

Methods

Collection and Preservation. *Neduba* are locally common insects that are incidentally collected by general entomologists. Many conventional and mass collecting techniques are not effective: only occasionally do individuals wander to collecting lights and beating vegetation is nonproductive. An entomologist interested in amassing a collection of these katydids must resort to specialized methods. Triangulating the source of calling songs at night is an efficient way to collect males but takes practice. Besides being challenging to localize, not everyone can hear the high frequency range of their songs, and the ability to hear the songs diminishes with age of the observer. Bat detectors may be employed to locate males that are not audible. Oatmeal trails laid down at night can be an effective way to attract individuals, especially females. Searching bark, logs, and leaf litter during the day may turn up small numbers of adults.

Gathering nymphs early in the season and raising them to maturity is a way of obtaining large samples. *Neduba* are easy to maintain in captivity, readily accept the orthopteran food mixture (Rentz 1996), are non-aggressive and thus may be group-housed, and are long-lived. None of us have successfully reared *Neduba* through an entire generation from eggs, a problem that until solved precludes detailed laboratory genetics and limits behavioral studies. Given their habitats, *Neduba* may require simulated winter conditions to break diapause (see Rentz 1973).

Quality specimen preservation requires taxidermy and/or freeze-drying to prevent shriveling of the body and loss of pigment colors. To taxidermize specimens we either (1) inserted forceps dorsally through the cervical membrane between the head and pronotum and removed the gut contents through the opening, and then inserted loosely balled cotton through the same opening, or (2) made an incision along an abdominal pleuron, removed the gut, and inserted cotton to reform the shape of the abdomen. Color preservation, especially for green tones, is improved by drying taxidermized specimens in a conventional, frost-free freezer. Freeze-drying is also effective for entire (i.e. non-gutted) specimens.

Molecular Phylogenetic Analysis. *DNA extraction, amplification, and sequencing.* Middle or hind legs, or in a few cases whole specimens, were directly frozen at -20°C or preserved in 95–100% ethanol. DNA was extracted using an ethanol precipitation method equivalent to the Puregene Extraction Kit (Gentra Systems, Inc.) or a commercially available extraction kit (DNEasy Blood and Tissue Kit, Qiagen, Inc., Valencia, CA, USA) according to the manufacturer's protocol for animal tissues. The polymerase chain reaction (PCR) was used to amplify five genes: the entire ribosomal *internal transcribed spacer 2* (ITS2, variable length), a 1030 bp fragment of *28S ribosomal RNA* (28S), a 384 bp fragment of *wingless* (*wg*), an 828 bp fragment of *cytochrome oxidase I* (COI), and a fragment of *cytochrome oxidase II* (COII) with 501 bp overlap after alignment. The first two regions are linked on the nuclear ribosomal cistrons and are collectively referred to as rDNA, while the last two loci are linked on the mitochondrial genome and are referred to as mtDNA henceforth. *wg* is a slow evolving gene (Campbell *et al.* 2000) that we included in our concatenated analysis. Primer sequences and PCR conditions are listed in Table 1. PCR reactions were performed in either 10 or 25 μ L volumes on a GeneAmp 9700 (Applied Biosystems) or MyCycler (BioRad) thermocycler using a Taq master mix (HotStar Plus Taq Master Mix, Qiagen Inc., Valencia, CA, USA) according to the cycling conditions in Table 1. Direct sequencing of PCR products followed enzymatic cleanup (ExoSAP-*it*, Affymetrix, Santa Clara, CA, USA).

Sequence alignment. Trace files were imported into Geneious v. 6.1.8 (created by Biomatters, available from <http://www.geneious.com/>) for contig assembly and editing. Protein coding COI, COII and *wg* genes were unambiguously aligned by amino acid sequence in Geneious. 28S was aligned using the *x-ins-i* algorithm (Katoh & Toh 2008) in MAFFT (Katoh *et al.* 2002; Katoh & Standley 2013). ITS2 alignment was aided by estimating secondary structures. The precise start and end points of each ITS2 sequence were annotated using a hidden Markov model (Eddy 1998) implemented through the ITS2 database (Koetschan *et al.* 2010). Secondary structures were estimated for batches of sequences using the TurboFold algorithm (Harmanci *et al.* 2010) as implemented in RNAstructure v. 5.3 (Reutters & Mathews 2010) set to default parameters. The resulting secondary structures were used to produce a simultaneous sequence and structure alignment in RNAsalsa (Stocsits *et al.* 2009).

Phylogenetic analysis. Phylogenetic analyses were run on the supercomputer resources available at the CIPRES Science Gateway (Miller *et al.* 2010). Models of sequence evolution and partitioning schemes were evaluated simultaneously for the combined dataset using Partition Finder v. 2 (Lanfear *et al.* 2016) using a greedy search algorithm (Lanfear *et al.* 2012) and PhyML (Guindon *et al.* 2010). Bayesian phylogenetic analysis was performed in MrBayes v. 3.2.6 (Huelsenbeck & Ronquist 2001; Ronquist *et al.* 2012; Ronquist & Huelsenbeck 2003). Branch lengths were unconstrained and all topologies were considered equally likely. Gaps in ITS2 secondary structure in-