

A revised classification of the genus *Matrona* Selys, 1853 using molecular and morphological methods (Odonata: Calopterygidae)

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An extensive review of the genus *Matrona* is presented based on mitochondrial (COI) and nuclear (ITS) sequences from 150 samples which cover all the known taxa of this genus. The separation of two main clades (*oreades* group: *M. oreades*, *M. corephaea* and *M. taoi*; *basilaris* group: *M. basilaris*, *M. nigripectus*, *M. cyanoptera*, *M. japonica* and *M. annina*) is strongly supported. The classification of all traditional recognized species is confirmed. The Hainan population separates very well from mainland *M. basilaris* populations, which is also confirmed by geometric morphometric analysis of wing shape. Given the implications of the molecular analysis the genus *Matrona* is grouped into two subgenera: subgen. *Matrona* (type species *M. basilaris*) and ***Divortia* subgen. nov.** (type species *M. oreades*). A new species, ***M. (M.) mazu* sp. nov.**, from Hainan is described. Brief taxonomic notes on the nine recognized species of the genus are given. Lectotype designations of *M. basilaris* and *M. nigripectus* are published.

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INTRODUCTION

Matrona Selys, 1853 is a genus of rather large, conspicuous and attractively coloured East Asian calopterygid damselflies. In the Asian mainland their range extends from Meghalaya in the west to Vietnam in the south and to Hebei province in the north. In addition, *Matrona* species inhabit Hainan and Taiwan islands and Okinawa and Amami islands in Japan (Fig. 1). Less than 15 years ago only one species (*M. basilaris*) with two named and one unnamed subspecies was known, but now eight species are recognized (Hämäläinen *in* Karjalainen & Hämäläinen, 2013). They are listed here in chronological order:

Matrona basilaris Selys, 1853, *M. nigripectus* Selys, 1879, *M. japonica* Förster, 1897, *M. cyanoptera* Hämäläinen and Yeh, 2000, *M. oreades* Hämäläinen, Yu and Zhang, 2011, *M. corephaea* Hämäläinen *et al.*, 2011, *M. taoi* Phan & Hämäläinen, 2011 and *M. annina* Zhang & Hämäläinen, 2012.

Although morphological characters suggested enough evidence to place *M. oreades*, *M. corephaea* and *M. taoi* in their own species group within the genus (Hämäläinen *et al.*, 2011; Phan & Hämäläinen, 2011; Zhang & Hämäläinen, 2012), the phylogenetic relationships of all taxa within *Matrona* have not then been studied. Three of the taxa (*M. basilaris*, *M. nigripectus* and *M. cyanoptera*) have been included in molecular studies by Bybee *et al.* (2008), Dumont *et al.* (2005), Dumont, Vierstraete & Vanfleteren (2007), Dumont, Vierstraete & Vanfleteren (2010) and Guan *et al.* (2012), which show *Matrona* closely related to *Atrocalopteryx* Dumont *et al.*, 2005 and *Neurobasis* Selys, 1853.

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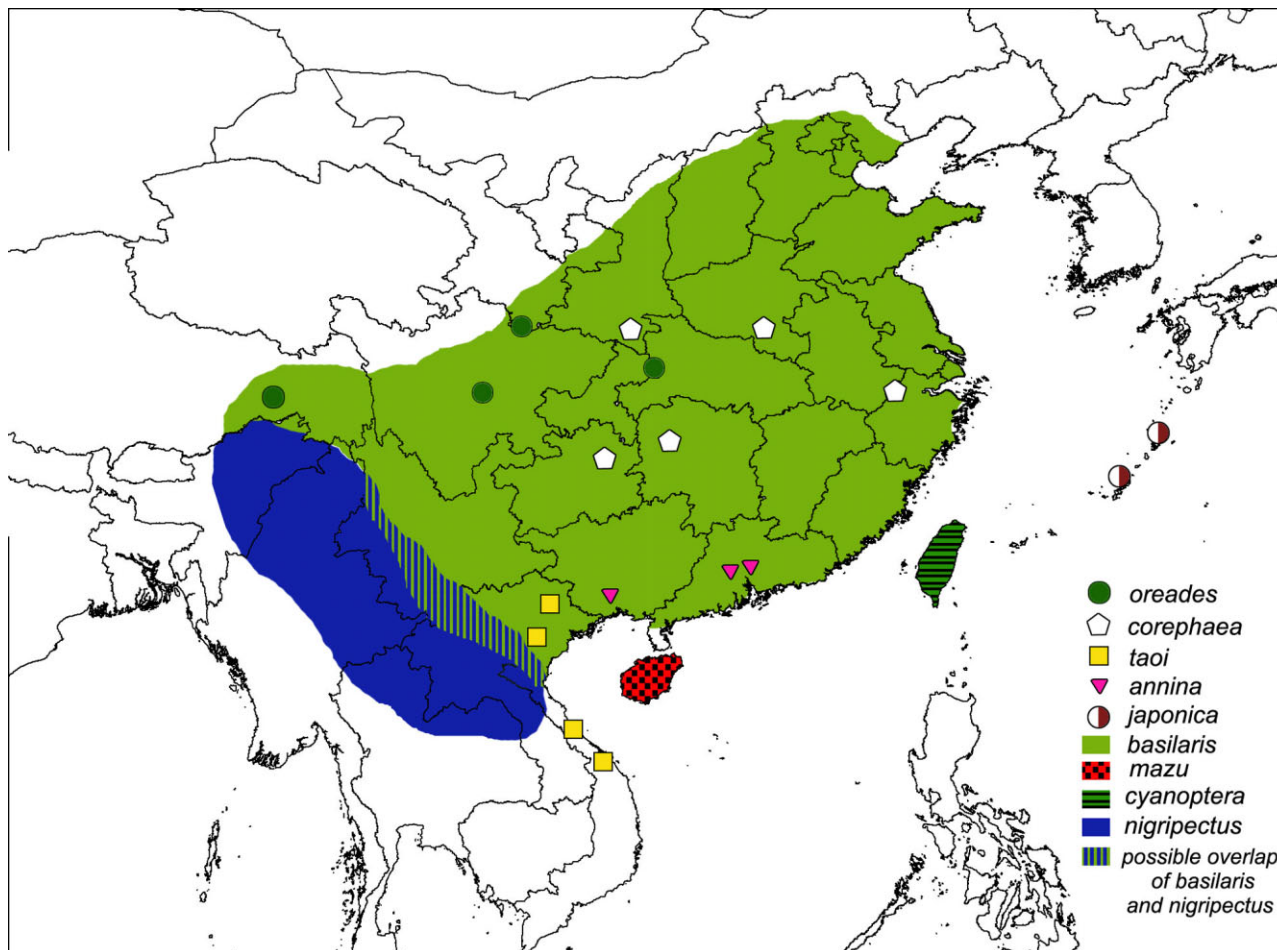


Figure 1. Approximate distribution ranges of known *Matrona* species.

The present phylogenetic analysis is based on analysis of the mitochondrial gene cytochrome *c* oxidase subunit 1 (COI) and combined nuclear gene of the ribosomal ITS1–5.8S–ITS2 region (ITS). Wing shape and colour have been subjected to intense sexual selection pressure in calopterygids and are thus more important than genitalia and caudal appendages in classification (Cordoba-Aguilar & Cordero-Rivera, 2005; Eroukhmanoff *et al.*, 2009; Outomuro, Bokma & Johansson, 2012; Outomuro, Adams & Johansson, 2013). Morphometric analysis of calopterygid wing shape can yield useful information (Sadeghi, Adriaens & Dumont, 2009; Outomuro *et al.*, 2013). The present study is the first to apply morphometric analysis to wings of *Matrona* to confirm the real division between closely related populations within the *a priori* *basilaris* group.

Our molecular results support two monophyletic clades of the genus *Matrona*, suggesting the division into two new subgenera *Divortia* and *Matrona*. We also describe a new species, *M. mazu* sp. nov., which is supported by our molecular and morphological assessments.

MATERIALS AND METHODS

MATERIAL ACQUISITION

A total of 188 samples were studied, 139 for molecular research and 63 for wing shape analysis (Supporting Information: S1). The samples were mainly collected by the first author (X.Y.), with the exception of a few specimens received from foreign colleagues. Paratypes of both *M. oreades* and *M. corephaea* were included in the molecular analysis. All the specimens have been deposited in the collections of the Institute of Entomology, College of Life Sciences, Nankai University, China. Voucher specimens were preserved in 75% ethanol immediately upon collection in the field. In most cases, one or two legs were isolated from the samples. Occasionally, dried specimens were used to obtain DNA data, although this was difficult. In addition, the following sequences were obtained from GenBank, *M. annina* (JX852701.1, JX852702.1), *M. cyanoptera* (AJ459205.1), *M. nigripectus* (AJ459206.1) and *M. japonica* (AB706444.1, JAB706443.1, AB706442.1,

AB706441.1, AB706440.1, AB706439.1 and AB706438.1). For wing shape analysis hind wings of a subsample of specimens were detached and photographed with a Nikon D700 digital camera.

In addition, for the taxonomic analysis of the genus, the third author (M.H.) studied numerous *Matrona* specimens in various museums and private collections. These include 19 specimens of *M. mazu* sp. nov. kept in his private collection, the syntype series of *M. basilaris* and *M. nigripectus* at IRSN (Brussels), and representative paratypes of all other *Matrona* taxa presently recognized as valid, with the exception of *M. japonica*.

DNA EXTRACTION AND AMPLIFICATION

Total genomic DNA was extracted using the protocol of the UniversalGen DNA Kit (Beijing ComWin Biotech). Small doses were used as a template for PCR amplification. Each PCR amplification was performed in 50- μ L reaction mixes containing 6 μ L 10 \times LA PCR BufferII (Mg²⁺ Plus), 6 μ L dNTP Mixture (2.5 mM), 2.5 U *TaKaRa* LA *Taq* (*TaKaRa* Biotechnology), 1 μ L of each primer (10 μ M), 2 μ L DNA template and 33.7 μ L distilled water. ITS was amplified with the primers detailed by Dumont *et al.* (2010), namely Vrain2F (5'-CTTTGTACAC ACCGCCGTCGCT-3') and Vrain2R (5'-TTTCACTC GCCGTTACTAAGGGAATC-3'). Newly designed primers COIf1 (5'-GRGCATGRGCAGGWATAGTNG-3') and COIr1 (5'-GGGTAGTCTGARTATCGTCGNGGT-3') were used for amplification of COI. The PCR cycling procedure was 2 min at 95 °C followed by 35 cycles of denaturation at 95 °C for 30 s, annealing temperature at 56 °C (ITS) or 52 °C (COI) for 30 s, and extension at 72 °C for 1 min, with a final single extra extension step at 72 °C for 8 min. All PCR products were visualized via 1% agarose gel electrophoresis and amplifications were purified using a gel extraction kit (Sangon Biotech), then sent to commercial companies (BGI TechSolutions or GENEWIZ) for sequencing based on Sanger's chain termination method. Fragments which failed in direct sequencing were cloned into a TA-cloning vector, pMD-18T (*TaKaRa*), and transformed into competent *Escherichia coli* DH5 α . Putative clones containing the PCR fragments were selected and sequenced.

SEQUENCE ANALYSES

Sequences obtained were edited and assembled in BioEdit v7.2.0 (Hall, 1999). Alignments of protein coding genes were translated to amino acids using MEGA v6.06 (Tamura *et al.*, 2013) to detect frameshift mutations and premature stop codons, which may indicate the presence of pseudogenes. Sequences were aligned using the ClustalX version 2.1 program package with default

settings and subsequently corrected manually to ensure the peak figure of each mutation loci was credible.

PHYLOGENETIC ANALYSES

Subsequent analyses were performed using optimality criteria including neighbour-joining (NJ), maximum-likelihood (ML) and the Bayesian inference algorithm (BI) to resolve the phylogenetic relationships. The distance method NJ tree was derived using BioEdit7.2.0 based on the Kimura two-parameter model with setting transition/transversion ratio = 2.0. ML analysis was performed using RAxML v8.0.0 (Stamatakis, 2014). Final ML tree searches were conducted under the GTR + I + G model for both COI and ITS selected as appropriate models of sequence evolution as implemented through ModelTest3.7 (Posada & Crandall, 1998). Bootstrap analyses were performed with the rapid algorithm. We obtained bootstrap support for each node from 1000 rapid bootstrap pseudoreplicates with every fifth tree used as starting point for subsequent ML optimization on the original dataset. Bayesian phylogenetic analyses were performed using MrBayes v3.1.2 (Huelsenbeck & Ronquist, 2001) with GTR + I + G model for COI and GTR + I for ITS. MrModeltest v2.3 (Nylander, 2004) was used to explore appropriate substitution models for both COI and ITS. All the acquired trees were set to 10 million generations and for every 1000 generations the chain was sampled. The Markov chain Monte Carlo (MCMC) process was run over four parallel chains, one cold and three incrementally heated. Theoretically, it could not be stopped until the average standard deviation of split frequencies was down to < 0.01 and was discarded as burn-in. Convergence diagnostic was determined with Tracer 1.5 (Rambaut & Drummond, 2007). Trees sampled after burn-in of the first 25% of each run from the four runs were combined and used to construct a 50% majority rule consensus tree. Trees were displayed with TreeView v1.6.6 (Page, 1996) and FigTree v1.4.0 (Rambaut, 2012). Four sequences, HLJH01 (*Atrocalopteryx atrata* [Selys, 1853]), HaNWZS01 (*Neurobasis chinensis* (Linnaeus, 1758)), SCYA05 *Vestalaria velata* (Ris, 1912) and SCYA06 (*Vestalaria velata*), were used as outgroups.

WING SHAPE ANALYSES

To test if there are distinct wing shape differences between *M. basilaris* specimens from Hainan Island and mainland China, a brief geometric morphometric analysis was conducted on hind wings as hind wing shape evolves faster than front wing shape in *Calopteryx* damselflies (Outomuro *et al.*, 2012, 2013). In total, five species or populations were involved in this analysis. Among them *cyanoptera*, *nigripectus* and *annina* have

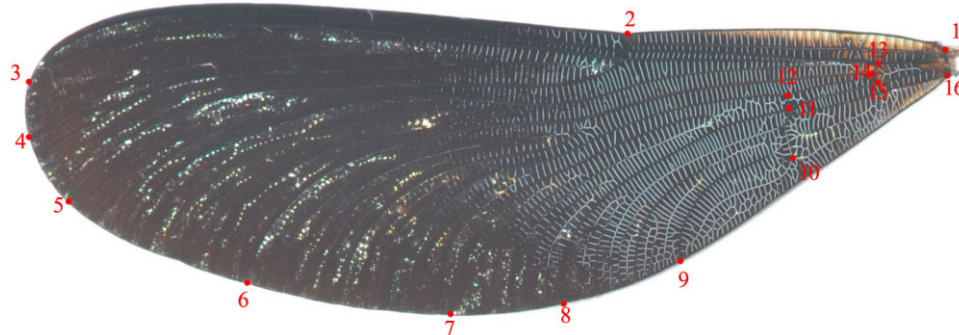


Figure 2. Landmarks of right hind wing (underside) of a *Matrona basilaris* male.

four samples each, and *basilaris* from mainland China and Hainan island has 45 and six samples, respectively. These methods allow for quantification of shape from landmark coordinates after the effects of non-shape variation (position, orientation and scale) have been mathematically held constant (Bookstein, 1991; Rohlf & Marcus, 1993). Sixteen landmarks were selected based on homologous sites (Sadeghi *et al.*, 2009), located at the wing base and along the wing margin where it is intersected by major wing veins, such as R2, IR2, R3, R4+5 and Cu1 (Fig. 2). A revised wing vein system of Riek & Kukalová-Peck (1984) was used (Rehn, 2003). The landmarks provide nearly complete coverage of the morphological changes in the hind wings. Wings of all species were photographed using a Nikon D700 camera. Images were entered in tps-UTILS 1.58 (Rohlf, 2013b) and Cartesian coordinates of landmarks were digitized with tps-DIG 2.17 (Rohlf, 2013a). Landmark configurations were scaled, translated and rotated against the consensus configuration using the GLS (generalized least squares) Procrustes superimposition method (Bookstein, 1991). The coordinates were analysed using tps-RELW 1.53 (Rohlf, 2013c) to calculate eigenvalues for each principal warp.

RESULTS

PHYLOGENY

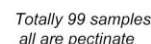
In total, 92 sequences of COI (1025 bp), 147 of ITS (616 bp) and 86 combined sequences of COI+ITS (1771 bp) were obtained. For each molecular marker all the phylogenetic trees (NJ, ML, BI) produced the same topological results, which strongly supported the division of the present monophyletic *Matrona* into two lineages, the *oreades* group (including *M. oreades*, *M. corephaea* and *M. taoi*) and the *basilaris* group (including *M. basilaris*, *M. nigripictus*, *M. cyanoptera*, *M. japonica* and *M. annina*), and within the *basilaris* group the Hainan populations represent a monophyletic group.

According to the ITS tree (Fig. 3), *M. oreades* was recovered as sister to *M. corephaea* with very strong BI posterior probability support (BPP = 0.99) and ML bootstrap value (MLB = 91). *Matrona taoi* was recovered as sister to *M. oreades* + *M. corephaea* with moderate support (BPP = 0.52, MLB = 32). In the *basilaris* clade, *M. nigripictus* was sister to all other groups (BPP = 0.89, MLB = 75). Both *M. cyanoptera* and the Hainan populations are confirmed as good monophyletic groups. The mainland *basilaris* populations also form a well-supported monophylum (BPP = 1.00, MLB = 89) with internal nodes showing an unparsed state, except *M. annina*, which occupies a sub-branch. *Matrona japonica* together with the mainland *basilaris* population forms a monophylum (BPP = 1.00, MLB = 89) with its inner nodes unparsed.

The result on the base of COI is slightly different from ITS. *Matrona oreades* and *M. corephaea* are not very well separated from each other as there was some overlap of individuals (Fig. 4). *Matrona cyanoptera* is sister to the continental *M. basilaris* with a high level of support (BPP = 1, MLB = 89). *Matrona japonica* was recovered as sister to all other members of the *basilaris* group (BPP = 0.93). The result produced by the combined COI + ITS data is similar to that from COI (Supporting Information: S5).

VARIATIONS OF MORPHOLOGY OF HIND WINGS

The first two relative warps of the 16 landmarks from 63 samples account for 58.13% of the variations among the species or populations (Supporting Information: S4). These were computed by a singular-value decomposition of the weight matrix (Rohlf, 1993). The first two relative warps were plotted to indicate variation along the two axes (Fig. 5). The shape changes among different species implied by variation along the first two relative warp axes and shape changes are shown as deformations of the GLS reference, using thin-plate splines (Fig. 5). The four splines, which show the deformation of the outlines in comparison with that of



the reference, indicate the most significant deformation, as it is situated furthest from the origin. The Hainan population is significantly different from the others but other groups cannot be clearly separated based on wing shape. The main deformation between the Hainan population and the mainland *basilaris* is

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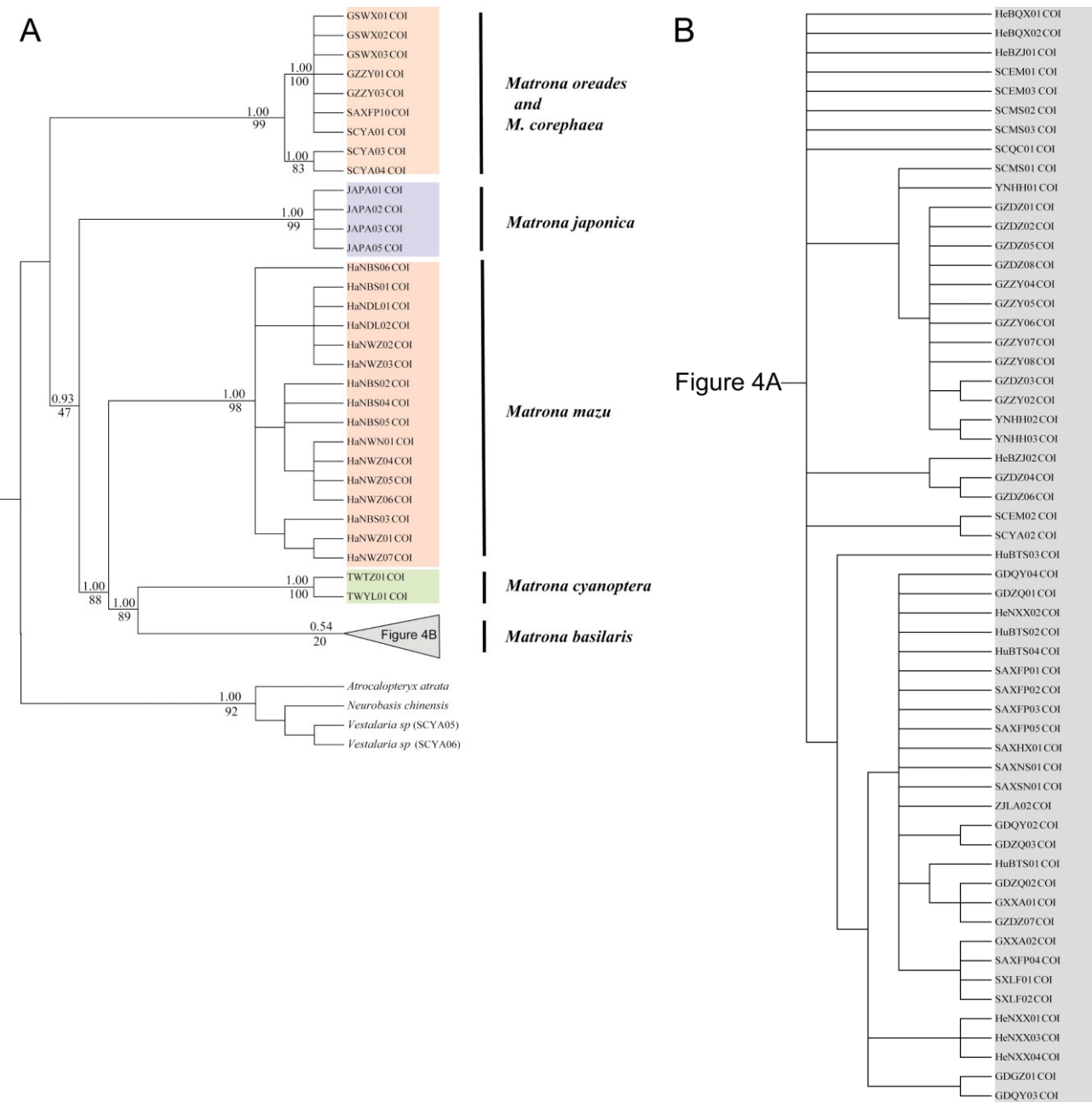


Figure 4. Phylogenetic reconstruction for 92 specimens from COI. Posterior probabilities (above) and bootstrap values (below) are shown. A, all the groups excluding *basilaris*; B, *basilaris*.

TAXONOMIC CONSIDERATIONS AND CONCLUSIONS

According to the results of phylogeny and morphology we divide the genus *Matrona* into two subgenera. A new subgenus *Divortia* Yu, Xue & Hämäläinen subgen. nov. is erected to accommodate the species *M. oreades*, *M. taoi* and *M. corephaea*. Within this group *M. taoi* was recovered as sister to *M. oreades* +

M. corephaea but with only moderate support from molecular analysis. The sister relationship of *M. oreades* and *M. corephaea* was strongly supported.

In the subgenus *Matrona*, the results confirm the specific status of all the five taxa listed as distinct species by Hämäläinen in Karjalainen & Hämäläinen (2013: 207). The Hainan populations, traditionally identified as *M. basilaris*, are considered to represent a new species, which is described here as *Matrona mazu*

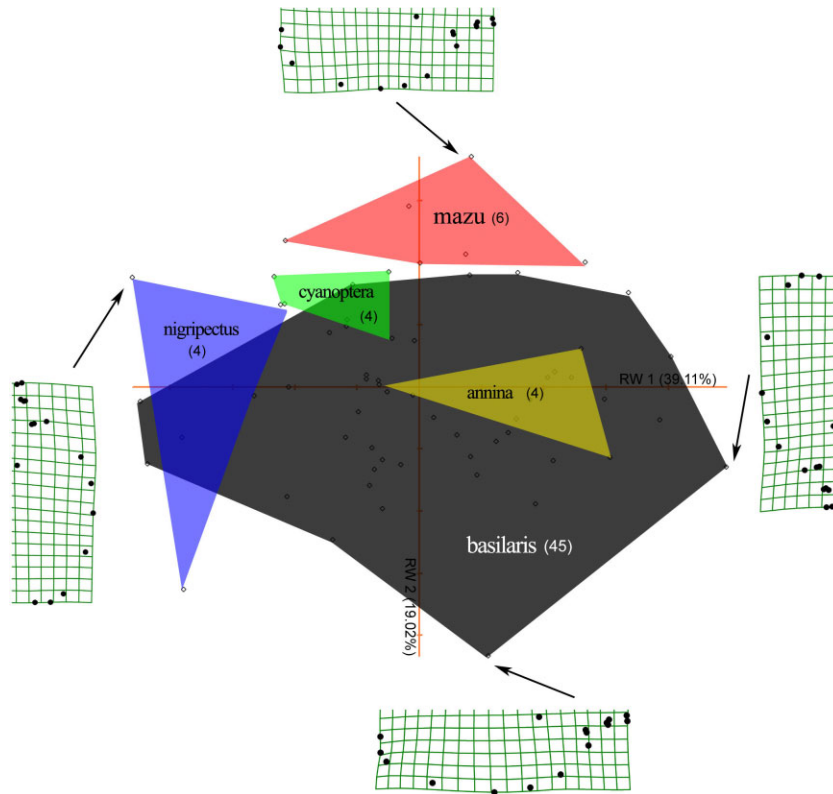


Figure 5. The first two relative warps (RW) computed from the landmark data set. RW 1 (x -axis) accounts for 39.11% of the variations, and RW 2 (y -axis) for 19.02%. Splines indicate deformation of the landmarks in comparison with the reference configuration. Numbers in parentheses show how many samples were used in each group.

Yu, Xue & Hämäläinen sp. nov. In the phylogenetic analyses of Dumont *et al.* (2007) and Guan *et al.* (2012), the Hainan population was also considered to be the sister group of the mainland Chinese *M. basilaris*.

The genus shows extraordinary diversity in mainland China and on adjacent islands. In addition to Hainan, Taiwan and the Japanese Okinawa and Amami Islands also have their own endemic species, namely *M. cyanoptera* and *M. japonica*. On the mainland, *M. annina* forms a branch on its own, although it is not far distant from *M. basilaris*, implying that the genetic distance between these two species is rather small (0.038 for ITS, the smallest among all the pairwise values compared; Supporting Information: S2). *Matrona nigripectus*, distributed in south-western Yunnan, north-eastern India, Burma, Thailand and Laos (Hämäläinen *et al.*, 2011), appears as sister to all others in the subgenus *Matrona*.

We have taken a combined molecular and morphological approach with a large number of samples in this study to provide a robust molecular framework for distinguishing all species of *Matrona*, most of which do not have clear structural differences in the anal appendages and penis, characters that have been used to distinguish species in many other odonate groups.

Generally the phylogeny recovered by ITS is even credible as it comprised all the species representatives. The phylogenetic trees structured by COI are not strictly identical to ITS as regards the relative position of *M. cyanoptera* and *M. japonica*, and they revealed overlap among several specimens of *M. oreades* and *M. corephaea*. We speculate that the reasons for this are that (1) the sample size (both on individual and species level) of COI is less than ITS, and (2) COI has an even faster rate of evolution compared with ITS at least in odonates, although both of them are quickly evolving genes. The result based on combined COI+ITS data is almost identical to that of COI, implying that sample size may strongly affect topology in short length (less than 10k bp) sequence analysis. Again the relative long sequence of COI (1025 bp) in the combined COI+ITS data (1771 bp) may also strongly affect the topological structure. Therefore ITS is confirmed to be a very useful marker for phylogenetic study on the genus and species level in Odonata whereas COI is more suitable for a population-level analysis, the same conclusion was also suggested by Sasamoto & Futahashi (2013) and Guan *et al.* (2012). Geometric morphometric analysis is a useful method especially for distinguishing otherwise morphologically cryptic species. We hope that this

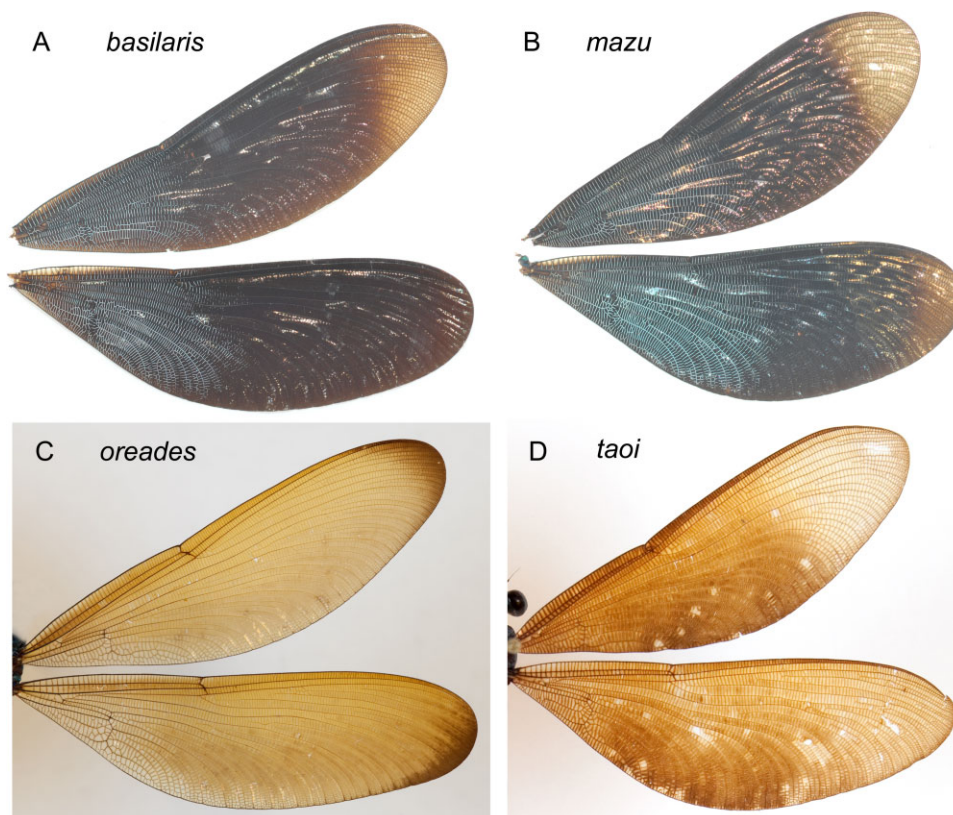


Figure 6. Right wings of males of *Matrona* species: A, *M. basilaris* (Qingliangfeng, Zhejiang); B, *M. mazu* sp. nov. (Baisha, Hainan; paratype, HaNBS02); C, *M. oreades* (Emeishan, Sichuan); D, *M. taoi* (Xuan Son, Vietnam; paratype).

research will help to provide a stable phylogeny and classification of the genus *Matrona* as a whole, and to reveal key taxa for future investigations.

CLASSIFICATION OF THE GENUS *MATRONA*

GENUS *MATRONA* SELYS, 1853

de Selys Longchamps, 1853: 17. – *Matrona*, defined as subgenus of *Calopteryx* Leach [1815]. Type species: *Matrona basilaris* Selys, 1853, as the only species.

Kirby, 1890: 100. – *Matrona* upgraded to genus.

Generic diagnosis: Rather large demoiselle damselflies with brilliant metallic green, slender body and long broad wings with rounded apices; male wings either wholly dark opaque or pale to medium brownish, lacking pterostigma; female wings brownish with creamy white pseudopterostigmata. Median space with partly reticulated cross-veins, a morphological synapomorphy that separates the genus both from *Calopteryx* Leach, 1815 and *Atrocalopteryx* Dumont *et al.*, 2005. Vein IA bifurcated, producing a branch basally. After diverging from RP3 (R4+5) the vein IR2 runs parallel to RA. Nodus situated nearer the base of wing than the apex.

Wings with numerous intercalated veins. Legs long with slightly curved middle and hind tibiae.

SUBGENUS *MATRONA* SELYS, 1853

Generic diagnosis: Male wings uniformly dark blue opaque or reddish brown with a distinctive area of the wing base, of variable size, which appears milky when viewed from an oblique angle, owing to the presence of bluish white cross-veins. The reticulation of the wings is dense to very dense, especially in the hind wings (Fig. 6A, B). Median space usually with 5–7 reticulated cross-veins.

SUBGENUS *DIVORTIA* YU, XUE & HÄMÄLÄINEN SUBGEN. NOV.

Type species: *Matrona oreades* Hämäläinen *et al.*, 2011

Generic diagnosis: Male wings variably pale to medium brown without any milky reticulation at the wing base. Wing apices either slightly darkened or subhyaline. The wing reticulation (Fig. 6C, D) is distinctly sparser than

in subgenus *Matrona*. Median space with 1–5 reticulated cross-veins. Species included: *M. oreades*, *M. corephaea* and *M. taoi*.

Etymology: *Divortia*, a noun derived from the Latin *divortium*, signifying the generic separation of these species from the remainder of *Matrona*.

LIST OF SPECIES

Subgenus *Matrona*

Matrona (Matrona) basilaris Selys, 1853

de Selys Longchamps, 1853: 17. – *Matrona basilaris*, De Selys; description of both sexes from ‘Nord de la Chine (Collect. Selys, Hagen, etc.)’.

Selys Longchamps in de Selys Longchamps & Hagen, 1854: 53. – Extended description.

de Selys Longchamps, 1888: Lii–Liii. – Statement on the type locality: . . . ‘*basilaris* dont les types proviennent de Shanghai et nord de la China’.

Designation of lectotype: The third author (M.H.) has studied all *Matrona* specimens in coll. Selys Longchamps (at IRSN, Brussels) in 2003 and 2008. Under the drawer label *Matrona basilaris* there are 5♂ and 4♀ specimens. Among the males, two specimens bear similar yellow labels in Selys’ handwriting ‘N. Chine,? Shang.’ The locality data in these two male specimens correspond the information given in the original description by Selys Longchamps (1853). These two specimens also carry a note on white paper handwritten by Dr Syoziro Asahina: ‘Probably one of the original Type series. Asahina 1968’. To fix the identity of the nominal taxon *Matrona basilaris* based on one specimen, we herewith designate the male specimen in best condition as lectotype. The specimen has been furnished with a printed label ‘LECTOTYPE. *Matrona basilaris* Selys, 1853. Designated by M. Hämäläinen 2003’.

Distribution: Eastern Laos (Lak Sao area), northern Vietnam, China (Yunnan, Guangxi, Guangdong, Guizhou, Hunan, Jiangxi, Fujian, south-east Xizang, Sichuan, Chongqing, Hubei, Anhui, Zhejiang, Shanghai, Shaanxi, Henan, Jiangsu, Shanxi, Hebei, Shandong, Beijing, Tianjin).

SYNONYM

Matrona kricheldorffi Karsch, 1892, junior synonym of *M. basilaris* Selys, 1953.

Karsch 1892: 456. – *Matrona kricheldorffi*; description of male holotype (at NHB, Berlin) from ‘Omi-shan p. Kiating, China occidentalis’ [Emeishan, Sichuan].

Needham 1930: 200. – Placed as junior synonym of *M. basilaris*.

Hämäläinen *et al.* 2011: 22. – Further notes on the synonymy.

Matrona (Matrona) nigripectus Selys, 1879

de Selys Longchamps, 1879: 355 – *Matrona nigripectus*, De Selys; description of both sexes from ‘Khasia Hills (Bengale), par M. Atkinsson en octobre (Coll. Selys.)’ with comment ‘Très-voisine de la *basilaris* dont elle n’est probablement qu’une race locale’.

Kirby 1890: 100. – Listed as good species.

de Selys Longchamps, 1891: 484. – Listed as *Matrona basilaris* race *nigripectus*.

Fraser 1934: 147. – Listed as *Matrona basilaris nigripectus*.

Hämäläinen *et al.*, 2011. – Ranked as a good species.

Designation of lectotype: In the collections of Selys Longchamps (IRSN) there are 2♂ and 2♀ specimens from the Khasia Hills under the drawer label *Matrona nigripectus*. The locality data in these specimens correspond the information given in the original description by Selys Longchamps (1853). To fix the identity of the nominal taxon *Matrona nigripectus* based on one specimen, we herewith designate the male specimen in best condition as lectotype. The specimen has the following attached labels: ‘Khasia Hill, Oct. 67’ [white handwritten label], ‘Atkinson’ [yellow printed label], ‘*Matrona nigripectus* S. ♂ Khasya’ [white handwritten label by Selys] and ‘117’ [white handwritten label]. The specimen has been furnished with a printed label: ‘LECTOTYPE. *Matrona nigripectus* Selys, 1879. Designated by M. Hämäläinen 2003’.

Distribution: North-eastern India (Meghalaya), Burma, Thailand, Laos, Vietnam, China (Yunnan).

Matrona (Matrona) cyanoptera Hämäläinen & Yeh, 2000

Ris, 1916: 6–7. – *Matrona basilaris*; differences in specimens from mainland China and Formosa are compared.

Asahina, 1962a: 8. – Listed as *Matrona basilaris* subsp.; the same name also in later papers by Asahina.

Lieftinck *et al.*, 1984: 11–12. – Listed as *Matrona basilaris* subsp.

Hämäläinen & Yeh, 2000: 2. – *Matrona cyanoptera* sp. nov.; description of both sexes from Taiwan. Type locality: Taipei, Neishwangsi. Holotype deposited at Taiwan Forestry Research Institute (TFRI).

Here we continue to treat *M. cyanoptera* as a good species based on its morphological characters, the phylogenetic analysis and its restricted distribution, even though the genetic distance of COI between it and *basilaris* is only 0.016 (Supporting Information: S2).



Figure 7. Male of *Matrona mazu* sp. nov.; photo taken by Graham T. Reels in Wuzhishan, Hainan, 16 April 2008.

Distribution: Confined to Taiwan Island, where it is widespread.

***Matrona (Matrona) mazu* Yu, Xue & Hämäläinen sp. nov.**

Type material: Holotype ♂: China, Hainan, Wuzhishan, Shuiman, alt. 620–720 m, 17.v.2007, Yu Xin leg. Deposited at Institute of Entomology, Life Sciences College of Nankai University, Tianjin, China. *Paratypes* (all from China, Hainan): 2 ♂, Wuzhishan, Shuiman, alt. 620–720 m, 17.v.2007, Yu Xin leg.; 1 ♀, Wuzhishan, Shuiman, alt. 700 m, 14.iv.2009, Xi Li leg.; 1 ♀, Wuzhishan, Shuiman, alt. 700 m, 13.vii.2009, Jiao Kelong leg.; 2 ♂, Wuzhishan, 30–31.iii.2008, Zhang Hao-miao leg.; 1 ♂, Wuzhishan, 17.iv.2009, Zhang Hao-miao leg.; 2 ♂, Wuzhishan, 9.v.2011, Zhang Hao-miao leg.; 1 ♀, Wuzhishan, 26.iv.2008, Zhang Hao-miao leg.; 7 ♂, 3 ♀, Jianfengling, alt. 850 m, 29–30.vi.1993, Colin G. Treadaway leg.; 1 ♂, Jianfengling, alt. 900 m, 7.vi.2007, Yu Xin leg.; 1 ♂, Bawangling, alt. 750 m, 9.vi.2007, Yu Xin leg.; 2 ♂, Diaoluoshan, 15.viii.2008, Xie Qiang leg.; 2 ♂, Diaoluoshan, 24.iv.2008, Zhang Hao-miao leg.; 1 ♂, Wanning, 13.xii.2010, Wang Rui leg.; 1 ♂, Yinggeling, Yinggezui, 28.iv.2011, Zhang Hao-miao leg.; 6 ♂, Yinggeling, 20–23.vii.2013, Yang Jie & Wang Yanhui leg.; 2 ♂, Yinggeling, 6.viii.2013, Yang Jie leg.

Etymology: Named after Mazu, a legendary Chinese woman Lin Moniang, who lived in Fujian in 960–987 during the Song Dynasty. In south-eastern coastal regions of China, including Hainan, Mazu is widely worshipped as a goddess of the sea, who protects fishermen and sailors.

DESCRIPTION OF MALE

Head: Eyes in life brown above, pale bluish-grey below (cf. Fig. 7). Labrum dark metallic, in some specimens

with a pair of tiny yellowish spots. Base of mandibles dark, with yellowish marking on outer side. Anteclypeus dark metallic green, with paler central greyish area basally. Postclypeus shining metallic green, frons and vertex metallic green. Antennae with anterior surface of scape obscurely pale, pedicel black with a distinct yellowish spot at base anteriorly, apical segments blackish brown.

Thorax: Prothorax shining metallic green. Synthorax shining metallic green, metepimeron partly narrowly bordered with yellowish-ochre. Basal border of metepisternum in front of stigma obscurely yellowish. Venter of synthorax a mixture of black and pale yellow-ochre. Legs black, with the exception of small pale dots on middle and hind trochanters and middle and hind coxa with lateral yellow stripes. Middle and hind tibiae moderately bent, hind tibiae more distinctly so (Fig. 8A).

Wings: Wings blackish opaque, the tip of forewing hyaline. In the basal half of both wings the reticulation is distinctly bluish-white, which gives the wing base a milky appearance when viewed from certain angles. Wings proportionally broad especially in hindwing; in the holotype the length/largest breadth ratio is 2.85 in hindwing. Median space of both wings with several reticulated cross-veins. In holotype the cubital field with 22–25 cross-veins in forewing and 29–29 in hindwing; quadrangle with 6–7 cross-veins in forewing and 7–7 in hindwing. Antenodals (the costal series) number 47–48 in forewing and 41–41 in hindwing.

Abdomen: Metallic green throughout, lower third of the side of S10 yellowish. Latero-ventral edge of S6–8 obscurely pale brownish. Ventral side of S8–10 pale brownish yellow. Appendages of typical shape for the genus; black, except the basal part of the inferiors which is pale on the underside.

Measurements (mm): Abdomen (incl. appendages) 53–56 (53 in holotype); hind wing 36–40 (36 in holotype).

DESCRIPTION OF FEMALE

Head: Eyes in life brown above, lower part extensively pale greenish. Labrum mainly yellowish with lower margin black; at middle of upper margin dark colour extends down forming a black circle. Clypeus, frons and vertex as in male. Antennae with scape and most of pedicel conspicuously creamy yellow, the apical segments black.

Thorax: Prothorax metallic green. Synthorax metallic green with metepimeron broadly bordered with pale

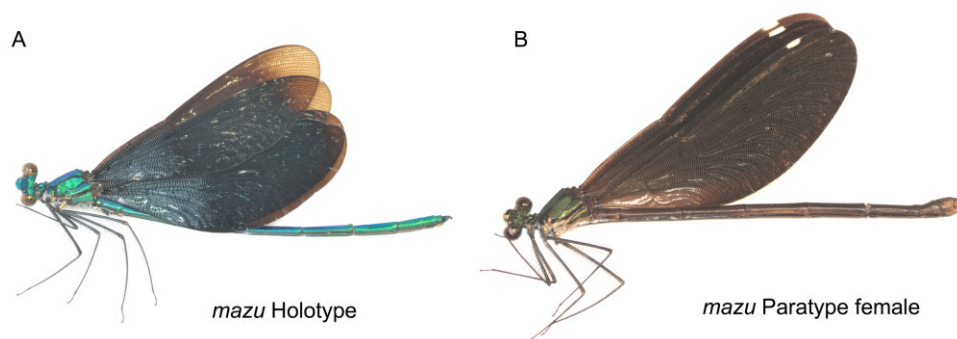


Figure 8. Habitus of *Matrona mazu* sp. nov.: A, holotype male; B, paratype female from Wuzhishan.

ochre. Pale markings on basal parts of metepisternum and mesepimeron more extensive than in male (Fig. 8B). Legs as in male.

Wings: Wings brown, with basal area obscurely subhyaline. Whitish pseudopterostigmata rather broad, crossed with 6–8 cross-veins. Venation resembles that in male.

Abdomen: Dark sepia brown throughout above and in basal segments, an obscure pale stripe ventro-laterally on S2. Lower lateral half of S9–10 obscurely pale. A distinct mid-dorsal pale stripe on S8–10, narrowest on S8.

Measurements (mm): Abdomen (incl. appendages) 49–51; hind wing 41.5–43.

Distribution: Confined to Hainan Island, where widespread.

DIFFERENTIAL DIAGNOSIS

Superficially resembles *M. basilaris*, but the male of *M. mazu* differs from it by its proportionally distinctly broader and shorter wings (cf. Fig. 6A, B and Supporting Information: S3).

In males there are also minor differences in the colour pattern. In *M. mazu* the scape and pedicel of antennae have pale yellowish markings, but are all black in *M. basilaris*. The yellow markings on the underside of the synthorax are more extensive in *M. mazu*. No clear distinguishing characters to separate females of these two species have been found.

Matrona (Matrona) japonica Förster, 1897
de Selys Longchamps, 1888: Lii. – ‘Le ♂ unique Loo-Choo . . . une race peu distincte de la *basilaris* . . .’.

Förster, 1897: 208. – *Neurobasis (Matrona) basilaris* Selys, Sous-race *japonica* Foerster; description of both sexes from ‘Japon’ based on one male and female specimen. Type series originates from Okinawa Islands

(Asahina, 1962b). The male syntype is deposited at UMMZ (Ann Arbor); the whereabouts of the female syntype is unknown (Garrison, von Ellenrieder & O’Brien, 2003).

In all references up to 2011 (in Japanese literature up to 2012) this taxon has traditionally been ranked as subspecies *M. basilaris japonica*.

Hämäläinen *et al.*, 2011: 20. – Ranked as a good species.

Ozono *et al.*, 2012: 48. – Ranked as a good species.

SYNONYM

Calopteryx okinawana Matsumura, 1931, junior synonym of *M. japonica* Förster, 1897.

Matsumura, 1931: 1454. – *Calopteryx okinawana* Mats.; description of female sex from Okinawa.

Asahina, 1989: 17. – listed as a synonym of *M. basilaris japonica* without details.

Hämäläinen, 2000: 83. – ranked as a synonym of *M. basilaris japonica*.

Distribution: Confined to Okinawa and Amami Islands in Japan.

Matrona (Matrona) annina Zhang & Hämäläinen, 2012

Zhang & Hämäläinen, 2012: 286. – *Matrona annina* sp. nov.; description of both sexes from Guangdong, China. Type locality: Shimentai Nature Reserve (in Qingyuan City). Holotype deposited at RMNH (Leiden).

Distribution: Known only from Guangdong and Guangxi in China. A rare species.

Note: This species has also been found co-occurring with *M. basilaris* in the same streams in Guangdong. However, the two species seem to prefer different microhabitats and behave differently (Zhang & Hämäläinen, 2012). Although the genetic distance between *annina* and mainland *basilaris* is rather small

(cf. Supporting Information: S2), *annina* deserves to be ranked as a good species for morphological and other reasons.

SUBGENUS *DIVORTIA*

Matrona (Divortia) oreades Hämäläinen *et al.*, 2011
Hämäläinen *et al.*, 2011: 21. – *Matrona oreades* Hämäläinen, Yu & Zhang, sp. nov.; description of both sexes from Gansu and Sichuan in China. Type locality: Bikou, Wenxian in Gansu. Holotype deposited at Institute of Entomology, Life Sciences College of Nankai University, Tianjin, China.

Distribution: Known from Gansu, south-east Xizang, Sichuan, Chongqing and Hupei in China.

Matrona (Divortia) corephaea Hämäläinen *et al.*, 2011

Hämäläinen *et al.*, 2011: 25. – *Matrona corephaea* Hämäläinen, Yu & Zhang, sp. nov.; description of both sexes from Zhejiang, Hunan and Guizhou in China. Type locality: West Tianmushan in Zhejiang. Holotype deposited at Institute of Entomology, Life Sciences College of Nankai University, Tianjin, China.

KEY TO THE MATURE MALES OF *MATRONA* SPECIES

1. Wing bases with an area with bluish white veins, which create a milky appearance when viewed from an oblique angle (cf. Fig. 6A, B); *Matrona* (subgen. *Matrona*).....2
- Veins at wing base similarly coloured as rest of wing, usually brown or black, never showing any bluish white (cf. Fig. 6C, D), *Matrona* (subgenus *Divortia*).....7
2. Basic wing colour dark blue opaque.....3
- Basic wing colour brown.....*M. annina*
3. Insular species occurring in islands of South China Sea or adjacent sea area.....4
- Continental species occurring in Asian mainland.....6
4. Underside of wings with bluish white cross-veins almost throughout the whole wing; species occurring in Taiwan.....*M. cyanoptera*
- Underside of wings with bluish white cross-veins only to the level of nodus.....5
5. Species occurring in Hainan.....*M. mazu*
- Species occurring in Okinawa and Amami Islands in Japan.....*M. japonica*
6. Ventral side of abdominal segments 8–10 black; reticulation at hind wing base rather open (cf. Fig. 9B).....*M. nigripictus*
- Ventral side of abdominal segments 8–10 largely yellowish; reticulation at hind wing base rather dense (cf. Fig. 9A).....*M. basilaris*
7. Wings brownish, with tips darkened.....8
- Wings brownish, tips paler than remainder (Fig. 6D).....*M. taoi*
8. Fore and hind wings similarly coloured (Fig. 6C); in both wings the veins are darker than the wing membrane; two basal segments of antennae wholly pale yellow.....*M. oreades*
- Hind wing darker than fore wing; in hind wing the veins are paler than the wing membrane; two basal segments of antennae black with a small pale dot.....*M. corephaea*

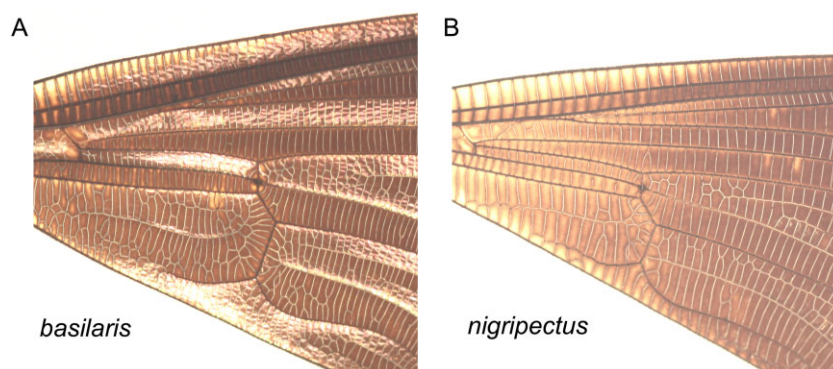


Figure 9. Venation at hind wing base: A, *M. basilaris* male (China, Guangdong, Luofushan); B, *M. nigripictus* male (Thailand, Chiang Mai, Doi Inthanon).

Distribution: Known from Zhejiang, Shaanxi, Hunan, Henan and Guizhou in China.

Matrona (Divortia) taoi Phan & Hämäläinen, 2011
Phan & Hämäläinen, 2011: 63. – *Matrona taoi* Phan & Hämäläinen, sp. nov.; description of both sexes from Phu Tho province in Vietnam. Type locality: Xuan Son National Park (Phu Tho, Vietnam). Holotype deposited at Vietnam National Museum of Nature (VNMN), Hanoi.

Distribution: Known from Quang Binh, Thua Thien Hue and Phu Tho provinces in Vietnam. Note: a long series of specimens were collected by Do Manh Cuong in Quang Binh province in central Vietnam in 2005–2006, earlier than the type series from Phu Tho, collected by Phan Quoc Toan in 2009–2010.

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SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article at the publisher's web-site:

- S1.** List of analysed samples.
- S2.** Estimates of evolutionary divergence over sequence pairs between species with the Kimura two-parameter (K2P) substitution model using MEGA 6. Left lower is based on ITS and right upper COI.
- S3.** Statistical analysis of the ratio of wing length to breadth between species.
- S4.** Morphometric analysis report of the 16 landmarks generated by tps-RELW.
- S5.** Phylogenetic reconstruction for 86 specimens from combined COI+ITS data. A, BI tree with posterior probabilities; B, ML tree with bootstrap values.