

above. File teeth were counted under a compound microscope (Zeiss, White Plains, NY, USA) at 500× magnification. The length of the file was measured in mm (across the curve) with an ocular micrometer as a straight line between the ends of the file. File length and tooth number were not significantly different across our specimen series; instead, the standardized measurement of tooth density, the ratio of the tooth count divided by file length, differs significantly between *Neduba* species and facilitates diagnosis.

Color patterns vary widely within and between species (Fig. 1). We offer images of living katydids as examples of common color patterns (Plates 1–3) but do not describe color patterns for each species for the following reasons: (1) preservation of color patterns is unreliable in museum specimens unless properly prepared (see Collection and Preservation above), (2) a single species may have multiple color patterns with slight variations, and (3) reliance on color pattern has in part led to the confusing taxonomy of *Neduba* (e.g. Caudell 1907; Tinkham 1944), and we emphasize characters that have diagnostic utility.

Song Recording and Analysis. The acoustic pair-formation mechanism employed by *Neduba* katydids permits inferences to be made about reproductive compatibility between populations and therefore about species status. Rates of sound production serve as species-specific mate recognition features in many acoustic animals (e.g. Gerhardt 2013; Gerhardt & Huber 2002; Gray *et al.* 2016; Rodriguez *et al.* 2006) including the related nedubine genus *Aglaothorax* (Cole 2016). Therefore, among the seven song characters we analyzed, rates of pulse train production ranked highly among potential mate recognition cues. Calling songs are only half of the pair formation equation; future research may test species boundaries inferred from calling song differences with female preference experiments (e.g. Cole 2016; Ritchie 1991; Rodriguez *et al.* 2006; Schul 1998; Shaw & Herlihy 1999). DBW recordings were made with a condenser microphone (model ME40 microphone and model K34 power module, Sennheiser Electronic Corp., Old Lyme, CT, USA) and reel-to-reel tape recorder (model 4000 Report LC, Uher, Munich, Germany) indoors where temperature was maintained at or near 25°C (24.3 ± 1.7°C). JAC digitized DBW analog recordings at a sampling rate of 44.1 kHz through a firewire interface (Cakewalk FA-66, Roland Corp., Los Angeles, CA, USA) into a MacBook Pro computer running Logic Pro v. 10.3.3 (Apple Inc.). JAC field recordings were made with a linear PCM recorder (model PCM-D50, Sony Corp., New York, NY, USA) with integral condenser microphones. This device recorded 16-bit audio at a sampling rate of 96 kHz. A low cut-off frequency of 75 Hz was set to reduce wind and other ambient noise. Together, the microphones and sampling parameters recorded a frequency range that extended to 40 kHz. High-frequency laboratory recordings were made by JAC in a semi-anechoic chamber at the University of Kansas in which temperature was maintained near 25°C (24.5 ± 0.7°C). High frequency equipment consisted of a 1/2 inch electret condenser microphone (model M51, Linear-X, Tualatin, OR, USA) and a PC computer running BatSound v. 3.3 (Pettersson Elektronik AB, Uppsala, Sweden) sampling at 150 kHz for 1 min. The high frequency laboratory recording apparatus captured frequencies up to 75 kHz, with a flat response from 10 Hz to 40 kHz.

A high pass filter set to 24 dB roll-off and a cutoff frequency from 1 to 6 kHz reduced tape transport and other noise, the higher cutoffs used for correspondingly greater intrusion of noise levels into higher frequencies of the recording. Cutoff frequencies did not affect the frequency range of *Neduba* calling songs, the minimum of which for all species seldom extended to 4 kHz. The complexity of *Neduba* calling song waveforms confounded automatic analysis of the temporal song features. Specifically, automatic waveform detection methods produced a dataset of high precision but low accuracy that underestimated pulse train lengths as automatic detection did not reliably find the start and end points of pulse trains. Manual measurement of song components was therefore undertaken using Audacity v. 2.1.0 (available from www.audacityteam.org). Six successive wingstroke cycles were randomly chosen and measured with the cursor to the nearest ms. Peak frequencies were measured from a portion of a randomly selected wingstroke cycle using a 256 Hz Fast Fourier transform algorithm and a Hanning window in Audacity. Oscillograms and spectrograms figured in this revision were generated with Raven Lite v. 2.0 (Cornell Laboratory of Ornithology, available from ravensoundsoftware.com). Recordings will be deposited in Macaulay Library–Cornell Lab of Ornithology and on Singing Insects of North America (<https://orthsoc.org/sina/>). Terms used to describe song characters are as follows, and the fundamental characters are shown graphically in Fig. 2:

PT = pulse train

MPT = major pulse train (always made by tegmina closing)

OPT = minor pulse train (usually made by tegmina opening, exceptions are species with multiple OPT in Sierranus Group (Fig. 2D–E) and *N. oblongata* sp. n. in the Carinata Group)

MPTL = major pulse train length, standardized by regression to 25°C

PTP = pulse train period (measured either from beginning to beginning or from end to end of MPT, depending on which measurement was more clearly identifiable on the oscillograms)

PTR = pulse train rate/s, standardized by regression to 25°C (= 1/(PTP × 1000))

PTdc = pulse train duty cycle (= MPTL/PTP)

PTF = frequency at maximum amplitude of major pulse train

PTN = pulse train number (counted for multiple OPT generated by partial wing closing and opening)

Statistical song analysis was performed in R v. 3.2.3 (available from www.r-project.org). Temperature dependent song parameters (PR, MPTL) were tested for significance between putative taxa using ANCOVA, with taxon as the factor and temperature as the covariate. A significant temperature × taxon interaction indicated different regression slopes. Population was also included in some ANCOVA models as a factor to test for interpopulation differences within taxa. Temperature-independent characters (PTdc, PTN) were tested with ANOVA.

Karyotypes. Chromosome rearrangements are frequently associated with species boundaries in animals ranging from grasshoppers (Weissman & Rentz 1980) to beetles (Maddison 2008) to velvet worms (Onychophora; Rockman & Rowell 2002) to rodents (Cross 1931). Diverse karyotypes are found in *Neduba* katydid. Apart from differences in autosome number and centromere locations, multiple sex chromosome systems have evolved: all males possess at least one X chromosome and in some species males have an additional X chromosome and/or a Y chromosome (Ueshima & Rentz 1979). We regard chromosomal differences between *Neduba* taxa as evidence for specific distinction, and we invite testing of our species hypotheses with population genetic analysis or crossing experiments.

Testes were removed from living males through an incision along the midline of the abdominal dorsum. Scissors were inserted underneath the tergite in front of the supra-anal plate and continued anteriorly. Excised testes were immediately stored in a freshly prepared 1:3 mixture of glacial acetic acid and 100% ethanol. Testes were prepared for light microscopy by squashing on microscope slides and staining according to the standard Schiff-Giemsa method. Karyotypes are reported in the descriptions as diploid counts followed by counts that are arranged by centromere location, where m = metacentric and t = telocentric. For example, a common *Neduba* karyotype is $2n\sigma = 26 (2m + 22t + XtYt)$, which denotes a diploid chromosome count of 26 that is composed of 2 metacentric autosomes, 22 telocentric autosomes, and a pair of telocentric sex chromosomes: one X and one Y. Where appropriate, the autosomes are noted to be large, medium, or small in size.

Results

Phylogenetic Analysis. GenBank accessions and voucher specimen information are reported in Supplementary Table 1. PartitionFinder results are summarized in Table 2. Bayesian analysis of the concatenated genetic data separated two major *Neduba* clades (posterior probability = 1) that are subdivided into six Species Groups (all posterior probabilities = 1): Carinata, Propsti, Castanea, Lucubrata, Sierranus, and Sequoia (Fig. 3). The Carinata Group consists of eight lineages, four of which are currently recognized species (*N. carinata*, *N. convexa*, *N. diabolica*, and *N. steindachneri*). The Carinata Group is comprised of two clades (posterior probability = 1): the Carinata Clade and the Convexa Clade. Although Convexa Clade lineages clearly cluster, the interrelationships of those lineages are poorly resolved. The Propsti and Lucubrata Groups contain one lineage each. The Castanea Group consists of reciprocally monophyletic lineages corresponding to *N. castanea* and *N. macneilli*. The Sierranus Group consists of four lineages, with a deep split across *N. sierranus* rendering that species paraphyletic. The Sequoia Group consists of four lineages that are not resolved by concatenated genetic data.

TABLE 2. Partitioning scheme for phylogenetic analysis as selected with PartitionFinder.

Partition	Gene fragment(s) and codon positions	model
1	wg 1st, wg 2nd, 28S	HKY + I
2	wg 3rd	HKY + Γ
3	COI 1st, COI 2nd, COII 1st, COII 2nd	HKY + I + Γ
4	COI 3rd, COII 3rd	GTR + Γ
5	ITS2	HKY + Γ