

Phylogeny and historical biogeography of Silky Lacewings (Neuroptera: Psychopsidae)

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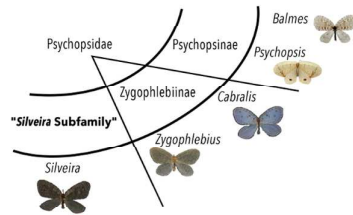
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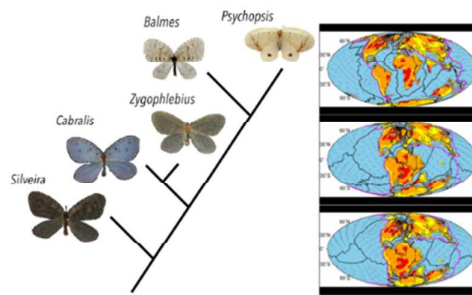
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Graphical Abstract

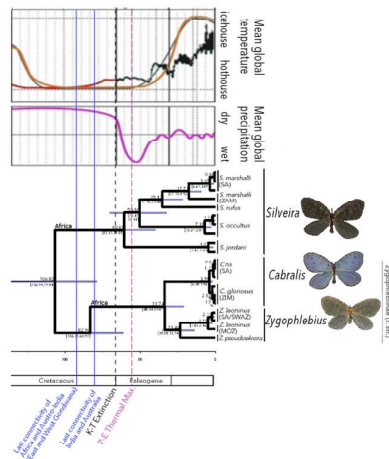
Highlight 1: Afrotropical *Silveira* represent an entirely unique lineage that warrants placement in its own subfamily.



Highlight 2: Current worldwide distribution resulted from vicariance events associated with Gondwanaland breakup, wherein India moved extant *Balmes* to their present day location in south-east Asia.



Highlight 3: Divergences between Afrotropical species are hypothesized as driven by adaptations to either arid or moist climates and associated habitat types.



Abstract

Psychopsidae (silky winged lacewings) are a small family of Neuroptera characterised by broad hirsute wings that impart a physical resemblance to moths. The fossil record includes many psychopsid-like taxa from the Late Triassic to Early Oligocene from all major continents. Extant species have a disjunct, tripartite distribution comprising Afrotropical, south-east Asian and Australian regions that is significant to historical biogeography. Two subfamilies are currently recognized: Zygophlebiinae in the Afrotropics, and Psychopsinae in Australia and south-east Asia. This study explores phylogeny and historical biogeography of Psychopsidae, using data from biogeography, comparative morphology and molecular sequences (16S, 18S, CAD, COI). Our results show that (1) the morphological phylogeny is incongruent with molecular data, (2) Afrotropical *Silveira* Navás, represent a separate lineage that warrants placement in its own subfamily, (3) the family originated in Pangea, and (4) the present genus level distribution resulted from two vicariance events associated with Gondwanan fragmentation.

Key words: Afrotropical, Lacewing, Phylogenetics, Ancestral area, Molecular phylogeny, Morphology, Neuroptera.

Introduction

Psychopsidae Handlirsch (Neuroptera) are a small family of lacewings, comprising about 26 extant species in five genera worldwide (Oswald, 1993). Members are characterized by broad moth-like wings with a parallel triplet-vein formation comprising the subcostal, radial and radial sector veins collectively known as the *vena triplica*. Extant species manifest a disjunct, tripartite distribution between the Afrotropical, Australian and south-east Asian regions. The Afrotropical fauna (Figs 1A, B, C, D, G, H) comprises three genera: *Cabralis* Navás; *Silveira* Navás and *Zygophlebius* Navás in Zygophlebiinae Navás, while Australian and south-east Asian fauna have only one genus each: *Psychopsis* Newman in Australia and *Balmes* Navás in south-east Asia, both in Psychopsinae Handlirsch. Larvae have been found in leaf debris surrounding trees, and two Australian species are known to live under bark of myrtaceous trees, preying on Microlepidoptera (Tillyard, 1919b; Tjeder, 1960). Adult females have a specialized oviposition strategy that involves grinding a substrate of sand or plant material with the toothed side of the gonocoxite stylus. The substrate is then transferred to the abdominal chamber and used to coat eggs, probably for camouflage and minimizing desiccation. Eggs are laid singly during flight (Tjeder, 1960; New, 1988; Oswald, 1993).

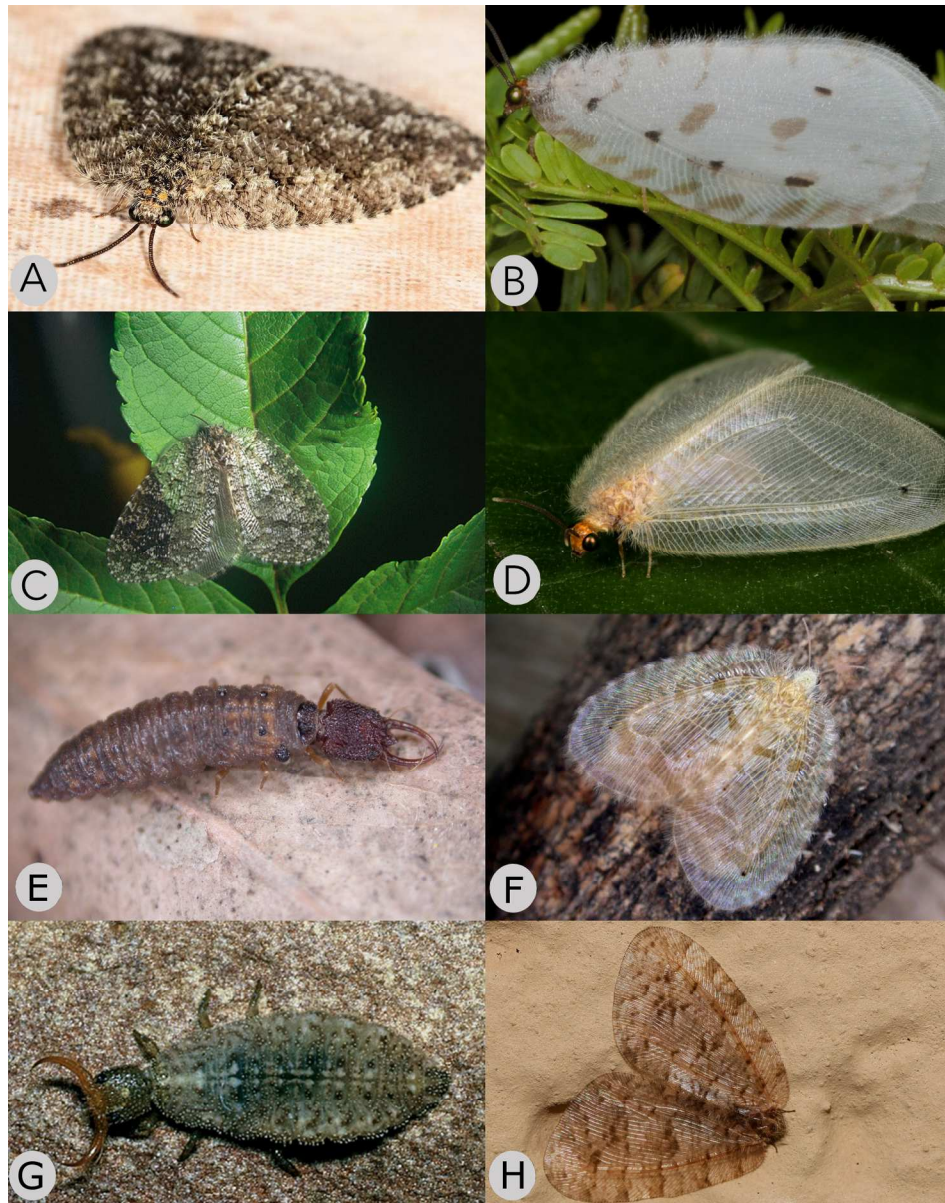


Figure 1. Diversity of Psychopsidae. A) *Silveira marshalli* (McLachlan, 1902) (Photo: Wynand Uys). B) *Cabralis cns* (Photo: Hennie de Klerk). C) *Silveira occultus* Tjeder, 1960 (Photo: Peter Duelli). D) *Zygophlebius leoninus* Navás, 1910 (Photo: Wynand Uys). E) *Psychopsis* sp. Larva (Photo: Shaun Winterton). F) *Psychopsis insolens* (Photo: Shaun Winterton). G) *Cabralis cns* larva (Photo: Peter Duelli). H) *Silveira rufus* (Photo: Penni Warncke).

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Fossil Psychopsidae manifest considerable disparity across a rich fossil record. The Kalligrammatidae Handlirsch, Aetheogrammatidae Ren & Engel and Osmylopsychopidae Martynova (syn. Brongniartiellidae Martynova; see Peng *et al.* 2016) are extinct families that share the closest relationship with extant Psychopsidae (Fig. 2 and Andersen, 2001). Makarkin (2010) placed these within Psychopsoidea Handlirsch which, in turn, is sister to Myrmeleontiformia (Withycombe, 1925; Mansell, 1992; Haring & Aspöck, 2004; Grimaldi & Engel, 2005; Beutel & Pohl, 2006; Badano *et al.*, 2016). Psychopsidae (s. str.) comprise fossil Triassopsychopsinae Tillyard along with extant taxa, and are considered a distinct lineage of Psychopsoidea united by a broad costal space (Makarkin, 1997; Andersen, 2001; Lambkin, 2014). Within Psychopsidae (s. str.), extant lineages have an abruptly closing *vena triplica* distally, while extinct *Baisopsychops* Makarkin and *Triassopsychops* Tillyard (Triassopsychopsinae) have this termination gradually tapering (Fig. 2). This supports division between all extant subfamilies, and extinct Triassopsychopsinae (Tillyard, 1922b; Makarkin, 1997; Andersen, 2001). Fossil *Propsychopsis* Krüger are an exception with a tapering *vena triplica* similar to *Triassopsychops*, but with similar adult characters to extant *Balmes* (MacLeod, 1970), and similar larval characters to extant *Psychopsis* (Tillyard, 1919a; MacLeod, 1970). These similarities place *Propsychopsis* near *Balmes* in Psychopsinae (Andersen, 2001).

Psychopsidae have previously been considered close related to, or the sister family of Nemopteridae Burmeister based on assessments of adult and larval morphology (Withycombe 1925; Aspöck *et al.* 2001). Recent studies however, which focused holistically on Neuropterida using molecular and morphological characters, have shown that Psychopsidae are closer to Nymphidae Rambur, placing Psychopsidae as sister to all other Myrmeleontiformia (Winterton *et al.*, 2010; Wang *et al.*, 2016). Extant species are divided into Zygophlebiinae and Psychopsinae based on a cladistic analysis of 60 morphological characters (Oswald, 1993). Psychopsinae comprise Australian and south-east Asian genera that appear closely related despite geographic distance (New, 1988; Oswald, 1993). Zygophlebiinae comprise all Afrotropical genera, with *Cabralis* and *Zygophlebius* closely related, and *Silveira* more plesiomorphic and distinct (Oswald, 1993).

Historical biogeography of extant Psychopsidae was investigated by Oswald (1993) in an area cladogram test (Nelson & Platnick, 1981; Humphries & Parenti, 1986) that was formulated to account for *Balmes* in south-east Asia. This test found most support for an Australian dispersal route based on the close relationship between *Balmes* and *Psychopsis*, and indicated that *Balmes* underwent north-western dispersal from Australia along the Malay Archipelago to mainland Asia. The second most-supported hypothesis was Indian drift, postulating that *Balmes* arrived in south-east Asia via tectonic movement of India towards

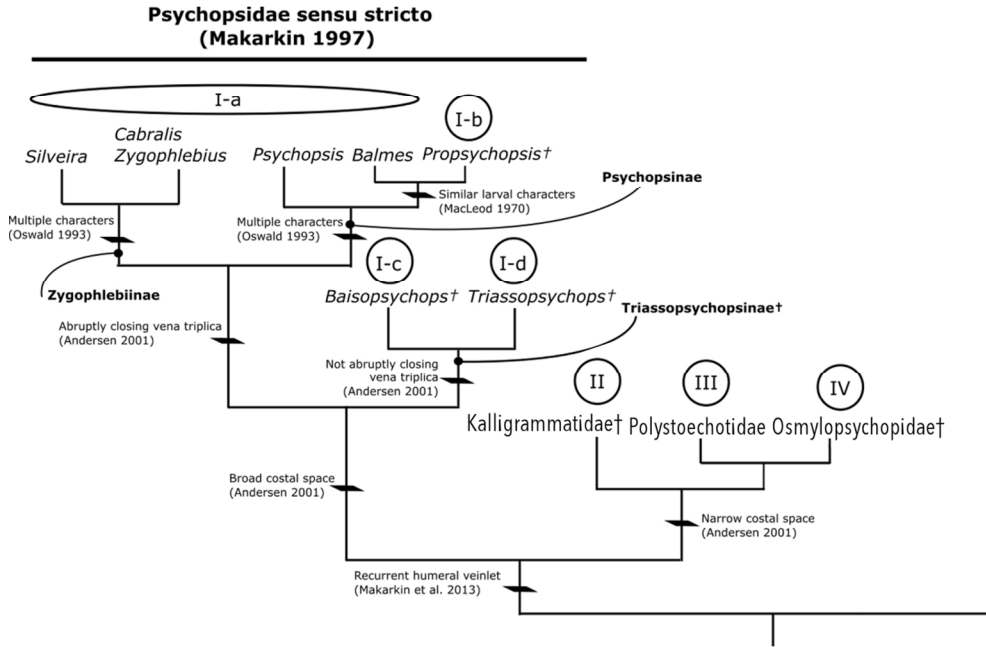


Figure 2. Cladogram of Psychopsoidea taxa including fossils, based on the text of Andersen (2001). Groups I-IV follow Andersen (2001) and represent relatedness to extant Psychopsidae (Group I-a). Synapomorphies explained in the text are shown uniting each clade with relevant literature cited. †

160x103mm (300 x 300 DPI)

Asia after Gondwanaland fragmented (Ali & Aitchison, 2008). Oswald (1993) also considered hypotheses for *Balmes* as a Laurasian relic and an African disperser, but these were rejected due to the close relationship between *Balmes* and *Psychopsis* (Oswald, 1993). This delimited Psychopsidae historical biogeography under two competing hypotheses. Uncertainty remains however, due to the limitation of not including timing data in the area-cladogram test (Oswald, 1993).

The aim of this study was to estimate phylogenetic relationships among extant Psychopsidae species and genera with reference to their historical biogeography. We tested the current taxonomy of the family using integrated morphological and molecular data. Secondly, vicariance or dispersal at each phylogenetic node was tested using estimates of divergence time and ancestral area based on extant distribution patterns. Proposed explanations for each divergence were sought in correlates of paleogeography, historical climate and habitat change.

Material and methods

Sampling and taxon selection

Taxa used in this study are listed alongside voucher and GenBank accession numbers in Supplementary Table S1. Twenty-three Psychopsidae specimens were collected either by hand-netting or at mercury vapour light traps, preserved in absolute ethanol and placed into -20°C storage. Sequences for *Psychopsis margarita* Tillyard were obtained from Genbank. The sample-set represents all extant genera and all major biogeographic regions of Psychopsidae. Afrotropical species were sampled extensively although fresh material of *Zygophlebius zebra* (Brauer) was not collected. Australian and south-east Asian faunas were represented by two species from each genus. Nine outgroup taxa were selected based on the phylogeny of Winterton *et al.* (2010) according to sister clade sampling (Hillis, 1998). The final dataset comprised 33 individuals representing thirteen Psychopsidae species. Specimens collected by the authors are housed in the South African National Collection of Insects, Pretoria (SANC) with accession numbers from the Palpares Relational Database (Mansell & Kenyon, 2002) as voucher numbers (Table S1).

Morphological characters

Ninety-eight morphological characters describing adult morphology of males and females were scored for phylogenetic analyses (Table S2). These characters include Oswald's (1993) dataset, although several were refined to improve states specific to Afrotropical taxa.

New characters specific to Afrotropical species were added. All morphological characters were treated as unordered, with gap or unknown characters coded as “-” or “?” respectively.

DNA extraction, sequencing and alignment

Four genes were selected for sequencing to represent a range of mutational rate changes over time, maximizing phylogenetically informative data, and testing whether gene trees reflect the species tree by congruence (Doyle, 1992; Maddison, 1997; Nichols, 2001). These comprised two ribosomal genes (16S rDNA and 18S rDNA) and two protein-encoding genes (mitochondrial cytochrome oxidase I; COI, and nuclear CPSase region of carbamoyl-phosphate synthetase-aspartate transcarbamoylase-dihydroorotase; CAD).

Two legs were removed from each specimen for genomic DNA extraction using the Macherey-Nagel NucleoSpin® Tissue kit (Düren, Germany). Previously published primer sequences were used to amplify genomic DNA was amplified in polymerase chain reactions (PCR) (Table S3). PCR mixtures were prepared to a final volume of either 50µL or 25µL containing 2.5mM MgCl₂, 20pmol of each primer, 10mM dNTPs, 1 x PCR buffer, with 1 unit of TaqDNA polymerase (Supertherm® DNA polymerase, Separation Scientific SA (Pty) Ltd, South Africa; or Emerald Amp®MAX HS PCRMastermix, Takara Bio Inc., Otsu, Shiga, Japan) in the presence of 100-500ng of extracted genomic DNA template. Cycling parameters varied for each gene (Table S3). Successful amplifications were purified using the Macherey-Nagel NucleoSpin® Gel and PCR Clean-up kit (Düren, Germany). Cycle sequencing reactions in both sequence directions used the BigDye® Terminator v3.1 Cycle Sequencing kit (Applied Biosystems, Foster City, USA). All sequences were viewed, assembled and edited in CLC Bio main Workbench Version 6.9 (<http://www.clcbio.com>). New sequences were submitted to GenBank (Table S1).

Alignments of edited sequences were estimated for each gene region based on primary structure (nucleotide bases) using MAFFT online (Katoh *et al.*, 2005; Katoh & Toh, 2008). The online tool was instructed to employ the ‘Auto’ strategy for alignment, inspect direction of nucleotide sequences and adjust according to the first sequence. Subsequently, the aligned sequences were inspected visually.

Phylogenetic analyses

A 50% reciprocal bootstrap test (Mason-Gamer & Kellogg, 1996; Reeb *et al.*, 2004) of the four gene partitions was conducted in PAUP*4.0b10 (Swofford, 2003) under maximum parsimony. This tested whether the four genetic datasets (16S, 18S, COI, CAD) showed congruence and could be combined. Neighbour network trees for ingroup taxa were

constructed in SplitsTree v4.14.3 (Huson & Bryant, 2006) to test the same assumption. Analyses were performed on the morphological and concatenated-molecular datasets separately, with trees inspected for congruence. Thereafter, combined molecular and morphological analyses were performed. Morphological, concatenated-molecular, and combined morphology/molecular datasets were analyzed under maximum parsimony in PAUP*4.0b10 (Swofford, 2003). The concatenated-molecular dataset and combined morphology/molecular dataset were analyzed under maximum likelihood in RAxML-HPC v8.1.20 (Stamatakis, 2014), and under Bayesian inference in MrBayes v3.2.5 (Ronquist & Huelsenbeck, 2003). Appropriate models for base pair substitution rates were estimated in jModel Test v2.1.6 (Posada, 2008) under the Akaike information criterion (Table S4).

Parsimony analyses employed heuristic tree search. All characters were equally weighted and unordered, uninformative characters excluded. A starting tree was obtained with the tree-bisection-reconnection (TBR) branch swapping algorithm using 10 replicates of random stepwise addition of sequences. Initial 'maxtrees' was set to 200 with automatic increase by 100. Bootstrap support values (Felsenstein, 1985) were calculated from heuristic search using TBR branch swapping pseudoreplicated in 1000 resampled datasets with simple addition of 10 sequences. Maximum likelihood analyses employed separate nucleotide substitution models for each partition with empirical base frequencies for each model estimated in RAxML-HPC. An unordered MK model for multistate characters was applied to the morphological partition. Bootstrap support was based on 1000 pseudoreplicates and was followed by thorough maximum likelihood search. Analysis was repeated five times, using different starting seeds to sample tree space effectively. Analyses used the rapid hill climbing algorithm (Stamatakis *et al.*, 2007). Bayesian inference was done using nucleotide substitution models selected by jModel Test for each gene (Table S4). Parameters for each partition were unlinked to obtain separate estimates, with rate prior set as variable from a flat Dirichlet distribution. Analysis was run by two Monte-Carlo-Markov-Chains (MC²) simultaneously for 30 million iterations sampling every 200th iteration, resulting in 150 000 trees. The first 37 500 (25%) trees from each MC² run were discarded as burn-in. Analysis was repeated, and all four runs were combined to produce a majority rule consensus tree with posterior probabilities at each node. Tracer v1.6 (Drummond & Rambaut, 2007) was used to assess convergence and sampling efficiency between runs. All ESS values were greater than 200 indicating effective sampling (Drummond *et al.*, 2006).

Divergence time estimates

Divergence times were estimated in BEAST v2.1.2 (Bouckaert *et al.*, 2014) using the concatenated-molecular dataset. A relaxed molecular clock was modelled under an

uncorrelated lognormal model with the Yule speciation process applied. Yule speciation assumes a pure birth process, but with branch length distribution similar to birth-death processes, and an equivalent net birth rate. Separate substitution models were used for each partition (Table S4) with model and clock parameters unlinked across all partitions. Analyses were set to 300 million iterations, with every 1000th iteration sampled. A starting tree was generated from random seed. Analysis was run four times to ensure effective sampling. The first 75 000 (25%) trees from each run were discarded as burn-in. Apart from node age constraints, default settings were used for all priors. LogCombiner v2.1.2 (Bouckaert *et al.*, 2014) was used to combine log and tree outputs from the four independent runs. Tracer assessed convergence and sampling efficiency between runs, with all ESS values greater than 200, indicating effective sampling. TreeAnnotator v2.1.2 (Bouckaert *et al.*, 2014) generated the maximum clade credibility tree and calculated node ages with $\pm 95\%$ High Posterior Density (HPD) confidence intervals.

Fossil dates from published literature served as calibrations (Table 1). Four outgroup nodes and two ingroup nodes were calibrated, each modelled under lognormal prior distributions to allow priors to serve as minimum age constraints, and vary towards including older divergence times. Each distribution was modelled with an offset, mean and standard deviation that set 95% of priors within minimum and maximum ages of fossil dates. The minimum age constraint on the most recent common ancestor (MRCA) of the ingroup (node A, Table 1) was set to the Late Triassic based on *Triassopsychops superbus* Tillyard (237–228 Mya), and was calibrated as a stem with a wide interval of priors to allow analyses to optimize divergence time estimates, rather than adhere to hard constraints. Uncertainty in phylogenetic placement and possibility of extinction in Psychopsidae motivated this decision (Fig. 2). The approximately 20 My difference between first appearances of *Triassopsychops*, and the estimated divergence time of extant *Psychopsis* (Winterton *et al.* 2010), is probably due to the crown node of *Psychopsis* being younger than the stem node of *Psychopsis* + *Triassopsychops* (Fig. 2). This makes divergence time recovered by Winterton *et al.* (2010) attributable to the later divergence of extant Psychopsidae from the rest of Myrmeleontiformia, instead of to the earlier divergence between Myrmeleontiformia and Triassopsychopsinae (as Psychopsidae stem).

Ancestral areas

Ancestral area probabilities were estimated in RASP v3.2 (Yu *et al.*, 2015) to test whether vicariance or dispersal was dominant, and which ancestral area per node was most likely. Evolutionary events were inferred as either dispersal, vicariance, extinction or standard speciation; the last serves as a null hypothesis that makes cladogenesis clock like (Kuhner &

Table 1. Fossil taxon information and prior distribution parameters for divergence time analysis.

Constraint	Most recent common ancestor (MRCA)	Fossil [geological period; age (Ma)]	References	Prior distribution parameters				
				Prior distribution	Offset	Log (mean)	Log (SD)	95% CI of priors
A	Psychopsidae (ingroup)	<i>Triassopsychops superbis</i> Tillyard (1922b) [Late Triassic (Carnian); 237 to 228]	Tillyard (1922b), Peng <i>et al.</i> (2011) and Lambkin (2014)	Lognormal	205	2.2	0.7	207.3–240.6
B	<i>B. birmanus</i> + <i>B. terissinus</i>	<i>Propsychopsis lapcidae</i> MacLeod (1970) [Eocene; 54.8 to 33.7]	MacLeod (1970)	Lognormal	30	2.2	0.5	33.4–54.0
C	<i>Apochrysa</i> + <i>Notiobiella</i>	<i>Lembochrysa miniscula</i> Ren & Guo (1996) [Late Jurassic; 160]	Ren & Guo (1996) and Nel <i>et al.</i> (2005)	Lognormal	160	1.7	0.5	162.1–174.6
D	<i>Polystoechotes</i> + <i>Ithone</i>	<i>Jurapolystoechotes melanolomusi</i> Ren, Engel & Lü (2002) [Middle Jurassic; 170]	Ren <i>et al.</i> (2002)	Lognormal	170	1.5	0.6	171.4–184.5
E	<i>Libelloides</i> + <i>Ululodes</i>	<i>Choromyrmeleon othneius</i> Ren & Guo (1996) [Late Jurassic; 150]	Ren & Guo (1996)	Lognormal	149	1.5	0.5	151.0–161.0
F	<i>Nymphes</i> + <i>Osmylops</i>	<i>Nymphes georgei</i> Archibald, Makarkin & Ansoerge (2009) [Early Eocene; 49.96–48.88]	Archibald <i>et al.</i> (2009)	Lognormal	48	1.2	0.6	49.0–58.0

Felsenstein, 1994). A dataset of 15 000 trees obtained from BEAST was used, and was pruned to exclude duplicate representatives and distantly related outgroup taxa to minimize unequal taxon bias. However, *Notiobiella* Banks (Hemerobiidae) and *Apochrysa* Schneider (Chrysopidae) were kept to provide deep resolution. The dataset was analyzed under two models of ancestral area estimation: S-DIVA (Yu *et al.*, 2010) and BayArea (Landis *et al.*, 2013). Parameters for each analysis are presented in Table S5. Areas were assigned to biogeographic regions of extant Psychopsidae: (A) Australia, (B) south-eastern Africa, (C) south-western Africa, and (D) south-eastern Asia. Combined area codes represent historical landmasses such as Gondwanaland (ABCD) and Austro-India (AD).

Results

Phylogenetic analyses

Morphological characters (90 parsimony informative) analyzed under parsimony yielded 156 equally most parsimonious trees (length = 160 steps, consistency index (CI) = 0.8125, retention index (RI) = 0.9506). The strict consensus tree of bootstrap support greater than 50% is presented in Supplementary Fig. S1A. The three Afrotropical genera, *Cabralis*, *Zygophlebius* and *Silveira*, were monophyletic (bootstrap support = 87%), with *Cabralis* recovered as sister to *Zygophlebius* with limited support (68%). *Cabralis* comprised two species, *C. gloriosus* Navás (87%) sister to an undescribed species, 'Cns' (Oswald, 1993) (58%). *Silveira* was monophyletic (100%) with the following six synapomorphies: (1) Presence of three ocellar/cranial pulvinae (character 1), (2) Male sternite IX narrow or parallel-sided (character 29: Table S2), (3) Female 9th gonocoxite with longitudinal row of stiff setae (character 83), (4) Female 9th gonocoxite with suprastylar setae <50% cochleariform (character 86), (5) Female 9th gonocoxite with stylus bearing sharp, long spines (character 88), and (6) Female spermatheca with ventral lobes narrow in lateral view (character 93). Within *Silveira*, *S. jordani* Kimmins was recovered as sister to the rest of the genus (100%), and *S. rufus* Tjeder was recovered as sister to *S. occultus* Tjeder + *S. marshalli* (McLachlan) (94%). *Silveira occultus* was sister to *S. marshalli* (98%), and monophyly of *S. marshalli* was likewise well supported (91%).

Sequence data yielded a total of 3746 nucleotide base pair characters after alignment. Model parameters and sequence information for each gene partition is summarized in Table S4. The 50% reciprocal bootstrap test and neighbour network trees for the four gene partitions showed congruence with major clades always present, confirming the assumption of phylogenetic congruence (Figs S1, S2: Supplementary material). Consequently, individual gene data were combined. Molecular data analyzed under parsimony yielded a single most

parsimonious tree (length = 3193 steps, CI = 0.5011, RI = 0.6756). The strict 50% consensus tree is presented in Fig. S3B alongside the morphological tree, highlighting incongruence. Parsimony analysis of the combined morphology/molecular dataset yielded one most parsimonious tree (length = 3355 steps, CI = 0.5156, RI = 0.7055). Maximum likelihood analyses recovered a single best tree, congruent with parsimony and Bayesian trees of the combined morphology/molecular data. This indicates that adding information from morphology to molecular data did not alter phylogenetic relationships, but marginally increased parsimony support for monophyly of ((*Balmes* + *Psychopsis*) + (*Cabralis* + *Zygophlebius*)) (parsimony bootstrap = 84% from combined data versus 80% from molecular data alone, see Figs 3 and S3B). The Bayesian consensus tree with posterior probability and bootstrap support is shown in Fig. 3. Bootstrap values of greater than 70%, and posterior probability values greater than 0.95, are considered strong support (Hillis & Bull, 1993; Rannala & Yang, 1996; Erixon *et al.*, 2003). Nodes were considered strongly supported when good support was received from at least two out of three analyses (Sheldon & Bledsoe, 1993; Miyamoto & Fitch, 1995).

Combined morphological and molecular data analyzed under parsimony, maximum likelihood and Bayesian inference recovered the family as monophyletic (parsimony bootstrap = 100%, maximum likelihood bootstrap = 100%, Bayesian posterior probability = 1; Fig. 3). Zygophlebiinae *s. lat.* (*Cabralis*, *Zygophlebius* and *Silveira*), was recovered as paraphyletic. *Cabralis* + *Zygophlebius* were monophyletic (100%, 100%, 1), and *Balmes* + *Psychopsis* (*i.e.* Psychopsinae) were also monophyletic but with limited support (64%, 59%, 0.88). The node comprising ((*Cabralis* + *Zygophlebius*) + (*Psychopsis* + *Balmes*)) was recovered with good support (84%, 65%, 0.99). *Silveira* was recovered as monophyletic, sister to ((*Cabralis* + *Zygophlebius*) + (*Psychopsis* + *Balmes*)) (100%, 100%, 1). Within *Silveira*, *S. jordani* was monophyletic (100%, 100%, 1), sister to the rest of the genus. *Silveira occultus* was monophyletic (100%, 100%, 1) and was sister to the monophyletic *S. rufus* + *S. marshalli* (71%, 100%, 0.99).

Divergence time estimates

The maximum clade credibility tree was congruent with parsimony, maximum likelihood and Bayesian trees from combined morphology/molecular and concatenated-molecular data. The divergence time estimates tree is shown in Fig. 4 with generally narrow 95% HPD intervals, indicating confidence in estimates.

Psychopsidae are estimated to have diverged from the rest of Neuroptera in the Late Triassic, 214 Mya (95% HPD interval = 224.50-206.17 Mya). *Silveira* diverged from the remaining Psychopsidae in the Mid Cretaceous about 107 Mya (136.94-79.04 Mya) and

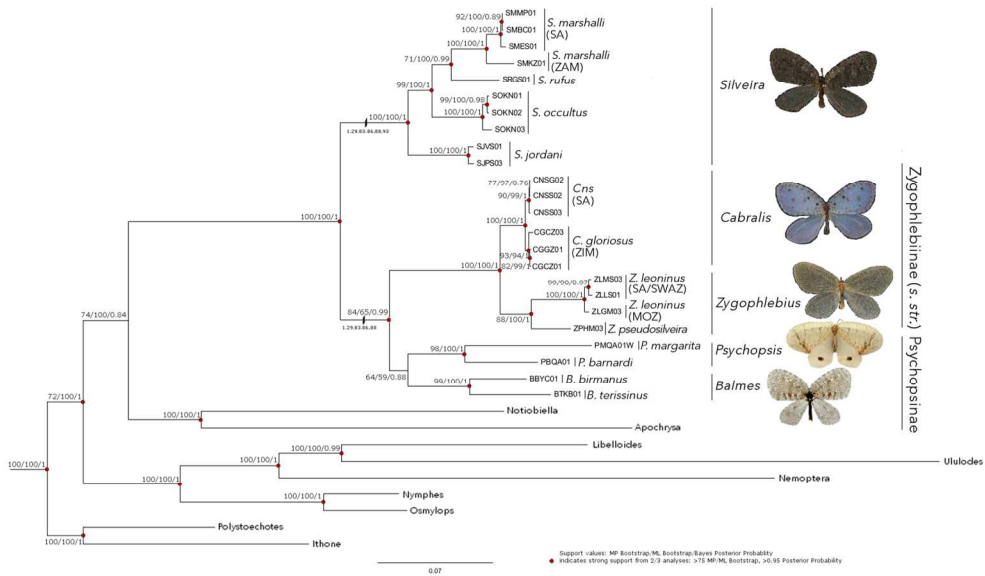


Figure 3. Bayesian consensus tree recovered from Bayesian analysis of combined morphological/molecular dataset. Bootstrap values from parsimony and maximum likelihood, as well as Bayesian posterior probabilities are displayed above nodes (MP/ML/Bayesian). Red nodes indicate strong support from at least two out of three analyses i.e. >70% bootstrap for parsimony and maximum likelihood, and >0.95% posterior probability for Bayesian analysis. Dashes with corresponding numbers on Silveira and Zygophlebiinae (s. str.) + Psychopsinae lineages refer to morphological synapomorphies for these lineages – numbers refer to characters in the morphological dataset (Table S2: supplementary material). Uppercase codes refer to specimen country of collection (MOZ – Mozambique, SA – South Africa, SWAZ – Swaziland, ZAM – Zambia, ZIM – Zimbabwe). Photo credits: Psychopsis – Graeme Cocks, Balmes – Hao Huang, Silveira, Cabralis and Zygophlebius – Deon Bakkes.

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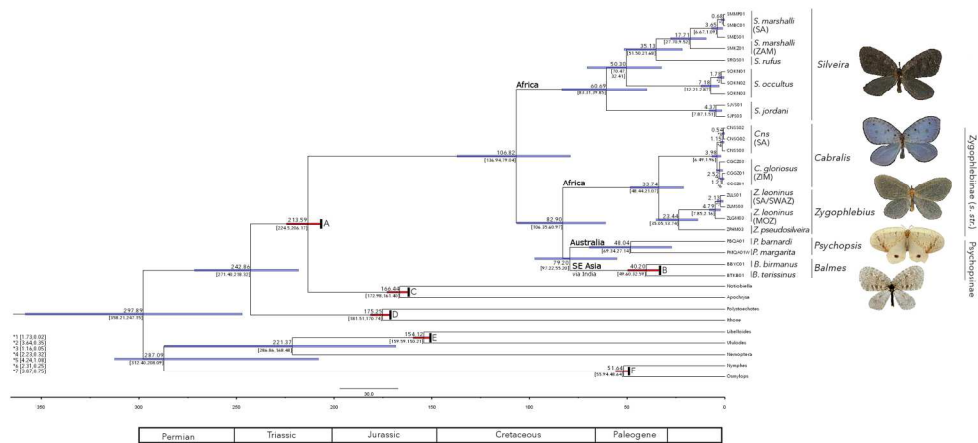


Figure 4. Divergence time estimates for Psychopsidae from analyses of the concatenated molecular dataset (16S, 18S, CAD, COI). Median node ages are displayed above nodes. 95% high posterior density (HPD) intervals are displayed in [] brackets below nodes and are represented by blue bars transecting each node. *HPD intervals in the bottom-left refer to those that were unable to fit below the node. Red nodes A, B, C, D, E and F are constrained based on relevant fossils for those taxa, discussed in the text. Time-scale units are in millions of years.

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underwent a series of radiations in the Late Cretaceous to Oligocene/Early Miocene about 61-35 Mya (83.31-21.68 Mya from combined 95% HPD). The *Cabralis* + *Zygophlebius* lineage diverged from the *Balmes* + *Psychopsis* lineage in the Mid to Late Cretaceous about 83 Mya (106.35-60.97 Mya), with *Balmes* and *Psychopsis* diverging from one another soon after at about 79 Mya (97.22-55.20 Mya). *Cabralis* and *Zygophlebius* diverged in the Eocene/Oligocene at 34 Mya (48.44-21.07 Mya). *Cabralis gloriosus* and *Cns* diverged in the Late Miocene/Pliocene, about 4 Mya (6.49-1.96 Mya).

Ancestral areas

Ancestral areas are summarized in Fig. 5 with probability charts of each model at each node, and dispersal-vicariance-extinction plots juxtaposed with the phylogeny. Plots were similar with the exception of a single extinction event in BayArea modelling. Dispersal was estimated to be dominant in deeper time (130-80 Mya) with vicariance playing a role more recently (90-40 Mya).

Psychopsidae are estimated to have originated in Gondwanaland, somewhere between Australia and parts of Africa (Fig. 5). Divergence of *Silveira* occurred in Gondwanaland by dispersal. *Silveira* subsequently underwent a series of divergences in Africa, the earliest two (*S. jordani* and *S. occultus*) were between south-east and south-west Africa (standard speciation). The *Cabralis* + *Zygophlebius* lineage diverged from the *Balmes* + *Psychopsis* lineage in Gondwanaland (between south-east Africa, Australia and south-east Asia), by a combination of standard speciation, vicariance and dispersal. *Balmes* and *Psychopsis* diverged in a similar manner (Fig. 5), however this result is contentious because node frequency is low (46%). This indicates limited taxon sampling and highlights the need for increased sampling of these genera.

Discussion

Psychopsidae were supported as being monophyletic and sister to the rest of Myrmeleontiformia (Fig. 3) in line with previous studies (Withycombe, 1925; Oswald, 1993; Aspöck *et al.*, 2001; Winterton *et al.*, 2010; Badano *et al.*, 2016; Wang *et al.* 2016). This suggests they form an entirely separate clade of Myrmeleontiformia, and if fossil Psychopsoidea are monophyletic (Makarkin, 2010; Makarkin *et al.*, 2013; Peng *et al.* 2016), then Psychopsidae represent the last remnant of fossil rich Psychopsoidea. Within Psychopsidae, Zygophlebiinae including *Silveira* (Zygophlebiinae *s. lat.*), are considered monophyletic (Oswald, 1993), but our results indicate paraphyly. Incongruence between morphological and molecular data manifests in Afrotropical genera and between *Silveira* species (Fig. S3). Morphology recovered *Silveira* in Zygophlebiinae (*s. lat.*), but *Silveira* fell

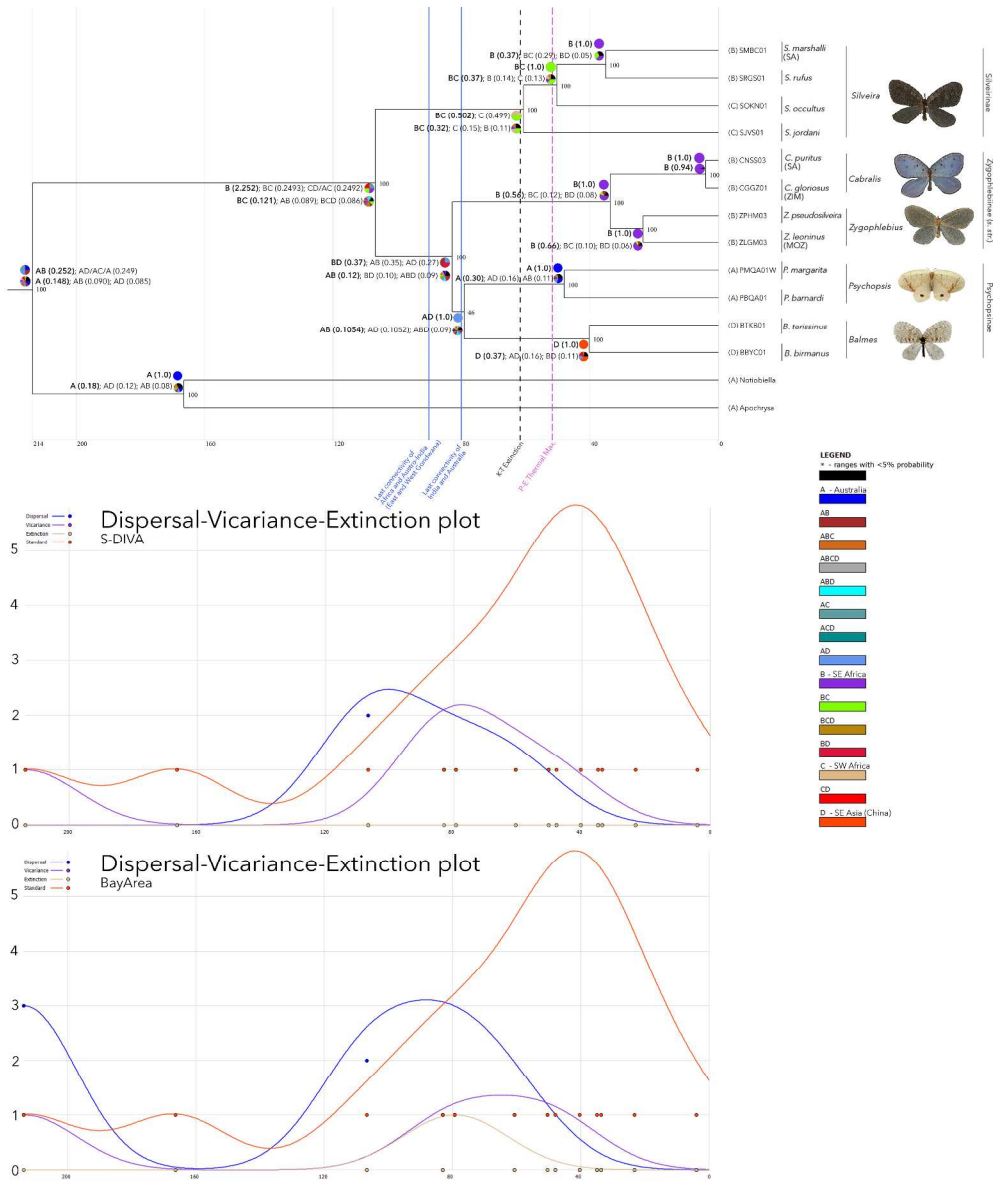


Figure 5. Ancestral area estimation of 15 000 BEAST trees. Statistics left of each node represent results from two models (top-to-bottom: S-DIVA and BayArea). Pie charts represent probabilities for ancestral area by colour. Text alongside each pie chart represents the probabilities of the three or four most-supported ancestral areas for the model, at that node. Ancestral area with best support per model is bolded at a given node. Mass extinction events are represented by black dashed lines, climatic events by purple dashed lines, and dates of last faunal connectivity between landmasses by solid blue lines. Dispersal-Vicariance-Extinction plots from S-DIVA and BayArea analyses are juxtaposed with the ancestral area estimation phylogeny. Time-scale units are in millions of years and the y-axis represents inferred events.

491x576mm (300 x 300 DPI)

significantly beyond the *Cabralis* + *Zygophlebius* (*Zygophlebiinae s. str.*) clade when molecular data were analyzed (Figs 3, S3B). This demonstrates that *Silveira* comprise a separately evolving lineage that warrants placement in a new subfamily. Revisiting morphology yields synapomorphies to support this (branch dashes in Fig. 3), and Tjeder (1960) considered *Silveira* basal, leaving a close relationship between *Cabralis*, *Nothopsychops* Tillyard ([sic.] synonym of *Zygophlebius*) and *Psychopsis*. His assertions were based on similar synapomorphies as highlighted here (Fig. 3).

The disjunct, tripartite distribution of extant Psychopsidae is significant to their historical biogeography, suggesting Gondwanan origin (Tillyard, 1919a). However, the earliest fossil, *T. superbus*, appears in the Carnian (220.7-227.4 Mya; Denmark Hill, Queensland, Australia) before Pangea fragmented (ca. 160 Mya) (Scotese, 2004; Schettino & Turco, 2009; Seton *et al.*, 2012). This study reveals that Psychopsidae diverged approximately 214 Mya (224.5-206.17 Mya; Fig. 4; corroborating Winterton *et al.*, 2010), pre-dating fragmentation of Pangea into Gondwanaland and Laurasia (ca. 180 Mya: Seton *et al.*, 2012). This supports the contention that Psychopsidae were widespread during the Mesozoic and inhabited most of Pangea during their radiation (Oswald, 1993; Anderson, 2001). Fossil *Baisopsychops* from Laurasia in the Cretaceous corroborate this, but these form a lineage separate from extant taxa considering wing vein morphology (Andersen, 2001). This indicates extant Psychopsidae may have been restricted to southern Gondwanaland producing the present distribution. Alternatively, extinction of all lineages in Laurasia may explain the present distribution apparently Gondwanan as derived. Indeed, the Cretaceous-Paleogene (K-Pg) extinction might have significantly decreased Psychopsidae abundance, especially in Laurasia (Makarkin & Archibald, 2014).

Dispersal and vicariance seem prevalent at different stages of psychopsid historical biogeography. Dispersal was generally dominant before Gondwanan fragmentation, with vicariance prevalent during fragmentation, and a combination of these two processes evident later (Fig. 5). Divergence of *Silveira* occurred at 107 Mya (136.94-79.04 Mya; Fig. 4) within African parts of Gondwanan, probably by dispersal (Fig. 5). This pre-dates last faunal connectivity between Africa and Austro-India at about 90 Mya and conclusively excludes vicariance (Bossuyt *et al.*, 2006). *Zygophlebiinae (s. str.)* in the Afrotropics and Psychopsinae in Australasia suggests Gondwanan vicariance. Their divergence occurred at 83 Mya (106.35-60.97 Mya, Fig. 4) in Gondwanan, concurring with last faunal connectivity between Africa and Australia, putatively indicating vicariance (Jokat, 2003; Scotese, 2004; Bossuyt *et al.*, 2006; Seton *et al.*, 2012). Dispersal is possible however, because 95% HPD (maximum age at 106.35 Mya; Fig. 4) encompasses up to 16 My before last faunal

connectivity was lost. Nonetheless, Gondwanan vicariance provides a reasonable explanation given the majority of estimates after this time.

Historical biogeography of *Balmes* can be narrowed to two possibilities (Oswald, 1993). Either dispersal from Australia along the Malay Archipelago, or vicariance with Indian drift after Austro-India fragmented. The former requires divergence time between *Psychopsis* and *Balmes* correlating with Malay Archipelago formation (ca. 25 Mya: Scotese, 2004; Seton *et al.*, 2012), while the latter requires such correlation with Austro-India fragmentation instead (ca. 80 Mya: Scotese, 2004; Bossuyt *et al.*, 2006; Ali & Aitchison, 2008; Seton *et al.*, 2012). This divergence occurred at 79 Mya (97.22-55.20 Mya, Fig. 4), which significantly pre-dates Malay Archipelago formation and approximates last faunal connectivity between India and Australia. This conclusively excludes Australian dispersal. This finding is corroborated by mitochondrial phylogenomics of Wang *et al.* (2016) with divergence time of *Psychopsis* at 99 Mya (204.26-43.66 Mya), although based on a different species. MacLeod (1970) speculated *Psychopsis* underwent prolonged and isolated evolution based on morphology. Indian drift supports such isolation. However, timing estimates again have 95% HPD up to 17 My before last faunal connectivity between India and Australia (maximum age limit at 97.22 Mya; Fig. 4), indicating a small chance of dispersal. Nonetheless, strong rejection of Australian dispersal from timing demonstrates that *Balmes* drifted on India toward mainland Asia.

Propsychopsis fossils that are closely related to *Balmes* based on morphological similarity (MacLeod, 1970; Andersen, 2001) provide additional clues for *Balmes* historical biogeography. These fossils are only known from Priabonian Baltic amber (37.2-33.9 Mya) (Peng *et al.*, 2011), which post-dates the India-Asia collision (ca. 55 Mya: Zhu *et al.*, 2005; Aitchison, *et al.* 2007; Ali & Aitchison, 2008; Seton *et al.*, 2012). This suggests *Propsychopsis* diverged from *Balmes* before the Eocene, became isolated in India, and similarly underwent Indian drift to reach Asia. Alternatively, tropical faunas were widespread at this time due to hot-house temperatures; hence *Propsychopsis* may be explained by Laurasian endemism. However, this seems unlikely given striking morphological similarity with *Balmes*, and the lack of *Propsychopsis* at other psychopsid rich fossil deposits before this time. Similar cases of putative Indian drift are found in fossil Ceratopogonidae (Diptera) of Cambay amber (55-52 Mya; Gujarat, India) that imply faunal exchange between India, Asia and Europe in the Early Eocene (Stebner *et al.*, 2017), as well as in extant *Thyridosmylus* Krüger (Osmylidae) that have sister species in Madagascar and south-east Asia, suggesting a close biogeographic connection (Wang *et al.*, 2011). India as a 'biotic ferry' has also been proposed for frogs and Eocene mammals (Bossuyt & Milinkovitch, 2001; Thewissen *et al.*, 2010).

Afrotropical Psychopsidae

An undescribed species of *Cabralis* (Cns) was confirmed as sister to *C. gloriosus* (Fig. 3), corroborating Oswald (1993). *Cabralis gloriosus* is restricted to Afromontane forests in the eastern Zimbabwean highlands, and Cns to Afromontane forests in the Soutpansberg mountains of South Africa. Allopatric speciation is hypothesized following fragmentation of Eocene forests. Divergence between *C. gloriosus* and Cns occurred 4 Mya (6.49-1.96 Mya; Fig. 4) in south-east Africa. Little support for either vicariance or dispersal was available (Fig. 5), although compelling evidence from disjunct distributions support vicariance. Climatically, this divergence closely post-dates decreasing global precipitation (Frakes, 1979), and coincides with final stages of Afromontane forest fragmentation (Axelrod & Raven, 1978; White, 1981; Lovett, 1988; Burgoyne *et al.*, 2005). This would have split presumably widespread *Cabralis* progenitors between respective Afromontane refugia, resulting in allopatric speciation. Another case of sister species in Afromontane refugia from a similar period is found in bats of the east African mountains (Taylor *et al.*, 2012).

Regarding relationships among *Silveira* species, morphology and molecular data were incongruent. Morphology places *S. jordani* and *S. rufus* as closely related, alongside a monophyletic *S. occultus* + *S. marshalli* (Fig. S3A) (Oswald 1993). However, molecular data place *S. jordani* and *S. occultus* as sister instead, with *S. rufus* + *S. marshalli* monophyletic (Figs S3B, 3). Molecular data accords with distribution patterns between groups on western (*S. jordani* + *S. occultus*) and eastern (*S. rufus* + *S. marshalli*) sides of the continent, and these distribution patterns mirror those of arid adapted plants (Jürgens, 1997) as well as Myrmeleontidae (Mansell, 1990). Divergence of *S. jordani* from the MRCA of (((*S. occultus*) + *S. rufus*) + *S. marshalli*) occurred at 61 Mya (83.31-39.85 Mya; Fig. 4), and divergence of *S. occultus* from the MRCA of *S. rufus* + *S. marshalli* occurred at 50 Mya (70.47-32.41 Mya; Fig. 4), both between south-west and south-east Africa. Timing coincides with climatic flux between arid and moist climates globally (Frakes, 1979), initiated by the Paleocene-Eocene Thermal Maximum (PETM; Wing *et al.* 2005). This stimulated forest expansion (Maley, 1996; Jacobs, 2004) and subsequently radiation of C₄ grasses characteristic of savannah and woodland habitats (Cerling *et al.*, 1997; Jacobs, 2004; Mannetje, 2007). Such evidence contends that during the Eocene, Afrotropical arid biomes gave way to forest expansion with the south-west coast remaining dry (Rayner *et al.*, 1991; Tankard & Rogers, 1978). Progenitors of *S. jordani* would have diverged from the MRCA of (((*S. occultus*) + *S. rufus*) + *S. marshalli*) following climatic and habitat change.

Conclusion

This study represents a comprehensive treatment of Psychopsidae evolutionary history that integrates morphological, molecular and biogeographic data. The main findings indicate that traditional morphological data are generally incongruent with molecular data, resulting in paraphyly of Zygophlebiinae (*s. lat.*). Afrotropical *Silveira* represent a divergent lineage of extant Psychopsidae. *Cabralis* and *Zygophlebius* (Zygophlebiinae *s. str.*) in the Afrotropics are monophyletic, and sister to Australasian Psychopsinae. Divergence time estimation and historical biogeography supports Psychopsidae origin in Pangea, and suggests that the present disjunct, tripartite distribution resulted from Gondwanaland vicariance and Indian drift. Beyond this, evolution of Afrotropical lineages were probably driven by adaptations to moist and arid habitats with associated expansions and contractions.

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Supplementary information

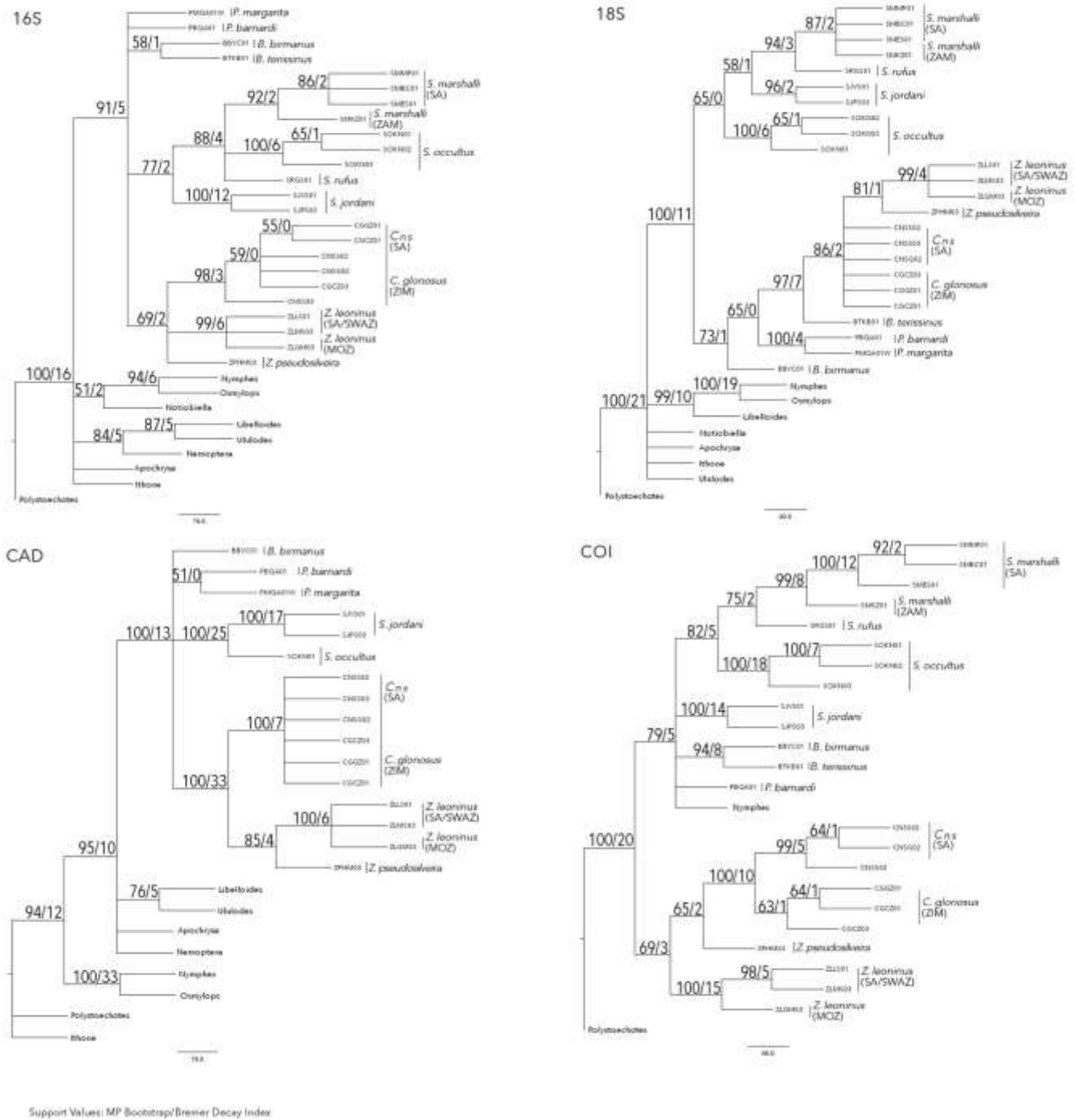


FIGURE S1. 50% bootstrap strict consensus trees recovered from parsimony analyses of individual gene datasets.

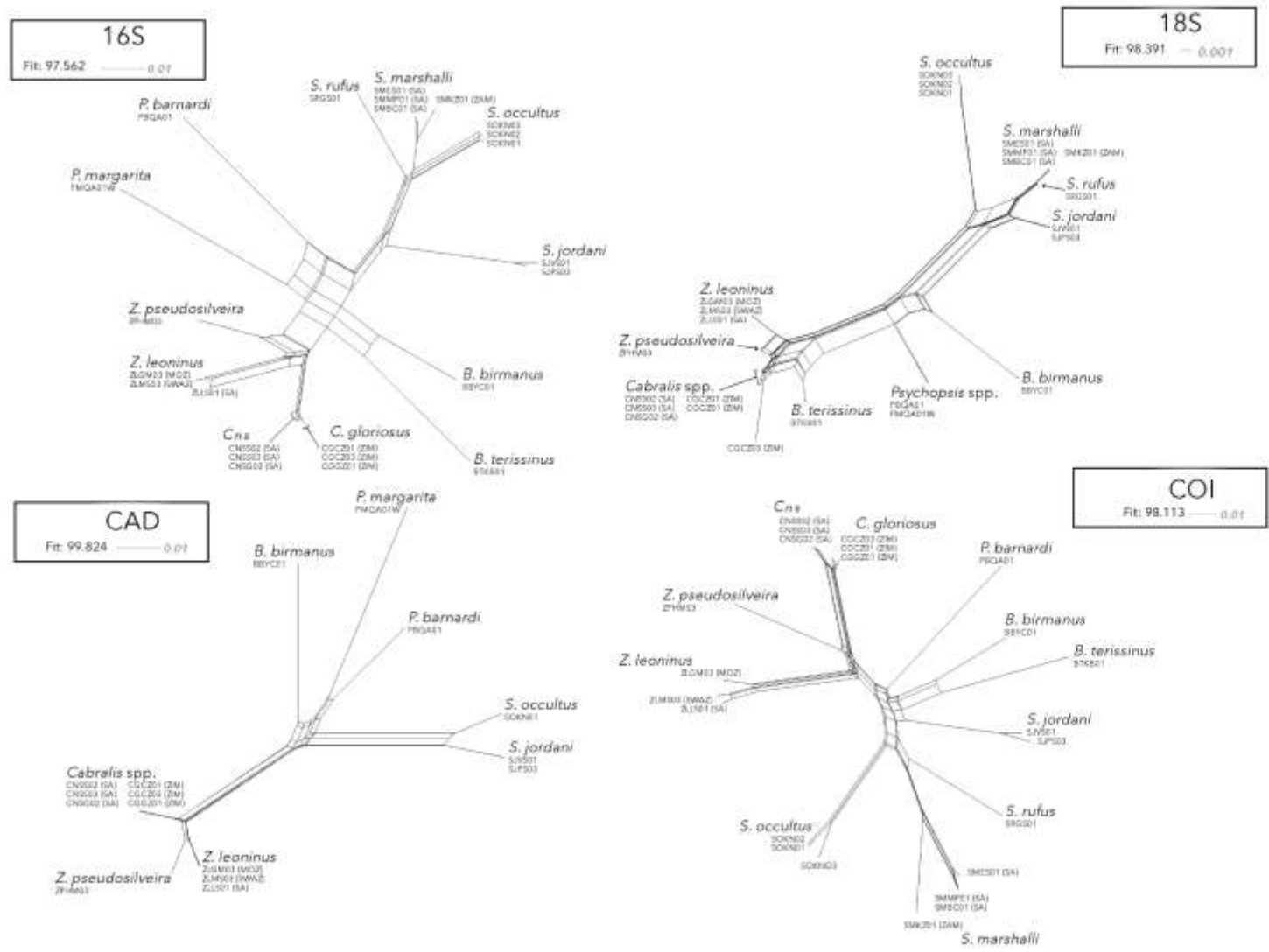


FIGURE S2. Neighbour-net splits trees recovered from SplitsTree analysis of individual gene datasets. Outgroups are excluded.

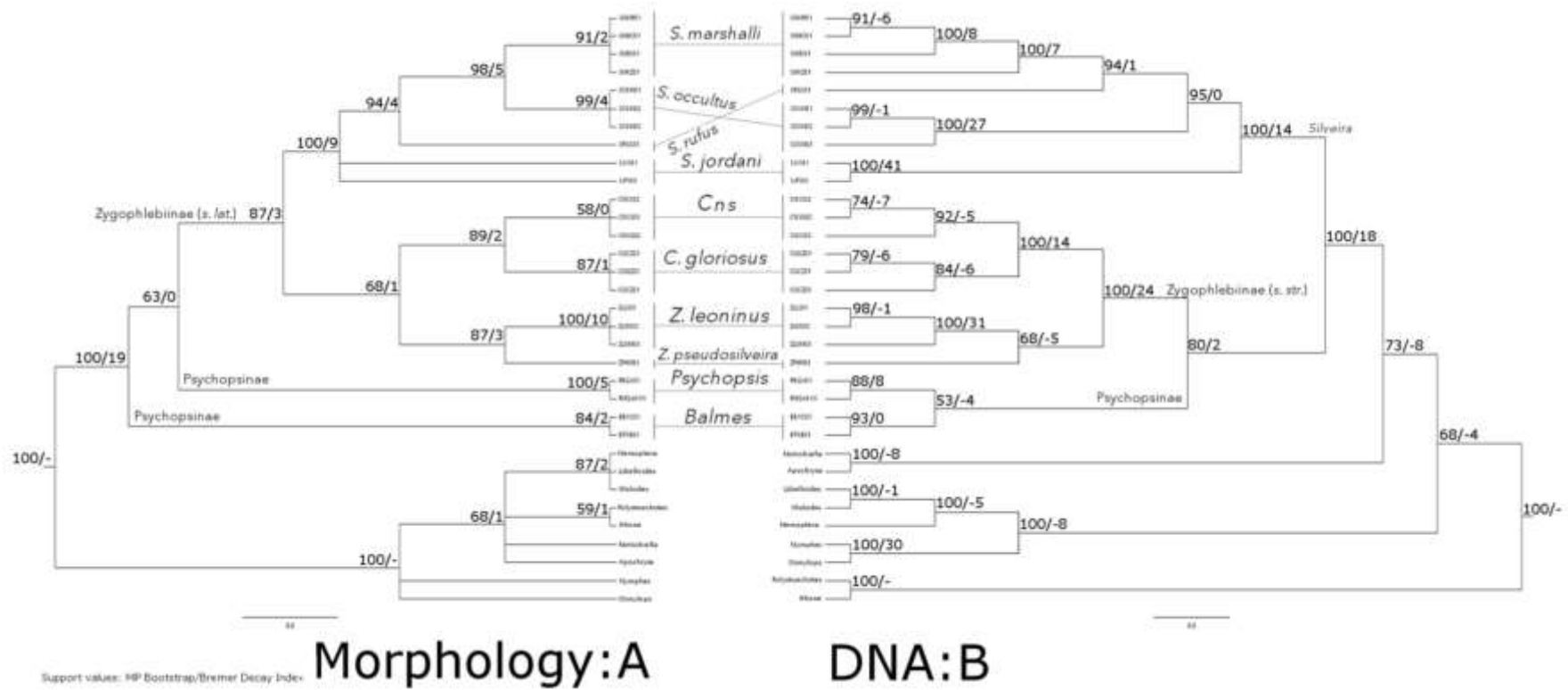


FIGURE S3. Strict consensus trees (bootstrap = 50%) recovered from parsimony analyses of (A) the morphological dataset and (B) the concatenated molecular dataset.

Table S1. Specimen, Database and GenBank accession numbers for specimens used in this study. Database accessions refer mainly to records contained in the Palpares Relational Database (Mansell & Kenyon, 2002).

Species	Sample ID	Locality	Palpares Relational Database Accession	GenBank Accessions			
				16S	18S	COI	CAD
<i>Silveira jordani</i>	SJVS01	Verlatenkloof, SA	NEUR52639	KT625698	KT625721	KT625759	KT625744
	SJPS03	Prince Albert, SA	NEUR52645	KT625699	KT625722	KT625760	KT625745
<i>Silveira occultus</i>	SOKN01	Khorixas, NAM	NEUR52666	KT625700	KT625723	KT625761	KT625746
	SOKN02	Khorixas, NAM	NEUR52667	KT625701	KT625724	KT625762	--
	SOKN03	Khorixas, NAM	NEUR52668	KT625702	KT625725	KT625763	--
<i>Silveira rufus</i>	SRGS01	Soutpansberg, SA	NEUR52637	KT625703	KT625726	KT625764	--
<i>Silveira marshalli</i>	SMMP01	Marloth Park, SA	NEUR52503	KT625704	KT625727	KT625765	--
	SMBC01	Blyde River Canyon, SA	NEUR52504	KT625705	KT625728	KT625766	--
	SMKZ01	Kumasamba, ZAM	WINT00001	KT625706	KT625729	KT625767	--
	SMES01	Ellisras, SA	NEUR52512	KT625707	KT625730	KT625768	--
<i>Zygophlebius leoninus</i>	ZLLS01	Lekgalameetse res., SA	NEUR52647	KT625708	KT625731	KT625769	KT625747
	ZLMS03	Mlawula res., SWAZ	NEUR52649	KT625709	KT625732	KT625770	KT625748
	ZLGM03	Gorongosa, MOZ	NEUR52658	KT625710	KT625733	KT625771	KT625749
<i>Zygophlebius pseudosilveira</i>	ZPHM03	Hoogenoeg, SA	NEUR52509	KT625711	KT625734	KT625772	KT625750
<i>Cabralis gloriosus</i>	CGCZ01	Chirinda Forest, ZIM	NEUR52651	KT625712	KT625735	KT625773	KT625751
	CGCZ03	Chirinda Forest, ZIM	NEUR52653	KT625713	KT625736	KT625774	KT625752
	CGGZ01	Gurunguhwe Mt., ZIM	NEUR52655	KT625714	KT625737	KT625775	KT625753
<i>Cns</i>	CNSS02	Soutpansberg, SA	NEUR52501	KT625715	KT625738	KT625776	KT625754
	CNSS03	Soutpansberg, SA	NEUR52507	KT625716	KT625739	KT625777	KT625755
	CNSG02	Soutpansberg, SA	NEUR52636	KT625717	KT625740	KT625778	KT625756
<i>Psychopsis margarita</i>	PMQA01W	Queensland, AUS	WINT00002	EU734897	EU815280	--	EU860149
<i>Psychopsis barnardi</i>	PBQA01	Queensland, AUS	WINT00003	KT625718	KT625741	KT625779	KT625757
<i>Balmes terrissinus</i>	BTKB01	Kuankuoshui res., CHI	LIUC00001	KT625719	KT625742	KT625780	--
<i>Balmes birmanus</i>	BBYC01	Kunming, CHI	WINT00004	KT625720	KT625743	KT625781	KT625758
<i>Polystoechotes punctatus</i>	Polystoechotes	Idaho, USA	CASENT8092171*	EU734893	EU815277	FJ171325 ¹	EU860146
<i>Notiobiella viridis</i>	Notiobiella	Queensland, AUS	CASENT8092205*	EU734883	EU815266	--	--
<i>Ithone fulva</i>	Ithone	Queensland, AUS	CASENT8092184*	EU734865	EU815247	--	EU860118
<i>Apochrysa lutea</i>	Apochrysa	Queensland, AUS	CASC203*	DQ399285 ²	EU815269	--	EU860139
<i>Nymphes myrmeleonides</i>	Nymphes	Queensland, AUS	CASENT8092181*	EU734884	EU815268	KJ461322 ³	EU860137
<i>Libelloides rhomboideus</i>	Libelloides	Peloponnisos, GREECE	CASENT8092187*	EU734868	EU815250	--	EU860121
<i>Nemoptera coa</i>	Nemoptera	Peloponnisos, GREECE	CASENT8092191*	EU734878	--	--	EU860131
<i>Osmylops armatus</i>	Osmylops	Queensland, AUS	CASENT8092182*	EU734886	EU815270	--	EU860140
<i>Ululodes quadripunctatus</i>	Ululodes	North Carolina, USA	CASENT8092188*	EU734854	EU815235	--	EU860109

*Specimens not contained in Palpares Relational database. ^{1,2,3}Sequences obtained from different studies for that taxon. ¹with different locality data ¹British Columbia, Canada.

Table S2: Matrix of morphological characters and states used in the study

	Head			Thorax				
	Vertex, ocellar/cranial pulvinae	Margin of torulus/ medial antennifer	Head Colour	Forewing, transverse bilineate fasciae	Forewing Markings, colour	Forewing Markings, patterning	Forewing, no. of Sc-R1 crossveins	Forewing, crossveins of costal gradate series
Taxon character states coded with a dash (-) belong to a state that is irrelevant for that character system and are thus excluded from analysis of that character.	0–3 pulvinae 1–2 pulvinae 2–0 pulvinae	0– absent 1– present	0 - light yellow 1 - darker yellow 2 - reddish brown 3 - dark brown 4 - pale testaceous 5 - dark brown testaceous	0– absent 1– present	0 - white 1 - yellowish hyaline 2 - brownish 3 - Pale grey brown	0 - strong dark spots on a white background 1 - weak dark spots on a white background 2 - sparse light brown transverse bars 3 - dense light brown transverse bars 4 - reddish contrast mottled 5 - dark contrast mottled	0– 1 (occasional adventitious crossveins) 1 –>4 (but rarely <10)	0 – absent (<6) 1 – numerous
	0 1	0 2	0 3	0 4	0 5	0 6	0 7	0 8
Australian/south east Asian taxa								
<i>Psychopsis barnardi</i>	1	0	4	1	1	-	1	1
<i>Psychopsis margarita</i>	1	0	4	0	1	-	1	1
<i>Balmes birmanus</i>	1	0	5	0	3	-	1	0
<i>Balmes terissinus</i>	1	0	5	0	3	-	1	0
Afrotropical taxa								
<i>Cabralis gloriosus</i>	1	1	1	0	0	0	1	1
<i>Cabralis puritus</i>	1	1	0	0	0	1	1	1
<i>Zygophlebius leoninus</i>	1	1	0	0	1	2	1	1
<i>Zygophlebius zebra</i>	1	1	0	0	1	2	1	1
<i>Zygophlebius pseudosilveira</i>	1	1	2	0	3	3	1	0
<i>Silveira jordani</i>	0	1	2	0	2	4	1	1
<i>Silveira rufus</i>	0	1	2	0	2	4	1	1
<i>Silveira occultus</i>	0	1	3	0	2	5	1	1
<i>Silveira marshalli</i>	0	1	3	0	2	5	1	1
Outgroup taxa								
<i>Polystoechotes punctatus</i>	2	0	-	-	-	-	0	0
<i>Notiobiella viridis</i>	2	0	-	-	-	-	0	0
<i>Apochrysa lutea</i>	2	0	-	-	-	-	0	0
<i>Ithone fulva</i>	2	0	-	-	-	-	0	0
<i>Nymphes myrmeleonides</i>	-	0	-	-	-	-	1	0
<i>Libelloides rhomboideus</i>	2	0	-	-	-	-	0	0
<i>Nemoptera coa</i>	2	0	-	-	-	-	0	0
<i>Osmylops armatus</i>	2	0	-	-	-	-	1	0
<i>Ululodes quadripunctatus</i>	2	0	-	-	-	-	0	0

Table S2: Matrix of morphological characters and states used in the study

	Thorax				Male terminalia				
	Pronotum patterning, broadly	Pronotum patterning, specifically	Pronotum Shape (dorsal view)	Body colour	8th somite, spiracles	8th sternite, posteromedian lobe	9th tergite, free posteroventral process	9th tergite, anterior margin of ectoproct articulation	9th tergite, costa bracing point of ectoproct articulation
	0 - 4 yellow spots and white median line	0 - pale spots/redish brown background	0 - rounded pronotum shape	0 - pale grey	0 – opening through pleural membrane	0 – absent	0 – absent	0 - anterior margin not hinged	0 – not braced by costa
	1 - white trident shape	1 - yellow spots/ dark background	1 - rectangular pronotum shape	1 - dark grey	1 – opening through sclerotized region of pleural membrane (continuous with 8th tergite)	1 – present	1 – present	1 - anterior margin broadly hinged to and articulate with the posterolateral margins of the 9th tergite	1 – braced by costa
	2 - streaky white/ long black transverse line w/ 2 spots (near indentation)	2 - small bulge in middle trident		2 - reddish brown					
		3 - large bulge in middle trident		3 - dark brown					
		4 - indistinct patterning		4 - yellow					
				5 - Pale testaceous					
				6 - dark brown testaceous	2 – opening through sclerotized free plate of pleural membrane (discontinuous with 8th tergite)				
	1	1	1	2	2	2	2	2	
	7	8	9	0	1	2	3	4	5
Australian/south east Asian taxa									
<i>Psychopsis barnardi</i>	-	-	0	5	1	0	0	1	0
<i>Psychopsis margarita</i>	-	-	0	5	1	0	0	1	0
<i>Balmes birmanus</i>	-	-	0	6	2	0	0	1	0
<i>Balmes terissinus</i>	-	-	0	6	1	0	0	1	0
Afrotropical taxa									
<i>Cabralis gloriosus</i>	1	2	0	1	1	1	1	1	0
<i>Cabralis puritus</i>	1	3	0	0	1	1	1	1	0
<i>Zygophlebius leoninus</i>	2	4	1	4	1	1	0	1	0
<i>Zygophlebius zebra</i>	2	4	1	4	1	1	0	1	0
<i>Zygophlebius pseudosilveira</i>	2	4	0	2	1	1	0	1	0
<i>Silveira jordani</i>	0	0	0	2	1	0	0	1	0
<i>Silveira rufus</i>	0	0	0	2	1	0	0	1	1
<i>Silveira occultus</i>	0	1	0	3	1	0	0	1	1
<i>Silveira marshalli</i>	0	1	0	3	1	0	0	1	1
Outgroup taxa									
<i>Polystoehotes punctatus</i>	-	-	-	-	0	0	0	0	-
<i>Notiobiella viridis</i>	-	-	-	-	0	0	0	0	-
<i>Apochrysa lutea</i>	-	-	-	-	0	0	0	0	-
<i>Ithone fulva</i>	-	-	-	-	0	0	0	0	-
<i>Nymphes myrmeleonides</i>	-	-	-	-	0	0	0	0	-
<i>Libelloides rhomboideus</i>	-	-	-	-	0	0	0	0	-
<i>Nemoptera coa</i>	-	-	-	-	0	0	0	0	-
<i>Osmylops armatus</i>	-	-	-	-	0	0	0	0	-
<i>Ululodes quadripunctatus</i>	-	-	-	-	0	0	0	0	-

Table S2: Matrix of morphological characters and states used in the study

Male terminalia								
9th tergite & sternite, articulation	9th sternite, anterolateral apodemes	9th sternite, anterolateral apodemes orientation	9th sternite, shape (ventral)	9th sternite lateral costa	9th sternite lateral costa, thickness	9th sternite, mesal spurs of lateral costae	9th sternite, configuration of apex, emargination presence	
0 – poorly developed 1 – well developed	0 – absent 1 – present	0 – situated dorsally from lateral midline of 9th sternite costa 1 – situated ventrally from lateral midline of 9th sternite costa	0 – not parallel sided or narrow 1 – narrow & parallel sided	0 – absent 1 – present	0 – thin/moderate 1 – thick /massive	0 – absent 1 – present	0 – apex without emargination 1 – apex with emargination (varied in size and form)	
	2	2	2	2	3	3	3	3
	6	7	8	9	0	1	2	3
Australian/south east Asian taxa								
<i>Psychopsis barnardi</i>	1	1	1	0	1	1	1	1
<i>Psychopsis margarita</i>	1	1	1	0	1	1	0	1
<i>Balmes birmanus</i>	1	1	1	0	1	0	0	0
<i>Balmes terissinus</i>	1	1	1	0	1	0	0	0
Afrotropical taxa								
<i>Cabralis gloriosus</i>	1	1	0	0	1	0	0	0
<i>Cabralis puritus</i>	1	1	0	0	1	0	0	0
<i>Zygophlebius leoninus</i>	1	1	0	0	1	0	0	1
<i>Zygophlebius zebra</i>	1	1	0	0	1	0	0	1
<i>Zygophlebius pseudosilveira</i>	1	1	0	0	1	0	0	0
<i>Silveira jordani</i>	1	1	0	1	1	0	0	1
<i>Silveira rufus</i>	1	1	0	1	1	0	0	1
<i>Silveira occultus</i>	1	1	0	1	0	0	0	1
<i>Silveira marshalli</i>	1	1	0	1	1	0	0	1
Outgroup taxa								
<i>Polystoehotes punctatus</i>	0	0	-	0	1	0	0	-
<i>Notiobiella viridis</i>	0	0	-	0	0	-	0	-
<i>Apochrysa lutea</i>	0	0	-	0	0	-	0	-
<i>Ithone fulva</i>	0	0	-	0	1	0	0	-
<i>Nymphes myrmeleonides</i>	0	0	-	0	0	-	0	0
<i>Libelloides rhomboideus</i>	0	0	-	0	0	-	0	-
<i>Nemoptera coa</i>	0	0	-	0	0	-	0	-
<i>Osmylops armatus</i>	0	0	-	0	0	-	0	-
<i>Ululodes quadripunctatus</i>	0	0	-	0	0	-	0	-

Table S2: Matrix of morphological characters and states used in the study

Male terminalia								
9th sternite, configuration of apex, emargination condition	9th sternite, subapical dorsomedial cavity	9th gonocoxites, ventral costae	9th gonocoxites, dorsolateral sclerotized plates, presence	9th gonocoxites, dorsolateral sclerotized plates, condition	9th gonocoxites, superprocesses, presence	9th gonocoxites, superprocesses, condition	9th gonocoxite, subprocesses	
0 – apex with shallow emargination setting off a pair of rounded parasagittal lobes	0 – absent 1 – present	0 – present 1 – absent	0 – absent 1 – present	0 – proximal portion not flared laterally 1 – proximal portion flared	0 – absent 1 – present	0 – pair of stout distal lobes 1 – pair of long, narrow, recurved processes	0 – absent 1 – present	
1 – apex with 2-4 protrudent lobes						2 – fused, transverse tumulus crossing fused gonocoxites distally		
	3	3	3	3	3	4	4	
	4	5	6	7	8	9	0	
Australian/south east Asian taxa								
<i>Psychopsis barnardi</i>	1	1	0	0	-	1	0	1
<i>Psychopsis margarita</i>	1	0	0	1	1	1	1	0
<i>Balmes birmanus</i>	-	0	1	1	1	1	2	0
<i>Balmes terissinus</i>	-	0	1	1	1	1	2	0
Afrotropical taxa								
<i>Cabralis gloriosus</i>	-	0	0	1	1	0	-	0
<i>Cabralis puritus</i>	-	0	0	1	1	0	-	0
<i>Zygophlebius leoninus</i>	0	0	0	1	2	0	-	0
<i>Zygophlebius zebra</i>	0	0	0	1	2	0	-	0
<i>Zygophlebius pseudosilveira</i>	-	0	0	1	2	0	-	0
<i>Silveira jordani</i>	0	0	0	1	1	0	-	0
<i>Silveira rufus</i>	0	0	0	1	1	0	-	0
<i>Silveira occultus</i>	0	0	0	1	1	0	-	0
<i>Silveira marshalli</i>	0	0	0	1	1	0	-	0
Outgroup taxa								
<i>Polystoechotes punctatus</i>	-	0	1	1	1	0	-	0
<i>Notiobiella viridis</i>	-	0	-	1	1	0	-	0
<i>Apochrysa lutea</i>	-	0	-	1	1	0	-	0
<i>Ithone fulva</i>	-	0	1	1	1	0	-	0
<i>Nymphes myrmeleonides</i>	-	0	0	1	1	0	-	0
<i>Libelloides rhomboideus</i>	-	0	0	1	1	0	-	0
<i>Nemoptera coa</i>	-	0	0	1	1	0	-	0
<i>Osmylops armatus</i>	-	0	0	0	-	0	-	0
<i>Ululodes quadripunctatus</i>	-	0	0	1	1	0	-	0

Table S2: Matrix of morphological characters and states used in the study

Male terminalia								
	9th gonocoxites, paired projecting venterolateral lobes	Ectoproct, posteroventral shape (lateral view)	Ectoproct Posteroventral shape, lower margin (ventral view)	Ectoproct Posteroventral shape, Inwards dilatation	Gonarcus, Extrahemigonal processes	Short stubble hairs on gonarcus	Hairs on mediuncus (likely to sense female copulatory fovea), presence	Hairs on mediuncus, amount (defined as length of mediuncus covered by hairs)
	0 – absent	0 – one apically rounded lobe	0 - simple (straight)	0 - inwards directed dilatation (broad)	0 – absent	0 - absent	0 - absent	0 - small tuft
	1 – present	1 – two narrowed lobes	1 - inwards directed dilatation	1 - inwards directed prong truncated at apex	1 – present	1 - present	1 - present	1 - medium tuft
			2 – two narrowed lobes					2 - large tuft
	4	4	4	4	4	4	4	4
	2	3	4	5	6	7	8	9
Australian/south east Asian taxa								
<i>Psychopsis barnardi</i>	0	1	2	-	0	1	1	0
<i>Psychopsis margarita</i>	0	0	0	-	0	1	1	0
<i>Balmes birmanus</i>	1	0	0	-	0	1	0	-
<i>Balmes terissinus</i>	0	0	0	-	0	1	0	-
Afrotropical taxa								
<i>Cabralis gloriosus</i>	0	0	0	-	0	1	1	0
<i>Cabralis puritus</i>	0	0	0	-	0	1	1	0
<i>Zygophlebius leoninus</i>	0	0	0	-	1	0	1	1
<i>Zygophlebius zebra</i>	0	0	0	-	1	0	1	1
<i>Zygophlebius pseudosilveira</i>	0	0	0	-	1	0	1	0
<i>Silveira jordani</i>	0	0	0	-	0	0	1	2
<i>Silveira rufus</i>	0	0	1	0	0	0	1	2
<i>Silveira occultus</i>	0	0	1	1	0	0	1	1
<i>Silveira marshalli</i>	0	0	1	0	0	0	1	1
Outgroup taxa								
<i>Polystoehotes punctatus</i>	0	0	0	-	0	0	0	-
<i>Notiobiella viridis</i>	0	0	0	-	0	0	0	-
<i>Apochrysa lutea</i>	0	0	0	-	0	0	0	-
<i>Ithone fulva</i>	0	0	0	-	0	0	0	-
<i>Nymphes myrmeleonides</i>	0	0	0	-	0	0	0	-
<i>Libelloides rhomboideus</i>	0	0	0	-	0	0	0	-
<i>Nemoptera coa</i>	0	0	0	-	0	0	0	-
<i>Osmylops armatus</i>	0	0	0	-	0	0	0	-
<i>Ululodes quadripunctatus</i>	0	0	0	-	0	0	0	-

Table S2: Matrix of morphological characters and states used in the study

Male terminalia								
Hairs on medicunus, surface position	Hairs on mediuncus, longitudinal position, broadly	Hairs on mediuncus, longitudinal position, specifically	Mediuncus, outline narrowing (dorsal view)	Mediuncus, relative tip length (dorsal view)	Mediuncus, proximodorsal lobes (lateral view), presence	Mediuncus, proximodorsal lobes (lateral view), condition	Mediuncus, proximodorsal lobes (dorsal view), groove length	
0 - On dorsal face of medicunus	0 - near or on apex	0 - slightly away from apex	0 - narrowed gradually towards apex	0 - tip shorter than base, stout	0 - no buldge/lobes in lateral view	0 - small round buldge/lobes visible in lateral view	0 - groove between lobes only	
1 - On ventral face of medicunus	1 - near middle or proximal tip	1 - on apex	1 - abruptly narrowed near mid-length	1 - tip longer than base	1 - buldge/lobes visible in lateral view	1 - small pointed buldge/lobes visible in lat view	1 - groove extending partially into apex of mediuncus	
2 - On lateral faces of mediuncus	2 - along entire mediuncus	2 - near middle/on lobes						
3 - on dorsal and ventral face		3 - on proximal tip					2 - groove extending fully into apex of mediuncus	
	5	5	5	5	5	5	5	5
	0	1	2	3	4	5	6	7
Australian/south east Asian taxa								
<i>Psychopsis barnardi</i>	3	0	0	0	-	0	-	-
<i>Psychopsis margarita</i>	0	0	2	0	-	1	0	1
<i>Balmes birmanus</i>	-	-	-	0	-	0	-	-
<i>Balmes terissinus</i>	-	-	-	0	-	0	-	-
Afrotropical taxa								
<i>Cabralis gloriosus</i>	1	0	1	0	1	0	-	-
<i>Cabralis puritus</i>	1	0	1	0	1	0	-	-
<i>Zygophlebius leoninus</i>	0	1	2	0	1	1	1	2
<i>Zygophlebius zebra</i>	0	0	0	0	1	1	1	2
<i>Zygophlebius pseudosilveira</i>	2	0	1	0	1	0	-	-
<i>Silveira jordani</i>	0	2	-	0	0	0	-	-
<i>Silveira rufus</i>	0	1	2	1	0	1	0	1
<i>Silveira occultus</i>	0	1	2	1	1	1	0	1
<i>Silveira marshalli</i>	0	1	2	1	1	1	0	0
Outgroup taxa								
<i>Polystoechotes punctatus</i>	-	-	-	0	-	0	-	-
<i>Notiobiella viridis</i>	-	-	-	0	-	0	-	-
<i>Apochrysa lutea</i>	-	-	-	0	-	0	-	-
<i>Ithone fulva</i>	-	-	-	0	-	0	-	-
<i>Nymphes myrmeleonides</i>	-	-	-	0	-	0	-	-
<i>Libelloides rhomboideus</i>	-	-	-	0	-	0	-	-
<i>Nemoptera coa</i>	-	-	-	0	-	0	-	-
<i>Osmylops armatus</i>	-	-	-	0	-	0	-	-
<i>Ululodes quadripunctatus</i>	-	-	-	0	-	0	-	-

Table S2: Matrix of morphological characters and states used in the study

Male terminalia								
	Mediuncus, (lateral view), shape	Mediuncus, (lateral view), decurvature extent	Mediuncus, distal portion decurvature (lateral view), shape	Mediuncus, distal portion recurvature (lateral view)	Mediuncus, apex (Lateral view)	Mediuncus, apodeme of adductor muscle, presence	Mediuncus, apodeme of adductor muscle, condition	Mediuncal accessory sclerites
	0 – linear or decurved 1 - recurved	0 - weakly decurved 1 - strongly decurved, forming hook	0 – strongly decurved, thick hook 1 - strongly decurved, thin hook	0 - recurved, recurvature near distal end 1 - recurvature near middle of mediuncus 2 - weakly recurved near middle	0 - apex unmodified 1 - distal point forming weakly downward directed lip 2 – distal point forming sharply downward directed lip	0 – absent 1 – present	0 – inserting proximoventrally on floor of mediuncus 1 - inserting at base of terminal mediuncal cleft	0 – absent 1 – present
	5	5	6	6	6	6	6	6
	8	9	0	1	2	3	4	5
Australian/south east Asian taxa								
<i>Psychopsis barnardi</i>	0	-	-	-	1	1	1	1
<i>Psychopsis margarita</i>	1	-	-	2	2	1	1	1
<i>Balmes birmanus</i>	0	-	-	-	0	1	1	1
<i>Balmes terissinus</i>	0	-	-	-	1	1	1	1
Afrotropical taxa								
<i>Cabralis gloriosus</i>	0	1	0	-	1	1	0	1
<i>Cabralis puritus</i>	0	1	1	-	1	1	0	1
<i>Zygophlebius leoninus</i>	1	-	-	1	2	1	0	1
<i>Zygophlebius zebra</i>	1	-	-	0	2	1	0	1
<i>Zygophlebius pseudosilveira</i>	0	-	-	2	2	1	0	1
<i>Silveira jordani</i>	0	0	-	-	1	1	0	1
<i>Silveira rufus</i>	0	0	-	-	1	1	0	1
<i>Silveira occultus</i>	0	0	-	-	1	1	0	1
<i>Silveira marshalli</i>	1	0	-	-	1	1	0	1
Outgroup taxa								
<i>Polystoechotes punctatus</i>	0	0	-	-	0	0	-	0
<i>Notiobiella viridis</i>	0	0	-	-	0	0	-	0
<i>Apochrysa lutea</i>	0	0	-	-	0	0	-	0
<i>Ithone fulva</i>	0	0	-	-	0	0	-	0
<i>Nymphes myrmeleonides</i>	0	0	1	-	0	0	-	0
<i>Libelloides rhomboideus</i>	1	-	-	2	0	0	-	0
<i>Nemoptera coa</i>	1	-	-	2	0	0	-	0
<i>Osmylops armatus</i>	0	-	-	-	0	0	-	0
<i>Ululodes quadripunctatus</i>	1	-	-	2	0	0	-	0

Table S2: Matrix of morphological characters and states used in the study

	Male terminalia					Female terminalia		
	Gonosacacal membrane, rounded spiculate lobes	7th sternite, posterior margin	Copulatory fovea, shape	Copulatory fovea, Cusp of Carina (ridge)	Copulatory fovea, Cusp of Carina (ridge), condition	Copulatory fovea, Cusp of Carina (ridge), shape	Copulatory fovea, Projection on sigattal Carina	Copulatory fovea, Projection on sigattal Carina, development
	0 – absent	0 – transverse & simple (copulatory fovea absent)	0 - spheroid	0 - absent	0 - thick	0 - straight, tapering	0 - absent	0 - short
	1 – present	1 – With a medial emargination (copulatory fovea present)	1 - posteriorly directed cavity placed internally in lateral view, formed with 8th sternite	1 - present	1 - projecting 2 - incipient	1 - bifurcating	1 - present	1 - long 2 - incipient
			2 - Furrow-like groove 3 - Pit divided by ridge					
	6	6	6	6	7	7	7	7
	6	7	8	9	0	1	2	3
Australian/south east Asian taxa								
<i>Psychopsis barnardi</i>	0	1	1	0	-	-	0	-
<i>Psychopsis margarita</i>	0	1	1	0	-	-	0	-
<i>Balmes birmanus</i>	1	1	1	0	-	-	0	-
<i>Balmes terissinus</i>	1	1	1	0	-	-	0	-
Afrotropical taxa								
<i>Cabralis gloriosus</i>	0	1	1	0	-	-	0	-
<i>Cabralis puritus</i>	0	1	1	0	-	-	0	-
<i>Zygophlebius leoninus</i>	0	1	2	1	1	0	1	1
<i>Zygophlebius zebra</i>	0	1	2	1	1	0	1	0
<i>Zygophlebius pseudosilveira</i>	0	1	0	1	1	0	1	2
<i>Silveira jordani</i>	0	1	2	0	-	-	0	-
<i>Silveira rufus</i>	0	1	3	1	2	0	0	-
<i>Silveira occultus</i>	0	1	3	1	0	1	0	-
<i>Silveira marshalli</i>	0	1	3	1	0	0	0	-
Outgroup taxa								
<i>Polystoehotes punctatus</i>	0	0	-	-	-	-	0	-
<i>Notiobiella viridis</i>	0	0	-	-	-	-	0	-
<i>Apochrysa lutea</i>	0	0	-	-	-	-	0	-
<i>Ithone fulva</i>	0	0	-	-	-	-	0	-
<i>Nymphes myrmeleonides</i>	0	0	-	-	-	-	0	-
<i>Libelloides rhomboideus</i>	0	0	-	-	-	-	0	-
<i>Nemoptera coa</i>	0	0	-	-	-	-	0	-
<i>Osmylops armatus</i>	0	0	-	-	-	-	1	2
<i>Ululodes quadripunctatus</i>	0	0	-	-	-	-	0	-

Table S2: Matrix of morphological characters and states used in the study

Female terminalia									
7th & 8th sternites	8th Sternite, condition	8th Sternite, medial cusp of anterior margin, presence	8th & 9th tergites	9th tergite, anterolateral apodemes	9th tergite, pilose posteroventral border	9th tergite, distal apodemes	9th gonocoxite, overall shape	9th gonocoxite, longitudinal costa	
0 – not fused (reduced)	0 – hemiannular, unmodified (outgroup)	0 – absent (or 8th sternite reduced)	0 – not fused	0 – absent	0 – absent	0 – absent	0 – reniform	0 – absent	
1 – fused	1 – small compact median sclerite with 1 or 2 distal lobes 2 – narrow, transversely elongated sclerite 3 – bilaterally symmetrical triangular sclerite	1 – present	1 – fused	1 – present	1 – present	1 – present	1 – spathulate	1 – present	
7	7	7	7	7	7	8	8	8	
4	5	6	7	8	9	0	1	2	
Australian/south east Asian taxa									
<i>Psychopsis barnardi</i>	0	3	1	1	1	1	1	1	1
<i>Psychopsis margarita</i>	0	3	1	1	1	1	1	1	1
<i>Balmes birmanus</i>	1	2	0	1	1	1	1	1	1
<i>Balmes terissinus</i>	1	2	0	1	1	1	1	1	1
Afrotropical taxa									
<i>Cabralis gloriosus</i>	0	1	0	1	1	1	1	1	1
<i>Cabralis puritus</i>	0	1	0	1	1	1	1	1	1
<i>Zygophlebius leoninus</i>	0	1	0	1	1	1	1	1	1
<i>Zygophlebius zebra</i>	0	1	0	1	1	1	1	1	1
<i>Zygophlebius pseudosilveira</i>	0	1	0	1	1	1	1	1	1
<i>Silveira jordani</i>	0	2	0	1	1	1	1	1	1
<i>Silveira rufus</i>	0	2	0	1	1	1	1	1	1
<i>Silveira occultus</i>	0	2	0	1	1	1	1	1	1
<i>Silveira marshalli</i>	0	2	0	1	1	1	1	1	1
Outgroup taxa									
<i>Polystoehotes punctatus</i>	0	0	0	0	0	0	0	0	0
<i>Notiobiella viridis</i>	0	0	0	0	0	0	0	0	0
<i>Apochrysa lutea</i>	0	0	0	0	0	0	0	0	0
<i>Ithone fulva</i>	0	0	0	0	0	0	0	0	0
<i>Nymphes myrmeleonides</i>	0	0	0	0	0	0	0	0	1
<i>Libelloides rhomboideus</i>	0	0	0	0	0	0	0	0	0
<i>Nemoptera coa</i>	0	0	0	0	0	0	0	0	0
<i>Osmylops armatus</i>	0	0	0	0	0	0	0	0	1
<i>Ululodes quadripunctatus</i>	0	0	0	0	0	0	0	0	0

Table S2: Matrix of morphological characters and states used in the study

Female terminalia								
9th gonocoxite, longitudinal row of stiff setae	9th gonocoxite, aggregation of setae near insertion of stylus	9th gonocoxite, suprastylar setae, presence	9th gonocoxite, suprastylar setae, composition proportion	9th gonocoxite stylus, diggnng setae	9th gonocoxite stylus, diggnng setae, condition	Bursa, number of bursal accessory glands	Bursa, lateral corniform diverticulae, presence	
0 – absent	0 – absent	0 – all setae acuminate and apically unmodified	0 – mostly unmodified with a few cochleariform (spoon-shaped) setae ventrally	0 – absent	0 – long, sharp	0 – 1 unpaired duct	0 – absent	
1 – present	1 – present	1 – cochleariform (spoon-shaped) setae present	1 – mostly cochleariform setae with a few unmodified setae dorsally	1 – present	1 – short, stout	1 – 1 pair of ducts 2 – 2 pairs of ducts	1 – present	
8	8	8	8	8	8	8	8	9
3	4	5	6	7	8	9	0	
Australian/south east Asian taxa								
<i>Psychopsis barnardi</i>	0	1	1	1	1	1	1	0
<i>Psychopsis margarita</i>	0	1	1	1	1	1	1	0
<i>Balmes birmanus</i>	0	0	1	1	1	1	0	0
<i>Balmes terissinus</i>	0	0	1	1	1	1	1	0
Afrotropical taxa								
<i>Cabralis gloriosus</i>	0	0	1	1	1	1	1	1
<i>Cabralis puritus</i>	0	0	1	1	1	1	1	1
<i>Zygophlebius leoninus</i>	0	0	1	1	1	1	2	1
<i>Zygophlebius zebra</i>	0	0	1	1	1	1	2	1
<i>Zygophlebius pseudosilveira</i>	0	0	1	1	1	1	1	1
<i>Silveira jordani</i>	1	0	1	0	1	0	1	0
<i>Silveira rufus</i>	1	0	1	0	1	0	1	0
<i>Silveira occultus</i>	1	0	1	0	1	0	1	0
<i>Silveira marshalli</i>	1	0	1	0	1	0	1	0
Outgroup taxa								
<i>Polystoehotes punctatus</i>	0	0	0	-	0	-	1	0
<i>Notiobiella viridis</i>	0	0	0	-	0	-	1	0
<i>Apochrysa lutea</i>	0	0	0	-	0	-	1	0
<i>Ithone fulva</i>	0	0	0	-	0	-	1	0
<i>Nymphes myrmeleonides</i>	0	0	0	-	0	-	1	0
<i>Libelloides rhomboideus</i>	0	0	0	-	0	-	0	0
<i>Nemoptera coa</i>	0	0	0	-	0	-	0	0
<i>Osmylops armatus</i>	0	0	0	-	0	-	1	0
<i>Ululodes quadripunctatus</i>	0	0	0	-	0	-	0	0

Table S2: Matrix of morphological characters and states used in the study

Female terminalia								
	Bursa, lateral corniform diverticulae, size	Spermatheca, ventrolateral lobes, presence	Spermatheca, ventrolateral lobes, shape	Spermatheca, ventrolateral lobes, position	Spermatheca, ventrolateral lobes, curvature	spermatheca, lamellate apodemes	Posterior abdominal chamber, presence	Posterior abdominal chamber, contents
	0 - incipient	0 - absent	0 - broad in lateral view	0 - placed far from apex	0 - directed inwards (arms-folded)	0 - absent	0 - absent	0 - filled with minerals
	1 - small	1 - preset				1 - present	1 - present	
	2 - large		1 - narrow and arm-like in lateral view	1 - placed close to apex	1 - directed forwards (arms straight)			1 - filled with wood/moss
					2-directed outwards (arms open)			
	9	9	9	9	9	9	9	9
	1	2	3	4	5	6	7	8
Australian/south east Asian taxa								
<i>Psychopsis barnardi</i>	-	0	-	-	-	1	1	1
<i>Psychopsis margarita</i>	-	0	-	-	-	1	1	1
<i>Balmes birmanus</i>	-	0	-	-	-	0	1	1
<i>Balmes terissinus</i>	-	0	-	-	-	0	1	1
Afrotropical taxa								
<i>Cabralis gloriosus</i>	0	1	0	0	-	0	1	1
<i>Cabralis puritus</i>	0	1	0	0	-	0	1	1
<i>Zygophlebius leoninus</i>	2	1	0	0	-	0	1	1
<i>Zygophlebius zebra</i>	2	1	0	0	-	0	1	1
<i>Zygophlebius pseudosilveira</i>	1	1	0	0	-	0	1	1
<i>Silveira jordani</i>	-	1	1	0	2	0	1	0
<i>Silveira rufus</i>	-	1	1	0	2	0	1	0
<i>Silveira occultus</i>	-	1	1	1	0	0	1	0
<i>Silveira marshalli</i>	-	1	1	0	1	0	1	0
Outgroup taxa								
<i>Polystoehotes punctatus</i>	-	0	-	-	-	0	0	-
<i>Notiobiella viridis</i>	-	0	-	-	-	0	0	-
<i>Apochrysa lutea</i>	-	0	-	-	-	0	0	-
<i>Ithone fulva</i>	-	0	-	-	-	0	0	-
<i>Nymphes myrmeleonides</i>	-	0	-	-	-	0	0	-
<i>Libelloides rhomboideus</i>	-	0	-	-	-	0	0	-
<i>Nemoptera coa</i>	-	0	-	-	-	0	0	-
<i>Osmylops armatus</i>	-	0	-	-	-	0	0	-
<i>Ululodes quadripunctatus</i>	-	0	-	-	-	0	0	-

Table S3. Table showing primer sequences and cycling parameters used in this study.

Gene	Primer	Direction	Primer sequence (5'→ 3')	Reference	Cycling Parameters
16S	LR-J-12887 LR-N-13398	forward reverse	CCG GTC TGA ACT CAG ATC ACG T CGC CTG TTT AAC AAA AAC AT	Simon <i>et al.</i> 1994	Initial denaturation: 95°C (5min); 33 cycles of 93°C (20s), 50±2°C (40s), 72°C (2min); final elongation: 72°C (5min)
18S	18S-intfw-ST12 18S-rev1	forward reverse	ATC AAG AAC GAA AGT TAG AG ATG GGG AAC AAT TGC AAG C	Simon <i>et al.</i> 1994	Initial denaturation: 94°C (2min); 30 cycles of 95°C (10s), 48±2°C (10s), 72°C (1min); final elongation: 72°C (5min)
COI	C1-J-2183 TL2-N-3014	forward reverse	CAA CAT TTA TTT TGA TTT TTT GG TCC AAT GCA CTA ATC TGC CAT ATT A	Simon <i>et al.</i> 1994	Initial denaturation: 95°C (5min); 33 cycles of 93°C (20s), 50±2°C (40s), 72°C (2min); final elongation: 72°C (5min)
CAD	581F2 1098r	forward reverse	GGW GGW CAA ACW GCW YTM AAY TGY GG TTN GGN AGY TGN CCN CCC AT	Moulton & Wiegmann 2004	Initial denaturation: 94°C (4min); [4 cycles of 94°C (30s), 51±2°C (30s), 72°C (2min)], [6 cycles of 94°C (30s), 47±2°C (1min), 72°C (2min)], [36 cycles of 94°C (30s), 42±2°C (20s), 72°C (2:30min)]; final elongation: 72°C (3min)
	787f 1278r	forward reverse	GGD GTN ACN ACN GCN TGY TTY GAR CC TCR TTN TTY TTW GCR ATY AAY TGC AT	Moulton & Wiegmann 2004	Initial denaturation: 94°C (4min); [4 cycles of 94°C (30s), 51±2°C (30s), 72°C (2min)], [6 cycles of 94°C (30s), 47±2°C (1min), 72°C (2min)], [36 cycles of 94°C (30s), 42±2°C (20s), 72°C (2:30min)]; final elongation: 72°C (3min)

Table S4. Model parameters used in maximum likelihood, Bayesian and divergence time estimates analyses (above), and sequencing information for each gene partition (below).

Parameter	16S	18S	COI	CAD
Best-fit model (AIC)	GTR+I+G	TVM+I+G	TIM2+I+G	TIM3+I+G
A frequency	0.3972	0.2245	0.3089	0.3596
C frequency	0.1267	0.2009	0.1114	0.1348
G frequency	0.0831	0.2780	0.1375	0.1772
T frequency	0.3931	0.2966	0.4422	0.3284
Gamma shape	1.1010	0.3110	1.1410	0.3570
p-inv	0.4120	0.4740	0.5580	0.2120
Base pairs	≈450	≈1006	747	1543
Conserved sites	242	628	493	910
Parsimony-informative sites	150	166	202	478

Table S5. Model parameters used in ancestral area estimation analysis.

Analysis model	Data Input	Areas	Range Constraints
S-DIVA	15 000 trees + 1 condensed tree (from BEAST)	A: Australia B: SE Africa C: SW Africa D: SE Asia	All ranges checked in ancestral range matrix. Max areas = 2. Extinction allowed.
BayArea	1 condensed tree (from BEAST)	A: -20.9175, 142.7027 B: -23.8320, 30.1358 C: -23.5618, 15.3041 D: 24.8801, 102.8329	Geodistance power = True Chain length 500 000 000 sampling every 1000 th iteration