

SUPPLEMENTARY MATERIALS OF

***Osmooka*, a new spider genus from Madagascar: a surprising relative of the Australian fauna (Araneae: Paraplectanoididae)**

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Additional Materials and Methods

To retest the phylogenetic placement of *Venomius* Rossi, Castanheira, Baptista & Framenau, 2023, we reanalyzed the large araneid Sanger matrix of Scharff et al. (2020), which included five genetic markers: cytochrome c oxidase subunit I (COI), 16S rRNA (16S), 28S rRNA (28S), 18S rRNA (18S), and Histone H3 (H3). We added additional sequences of some terminals not used in Scharff et al. (2020), e.g., two *Cyphalonotus* Simon, 1895 species (Yu et al. 2022), *Megaraneus gabonensis* (Lucas, 1858) (own data), and *Venomius tomhardyi* Rossi, Castanheira, Baptista & Framenau, 2023. The latter was sequenced for COI, 16S, 28S, and 18S and provided in unpublished form (Pedro de Souza Castanheira, personal comm.). We edited the sequences in Geneious Prime v.2019.2.3 and aligned the matrix of each gene

with MAFFT v.7 (Katoh et al. 2019, available from <https://mafft.cbrc.jp/alignment/server/index.html>). We then concatenated matrices of all genes using Mesquite v.3.81 (Maddison and Maddison 2023).

We first partitioned the matrix into six sections following Scharff et al. (2020), then reconstructed phylogenies using maximum likelihood (ML) and Bayesian inference (BI). We used IQTREE v.2.3.2 (Minh et al. 2020) to perform ML analysis. We set the substitution model as follows, suggested by ModelFinder (Kalyaanamoorthy et al. 2017), COI: GTR+F+I+R6; 16S: GTR+F+I; 28Sc: TIM3+F+I+R4; 28Sv: TVM+F+R5; 18S: TNe+FQ+I+R4; H3: TPM2+FQ+R4. We obtained node supports from 1,000 replicates of ultrafast bootstrap approximation (UFBoot; Hoang et al. 2017, Minh et al. 2013) and SH-like approximate likelihood ratio test (SH-aLRT; Guindon et al. 2010). We performed two BI analyses in MrBayes (Huelsenbeck and Ronquist 2001) via the CIPRES Science Gateway (Miller et al. 2010), each with two million generations and a 20% burn-in, one using GTR model and the other using simple model.

Supplementary Table

Table S1. GenBank accession numbers for the raw reads of the terminals used in phylogenomic analyses.

Taxon	Accession
<i>Anapistula</i> sp.	SRR10291857
<i>Anelosimus analyticus</i>	SRR10291873
<i>Baalzebub</i> sp.	SRR10291818
<i>Cyclosa conica</i>	SAMN50253285
<i>Deinopis</i> sp.	SRR10291868
<i>Euryopis</i> sp.	SRR10291866
<i>Frontinella pyramitela</i>	SRR10291865
<i>Gelanor</i> sp.	SRR10291864
<i>Herennia multipuncta</i>	SAMN50253286
<i>Laminacauda</i> sp.	SRR10291858
<i>Leucauge venusta</i>	SRR10291856
Malkaridae GH09 sp.	SRR10291855
<i>Megadictyna</i> sp.	SRR10291852
<i>Microdipoena guttata</i>	SRR10291851
<i>Nephila pilipes</i>	SAMN50253287
<i>Nephilengys malabarensis</i>	SAMN50253288
<i>Nephilingis cruentata</i>	SAMN50253289
<i>Nesticella</i> sp.	SRR10291849
<i>Patu</i> sp.	SRR10291844
<i>Pimoa breviata</i>	SRR10291842
<i>Rungo</i> cf. <i>flora</i>	SRR10291846
<i>Synotaxus</i> sp.	SRR10291835
<i>Tekelloides</i> sp.	SRR10291834
<i>Theridiosoma</i> sp.	SRR10291833
<i>Trichonephila fenestrata</i>	SAMN50253290
<i>Araneus marmoreus</i>	SRR10291862
<i>Argiope lobata</i>	SAMN50253291
<i>Australomimetes</i> sp.	SRR10291839
<i>Caerostris sexcuspidata</i>	SRR10291817

<i>Leviellus thorelli</i>	SAMN50253292
<i>Phonognatha graeffei</i>	SAMN50253293
<i>Poltys</i> sp.	SAMN50253294
<i>Trichonephila antipodiana</i>	SAMN50253295
<i>Zygiella</i> sp.	SRR10291827
<i>Crassanapis chilensis</i>	SRR10291870
<i>Arkys</i> sp.	SRR10291850
<i>Paraplectanoides crassipes</i>	SRR10291845
<i>Singafrotypa</i> sp.	SAMN50253296
<i>Singa hamata</i>	SAMN50253297
<i>Anapisona hamigera</i>	SRR10291824
<i>Cyatholipus</i> sp.	SRR10291823
Physoglenidae	SRR10291821
<i>Anapis</i> sp.	SRR10291825
<i>Clitaetra irenae</i>	SAMN50253298
<i>Osmooka aphana</i>	SAMN50253299

Supplementary Figures

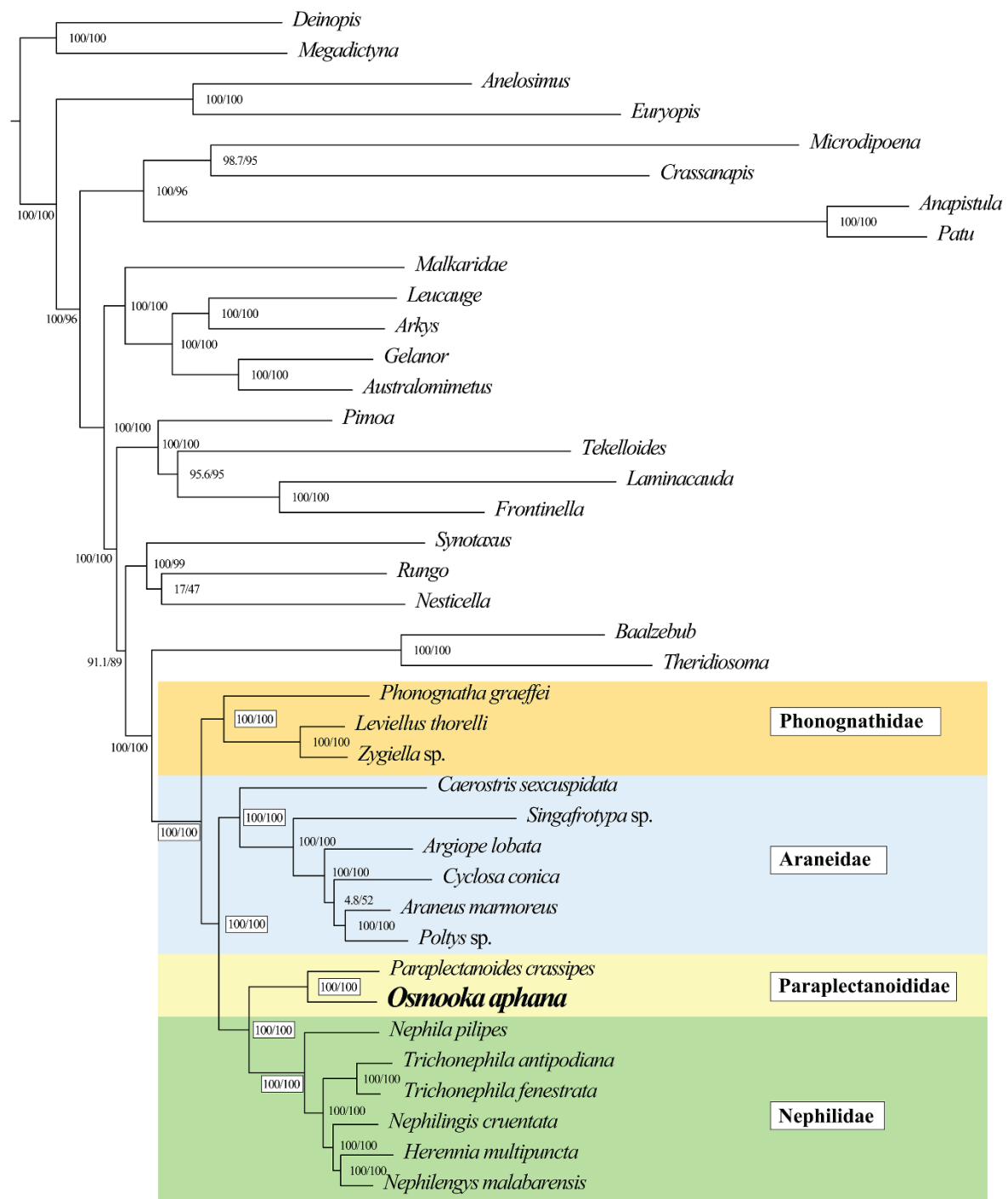


Figure S1. The UCE-based phylogenetic placement of *Osmooka aphana* gen. nov., sp. nov. is recovered with full support as sister to the Australian *Paraplectanoides* Keyserling, 1886. The tree was inferred in IQTREE model finder with partition merge along with UF bootstrap and SH-aLRT (both values on nodes). Relevant family level clades are color coded as Paraplectanoididae Kuntner, Coddington, Agnarsson & Bond, 2023; Nephilidae Simon, 1894; Araneidae Clerck, 1757; Phonognathidae Simon, 1894. See Taxonomy and Fig. 3 for details.

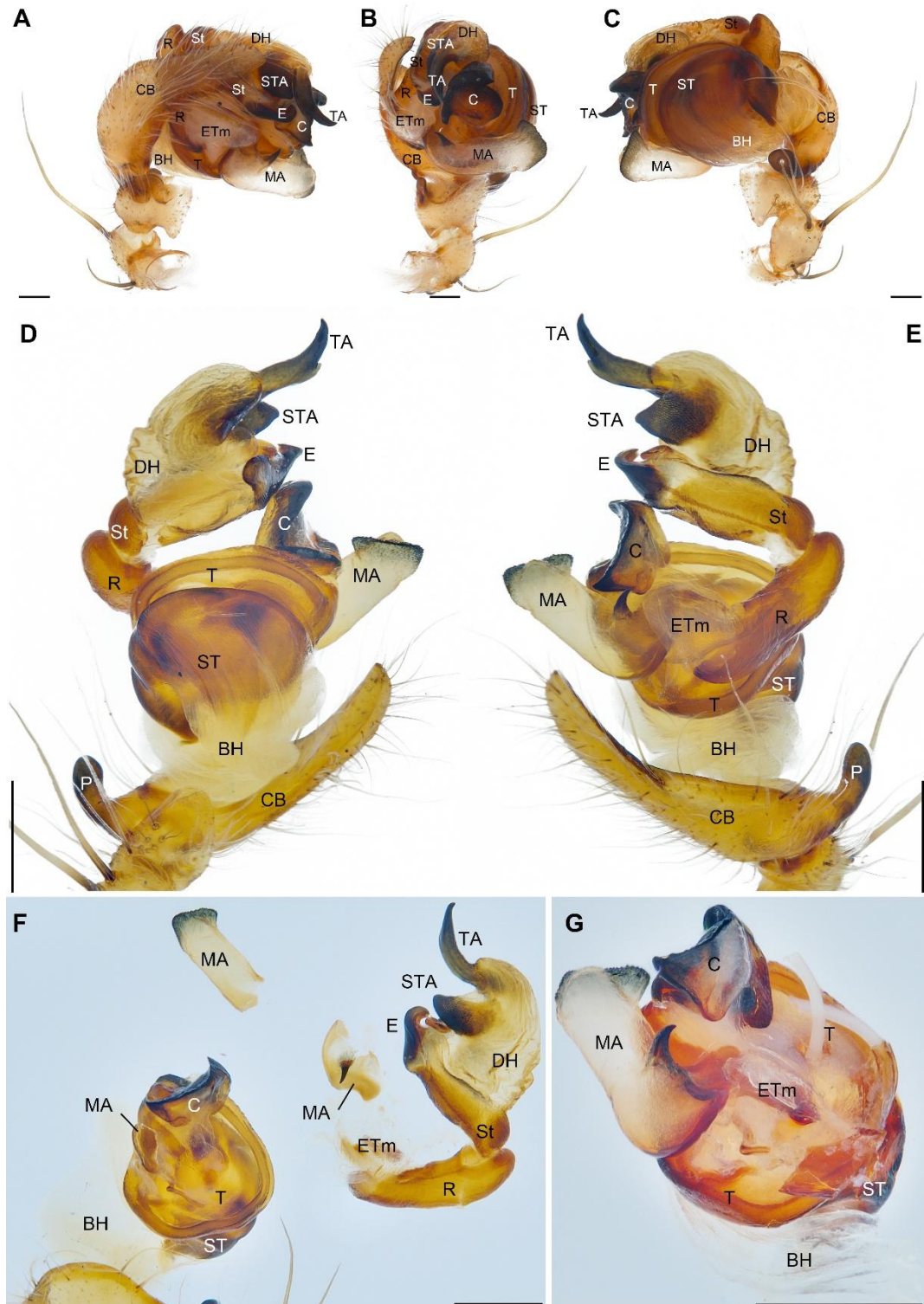


Figure S2. Palp of *Araneus diadematus* (Clerck, 1757) (Araneidae). A-C, intact left palp in mesal (A), apical (B) and ectal (C) views; D-E, treated and expanded right palp in mesal (D) and ectal (E) views; F, dissected, expanded right palp after removing the embolic division, showing a part of the median apophysis still connected to the tegulum; G, dissected, unexpanded left palp after removing the embolic division, showing the entire median apophysis connected to the tegulum. Scale bars: 0.5 mm.

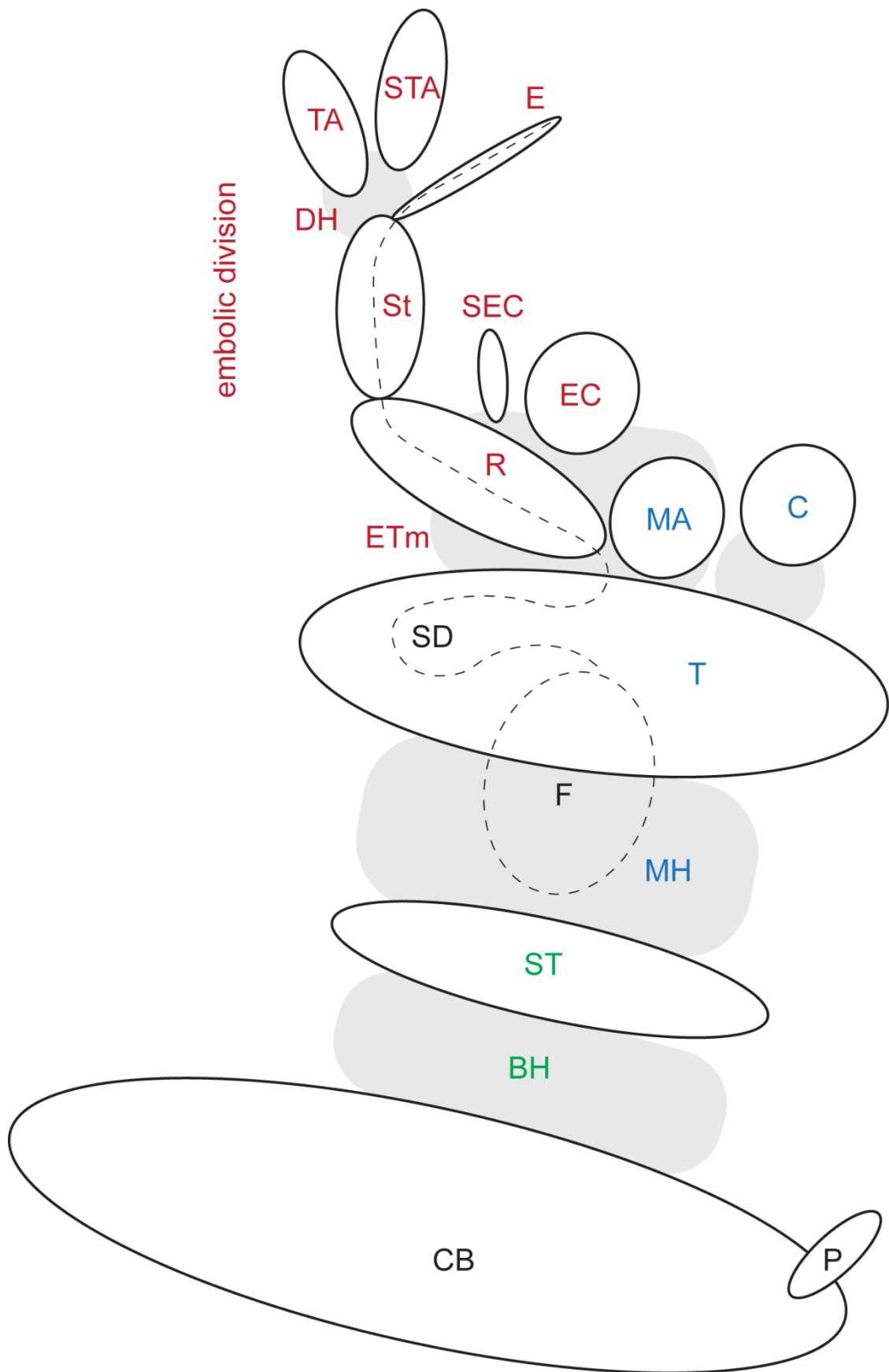


Figure S3. Palpal features that can be present in Orbipurae. The schematic drawing is of a hypothetical taxon/palp. See Results for details of the homology analysis.

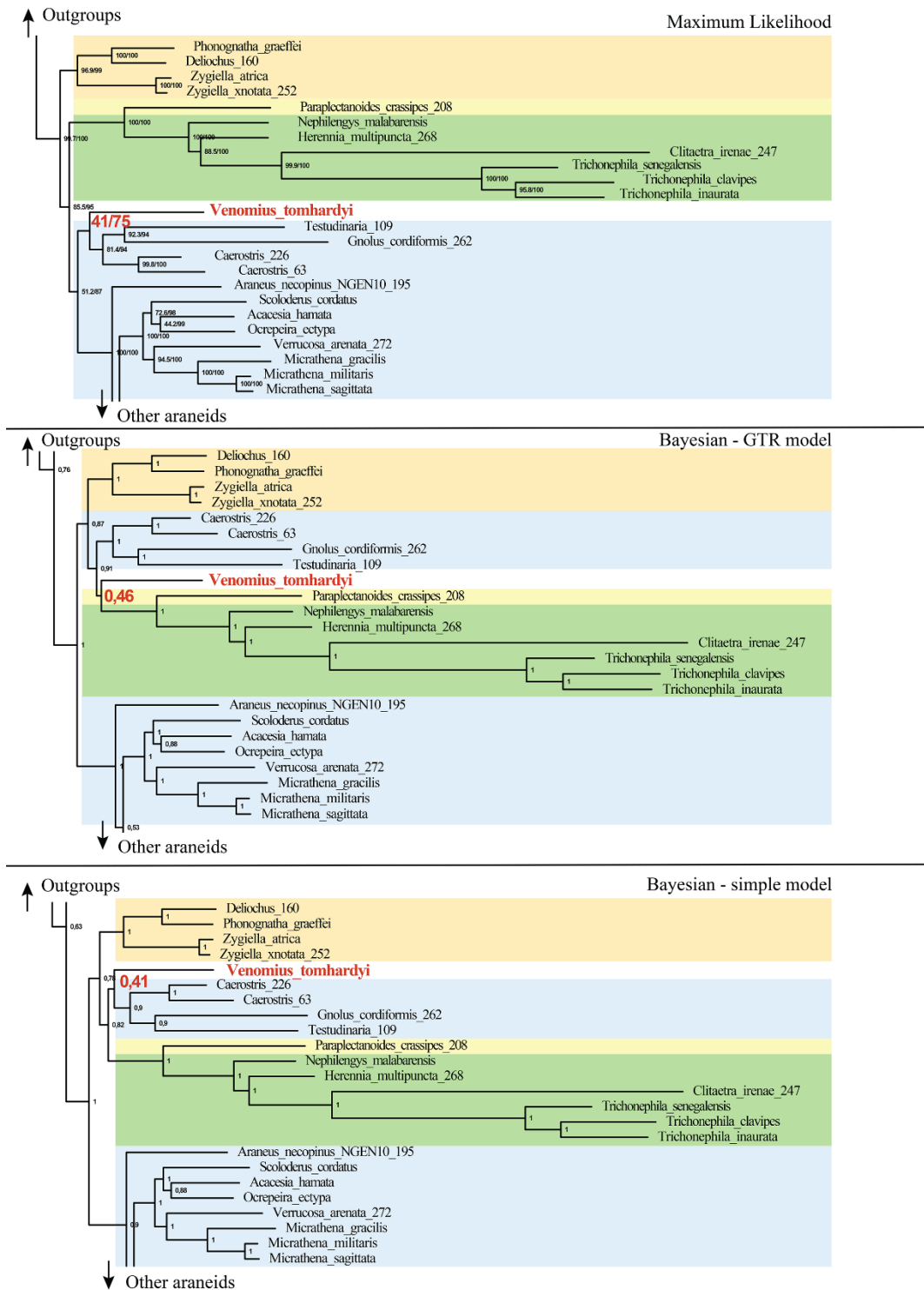


Figure S4. Re-analyses of the araneid five gene Sanger matrix from Scharff et al. (2020) with additional sequences. Analyses used maximum likelihood (tree above), as well as Bayesian inference using GTR model (tree in center) and simple model (tree below). These trees show discordance in the hypothetical placement of *Venomius* Rossi, Castanheira, Baptista & Framenau, 2023 and inadequate node supports (red fonts). Clade color codes match those in Fig. 3.



Figure S5. Palp of *Trichonephila antipodiana* (Walckenaer, 1841) (MK_116446441, NIB) (Nephilidae). A-B, unexpanded left palp in mesal (A) and ectal (B) views; C-F, treated and expanded palp in complementary views to reveal the relationships among sclerites and membranes. Scale bars: 0.2 mm.



Figure S6. Palp of *Caerostris darwini* Kuntner & Agnarsson, 2010 (ARA_8093, NIB) (Araneidae). A-C, unexpanded left palp in mesal (A), ventral (B), and ectal (C) views; D-G, treated and expanded palp in complementary views to reveal the relationships among sclerites and membranes. Scale bars: 0.2 mm.

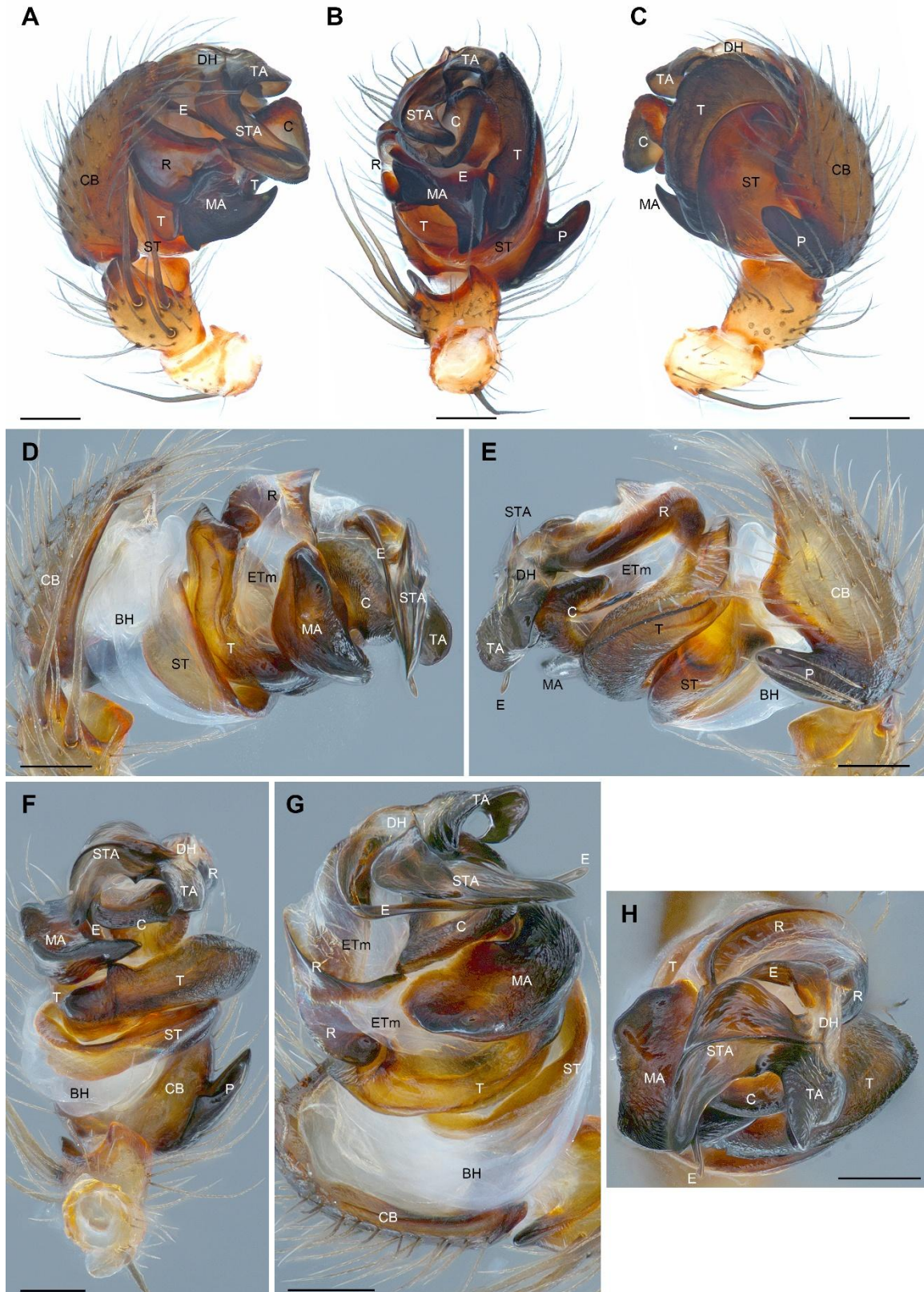


Figure S7. Palp of *Venomius tomhardyi* Rossi, Castanheira, Baptista & Framenau, 2023 (HB_N32479_4, NIB) (cf. Araneidae). A-C, unexpanded left palp in mesal (A), ventral (B), and ectal (C) views; D-H, treated and expanded palp in complementary views to reveal the relationships among sclerites and membranes. Scale bars: 0.2 mm.



Figure S8. Palp of *Artiphex melanopyga* (L. Koch, 1871) (QM_S86546, QM) (Phonognathidae). A-C, unexpanded left palp in mesal (A), ventral (B), and ectal (C) views; D-F, treated and expanded palp in complementary views to reveal the relationships among sclerites and membranes. Scale bars: 0.2 mm.

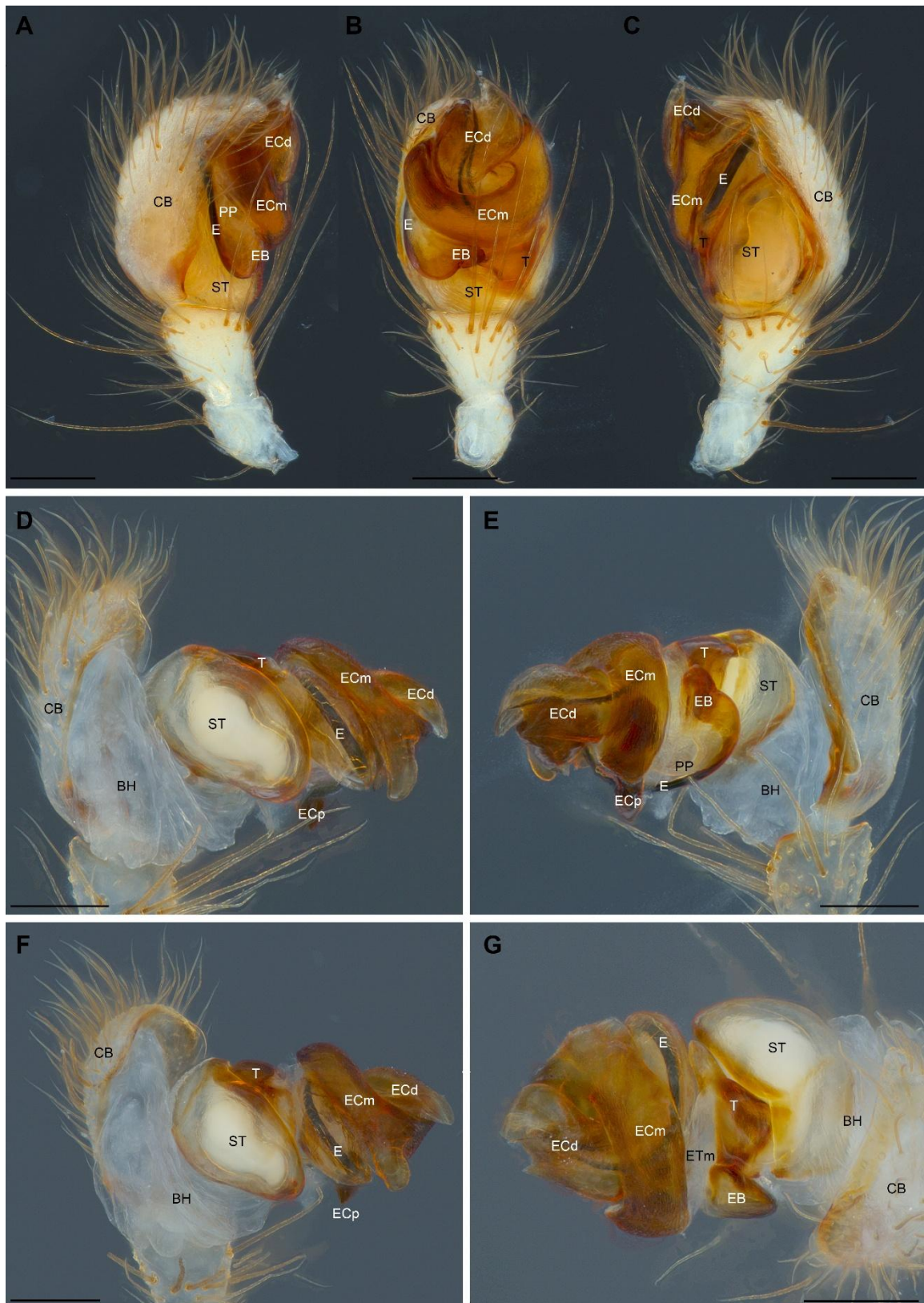


Figure S9. Palp of *Deliochus humilis* (L. Koch, 1867) (QM_S56345, QM) (Phonognathidae). A-C, unexpanded right palp (mirrored) in mesal (A), ventral (B), and ectal (C) views; D-G, treated and expanded palp in complementary views to reveal the relationships among sclerites and membranes. Scale bars: 0.2 mm.

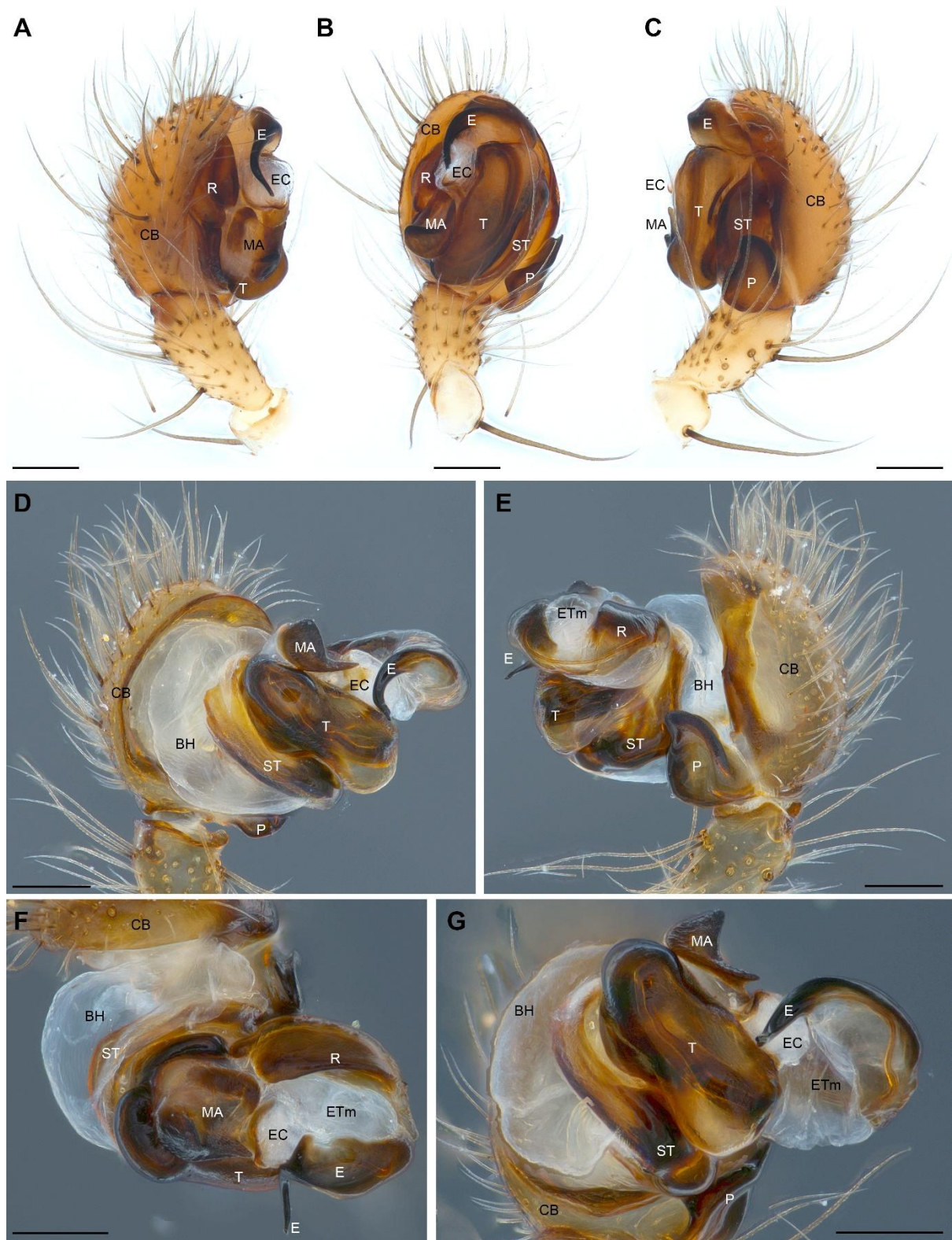


Figure S10. Palp of *Zygiella x-notata* (Clerck, 1757) (ARA_8061, NIB) (Phonognathidae). A-C, unexpanded left palp in mesal (A), ventral (B), and ectal (C) views; D-G, treated and expanded palp in complementary views to reveal the relationships among sclerites and membranes. Scale bars: 0.2 mm.



Figure S11. Palp of *Leviellus thorelli* (Ausserer, 1871) (MK_LC_collection, NIB) (Phonognathidae). A-C, unexpanded left palp in mesal (A), ventral (B), and ectal (C) views; D-F, treated and expanded palp in complementary views to reveal the relationships among sclerites and membranes. Scale bars: 0.2 mm.

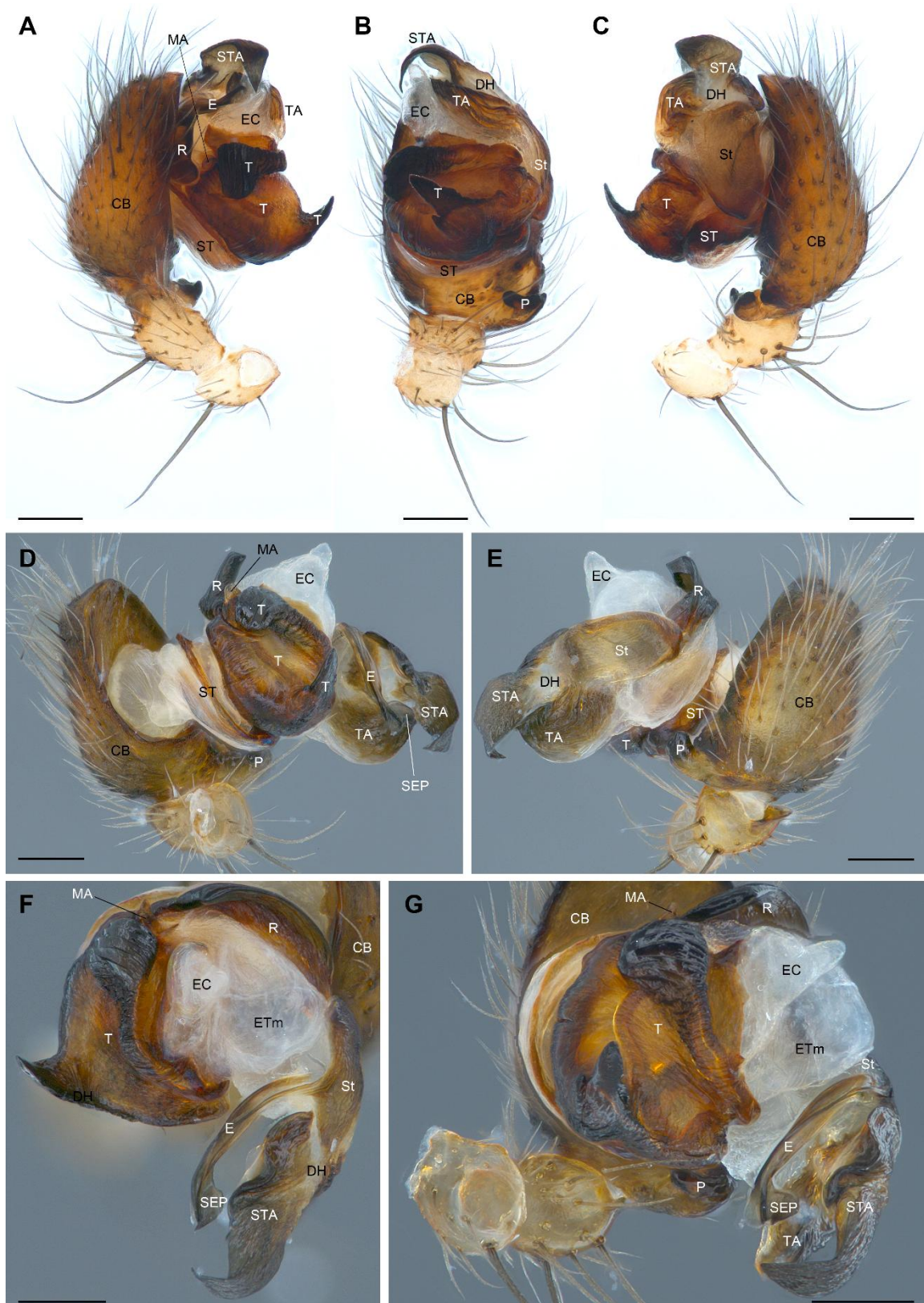


Figure S12. Palp of *Leviellus stroemi* (Thorell, 1870) (ARA_8382, NIB) (Phonognathidae). A-C, unexpanded left palp in mesal (A), ventral (B), and ectal (C) views; D-G, treated and expanded palp in complementary views to reveal the relationships among sclerites and membranes. Scale bars: 0.2 mm.

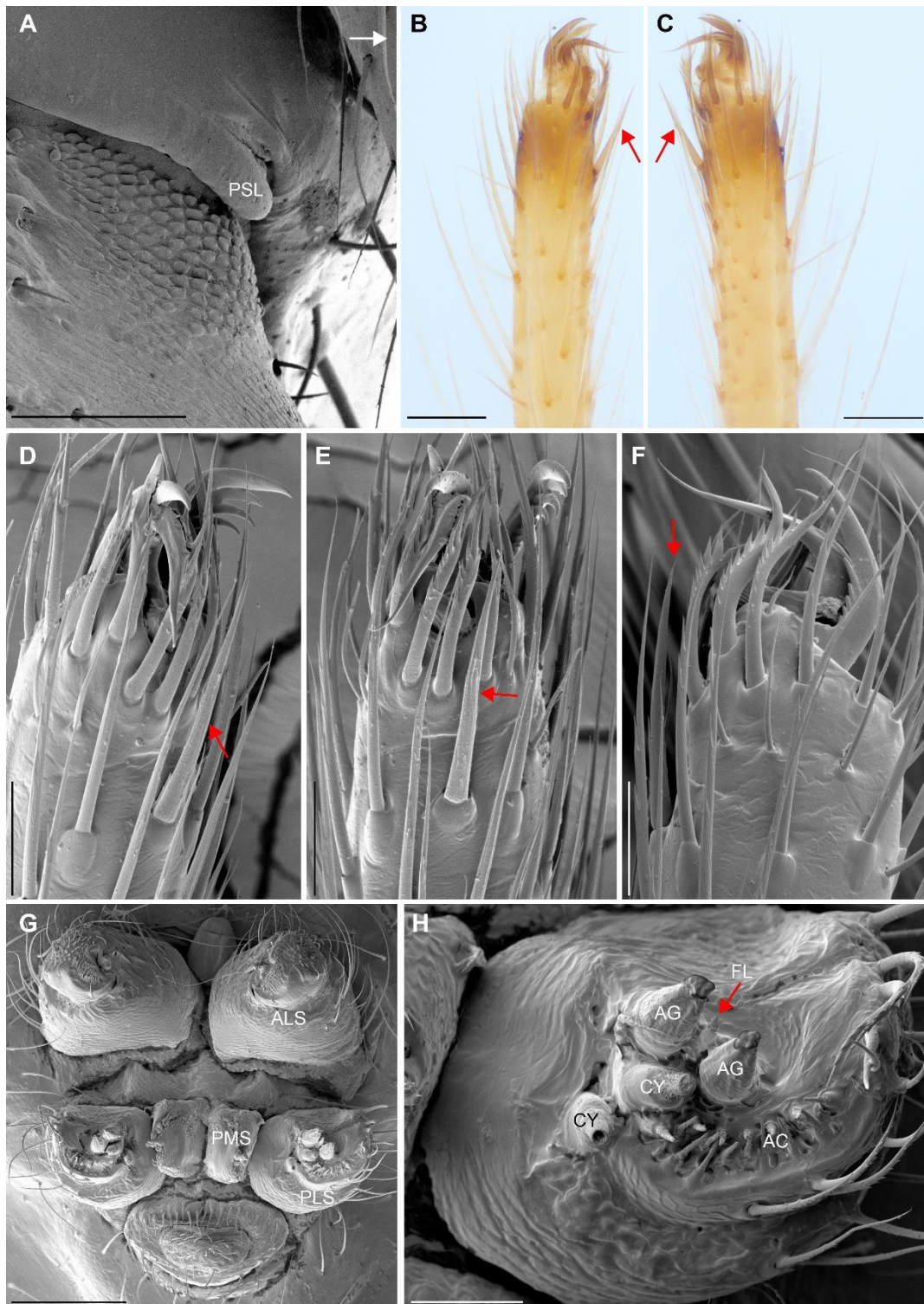


Figure S13. General female anatomy of *Osmooka aphana*. A, striated cheliceral boss of MAD-24-023 (white arrow indicates frontal direction); B-C, left fourth tarsus of the paratype (red arrow points to sustentaculum); D-F, left fourth tarsus of MAD-24-023 (red arrow points to sustentaculum); G, spinnerets of MAD-24-023; H, detail of the posterior lateral spinneret (red arrow points to flagelliform spigot). Scale bars: A-C = 0.1 mm; B-E, G = 0.05 mm; F = 0.2 mm.

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