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Transcriptome-based phylogenomics of Auchenorrhyncha (Hemiptera)

R.K. Skinner, C.H. Dietrich, J.M. Allen & K.P. Johnson. Illinois Natural History Survey, Prairie Research Institute, University of Illinois, Champaign, IL 61820 USA

K.K.O. Walden & H.M. Robertson. Department of Entomology, University of Illinois, Urbana, IL USA

M. Petersen. Zoologisches Forschungsmuseum Alexander Koenig, Bonn, Germany

L. Podsiadlowski. University of Memphis, Memphis, TN USA

C. Simon. Department of Ecology and Evolutionary Biology, 75 N. Eagleville Rd., Storrs, CT 06269 USA

D.M. Takiya. Laboratório de Entomologia, Departamento de Zoologia, Instituto de Biologia, UFRJ, Caixa Postal 68044, Rio de Janeiro, 21941-971, RJ, Brazil

The phylogenetic status and relationships of Auchenorrhyncha have been controversial since phylogenies of Hemiptera based on 18S rDNA sequence data suggested that the group was paraphyletic with respect to Heteroptera (Campbell *et al.* 1995, von Dohlen & Moran 1995). Subsequent analyses incorporating data from additional loci (Cryan & Urban 2012) supported Auchenorrhyncha monophyly but incompletely resolved relationships within the suborder. A recent large-scale phylogenomic analysis of Insecta recovered Coleorrhyncha as sister to monophyletic Auchenorrhyncha with strong support but included only 3 Auchenorrhyncha (Misof *et al.* 2014). New relatively inexpensive next-generation DNA sequencing methods enabled us to examine relationships among major lineages of Auchenorrhyncha in some detail.

Methods. RNA was extracted from specimens of 83 species from all 12 extant families of Cicadomorpha and 15 of the 20 extant families of Fulgoromorpha, with 4 Coleorrhyncha and 6 Heteroptera added as outgroups. Transcriptomes were sequenced using Illumina HiSeq from cDNA libraries and assembled using SOAPdenovo trans with orthologs identified using Orthograph (Petersen *et al.* 2017). Amino acid sequences from >2000 orthologs were aligned using MAFFT (Katoh & Standley 2013). The concatenated dataset comprised >1,400,000 characters. PartitionFinder2 (Lanfear *et al.* 2016) was used to select substitution models and partitioned maximum likelihood analysis was performed using RAXML (Stamatakis 2014).

Results and Discussion. Maximum likelihood analysis of the concatenated dataset yielded a well-resolved phylogeny with nearly all branches receiving 100% bootstrap support. The topology was generally congruent with the results of Misof *et al.* (2014) and Cryan & Urban (2012) with Auchenorrhyncha monophyletic and sister to Coleorrhyncha, Fulgoromorpha sister to Cicadomorpha, and Cicadoidea+Cercopoidea sister to Membracoidea. Within Fulgoroidea, Cixiidae+Delphacidae were sister to a clade comprising the remaining families and Achilidae+Derbidae were sister to a clade comprising the sister groups Dictyopharidae+Fulgoridae and the remaining included families. Caliscelidae were recovered as sister to Tettigometridae and this clade was sister to a clade comprising Acanaloniidae, Eurybrachidae, Flatidae, Issidae, Nogodinidae and Ricaniidae. Nogodinidae were not monophyletic, with *Bladina* sister to Eurybrachidae and *Lipocallia* sister to Ricaniidae. Within Cercopoidea, Clastopteridae and Machaerotidae formed a paraphyletic grade leading to the remaining families, with Aphrophoridae paraphyletic with respect to Cercopidae and

Epipygidae. Within Cicadoidea, *Tettigarcta* was sister to the remaining cicadas and Tibicininae were sister to Cicadinae+Cicadettinae. Within Membracoidea, Myerslopiidae were sister to a clade comprising the remaining taxa and the three treehopper families were derived from within Cicadellidae with Megophthalminae+Ulopinae recovered as sister to the treehopper lineage. In contrast to a separate analysis of Membracoidea based on anchored hybrid enrichment data (Dietrich *et al.* 2017), the more character-rich transcriptome data resolve relationships among major lineages of Membracoidea with high support. Nevertheless, although the transcriptome data appear to be more decisive, some unexpected relationships, such as derivation of Melizoderidae from within Membracidae, will require further investigation.

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