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SHORT COMMUNICATION

Caddo agilis and *C. pepperella* (Opiliones, Caddidae) diverged phylogenetically before acquiring their disjunct, sympatric distributions in Japan and North America

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Abstract. The harvestmen *Caddo agilis* Banks 1892 and *C. pepperella* Shear 1975 (Caddidae, Caddinae) share a disjunct distribution in eastern Asia and eastern North America that has been attributed to either recent (Pleistocene) evolution of a *C. pepperella* morph from *C. agilis* in each region or to a pre-glacial separation within each of two established species. The present study used 2,130-base sequences from two nuclear protein-coding genes (EF1α, Pol II) to test the phylogenetic predictions of both hypotheses using representatives from the two *Caddo* species from both regions and two acropsopilionine outgroup species. The results supported the hypothesis that the two *Caddo* species were distinct prior to their respective biogeographic disjunctions; *C. agilis* and *C. pepperella* were each recovered as monophyletic and each appears to have undergone separation into Asian and North American groups.

Keywords: Harvestmen, phylogeny, elongation factor-1α, RNA polymerase II

This study focuses on the phylogeny and biogeography of the two extant species of *Caddo*, *C. agilis* Banks 1892 and *C. pepperella* Shear 1975 (Caddidae, Caddinae), both of which occur in eastern North America and Japan. Two scenarios have been offered in the arachnological literature to explain the disjunct sympatric distribution of the two species: the parallel-evolution hypothesis (Shear 1975, 1996, 2004) and the habitat-fragmentation hypothesis (Suzuki 1976). Shear (1975) originally suggested that the smaller *C. pepperella* evolved in North America as a paedomorphic (progenetic) variant of *C. agilis*, perhaps as an adaptation to shorter growing seasons associated with glacial conditions during the Pleistocene. The subsequent discovery of *C. pepperella* in Japan (Suzuki 1976) was inconsistent with Shear's hypothesis, as it seemed to require an improbable recent dispersal between eastern North America and Japan. Shear (1996, 2004) countered that a *C. pepperella* morph may have evolved independently in North America and Japan during the Pleistocene. In contrast, Suzuki (1976) proposed that both species originally inhabited an ancient circumboreal ecosystem that now consists of isolated postglacial fragments in eastern Asia and eastern North America.

The parallel-evolution and habitat-fragmentation hypotheses can be tested by means of molecule-based phylogenetic analysis, with the former predicting diphyly within *C. pepperella* and the latter predicting monophyly of both *C. agilis* and *C. pepperella* across their ranges. Here we test these hypotheses using 2,130 base pairs of the nuclear protein-coding genes elongation factor-1α) (EF-1α) and RNA polymerase II (Pol II) from representative *C. agilis* and *C. pepperella* from North America and Japan as well as two acropsopilionine outgroup species, *Austropsopilio sudamericanus* Shultz & Cekalovic 2003 and *Acropsopilio chilensis* Silvestri 1904. Our results corroborate Suzuki's habitat-fragmentation hypothesis.

METHODS

Terminal taxa and sequences.—Specimens were collected alive and preserved in > 95% ethanol. They were stored in > 95% ethanol at −20° C up to 2 yr prior to RNA extraction. The analysis was based on sequences from six specimens, with collection data and GenBank accession numbers as follows:

1. *Caddo agilis* Banks 1892. USA: *New Hampshire*: Cheshire County, Pisgah State Park, 42.868°N, 72.448°W, 7–11 July 2001, J.W. Shultz (EF-1α: FJ361272; Pol II: FJ476262–FJ476264).
2. *Caddo agilis* Banks 1892. JAPAN: *Tottori Prefecture*: Chizu-cho, Ashizu Tunnel, 660 m, 20 June 1998, N. Tsurusaki (EF-1α: AF240838; Pol II: AH010430).
3. *Caddo pepperella* Shear 1975. USA: *New Hampshire*: Cheshire County, Pisgah State Park, 42.868°N, 72.448°W, 7–11 July 2001, J.W. Shultz (EF-1α: FJ361272; Pol II: FJ476265–FJ476267).
4. *Caddo pepperella*. JAPAN: *Tottori Prefecture*: Mt. Nagi, 630 m elev., 20 June 1998, N. Tsurusaki (EF-1α: AF240863; Pol II: AH010457).
5. *Acropsopilio chilensis*. CHILE: *Provinica de Concepcion*: Cerro Caracol, 5 October 2003, T. Cekalovic (EF-1α: FJ361275; Pol II: FJ476256 - FJ476258).
6. *Austropsopilio sudamericanus*. CHILE: *Provincia de Valdivia*: Cerro Oncol, April 2001, T. Cekalovic (EF-1α: FJ361274; Pol II: FJ476259–FJ476261).

A voucher specimen of each species is deposited in the National Museum of Natural History (Smithsonian Institution) except for *C. pepperella* from Japan, because the only specimen available was consumed in genomic extraction.

Molecular methods.—Detailed procedures for generating sequence data, including primer sequences, have been published elsewhere (Regier & Shultz 1997). In brief, total nucleic acids were isolated; complementary DNA of EF-1α and Pol II mRNA was made by reverse transcription; ds-DNA copies were amplified by PCR and subsequently gel isolated; the resulting PCR fragments were used as templates for another round of PCR amplification with nested primers; and the resulting fragments were gel isolated and sequenced. When the resulting fragment concentration was too low to sequence directly, it was either concentrated or reamplified using the M13 sequences present at the 5′ ends of all primers. The same M13

sequences were also used as primers for thermal cycle/dideoxy sequencing. Sequencing reactions were fractionated and preliminary analyses were performed with Perkin-Elmer/ABI automated DNA sequencers. Automated DNA sequencer chromatograms were edited and contigs were assembled using the pregap and gap4 programs within the Staden software package (Staden et al. 1999). Sequences were aligned and Nexus-formatted nucleotide data sets were constructed using the Genetic Data Environment, version 2.2 (Smith et al. 1994). All sequences lacked indels. Amino acid data were inferred from nucleotide sequences using the universal nuclear genetic code option in MacClade, ver. 3.08 (Maddison & Maddison 1992).

Phylogenetic analysis.—Parsimony analyses of three data sets [all nucleotides (*nt1-3*), third codon positions (*nt3*) and inferred amino acids (*aa*)] were performed in PAUP4.0 (Swofford 1998) using unordered, equally weighted characters. Analyses consisted of exhaustive searches followed by bootstrap analyses (Felsenstein 1985) based on branch-and-bound searches of 1,000 pseudoreplicates. In conducting maximum-likelihood (ML) analysis, the program Modeltest, ver. 3.7 (Posada & Crandall 1998) was used to choose a model for the *nt1-3* and *nt3* data sets using AIC (Posada & Buckley 2004), with specific parameter values being estimated during subsequent phylogenetic analysis. The ML analyses were conducted in PAUP* using exhaustive searches and nonparametric bootstrap analyses were performed using branch-and-bound searches of 1,000 pseudoreplicates.

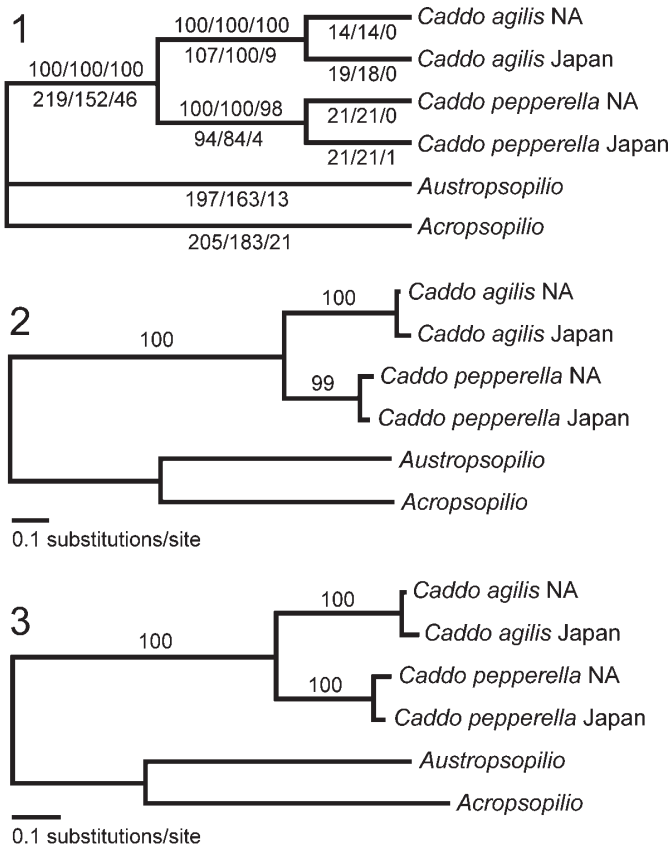
RESULTS

Parsimony analyses of all nucleotides (*nt1-3*), 3rd codon positions (*nt3*) and inferred amino acids (*aa*) produced identical, fully resolved topologies with bootstrap percentages (BP) of 98–100 for all internal nodes (Fig. 1) (*nt1-3*: 2,130 characters, 398 informative, length = 897, CI = 0.8305; *nt3*: 710 characters, 317 informative; length = 756, CI = 0.816; *aa*: 710 characters, 55 informative; length = 94, CI = 0.9492). *Caddo agilis* and *C. pepperella* were each recovered as monophyletic and reconstructed as sister groups with respect to the acropsopilionines, *Acropsopilio chilensis* and *Austropsopilio sudamericanus*.

Comparison of alternative likelihood models in Modeltest indicated that the *nt1-3* data should be analyzed using a GTR+Γ_4+I model and that the *nt3* matrix should be analyzed under the TVM+Γ_4 model. Exhaustive likelihood searches using these models recovered topologies identical to those derived from parsimony-based analyses (*nt1-3*: -ln likelihood = 6649.2923; *nt3*, -ln likelihood = 3391.9731). Specifically, the clades *C. agilis*, *C. pepperella*, and *Caddo* were each recovered as monophyletic with strong support (BP 99–100%) (Figs. 2, 3), a result predicted by Suzuki's hypothesis. The two data sets were reanalyzed under their respective models with the analyses constrained to yield Shear's hypothesis of independent evolution of *C. pepperella* from *C. agilis* in Asia and North America and Suzuki's hypothesis of monophyly of both *C. agilis* and *C. pepperella* throughout their ranges. Kishino-Hasegawa tests (Kishino & Hasegawa 1989) showed the trees constrained to the prediction of Shear's hypothesis to be significantly less likely than those constrained to Suzuki's hypothesis ($P < 0.001$).

DISCUSSION

Our results indicate that *Caddo agilis* and *Caddo pepperella* are monophyletic species that diverged phylogenetically before each acquired a disjunct geographic distribution in Japan and eastern North America. This supports Suzuki's (1976) habitat-fragmentation hypothesis and is inconsistent with Shear's (1975, 1996, 2004) parallel-evolution hypothesis. These findings are consistent with current understanding of climatic and biogeographic events during the late Tertiary (Sanmartín et al. 2001). The eastern Asia-eastern North America disjunction exemplified by *Caddo* parallels a long-known biogeographic pattern among flowering plants (Wen 1999; Xiang et al. 2000). During the mid-Cenozoic, eastern Asia and eastern North America were spanned by mesophytic forests that were eventually separated into Asian and American components during the early Pliocene, a culmination of long-term trends in the cooling and drying of central and northern North America. As a consequence, many plant genera have representative species in both eastern Asia and eastern North America, and relative rates tests conducted on 12 species pairs using the *rbcL* gene indicated a divergence time of 5.4 ± 2.6 million years ago (Xiang et al. 2000). Zoologists have not explored the Asian-North American disjunction to the same extent as botanists, but several examples are known among animals (Sanmartín et al. 2001), including the non-caddine harvestmen *Acropsopilio boopis* (Crosby 1904) and *Crosbycus dasycnemus* (Crosby 1911), *Okeantobates* millipedes (Enghoff 1993), plethodontid salamanders (Min et al. 2005) and, among late Tertiary fossils, lesser pandas and meline badgers (Tedford & Harington 2003; Wallace & Wang 2004).



Figures 1–3.—Results of phylogenetic analysis. 1. Parsimony tree based on separate analysis of all nucleotides, third codon positions and inferred amino acids, respectively. Numbers above branches are non-parametric bootstrap percentages based on 1000 pseudoreplicates. Numbers below branches are estimated branch lengths under acctran optimization. 2. Maximum-likelihood tree based on all nucleotides using the GTR + Γ_4 + I model. 3. Maximum-likelihood tree based on third codon positions using the TVM + Γ_4 model.

In contrast, because the two *Caddo* species are roughly sympatric and often syntopic in both North America and Japan, vicariant or climatic events cannot readily explain their phylogenetic divergence or morphological differences. It is possible that the two extant *Caddo* species diverged due to resource or habitat partitioning, as *C. agilis* tends to occupy exposed surfaces (e.g., tree trunks, logs, stones) and *C. pepperella* occurs on the ground in the leaf litter and under fallen objects (Suzuki 1976; Shultz, unpubl. obs.). Given the similarity between *C. pepperella* and juvenile *C. agilis*, it is possible that such habitat specialization produced morphological differences between the

two species via heterochrony, in a manner similar to that proposed by Shear (1975, 1996, 2004). Still, there is no clear evidence as to whether *C. agilis* is peramorphic with respect to its ancestor, whether *C. pepperella* is paedomorphic with respect to its ancestor, both, or neither. Outgroup comparison with the small soil- or litter-dwelling acropsopilionines (Caddidae) would seem to favor *C. pepperella* as the better model for the common ancestor of extant *Caddo* and thus evolution of *C. agilis* via hypermorphosis. However, without relevant information about the morphology and development of ancestral and extant *Caddo*, this matter will remain an exercise in speculation.

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