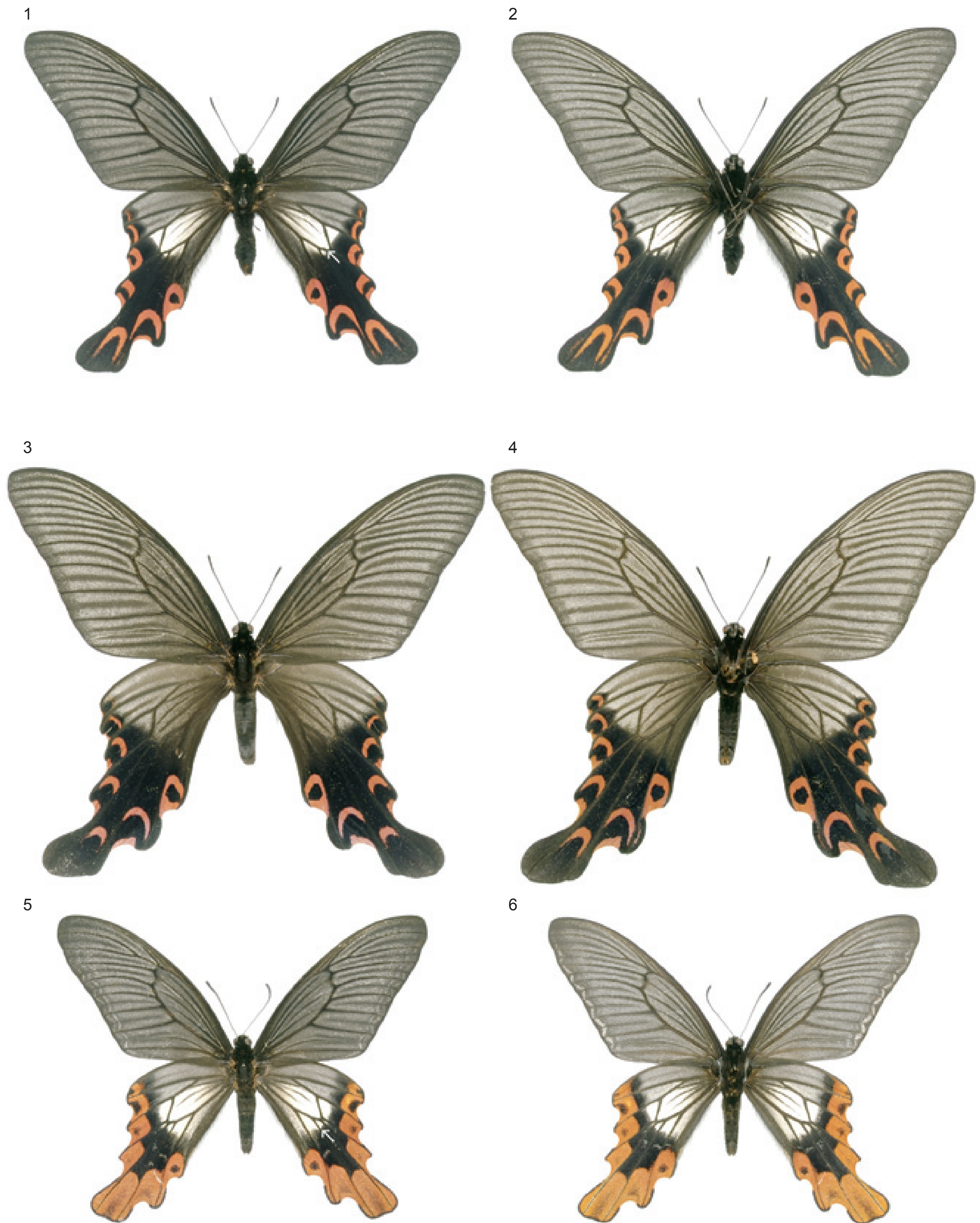
Chih-Chien Lu, Li-Wei Wu, Guo-Fang Jiang, He-Li Deng, Li-Hao Wang, and Yu-Feng Hsu (2009) Systematic status of *Agehana elwesi* f. *cavaleriei* based on morphological and molecular evidence. *Zoological Studies* **48**(2): 270-279. *Agehana* swallowtail butterflies are unusual in having 2 veins present in the broad lobe-like “tail” of their hindwing. Of the 2 species currently recognized in this genus, *A. maraho* is generally considered an endangered taxon endemic to Taiwan. The other species, *A. elwesi*, which is widespread in the southern part of continental China, contains a form superficially similar to *A. maraho*. This form is termed f. *cavaleriei*, and its distribution is restricted to southwestern China. The relationship between this form and *A. maraho* has not been critically examined using either morphology or molecular characters. The male genitalia and a 1530 bp fragment of mitochondrial DNA including the cytochrome oxidase I barcoding region suggest that f. *cavaleriei* is a form of *A. elwesi* rather than *A. maraho*. http://zoolstud.sinica.edu.tw/Journals/48.2/270.pdf

Key words: *Agehana maraho*, COI sequence, China, Taiwan, Swallowtail.

The swallowtails of the genus *Agehana* (or *Papilio* subgenus *Agehana*) are famous for possessing an unusually broad lobe-like “tail” in the hindwing, in which both M3 and CuA1 veins penetrate. This character is shared by only 3 taxa described from the southeastern part of continental Asia and Taiwan (D’Abrera 1982). Although there is little doubt that these taxa share a most recent common ancestor as their immature morphology and behavior are indistinguishable from each other (Igarashi 1979); the taxonomic identities of these taxa are a subject of controversy, especially that of populations of *Agehana* in southwestern China, which possess prominent white markings on the hindwings (Figs. 1, 2). This “form” was originally described as a subspecies of *A. elwesi* (Leech) (Figs. 3, 4) by Le Cerf (1923), and some subsequent authors accepted this treatment (e.g., Zhou and Zhang 1981). However, Li and Zhang (1984) suggested that this taxon should represent an infraspecific form of *A. elwesi*, and this opinion has been followed by subsequent authors (e.g., Huang et al. 1993, Chou 1994).

Besides the continental *A. elwesi*, another taxon *A. maraho* (Figs. 5, 6) occurs in Taiwan (Fig. 7). It is usually considered a rare species endemic to the island, and was listed as a vulnerable species in the International Union for the Conservation of Nature’s *IUCN Red Data Book* as early as 1985 (Collins and Morris 1985). It has been listed as an endangered species locally since 1994 (Cheng et al. 1996, Yen and Yang 2000,

*To whom correspondence and reprint requests should be addressed. Chih-Chien Lu and Li-Wei Wu contributed equally to this work. Tel: 886-2-29326234 ext. 338. Fax: 886-2-29312904. E-mail:t43018@cc.ntnu.edu.tw



Figs. 1-6. Male adults of *Agehana* taxa. **1.** *Agehana elwesi* f. *cavaleriei*, upperside; **2.** ditto, underside; **3.** "typical" *A. elwesi*, upperside; **4.** ditto; underside; **5.** *A. maraho*, upperside; **6.** ditto, underside. Scale bar = 1 cm.

Yen and Yang 2001), and this taxon was later assessed as a species in the category of "nearly threatened" (LR/nt) in 1996 by the IUCN (Gimenez Dixon 1996). The relationship between *A. maraho* and *A. elwesi* f. *cavaleriei* has been questioned as both taxa possess a white patch on the hindwing (Figs. 1, 2, 5, 6), whereas such a white patch is absent from the typical *A. elwesi* (Figs. 3, 4). These shared white patches are present in the same portions of the wings, i.e., in the discoidal cells of the hindwings plus areas surrounding the cells, (Figs. 1, 2, 5, 6), leading some to speculate that the 2 taxa may be closely related. There have been some discussions about the status of *A. elwesi* f. *cavaleriei* with regard to *A. elwesi* without a white patch (referred to as the "typical" *A. elwesi* in the present article), and between *A. elwesi* and *A. maraho*. However, *A. elwesi* f. *cavaleriei* has not been directly compared to *A. maraho* in terms of genital structure or molecular data. Considering the conservation status of *A. maraho*, it seems important to elucidate whether *A. elwesi* f. *cavaleriei* is truly an infraspecific taxon within *A. elwesi* and not a continental race of *A. maraho*.

The utility of gene barcoding using a fragment of the cytochrome oxidase I (COI) sequence has proven to be effective in species delimitation issues for a variety of organisms (Costa et al. 2007), including lepidopterous insects (e.g., Brown et al. 2003, Hajibabaei et al. 2006, van Velzen 2007). We attempted to resolve the systematic

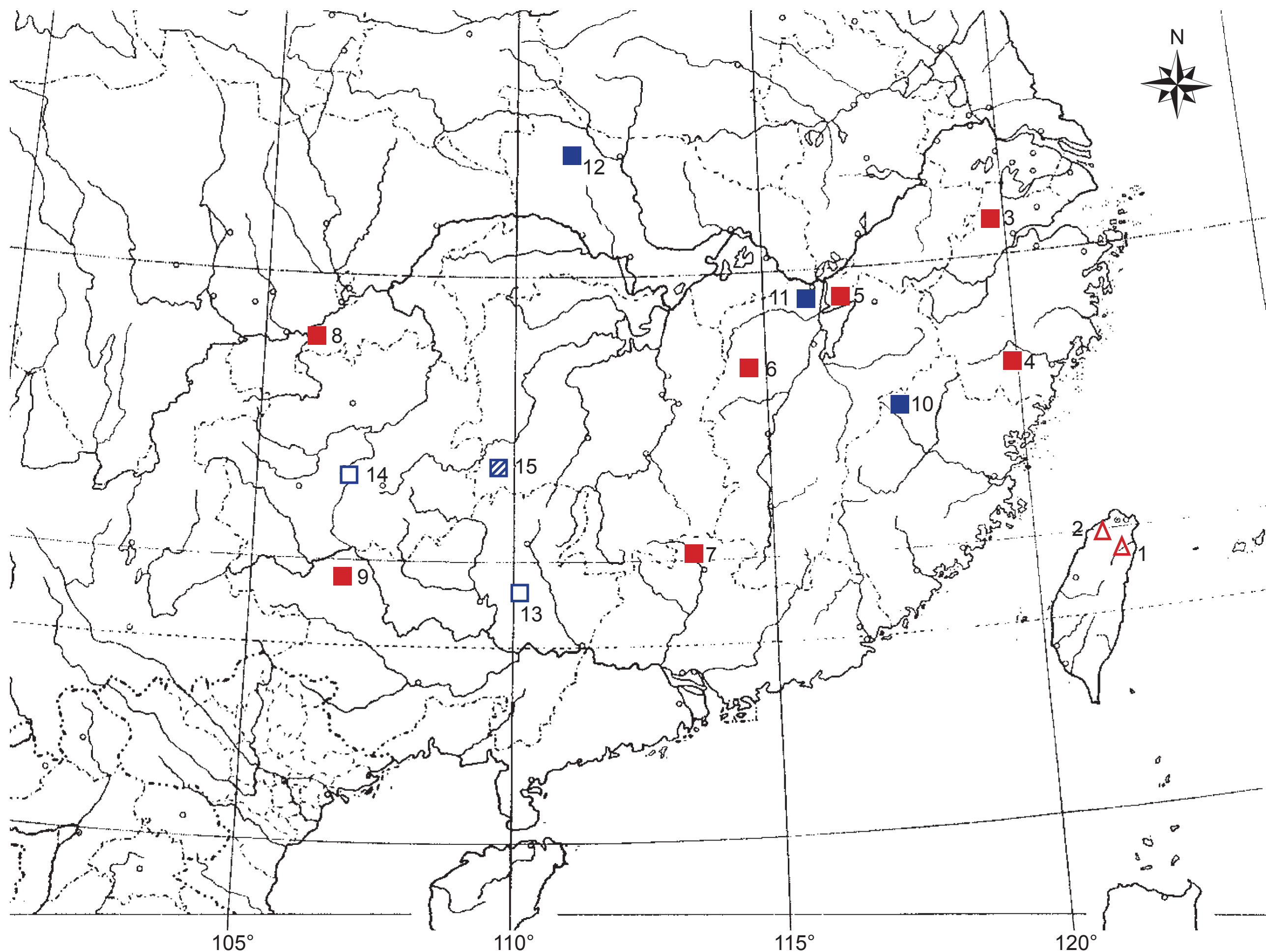


Fig. 7. Distribution of *Agehana* taxa. Open squares denote *A. elwesi* f. *cavaleriei*, solid squares "typical" *A. elwesi*, and triangles *A. maraho*. Localities: **1.** Taiwan: Taipingshan; **2.** Taiwan: Mingchi; **3.** Zhejiang, China: Longwangshan; **4.** Zhejiang, China: Chimushan; **5.** Jiangxi, China: Lushan; **6.** Jiangxi, China: Yifeng; **7.** Guangdong, China: Nanling; **8.** Chongqing, China: Simianshan; **9.** Guangxi, China: Huaping; **10.** Fujiang, China: Guangze; **11.** Jiangxi, China: Jiujiang; **12.** Hubei, China: Fang Xian; **13.** Guangxi, China: Dayaoshan; **14.** Guizhou, China: Guiyang; **15.** Hunan, China: Tongdao. Red symbols denote samples used in the present study; blue symbols denote records in the literature.

status of *A. elwesi* f. *cavaleriei* by sequencing the entire COI gene sequence including the barcoding region.

MATERIALS AND METHODS

Field observations and rearing procedures

Immatures of *Agehana* were collected from natural host plants. Rearing was performed under laboratory condition in Chongqing, China (for *A. elwesi*) and Taipei, Taiwan (for *A. maraho*). Immatures were reared in plastic containers (15 x 8 x 4.5 cm). Rearing protocols followed the system developed by Powell and De Benedictis (1995). Each collection of immatures was labelled according to the collecting year and month: e.g., HSU 02F4 refers to the 4th collection (4) in June (F) 2002 (02), with the month indicated by a letter. Leaves of its natural host were given to larvae reared in cavity. Only larval hosts found under natural conditions were recorded as confirmed hosts in the present study.

Morphological study

Samples of *Agehana* adult specimens from various localities, including field-collected and reared-out individuals, were compared with each other, and include: "typical" *A. elwesi* (10 ♂♂, 10 ♀♀, Guangdong Prov.; 2 ♂♂, 3 ♀♀, Guangxi Prov.; 2 ♂♂, 1 ♀, Jiangxi Prov.; 2 ♂♂, 3 ♀♀, Zhejiang Prov., China), *A. elwesi* f. *cavaleriei* (7 ♂♂, 8 ♀♀, Chongqing City, China), and *A. maraho* (7 ♂♂, 9 ♀♀, Taiwan). Dissections of genitalia were performed by removing the entire abdomen and placing it in 10% KOH at room temperature for 24 h to dissolve the soft tissue, then transferring it to Cellusolve (Dowanol EE; $C_2H_5OC_2H_4OH$) for another 24 h for descaling, before finally placing it in 70% ethanol for dissection. The dissected parts were preserved in 70% ethanol. Terminology for genitalia follows Klots (1970). Vouchers were deposited in the Department of Life Science, National Taiwan Normal University, Taipei, Taiwan (NTNU).

Molecular study

DNA extraction, polymerase chain reaction (PCR), and sequencing

Genomic DNA was obtained from a leg muscle using the Purgene DNA Isolation kit (Gentra Systems, Minnesota, USA), following the extraction protocol of the manufacturer. Precipitated DNA was resuspended in 50 μl of double-distilled (dd)H_2O and used to amplify the entire mitochondrial COI gene sequence by PCR. Each PCR was carried out in a final volume of 25 μl with 0.8 μl of 10 μM dNTP, 1.5 μl of 25 mM $MgCl_2$, 0.5 μl of each 10 μM of primer, 2.5 μl of 10x *Taq* buffer, and 0.1 μl of Amersham *Taq* (Amersham Biosciences, Buckinghamshire, UK). PCR conditions were as follows: an initial denaturation step of 94°C (2 min), then 35 cycles consisting of denaturation at 94°C (30 s), annealing at 50-60°C (30 s), and extension at 72°C (2 min), followed by an final extension step at 72°C (7 min). Different annealing temperatures were used to improve the PCR quality. The primers used in this study are listed in table 1. The PCR products were run on 1.0% agarose gels in 1x TBE buffer to ensure correct amplification. PCR products were cleaned using a Gel/PCR DNA Fragments Extraction kit (Geneaid, Taipei, Taiwan) when only a single DNA band was visible in a gel. Sequencing reactions were conducted using a 96-well Gel/PCR Clean Up kit (Geneaid) on an ABI3730 DNA Analyzer (Applied Biosystems, Taipei, Taiwan). Both strands were sequenced, and the sequences were checked and assembled into contiguous arrays using Sequencher 4.5 (GeneCode, Boston, USA)

ANALYSES

Genetic information

The population variation was described

Table 1. Primers used in this study (designed for the present study except where indicated)

Name	Sequence (5' to 3')
C1-J-1460[a]	TACAATTTATCGCCTAAACTTCAGCC
C1-N-2191[a]	CCCGGTAAAATTAAAATATAAACTTC
Efcox-J-2100	CTGCTGGAGGAGGCGATCC
Efcox-J-2600	GCAGTATTTGCTATTTTTGGAGG
Chcox-J-2600	TCTATRGGAGCYGTATTTGCTATT
Cppcox-N-2770	TGGATAATCAGAATATCGTCGAGG
Agcox-N-3300	CGTAATGAAGGTAAGGCAA

[a]Modified from k698-J-1460 and Nancy-N-2192 in Caterino and Sperling 1999.

by general statistics: the number of unique haplotypes, variable nucleotide positions, and measured genetic diversity which included nucleotide diversity (π), average number of nucleotide differences per site between 2 randomly chosen sequences, and haplotype diversity (h) of 2 randomly chosen sequences from a sample that differed. These statistical analyses were performed using DNASP 4.10 (Rozas et al. 2003).

Phylogenetic analyses

In order to determine the relationship among the 3 *Agehana* taxa, haplotypes were used to infer the phylogenies by maximum parsimony (MP), maximum likelihood (ML), and Bayesian inference (BI). MP analyses were performed using PAUP* 4.0b10 (Swofford 2002). Heuristic searches used the following settings: starting trees were determined by 10,000 random taxon additions, the tree bisection-reconnection (TBR) branch swapping algorithm was used, gaps were treated as missing data, multiple character states were treated as uncertainties, and all characters were given equal weights. *Chilasa epycides* and *C. agestor* were used as outgroups. Bootstrapping values (Felsenstein 1985) and Bremer support (Bremer 1988 1994) were used to evaluate the clade support. Bootstrapping values were determined using PAUP* with 100 random taxon addition and 1000 repetitions (Swofford 2002). The data matrix in this analysis used only parsimonious characters to raise the variability. Decay values were calculated using the program TreeRot v2c (Sorenson 1999) in conjunction with PAUP* 4.0b10 (Swofford 2002).

ML analyses were performed using PhyML (Guindon and Gascuel 2003). The best substitution models were computed by the program Modletest 3.06 (Posada and Crandall 1998), and the bootstrap analysis was also executed with 1000 repetitions.

The Bayesian analysis was performed using COI data with MrBayes 3.1.2 (Ronquist and Huelsenbeck 2003). The GTR+G+I (general time reversible + gamma distribution + proportion of invariable sites) model which is the most complicated model for all kinds of molecular sequence data was used in this study. We ran 4 chains (3 heated and 1 cold), and each chain initially began from a random tree and ran for 10^6 generations. The log-likelihood scores were sampled every 100 generations, and were plotted against generation time to determine when they became stationary. All sample points which were not stationary were discarded. Finally, we ran the model 2 additional times to ensure that the topologies were not in local optima.

Haplotype network

Conspecific population levels often have lower divergences than species levels. Therefore, we also created a haplotype network to study close relationships (Posada and Crandall 2001). The haplotype network based on maximum parsimony was constructed using the TCS 1.21 software (Clement et al. 2000). Each branch in the network was supported with a 0.95 probability (> 0.95). This setting provided plausibility for the uncertainty of the exact cladogram when only a part of the dataset was used (Templeton et al. 1992).

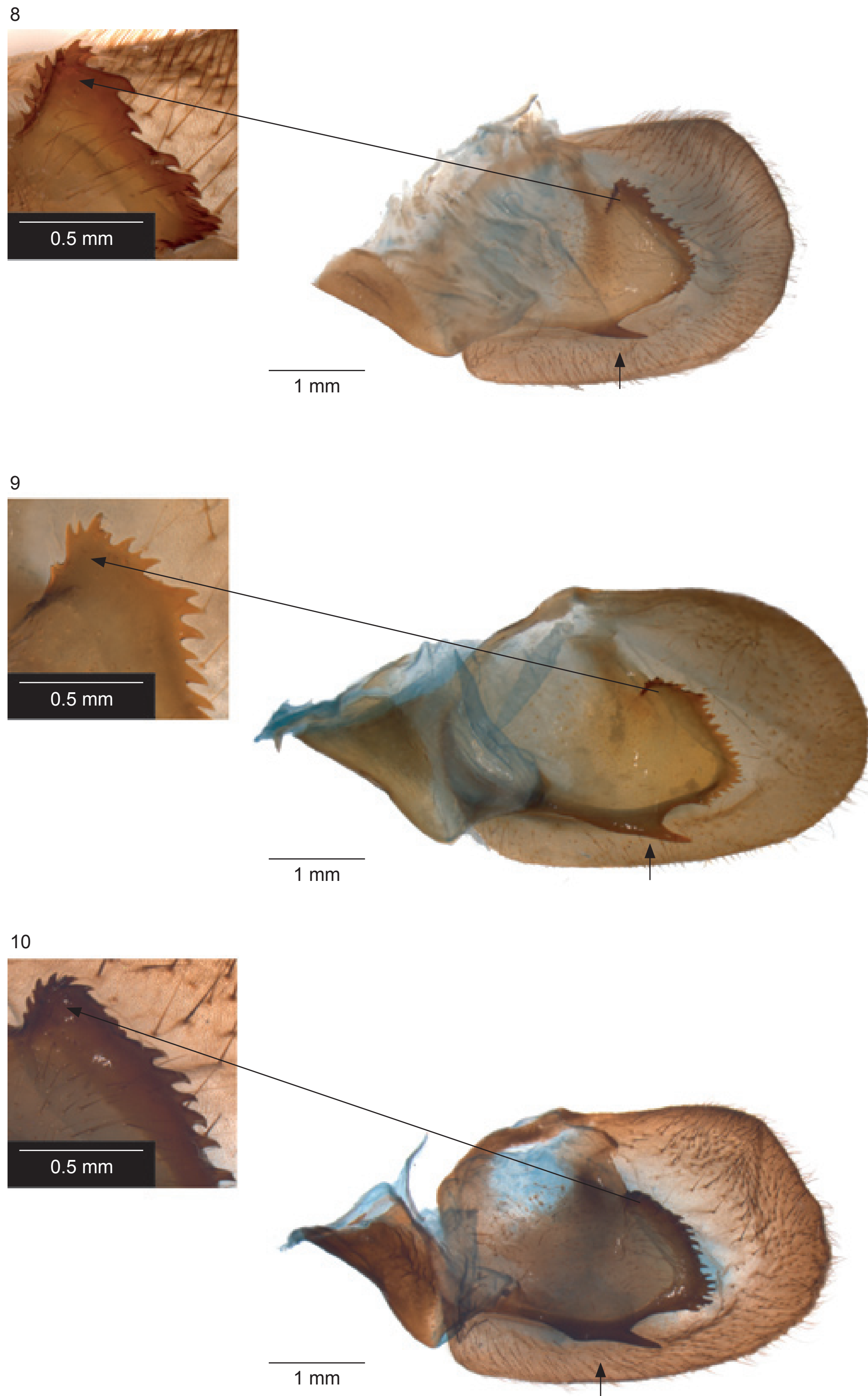
RESULTS

Host plants of *Agehana* swallowtails confirmed in the wild

During the investigation, immatures of *Agehana* swallowtails were found on 4 different host plants in 2 families: “typical” *A. elwesi* was found on *Sassafras tsumu* (Lauraceae) (Zhejiang Prov.: HSU 01G01, 01H55; Guangdong Prov.: HSU 98E60, 99F15, 00F18, 01H12, 06F16; Guangxi Prov.: 06E23), *Liriodendron chinense* (Magnoliaceae) (Jiangxi Prov.: HSU 01F20, 01H54), and *Magnolia officinalis* (Magnoliaceae) (Zhejiang Prov.: HSU 01G3); *A. elwesi* f. *cavaleriei* was taken from *S. tsumu* (Lauraceae) (Chongqing City: 01F21, 01H56, 01H57, 02E59, 02H10; Guangxi Prov.: 99F17), and *A. maraho* was found on *S. randaiense* (Lauraceae) (Taiwan: HSU 98F36, 98H24, 99G7, 00F15, 00G01, 01G7, 01G8, 02E19, 02E78, 02F4, 02H26, 02J7, 03D72, 05E22, 05E35, 05G14, 06E13, 06F10, 06F11, 06F4, 06F41, 06G1, 07F28).

Male genitalia of *Agehana* swallowtails

The male genitalia of all 3 taxa of *Agehana* were similar, with lobe-like valva. Variation was mainly found in the form of the harpe. Harpe of all 3 *Agehana* taxa bear a prominent conical process ventrad, with the base of the process not enlarged in *A. elwesi* f. *cavaleriei* (*n* = 5, Fig. 8, genitalia number: YFH 1427, 1428, 1435, 1438, 1439) and “typical” *A. elwesi* (*n* = 5, Fig. 9,



Figs. 8-10. Right valva of *Agehana taxa*, with magnification of the dorsal lobe shown in the upper left corner of each figure. **8.** *Agehana elwesi* f. *cavaleriei* (based on genitalic dissection YFH 1428); **9.** "typical" *A. elwesi* (based on genitalic dissection YFH 1425); **10.** *A. maraho* (based on genitalic dissection YFH 1431). Arrows point to the conical, ventral process of the harpe.

genitalia number: 1425, 1432, 1433, 1434, 1436), but enlarged in *A. maraho* (*n* = 5, Fig. 10, genitalia number: YFH 1426, 1429, 1430, 1431, 1437). Harpe of all 3 taxa of *Agehana* are serrate, with the dorsal end lobe-like, extending inwardly (Figs. 8-10). The number of teeth on the harpe of *A. maraho* was 21-29 (24 ± 3, *n* = 5), with dorsal lobe bearing 7-10 (8 ± 1, *n* = 5) teeth. The number of teeth on the harpe of typical *A. elwesi* was 15-29 (24 ± 5, *n* = 5), with the dorsal lobe bearing 6-9 (8 ± 1, *n* = 5) teeth. The number of teeth on the harpe of *A. elwesi* f. *cavaleriei* was 20-33 (28 ± 5, *n* = 5), with dorsal lobe bearing 4-10 (9 ± 3, *n* = 5) teeth. The number of teeth along the distal margin of the entire length of the harpe or on its dorsal lobe did not significantly differ among the 3 *Agehana* taxa (Tables 2, 3).

Gene sequence data

In terms of the COI gene sequence including the barcoding region, 3 haplotypes were identified from samples of *A. maraho*, 10 from typical *A. elwesi*, and 2 from *A. elwesi* f. *cavaleriei* (Table 4).

In total, 1531 base pairs (bp) of the COI gene region were sequenced from 56 specimens of *Agehana* (GenBank accession nos.: EU559043-EU559054) plus 2 outgroups *Chilasa epycides* (GenBank accession no.: EU559041) and *C. agestor* (GenBank accession no.: EU559042). The sequence data used in this study included 1530 bp; the terminal T (no. 1531) was excluded as it was part of the stop codon. No deletions or premature stop codons were found in the dataset. There were 196 mutation sites with 92 parsimoniously informative characters. However, when we excluded the outgroup sequences, only 15 variable sites with 8 parsimoniously informative sites among sampled specimens remained. The mean AT proportion was 72%.

The overall nucleotide diversity was relatively low, and haplotype diversity was variable among locations (Table 4). The Nanling site of Guangdong Prov., southern China hosted the highest diversity among the sampled sites.

In total, 12 unique haplotypes of *Agehana* were obtained from the sampled specimens (Table 4). Haplotypes A and B were only present in *A.*

Table 2. Analysis of the variance in the number of teeth on the distal end of the harpe in 3 *Agehana* taxa

Source	*d.f.*	Sum of squares	*F* value	*p* value
Between	2	49.733	1.217	0.3302
Within	12	245.2		
Total	14	294.933		

Table 3. Analysis of the variance in the number of teeth on the dorsal lobe of the harpe in 3 *Agehana* taxa

Source	*d.f.*	Sum of squares	*F* value	*p* value
Between	2	2.8	0.447	0.6499
Within	12	37.6		
Total	14	40.4		

Table 4. Locations, sample number, and genetic diversities of *Agehana* samples

Locality	*n*	Haplotype	Haplotype diversity	Nucleotide diversity
Agehana maraho				
Taipingshan, Taiwan	6	A, B	0.333	0.00022
Mingchi, Taiwan	4	A	0	0
"typical" *A. elwesi*				
Nanling, China	23	C, D, E, F, G, H, I, J, K	0.87	0.00141
Longwangshan, China	5	I, K	0.6	0.00039
Chimushan, China	9	I, J, K, L	0.75	0.00084
Lushan, China	2	I	0	0
Yifeng, China	1	I	-	-
A. elwesi f. *cavaleriei*				
Simianshan, China	6	J, K	0.333	0.00022

maraho, and the other 10 haplotypes were found in *A. elwesi*. Only 2 haplotypes, namely K and J, were found in f. *cavaleriei*, and both were shared with samples of "typical" *A. elwesi* (Table 4). The phylogenetic relationship among the haplotypes is shown in figure 10. The haplotype network is shown in figure 11.

Genetic distances among the 3 *Agehana* taxa were low. The mean *P* distance was 0.004 between *A. maraho* (n = 10) and "typical" *A. elwesi* (n = 40), 0.003 between *A. maraho* and *A. elwesi* f. *cavaleriei* (n = 6), and 0.001 between "typical" *A. elwesi* and *A. elwesi* f. *cavaleriei*.

DISCUSSION

It has been well documented that *A. elwesi* is oligophagous as larva and is associated with a variety of plants in the Lauraceae and Magnoliaceae (Chen and Huang 1993, Huang et al. 1993), whereas *A. maraho* is monophagous on *S. randaiense* (Igarashi 1979). Zhou and Zhang (1981) reported that both *S. tsumu* and *L. chinense* may serve as larval host plants of *A. elwesi* f. *cavaleriei*. The present study confirmed that *A. maraho* is monophagous on *S. randaiense*, whereas immatures of *A. elwesi* were obtained from 3 species of host plants in the families Lauraceae and Magnoliaceae, confirming the host usage information described in the literature referred to above. Immatures of *A. elwesi* f. *cavaleriei*, however, were obtained only from *S. tsumu* (Lauraceae) (Chongqing, China), revealing a host usage similar to that of *A. maraho*.

It was demonstrated that the male genitalia of *A. maraho* and "typical" *A. elwesi* are similar to each other, differing only by the ventral conical process of the harpe of the former being enlarged, whereas that of the latter is not (Huang et al. 1993, Bai and Wang 1998). In samples of male genitalia of *A. elwesi* f. *cavaleriei* examined in the present study, the base of the ventral conical process on the harpe of none was enlarged (Fig. 8), thus being similar to that of the "typical" *A. elwesi* (Fig. 9). Huang et al. (1993) considered that the number of teeth on the harpe of *A. elwesi* was considerably more numerous than that of *A. maraho* (31 vs. 17), and that the teeth number of the "the inner lobe" (termed the dorsal lobe of the harpe in the present study) of *A. elwesi* was 9, in contrast to none in *A. maraho*. We found, however, that the numbers of teeth along the distal margin and dorsal lobe of the harpe were both variable in all 3 *Agehana* taxa, and that all samples of *A. maraho* bore teeth on the dorsal lobe of the harpe (Table 3, Fig. 10). Moreover, the number of teeth on

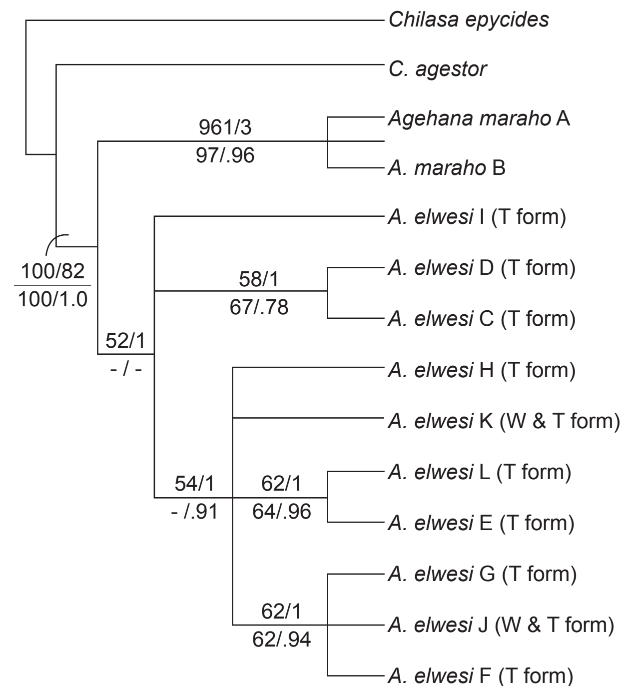
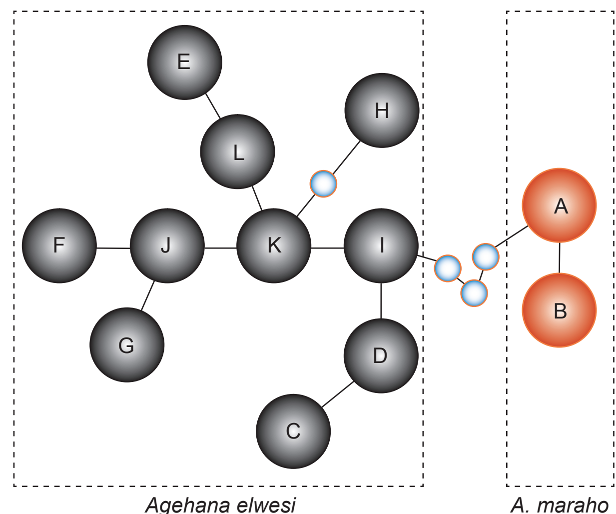


Fig. 11. Phylogenetic relationship among *Agehana* haplotypes based on 1530 bp of the cytochrome oxidase I (COI) fragment, showing only the most parsimonious tree found in the analysis (TL: 209, CI: 0.981, RI: 0.961). Bootstrap support/Bremer support values are plotted above and maximum likelihood/Bayesian posterior probabilities are below the branches. Only support values of > 50 or > 0.5 are shown on the branches. "T form" refers to typical *A. elwesi*, whereas "W form" refers to *A. elwesi* f. *cavaleriei*.



Figs. 12. Haplotype network of *Agehana* swallowtails.

the harpe did not significantly differ among the 3 *Agehana* taxa (Tables 2, 3), thus failing to provide useful diagnostic characters for these taxa. Our observations indicate that the 3 *Agehana* taxa cannot be satisfactorily distinguished by the form of their male genitalia except in the lack of a ventral conical process of the harpe shared by "typical" *A. elwesi* and *A. elwesi* f. *cavaleriei*.

The gene sequence data revealed that the 2 COI haplotypes found in the samples of *A. elwesi* f. *cavaleriei* were identical to two of the 10 haplotypes found in samples of "typical" *A. elwesi*. By contrast, the 2 COI haplotypes from samples of *A. maraho* were not found in the any sample of "typical" *A. elwesi* nor *A. elwesi* f. *cavaleriei*. The phylogenetic relationship of among these COI haplotypes revealed that *A. maraho* formed a monophyletic assemblage sister to the other monophyletic assemblage formed by "typical" *A. elwesi* plus *A. elwesi* f. *cavaleriei* (Fig. 11), and differing by 3 bp (Fig. 12). Both the morphological features of the genitalia and DNA sequence data thus supported *A. elwesi* f. *cavaleriei* being placed within *A. elwesi*, as suggested by Li and Zhang (1984), and that it is not more closely related to *A. maraho* than to the "typical" *A. elwesi*. Thus its systematic status does not affect the conservation status of *A. maraho*, which remains a rare taxon endemic to Taiwan. Nevertheless, as the lineage support of *A. maraho* + *A. elwesi* was very robust, in contrast to a relatively low support of the *A. maraho* lineage and *A. elwesi* lineage (Fig. 11), whether these 2 taxa are conspecific remains an open question to be investigated in the future.

Acknowledgments: We thank S.M. Wang (Conservation and Recreation Division, Taiwan Forestry Bureau, Council of Agriculture (COA), Taipei, Taiwan) for issuing collecting permits for the *Agehana maraho* samples. H.H. Hsu and J.S. Shia (Taiwan Forestry Bureau, Council of Agriculture, Taipei, Taiwan) assisted with administrative issues for our project. T.T. Huang (Hsinchu Forest District Office, Forestry Bureau, Council of Agriculture, Hsinchu, Taiwan), P. Yang (Chongqing Station of Pests Control and Quarantine, Chongqing, China), and Z.q. Qian (Institute of Agricultural Sciences of Lishui, Lishui, China) assisted with field work. C.R. Chen and T.W. Chen (National Taiwan Normal University, Taipei, Taiwan) assisted with the preparation of the figures. D. Kain (Merced College, Merced, California, USA) kindly read our manuscript and greatly improved it. We also thank 2 anonymous reviewers who gave many helpful comments. This study was supported by COA grants 91AS-2.1.4-FC-R1, 92AS-4.1.4-FC-R1, 93AS-4.1.1-FB-e2, and 94AS-9.1.7-FB-e1.

REFERENCES

Bai W, HY Wang. 1998. Swallowtail butterflies in China. Taipei, Taiwan: Sueshin Publisher. (in Chinese)

Bremer K. 1988. The limits of amino acid sequence data in angiosperm phylogenetic reconstruction. Evolution **42:** 795-803.

Bremer K. 1994. Branch support and tree stability. Cladistics **10:** 295-304.

Brown JW, S Miller, M Horak. 2003. Studies on New Guinea moths. 2. Description of a new species of *Xenothictis* Meyrick (Lepidoptera: Tortricidae: Archipini). Proc. Entomol. Soc. Wash. **105:** 1043-1050.

Chen H, S Huang. 1993. Study on the bionomics of *Agehana elwesi* Leech. J. Zhejiang Agric. Univ. **19:** 128. (in Chinese)

Cheng HC, CT Yao, HC Lin, TW Li, LH Lin, CF Lu. 1996. A guide of endangered wild animals. Chichi, Taiwan: Taiwan Endemic Species Research Institute. (in Chinese)

Chou I. 1994. Monographia Rhopalocerorum Sinensium. Zhenzhou, China: Henan Scientific and Technological Publishing House. (in Chinese)

Clement M, D Posada, K Crandall. 2000. TCS: a computer program to estimate gene genealogies. Mol. Ecol. **9:** 1657-1660.

Collins NM, MG Morris. 1985. Threatened swallowtail butterflies of the world. The IUCN red data book. Gland, Switzerland and Cambridge, UK: International Union for the Conservation of Nature (IUCN).

Costa FO, JR de Waard, JB Boutillier, S Ratnasingham, R Dooh, M Hajibabaei, PD Hebert. 2007. Biological identification through DNA barcodes: the case of the Crustacea. Can. J. Fish. Aquat. Sci. **64:** 272-295.

D'Abrera B. 1982. Butterflies of the Oriental Region, Part I Papilionidae, Pieridae & Danaidae. Victoria, Australia: Hill House.

Felsenstein J. 1985. Confidence limits on phylogenies: an approach using the bootstrap. Evolution **39:** 783-791.

Gimenez Dixon M. 1996. *Papilio maraho*. *In* IUCN 2007. 2007 IUCN red list of threatened species. Available at www.iucnredlist.org. Accessed 9 Mar. 2008.

Guindon S, O Gascuel. 2003. A simple, fast, and accurate algorithm to estimate large phylogenies by maximum likelihood. Syst. Biol. **52:** 696-704.

Hajibabaei M, DH Janzen, JM Burns, W Hallwachs, PDN Hebert. 2006. DNA barcodes distinguish species of tropical Lepidoptera. Proc. Natl. Acad. Sci. USA **103:** 968-971.

Huang B, S Qi, X Wu. 1993. Butterflies of *Agehana* on the two sides across the Taiwan Strait. Entomol. J. East China **2:** 5-9. (in Chinese with English abstract)

Igarashi S. 1979. Papilionidae and their early stages. Tokyo: Kodansha. (in Japanese)

Klots AB. 1970. Lepidoptera. *In* SL Tuxen, ed. Taxonomist's glossary of genitalia in insects, 2nd edn. Copenhagen: Munksgaard, pp. 115-130.

Le Cerf MF. 1923. Descriptions de formes nouvelles de Lépidoptères Rhopalocères. Bull. Mus. hist. Nat. **24:**

360-367.
Li C, L Zhang. 1984. A review of the Chinese rare papilionid butterfly *Agehana* elwesi (Leech). Acta Zootaxonom. Sin. **9:** 335. (in Chinese)
Posada D, KA Crandall. 1998. Modeltest: testing the model of DNA substitution. Bioinfo. Applic. Note **14:** 817-818.
Posada D, KA Crandall. 2001. Intraspecific gene genealogies: trees grafting into networks. Trends Ecol. Evol. **16:** 37-45.
Powell JA, JA De Benedictis. 1995. Biological relationships: host tree preferences and isolation by pheromones among allopatric and sympatric populations of western *Choristoneura*. Univ. Calif. Publ. Entomol. **115:** 21-68.
Ronquist F, JP Huelsenbeck. 2003. MrBayes 3: Bayesian phylogenetic inference under mixed models. Bioinformatics **19:** 1572-1574.
Rozas J, JC Sanchez-DelBarrio, X Messeguer, R Rozas. 2003. DnaSP, DNA polymorphism analyses by the coalescent and other methods. Bioinformatics **19:** 2496-2497.
Sorenson MD. 1999. TreeRot, vers. 2. Boston, MA: Boston Univ.
Swofford DL. 2002. PAUP: phylogenetic analysis using parsimony (and other methods), vers. 4.0 b10. Sunderland, MA: Sinauer Associates.
Templeton AR, KA Crandall, CF Sing. 1992. A cladistic analysis of phenotypic associations with haplotypes inferred from restriction endonuclease mapping and DNA sequence data. III. Cladogram estimation. Genetics **132:** 619-633.
van Velzen R, F Bakker, JJA van Loon. 2007. DNA barcoding reveals hidden species diversity in *Cymothoe* (Nymphalidae). Proc. Netherl. Entomol. Soc. Meet. **18:** 95-103.
Yen SH, PS Yang. 2000. Illustrated guide to insects protected by the *Wildlife Conservation Law* (including species protected by CITES appendices). Taipei, Taiwan: Council of Agriculture, Executive Yuan. (in Chinese)
Yen SH, PS Yang. 2001. Illustrated identification guide to insects protected by the CITES and *Wildlife Conservation Law* of Taiwan, R.O.C. Taipei, Taiwan: Council of Agriculture, Executive Yuan.
Zhou L, L Zhang. 1981. A preliminary observation on *A. elwesi cavaleriei*. Insect Knowl. **18:** 254-255. (in Chinese)