



## A basal phylogenetic placement for the salticid spider *Eupoa*, with descriptions of two new species (Araneae: Salticidae)

WAYNE P. MADDISON<sup>1</sup>, J.X. ZHANG<sup>2</sup>, & MELISSA R. BODNER<sup>3</sup>

<sup>1</sup>Departments of Zoology and Botany and Centre for Biodiversity Research, University of British Columbia, 6270 University Boulevard, Vancouver, British Columbia, V6T 1Z4, Canada, and Wissenschaftskolleg zu Berlin, Wallotstraße 19, Berlin, 14193, Germany.

E-mail: wmaddisn@interchange.ubc.ca

<sup>2</sup>Department of Zoology, University of British Columbia, 6270 University Boulevard, Vancouver, British Columbia, V6T 1Z4, Canada.

E-mail: jxzhang@interchange.ubc.ca

<sup>3</sup>Department of Zoology, University of British Columbia, 6270 University Boulevard, Vancouver, British Columbia, V6T 1Z4, Canada.

E-mail: mbodner9@interchange.ubc.ca

### Abstract

The southeast Asian genus *Eupoa* includes small salticid spiders with unusual palpi. Two new species of the genus are described. Two morphological characters (presence of a median apophysis in the male palp and of a tarsal claw in the female palp) suggest it is excluded from the main clade of salticids (the Salticoida). Sequences of nuclear and mitochondrial gene regions (28S, 18S, 16S–ND1, CO1), analyzed by parsimony and Bayesian methods, agree that *Eupoa* is a basal (non-salticoid) salticid, but fail to find a clear placement. *Eupoa* may represent a deep-branching lineage long separate from the lyssomanines, spartaeines, and other basal groups.

**Key words:** Araneae, Salticidae, *Eupoa*, lapsiines, Spartaeinae, Lyssomaninae, Hispaninae, jumping spider, basal groups, phylogeny

### Introduction

Recent work on phylogeny of salticid spiders has shown that most species of this large group (> 5000 species) fall within a single large clade, the Salticoida (Wanless 1980, 1982, 1984; Rodrigo & Jackson 1992; Maddison 1988, 1996; Wijesinghe 1992, 1997; Maddison & Hedin 2003; Maddison & Needham 2006). The "basal" groups outside of the Salticoida are of particular value for interpreting the early evolution of salticids (Jackson & Pollard 1996; Blest *et al.* 1990; Maddison & Needham 2006). However, relatively few species (ca. 260 by Platnick 2006) of these basal groups survive. Long known to be basal are the lyssomanines, spartaeines, and the *Cocalodes* group (Wanless 1980, 1982, 1984; Rodrigo & Jackson 1992; Wijesinghe 1992, 1997), while recently demonstrated as basal are the lapsiines and hisponines (Maddison & Needham 2006). Here we present data from recently collected material showing that a little-known salticid, *Eupoa* Zabka, is also among the basal groups. Because of its atypical size and ecology, *Eupoa* will provide an interesting challenge for any interpretation of ancestral salticid behaviour.

### Material and methods

**Morphology.** Specimens were examined under both dissecting microscopes and a compound microscope with reflected light. Drawings were made with a drawing tube on a Nikon ME600L compound microscope.

Terminology is standard for Araneae. All measurements are given in millimeters. Carapace length was measured from the base of the anterior median eyes not including the lenses to the rear margin of the carapace medially. The following abbreviations are used: ALE, anterior lateral eyes; AME, anterior median eyes; PLE, posterior lateral eyes; PME, posterior median eyes (the "small eyes").

Specimens are deposited in the Spencer Entomological Museum of the University of British Columbia (UBC-SEM, K. Needham).

**Sampling and Sequencing.** DNA sequences were obtained from 2 specimens of *Eupoia nezha* Maddison & Zhang new species, and specimens of 17 other salticid species and three outgroup species. Specimens were preserved in 95% or 100% ethanol. Because of its small size, an entire male of *E. nezha* was used for DNA extraction, except its palpi, which were saved as a voucher (voucher #d220). The entire abdomen of a second male (voucher #MRB102) was also used. The other specimens sequenced are listed in Table 1, which also includes authors of species names. Voucher specimens are stored in the Spencer Entomological Museum, UBC, accompanied by labels with their voucher numbers (as indicated in the species descriptions); the label is marked "WPM voucher DNA" or "DNA voucher MRB". Genomic DNAs were extracted from tissues using the QIAGEN DNeasy extraction kit or using the Puregene Genomic DNA Purification Kit (Gentra Systems).

**TABLE 1.** Specimens from which 28S sequences were obtained. Locality data for same specimens given by Maddison & Needham (2006). These represent extended sequences (from ZX1 primer to C primer) compared to those used by Maddison & Needham (2006). The *Ghelna canadensis* specimen had been misidentified by Maddison & Needham (2006) as *G. castanea*.

Species	Voucher number	Bases	Genbank
<i>Dendryphantès hastatus</i> Clerck	d043	1046	EF201646
<i>Euophrys monadnock</i> Emerton	d029	1024	EF201647
<i>Galianora bryicola</i> Maddison	d124	1100	EF201650
<i>Galianora sacha</i> Maddison	d116	1059	EF201649
<i>Ghelna canadensis</i> C.L. Koch	d005	1083	EF201651
<i>Goleba lyra</i> Maddison & Zhang	d051	1086	EF201652
<i>Helophanus cupreus</i> Walckenaer	d044	981	EF201653
<i>Holcolaetis</i> sp.	d036	1014	EF201654
<i>Massagris schisma</i> Maddison & Zhang	d081	1086	EF201655
<i>Massagris</i> cf. <i>honesta</i> Wesolowska	d082	1019	EF201656
<i>Pellenes peninsularis</i> Emerton	d057	1053	EF201657
<i>Portia</i> cf. <i>schultzi</i> Karsch	d131	983	EF201658
<i>Salticus scenicus</i> Clerck	d003	1061	EF201659
<i>Sitticus palustris</i> Peckham and Peckham	d030	1068	EF201660
<i>Thrandina parocula</i> Maddison	d123	1102	EF201661
<i>Tomocyrrba andasibe</i> Maddison & Zhang	d127	1078	EF201662
<i>Cesonia</i> sp.	S319	1086	EF201663
<i>Cheiracanthium</i> sp.	S321	1029	EF201664
<i>Xysticus</i> sp.	S316	1084	EF201665

Four gene regions (nuclear 28S and 18S and mitochondrial 16S–ND1 and CO1) were amplified by PCR and sequenced from *Eupoia nezha*; only the 28S region was amplified from the other species. We used the same protocol as Maddison and Needham (2006), except for a few minor deviations. For all of the species sequenced we used a different forward primer (ZX1 from Mallatt & Sullivan 1998) paired with 28SC to yield a larger piece of 28S. Because we now have a longer piece, we present new 28S sequences for the 20 species

apart from *Eupoa*. This sequencing also allowed resolution of a few ambiguities in Maddison & Needham's sequences near the 28SO primer. In addition to these protocol changes, to amplify the 28S gene region from *Eupoa* we used "Buffer J" from the MasterAmp PCR Optimization Kit (Epicentre Technologies) and three new 28S reverse primers in addition to 28SC: 28S-WMD (5'-TTGGTCCGTGTTTCAAGACGG-3'); 28S-WME (5'-CATAGTTCACCATCTTTCGG-3'); 28S-WMG (5'-ATCTGACGATCGATTTGCAC-3').

Sequencing from the PCR products was done by MacroGen, Inc. (ABI 3730 sequencer). The primers used in PCR were also used for sequencing. 28S proved particularly difficult to sequence in *Eupoa*, and as a result we used additional internal primers to aid with sequencing. The 28S sequence presented is obtained from reads using the forward primers 28S-ZX1, 28SO, 28S-ZR1 (Mallatt & Sullivan 1998), 28S-ZR3 (Mallatt & Sullivan 1998), and the reverse primers 28SC, 28S-WMD, 28S-WME, and 28S-WMG. Most base calls were confirmed by reads in both directions. Because we exhausted the extracted DNA from our first specimen (voucher #d220), we used a second (#MRB102) for additional sequencing attempts for 28S. The 28S sequences presented for *Eupoa* are thus composed of reads from both specimens. In the extensive regions of overlap no differences between the specimens were found.

Sequences were obtained from the chromatogram files using Phred (Ewing & Green 1998; Ewing *et al.* 1998; Green & Ewing 2002) and Phrap (Green 1999) as operated via the chromaseq package (D. Maddison & W. Maddison in prep.) for Mesquite (Maddison & Maddison 2006), following the procedures used by Maddison and Needham (2006). Final sequences were submitted to GenBank. Accession numbers for the *Eupoa* sequences are given in Results; those for species other than *Eupoa* in Table 1.

For phylogenetic analyses, the sequences of *Eupoa* were added to the matrices used by Maddison and Needham (2006). The one exception is for 28S, where we replaced their sequences for our longer sequences indicated in Table 1. Most of our analyses were done with Maddison & Needham's small taxon sample matrices (6 to 9 salticoid genera, 10–13 basal salticoid genera, four non-salticoid dionychans) supplemented with *Eupoa*. In addition, two analyses were done with their large taxon sample matrix (with considerably more salticoids) supplemented with *Eupoa*.

Sequences for each gene region were re-aligned with *Eupoa* included, using ClustalX (Thompson *et al.* 1997) following the methods described by Maddison and Needham (2006), with one modification. One analysis used amino acid sequences for CO1 and ND1 (inferred from the nucleotide sequence using the standard invertebrate mitochondrial genetic code, using MacClade, Maddison & Maddison 2005). To generate the amino acid sequence of ND1 from the nucleotide sequences we had to realign the start of ND1 because the automatic alignment had dissociated the first few codons in some sequences. While we realigned to get the amino acid sequence, we did not use this realignment in the nucleotide analyses, because our hand-realignment left the adjacent noncoding region with arbitrary and non-optimized gaps. For the nucleotide analyses we felt it better to use the objectively optimized alignment even though it was biologically problematical in dissociating a few codons. The aligned all-genes matrix used in the Bayesian analysis for Figure 15 is deposited in TreeBASE (treebase.org).

**Phylogenetic analysis.** Phylogenetic analyses were performed on the gene regions separately and combined, and using parsimony and Bayesian methods.

Bayesian analyses were done using MrBayes 3.1.2 (Huelsenbeck & Ronquist 2001; Ronquist & Huelsenbeck 2003). The GTR invariant-gamma model was used throughout (nst=6 rates=invgamma). Models were permitted to vary among data partitions, with the partitions defined as follows: 28S; 18S; 16S; ND1 first, second, and third codon positions; CO1 first, second, and third codon positions. When ND1 and CO1 were included together in an analysis, they were united to yield 3 partitions (ND1+CO1 first, second, and third codon positions) instead of 6. Each analysis was run for 10 million generations via the command "mcmc ngen= 10000000 printfreq=1000 samplefreq=1000 nchains=4 savebrlens=yes;". Results are summarized by a majority rules consensus tree of sampled trees, having discarded generations up to 100,000 (by which point the posterior probabilities had stabilized).

Parsimony analyses were done using PAUP\* (Swofford, 2002), treating character states as unordered (one step for any state change). Gaps were treated as missing data. Analyses followed the methods of Maddison and Needham (2006), with small taxon sample analyses based on 5,000 random addition sequence replicates, and large sample analysis based on 20,000.

## Descriptions of new species

*Eupoa* was described by Zabka (1985) for a species from Vietnam; a second species has been described by Peng & Li (2006). Although figures of three other species are available, there is doubt as to whether their descriptions have been validly published (Platnick 2006). Both of the new species described are closely similar to *E. prima* Zabka in genital morphology; neither matches *E. liaoi* Peng & Li or the three doubtfully published species.

### *Eupoa nezha* Maddison & Zhang, new species

Figs 1–7

**Type material:** Holotype male in UBC-SEM from CHINA: *Guangxi*: Pingxiang City, Daqingshan Park. N 22°07'18" E 106°43'59", 247m, 14 May 2006. J.X. Zhang, M.S. Zhu, W.G. Lian, H.Q. Ma (JXZ06#004).

Paratypes: 3 males, 1 female, one juvenile, same data as holotype. Two of the paratype males were used for DNA extraction. Of one (WPM DNA Voucher d220), the entire body and appendages were used except for the palpi; of the other (DNA Voucher MRB102) the abdomen was used.

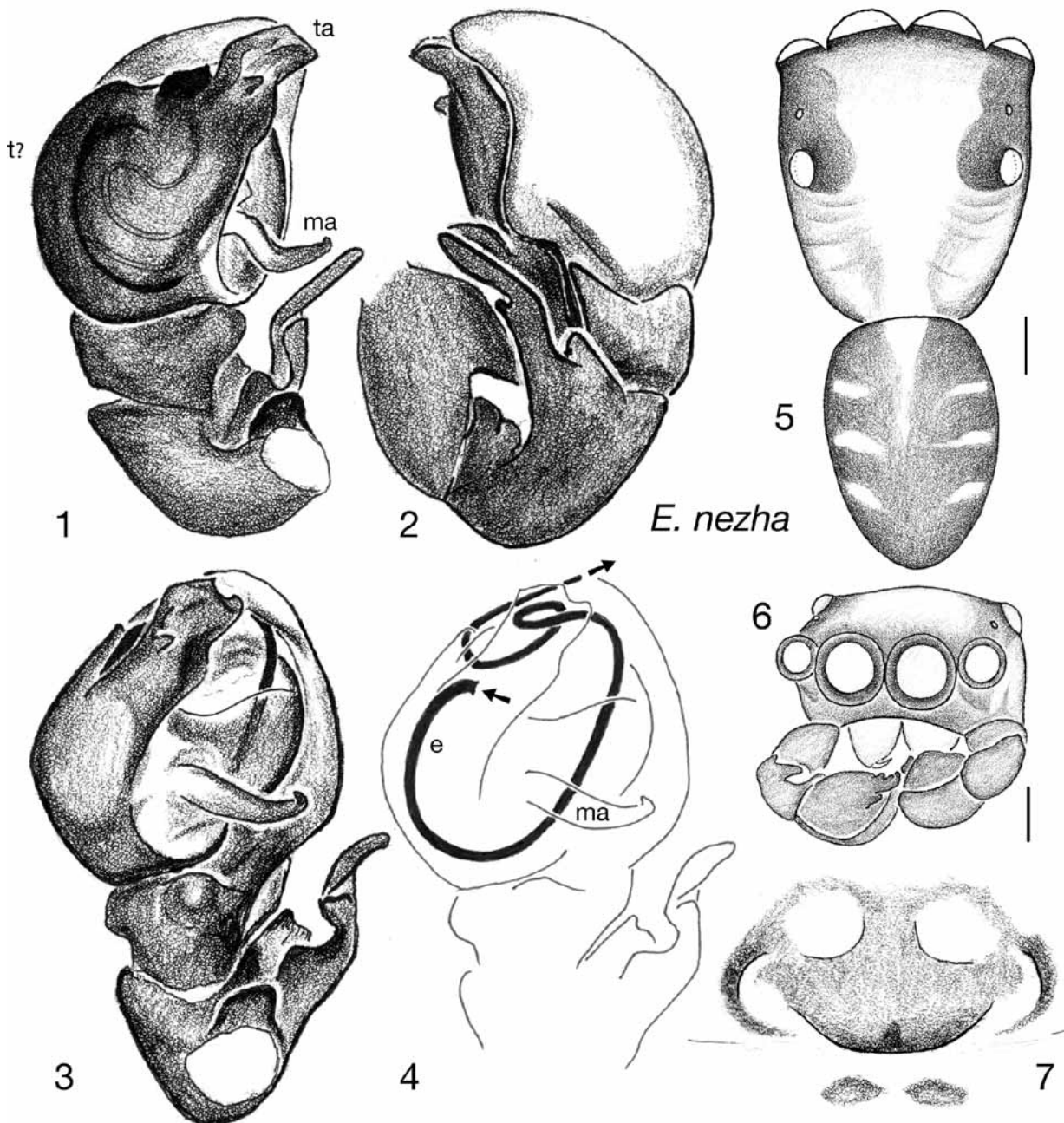
**Etymology.** This species and *E. jingwei*, small and mysterious forest-dwellers, are named after spirit creatures in Chinese mythology. Nezha fought the son of the dragon king, and was later reborn from a water lily as a powerful spirit.

**Diagnosis.** Distinguished from *Eupoa prima* and *E. jingwei* by genitalic characters. Male palp patellar apophysis more folded than in *E. prima*, and more delicate than in *E. jingwei*. Tip of tegular apophysis not as bifurcate as in *E. prima*, and shorter than in *E. jingwei*. In epigynum, shield behind openings smaller compared to openings than in *E. prima* and *E. jingwei*.

**Description. Male:** Total length 1.5–1.6. Holotype total length 1.5; carapace 0.9 long, 0.7 wide, 0.4 high; abdomen 0.7 long, 0.6 wide. Carapace (Fig. 5) yellow brown with gray brown lateral margins; eyes with dark surroundings; eye region gray brown. Clypeus (Fig. 6) gray brown with only a few long hairs. Eye sizes: AME 0.24, ALE 0.16, PME 0.03, PLE 0.14. First eye row 0.80 wide, second eye row 0.60 wide, third eye row 0.68 wide, eye area 0.50 long. Clypeus height 0.06. Fovea absent. Chelicerae small and yellow; promargin with two teeth; retromargin with three teeth and three denticles; both margins with long setae. Endites, labium and sternum yellow with gray pigments. Legs gray with yellow longitudinal stripes. Metatarsus I with three pairs of ventral spines, and tibia I without ventral spines. Measurements of legs: I 1.4, II 1.3, III 1.3, IV 1.9. Leg formula: 412=3. Abdomen (Fig. 5) with dorsal scutum, dorsum dark and venter yellow. One male has paired yellow patches on dorsum as in female. Palp (Figs 1–4): complex and difficult to interpret. A large sclerite on the prolateral ventral side, which appears to be the tegulum, bears a broad projection distally that is not the embolus; we will refer to it as the tegular apophysis (Fig. 1, ta). Rather, the embolus (Fig. 4) begins hidden behind the tegulum just prolateral to the aforementioned projection, then proceeds proximally beneath the tegulum, then curls up the prolateral side, becoming externally visible for a short length (Fig. 3) before curling in the distal portion of the palp. The path of the embolus is shown in Fig. 4. A prominent fingerlike sclerite projects retrolaterally from a membranous region retrolateral to the tegulum (Figs 1, 3, 4). This is presumed to be the median apophysis (Figs 1, 4, ma). The tibia has a modest blunt retrolateral apophysis. The

dominant apophysis is a retrolateral patellar apophysis (Figs 2, 3), which is twisted and folded.

**Female:** Total length 1.8; carapace 0.9 long, 0.8 wide, 0.5 high; abdomen 0.9 long, 0.8 wide. Carapace coloration and patches similar to those of male. Eye sizes: AME 0.24, ALE 0.16, PME 0.04, PLE 0.14. First eye row 0.86 wide, second eye row 0.70 wide, third eye row 0.76 wide, eye area 0.52 long. Clypeus height 0.06. Measurements of legs: I 1.6, II 1.3, III 1.4, IV 2.0. Leg formula: 4132. Abdomen gray brown dorsally with paired yellow patches, front margin with a few long black setae, venter yellow. Epigynum (Fig. 7): Difficult to interpret, with two circular less sclerotized areas anteriorly that we presume include the openings. Behind them is a shield that overlaps the epigastric furrow.



**FIGURES 1–7.** *Eupoa nezha* Maddison & Zhang, new species. 1–4 Palp of male holotype (1 ventral-prolateral view, 2 retrolateral, 3 ventral-retrolateral, 4 view as Figure 3, but highlighting path of embolus. Arrows show exit path of sperm, first into embolus then out of embolus opening); 5–6 Male paratype; 7 epigynum of paratype female, ventral view. e = embolus; ma = median apophysis; t? = tegulum?; ta = tegular apophysis. Scale bars 0.2mm.

*Eupoa jingwei* Maddison & Zhang, new species

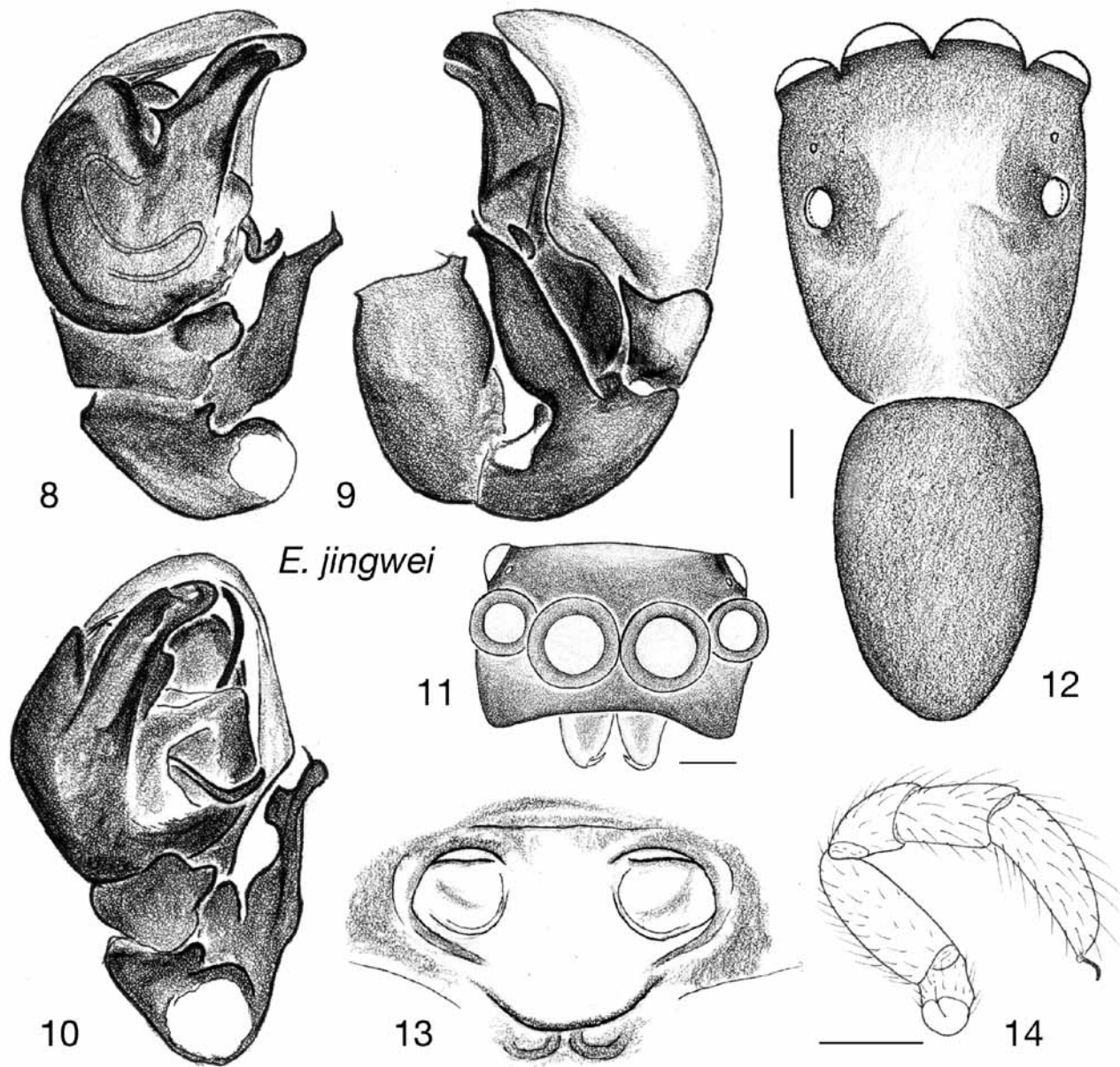
Figs 8–14

**Type material:** Holotype male in UBC-SEM from **CHINA: Guangxi:** Pingxiang City, Daqingshan Park. N 22°07'18" E 106°43'59", 247m, 14 May 2006. J.X. Zhang, M.S. Zhu, W.G. Lian, H.Q. Ma, (JXZ06#004).

Paratypes: 1 male and 1 female, same data as holotype.

**Etymology.** In an old Chinese story, the emperor's daughter Jingwei died at sea to be reborn into a bird who, in revenge, attempted to fill the sea with pebbles.

**Diagnosis.** Differs from *E. prima* and *E. nezha* in the robust and relatively straight patellar apophysis in the male palp. The median apophysis is relatively smaller than in those two species. The female differs from the other species in details of the epigynum.



**FIGURES 8–14.** *Eupoa jingwei* Maddison & Zhang, new species. 8–10 Palp of male holotype (8 ventral-prolateral view, 9 retrolateral, 10 ventral-retrolateral); 11–12 Male holotype; 13 epigynum of female paratype, ventral view; 14 palpus of female paratype. Scale bars 0.2mm.

**Description. Male:** Total length 1.8–1.9. Holotype total length 1.8: carapace 1.0 long, 0.8 wide, 0.5 high; abdomen 0.9 long, 0.6 wide. Carapace (Fig. 12) dark brown with eye region dark, and a triangular yellow brown patch from between PLEs to posterior end of carapace. Clypeus (Fig. 11) yellow brown, with lots of long white hairs. Eye sizes: AME 0.26, ALE 0.18, PME 0.04, PLE 0.14. First eye row 0.94 wide, second eye row 0.72 wide, third eye row 0.76 wide, eye area 0.50 long. Clypeus height 0.12. Fovea absent. Chelicerae small and yellow, with front face gray, promargin with two teeth, retromargin with three teeth and two denticles, both margins with long setae. Endites and labium yellow brown. Sternum gray brown with many yellow speckles. Femora, patellae and tibiae gray, with yellow longitudinal stripes. Other segments yellow brown, with gray pigments. Metatarsus I with three pairs of ventral spines and tibia I with two pairs of ventral spines. Measurements of legs: I 1.8, II 1.6, III 1.5, IV 2.2. Leg formula: 4123. Abdomen (Fig. 12) dark with dorsal scutum, front margin with a few long setae, venter with a yellow longitudinal band behind genital groove. Palp (Figs 8–10): Similar in structure to that of *E. nezha*, although the tegular apophysis is relatively longer, the median apophysis smaller, and the patellar apophysis more robust.

**Female:** Total length 2.5; carapace 1.2 long, 1.0 wide, 0.6 high; abdomen 1.2 long, 1.1 wide. Carapace and abdomen coloration and patches similar to those of male. Clypeus dark brown with yellow brown patches. Eye sizes: AME 0.28, ALE 0.20, PME 0.04, PLE 0.14. First eye row 0.96 wide, second eye row 0.74 wide, third eye row 0.86 wide, eye area 0.58 long. Clypeus height 0.12. Measurements of legs: I 1.8, II 1.5, III 1.6, IV 2.4. Leg formula: 4132. Epigynum (Fig. 13): Difficult to interpret, with two circular areas that we presume include the openings, lying anterior to a shield that overlaps the epigastric furrow.

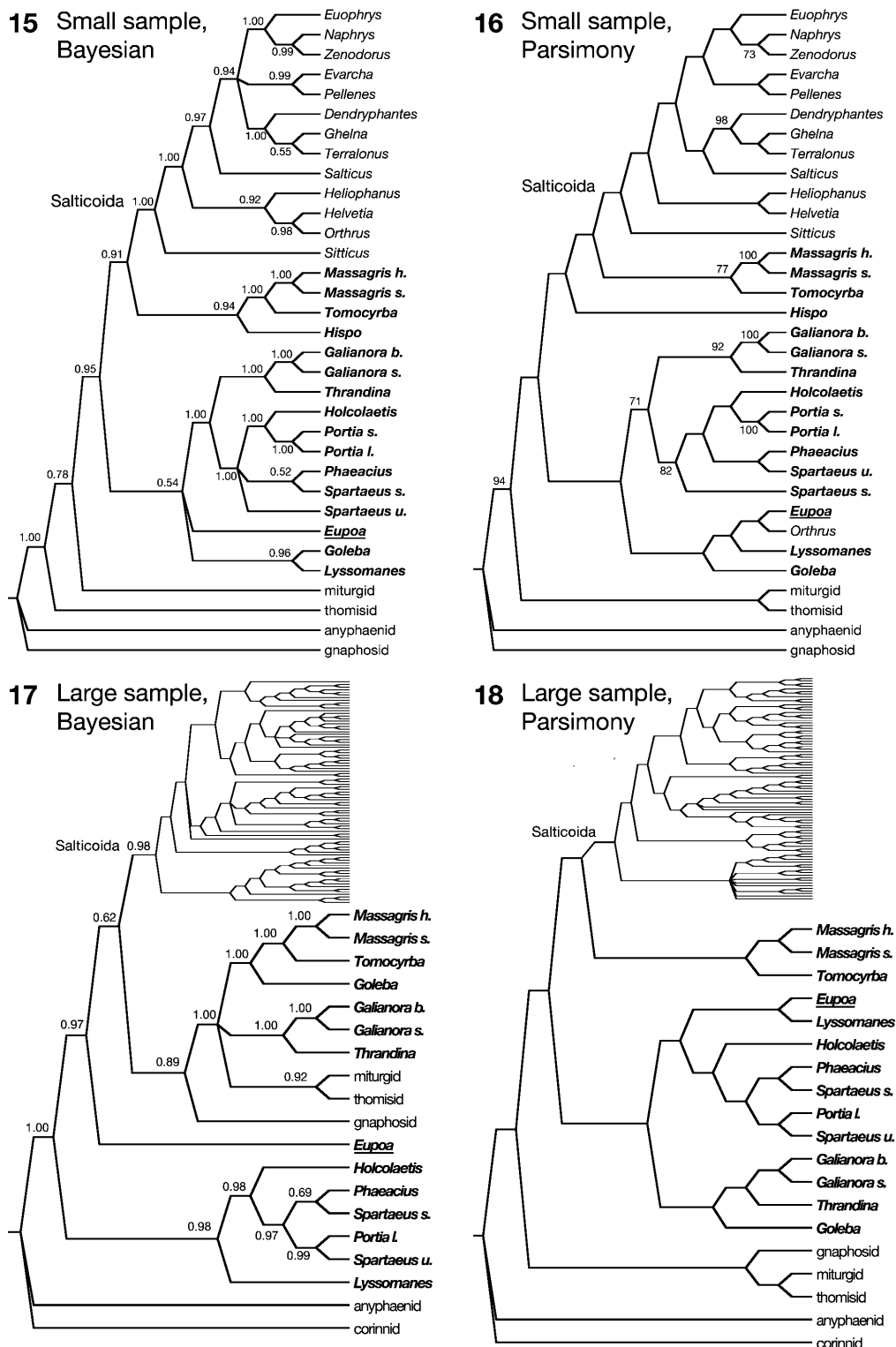
## Phylogenetic placement

**Morphology.** Two aspects of *Eupoa* morphology suggest strongly that they are excluded from the Salticoida. First, the palp has a distinct fingerlike sclerite arising from a membranous area in the retrolateral region of the tegulum (Figs 1 ma, 3, 4 ma, 9). This sclerite therefore appears to be the median apophysis, as seen in basal salticids such as lapsiines, hisponines and the *Cocalodes* group (Maddison & Needham, 2006). The loss of the median apophysis characterizes the Salticoida (Maddison & Needham, 2006). Second, the female palp of *Eupoa* has a distinct tarsal claw (Fig. 14; observed in both *E. jingwei* and *E. nezha*), which also is absent in the Salticoida.

**Molecular data.** Sequences for species other than *Eupoa* were obtained as listed in Table 1. Sequences were obtained from *Eupoa nezha* for 28S (960 bases from vouchers #d220 and MRB102, Genbank EF201648), 18S (1201 bases from voucher #d220, Genbank EF201666), 16S-ND1 (865 bases from voucher #d220, Genbank EF201667), and CO1 (959 bases from voucher #d220, Genbank EF201668). The sequence of 28S as noted above was difficult to obtain. Three short regions of 46, 17, and ca. 20 base pairs were quite difficult to read. Ambiguous reads for the first two of these were left in the sequence for alignment so that the alignment routine did not have to account for these with gaps, but after alignment they were converted to '?'s for analysis. The third region was so ambiguous that it was cut out before alignment. Thus, the 28S sequence of *Eupoa* is incomplete in having these three regions missing. The sequence of 18S is also incomplete as it is missing part of the 5' region.

The results of the phylogenetic analyses are summarized in Figures 15–18, which combine all genes in Bayesian and parsimony analyses. In each case *Eupoa* is well supported as being outside of the Salticoida — the estimated posterior probabilities for the salticoid node (excluding *Eupoa*) are 1.0 for the small taxon sample analysis, 0.98 for the large taxon sample analysis. *Eupoa*, however, does not fall in a consistent place. In the small taxon sample Bayesian analysis *Eupoa* is weakly supported as near the lapsiine+spartaeine clade, but by parsimony it falls weakly with the lyssomanines. In the large taxon sample Bayesian analysis *Eupoa* branches as a deep independent lineage, but by parsimony it is sister to *Lyssomanes*. There are some likely

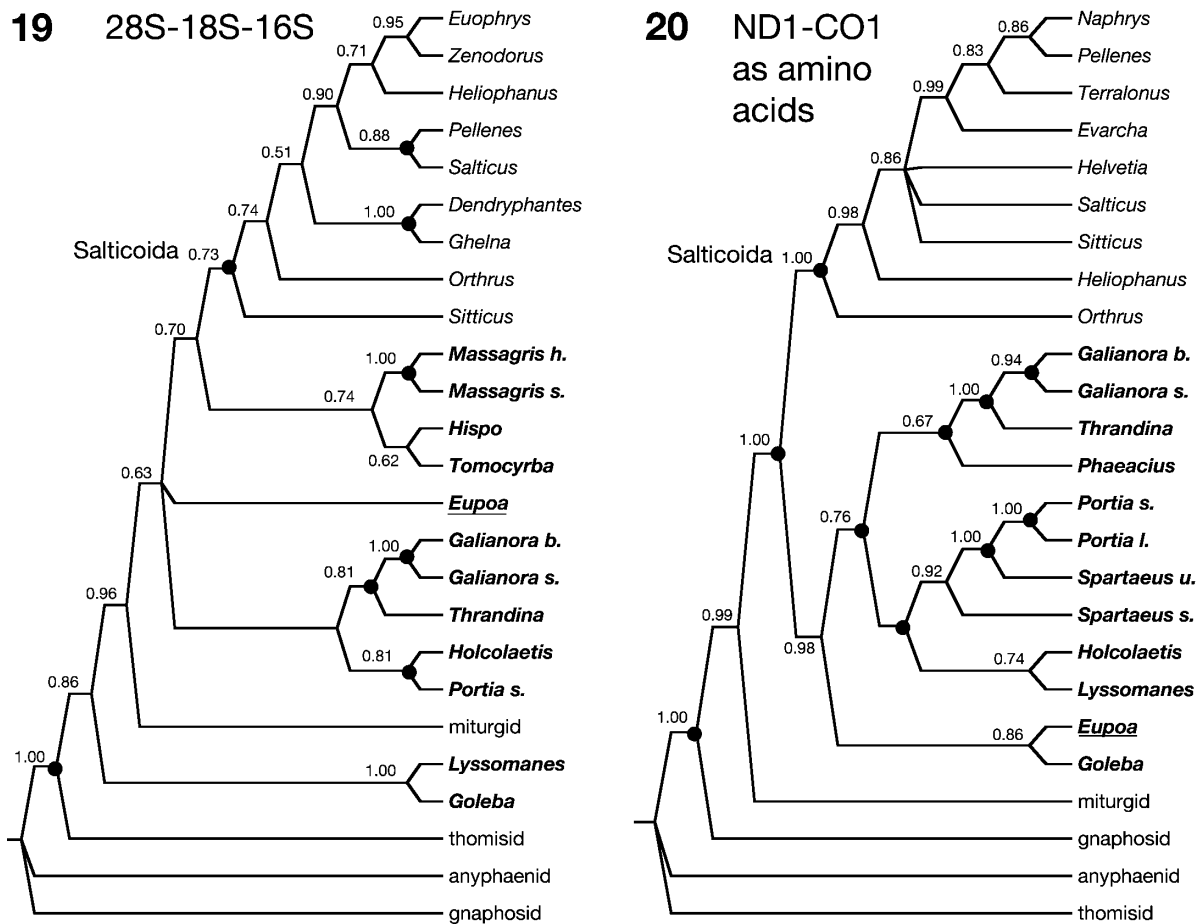
errors in these trees, such as the placement of the salticoid *Orthrus* with *Eupoa* in the small sample parsimony analysis (Fig. 16) and the non-monophyly of salticids in the large sample Bayesian analysis (Fig. 17).



**FIGURES 15–18.** Analyses using all genes for both small sample of salticoids (28S + 18S + 16S-ND1 + CO1) and large sample (28S + 16S-ND1 + CO1). The salticoid taxa in the large sample are unnamed; they are those used by Maddison & Needham (2006). 15 Small sample, majority rule consensus tree of 9900 trees sampled from 10 million generation Bayesian analysis; shown are estimated posterior probabilities; 16 Small sample, single most parsimonious tree found (tree-length 7375 steps); shown are bootstrap values (Felsenstein 1985), 1000 replicates; 17 Large sample, majority rule consensus tree of 9900 trees sampled from 10 million generation Bayesian analysis, with estimated posterior probabilities; 18 Large sample, strict consensus of 2 most parsimonious trees found (treelength 22326 steps).

The individual gene regions analyzed separately provide independent support for the basal position of *Eupoa*. Figures 19 and 20 show analyses with data broken into two independent partitions: non-coding and protein coding regions. The non-coding regions place *Eupoa* as branching deep and outside of the Salticoida (Fig. 19), while the coding regions place it as sister to *Goleba* (Fig. 20). We also ran analyses of each sequenced gene region separately for the small taxon sample: *Eupoa* is placed outside of the Salticoida by Bayesian and parsimony analyses of 28S, 16SND1, and CO1 separately, although there are some misplaced taxa (gnaphosid next to *Sitticus* by 28S Bayesian and parsimony; *Helvetia* basal by 16SND1 Bayesian; *Orthrhus* basal by CO1 parsimony). 18S placed *Eupoa* as a salticoid, but for that gene the sequence is incomplete in *Eupoa*.

At present we can conclude that *Eupoa* is a basal salticid (i.e. not a salticoid), but we cannot determine its relationships. *Eupoa* falls outside the spartaeines by both the molecular data (Figs 15–18) and morphological data (in having a median apophysis and lacking a recognized tegular furrow). *Eupoa*'s sequences in general are quite divergent from those of other salticids, and we would not be surprised if it has a very deep divergence from other salticids.



**FIGURES 19–20.** Results from analyses on separate data partitions. Each is the majority rule consensus tree from sampled trees from Bayesian analysis, with posterior probabilities at nodes. Filled spots on nodes indicate clade was recovered also in parsimony analysis. 19 Ribosomal genes 28S, 18S and 16S plus the non-coding region between 16S and ND1; 20 Mitochondrial protein coding genes ND1 and CO1, analyzed after translating to amino acids.

Our interpretations of early evolution of salticids (e.g., Jackson & Pollard 1996, Blest *et al.* 1990) depend on observations of basal salticids. Finding any new deeply branching lineage may influence these interpretations, but *Eupoa* may provide an especially important lineage in this respect. If our sample of surviving basal salticids is poor, then we may mistakenly associate with their early evolution a common trait that in fact is a

more recent derivation associated with a uniform ecology. There is reason to believe that *Eupoia* has a distinctive ecology, and thus would provide a strong test of what traits are ancestral. *Eupoia* is a minute leaf-litter dweller (Zabka, 1985; J.X. Zhang, personal observations). This is unlike other known basal salticids, which are larger salticids and typically live in foliage, on tree trunks, or on rocks. It will be particularly interesting to learn more about *Eupoia* diversity, ecology and phylogeny to understand what insights it may give into the family's early evolution.

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