

Hoybjerg, Christian James

A comparison of the phylogeny and drumming characteristics in *Isogenoides*

Faculty Mentor: Michael F. Whiting, Integrative Biology

Introduction

Isogenoides is a genus of Plecoptera (stoneflies) from the family Perlodidae. The genus is composed of seven separate species and is endemic to North America. Each species has a limited distribution and a distinct system of courtship behavior. The male taps his abdomen on the substrate with a specific “drumming” pattern until he attracts a mate. The female will respond with her own drumming pattern and, in most species, the male confirms the acceptability of the female with a final, distinct answer. This behavior has been hypothesized as both a barrier to cross-species mating and as a mechanism of speciation. Despite the implications of this very little is known regarding the evolution of these signals.

The project consisted of sequencing six genes (18S, 28S, Histone-3, and three mitochondrial genes: COII, 12S, 16S) from each of the seven species and constructing the first robust phylogeny for the genus. The constructed phylogeny is now enabling us to evaluate discrete characteristics of the call-response-answer series observed in *Isogenoides*, and determine how these signals have evolved.

Methods

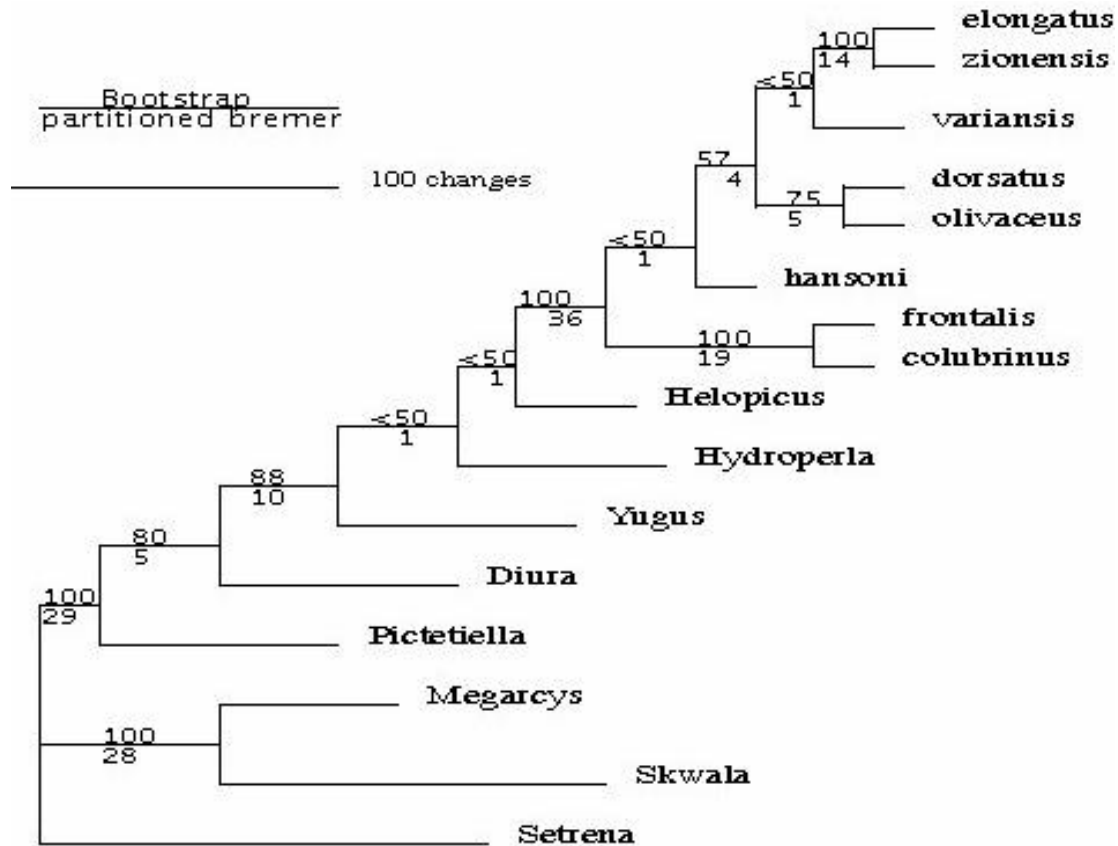
All specimens were preserved in 100% and stored at -80°C . The thorax or leg of the insect specimen was used for extraction of genomic DNA. Extraction was performed using Qiagen Dneasy Tissue Kit (Qiagen Inc., Valencia, CA). 18S and 28S nuclear ribosomal DNA, Histone-3 protein coding gene and 16S, 12S and COII mitochondrial genes were amplified using AmpliTaq Gold DNA Polymerase (Applied Biosystems, Foster City, CA) from primers designed from insects. Standard amplification temperature profiles were employed. PCR products were examined via agarose gel electrophoresis and subsequently purified using Montage PCR Cleanup Kit (Millipore) and cycle-sequenced using BigDye Terminator chemistry (ABI). Sequences were generated using an ABI 3730xl DNA Analyzer at the DNA Sequencing Center, BYU. The alignment for the six genes was generated manually in Sequencher 4.1 (Genecodes, 1999). All partitions were analyzed via Optimizaiton Alignment in POY (Galdstein, 1997). Partition Bremer support values were computed in TreeRot.v2a (Sorenson, 1999) using PAUP 4.0b.7 (Swofford, 2001). Likelihood analysis was performed by using Modeltest (Posada, 1998).

Results

The first robust phylogeny for *Isogenoides* is found below. The constructed phylogeny supports that the most closely related genera to *Isogenoides*, are *Helopicus*, *Hydroperla* and *Yugus*, which is insightful due to that fact that the closest related genus to *Isogenoides* is unknown. All genes sequenced support *Isogenoides* as being monophyletic, with a bootstrap (BS) of 100 and a Partition Bremer Support (PBS) (Baker, 1997) of 36. The analysis supports that *Dorsatus* and *Olivaceus* are sister groups with a BS of 75 and a PBS of 5. *Frontalis* and *Colubrinus* also are sister groups, as supported by the analysis (BS of 100 and a PBS of 19). These data also support the relationship between *Elongatus* and *Zionensis* as being sister groups with a BS of 100 and a

PBS of 14. The later relationship is very interesting due to the fact that these two genera also express the most complex drumming behaviors. This study has implications for both Plecoptera, as many other stoneflies also drum to signal mates, and for other insects that exhibit pre-mating signals.

Table



References

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