Alien species revises systematic status: integrative species delimitation of two similar taxa of *Symbrenthia* Hübner, [1819] (Lepidoptera, Nymphalidae)

Yu-Feng Hsu [Corresp., 1], **Zong-Yu Shen** [1, 2, 3], **Hang-Chi Huang** [4], **Chih-Wei Huang** [1], **Chen-Chih Lu** [5]

[1] Department of Life Science, National Taiwan Normal University, Taipei, Taiwan, R.O.C.

[2] Biodiversity Research Center, Academia Sinica, Taipei, Taiwan, R.O.C.

[3] Biodiversity Program, Taiwan International Graduate Program, Academia Sinica and National Taiwan Normal University, Taipei, Taiwan, R.O.C.

[4] Butterfly Conservation Society of Taiwan, Taipei, Taiwan, R.O.C.

[5] Fu-Xing Elementary School, New Taipei City, Taiwan, R.O.C.

Corresponding Author: Yu-Feng Hsu
Email address: t43018@ntnu.edu.tw

Introduction of organisms to new range may impose detrimental effects on local organisms, especially when closely related species are involved. Species delimitation employing an integrative taxonomy approach may provide a quick assessment for the species status between taxa of interest, and to infer ecological competition and/or introgression that may be the associated with the introduction. A nymphalid butterfly, *Symbrenthia lilaea lunica*, was recently introduced to Taiwan, where a closely related local taxon, *S, l. formosanus*, can be found. We employed multiple species delimitation methods to study the species status between the two taxa, and the results revealed that they can be recognized as two distinct species. We further performed a niche modeling approach to investigate the ecological interaction between the two species. The taxonomic status of the two taxa, now elevated to species, has been revised and conservation facing rapid expansion of the introduced species discussed.

Alien species revises systematic status: integrative species delimitation of two similar taxa of *Symbrenthia* Hübner, [1819] (Lepidoptera, Nymphalidae)

Yu-Feng Hsu[1*], Zong-Yu Shen[1,2,3], Hang-Chi Huang[4], Chih-Wei Huang[1] and Chen-Chih Lu[5]

[1]Department of Life Science, National Taiwan Normal University, Taipei, Taiwan, R. O. C.

[2]Biodiversity Research Center, Academia Sinica, Taipei, Taiwan, R. O. C.

[3] Biodiversity Program, Taiwan International Graduate Program, Academia Sinica and National Taiwan Normal University, Taipei, Taiwan, R. O. C.

[4]Butterfly Conservation Society of Taiwan, Taipei, Taiwan, R. O. C.

[5]Fu-Xing Elementary School, Zhonghe Dist., New Taipei City, Taiwan, R. O. C.

*Corresponding Author: Yu-Feng Hsu (t43018@ntnu.edu.tw)

Orcid ID: 0000-0003-2091-7775

Tel: +886-2-77496340

Address: No. 88, Sec. 4, Tingzhou Road, Wenshan Dist., Taipei 116, Taiwan, R. O. C.

ABSTRACT

Introduction of organisms to new range may impose detrimental effects on local organisms, especially when closely related species are involved. Species delimitation employing an integrative taxonomy approach may provide a quick assessment for the species status between taxa of interest, and to infer ecological competition and/or introgression that may be the associated with the introduction. A nymphalid butterfly, *Symbrenthia lilaea lunica*, was recently introduced to Taiwan, where a closely related local taxon, *S. l. formosanus*, can be found. We employed multiple species delimitation methods to study the species status between the two taxa, and the results revealed that they can be recognized as two distinct species. We further performed a niche modeling approach to investigate the ecological interaction between the two species. The taxonomic status of the two taxa, now elevated to species, has been revised and conservation facing rapid expansion of the introduced species discussed.

Keywords Systematics, Species delimitation, Biological introduction, Species interaction, Niche modeling

Introduction

Biological invasions and range expansion of organisms usually impose unfavorable effects on local organisms that share similar ecological requirements (*Mooney & Cleland, 2001*), particularly when the expanding form is of continental origin and entering insular areas (*Sax & Gaines, 2008*). Competition may occur between native taxa and the invading one, notably when the counter taxa are closely related, where they are expected to share resource requirements (*Zwerschke et al., 2018*). This kind of scenarios have been well-documented in various organisms, such as ants (*von Aesch & Cherix, 2005*), birds (*Koenig, 2003*), molluscs (*Zwerschke et al., 2018*), plants (*Leger & Espeland, 2010*; *Čuda et al., 2015*; *Sheppard & Brendel, 2021*), etc., and even inspired E. O. Wilson to coin in his "taxon cycles" hypothesis (*Wilson, 1961*). Alternatively, if the involved taxa were previously allopatric populations or subspecies, gene flow may occur between taxa, resulting in changes in the genetic make-up of the local population (*Rhymer & Simberloff, 1996*). Consequently, whether the exotic taxon represents interspecific or intraspecific entity to the local taxon may lead to different types of ecological and evolutionary impacts to local community. Species delimitation employing an integrative taxonomy approach has becoming popular to help with taxonomic treatments/decisions among closely related taxa and to detect cryptic species (*Moraes et al., 2021*). This approach can also help with assessing whether an invading taxon is conspecific or not to the local taxon.

A case of recent invasion, either by anthropogenic introduction or by natural means such as wind, of a nymphalid butterfly currently recognized as *Symbrenthia lilaea lunica* Bascombe, Johnston & Bascombe, 1999 (Fig. 1A－1D) into Taiwan has been reported (*Lu & Chen*, *2014*; *Hsu et al.*, *2022*). In Taiwan, there is a local native *Symbrenthia* Hübner, [1819] taxon, *S, l. formosanus* Fruhstorfer, 1908 (Fig, 1E－1H; *Shirôzu & Ueda, 1992*; *Hsu et al.*, *2022*). The taxon *lunica* Bascombe, Johnston & Bascombe, 1999 is a replacement name for *Papilio lucina* Stoll, 1780; in Cramer, De uitlandsche kapellen 4(26b-28): 82 (*Bascombe et al., 1999*), distributed from northern Indochina to southern China according to Tsukada & Nishiyama (1985). Although some authors (e. g. *Bozano & Floriani, 2012*; *Ek-Amnuay, 2012*; *Lang, 2012*; *Monastyrskii, 2019*) regarded *lucina* a synonym of the nominotypical *lilaea* described from India but have not formally synonymized these two names, others treat them as separate subspecies (e. g. *Tsukada & Nishiyama, 1985*; *Hsu et al., 2022*; *Fric et al., 2022*). *Symbrenthia lilaea lunica* from continental Asia was first documented in 2004 in Taiwan *(Lu & Chen*, *2014)*. Natural observations in Taiwan on immature biology and hostplant usage of both *S. l. lunica* and *S. l. formosanus* have documented that the two taxa use the same larval hostplants (*Lu & Chen, 2014*). The invading or introduction event represents a ‘natural experiment’ in which to examine whether these two taxa are conspecific as current classification suggests, or if they represent distinct biological species. The answer to this natural experiment may help clarify what

ecological effect the introduced taxon may impose on the native taxa. If they turn out to represent distinct species, competition between them may occur as they do not differ in larval hostplant usage. If they turn out to be conspecific, gene introgression may occur since hybridization is liable to occur.

We investigated the species status between the two now sympatric taxa in Taiwan using multiple coalescent model based species delimitation methods and distance based species delineation, under a scheme for testing hypothesis of taxonomic status developed by *Braby et al. (2012)*, following by a niche modeling survey to understand the ecological interaction between two taxa.

Materials and Methods

Sampling

The mitochondrial cytochrome oxidase subunit I (*COI*) gene has been successfully applied as a helpful marker with which to delimit closely related species (*Hebert et al., 2003*). We included a total of 13 specimens collected from various localities in our *COI*-based study. Four specimens of *S. l. formosanus* were collected around Taiwan, and six specimens of *S. l. lunica* were collected in Taiwan (mainland and Matsu archipelago), China, and Thailand. All samples were preserved in 70% ethanol and kept at -20℃ for the subsequent molecular study. Moreover,

92 additional *COI* sequences of *S. l. formosanus* (AY788679) from Taiwan, *S. l. lunica* (EU368155,
93 KJ649017, KX300094) from China, Vietnam, Myanmar respectively, and the nominate
94 subspecies *S. l. lilaea* (Hewitson, 1864) (KP644228, KP644229) from India were obtained from
95 GenBank. For the phylogenetic analyses, we used one sequence *of S. brabira* Moore, 1872
96 (EU368154) as an outgroup, which was also obtained from GenBank.
97
98 **DNA extraction, PCR amplification and DNA sequencing**
99 Genomic DNA was extracted from one leg of specimens using the Gentra Puregen tissue kit
100 form QIAGEN (QIAGEN, Maryland, USA), following the manufacturer's protocol. A partial
101 fragment from the *COI* gene was targeted for amplification by polymerase chain reaction (PCR).
102 The *COI* gene was amplified using the universal primers COX-J-1460 (5'-
103 TACAATTTATCGCCTAAACTTCAGCC-3') and COX-N-2191 (5'-
104 CCCGGTAAAATTAAAATATAAACTTC-3'). PCR reactions were performed in a 30μL
105 volume eppendorf, containing 1μL of extracted DNA, 23.5μL of ddH_2O, 3μL of 10X PCR
106 reaction buffer, 0.6μL of each primer and 0.3μL of Power Taq (Genomics Biosci & Tech,
107 Taiwan). The following PCR protocol was used: an initial denaturation at 95℃ for 5 min,
108 followed by 40 cycles of 30s denaturation at 95℃, 30s annealing at 50℃ and 45s extension at
109 72℃, and a final extension at 72℃ for 10 min. Automatic sequencing was preformed using an

ABI 3730XL DNA Analyzer (Applied Biosystems).

Sequence Analyses and Phylogenetic Reconstruction

Sequences were edited and assembled using Sequencher 4.10.1 (Gene Codes Corporation, Ann Arbor, USA), and sequence alignments were performed using MUSCLE in MEGA 11(*Tamura et al., 2021*), and pairwise genetic distances between different populations of *S. l. formosanus* and *S. l. lunica* were also measured using MEGA 11 with the Kimura 2-parameter model.

The best-fit nucleotide substitution model for phylogenetic analysis was inferred using jModelTest 2.1.10 (*Posada, 2008*) based on Akaike information criterion (AIC). Phylogenetic trees were reconstructed under maximum likelihood (ML) and Bayesian inference (BI). ML analysis was performed using RAxML v8.2.10 (*Stamatakis, 2014*) with 1000 bootstrap replicates to assess the reliability of the tree. BI analysis was performed using MrBayes 3.2.6 (Ronquist *et al*., 2012). For MrBayes, the substitution model inferred from jModelTest was applied. The Bayesian Markov Chain Monte Carlo (MCMC) analysis for 10^9 generations with sampling every 1000 generations was run to ensure the average standard deviation of split frequencies were below 0.01. The first 30% of trees were discarded as burn-in. FigTree v1.4.4 was used to visualize the consensus tree.

Molecular species delimitation analyses

Many molecular species delimitation programs have been proposed and broadly applied in speciation studies, which provides important evidence for integrative taxonomy. Among molecular species delimitation programs, the Poisson Tree Processes model (PTP) (*Zhang et al., 2013*), the Automatic Barcode Gap Discovery (ABGD) (*Puillandre et al., 2012*), and the Generalized Mixed Yule Coalescent model (GMYC) (*Fujisawa & Barraclough, 2013*) were developed as single locus-based approaches for species delimitation. Therefore, we delineated species limits among *S. l. formosanus*, *S. l. lunica*, and *S. l. lilaea* by employing the Molecular Operational Taxonomic Unit concept set by these three programs.

For PTP, we used the tree inferred by MrBayes as input tree on the web server (https://species.h-its.org/ptp/), with 100000 MCMC generations and 100 thining. Subsequently, PhyloMap was used to visualize the results of PTP. For ABGD, we performed the analyses on the web version of ABGD (https://bioinfo.mnhn.fr/abi/public/abgd/), with default settings of relative gap width (X=1.5) and the Kimura two-parameter (K2P) model for nucleotide substitution. For GMYC, we used the phylogenetic tree inferred by MrBayes 3.2.6. The results from MrBayes were forced bifurcated by the “multi2di” and “chronos” function in the package “ape” in R 4.1.2. A single-threshold GMYC analysis was performed in the R package splits v1.0-20. We chose the single-threshold model because of the limited improvements of multiple-

146 threshold model.

147

148 **Species distribution model of *S. l. formosanus* and *S. l. lunica* in Taiwan**

149 *Symbrenthia lilaea lunica* was not known to occur in Taiwan until recently, although it inhabits
150 Matzu and Kinmen, which are small outlying islands of Taiwan and close to mainland Asia.
151 However, *S.l. lunica* arrived to the main island of Taiwan due to anthropogenic activities or via
152 natural dispersal, with the first credible record found in Xinzhu in northwestern Taiwan in 2004
153 (*Lu & Chen, 2014*). Since then, the range of *S. l. lunica* has expended quickly, and is currently
154 found in lowland areas throughout Taiwan (*Lu & Chen, 2014*; *Hsu et al., 2022*). It is an
155 interesting issue whether competitive exclusion has happened between *S. l. lunica* and native *S. l.*
156 *formosanus*, especially if the species delimitation analyses decide they represent different
157 species.

158

159 The occurrence data of *S. l. formosanus* and *S. l. lunica* were obtained from the Global
160 Biodiversity Information Facility (GBIF) (https://gbif.org/, accessed 26 July 2021), Taiwan Moth
161 Information Center (https://twmoth.tersi.gove.tw/, accessed 26 July 2021), and the specimen
162 collection at National Taiwan Normal University. To test the interaction between these two
163 species, we separated the occurrence data into two stages based on year. Because the first

documentation of *S. l. lunica* was in 2004, we divided the time period based on the median year (2012). The early invasion was defined as the data recorded from 1911－2012, and the late invasion was defined as the data recorded from 2013－2021. Repeated data was excluded using R 4.1.2, and we ensured the presences of only one presenting point in each raster to avoid overfitting. In total, 48 and 43 localities were obtained during the early invasion stage for *S. l. formosanus* and *S. l. lunica*, respectively (Fig. 2A), and 132 and 192 localities were obtained during the later invasion stage for *S. l. formosanus* and *S. l. lunica*, respectively (Fig. 2B). These data were organized using Microsoft Excel for the subsequent analyses.

A total 19 bioclimate variables (period: 1979－2013) were collected from CHELSA (https://chelsa-climate.org/, accessed on 14 July 2021) at a spatial resolution of 30 arc-seconds ($1km^2$). These bioclimate variables were derived from temperature and precipitation, which are considered to be related to the distribution and survival of small arthropods and have been widely used in the prediction of species distribution (*De Meyer et al., 2010; Xu et al., 2020*). In order to avoid the effect of multicollinearity, these 19 variables were selected by the "vifstep" and "vifcor" function with the threshold of 10 and 0.6 separately in "usdm" package in R 4.1.2 (selected variables shown in Fig. 3).

MaxEnt (3.4.4) (*Phillips et al., 2006*) was applied to predict the habitat suitability of *S. l. formosanus* and *S. l. lunica* based on the occurrence data. 10% of the data were selected to run a

182 random test and the remaining data were run following the default settings. Presence-only data
183 were generated pseudo-absences and 10000 random background points were randomly selected
184 by the MaxEnt model. The results were output after 10 cross-validation replicates.
185 The predictions generated from MaxEnt modeling were evaluated according to the
186 threshold independent area under the receiver operating characteristic (ROC) curve (AUC)
187 values. ROC curves were used to plot the true-positive rate against the false-positive rate and the
188 AUC was used as a measure of the goodness of fit of the model. The AUC value ranges from 0
189 to 1, with higher values indicating higher predictive performance. The logistic output was chosen
190 as an estimate of the probability of presence conditioned by bio-environmental variables per grid
191 cell. Jackknifing was used to screen for the contribution of each bio-environmental variable used
192 in the model.
193 We performed principal component analyses (PCA) to test the niche overlap of these two
194 species in both the early invasion stage and late invasion stage. The 19 bioclimatic variables
195 were obtained from the CHELSA database based on the GPS of each observation point. The
196 analyses were conducted in R 4.1.2 using the function “prcomp”, with scatterplots built using the
197 function “ggbiplot”. Additionally, in order to evaluate the niche shift pattern between the two
198 *Symbrenthia* species in Taiwan, we apply methods modified from *Bates et al. (2020)* to quantify
199 the niche shift between *S. l. formosanus* and *S. l. lunica* by calculating niche overlap, presented

by Schoener's D, and niche expansion of *S. l. lunica*.

Results

Taxonomic decisions

Phylogenetic reconstruction of *Symbrenthia COI* samples (Fig. 4) revealed that all samples of *lunica+lilaea* form a monophyletic group sister to *formosanus* samples, which also formed a monophyletic group. The p-distance was 0.0017 between *lunica* and *lilaea* and 0.0505－0.0525 between *lunica+lilaea* and *formosanus*. PTP, ABGD and GMYC all recognize a two species scenario, with *lunica+lilaea* and *formosanus* each representing a distinct species. Therefore, *formosanus* is recognized as a species distinct from *lunica+lilaea*, with the combination as *Symbrenthia formosanus* Fruhstorfer, 1908 (**stat. rev.**). The taxon *S. l. lunica* Bascombe, Johnston & Bascombe, 1999 is proposed to be lumped with *S. l. lilaea*, Hewitson, 1864 (**syn. nov.**) herein as the two may not be distinguished by *COI* barcode nor adult and immature morphology. We thus will call them *S. formosanus* and *S. lilaea* respectively in the remaining text of this article.

Environmental factors which contribute to the distribution of *Symbrenthia* species in question

After applying "usdm" package to remove those highly correlated factors, “bio 2”, “bio 8”, “bio 12” (Fig. 3A & 3B) are used to construct the species distribution model of “early invasive stage”, while “bio 7”, “bio 8”, “bio 9”, “bio 12”, “bio 18” (Fig. 3C & 3D) are used to construct the species distribution model of “late invasive stage”. According to the results of the jackknife test, the factors show different contribution patterns in the early invasive stage. In the early invasive stage, “bio 2” (annual precipitation) and “bio 12” (air temperature) contribute reversely between these two species; annual precipitation contributes more than mean diurnal air temperature range in the distribution model of *S. formosanus,* whereas mean diurnal air temperature range contributes more than annual precipitation amount in the model of *S. lilaea*.

Comparing the jackknife results of both species between the two invasive stages, “bio 8” contributes the most among all models. According to this, the mean daily air temperatures of the wettest quarter may play a key role in the distribution of these two *Symbrenthia* species in Taiwan.

The species distribution model and niche shifting of the two *Symbrenthia* species in different time stages

According to the species distribution model results, *S. formosanus* does not show an obvious change between the early and late invasive stages (Fig. 5A & 5C). For both invasive stages, the

presence probability of *S. formosanus* seems to be higher in the suburban areas and places with less human activity. For *S. lilaea*, the distribution model presents different results between the two time stages (Fig. 5B & 5D). Particularly, presence probability in the southwest part of Taiwan is higher in the later invasive stage (Fig. 5D). The SDM results of both species show that the presence probability decreases in the Pingtung area, the southernmost county of Taiwan. Although there may be biological importance to this observation, it is most likely a result of uneven presence observation point density in the later stage. Most of the presence points for the late invasive stage SDM are from northern Taiwan.

From the results of the early and late invasive stages (Fig 6), the niche overlap value increased during the recent years (past-2012 D: 0.48; 2013-2021 D: 0.64), and the niche expansion value of *S. lilaea* remained zero between the two different time stages. Together, these mean that, during these two periods of time, the niche of this alien species did not extend beyond the niche of the native species. According to the ENM model and the niche shift results, competitive exclusion seems to not be occurring between these two species over these 18 years.

Discussion

Taxonomic status of the introduced and native *Symbrenthia* butterflies

The introduced and native *Symbrenthia* butterflies in question of the study were regarded as

conspecific subspecies prior to the present study (e. g., *Hsu et al., 2022*; *Fric et al.*, *2022*). It has been argued that species delimitation is difficult for allopatric populations or subspecies of similar forms (*King, 1993*; *Braby et al., 2012*), but in the present case, the introduction of continental *S. lilaea* to Taiwan has proven that insular *S. formosanus* ought to represent a species endemic to the island, instead of being a geographical race of the former. Distinctions between them include: 1) distal band on hindwing uppersides of both sexes form a continuous orange stripe in *S. lilaea* (Fig. 1A & 1C), whereas it is interrupted by darkened veins in *S. formosanus* (Fig. 1E & 1G)*;* 2) distal tip of uncus is acute in *S. lilaea* (Fig. 7A), whereas it is blunt in *S. formosanus* (Fig. 7D); 3) distal margin of valva is rounded in S. *lilaea* (Fig. 7A), whereas it is angled, somewhat squared in *S. formosanus* (Fig. 7D); 4) ampulla is stout, slightly down-curved in *S. lilaea* (Fig. 7A), whereas it is slender, strongly bent downwards in *S. formosanus* (Fig. 7D); 5) posterior margin of sterigma is concave in *S. lilaea* (Fig. 7C), whereas it is truncate in *S. formosanus* (Fig. 7F); 6) yellow eggs are laid in cluster in *S. lilaea* (Fig. 8A), in contrast to green eggs laid singly in *S. formosanus* (Fig. 8D); 7) larvae are gregarious in *S. lilaea* (Fig. 8B-8C), but solitary in *S. formosanus* (Fig. 8E－8F).

Vouchers.

***Symbrenthia formosanus* Fruhstorfer, 1908**

Ssp. *formosanus*: **XINBEI CITY [= TAIPEI Co.]:** 1♀, Xindian, Sikanshui, 5. X. 2001 (H. S. Que leg.); 1♂, Wulai, 150m, reared from *Boehmeria nivea*,emerged 8. XI. 2004, HSU 04L21 (Y. F. Hsu leg.); 1♂, Wulai, 13. II. 2005, reared from *Debregeasia orientalis*, emerged 12. III. 2005, HSU 05B6 (L. H. Wang leg.); 1♀, same locality, 5. VI. 2005, reared from *B. densiflora*,emerged 23. VI. 2005, HSU 05F9 (Y. F. Hsu leg.); 1♂, same locality, 5. VI. 2005, reared from *D. orientalis*, emerged 5. VI. 2005, HSU 05F10 (Y. F. Hsu leg.); 1♂, same locality, 13. VI. 2005, reared from *B. nivea*, emerged 2. VII. 2005, HSU 05F36 (Y. F. Hsu leg.); 1♀, same locality, 16. I. 2007 (Y. F. Hsu leg.); 1♂, Wulai, ca 200m, 5. VI. 2005 (Y. F. Hsu leg.). **TAIPEI CITY:** 1♀, Daan, Baozangyan, 28. III. 2019 (L. Huang leg.). **TAOYUAN CITY [= TAOYUAN Co.]:** 1♂, Fuxing, Xuanyuan, ca 1000m, 16. VI. 2005 (L. H. Wang leg.); 1♀, Fuxing, Daman, 10. VI. 2005, reared from *B. nivea*, emerged 22-23. VI. 2005, HSU 05F28 (Y. F. Hsu leg.); 1♀, same locality, 10. VI. 2005, reared from *D. orientalis*, emerged 23. VI. 2005, HSU 05F29 (Y. F. Hsu leg.); 1♀, Fuxing, Gaoyi, 600m, 7. III. 2008 (Y. F. Hsu leg.). **YILAN Co.:** 1♂, 2♀, Nanao, 14Km S. Nanao, 6. VI. 2005, reared from *B. nivea*, emerged 8-17. VI. 2005, HSU 05F17 (Y. F. Hsu leg.); 3♂, 1♀, Datong, Qilan, 1. XII. 2005, reared from *B. nivea*, emerged III. 2006, HSU 05M7 (C. C. Lu & H. Y. Lee leg.). **XINZHU Co.:** 2♂, Jianshi, Xiuluan, 800m, 21. VI. 2005 (L. H. Wang leg.); 1♀, Jianshi, Guanwu, Dalu Forest Trail, 2. X. 2006 (L. H. Wang leg.); 1♂, Jianshi, Yulao, ca 1500m, 25. II. 2009 (C. K. Wang leg.). **MIAOLI Co.:** 1♂, Zhuolan, Liyutan,

300m, 2. VII. 2006 (Y. F. Hsu leg.). **TAIZHONG CITY [= TAICHUNG Co.]:** 1♀, Heping, Shangguguan, 900m, 30. X. 2006 (Y. F. Hsu leg.); 1♂, same locality, 29. VII. 2007 (Y. F. Hsu leg.); 1♂, Heping, Guguan, 700m, 5. VII. 1998 (Y. T. Lo leg.); 1♂, Heping, Guguan, IV. 2005 (L. H. Wang leg.). **NANTOU Co.:** 1♂, Renai, Tunyuan/ Tianchi, 2000/2800m, 31. VII- 1. VIII. 1998 (Y. T. Lo leg.); 1♂, 1♀, Renai, Meiyuan, ca 400m, 22. XI. 2004, reared from *B. nivea*, emerged 16-22. XII. 2004, HSU 04L49 (Y. F. Hsu leg.); 1♀, Yuchi, Shiguanyin, 480m, 22. XI. 2004, reared from *B. densiflora*, emerged 21. XII. 2004, HSU 04L52 (Y. F. Hsu leg.); 1♂, Lugu, Fenghuanggu, 600m, 4. XII. 2004, reared from *B. densiflora*,emerged 23. XII. 2004, HSU 04M5 (Y. F. Hsu leg.); 1♂, 3♀, Lugu, Fenghuanggu, 4. XII. 2004, reared from *B. nivea*, emerged 17-20. XII. 2004, HSU 04M4 (Y. F. Hsu leg.), 1♀, same locality, 11. XII. 2004, reared from *B. densiflora*, emerged 14. II. 2005, HSU 04M14 (Y. F. Hsu leg.), 1♂, same locality, 12. XII. 2004 (Y. F. Hsu leg.); 1♂, Renai, Beidongyanshan, ca 1800m, 17. XII. 2005 (L. W. Wu & Y. F. Hsu leg.) (genitalia preparation YFH 1583), 1♀, same locality, 17. XII. 2005, reared from *D. orientalis*, emerged 4. I. 2006, HSU 05M32 (Y. F. Hsu leg.); 3♂, Renai, Tunyuan, 1550m, 28. VII. 2006 (Y. F. Hsu leg.); 1♀, Renai, Nanshanxi, ca 900m, 1. IX. 2007 (Y. F. Hsu leg.); 1♂, Lugu, ca 600m, 19. VII. 2006, reared from *B. nivea*, emerged 4. VIII. 2006, HSU 06G12 (Y. F. Hsu leg.); 1♂, 2♀, Lugu, ca 500m, 1. I. 2007, reared from *B, nivea*, emerged 18. I. 2007, HSU 07A7 (Y. F. Hsu leg.); 1♀, Renai, Huisun, 600-700m, 20. II. 2010 (Y. F. Hsu & H. C. Huang

leg.). **HUALIAN Co.:** 2♂, Xiulin, Huitouwan, 23. VII. 2005 (Y. F. Hsu leg.), 1♂, same locality, 30. I. 2007 (Y. F. Hsu leg.); 4♂, 3♀, Xiulin, Zuocang Trail, 7. III. 2006 (Y.F. Hsu leg.); 1♀, Xiulin, Guangbeibabiao, 2200m, 28. V. 2007 (Y. F. Hsu leg.); 1♀, Xiulin, Huoranting, 1000m, 10. XII. 2007 (M. H. Sun leg.); 1♂, Xiulin, Wujiabengshan, 2000m, 7. VI. 2008 (L.H. Wang leg.); 1♀, Zhouxi, Zhongping Forest Trail, 1. VI. 2007 (L. H. Wang leg.). **TAINAN CITY [= TAINAN Co.]:** 1♀, Dongshan, Kantoushan, 600-800m, 4. II. 2010 (Y. F. Hsu leg.).

PINGDONG Co.: 1♂, Shizi, Nunaishan, 200m, 26. III. 2006 (Y. F. Hsu leg.); 1♂, Fangliao, Yuquan, 26. II. 2006 (Y. F. Hsu leg.); 2♂, 1♀, Shizi, Lilongshan, 500m, 17. II. 2008 (Y. F. Hsu leg.); 1♂, same locality, 1. III. 2008 (Y. F. Hsu leg.); 1♀, Wutai, Jiudawu, 500m, 11. V. 2008 (C. L. Huang leg.); 1♀, Sandimen, ca 300m, 2. I. 2009 (Y. F. Hsu leg.). **TAIDONG Co.:** 1♀, Yanping, Hongye, 11. I. 2009 (C. H. Lin, jr leg.); 1♀, Taidong, Pipa Lake, 4. VI. 2006 (Y. F. Hsu leg.); 1♀, Haiduan, Liyuan, 7. VI. 2020 (Y. F. Hsu leg.) (genitalia preparation JYL1036).

***Symbrenthia lilaea* (Hewitson, 1864)**

JILONG CITY: 1♀, Longgang Trai, 26. IX. 2006 (Y. F. Hsu leg.); 2♀, same locality, 2. X. 2006 (Y. F. Hsu leg.);1♀, same locality, 7. II. 2007 (C. K. Wang leg.); 1♂, Shenao, Sea Level, 20. VI. 2006, reared from *B. nivea*,emerged 2. VII. 2006, HSU 06F17 (Y. F. Hsu leg.); 2♂, Haimentianxian, 26. IX. 2006 (Y. F. Hsu leg.). **XINBEI CITY [= TAIPEI Co.]:** 7♂, 7♀, Wulai, 150m, 8. XI. 2004, reared from *B. nivea*, emerged 29. XI-5. XII. 2004, HSU 04L20 (Y. F. Hsu

leg.); 1♂, Wulai, 11. III. 2005 (C. R. Chen leg.), 1♀, same locality, 5. VI. 2005 (Y. F. Hsu leg.), 1♂, 1♀, same locality, 11. VIII. 2005, reared from *B. densiflora*,emerged 25. VIII. -1. IX. 2005, HSU 05H8 (Y. F. Hsu leg.), 1♂, same locality, 23. IX. 2005, reared from *B. nivea*, emerged 4. X. 2005, HSU 05J72 (J. R. Chen leg.), 3♀, same locality, 6. X. 2006, reared from *B. nivea*, emerged 3-15. XI. 2006, HSU 06L3 (Y. F. Hsu leg.); 1♂, Wulai, Fushan, 700m, 4. VII. 2005, reared from *B. nivea*, emerged 15. VII. 2005 (C. L. Huang leg.); 1♀, Wulai, Fushan, 16. VI. 2005 (C. L. Huang leg.); 1♂, Wulai, Baoqing Temple, ca 620m, 4. VIII. 2005 (J. R. Chen leg.); 1♀, Xindian, Yinhedong, 250m, 14. VI. 2005 (Y. F. Hsu leg.); 8♂, 1♀, Pinglin, Yuguang, 2. XI. -11.XII 2005, reared from *B. formosana*, emerged 29. XI. /18. XII. 2005, HSU 05L5 (C. C. Lu leg.); 1♀, Pinglin, Zhongxinlun, 2. XI. 2005 (C. C. Lu leg.). **TAIPEI CITY:** 1♀, Beitou, Guizikeng, 24. X. 2004 (H. C. Huang & P. Lo leg.); 2♀, Wenshan, Xianjiyan, 16. V. 2005 (Y. F. Hsu leg.); 2♀, Daan, NTNU campus, 9. VI. 2005, reared from *B. nivea*, emerged 23-24. VI. 2005, HSU 05F18 (Y. F. Hsu leg.); 5♀, Wenshan, Gongguan campus, NTNU, 9. VI. 2005, reared from *B. nivea*, emerged 21-24. VI. 2005, HSU 05F18 (Y. F. Hsu leg.); 3♀, same locality, 15. I. 2007, reared from *B. nivea*, emerged 31. I. 2007, HSU 07A20 (Y. F. Hsu leg.); 1♂, Shilin, Tianxiyuan, 17. VII. 2011 (H. C. Huang leg.); 1♂, Neihu, Dagouxi, 25. I. 2014 (L. Huang leg.). **TAOYUAN CITY [= TAOYUAN Co.]:** 2♀, Fuxing, Kapu, 800m, 14. X. 2007, reared from *B. densiflora*, emerged 29. XI. 2007, HSU 07K7 (Y. F. Hsu & H. C. Huang leg.). **YILAN Co.:** 1♂, Yuanshan,

Fushan Botanical Garden, ca 700m, 4-5. VIII. 2006 (Y. F. Hsu & H. C. Huang leg.); 1♂, 1♀, Toucheng, Guishan Is., 4-5. III. 2006 (H. C. Huang & C. L. Huang leg.); 3♀, same locality, 24. VI. 2006 (H. C. Huang leg.); 1♀, same locality, 24. VI. 2006 (C. L. Huang leg.); 2♀, same locality, 4. VIII. 2007 (H. C. Huang leg.). **XINZHU Co.:** 1♂, Qionglin, Feifengshan, ca 80m, 19. I. 2005, reared from *B. densiflora*, emerged 14. II. 2005, HSU 05A4 (Y. F. Hsu leg.); 1♂, Qionglin, Feifengshan, 10-80m, 19. I. 2005, reared from *B. nivea*, emerged 8. II. 2005, HSU 05A3 (Y. F. Hsu leg.); 1♀, Zhudong, 11. IV. 2006 (Y. F. Hsu leg.); 2♀, Zhudong, 100m, 28. III. 2008 (Y. F. Hsu leg.). **MIAOLI Co.:** 8♂, 2♀, Nanzhuang, 200m, 28. XI. 2004, reard from *B. densiflora*, emerged 11-20. XII. 2004, HSU 04L68 (Y. T. Lo leg.); 1♂, Touwu, Xiangshan Village, Jinshui, 60m, 27. II. 2005, reared from *B. nivea*, emerged 7. III. 2005, HSU 05B36 (Y. F. Hsu leg.); 5♂, 4♀, Sanwan, Emei Bridge, 14. I. 2006, reared from *B. nivea*, emerged 3-7. II. 2006, HSU 06A12 (Y. F. Hsu leg.); 1♂, Zhuolan, Liyutan, 2. VII. 2006 (Y. F. Hsu leg.). **NANTOU Co.:** 2♂, 3♀, Lugu, 500m, 1. I. 2007, reared from *B. nivea*, emerged 17-31. I. 2007, HSU 07A6 (Y. F. Hsu leg.). **HUALIAN Co.:** 1♀, Wanrong, Wanrong Forest Trail, 29. VIII. 2010 (Z. H. Yen leg.). **TAINAN CITY [= TAINAN Co.]:** 2♀, Xinhua, Dakeng, 80m, 10/11. II. 2006 (Y. F. Hsu leg.); 1♀, Xinhua, 4. II. 2006, reared from *B. nivea*, emerged 13. III. 2006, HSU 06J41 (Y. F. Hsu leg.), 2♂, same locality, 20. I. 2007 (Y. F. Hsu leg.). **KAOHSIUNG CITY:** 3♂, 1♀, Jiaxian Dist., Butingshan, ca 700m, 26. VII, 2022, reared from *Pouzolzia elegans*,

362 emerged 14. VIII. 2022, HSU 22G36 (Y. F. Hsu leg.). **LIANJIANG Co.:** 1♂, 1♀, Nangan, 10.
363 VII. 2003, reared from *B. nivea*, emerged 25. VII. 2003, HSU 03G20 (L. W. Wu leg.); 1♂,
364 Nangan, Shengli Dam, 9-10. XII. 2006, reared from *B. nivea*, emerged 6. I. 2007, HSU 06M2
365 (H. C. Huang leg.); 1♂, 1♀, Beigan, Qinbi, 22. IV. 2007 (Y. F. Hsu & H. C. Huang leg.).
366
367 **Niche overlap between two *Symbrenthia* species in Taiwan**
368 Many studies have reported that when a newly introduced species is present, competitive
369 exclusion could be observed between the alien and similar native species (*Mooney &*
370 *Cleland,2001; Paini et al., 2008; Muthukrishnan et al., 2018*). However, our study presents a
371 different aspect of this interaction. Both the SDM and the niche overlap results showed that the
372 degree of overlap of the presenting area between these two species increases over time (Fig. 6).
373 This means that competitive exclusion may not be present between the alien and the native
374 species. This result may be explained by the following two alternative scenarios:
375 Firstly, obvious competition for the two species may not be observed due to insufficient
376 time of introduction of the alien species. *S. lilaea* was first found on the main island of Taiwan as
377 recently as 2004, and thus may still be in the process of population establishment and early
378 growing stages (*McGeoch & Jetz, 2019*). Consequently, the competitive exclusion effect
379 between these two species may have not yet occurred or not yet occurred to a level observable by

our available data.

Secondly, perhaps no competitive exclusion will occur between the two *Symbrenthia* species due to abundant host plant resources. Some studies have shown that host plants are much more important for the distribution of herbivorous insects when compared with the abiotic environmental factors (*Wiens et al., 2010; Simões & Peterson, 2018*). These two butterfly species feed on several species in the family Urticaceae, and most of these host plants are common and abundant in Taiwan (*Yang et al., 1996*). The food supply to the caterpillars of *Symbrenthia* may therefore be beyond the demand of both species combined, resulting in the absence of interspecific competition.

It awaits to be seen which scenario is more likely to occur, but it may be worthwhile to notice that *S. formosanus* was abundant in the Yangmingshan National Park of northern Taiwan *(Chang, 1994*), but in a butterfly survey conducted with sampling on monthly basis there from the beginning of 2021 to date, only *S. lilaea* has been recorded (*Hsu et al., unpublished data*). This observation suggests competition between the two species may actually have initiated.

Distribution difference of *S. lilaea* between two different invasive stage

The SDM of *S. lilaea* shows different result patterns between the early and late invasive time stages (Fig. 5B & 5D). Especially in southwest portion of Taiwan, the presence probability

398 increases significantly in the late invasive stages. This phenomenon can be the result of the
399 expansion of the distribution area of this alien species. The first record of *S. lilaea* is in Xinzhu
400 county located in the northwest part of Taiwan. The distribution area of this species gradually
401 expanded during the 18 years since it was first observed in Taiwan, and this butterfly species can
402 today be observed in nearly all lowland areas around Taiwan.

403 The species distribution model (SDM) has been widely used as a tool to detect the potential
404 invasive area of invasive species (*Wiens et al., 2010; Ahmed et al., 2020*). Based on the niche
405 conservatism of the invasive species, we are usually able to predict the invasive area based on
406 areas in the invaded region with similar environment to the source area from which the species
407 originated. However, the SDM difference of *S. lilaea* between two different invasive stages
408 suggests that the SDM may have inaccurately predicted potential areas of invasive species
409 presence. The niche may be hard to quantify, even though some studies have suggested methods
410 to measure it (*Framiout & Monnet, 2018; Lei et al., 2019*), but introduced species usually still
411 undergo niche expansion in the newly invaded area (*Datta et al., 2019; Bates et al., 2020*).
412 Although SDM is still a widely used tool to evaluate potential impact of invasive species, the
413 inaccuracy of the model is inevitable due to the reasons addressed above. We suggest to include
414 more biotic factors of the invasive species when predicting potential invasive regions rather than
415 relying on the SDM results alone. By combining the biotic variables with the SDM constructed

by the abiotic variables, the results should be closer to the realistic distribution pattern.

CONCLUSIONS

Species delimitation employing an integrative taxonomy approach has helped to clarify taxonomic entities of an introduced and a native *Symbrenthia* butterfly taxa regarded conspecific to date, leading to a decision to recognize each as a distinct species. This result suggests that interspecific competition may occur by the introduction of the alien species, rather than gene introgression. Subsequently, a niche modeling investigation was following, and the result showed that competition between the two species interest has not yet occurred or just initiated.

ACKNOWLEDGEMENTS

We are grateful to Dr. Jen-Pan Huang, Mr. Ming-Hsun Chou (both from Academia Sinica) and Mr. Trevor Padgett (Academia Sinica & Tunghai University) for reading and improving the manuscript of this article. We express our gratitude to Mr. Min-Wei Chai (National Taiwan Normal University) for giving us lots of advice on SDM. We also thank Dr. Masaya Yago (The University Museum, University of Tokyo) for helping us with valuable literatures. Also, we express our gratitude for the DNA Sequencing Core Facility of the Institute of Biomedical Sciences of Academia Sinica, Taipei, for providing DNA sequencing service.

434

435 **Data Availability**

436 The following information was supplied regarding data availability:

437 Voucher specimens are housed at Department of Life Science, National Taiwan University,

438 Taipei, R. O. C. and Biodiversity Research Center, Academia Sinica, Taiwan, R. O. C. The *COI*

439 sequences described here are accessible via GenBank accession number LC727399 to

440 LC727411.

441

442 **REFERENCES**

443 **Ahmed N, Atzberger C, Zewdie W. 2020.** Integration of remote sensing and bioclimatic data

444 for prediction of invasive species distribution in data-poor regions: a review on challenges and

445 opportunities. *Environmental Systems Research* **9**: 32 DOI: 10.1186/s40068-020-00195-0.

446 **Bascombe MJ, Johnston G, Bascombe FS. 1999.** *The Butterflies of Hong Kong*. London:

447 Academic Press. **Bates OK, Ollier S, Bertelsmeier C. 2020.** Smaller climatic niche shifts in

448 invasive than non-invasive alien ant species. *Nature Communications* **11**: 5213 DOI:

449 10.1038/s41467-020-19031-1.

450 **Bozano GC, Floriani. A. 2012.** *Guide to the Butterflies of the Palearctic Region. Nymphalidae.*

451 *Part V. Subfamily Nymphalinae.* Milano: Omnes Artes.

Braby MF, Eastwood R, Murray N. 2012. The subspecies concept in butterflies: has its application in taxonomy and conservation biology outlived its usefulness? *Biological Journal of the Linnean Society* **106(4)**: 699–716. DOI: 10.1111/j.1095-8312.2012.01909.x.

Chang, JR. 1994. *Observation of Butterflies in Yangmingshan National Park*. Taipei: Yangmingshan National Park Headquarter…

Čuda J, Skálová H, Janovský Z, Pyšek P. 2015. Competition among native and invasive impatiens species: the roles of environmental factors, population density and life stage. *AoB Plants* **7**: plv033. DOI: 10.1093/aobpla/plv033.

Datta A, Schweiger O, Kühn I. 2019. Niche expansion of the invasive plant species *Ageratina adenophora* despite evolutionary constraints. *Journal of Biogeography* **46**: 1306–1615 DOI: 10.1111/jbi.13579.

De Meyer M, Robertson MP, Mansell MW, Eksei S, Tsuruta K, Mwaiko W, Vayssiéres J-F, Peterson AT. 2010. Ecological niche and potential geographic distribution of the invasive fruit fly *Bactrocera invadens* (Diptera, Tephritidae). *Bulletin of Entomological Research* **100**: 35-48 DOI: 10.1017/S0007485309006713.

Drummond AJ, Rambaut A. 2007. BEAST: Bayesian evolutionary analysis by sampling trees. *BMC Evolutionary Biology* **7**: 214 DOI: 10.1186/1471-2148-7-214.

Ek-Amnuay P. 2012. *Butterflies of Thailand, 2nd edition.* Bangkok: B aan Lae Suan Amarin

Printing and Publishing.**Fraimout A, Monnet A. 2018.** Accounting for intraspecific variation to quantify niche dynamics along the invasion routes of *Drosophila suzukii*. *Biological Invasions* **20**: 2963–2979 DOI: 10.1007/s10530-018-1750-z.

Fric ZF., Martinkova B, Rindos, M, Suchackova Bartonova A., Wahlberg N., Papp Maresova J. 2022. Molecular phylogeny and biogeography of the genus *Symbrenthia* (Lepidoptera, Nymphalidae) correlates with the past geography of the Oriental region, *Molecular Phylogenetics and Evolution* **177**: 107605 DOI: 10.1016/j.ympev.2022.107605.

Fujisawa T, Barraclough TG. 2013. Delimiting Species Using Single Locus Data and the Generalized Mixed Yule Coalecent Approach: A Revised Method and Evaluation on Simulated Data Sets. *Systematic Biology* **62(5)**: 707–724 DOI: 10.1093/sysbio/syt033.

Hebert PDN, Cywinska A, Ball SL, deWaard JR. 2003. Biological identifications through DNA barcodes. *Proceedings of the royal society B Biological Sciences* **270(1512)**: 313–321 DOI: 10.1098/rspb.2002.2218.

Holt A. 1998. A conservation vision for the 21th century. The Nature Conservancy of Hawaii Newsletter **17:** 4-5.

Hsu YF, Liang CY, Huang CW, Shen ZY. 2022. *Butterfly Fauna of Taiwan. Vol V. Nymphalidae*. Taipei: Forestry Bureau, Council of Agriculture, Executive Yuan, Taiwan, ROC.

Koenig WD. 2003. European starlings and their effect on native cavity-nesting birds. *Conservation Biology* **17(4)**: 1134-1140. Doi: 10.1046/j.1523-1739.2003.02262.x.

Lang S. 2012. *The Nymphalidae of China (Lepidoptera, Rhopalocera). Part I. Libytheinae, Danainae, Calinaginae, Morphinae, Heliconiinae Nymphalinae, Charaxinae, Apaturinae, Cyrestinae, Biblidinae, Limenitinae*. Pardubice: Tshikolovets Publications.

Leger EA, Espeland, EK. 2010. Coevolution between native and invasive plant competitors: implications for invasive species management. *Evolutinary Applications* **3(2)**: 169–178. Doi: 10.1111/j.1752-4571.2009.00105.x.

Lei J, Jia Y, Wang Y, Lei G, Lu C, Saintilan N, Wen L. 2019. Behavioural plasticity and trophic niche shift: How wintering geese respond to habitat alteration. *Freshwater Biology* **64(6)**: 1183-1195 DOI: 10.1111/fwb.13294.

Lin CT. 2018. QGIS template for displaying species distribution by horizontal and vertical view in Taiwan. URL: https://github.com/mutolisp/distrmap_tw.qgis. DOI: 10.5281/zenodo.1493690.

Lu CC, Chen JR. 2014. *The Life History of Butterflies in Taiwan*. Taichung: Morning Star Publishing Inc.

McGeoch M, Jetz W. 2019. Measure and Reduce the Harm Caused by Biological Invasions. *One Earth* **1(2)**: 171-174 DOI: 10.1016/j.oneear.2019.10.003.

506 **Monastyrskii A. 2019.** *Butterflies of Vietnam. Vol 4(I) Nymphalidae: Libytheinae, Calinaginae,*
507 *Apaturinae, Charaxinae, Nymphalinae, Biblidinae, Pseudergolinae, Cyrestinae, Heliconiinae.*
508 Hanoi: Plan-o-rama Media Co.
509 **Mooney HA, Cleland EE. 2001.** The evolutionary impact of invasive species. *PNAS* **98(10)**:
510 5446-5451 DOI: 10.1073/pnas.091093398.
511 **Moraes SS, Montebello Y, Stanton MA, Yamaguchi LF, Kato MJ, Freitas AVL. 2021.**
512 Description of three new species of Geometridae (Lepidoptera) using species delimitation in an
513 integrative taxonomy approach for a cryptic species complex. *PeerJ* **9**: e11304. DOI:
514 10.7717/peerj.11304.
515 **Muthukrishnan R, Hansel-Welch N, Larkin DJ. 2018.** Environmental filtering and
516 competitive exclusion drive biodiversity-invasibility relationships in shallow lake plant
517 communities *Journal of Ecology* **106**: 2058-2070 DOI: 10.1111/1365-2745.12963.
518 **Paini DR, Funderburk JE, Reitz SR. 2008.** Competitive exclusion of a worldwide invasive
519 pest by a native. Quantifying competition between two phytophagous insects on two host plant
520 species. *Journal of Animal Ecology* **77**: 184–190 DOI: 10.1111/j.1365-2656.2007.01324.x.
521 **Phillips SJ, Anderson RP, Schapire RE. 2006.** Maximum entropy modeling of species
522 geographic distributions. *Ecological Modelling* **190**: 231-259 DOI:
523 10.1016/j.ecolmodel.2005.03.026.

Posada D. 2008. jModelTest: Phylogenetic Model Averaging. *Molecular Biology and Evolution* **25(7)**: 1253-1256 DOI: 10.1093/molbev/msn083.

Puillandre N, Lambert A, Brouillet S, Achaz G. 2012. ABGD, Automatic Barcode Gap Discovery for primary species delimitation. *Molecular Ecology* **21**: 1864–1877 DOI: 10.1111/j.1365-294X.2011.05239.x.

Rambaut A, Drummond AJ, Xie D, Baele G, Suchard MA. 2018. Posterior Summarization in Bayesian Phylogenetics Using Tracer 1.7. *Systematic Biology* **67(5)**: 901–904 DOI: 10.1093/sysbio/syy032.

Rhymer JM, Simberloff D. 1996. Extinction by hybridization and introgression. *Annual Review of Ecology and Systematics* **27**: 83–109

Ronquist F, Teslenko M, Mark PVD, Ayres DL, Darling A, Höhna S, Larget B, Liu L, Suchard MA, Huelsenbeck J. 2012. MrBayes 3.2: Efficient Bayesian Phylogenetic Inference and Model Choice Across a Large Model Space. *Systematic Biology* **61(3)**: 539–542 DOI: 10.1093/sysbio/sys029.

Sax DF, Gaines, SD. 2008. Species invasions and extinction: The future of native biodiversity on islands. *PANS* **105 (supplement 1)**: 11490–11497 DOI: 10.1073/pnas.0802290105.

Sheppard CS, Brendel MR. 2021. Competitive ability of native and alien plants: effects of

542 residence time and invasion status. *NeoBiota* **65**: 47–69 DOI: 10.3897/neobiota.65.63179.

543 **Shirôzu T, Ueda K. 1992.** Nymphalidae. In: Heppner JN, Inoue H, eds. *Lepidoptera of Taiwan,*

544 *Vol. 1, part 2: checklist*. Gainesville, FL: Association for Tropical Lepidoptera, 140–150.

545 **Simões MVP, Peterson AT. 2018.** Importance of biotic predictors in estimation of potential

546 invasive areas: the examples of the tortoise beetle *Eurypedus nigrosignatus*, in Hispaniola.

547 *PeerJ* **6**: e6052 DOI: 10.7717/peerj.6052.

548 **Stamatakis A. 2014.** RAxML version 8: a tool for phylogenetic analysis and post-analysis of

549 large phylogenies. *Bioinformatics* **30(9)**: 1312–1313 DOI: 10.1093/ioinformatics/btu033.

550 **Tamura K, Stecher G, Kumar S. 2021.** MEGA11: Molecular Evolutionary Genetics Analysis

551 Version 11. *Molecular Biology and Evolution* **38(7)**: 3022–3027 DOI:

552 10.1093/molbev.msab120.

553 **Tsukada E, Nishiyama Y. 1985.** Genus *Symbrenthia*. In Tsukada E, ed. *Butterflies of the South*

554 *East Asian Islands. Vol. IV. Nymphalidae (I)*. Tokyo: Plapac Co. Ltd., 308-323.

555 **Vestbo S, Obst M, Fernandez FJQ, Intanai I, Funch P. 2018.** Present and potential future

556 distributions of Asian Horseshoe crabs determine areas for conservation. *Frontiers in Marine*

557 *Science* **5**: 164 DOI: 10.3389/fmars.2018.00164.

558 **von Aesch L, Cherix D. 2005.** Introduced ant species and mechanisms of competition on

559 Floreana Island (Galápagos, Ecuador). *Sociobiology* **45(2)**: 1–19.

Wiens JJ, Ackerly DD, Allen AP, Anacker BL, Buckley LB, Cornell HV, Damschen EI, Davies TJ, Geytnes J, Harrison SP, Hawkins BA, Holt RD, McCain CM, Stephens PR. 2010. Niche conservatism as an emerging principle in ecology and conservation biology. *Ecology Letters* **13**: 1310–1324 DOI: 10.1111/j.1461-0248.2010.01515.x.

Wilson EO. 1961. The nature of the taxon cycle in the Melanesian ant fauna. *The American Naturalist* **95(882)**: 169–193.

Xu D, Li X, Jin Y, Zhuo Z, Yang H, Hu J, Wang R. 2020. Influence of climatic factors on the potential distribution of pest *Heortia vitessoides* Moore in China. *Global Ecology and Conservation* **23**: e01107. DOI: 10.1016/j.gecco.2020.e01107.

Yang YP, Lin BL, Liu HY. 1996. Urticaceae. In: Huang TC, ed. *Flora of Taiwan, second edition.* Taipei: National Science Council, Republic of China, 197-257

Zhang J, Kapli P, Pavlidis P, Stamatakis A. 2013. A general species delimitation method with applications to phylogenetic placements. *Bioinformatics* **29(22)**: 2869-2876 DOI: 10.1093/bioinformatics/btt499.

Zwerschke N, van Rein H, Harrod C, Reddin C, Emmerson MC, Roberts D, O'Connor NE. 2018. Competition between co-occurring invasive and native consumers switches between habitats. *Functional Ecology* **32**: 2717–2729 DOI: 10.1111/1365-2435.13211.

578 **Figure 1 Adult specimens of *Symbrenthia lilaea lunica* and *S. l. formosanus*. (**A－B) Male
579 specimen of *S. l. lunica*, TAIWAN: Miaoli County, Sanmwan, Emei Bridge. (C－D) Female
580 specimen of *S. l. lunica,* TAIWAN: Xinzhu County, Zhudong, 100m. (E－F) Male specimen of
581 *S. l, formosanus*, TAIWAN: New Taipei City, Wulai, 150m. (G－H) Female specimen of *S. l.*
582 *formosanus*, TAIWAN: Taoyuan City, Fuxing, Daman. Scale bar = 1cm (A－H).
583
584 **Figure 2 Distribution points of *Symbrenthia lilaea formosanus* and *S. lilaea lunica* during**
585 **two different invasive stages in Taiwan.** (A) The distribution points of *S. l. formosanus* and *S.*
586 *l. lilaea* in the early invasive stage (1911-2012). (B) The distribution points of *S. l. formosanus*
587 and *S. l. lilaea* in the late invasive stage (2013-2021).
588
589 **Figure 3 The Jackknife of regularized training gain of different bioclimatic factors of**
590 ***Symbrenthia formosanus* and *S. lilaea* between different invasive time stages.** (A) represents
591 the jackknife results of *S. formosanus* in the early invasive stage. (B) represents the jackknife
592 results of *S. lilaea* (=*lunica*) in the early invasive stage. (C) represents the jackknife results of *S.*
593 *formosanus* in the late invasive stage. (D) represents the jackknife results of *S. lilaea* (=*lunica*) in
594 the late invasive stage.
595

596 **Figure 4 Systematic and species delimitation results of *Symbrenthia lilaea* and *S.***
597 ***formosanus*.** (A) Phylogenetic tree reconstructed by BI analysis of 20 *COI* sequences of
598 *Symbrenthia.* Nodal support from the BI posterior probabilities is along with ML bootstrap
599 pseudo-replicates (BI posterior probabilities/MLbootstrap). Columns after taxon names
600 correspond to the results of single-locus species delimitation analyses: Poisson Tree Processes
601 model (PTP), Automatic Barcode Gap Discovery (ABGD) and Generalized Mixed Yule
602 Coalecent model (GMYC). (B) PhyloMap visualization the result of PTP species delimitation,
603 including 20 taxa of *Symbrenthia*. The first axis (horizontal) explained 62.64% and the second
604 axis (vertical) explained 36.08% of sequence variance among samples.
605
606 **Figure 5 The Species Distribution Model (SDM) of *Symbrenthia formosanus* and *S. lilaea***
607 **between different invasive time stages predicted from MaxEnt.** (A) represents the SDM of *S.*
608 *formosanus* in the early invasive stage. (B) represents the SDM of *S. lilaea* in the early invasive
609 stage. (C) represents the SDM of *S. formosanus* in the late invasive stage. (D) represents the
610 SDM of *S. lilaea* in the late invasive stage.
611
612 **Figure 6 Shifts in climatic niche between alien (*Symbrenthia lilaea*) and native (*S.***
613 ***formosanus*) species.** (A) The climatic niche shift between two *Symbrenthia* species in the early

614 invasive stage. (B) The climatic niche shift between two *Symbrenthia* species in the late invasive
615 stage. Red describes the climatic niche of *S. lilaea* and blue describes the climatic niche of *S.*
616 *formosanus*.

617

618 **Figure 7 Genitalia of *Symbrenthia lilaea* and *S. formosanus*.** (A－B) Male genitalia of *S.*
619 *lilaea* (Gen. Prep. JYL-877, NTNU). (C) Female genitalia of *S. lilaea* (Gen. Prep. JYL-880,
620 NTNU). (D－E) Male genitalia of *S. formosanus* (Gen. Prep. YFH-1583, NTNU). (F) Female
621 genitalia of *S. formosanus* (Gen. Prep. JYL-1036, NTNU). Scale bar = 1mm (A－B; D－E),
622 1mm (C; F)

623

624 **Figure 8 Eggs and immature stages of *Symbrenthia lilaea* and *S. formosanus*.** (A) Egg mass
625 of *S. lilaea*. (B) Young larvae of *S. lilaea*. (C) mature larvae of *S. lilaea*. (D) Egg of *S.*
626 *formosanus*. (E) Young larvae of *S. formosanus*. (F) mature larvae of *S. formosanus*.

627

628 **Figure S1 The niche overlap results infer from the 19 bioclimatic variables by principal**
629 **component analyses (PCA).** (A) The niche overlap results of the early invasive stage. (B) The
630 niche overlap results of the late invasive stage.

631

Figure S2 The receiver operating characteristic curve (ROC) and the thresholdindependent area under the ROC curve (AUC) values of the SDM. (A) the ROC and AUC of *S. formosanus* in the early invasive stage. (B) the ROC and AUC of *S. lilaea* (=*lunica*) in the early invasive stage. (C) the ROC and AUC of *S. formosanus* in the late invasive stage. (D) the ROC and AUC of *S. lilaea* (=*lunica*) in the late invasive stage.

Table S1 pairwise distance of *Symbrenthia* samples

Table S2 *Symbrenthia* samples information

Figure 1

Adult specimens of *Symbrenthia lilaea lunica* and *S. l. formosanus*

(A - B) Male specimen of *S. l. lunica*, TAIWAN: Miaoli County, Sanmwan, Emei Bridge. (C - D) Female specimen of *S. l. lunica,* TAIWAN: Xinzhu County, Zhudong, 100m. (E - F) Male specimen of *S. l, formosanus*, TAIWAN: New Taipei City, Wulai, 150m. (G - H) Female specimen of *S. l. formosanus*, TAIWAN: Taoyuan City, Fuxing, Daman. Scale bar = 1cm (A - H).

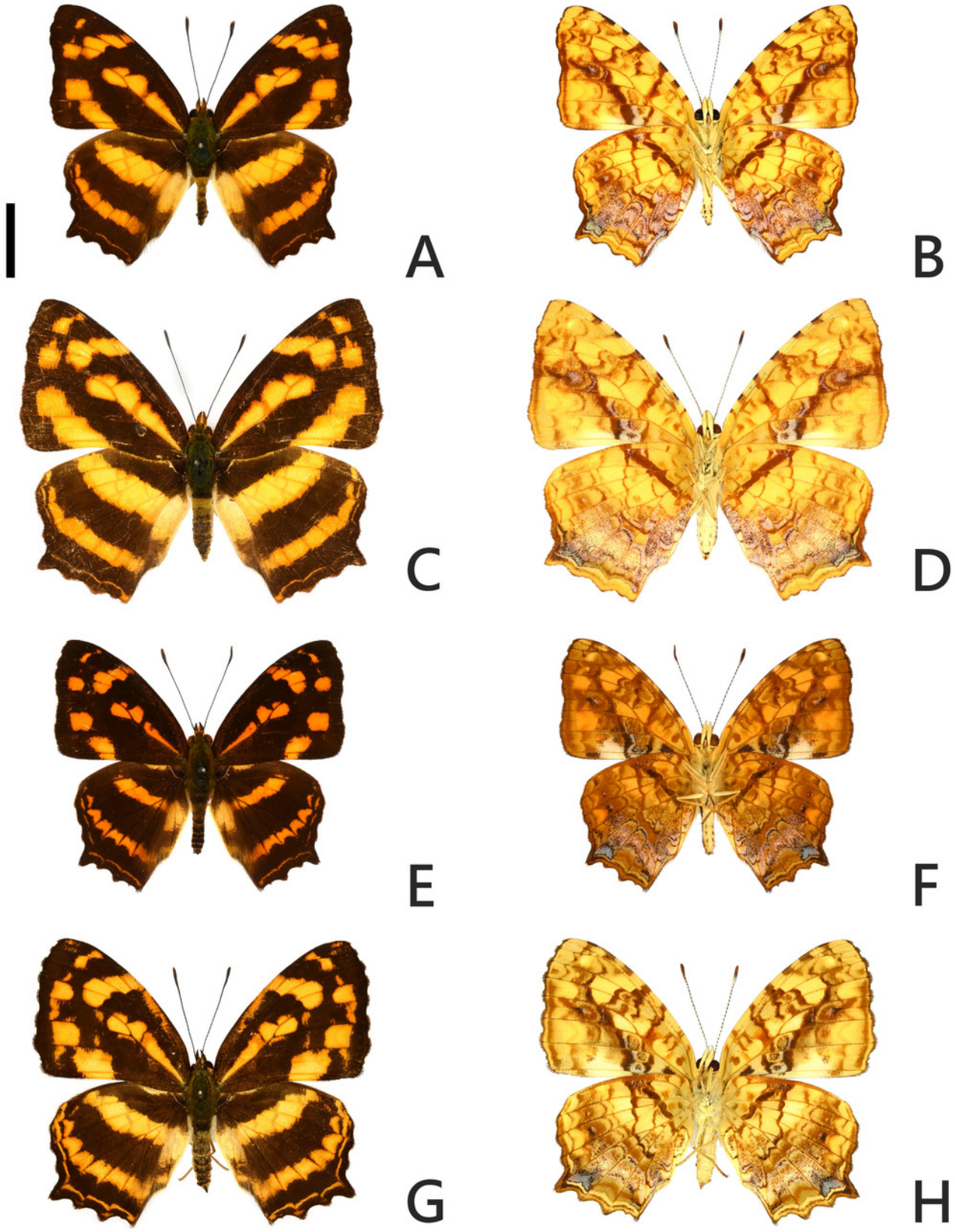

A
B
C
D
E
F
G
H

Figure 2

Distribution points of *Symbrenthia lilaea formosanus* and *S. lilaea lunica* during two different invasive stages in Taiwan

(A) The distribution points of *S. l. formosanus* and *S. l. lilaea* in the early invasive stage (1911-2012). (B) The distribution points of *S. l. formosanus* and *S. l. lilaea* in the late invasive stage (2013-2021).

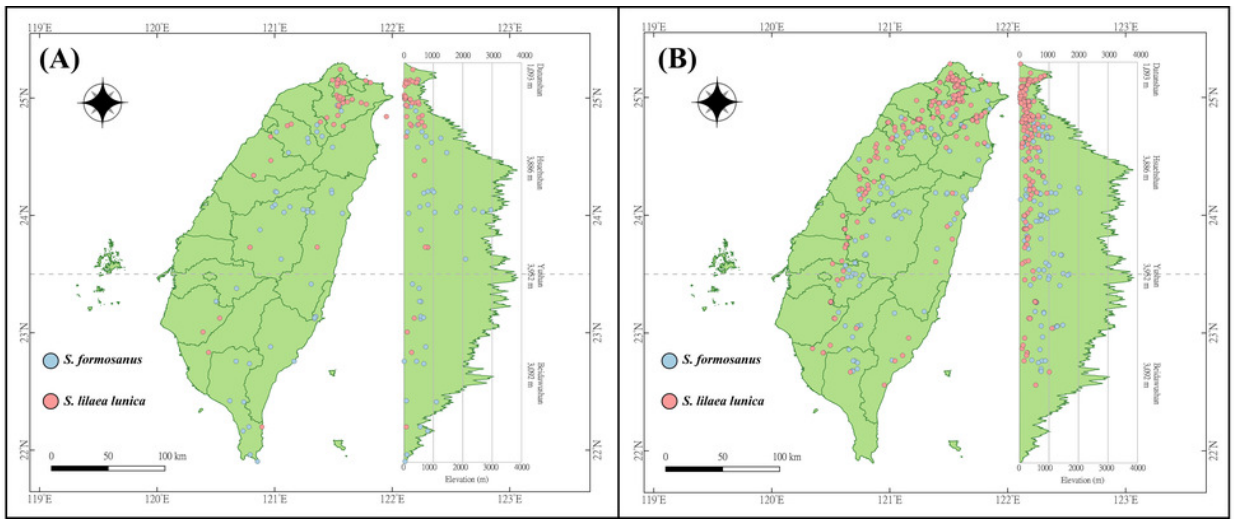


Figure 3

The Jackknife of regularized training gain of different bioclimatic factors of *Symbrenthia formosanus* and *S. lilaea* between different invasive time stages

(A) represents the jackknife results of *S. formosanus* in the early invasive stage. (B) represents the jackknife results of *S. lilaea* (=*lunica*) in the early invasive stage. (C) represents the jackknife results of *S. formosanus* in the late invasive stage. (D) represents the jackknife results of *S. lilaea* (=*lunica*) in the late invasive stage.

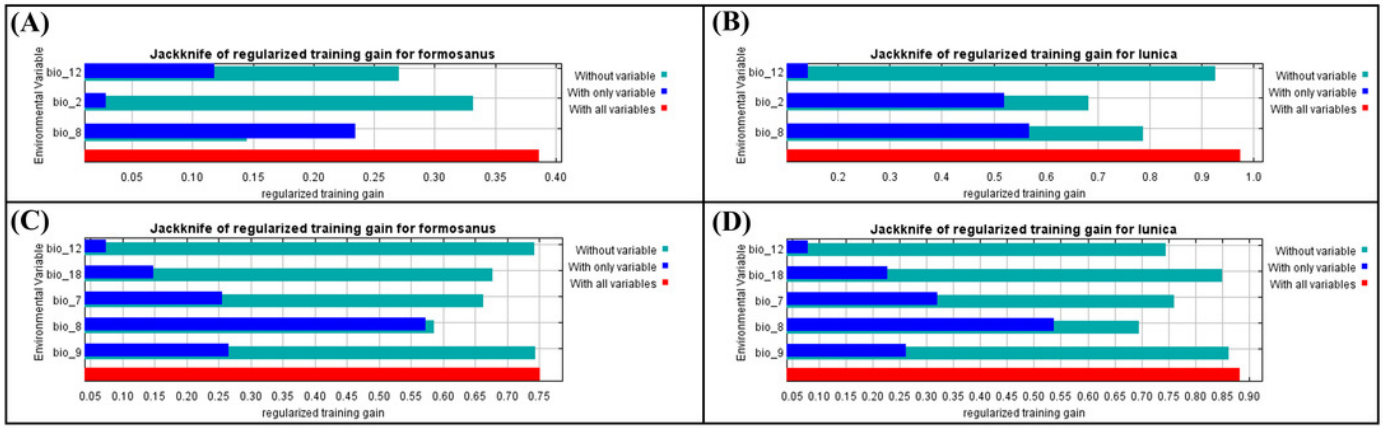


Figure 4

Systematic and species delimitation results of *Symbrenthia lilaea* and *S*. *formosanus*

(A) Phylogenetic tree reconstructed by BI analysis of 20 *COI* sequences of *Symbrenthia.* Nodal support from the BI posterior probabilities is along with ML bootstrap pseudo-replicates (BI posterior probabilities/MLbootstrap). Columns after taxon names correspond to the results of single-locus species delimitation analyses: Poisson Tree Processes model (PTP), Automatic Barcode Gap Discovery (ABGD) and Generalized Mixed Yule Coalecent model (GMYC). (B) PhyloMap visualization the result of PTP species delimitation, including 20 taxa of *Symbrenthia*. The first axis (horizontal) explained 62.64% and the second axis (vertical) explained 36.08% of sequence variance among samples.

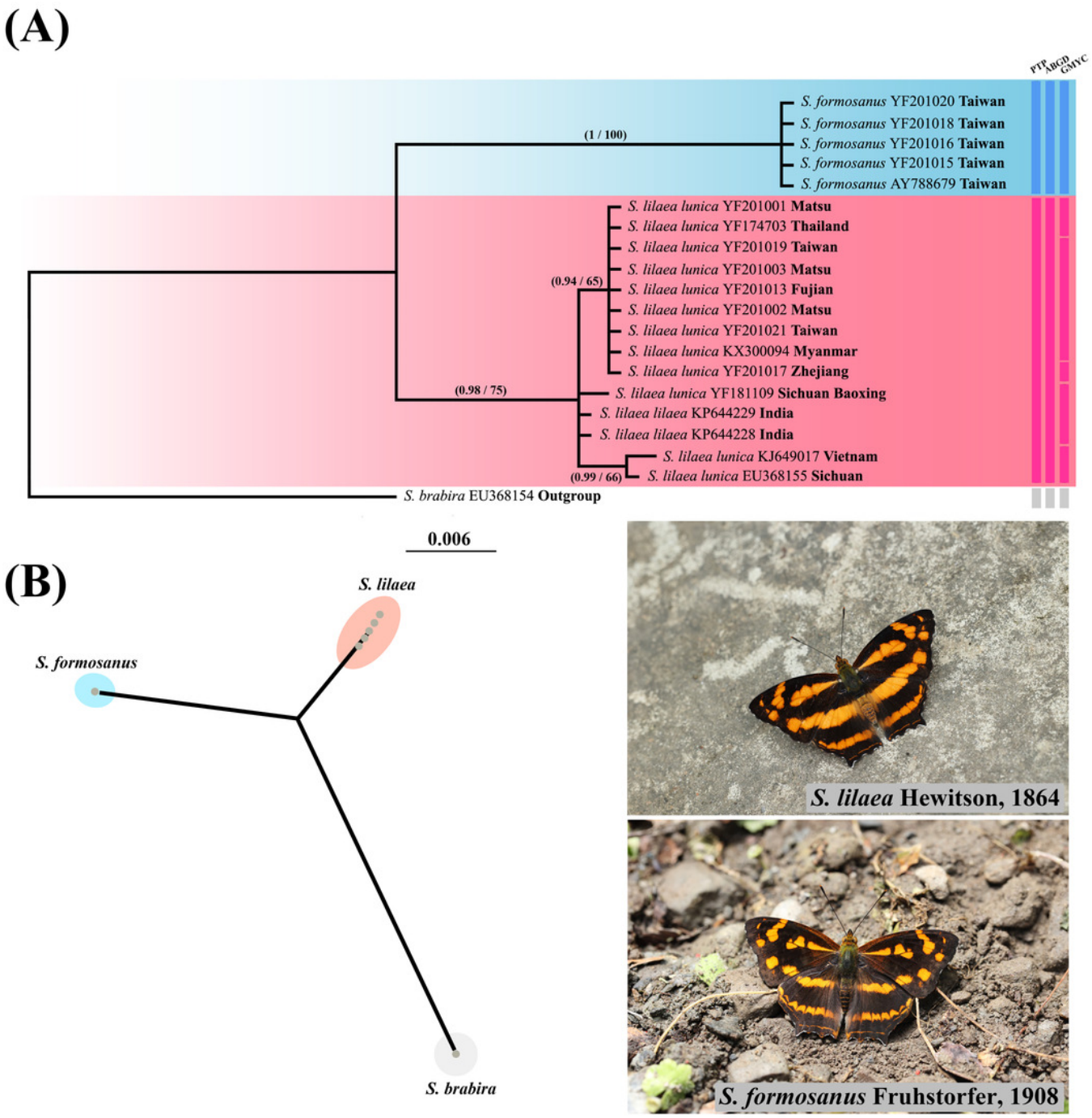

(A)
PTP
ABGD
GMYC
S. formosanus YF201020 Taiwan
S. formosanus YF201018 Taiwan
S. formosanus YF201016 Taiwan
S. formosanus YF201015 Taiwan
S. formosanus AY788679 Taiwan
(1 / 100)
S. lilaea lunica YF201001 Matsu
S. lilaea lunica YF174703 Thailand
S. lilaea lunica YF201019 Taiwan
S. lilaea lunica YF201003 Matsu
(0.94 / 65)
S. lilaea lunica YF201013 Fujian
S. lilaea lunica YF201002 Matsu
S. lilaea lunica YF201021 Taiwan
S. lilaea lunica KX300094 Myanmar
S. lilaea lunica YF201017 Zhejiang
(0.98 / 75)
S. lilaea lunica YF181109 Sichuan Baoxing
S. lilaea lilaea KP644229 India
S. lilaea lilaea KP644228 India
S. lilaea lunica KJ649017 Vietnam
(0.99 / 66)
S. lilaea lunica EU368155 Sichuan
S. brabira EU368154 Outgroup
0.006
(B)
S. lilaea
S. formosanus
S. brabira
S. lilaea Hewitson, 1864
S. formosanus Fruhstorfer, 1908

Figure 5

The Species Distribution Model (SDM) of *Symbrenthia formosanus* and *S. lilaea* between different invasive time stages predicted from MaxEnt

(A) represents the SDM of *S*. *formosanus* in the early invasive stage. (B) represents the SDM of *S*. *lilaea* in the early invasive stage. (C) represents the SDM of *S*. *formosanus* in the late invasive stage. (D) represents the SDM of *S*. *lilaea* in the late invasive stage.

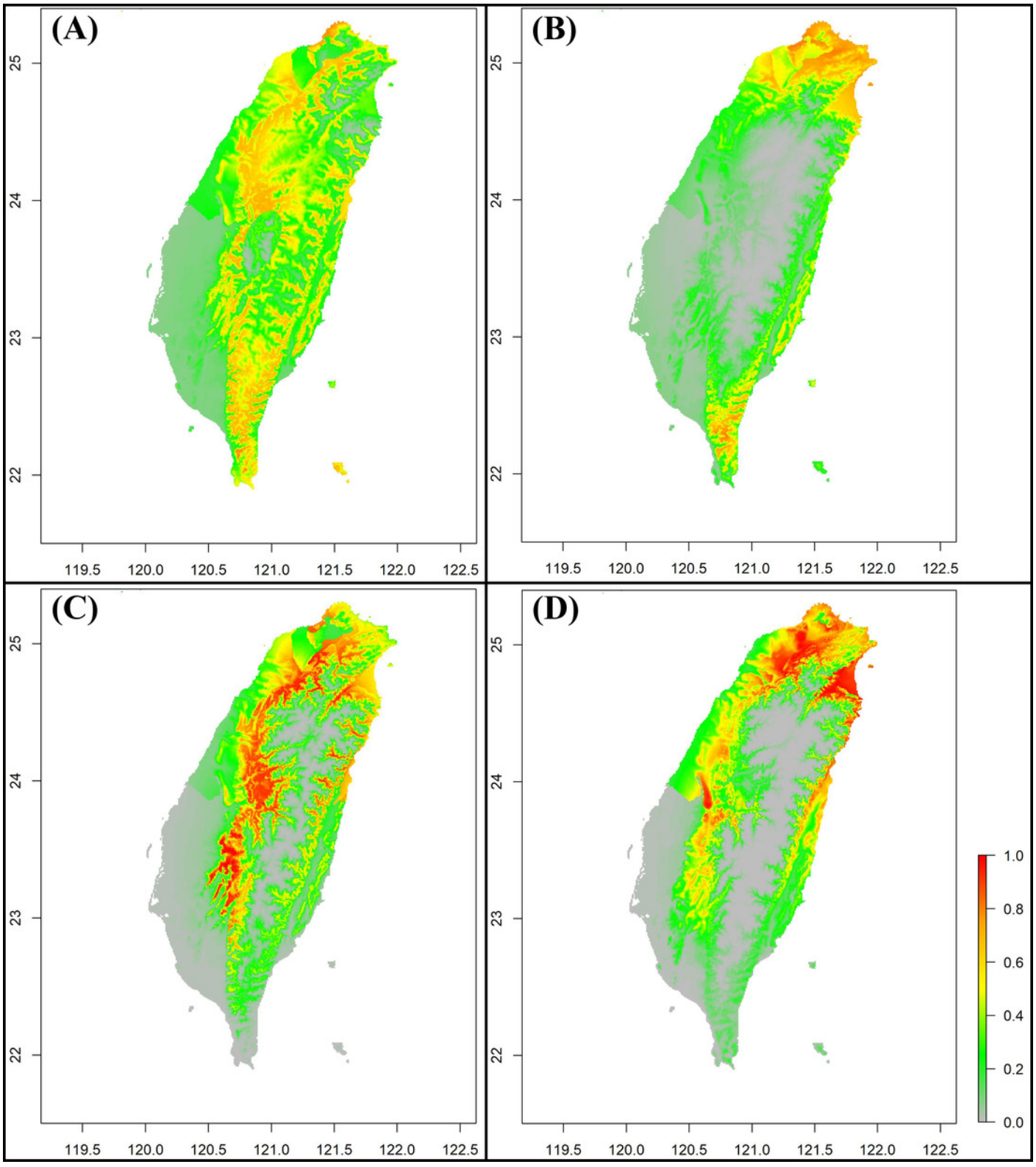
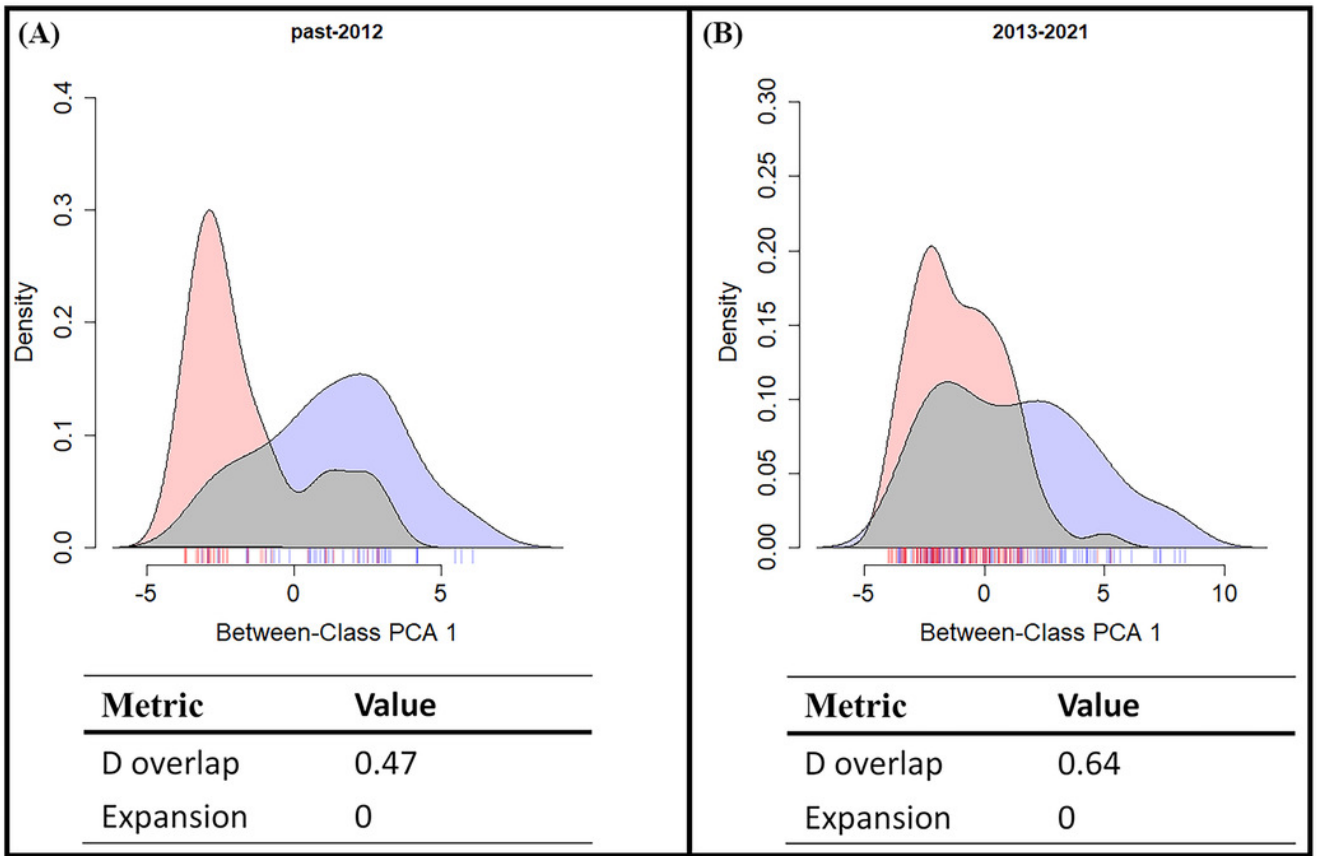

(A)
(B)
(C)
(D)
25
24
23
22
119.5
120.0
120.5
121.0
121.5
122.0
122.5
1.0
0.8
0.6
0.4
0.2
0.0

Figure 6

Shifts in climatic niche between alien (*Symbrenthia lilaea*) and native (*S. formosanus*) species

(A) The climatic niche shift between two *Symbrenthia* species in the early invasive stage. (B) The climatic niche shift between two *Symbrenthia* species in the late invasive stage. Red describes the climatic niche of *S. lilaea* and blue describes the climatic niche of *S. formosanus*.



Metric	Value
D overlap	0.47
Expansion	0

Metric	Value
D overlap	0.64
Expansion	0

Figure 7

Genitalia of *Symbrenthia lilaea* and *S*. *formosanus*

(A - B) Male genitalia of *S*. *lilaea* (Gen. Prep. JYL-877, NTNU). (C) Female genitalia of *S. lilaea* (Gen. Prep. JYL-880, NTNU). (D - E) Male genitalia of *S*. *formosanus* (Gen. Prep. YFH-1583, NTNU). (F) Female genitalia of *S*. *formosanus* (Gen. Prep. JYL-1036, NTNU). Scale bar = 1mm (A - B; D - E), 1mm (C; F).

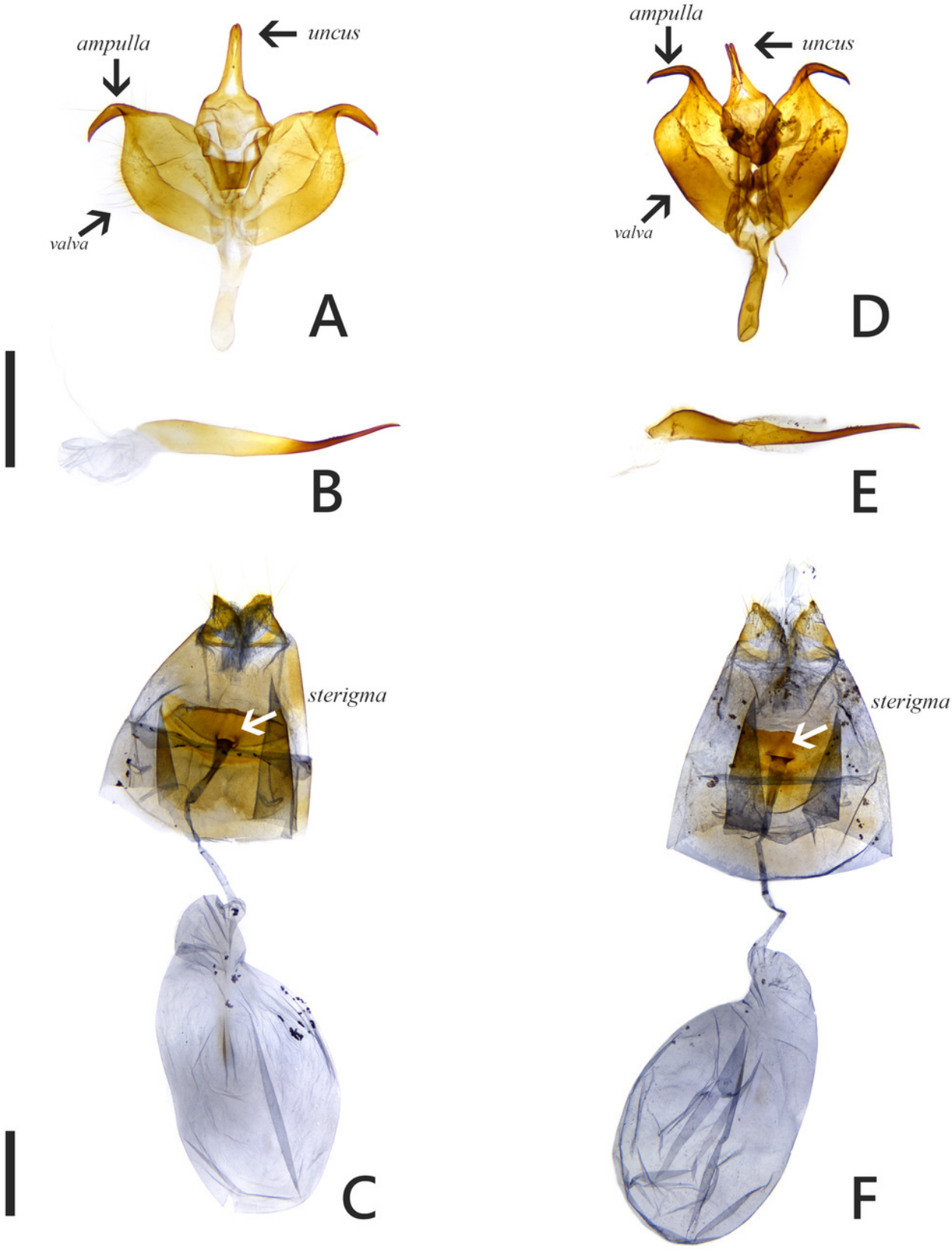
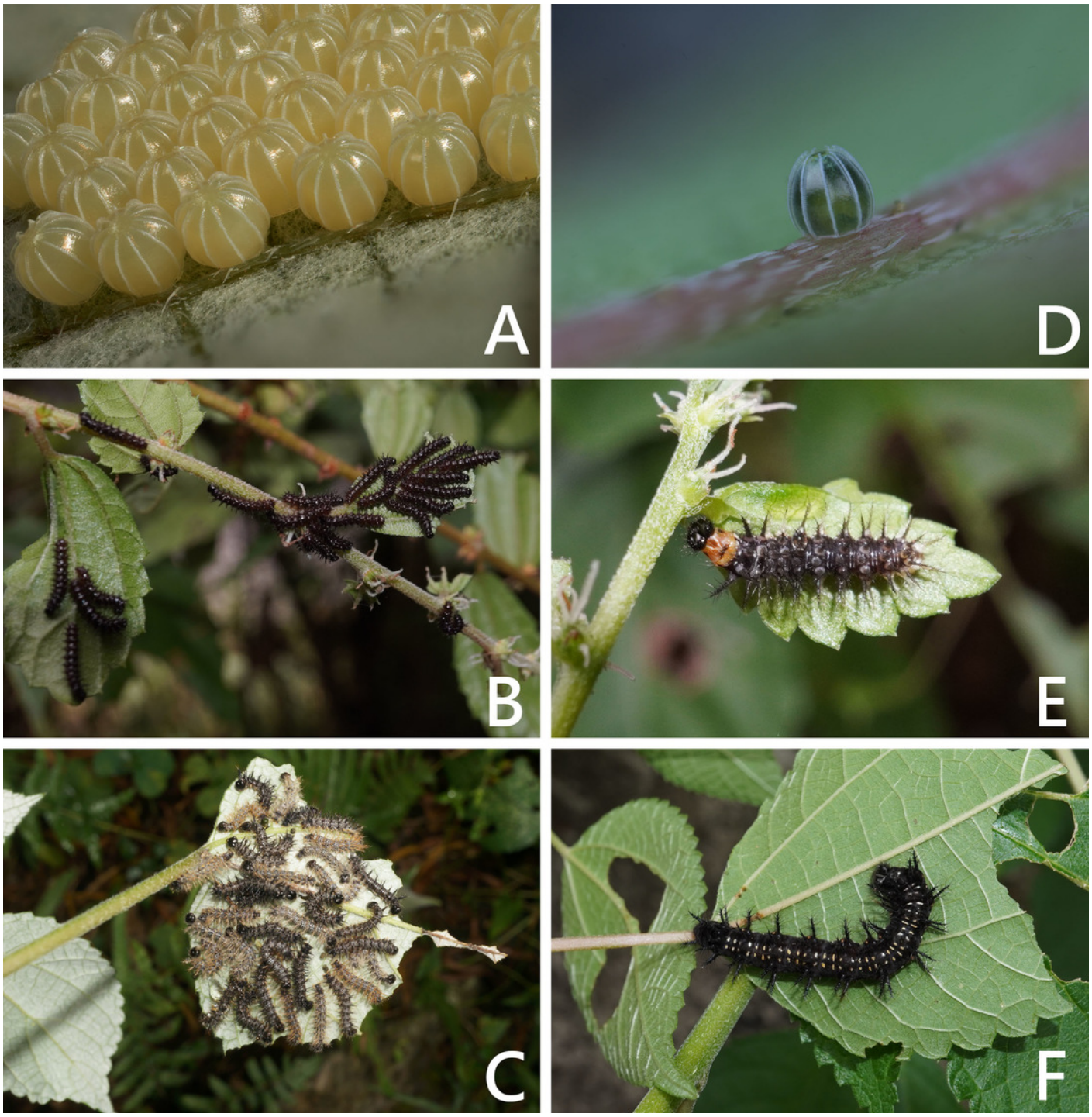

ampulla
uncus
valva
A
B
ampulla
uncus
valva
D
E
sterigma
C
sterigma
F

Figure 8

Eggs and immature stages of *Symbrenthia lilaea* and *S*. *formosanus*

(A) Egg mass of *S*. *lilaea*. (B) Young larvae of *S*. *lilaea*. (C) mature larvae of *S*. *lilaea*. (D) Egg of *S*. *formosanus*. (E) Young larvae of *S*. *formosanus*. (F) mature larvae of *S*. *formosanus*.


A
D
B
E
C
F