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

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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.

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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*; *Cuda 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* (Fig. 1A－1D)

into Taiwan has been reported (*Lu & Chen 2014*; *Hsu et al. 2022*). In Taiwan, there is a local native *Symbrenthia* taxon, *S, l. formosanus* (Fig, 1E－1H; *Shirôzu & Ueda, 1992*; *Hsu et al. 2022*). *S. l. 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 *Symbrenthia lilaea formosanus* were collected around Taiwan, and six specimens of *S. l. lunica* were collected in Taiwan (mainland and Matsu archipelago), China, and Thailand. located in the Northwest of Taiwan. All samples were preserved in 70% ethanol and kept at -20℃ for the subsequent molecular study. Moreover, additional COI sequences of *S. l. formosanus* (AY788679) from Taiwan, *S. l. lunica* (EU368155, KJ649017, KX300094) from China, Vietnam, Myanmar respectively, and the nominate subspecies *S. l. lilaea* (KP644228, KP644229) from India were obtained from GenBank. For the phylogenetic analyses, we used one sequence *of S. brabira* (EU368154) as an outgroup.

DNA extraction, PCR amplification and DNA sequencing

Genomic DNA was extracted from one leg of specimens using the Gentra Puregen tissue kit form QIAGEN (QIAGEN, Maryland, USA), following the manufacturer's protocol. A partial fragment from the *COI* gene was targeted for amplification by polymerase chain reaction (PCR).

The *COI* gene was amplified using the universal primers COX-J-1460 (5'-TACAATTTATCGCCTAAACTTCAGCC-3') and COX-N-2191 (5'-CCCGGTAAAATTAAAATATAAACTTC). PCR reactions were performed in a 30μL volume eppendorf, containing 1μL of extracted DNA, 23.5μL of ddH_2O, 3μL of 10X PCR reaction buffer, 0.6μL of each primer and 0.3μL of Power Taq (Genomics Biosci & Tech, Taiwan). The following PCR protocol was used: an initial denaturation at 95℃ for 5 min, followed by 40 cycles of 30s denaturation at 95℃, 30s annealing at 50℃ and 45s extension at 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-threshold model.

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

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

146 especially if the species delimitation analyses decide they represent different species.

147

148 The occurrence data of *S. l. formosanus* and *S. l. lunica* were obtained from the Global
149 Biodiversity Information Facility (GBIF) (https://gbif.org/, access 26 July 2021), Taiwan Moth
150 Information Center (https://twmoth.tersi.gove.tw/, access 26 July 2021), and the specimen
151 collection at National Taiwan Normal University. To test the interaction between these two
152 species, we separated the occurrence data into two stages based on year. The early invasion was
153 defined as the data recorded from 1911—2012, and the late invasion was defined as the data
154 recorded from 2013—2021. Repeated data was excluded using R 4.1.2, and we ensured the
155 presences of only one presenting point in each raster, as shown in Fig. 1, to avoid overfitting. In
156 total, 48 and 43 localities were obtained during the early invasion stage for *S. l. formosanus* and
157 *S. l. lunica*, respectively (Fig. 2A), and 132 and 192 localities were obtained during the later
158 invasion stage for *S. l. formosanus* and *S. l. lunica*, respectively (Fig. 2B). These data were
159 organized using Microsoft Excel for the subsequent analyses.

160 A total 19 bioclimate variables (period: 1979—2013) were collected from CHELSA (https:/
161 /chelsa-climate.org/, accessed on 14 July 2021) at a spatial resolution of 30 arc-seconds (1km^2).
162 These bioclimate variables were derived from temperature and precipitation, which are
163 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. 7).

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 random test and the remaining data were run following the default settings. Presence-only data were generated pseudo-absences and 10000 random background points were randomly selected by the MaxEnt model. The results were output after 10 cross-validation replicates.

The predictions generated from MaxEnt modeling were evaluated according to the threshold independent area under the ROC curve (AUC) values. Receiver operating characteristic (ROC) curves were used to plot the true-positive rate against the false-positive rate and the AUC was used as a measure of the goodness of fit of the model. The AUC value ranges from 0 to 1, with higher values indicating higher predictive performance. The logistic output was chosen as an estimate of the probability of presence conditioned by bio-environmental variables per grid cell. Jackknifing was used to screen for the contribution of each bio-environmental variable used in the model.

We performed principal component analyses (PCA) to test the niche overlap of these two

182 species in each of the two stages. The 19 bioclimatic variables were obtained from the CHELSA
183 database based on the GPS of each observation point. The analyses were conducted in R 4.1.2
184 using the function "prcomp", with scatterplots built using the function "ggbiplot". Additionally,
185 in order to evaluate the niche shift pattern between the two *Symbrenthia* species in Taiwan, we
186 apply methods modified from *Bates et al., 2020* to quantify the niche shift between *S. l.*
187 *formosanus* and *S. l. lunica* by calculating niche overlap, presented by Schoener's D, and niche
188 expansion of *S. l. lunica*.
189
190 **Results**
191 **Taxonomic decisions**
192 Phylogenetic reconstruction of *Symbrenthia* COI samples (Fig. 5) revealed that all samples of
193 *lunica+lilaea* form a monophyletic group sister to *formosanus* samples, which also formed a
194 monophyletic group. The p-distance was 0.0017 between *lunica* and *lilaea* and 0.0505－0.0525
195 between *lunica+lilaea* and *formosanus*. PTP, ABGD and GMYC all recognize a two species
196 scenario, with *lunica+lilaea* and *formosanus* each representing a distinct species. Therefore,
197 *formosanus* is recognized as a species distinct from *lunica+lilaea*, with the combination as
198 *Symbrenthia formosanus* Fruhstorfer, 1908 (**stat. rev.**). The taxon *lunica* Bascombe, Johnston &
199 Bascombe, 1999 is proposed to be lumped with *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. 7A & 7B) are used to construct the species distribution model of “early invasive stage”, while “bio 7”, “bio 8”, “bio 9”, “bio 12”, “bio 18” (Fig. 7C & 7D) 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. 6A & 6C). 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. 6B & 6D). Particularly, presence probability in the southwest part of Taiwan is higher in the later invasive stage (Fig. 6D). 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 8), 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,

236 competitive exclusion seems to not be occurring between these two species over these 18 years.

237

238 **Discussion**

239 **Taxonomic status of the introduced and native *Symbrenthia* butterflies**

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

241 conspecific subspecies prior to the present study (e. g. *Hsu et al., 2022*; *Fric et al. 2022*). It has

242 been argued that species delimitation is difficult for allopatric populations or subspecies of

243 similar forms (*King, 1993*; *Braby et al., 2012*), but in the present case, the introduction of

244 continental *S. lilaea* to Taiwan has proven that insular *S. formosanus* ought to represent a species

245 endemic to the island, instead of being a geographical race of the former. Distinctions between

246 them include: 1) distal band on hindwing uppersides of both sexes form a continuous orange

247 stripe in *S. lilaea* (Fig. 1A & 1C), whereas it is interrupted by darkened veins in *S. formosanus*

248 (Fig. 1E & 1G)*;* 2) distal tip of uncus is acute in *S. lilaea* (Fig. 3A), whereas it is blunt in *S.*

249 *formosanus* (Fig. 3D); 3) distal margin of harpe+sacculus is rounded in S. *lilaea* (Fig. 3A),

250 whereas it is angled, somewhat squared in *S. formosanus* (Fig. 3D); 4) ampulla is stout, slightly

251 down-curved in *S. lilaea* (Fig. 3A), whereas it is slender, strongly bent downwards in *S.*

252 *formosanus* (Fig. 3D); 5) posterior margin of sterigma is concave in *S, lilaea* (Fig. 3C), whereas

253 it is truncate in *S. formosanus* (Fig. 3F); 6) yellow eggs are laid in cluster in *S. lilaea* (Fig. 4A),

254 in contrast to green eggs laid singly in *S. formosanus* (Fig. 4D); 7) larvae are gregarious in *S.*

255 *lilaea* (Fig. 4B-4C), but solitary in *S. formosanus* (Fig. 4E－4F).

256

257 **Vouchers.**

258 ***S. formosanus*.**

259 Ssp. *formosanus*: **XINBEI CITY [= TAIPEI Co.]:** 1♀, Xindian, Sikanshui, 5. X. 2001 (H. S.

260 Que); 1♂, Wulai, 150m, reared from *Boehmeria nivea*, emgd. 8. XI. 2004, HSU 04L21 (Y. F.

261 Hsu); 1♂, Wulai, 13. II. 2005, reared from *Debregeasia orientalis*, emgd. 12. III. 2005, HSU

262 05B6 (L. H. Wnag); 1♀, same locality, 5. VI. 2005, reared from *B. densiflora*, emgd. 23. VI.

263 2005, HSU 05F9 (Y. F. Hsu); 1♂, same locality, 5. VI. 2005, reared from *D. orientalis*, emgd. 5.

264 VI. 2005, HSU 05F10 (Y. F. Hsu); 1♂, same locality, 13. VI. 2005, reared from *B. nivea*, emgd.

265 2. VII. 2005, HSU 05F36 (Y. F. Hsu); 1♀, same locality, 16. I. 2007 (Y. F. Hsu); 1♂, Wulai, ca

266 200m, 5. VI. 2005 (Y. F. Hsu). **TAIPEI CITY:** 1♀, Daan, Baozangyan, 28. III. 2019 (L.

267 Huang). **TAOYUAN CITY [= TAOYUAN Co.]:** 1♂, Fuxing, Xuanyuan, ca 1000m, 16. VI.

268 2005 (L. H. Wang); 1♀, Fuxing, Daman, 10. VI. 2005, reared from *B. nivea*, emgd. 22/23. VI.

269 2005, HSU 05F28 (Y. F. Hsu); 1♀, same locality, 10. VI. 2005, reared from *D. orientalis*, emgd.

270 23. VI. 2005, HSU 05F29 (Y. F. Hsu); 1♀, Fuxing, Gaoyi, 600m, 7. III. 2008 (Y. F. Hsu).

271 **YILAN Co.:** 1♂, 2♀, Nanao, 14Km S. Nanao, 6. VI. 2005, reared from *B. nivea*, emgd. 8/17.

VI. 2005, HSU 05F17 (Y. F. Hsu); 3♂, 1♀, Datong, Qilan, 1. XII. 2005, reared from *B. nivea*, emgd. III. 2006, HSU 05M7 (C. C. Lu & H. Y. Lee). **XINZHU Co.:** 2♂, Jianshi, Xiuluan, 800m, 21. VI. 2005 (L. H. Wang); 1♀, Jianshi, Guanwu, Dalu Forest Trail, 2. X. 2006 (L. H. Wang); 1♂, Jianshi, Yulao, ca 1500m, 25. II. 2009 (C. K. Wang). **MIAOLI Co.:** 1♂, Zhuolan, Liyutan, 300m, 2. VII. 2006 (Y. F. Hsu). **TAIZHONG CITY [= TAIZHONG Co.]:** 1♀, Heping, Shangguguan, 900m, 30. X. 2006 (Y. F. Hsu); 1♂, same locality, 29. VII. 2007 (Y. F. Hsu); 1♂, Heping, Guguan, 700m, 5. VII. 1998 (Y. T. Lo); 1♂, Heping, Guguan, IV. 2005 (L. H. Wnag). **NANTOU Co.:** 1♂, Renai, Tunyuan/ Tianchi, 2000/2800m, 31. VII- 1. VIII. 1998 (Y. T. Lo); 1♂, 1♀, Renai, Meiyuan, ca 400m, 22. XI. 2004, reared from *B. nivea*, emgd. 16/22. XII. 2004, HSU 04L49 (Y. F. Hsu); 1♀, Yuchi, Shiguanyin, 480m, 22. XI. 2004, reared from *B. densiflora*, emgd. 21. XII. 2004, HSU 04L52 (Y. F. Hsu); 1♂, Lugu, Fenghuanggu, 600m, 4. XII. 2004, reared from *B. densiflora*, emgd. 23. XII. 2004, HSU 04M5 (Y. F. Hsu); 1♂, 3♀, Lugu, Fenghuanggu, 4. XII. 2004, reared from *B. nivea*, emgd. 17/20. XII. 2004, HSU 04M4 (Y. F. Hsu), 1♀, same locality, 11. XII. 2004, reared from *B. densiflora*, emgd. 14. II. 2005, HSU 04M14 (Y. F. Hsu), 1♂, same locality, 12. XII. 2004 (Y. F. Hsu); 1♂, Renai, Beidongyanshan, ca 1800m, 17. XII. 2005 (L. W. Wu & Y. F. Hsu) (genitalia preparation YFH 1583), 1♀, same locality, 17. XII. 2005, reared from *D. orientalis*, emgd. 4. I. 2006, HSU 05M32 (Y. F. Hsu); 3♂, Renai, Tunyuan, 1550m, 28. VII. 2006 (Y. F. Hsu); 1♀, Renai, Nanshanxi, ca 900m, 1. IX. 2007

(Y. F. Hsu); 1♂, Lugu, ca 600m, 19. VII. 2006, reared from *B. nivea*, emgd. 4. VIII. 2006, HSU 06G12 (Y. F. Hsu); 1♂, 2♀, Lugu, ca 500m, 1. I. 2007, reared from *B, nivea*, emgd. 18. I. 2007, HSU 07A7 (Y. F. Hsu); 1♀, Renai, Huisun, 600/700m, 20. II. 2010 (Y. F. Hsu & H. C. Huang). **HUALIAN Co.:** 2♂, Xiulin, Huitouwan, 23. VII. 2005 (Y. F. Hsu), 1♂, same locality, 30. I. 2007 (Y. F. Hsu); 4♂, 3♀, Xiulin, Zuocang Trail, 7. III. 2006 (Y.F. Hsu); 1♀, Xiulin, Guangbeibabiao, 2200m, 28. V. 2007 (Y. F. Hsu); 1♀, Xiulin, Huoranting, 1000m, 10. XII. 2007 (M. H. Sun); 1♂, Xiulin, Wujiabengshan, 2000m, 7. VI. 2008 (L.H. Wang); 1♀, Zhouxi, Zhongping Forest Trail, 1. VI. 2007 (L. H. Wang). **TAINAN CITY [= TAINAN Co.]:** 1♀, Dongshan, Kantoushan, 600/800m, 4. II. 2010 (Y. F. Hsu). **PINGDONG Co.:** 1♂, Shizi, Nunaishan, 200m, 26. III. 2006 (Y. F. Hsu); 1♂, Fangliao, Yuquan, 26. II. 2006 (Y. F. Hsu); 2♂, 1♀, Shizi, Lilongshan, 500m, 17. II. 2008 (Y. F. Hsu); 1♂, same locality, 1. III. 2008 (Y. F. Hsu); 1♀, Wutai, Jiudawu, 500m, 11. V. 2008 (C. L. Huang); 1♀, Sandimen, ca 300m, 2. I. 2009 (Y. F. Hsu). **TAIDONG Co.:** 1♀, Yanping, Hongye, 11. I. 2009 (C. H. Lin, jr); 1♀, Taidong, Pipa Lake, 4. VI. 2006 (Y. F. Hsu); 1♀, Haiduan, Liyuan, 7. VI. 2020 (Y. F. Hsu) (genitalia preparation JYL1036).

S. lilaea.

JILONG CITY: 1♀, Longgang Trai, 26. IX. 2006 (Y. F. Hsu); 2♀, same locality, 2. X. 2006 (Y. F. Hsu);1♀, same locality, 7. II. 2007 (C. K. Wang); 1♂, Shenao, Sea Level, 20. VI. 2006,

reared from *B. nivea*, emgd. 2. VII. 2006, HSU 06F17 (Y. F. Hsu); 2♂, Haimentianxian, 26. IX. 2006 (Y. F. Hsu). **XINBEI CITY [= TAIPEI Co.]:** 7♂, 7♀, Wulai, 150m, 8. XI. 2004, reared from *B. nivea*, emgd. 29. XI-5. XII. 2004, HSU 04L20 (Y. F. Hsu); 1♂, Wulai, 11. III. 2005 (C. R. Chen), 1♀, same locality, 5. VI. 2005 (Y. F. Hsu), 1♂, 1♀, same locality, 11. VIII. 2005, reared from *B. densiflora*, emgd. 25. VIII. /1. IX. 2005, HSU 05H8 (Y. F. Hsu), 1♂, same locality, 23. IX. 2005, reared from *B. nivea*, emgd. 4. X. 2005, HSU 05J72 (J. R. Chen), 3♀, same locality, 6. X. 2006, reared from *B. nivea*, emgd. 3/15. XI. 2006, HSU 06L3 (Y. F. Hsu); 1♂, Wulai, Fushan, 700m, 4. VII. 2005, reared from *B. nivea*, emgd. 15. VII. 2005 (C. L. Huang); 1♀, Wulai, Fushan, 16. VI. 2005 (C. L. Huang); 1♂, Wulai, Baoqing Temple, ca 620m, 4. VIII. 2005 (J. R. Chen); 1♀, Xindian, Yinhedong, 250m, 14. VI. 2005 (Y. F. Hsu); 8♂, 1♀, Pinglin, Yuguang, 2. XI. /11.XII 2005, reared from *B. formosana*, emgd. 29. XI. /18. XII. 2005, HSU 05L5 (C. C. Lu); 1♀, Pinglin, Zhongxinlun, 2. XI. 2005 (C. C. Lu). **TAIPEI CITY:** 1♀, Beitou, Guizikeng, 24. X. 2004 (H. C. Huang & P. Lo); 2♀, Wenshan, Xianjiyan, 16. V. 2005 (Y. F. Hsu); 2♀, Daan, NTNU campus, 9. VI. 2005, reared from *B. nivea*, emgd. 23/24. VI. 2005, HSU 05F18 (Y. F. Hsu); 5♀, Wenshan, Gongguan campus, NTNU, 9. VI. 2005, reared from *B. nivea*, emgd. 21/24. VI. 2005, HSU 05F18 (Y. F. Hsu); 3♀, same locality, 15. I. 2007, reared from *B. nivea*, emgd. 31. I. 2007, HSU 07A20 (Y. F. Hsu); 1♂, Shilin, Tianxiyuan, 17. VII. 2011 (H. C. Huang); 1♂, Neihu, Dagouxi, 25. I. 2014 (L. Huang). **TAOYUAN CITY [=**

TAOYUAN Co.]: 2♀, Fuxing, Kapu, 800m, 14. X. 2007, reared from *B. densiflora*, emgd. 29. XI. 2007, HSU 07K7 (Y. F. Hsu & H. C. Huang). **YILAN Co.:** 1♂, Yuanshan, Fushan Botanical Garden, ca 700m, 4/5. VIII. 2006 (Y. F. Hsu & H. C. Huang); 1♂, 1♀, Toucheng, Guishan Is., 4/5. III. 2006 (H. C. Huang & C. L. Huang); 3♀, same locality, 24. VI. 2006 (H. C. Huang); 1♀, same locality, 24. VI. 2006 (C. L. Huang); 2♀, same locality, 4. VIII. 2007 (H. C. Huang). **XINZHU Co.:** 1♂, Qionglin, Feifengshan, ca 80m, 19. I. 2005, reared from *B. densiflora*, emgd. 14. II. 2005, HSU 05A4 (Y. F. Hsu); 1♂, Qionglin, Feifengshan, 10/80m, 19. I. 2005, reared from *B. nivea*, emgd. 8. II. 2005, HSU 05A3 (Y. F. Hsu); 1♀, Zhudong, 11. IV. 2006 (Y. F. Hsu); 2♀, Zhudong, 100m, 28. III. 2008 (Y. F. Hsu). **MIAOLI Co.:** 8♂, 2♀, Nanzhuang, 200m, 28. XI. 2004, reard from *B. densiflora*, emgd. 11/20. XII. 2004, HSU 04L68 (Y. T. Lo); 1♂, Touwu, Xiangshan Village, Jinshui, 60m, 27. II. 2005, reared from *B. nivea*, emgd. 7. III. 2005, HSU 05B36 (Y. F. Hsu); 5♂, 4♀, Sanwan, Emei Bridge, 14. I. 2006, reared from *B. nivea*, emgd. 3/7. II. 2006, HSU 06A12 (Y. F. Hsu); 1♂, Zhuolan, Liyutan, 2. VII. 2006 (Y. F. Hsu). **NANTOU Co.:** 2♂, 3♀, Lugu, 500m, 1. I. 2007, reared from *B. nivea*, emgd. 17/31. I. 2007, HSU 07A6 (Y. F. Hsu). **HUALIAN Co.:** 1♀, Wanrong, Wanrong Forest Trail, 29. VIII. 2010 (Z. H. Yen). **TAINAN CITY [= TAINAN Co.]:** 2♀, Xinhua, Dakeng, 80m, 10/11. II. 2006 (Y. F. Hsu); 1♀, Xinhua, 4. II. 2006, reared from *B. nivea*, emgd. 13. III. 2006, HSU 06J41 (Y. F. Hsu), 2♂, same locality, 20. I. 2007 (Y. F. Hsu). **KAOHSIUNG CITY:** 3♂, 1♀, Jiaxian Dist.,

Butingshan, ca 700m, 26. VII, 2022, reared from *Pouzolzia elegans*, emgd. 14. VIII. 2022, HSU 22G36 (Y. F. Hsu). **LIANJIANG Co.:** 1♂, 1♀, Nangan, 10. VII. 2003, reared from *B. nivea*, emgd. 25. VII. 2003, HSU 03G20 (L. W. Wu); 1♂, Nangan, Shengli Dam, 9/10. XII. 2006, reared from *B. nivea*, emgd. 6. I. 2007, HSU 06M2 (H. C. Huang); 1♂, 1♀, Beigan, Qinbi, 22. IV. 2007 (Y. F. Hsu & H. C. Huang).

Niche overlap between two *Symbrenthia* species in Taiwan

Many studies have reported that when a newly introduced species is present, competitive exclusion could be observed between the alien and similar native species (*Mooney & Cleland.,2001; Paini et al., 2008; Muthukrishnan et al., 2018*). However, our study presents a different aspect of this interaction. Both the SDM and the niche overlap results showed that the degree of overlap of the presenting area between these two species increases over time (Fig. 8). This means that competitive exclusion may not be present between the alien and the native species. This result may be explained by the following two alternative scenarios:

Firstly, obvious competition for the two species may not be observed due to insufficient time of introduction of the alien species. *S. lilaea* was first found on the main island of Taiwan as recently as 2004, and thus may still be in the process of population establishment and early growing stages (*McGeoch et al., 2019*). Consequently, the competitive exclusion effect 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. 6B & 6D). Especially in southwest portion of Taiwan, the presence probability increases significantly in the late invasive stages. This phenomenon can be the result of the expansion of the distribution area of this alien species. The first record of *S. lilaea* is in Xinzhu county located in the northwest part of Taiwan. The distribution area of this species gradually expanded during the 18 years since it was first observed in Taiwan, and this butterfly species can today be observed in nearly all lowland areas around Taiwan.

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

398 relying on the SDM results alone. By combining the biotic variables with the SDM constructed
399 by the abiotic variables, the results should be closer to the realistic distribution pattern.
400

401 **CONCLUSIONS**

402 Species delimitation employing an integrative taxonomy approach has helped to clarify
403 taxonomic entities of an introduced and a native *Symbrenthia* butterfly taxa regarded conspecific
404 to date, leading to a decision to recognize each as a distinct species. This result suggests that
405 interspecific competition may occur by the introduction of the alien species, rather than gene
406 introgression. Subsequently, a niche modeling investigation was following, and the result
407 showed that competition between the two species interest has not yet occurred or just initiated.
408

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Data Availability

The following information was supplied regarding data availability:

Voucher specimens are housed at Department of Life Science, National Taiwan University, Taipei, R. O. C. and Biodiversity Research Center, Academia Sinica, Taiwan, R. O. C.

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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).

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. formosanus* and *S.*

557 *lilaea* in the early invasive stage (1911-2012). (B) The distribution points of *S. formosanus* and
558 *S. lilaea* in the late invasive stage (2013-2021).
559
560 **Figure 3 Genitalia of *Symbrenthia lilaea* and *S. formosanus*.** (A－B) Male genitalia of *S.*
561 *lilaea* (Gen. Prep. JYL-877, NTNU). (C) Female genitalia of *S. lilaea* (Gen, Prep, JYL-880,
562 NTNU). (D－E) Male genitalia of *S. formosanus* (Gen. Prep. YFH-1583, NTNU). (F) Female
563 genitalia of *S. formosanus* (Gen. Prep. JYL-1036, NTNU). Scale bar = 1mm (A－B; D－E),
564 1mm (C; F)
565
566 **Figure 4 Eggs and immature stages of *Symbrenthia lilaea* and *S. formosanus*.** (A) Egg mass
567 of *S. lilaea*. (B) Young larvae of *S. lilaea*. (C) mature larvae of *S. lilaea*. (D) Egg of *S.*
568 *formosanus*. (E) Young larvae of *S. formosanus*. (F) mature larvae of *S. formosanus*.
569
570 **Figure 5 Systematic and species delimitation results of *Symbrenthia lilaea* and *S.***
571 ***formosanus*.** (A) Phylogenetic tree reconstructed by BI analysis of 20 COI sequences of
572 *Symbrenthia.* Nodal support from the BI posterior probabilities is along with ML bootstrap
573 pseudo-replicates (BI posterior probabilities/MLbootstrap). Columns after taxon names
574 correspond to the results of single-locus species delimitation analyses: Poisson Tree Processes

575 model (PTP), Automatic Barcode Gap Discovery (ABGD) and Generalized Mixed Yule
576 Coalecent model (GMYC). (B) PhyloMap visualization the result of PTP species delimitation,
577 including 20 taxa of *Symbrenthia*. The first axis (horizontal) explained 62.64% and the second
578 axis (vertical) explained 36.08% of sequence variance among samples.
579
580 **Figure 6 The Species Distribution Model (SDM) of *Symbrenthia formosanus* and *S. lilaea***
581 **between different invasive time stages predicted from MaxEnt.** (A) represents the SDM of *S.*
582 *formosanus* in the early invasive stage. (B) represents the SDM of *S. lilaea* in the early invasive
583 stage. (C) represents the SDM of *S. formosanus* in the late invasive stage. (D) represents the
584 SDM of *S. lilaea* in the late invasive stage.
585
586 **Figure 7 The Jackknife of regularized training gain of different bioclimatic factors of**
587 ***Symbrenthia formosanus* and *S. lilaea* between different invasive time stages.** (A) represents
588 the jackknife results of *S. formosanus* in the early invasive stage. (B) represents the jackknife
589 results of *S. lilaea* in the early invasive stage. (C) represents the jackknife results of *S.*
590 *formosanus* in the late invasive stage. (D) represents the jackknife results of *S. lilaea* in the late
591 invasive stage.
592

Figure 8 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*.

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

Figure S2 The receiver operating characteristic curve (ROC) and the threshold independent 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* 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* in the late invasive stage.

Table S1 pairwise distance of *Symbrenthia* samples

611 **Table S2 *Symbrenthia* smaples 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).

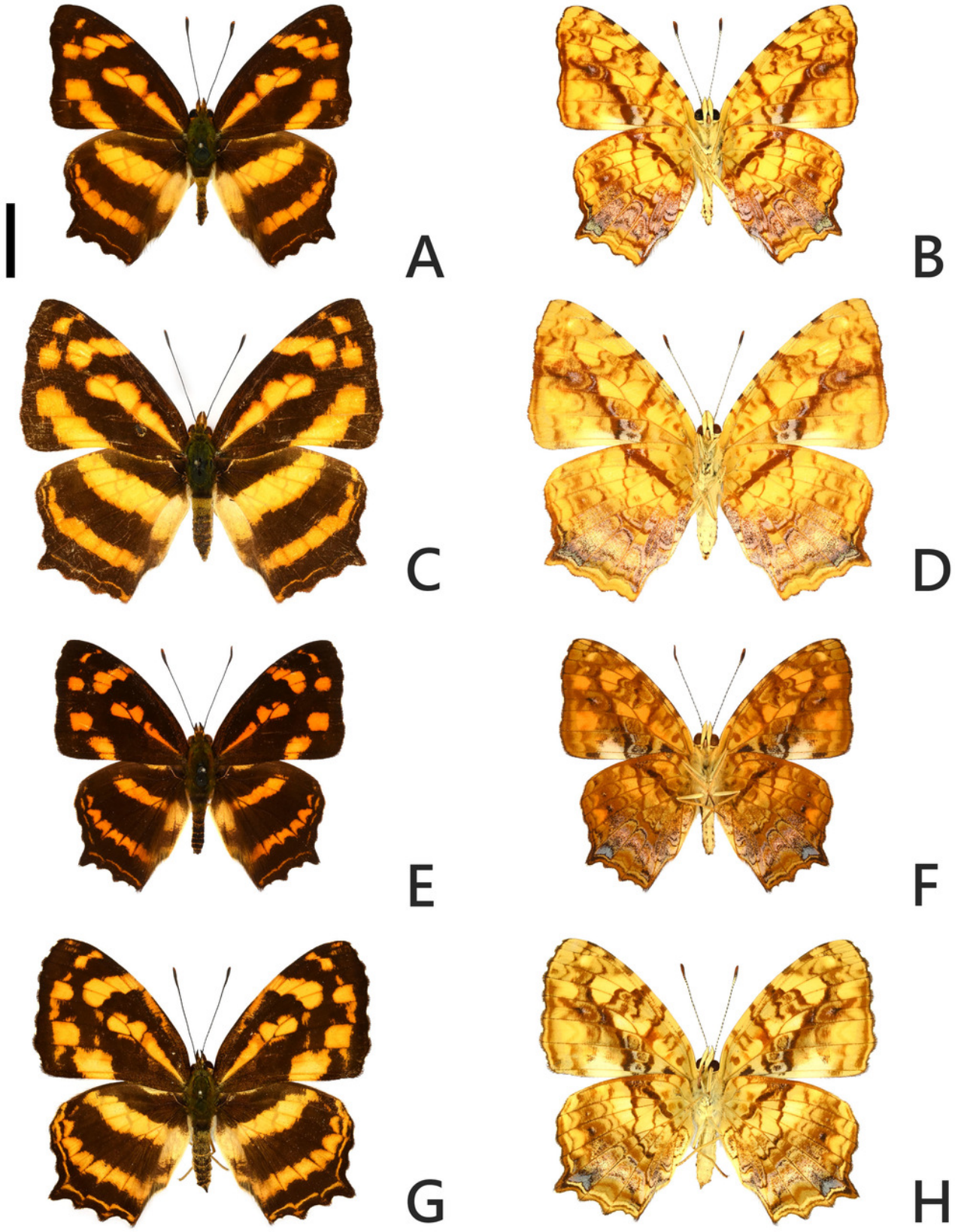
A
B
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Figure 2

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

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

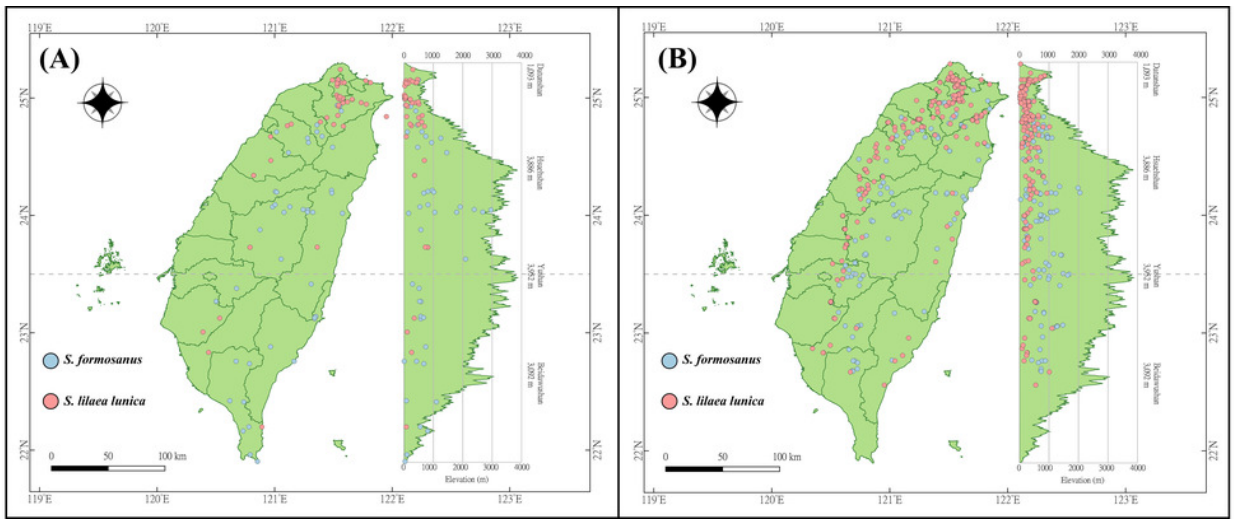


Figure 3

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)

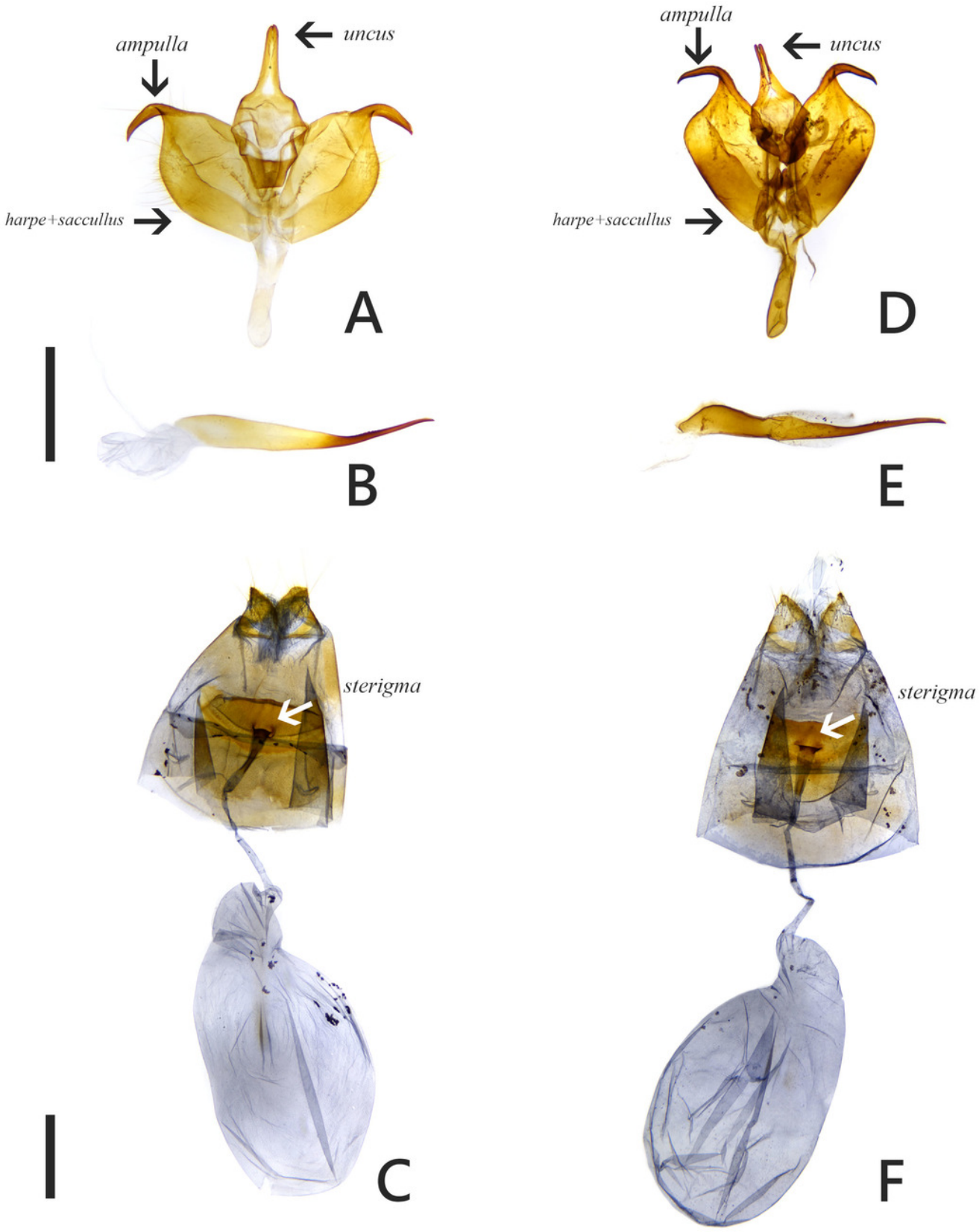

ampulla
uncus
harpe+saccullus
A
B
ampulla
uncus
harpe+saccullus
D
E
sterigma
C
sterigma
F

Figure 4

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*.

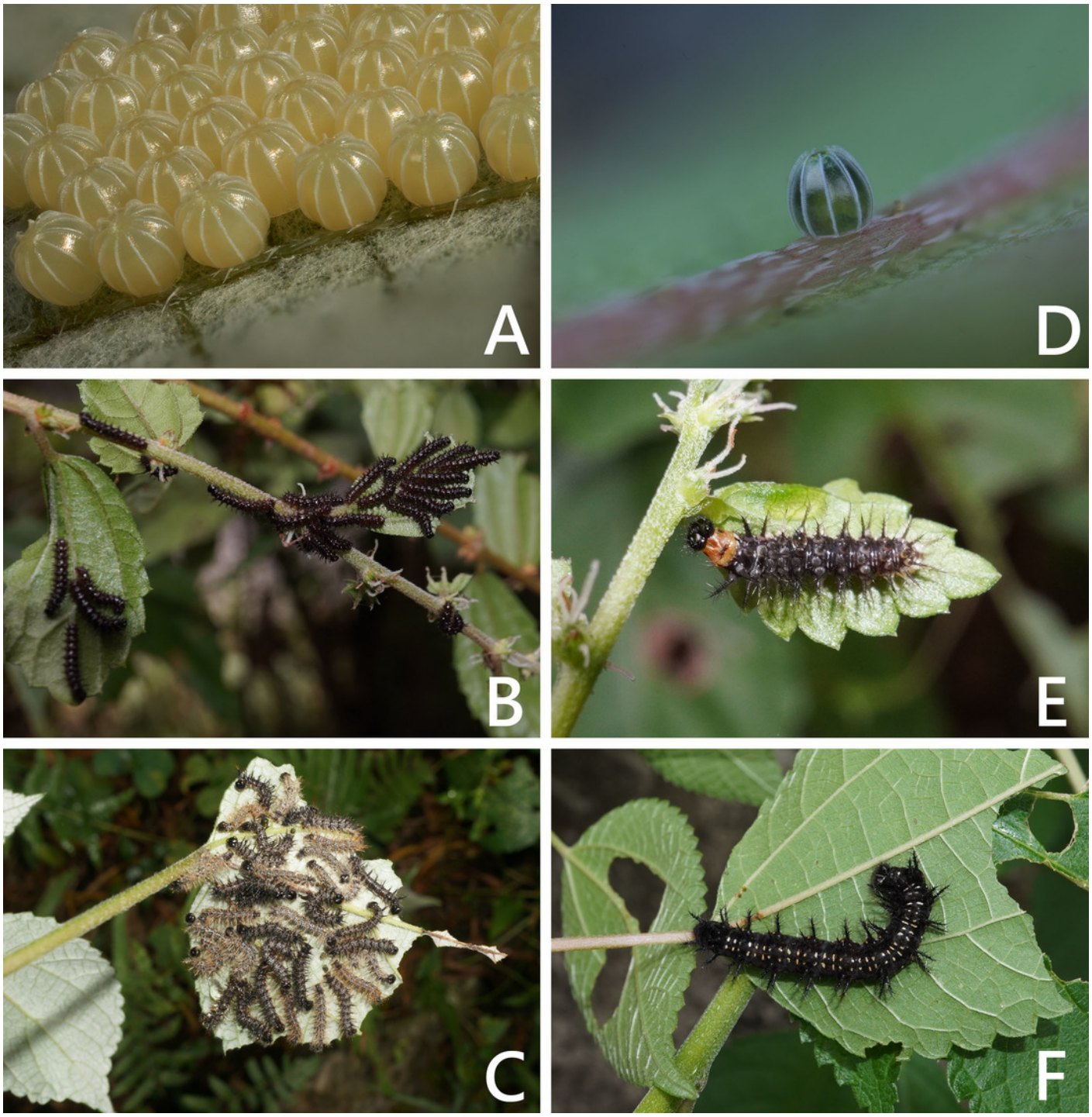

A
D
B
E
C
F

Figure 5

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.

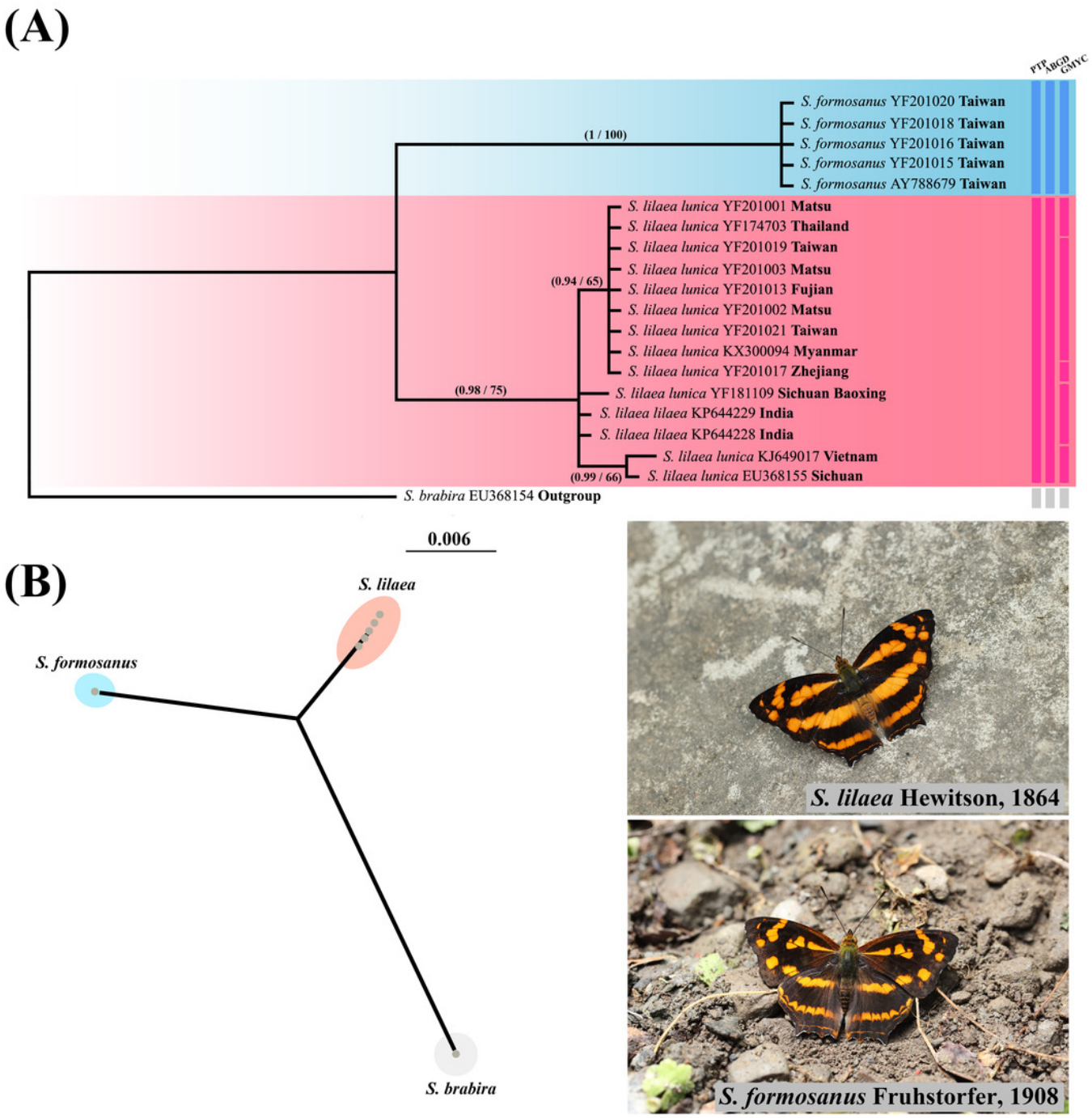

(A)
PTP
ABGD
GMYC
S. formosanus YF201020 Taiwan
S. formosanus YF201018 Taiwan
S. formosanus YF201016 Taiwan
S. formosanus YF201015 Taiwan
S. formosanus AY788679 Taiwan
(1 / 100)
S. lilaea lunica YF201001 Matsu
S. lilaea lunica YF174703 Thailand
S. lilaea lunica YF201019 Taiwan
S. lilaea lunica YF201003 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 6

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.

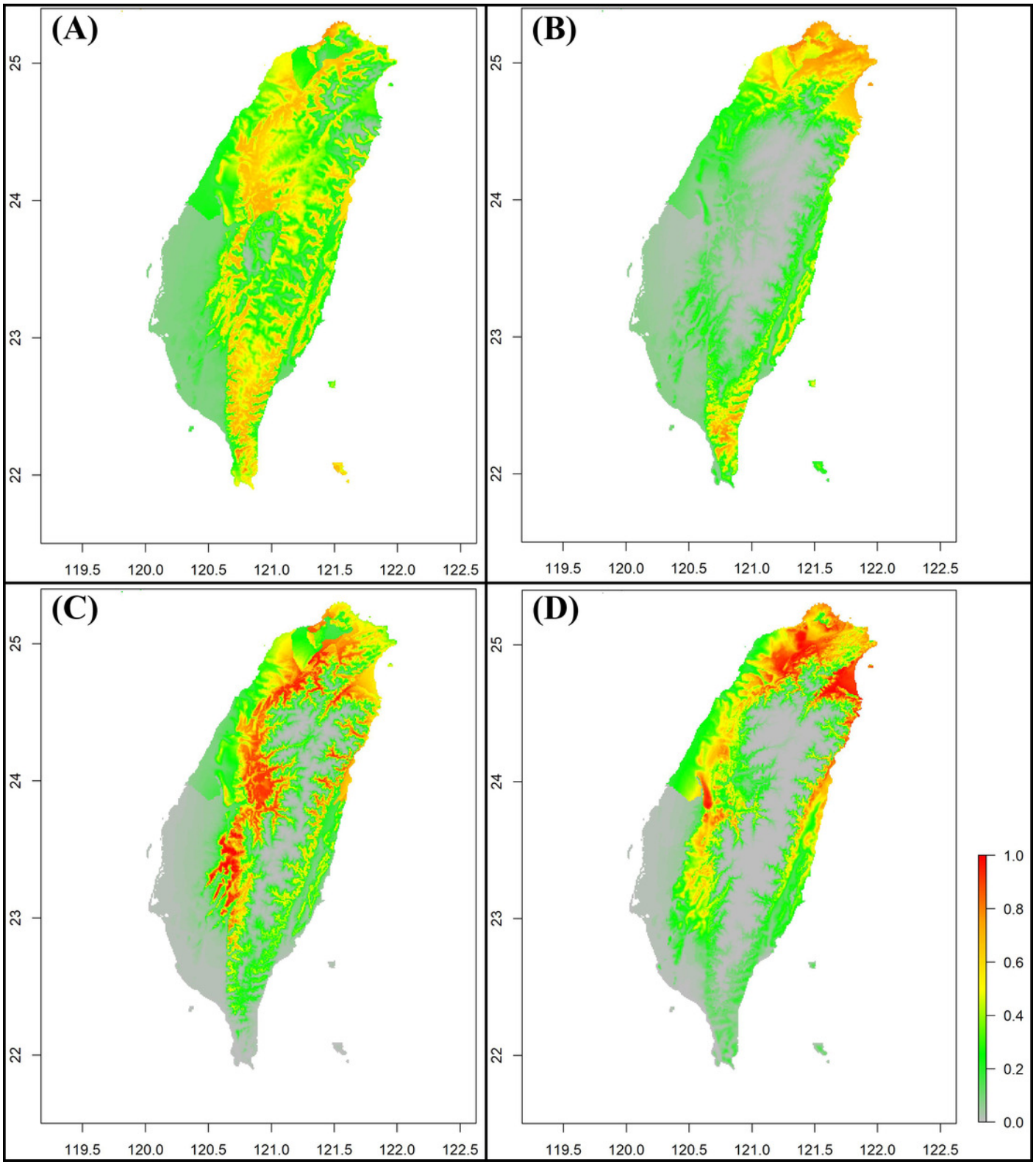

(A)
(B)
(C)
(D)
25
24
23
22
119.5
120.0
120.5
121.0
121.5
122.0
122.5
1.0
0.8
0.6
0.4
0.2
0.0

Figure 7

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* 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* in the late invasive stage.

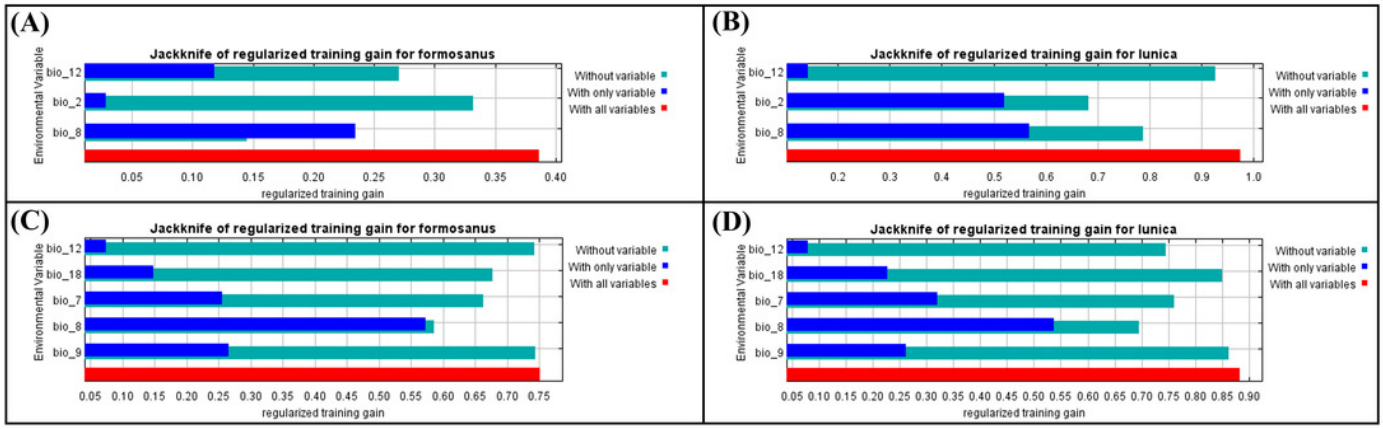
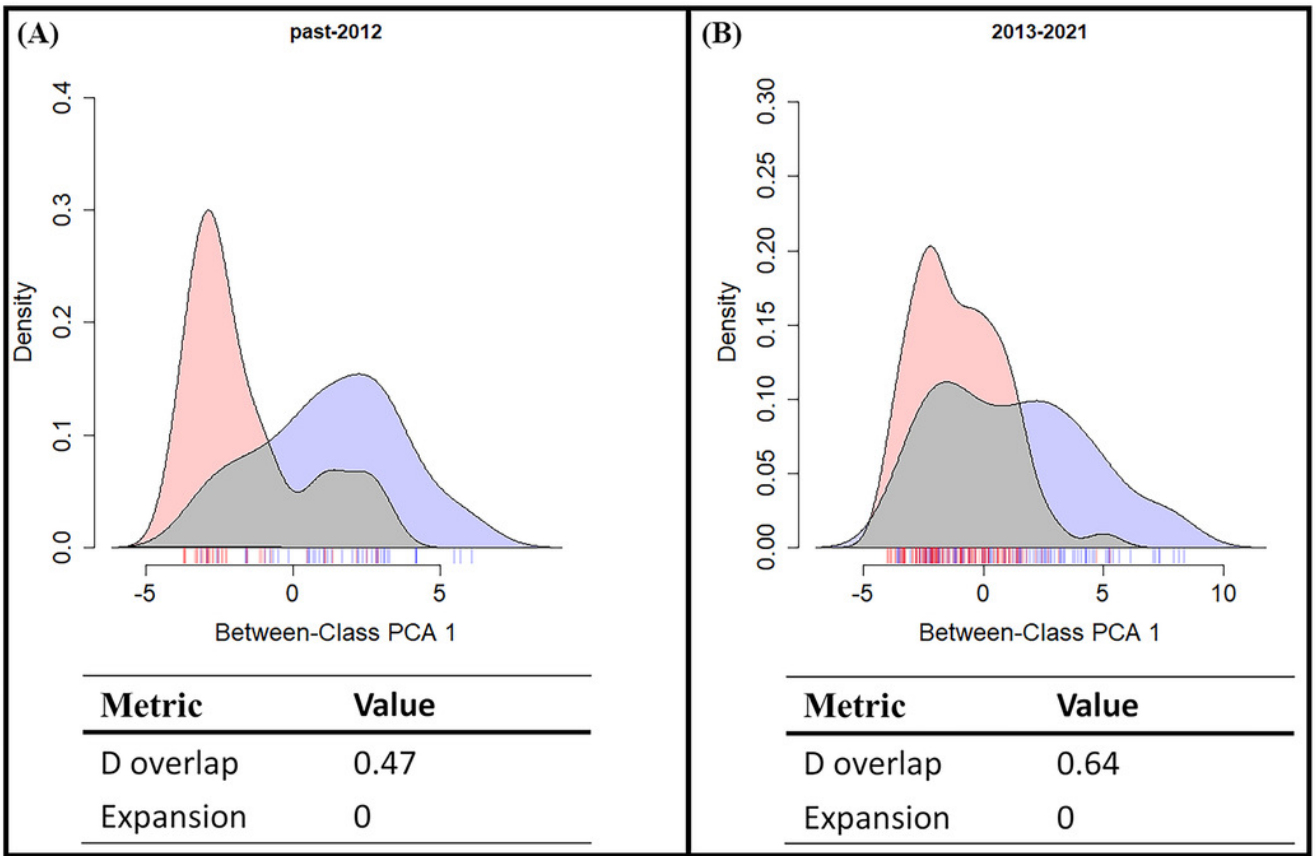


Figure 8

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