Introduction

Shield-back katydids (Orthoptera: Tettigoniidae: Tettigoniinae) of the genus *Neduba* belong to the Nearctic members of the katydid tribe Nedubini (Cole & Chiang 2016; Rentz & Colles 1990) along with *Aglaothorax* and *Phymonotus* (Lightfoot *et al.* 2011). *Neduba* are a common but understudied component of the rich orthopteran fauna of the Pacific Coast of North America. A diversity of cryptic color patterns, conservative morphology, and complex high-frequency callings songs together conspired against a thorough systematic understanding thus far. The present revision clarifies 150 years of taxonomic confusion, reveals over twice the number of species than were previously described, illustrates the strong affinity of taxa with the geography of the Pacific Coast of North America, details their complex acoustic behavior, and offers hypotheses that explain the evolution of their calling songs.

Biology. *Neduba* are large, flightless inhabitants of the understory and leaf litter of western North American chaparral, oak woodlands, and conifer forests (Rentz & Birchim 1968). During the day individuals remain motionless in bark crevices (Rehn & Hebard 1909), in leaf litter, in tangles of vegetation, or in pack rat nests (J.A. Cole (JAC) and D.B. Weissman (DBW), pers. obs.). The katydids move little, even when disturbed, and are reluctant to jump. Individuals from the same population can vary greatly from one another in color pattern, for example a morph with a mottled dorsum (Fig. 1A) and a morph with a middorsal stripe (Fig. 1B). Color patterns are also shared among multiple species (Fig. 1C–D) and species Groups, such as a countershaded morph with strong postocular bands (Fig. 1E–F). Color pattern diversity and the fact that color patterns are shared among species minimizes its utility in species identification.

Feeding, singing, courtship, mating, and oviposition take place at night. *Neduba* are omnivores, feeding primarily on the foliage of common plants in their habitats. Most species are associated with oak and conifer forests, and like the related *Phymonotus* some may feed on conifers (Lightfoot *et al.* 2011), a food preference that is rare among Orthoptera (Hojun Song pers. comm.) and that stems from nedubine ancestors that inhabited the ancient coniferous forests of western North America (Cole & Chiang 2016). *Neduba* may scavenge on dead insects but, at least in captivity, show no predaceous tendencies and do not behave aggressively towards each other, in contrast to other North American shield-back katydids like *Capnobotes* that may be predaceous, aggressive, and cannibalistic (Rentz & Birchim 1968; Tinkham 1944).

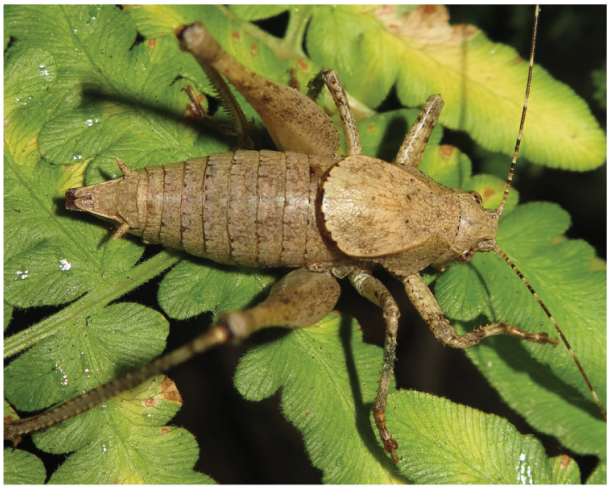
The common name of shield-back katydid describes aptly the enlarged male pronotum in *Neduba* (Figs. 1, 2F) that largely or entirely conceals the small oval front wings (tegmina; Fig. 2F). The male tegmina are used only for sound production and females are completely apterous. Male *Neduba* produce continued high frequency calling songs of appreciable complexity (Fig. 2) that may be heard at night throughout summer and fall in suitable habitats. The enlarged pronotum, together with the dorsum of abdominal segments I–III act as an acoustic horn to amplify the calling songs produced by males (Morris *et al.* 1975). Females are mute and move towards calling males to form pairs (phonotaxis; Fig. 2G). Calling typically begins at dusk and continues throughout the night as long as temperatures do not dip too low, although a few species that inhabit northern latitudes or high elevations are adapted for activity at low temperatures (see Results). Males spend a great deal of time singing: duty cycles are frequently over 50% and may be as high as 90% (see Results). *Neduba* songs are broadband signals with peak frequencies centered high on the audible spectrum (range 11–17 kHz) and with sound energy extending well into the ultrasonic (Fig. 2C, limit of human hearing at white dashed line). Song structure complexity arises from alternating major and minor pulse trains (Fig. 2A, B; Cole 2010; Morris *et al.* 1975) that are produced during closing and opening of the tegmina, respectively. All Sierranus Group species and *N. oblongata* **sp. n.** in the Carinata Group have elaborated this pattern, producing multiple minor pulse trains that are produced by partial closing and opening wingstrokes (Fig. 2D-E).

Systematic History. Reliance on habitus and color pattern characters have produced a confusing systematic history concerning *Neduba* and *Aglaothorax*. The first described species were *Neduba carinata* from California (Walker 1869) and *Arytropteris steindachneri* from "Fox Island, Pouget Sound" (Herman 1874). Samuel H. Scudder moved *steindachneri* to *Tropizaspis* (Scudder 1894) and described four new species under that genus: *castanea*, *diabolica*, *ovata*, and *picturata* (Scudder 1899). Caudell (1907) rearranged the nomenclature considerably in his revision of the North American Decticinae. He synonymized *steindachneri* under *carinata* and the *nomen nudum Tropizaspis* under *Neduba.* In that work Caudell also described *Neduba morsei* and recognized two varieties of *carinata*: Scudder's *picturata* and a new variety *convexa*. Based on the type species *ovata*, Caudell erected *Aglaothorax* for the species with broad, ovate pronota and short hind femora, in which he placed *castanea* and *diabolica*, the latter of which paradoxically lacks both of those character states. The remaining species he retained in *Neduba* but cautioned that due to intermediate morphologies this split was imperfect. Rehn and Hebard (1909) described *Aglao-*

thorax sierranus. Tinkham (1944) haphazardly and erroneously applied five species names to scattered populations of *Neduba*, but correctly placed *diabolica* and *sierranus* in *Neduba* rather than in *Aglaothorax* based on songs and morphology.

A. *Neduba cascadia* sp. n. PARATOPOTYPE
OR: Jackson Co.

B. *Neduba cascadia* sp. n. PARATOPOTYPE
OR: Jackson Co.



C. *Neduba sierranus*
CA: Madera Co.

D. *Neduba radocantans* sp. n. PARATYPE
CA: El Dorado Co.

E. *Neduba ambagiosa* sp. n. PARATOPOTYPE
CA: Lake Co.

F. *Neduba inversa* sp. n. PARATYPE
CA: Fresno Co.

FIGURE 1. Color pattern variation in *Neduba*. Multiple color patterns are found within species (A and B), and color patterns may be shared among different species (C and D) and species Groups (E and F).

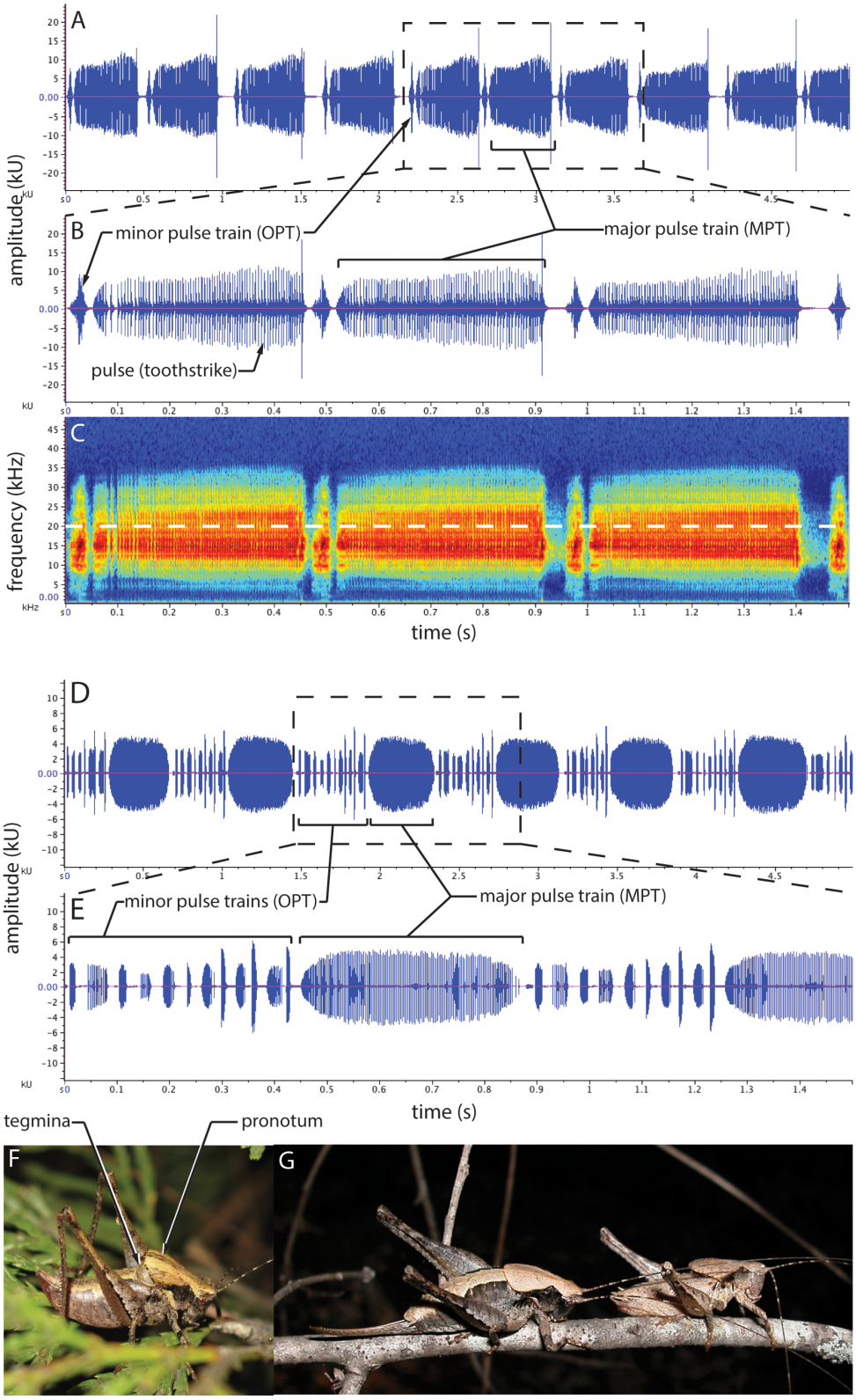


FIGURE 2. *Neduba* typical male calling song structure exemplified by *N. convexa*, recording JCR130808_01, 23.3°C (A-C), and song structure with multiple minor pulse trains exemplified by *N. sierranus* recording JCR120805_00, 21.7°C (D-E). A. 5 s oscillogram window showing 9 full wingstroke cycles (and a partial 10th major pulse train); B. 1.5 s oscillogram window showing individual pulses within major pulse trains; C. spectrogram of B with dashed line at limit of human hearing at 20 kHz; D. 5 s oscillogram window showing 6 full wingstroke cycles (and a partial series of minor pulse trains); E. 1.5 s oscillogram window of D showing individual pulses within major pulse trains; F. Male *N. sierranus* singing, Madera Co., CA. Note elevated pronotum exposing tegmina beneath; G. Phonotaxis in *N. ambagiosa* **sp. n.**, Lake Co., CA.

The most comprehensive treatment of *Neduba* to date is that of Rentz and Birchim (1968). Based on examination of the internal genitalia of topotypes and on qualitative features of songs, they rearranged species between *Neduba* and *Aglaothorax*. The latter name was relegated to a subgenus of the former based on the aforementioned intergradation. They moved the small *morsei* to *Neduba* (*Aglaothorax*) and described *macneilli*, placing it under *Neduba* (*Neduba*) along with *castanea*, *convexa*, *diabolica*, *sierranus*, and *steindachneri*. The last species was resurrected from synonymy based on the geographic distance between its type locality and those of all other known taxa, but without examination of specimens. The descriptions of *extincta* (Rentz 1977) and *propsti* (Rentz & Weissman 1981) brought the total number of *Neduba* (*Neduba*) species up to 9 (eight extant species). Vickery & Kevan (1985) treated *N. steindachneri*, the sole species found in Canada. Gorochov (1988) reviewed the Nedubini as part of a worldwide treatise on Orthoptera evolution. *Neduba* and *Aglaothorax* were reinstated to genus rank after considering the worldwide shield-back katydid fauna (Rentz 1988). Finally, generic rank for the three Nearctic nedubine taxa was supported by bioacoustical (Cole 2010), morphological (Lightfoot *et al.* 2011), and molecular phylogenetic (Cole & Chiang 2016) investigations.

Together with *Aglaothorax* and *Phymonotus* (Lightfoot *et al.* 2011), the Nearctic Nedubini form a distinctive morphological unit that is monophyletic (Cole & Chiang 2016) due to (1) the modification of the male paraprocts into clasping organs (pseudocerci; Rentz & Birchim 1968) instead of the reduced, conical cerci (Rentz & Colless 1990) and (2) the ambidextrous condition of the tegmina (Cole & Chiang 2016; Morris *et al.* 1975; Pemberton 1911). Female nedubines are the only North American shield-back katydids with serrated ovipositors, which suggests that the oviposition substrates may include wood or other plant tissue in addition to soil, which is typical for Tettigoniinae. Although the Nedubini remain in the subfamily Tettigoniinae, which includes the worldwide shield-back katydid fauna, this subfamily is polyphyletic (Cole & Chiang 2016; Mugleston *et al.* 2013). The Nearctic Nedubini are the most basal extant clade of katydids worldwide and neither share recent common ancestors with "nedubines" from South America and Australia nor with the Holarctic shield-back fauna (Cole & Chiang 2016); thus, the shield-back body plan has evolved multiple times. We agree with Rentz (1988) and Lightfoot *et al*. (2011) that *Neduba*, *Aglaothorax*, and *Phymonotus* are of genus rank, given the date of cladogenesis of the three Nearctic nedubine lineages (~36 Ma) and given that the morphologically distinct *Phymonotus* is sister to *Aglaothorax* (Cole & Chiang 2016). Systematics of subfamilies and tribes must await phylogenetic analyses with more thorough taxon sampling than have been accomplished to date.

Philosophy and Characters Studied. Systematics that increases causal understanding of biological diversity is congruent with the overall goal of science (Fitzhugh 2013). To this end, our species hypotheses are informed by biological processes that may have generated diversity in the past and that may currently maintain it. Geography is immediately implicated as an isolating mechanism as *Neduba* generally occur in allopatric or parapatric populations that replace one another in different biogeographical regions (see Results). Whereas most species concepts perform poorly when faced with allopatric taxa (Coyne & Orr 2004), the phylogenetic or genealogical species concept (Baum & Donoghue 1995; Shaw 2001) may identify independently evolving lineages. Over the course of speciation geography may not remain constant (Butlin 2008; Fitzpatrick *et al.* 2009); thus, while we acknowledge the apparently large contribution of geography to *Neduba* diversification, we favor characters that are involved in the evolution of reproductive isolation in accordance with the biological species concept, which defines species as "...groups of interbreeding natural populations that are reproductively isolated from other such groups" (Mayr 1995). The evolution of reproductive isolation takes time, and our results suggest that several *Neduba* species are in early stages of divergence and that others are composed of formerly diverging populations that have homogenized. The version of the biological species concept employed here (Coyne & Orr 2004) allows a modicum of gene flow between taxa, which is expected in incipient species and for which there is evidence in *Neduba* (see Results).

Our systematic methodology began with the inference of phylogenetic hypotheses from multiple nuclear and mitochondrial loci. The resulting clades were then evaluated for potential reproductive isolation by observing differentially shared characters in male calling song, morphology, and karyotypes. The goal of systematics is systematization, not classification (Fitzhugh 2013), and to that end we propose species hypotheses consistent with total evidence: genetically distinct entities that are reproductively isolated by mating signals, chromosome rearrangements, and geography. Valid, behaviorally isolated biological species may be morphologically cryptic (Bickford *et al.* 2006; Wells & Henry 1998), a familiar concept to orthopterists who study bioacoustics (Walker 1964). We sought correlated characters that allow morphological diagnoses of our biological species and conclude that the majority of *Neduba* species are morphologically diagnosable, but the informative morphological characters are often quantitative and subject to continuous variation, and thus best used in conjunction with calling songs and genetics.

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 uL 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 (Reuters & 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-

TABLE 1. Primers and PCR conditions. Primers marked with asterisks (*) were used in nested PCR reactions.

Locus	Primer name	Primer sequence	Annealing conditions	Extension time (s)	Cycles	Reference
ITS2	3S	GGTACCGGTGGATCACTGGGCTCGTG	53°C 15 s	60	30	(Slaney & Blair 2000)
	BD2	TATGCTTAAATTCAGCGGGT				(Slaney & Blair 2000)
28S	NLF184-21	ACCCGCTGAAYTTAAGCATAT	54°C 30 s	60	30	(Van der Auwera *et al*. 1994)
	LS1041R	TACGGACRTCCATCAGGGTTTCCCCTGACTTC				(Wild & Maddison 2008)
wg	LEPWG1	GARTGYAARTGYCAYGGYATGTCTGG	59°C 15 s	60 s	40	(Brower & DeSalle 1998)
	modLEPWG2	ACTICGCARCACCARTGGAATGTRCA				
	WG550Fkt	ATGCGTCAGGARTGYAARTGY	46°C 30 s	45 s	30	(Mugleston *et al.* 2013)
	WgAbRz	CACTTNACYTCRCAR CACCARTG				(Wild & Maddison 2008)
	*WG1TET	GAGTGTAAGTGTCATGGTATGTCTGG	50°C 30 s	45 s	30	(Cole & Chiang 2016)
	*WgAbRNed	ACTGCGCAGCACCARTGGAA				This study.
COI	COI-J-2195	TTGATTTTTTGGTCATCCAGAAGT	50°C 60 s	60 s	35	(Simon *et al.* 1994)
	TL2-N-3014	TCCAATGCACTAATCTGCCATATTA				(Simon *et al.* 1994)
	Jerry	CAACAYTTATTTTGATTTTTTGG	50–52°C 60 s	60–75 s	30–35	(Simon *et al.* 1994)
	Pat	ATCCATTACATATAATCTGCCATA				(Simon *et al.* 1994)
COII	C2-J-3279	GGACAAACAATTGAGTTAATTGGAAC	45–47°C 60 s	60 s	30	(Simon *et al.* 1994)
	TD-N-3862	TTTAGATTGACATTCTAATGTTAT				(Simon *et al.* 1994)
	COII Flue	TCTAATATGGCAGATTAGTGC	46–50°C 30–60 s	75 s		(Svenson & Whiting 2004)
	COII Rlys	GAGACCAGTACTTGCTTTCAGTCATC				(Svenson & Whiting 2004)
	*COII 2a	ATAGAKCWTCYCCHTTAATAGAACA	46–51°C 30–60 s	75 s		(Mugleston *et al.* 2013)
	*CPOO 9b	GTACTTGCTTTCAGTCATCTWATG				(Mugleston *et al.* 2013)

formation were modeled as binary restriction data (1 = nucleotide, 0 = gap) with variable coding. Four runs of four chains each were continued for 5×10^7 generations, after which convergence was inferred from a standard deviation of split frequencies of < 0.02. Consensus trees were calculated from the posterior probability distributions after burnin fractions of 0.25 were discarded.

Morphology. Specimens were examined in the collections of the California Academy of Sciences (CAS), the Natural History Museum of Los Angeles County (LACM), the Bohart Museum of Entomology (BMED) at UC Davis, California State University Northridge (CSUN), the personal collection of the senior author (JAC), and the California State Collection of Arthropods (CSCA) in Sacramento, CA. Localities on museum specimen labels were georeferenced using the batch client at GeoLocate (http://www.museum.tulane.edu/geolocate/). Distribution maps were generated from georeferenced data using ArcMap v. 10.5.1 (www.esri.com). The authors collected the bulk of the material examined in this revision. Holotypes are deposited at CAS.

A system of codes appears on our labels and are referred to throughout this manuscript to organize specimen data. Many codes take the form two-digit year—(hyphen) event numbered in sequence for that year:

S = DBW stop, i.e. collecting event, e.g. S82-37, the 37th collecting event in 1982
R = DBW recording number
JCS = scanning electron microscope (SEM) preparation
T = DBW testes preparation for karyotyping
JCT = JAC testes preparation for karyotyping

Other codes include:

A (adult) = eclosion date of adult reared from nymph
D = number associating DBW voucher specimen with tissue, usually right middle leg
DNA = number associating JAC voucher specimen with tissue
JAC = specimen barcode, 9-digit number
JCR = JAC recording date_(underscore) number, e.g. 160729_03, the 3rd recording on 29-VII-2016.
SING = DNA extraction, numbered sequentially

Morphological measurements were made with Vernier calipers and are a subset of the measurements established for *Phymonotus* (Lightfoot *et al.* 2011) as relevant for Nedubini. All measurements are expressed in mm. Photographs of habitus and terminalia were made with a digital camera (model TG-5, Olympus Inc., Center Valley, PA, USA) set to image stacking in microscope mode.

Preserved males were relaxed for genitalia dissection by immersing the abdomen tip in boiling water for a few minutes. The subgenital plate was then reflexed and the entire genital capsule was removed with fine forceps. Soft tissue was cleared by immersion in 10% KOH for 0.5–1.0 d followed by neutralization in distilled white vinegar for 0.5 d. Genitalia for light microscopy are stored in glycerol-filled genitalia vials that accompany the specimens. Genitalia preparations were illustrated by hand with the aid of a camera lucida attached to a stereo zoom microscope set to 10×zoom.

Genitalia for SEM preparations were chemically dried by immersion in 70% ethanol for 1 d, 95% ethanol for 1 d, and then two changes of hexamethyldisilazane (HMDS) for 20 min each. Chemically dried specimens were adhered onto pennies with carbon stickers and sputter-coated with a 60% Au/40% Pd mixture. Imaging was performed on a scanning electron microscope (model Phenom X, Thermo-Fisher Scientific, Waltham, MA, USA) set to 10 kV beam strength. An additional carbon sticker adhered the penny with the sample onto an SEM stub so that the stub could be reused.

Surprisingly, study of the stridulatory file, which is commonly employed in the systematics of ensiferan Orthoptera (e.g. Walker & Funk 2014; Weissman & Gray 2019), has been neglected in *Neduba* and turns out to be an important diagnostic character for species Groups and species (see Results). Stridulatory files were studied on excised tegmina. The tegmina of *Neduba* are ambidextrous (Cole & Chiang 2016; Pemberton 1911) but do not switch position while singing (Morris *et al.* 1975); thus the dorsal tegmen, which will have the functional stridulatory file if there is any asymmetry in tegminal development, was excised and mounted upside down on a microscope slide. Excision was more easily accomplished when the specimens were fresh. Dried specimens were relaxed as described

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 25ºC (= 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* katydids. 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♂ = 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