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Enhancing Phylogenetic Resolution with Taxonomic and Geographic Characterization of the Chilean Species of the Cicada Genus Tettigades

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AUTHORS DISCLAIMER:

All taxonomic actions in this work are hereby disclaimed for nomenclatural purposes, as recommended in Article 8 of the International Code of Zoological Nomenclature (Ride et al., 1999).

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APPROVAL PAGE

Master of Science Thesis

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Abstract

The Patagonian cicada genus *Tettigades,* although abundant and diverse, was poorly documented until Torres (1958) reviewed the species of the genus, describing 17 new species and redescribing others. His review provided useful insights on variation and distribution of species in the genus. However, despite Torres' efforts, some species descriptions remained vague and many lacked descriptions of morphological variation within or among populations.

The goal of this thesis is to compile, evaluate, and integrate previous work with new data to serve as a reference for future studies of this genus. Morphological, geographical, and molecular data are combined in order to treat some of the Chilean species of *Tettigades* and to provide insight on potential species new to science. Argentine and other Chilean species will be included in future studies. The described species that will be treated in this thesis include the following Chilean species: *T. auropilosa, T. chilensis, T. distanti, T. lacertosa, T. limbata, T. opaca, T. ulnaria*, and *T. undata*.

Chapter 1

The Taxonomic History of the Patagonian Cicada Genus *Tettigades*

Introduction: Andean uplift and Patagonian cicada diversity

The uplift of the modern Andes is thought to have begun 23Mya (Woodburne et al. 2014). As a result of collision in the northern Andes and subduction in the southern Andes (Folguera et al. 2011), the Andean uplift underwent several phases of mountain building in the late Oligocene to early Miocene, the late middle Miocene, and the early Pliocene, leading to the shape of the Andes seen today (Hoorn et al. 2010; Folguera et al. 2011). Climatic oscillations, glaciation, and volcanic events during the complex geological history of the Andes has resulted in restricted gene flow between populations, shaping the diversity and distribution of lineages through time, throughout South America (Turchetto-Zolet et al. 2012; See Chapter 2), but of course less so for mountain species and less so in the south where the Andes are lower in elevation.

The cicada fauna of South America is composed of a diverse array of species from multiple subfamilies and tribes. However, Patagonia is dominated by one tribe of cicadas, Tettigadini Distant, 1905 in the subfamily Tibicininae Distant, 1905, which is sister to all other subfamilies within the family Cicadidae Latreille, 1802 (Moulds 2005). Moulds (2005) revised Cicadidae subfamiles and named the subfamily to which *Tettigades* belongs, “Tettigadinae"; however, according to the principle of priority in the Code of Zoological Nomenclature (ICZN, 1999 - Article 23), the name Tibicininae takes priority (as used in the most recent catalogue of the Cicadoidea (Sanborn 2013)).

The most diverse genus in the tribe Tettigadini is the type genus *Tettigades* Amyot & Audinet-Serville, 1843 which comprises 27 described species as catalogued in Metcalf (1963), Duffels & Van der Laan (1985) and Sanborn (2013). Although the species are found throughout Patagonia, little is known about this genus. Most of the work on *Tettigades* consists of species descriptions based solely on morphological data and comparatively few specimens. As discussed below, recent molecular studies such as those of Van Leuven et al. *(*2014) and Lukasik et al. (2017) have shed light on the coevolution of cicadas (including *Tettigades*) and their obligate bacterial endosymbionts.

History of the genus *Tettigades*

The genus *Tettigades* was first described as monotypic by Amyot & Audinet-Serville (1843) based on the type species, *Tettigades chilensis*. *Tettigades* was distinguished from other known genera at the time by the presence of: [translated from French - Fig. S1/S2]

> "Body (black) with long fine hairs. Head almost as wide as the prothorax, very short. Eyes small, round. Prothorax a transverse narrow band, with slightly but distinctly expanded edges rounded on each side. Fore and hind wings transparent (veins shaded dark brown). Tarsi three segmented."

The type species, *Tettigades chilensis* was described as inhabiting Chile: [translated from French – Fig. S1/S2]

> "Length 0.025m. Body entirely black with fawn or gray hairs. The veins of the apical ends of the fore and hind wings shaded with intense black; veins of the wing base, blood red; Ventral surface of body, hairy. Legs the same color as the body and hairy. Female."

The problem with early work on *"Tettigades chilensis"*

The 1800's was the era that has caused much of the confusion surrounding *Tettigades chilensis* today. This confusion has led to countless misidentifications and created a misleading notion of the diversity of the genus. During the 1850's, several species would be described that would be mistakenly synonymized with *T. chilensis,* such as *Cicada rubrolineata* Spinola, 1852; *Fidicina crassivena* Walker, 1858; and *Cicada eremophila* Philippi, 1860 (Sanborn 2013). Even species that were morphologically distinct were synonymized with *T. chilensis*, for example *Tettigades lebruni* by Signoret (1863). At various times, *Tettigades crassimargo* Signoret, 1863 has been placed in the genus *Tibicen* Latreille, 1825*, Cicada* Linnaeus, 1758, *or Tibicina* Kolenati, 1857*.* It is now treated as *Chilecicada occidentis* Walker, 1850 and held as the type genus of the tribe Chilecicadini (Sanborn 2014a).

Other *Tettigades* work in the 1800s

After the initial characterization of the genus in 1843, one of the smallest species in the genus, *Tettigades compacta* Walker, 1850 was described based on its distinct cloudy wings and small body size. Its type locality was listed vaguely as "West coast of America." *Tettigades cinnabarina* Berg, 1879 was described and also stood out due to its distinct red body color which was inconsistent with the original concept of the genus. It would take 79 years before it was recognized not to be a species belonging to the genus *Tettigades* (Torres 1958b).

By 1881, Distant concluded that *Tettigades* was restricted to the Neotropics. Therefore, he alleged that the habitat given by Walker for the locality of *T. compacta*

was likely referring to Central America (Distant 1881). Distant also described a specimen labeled as inhabiting Mexico, *Tettigades mexicana* Distant, 1881 (Fig. S3/S4).

Shortly thereafter *Tettigades papa* Berg, 1882 was described and became for a while the largest and first Argentine *Tettigades*. However, it would be moved later to the genus *Chonosia* Distant, 1905. Distinguishable by its slender body, the Argentine *Tettigades parva* Distant, 1892 was the last species described during this era.

Tettigades work during the early 1900's

The next 100 years would lead to the introduction of many new species, illustrating both the diversity and large geographic range of *Tettigades*. In 1906, three new *Tettigades* species were described by Distant: from Argentina, *Tettigades lebruni* and *Tettigades varivenosa*; and from Chile, *Tettigades ulnaria.* The next *Tettigades* species to be described was *Tettigades opaca* Jacobi, 1907, which closely resembles *T. compacta* in its cloudy wings and small body size. *Tettigades lizeriana* Delétang, 1919 was recognized and named after a friend of the describer, engineer Carlos Lizer. However, the description was restricted to a statement in a dichotomous key, therefore, not considered a valid taxonomic act. Interestingly, *Tettigades dumfriesi* Distant, 1920 was named after the Earl of Dumfries who captured two Argentine specimens that flew into his railway carriage at about 6000 feet as he traveled across the Andes.

Some of the confusion surrounding *T. chilensis* and other *Tettigades* species continued during this time period. Jacobi insisted that *Tettigades crassimargo* was synonymous with *T. chilensis*, however, this idea was rejected by Distant. In addition, Jacobi noted that the reference to *T. mexicana* in Mexico may have possibly been an

error (i.e., mislabeled specimen). *Tettigades porteri* Bréthes, 1920 was described but was synonymized with *T. opaca. T. lebruni* was assigned to a new genus, *Edholmbergia*, by Delétang (1919).

The only documentation on the life history of any *Tettigades* species was recorded in Pirion (1929) who suggested that *Tettigades "chilensis"* in Chile emerged at least every 15 years. However, it is unclear whether this is the species we call *T. chilensis* today.

The work of Belindo A. Torres (1940s-1960s)

Tettigades would be at the center of an ambitious revisionary project during the 1940's and 1950's by Belindo A. Torres. Torres, an Argentinian entomologist, who not only worked on *Tettigades* but described and redescribed a total of nine cicada genera including phylogenetic cousins of *Tettigades* such as *Tettigotoma* 1942, *Chonosia* 1945, *Alarcta* 1958c, and *Calliopsida* 1958b. Torres would travel to museums in both Argentina and Chile, examining and describing museum specimens. It appears that Torres did no field work himself because the three major type-holding museums in Chile and Argentina I visited (el Museo Natural de Historia Natural in Chile, el Museo de La Plata, and el Museo Argentino de Ciencias Naturales "Bernardino Rivadavia" in Argentina), where Torres examined specimens, have no specimens collected by Torres.

In 1942, Torres described two Argentine *Tettigades*, *T. bosqi* and *T. lizeri.* Torres (1944) revised the description of *T. chilensis* by providing a more detailed description and new diagnostic characters not used by Amyot & Audinet-Serville in their original description (*See Chapter 3*). He argued that Argentine references to *T. chilensis* and *T. ulnaria* were actually two undescribed species. In 1944, he would also describe from

Argentina: *T*. *major* and *T. sarcinatrix*; and from Chile: *T. auropilosa, T. lacertosa*, and *T. sordida*.

In Torres (1958c), he described and published on the new genus *Alarcta*. Several of the species within *Alarcta* were originally described by Torres in the 1940's under *Tettigades*. Using the ratio of wing length to width and other characters, Torres moved the following species from *Tettigades* to *Alarcta*: *A. bahiensis* (1942), *A. blanchardi* (1948), *A. albicerata* (1949), *A. macrogina* (1949), and *A. minuta* (1949).

Torres continued to publish short papers on new species, but in 1958, along with introducing 10 new species to the genus, Torres compiled the most comprehensive revision of *Tettigades* to date. Torres delimited species using new morphological characters such as the coloration of the vannal area of the hindwing and the morphology of the stridulatory organ of the mesonotum. Torres provided a more detailed description of the genus: [Translated from Spanish]

> "Head including eyes slightly wider or the same width as the base of mesonotum; Postclypeus scarcely or not projected beyond the anterior edges of the vertex, almost as long as the sum of the latter plus the front to the height of the area of the ocelli; Moderate pronotal expansions rounded and non-angular; Wings hyaline, the first with some veins especially the transverse ones and anastomosis and apical area being shown to be very finely shaded; Wings with 4 convex transverse veins; Apical areas 5 and 6 almost equal in length; 7^{th} apical cell, in most cases much longer than 6^{th}; Relationship between length and width of wings 2.5: 1, and varying between 2.2: 1 and 2.7: 1; Body generally covered with long hair."

Much terminology for external and internal morphology of the Cicadoidea has been standardized by Moulds (2005), due to differences in structural interpretation of previous in-depth studies of cicada morphology. In Torres' description of the genus, the

apical areas (R_2, 1^aR_3, 2^aR_5, M_1, 2^aM_2, M_3, M_4, and CU_1 – Fig. S5) are now known as apical cells 1-8 according to Moulds (2005).

In that same publication, Torres commented on several previous species descriptions and references. He noted Signoret's (1863) suggestion that *Cicada crassimargo* was synonymous to *T. chilensis* as incorrect. The reference to an Argentine *T. opaca* was a misinterpretation of Brèthes (1920) where Argentina was listed as the site of publication not the type locality. Jacobi (1907) expressed concern over whether *T. opaca* was a valid species or synonymous with T*. chilensis*. However, Torres concluded that *T. opaca* was indeed distinct from T*. chilensis* (*see descriptions later in this thesis – Chapter 3*).

After observing images of the type specimen, Torres recognized *Tettigades mexicana*, as a valid species and noted the reference to the possibility that it may represent a mislabeled specimen. Torres suggested that *T. mexicana* belonged to the Chilean fauna and was morphologically similar to *T. curvicosta*. *T. varivenosa* was described by Torres as having multiple morphotypes which varied by color. *Tettigades cinnabarina* was moved to the genus *Calliopsida* in 1958b. Torres also reassigned *Edholmbergia lebruni* back to *Tettigades*. During his career, Torres would describe 17 new species of Tettigades: *T. bosqi* and *T. lizeri* in 1942; T. *auropilosa*, *T. lacertosa*, *T. major*, *T. sarcinatrix*, *and T. sordida* in 1944; and *T. angularis*, *T. brevicauda*, *T. crassa*, *T. curvicosta*, *T. distanti*, *T. limbata*, *T. pauxill*a, *T. procera*, *T. undata*, and *T. unipuncta* in 1958.

Modern work (2010-Present)

After Torres' monograph in 1958, it would be 56 years until any new work on *Tettigades* was done. In a historical overview of the Neotropical tribe Zammarini, Goemans (2010) cleared up much of the confusion surrounding the name *Tettigades lebruni*. He noted that there were two *lebruni* species described, one by Distant and the other by Delétang. The description by Distant was determined to be a *Tettigades* species. Together with *Adusella signata*, Delétang's descriptions were regarded to be junior synonyms of *Odopoea venturii* (Goemans 2010).

In their checklist of Argentine cicadas, Sanborn & Health (2014), also discussed the confusion surrounding the name *Tettigades lebruni*. They noted that Delétang incorrectly transferred *Tettigades lebruni* to a new genus, *Edholmbergia*. The illustration of *E. lebruni* in Delétang (1919) did not match the type specimen of *T. lebruni* held at the British Museum. *Adusella signata* Haupt, 1918 was determined to be a valid species and removed as a junior synonym of *T. lebruni* and reassigned to the genus *Odopoea* Stål, 1861.

Sanborn and Heath (2014) also noted that Metcalf (1963) misreported *T. compacta, T. opaca,* and *T. ulnaria* from both Argentina and Chile. Argentine references to *T. compacta* and *T. ulnaria* were determined to be a misinterpretation of Delétang (1923). The reference to *T. opaca* was a misreading of the publication locality. They also demonstrated that *Tettigades lizeriana* Delétang, 1919 was an invalid name, and provided new records for several *Tettigades* species. The description of *T. lizeriana* was restricted to a statement in a dichotomous key to distinguish *T. lizeriana* from *T. chilensis*. This did not meet the provisions for availability under the Code (ICZN, 1999).

Endosymbiont Work (2014-Present)

Van Leuven et al. (2014) recently revealed that the obligate nutritional bacterial endosymbionts of *Tettigades* have experienced unprecedented genome duplication and parallel/co-dependent genome reduction similar to those of chloroplast and mitochondria. Instead of two endosymbionts (as commonly found in other plant sap sucking bugs), three were found in *Tettigades undata*. The smaller endosymbiont, *Candidatus* Hodgkinia *cicadicola* had split into two mutually dependent symbionts each of which performed only some of the necessary functions but together they supplied the necessary gene products for cicadas to function. Lukasik et al. 2017 sampled cicadas from the genus *Tettigades* more broadly to better understand the frequency, timing, and structural outcomes of *Hodgkinia* lineage splitting. They discovered that while some species had a single *Hodgkinia* lineage, others had split from between one and six independent times, leading to complex *Hodgkinia* populations of up to six lineages per cicada host.

Where the genus stands today

Tettigades currently comprises of 27 species (Sanborn 2013). Nine species are found in Argentina, seventeen in Chile, and the exact locality of *T. mexicana* unknown (Table 1). The name *Tettigades lizeriana* remains as nomen nudum. Recent collections from Chile suggests *T. sarcinatrix* is found both in Argentina and Chile (collected at 887 meters in the Andes 35 kilometers from the border of Argentina), not just Argentina as previously suggested. No doubt, other *Tettigades* will be found to inhabit both countries.

The existence of *T. mexicana* in Central America remains in doubt. Collections throughout the world do not have any specimens resembling this species. After

examining images of museum specimens in the United State National Museum with localities labeled Cochise County, Arizona, it appears as though they may have been mislabeled and misidentified. USNM specimens are identified as *T. mexicana* but do not match the drawing and description of *T. mexicana* by Distant (1881) nor characters described by Torres (1958) in his redescription. The collecting locality of these specimens is “near Buenos Aires National Wildlife Reserve” which may have led to some confusion. Museum specimens with locality labels such as “Patagonia” might have been confused with the town of Patagonia in Santa Cruz County, Arizona. However, *Tettigades* is not known to occur in North America and members of the genus do not resemble any species found in Arizona.

Table 1. Checklist of species currently included within the genus *Tettigades.*

Family Cicadidae Latreille, 1802 **Subfamily** Tibicininae Distant, 1905 **Tribe** Tettigadini Distant, 1905 **Genus** *Tettigades* Amyot & Serville 1843 = *Tettigodes* (sic) = *Tettigates* (sic) **Type species** *Tettigades chilensis* Amyot & Serville 1843				
Species	Original Describer	Type Locality	Country	Synonyms
T. angularis	Torres, 1958	Lag. La Laja - (Los Barros) 1500m	Chile	None
T. auropilosa	Torres, 1944	Queltehues*	Chile	None
T. bosqi	Torres, 1942	Mendoza	Argentina	None
T. brevicauda	Torres, 1958	Cord. Chillán	Chile	None
T. chilensis	Amyot & Audinet-Serville, 1843	Exact locality not provided	Chili (sic)	=*T. chiliensis*(sic)= *Cicada rubrolineata* Spinola, 1852=*T. rubrolineata*=*Fidicina crassivena* Walker, 1858=*Cicada eremophilia* Philippi, 1866
T. compacta	Walker, 1850	West Coast of America [Central America] **	Chile	None
T. crassa	Torres, 1958	Colchagua	Chile	None
T. curvicosta	Torres, 1958	Exact locality not provided	Chile	None
T. distanti	Torres, 1958	Los Andes	Chile	None
T. dumfriesi	Distant, 1920	Argentine side of the Andes, about 6000ft above sea level	Argentina	= *T. dumfriesii* (sic)
T. lacertosa	Torres, 1944	Valparaíso*	Chile	None
T. lebruni	Distant, 1906	Patagonia, Santa Cruz	Argentina	None
T. limbata	Torres, 1958	Los Valdes, Santiago (Elevation 1800m)	Chile	None
T. lizeri	Torres, 1942	Villavincencio Mend. (1980m)	Argentina	None
T. lizeriana nomen nudum	Delétang, 1919	Mendoza province	Argentina	None

T. major	Torres, 1944	Bajo de Sta. Rosa (Río Negro)	Argentina	None
T. mexicana	Distant, 1881	Exact locality not provided***	Probably Chile	None
T. opaca	Jacobi, 1907	No data provided	Chile	=*T. porteri* Bréthes, 1920
T. parva	Distant, 1892	Argentine Republic	Argentina	*T.* parvus (sic)=*T. brevivenosa* Torres, 1942
T. pauxilla	Torres, 1958	Cura Cautin (sic)	Chile	None
T. procera	Torres, 1958	Valdivia	Chile	None
T. sarcinatrix	Torres, 1944	San Carlos de Bariloche (Río Negro)/Nahuel Huapi (Río Negro)/Isla Victoria (Neuquén)*	Argentina [Chile]	=*sarsinatrix*(sic)
T. sordida	Torres, 1944	Exact locality not provided	Chile	None
T. ulnaria	Distant, 1906	Exact locality not provided	Chili (sic)	=*T. urnaria* (sic)
T. undata	Torres, 1958	San Bernando	Chile	None
T. unipuncta	Torres, 1958	Exact locality not provided	Chile	None
T. varivenosa	Distant, 1906	Argentine, Gob. Rio Negro	Argentina	None

* Described using cotypes.
** Distant (1881) suggests Walker was referring to Central America in his description of the species.
*** Reports of *T. mexicana* inhabiting Mexico are probably due to a mislabeled specimen (as noted in Jacobi 1907, Torres 1958a, and Sanborn 2014b).

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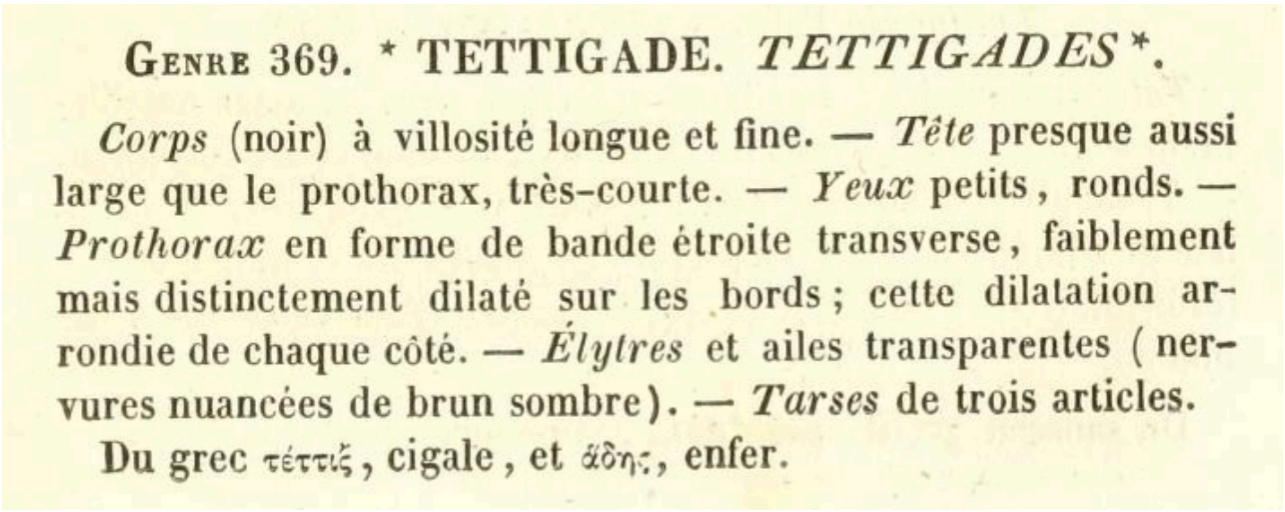
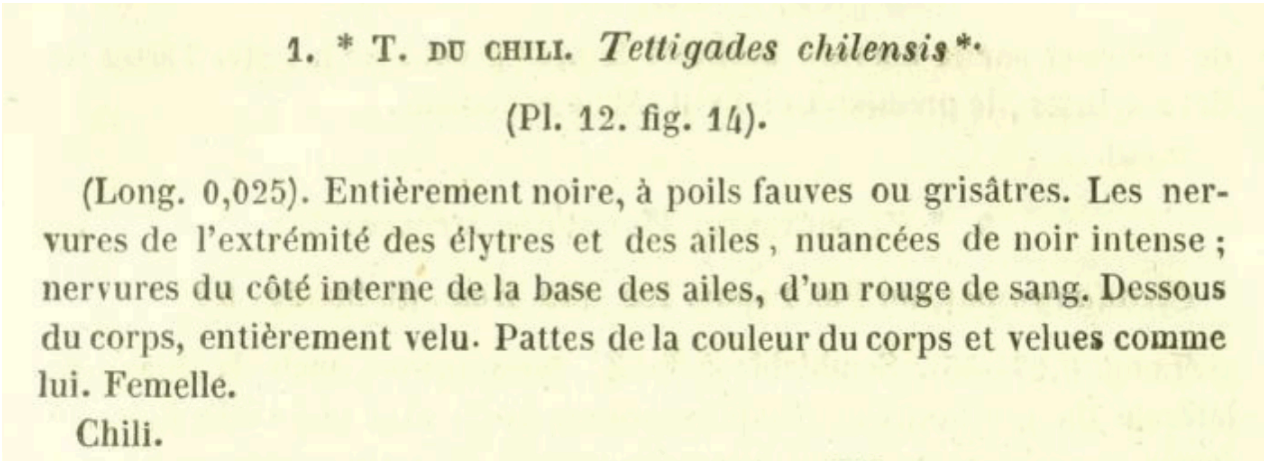
Supplemental Material



GENRE 369. * TETTIGADE. *TETTIGADES* *.

Corps (noir) à villosité longue et fine. — *Tête* presque aussi large que le prothorax, très-courte. — *Yeux* petits, ronds. — *Prothorax* en forme de bande étroite transverse, faiblement mais distinctement dilaté sur les bords; cette dilatation arrondie de chaque côté. — *Élytres* et ailes transparentes (nervures nuancées de brun sombre). — *Tarses* de trois articles.

Du grec τέττιξ, cigale, et ἅδης, enfer.



1. * T. DU CHILI. *Tettigades chilensis* *.

(Pl. 12. fig. 14).

(Long. 0,025). Entièrement noire, à poils fauves ou grisâtres. Les nervures de l'extrémité des élytres et des ailes, nuancées de noir intense; nervures du côté interne de la base des ailes, d'un rouge de sang. Dessous du corps, entièrement velu. Pattes de la couleur du corps et velues comme lui. Femelle.

Chili.

Figure S1. (Top) Original description of the genus *Tettigades* and (Bottom) *Tettigades chilensis* by Amyot & Audinet-Serville, 1843.

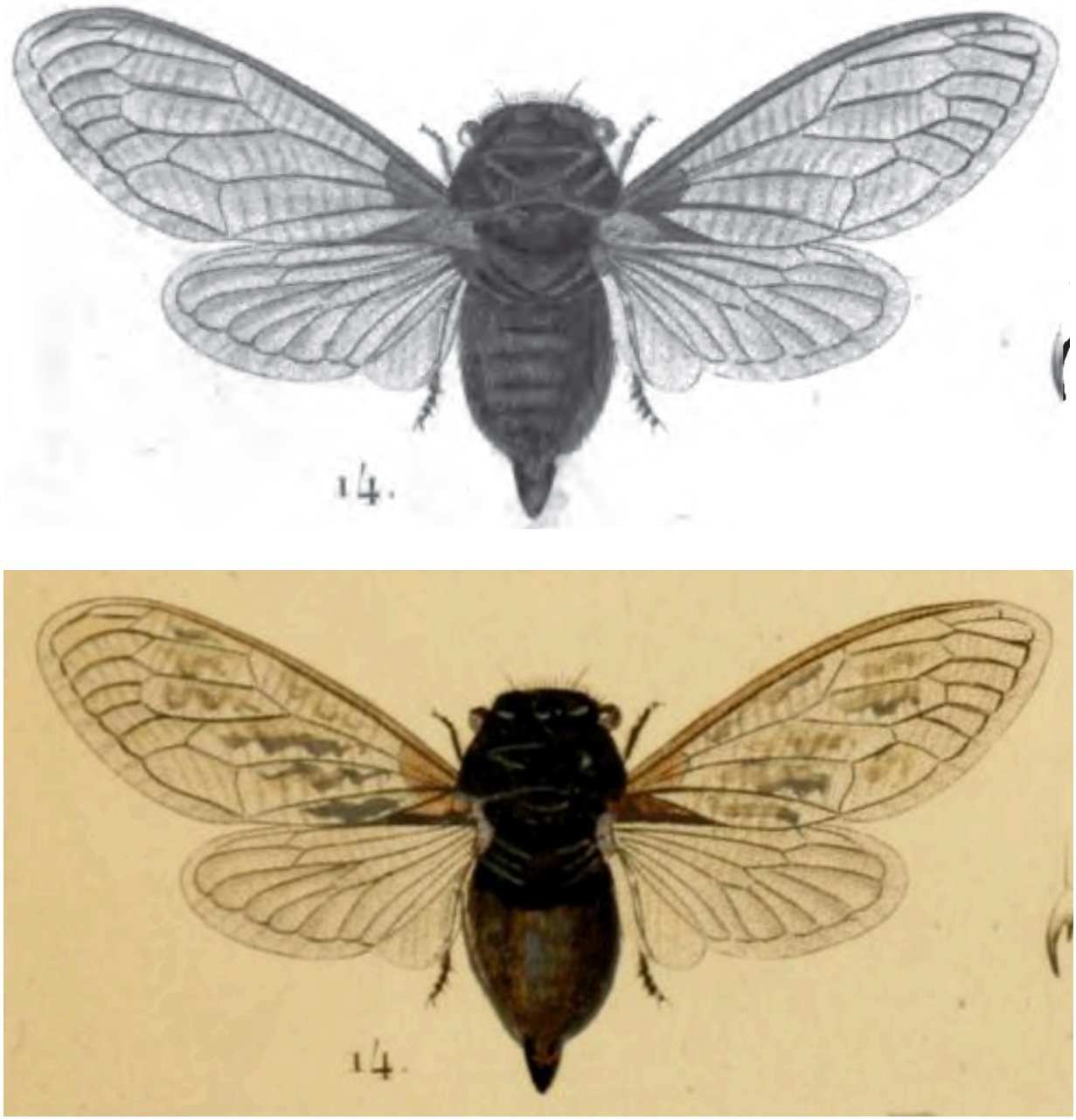


Figure S2. Drawing of *Tettigades chilensis* by original describers Amyot & Audinet-Serville, 1843. (Top) Gray scale image; (Bottom) Colored image of the holotype.

Figure S3. Drawing of *Tettigades mexicana* by original describer Distant (1881) of *Tettigades mexicana.*

Figure S4. Dorsal and ventral view of the holotype of *Tettigades mexicana* deposited at ZMHB (Berlin, Germany). Image courtesy of ZMHB.

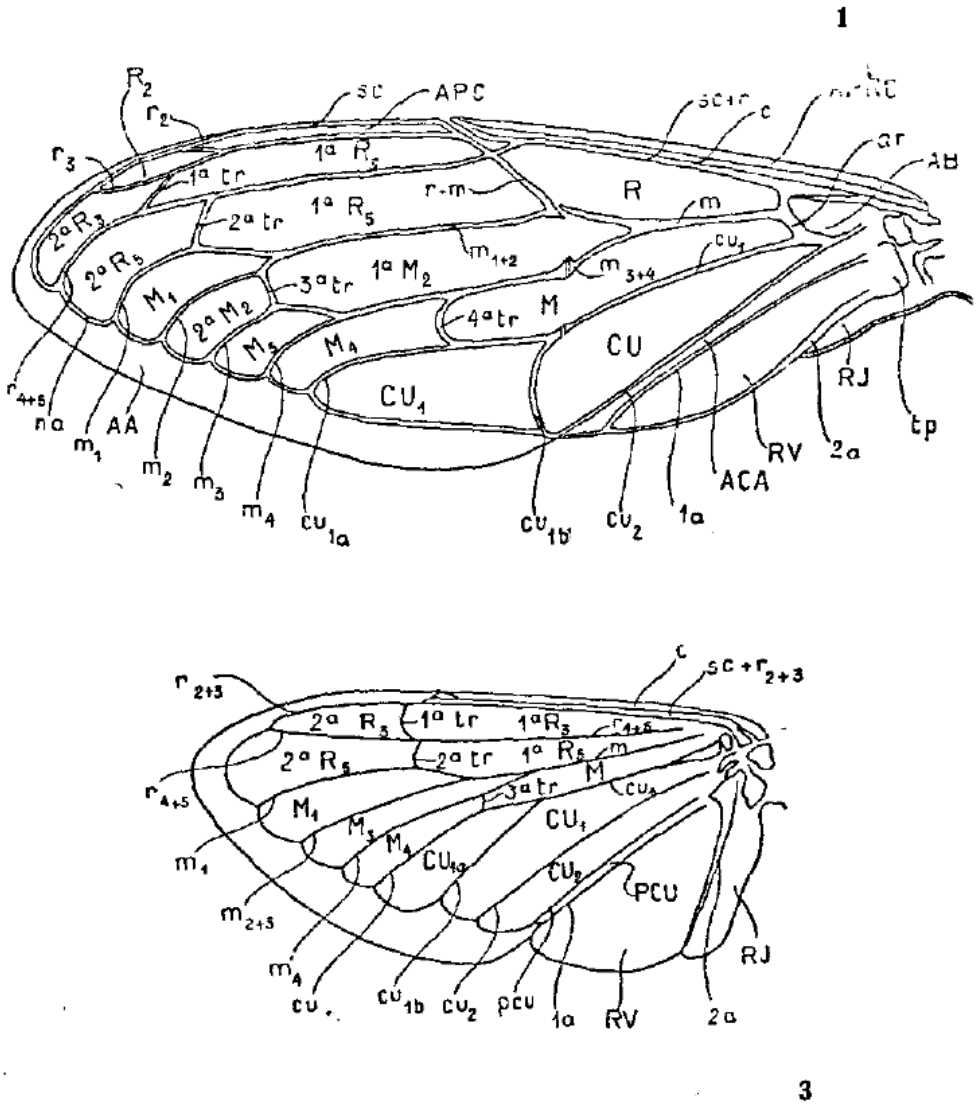


Fig. 1, Cabeza de cicádido en vista dorsal ; a : postclípeo; b : frente; c: vértex. Fig. 2, Tégmina mostrando sus áreas y nervaduras. AA : área ambiente ; AB : área basal ; ACA : área cúbito anal ; APC : área postcostal ; APRC : área precostal ; CU : área cubital ; M : área mediana ; R : área radial ; RJ : región jugal ; RV : región *vannal* ; áreas 1ª R_3, 1ª R_5, 1ª M_2, M_1 y CU = áreas ulnares ; áreas R_2, 2ª R_3, 2ª R_5, M_3, 2ª M_2, M_3, M_4 y CU_1 = áreas apicales ; ar : *areulus* ; 1a y 2a : nervaduras anales ; c : nervadura costal; cu_1 y cu_2 : nervaduras cubitales ; m : nervadura mediana ; na : nervadura ambiente ; sc : nervadura subcostal ; sc + r : nervadura subcostal + radial ; 1ª tr — 4ª tr : nervaduras 1ª — 4ª transversales ; tp : tuberosidad posterior o *plectrum*. Fig. 3, Alas con sus áreas y nervaduras. (Tanto en las tégminas como en las alas las áreas están indicadas con letras mayúsculas y las nervaduras con minúsculas).

Figure S5. Wing notation as described by Torres (1958a).

Chapter 2

Phylogenetic Resolution of the Chilean Species of the Cicada Genus Tettigades using Targeted Gene Capture

Introduction

After describing 17 new species of *Tettigades* in 1958, Belindo A. Torres noted in his general considerations of the genus *Tettigades*:

> "The difficulties encountered by the study of the genus *Tettigades* do not fail to be numerous, because their representatives do not show as in other cases a series of characters that facilitate their identification such as the presence of shape and color characteristics in the different parts of the body, since in the genus there is a predominance of a general black color with very few areas of ochre brown distributed in very similar form in each of the different species."

Although highly abundant and attractive, the genus *Tettigades* has had little attention since its last revision by Torres (1958a). While recent molecular studies on the genus have been conducted such as Van Leuven et al. (2014), the primary focus of the studies has been the endosymbionts on which these cicadas rely for nutrition. Little is known about the evolutionary and life history of the genus. The majority of species descriptions remain vague and many lack descriptions of morphological variation within or among possible intraspecific clades.

Multiple sources of data such as morphology, geography, and DNA are often combined to delimit cicada species, especially for those that are difficult to distinguish (Hertach et al. 2016; Owen & Moulds 2016). Here we present nuclear and mitochondrial phylogenies, in addition to geographic and morphological data, to further understand the Chilean species within *Tettigades.* Although DNA barcodes such as mitochondrial cytochrome *c* oxidase (COI) have been used to assist in species identification (Ratnasingham & Herbert 2003), genetic data have been found to conflict with current

taxonomic classification of cicadas (Nunes et al. 2013), requiring additional genes and other taxonomic data and analyses. Due to the common occurrence of nuclear mitochondrial DNA segments (numts) when sequencing COI from several *Tettigades* species, the time consuming and expensive methods necessary to avoid such numts (Łukasik et al. 2017), and the number of specimens in this study, we chose to create a phylogeny for Chilean *Tettigades* species using a targeted gene capture approach. Targeted gene capture allows for higher reproducibility and a lower variance in target coverage compared to other genome-partitioning approaches (Jones & Good 2015).

The phylogenetic relationships of 152 specimens within the cicada genus *Tettigades* and two outgroups (*Chonosia* and *Alarcta*) were explored using molecular data from within the exonic regions of 742 single-copy protein-coding genes conserved in Arthropoda (941,450bp) and from all 13 mitochondrial protein coding genes (10,266bp). Nine previously described Chilean species of the genus were included in the analyses (Fig. S1): *T. auropilosa, T. chilensis, T. distanti, T. lacertosa, T. limbata, T. opaca, T. sarcinatrix, T. ulnaria*, and *T. undata*. This study represents the basis for a future revision of the genus, the first since it was last revised 59 years ago. Argentine and other Chilean species will be treated in future studies.

Materials and Methods

Specimen Acquisition

Due to the limited amount of material available for molecular analyses two field trips were made to Chile to collect fresh specimens. These trips focused on obtaining samples from a variety of habitats, known species localities, and new localities. A total of 152 specimens from the genus *Tettigades were* collected from 59 localities in Chile

during two field-collecting trips, one in December 2014 and the second from December 2015 to January 2016 (Table S1). During the second field-collecting trip, specimens from the genus *Alarcta* and *Chonosia*, were collected and used as the outgroup. Specimens were also obtained from natural history museums and private collections. Representative specimens were identified based on morphological characters provided by original descriptions and redescriptions, as well as by examining type material. Most specimens were directly preserved whole in 70% or 95% ethanol. Others specimens had their abdomens dissected and preserved in RNAlater, formaldehyde solution, or glutaraldehyde solution, while the remainder of the body was placed in 95% ethanol. A representative from each clade in this study will be vouchered and deposited at el Museo Natural de Historia Natural (MNNC, Santiago, CL).

The numbering system used by the McCutcheon Lab at the University of Montana, “PL”, “KN”, and “JM” values, are the specimen numbers associated with each individual collected. The first two letters of the numbering system are the initials of one of the collectors during one of the collecting trips.

A detailed description of specimen acquisition is provided in Chapter 3 of this thesis.

Library Preparation and Targeted Capture

Genomic DNA was extracted from dissected bacteriome tissue stored in RNAlater or from one cicada leg stored in 95% or 70% ethanol, all at -80°C, using the Qiagen (Qiagen, Valencia, CA, USA) DNeasy Blood & Tissue kit (DY14 Aug-06), according to the manufacturer’s instructions. For both bacteriome and leg DNA

extractions, at least one individual from each location was selected. In some cases, when species identity was unknown or variation was expected, more than one individual was included.

As a positive control for all library preparation steps, we used the PCR-amplified partial RNA polymerase subunit B (rpoB) gene of the *Hodgkinia* endosymbiont of *Tettigades distanti*, of an approximate length of 540bp (including primers). As a negative control for all steps, we used molecular-grade water. Library preparation was performed following the protocol from Meyer and Kircher (2010) with the following modifications and devices. DNA quantification was performed using a Qubit 2.0 fluorometer (Invitrogen, Life Technologies). Shearing was performed using either 1.0 µg, 0.8 µg, or 0.5 µg of purified DNA, on a Covaris LE220 sonicator. Fragments varied in size from an average of 340-460bp and were verified using a Tapestation D1000 High Sensitivity kit (Agilent Technologies). Blunt-end repair was performed with the following modification: for DNA from cicada legs 30µLof DNA and 20 µL of water were used; for DNA from bacteriomes 20µLof DNA and 30 µL of water were used. For indexing PCR, Q5 polymerase, P5 primer, and P7 primer were used; however, after testing various polymerases, all indexing of final libraries was performed using MyTaq™ polymerase. For pooling, DNA from legs and bacteriomes were pooled separately. If available, 100ng from each library were used for pooling and about 30 samples were pooled together.

A targeted nuclear gene capture was performed using the MYcroarray (Ann Arbor, MI) MYbaits® protocol (version 3.01 August 2015) without modifications. The kit consisted of probes created by P. Łukasik for 742 transcripts of a reference

transcriptome of *Tettigades chilensis*, specimen PL470. Each transcript was a unique hit to one of the BUSCO-Arthropod protein-coding genes, of similar length, ranging from 1-3kb. Prior to capture, size selection of about 400bp was performed using Bluepippin (Sage Science). Samples were then sent to Novogene (Chula Vista, CA) to be sequenced on one lane of an Illumina HiSeq 4000.

Phylogenetic Analysis

The diversity and phylogenetic relationships of 152 *Tettigades* specimens and 2 outgroups (*Alarcta* and *Chonosia*) were estimated (Fig. 1). Quality-trimmed reads were aligned against transcripts from *Tettigades chilensis*, specimen PL470 [nuclear phylogeny] or protein-coding mitochondrial genes from TETUND (Van Leuven et al. 2014) [mitogenomes-capture bycatch] using bwa (v.0.7.12). The resulting SAM alignment file was processed using samtools (v. 0.1.19) such that only successfully mapped reads remained. These alignment files were processed using custom Python scripts by Piotr Łukasik (Łukasik unpublished) relying on a pysam module (https://github.com/pysam-developers/pysam).

A RAxML analysis was generated using 941,450 nucleotide positions within exonic regions of 742 single-copy protein-coding genes conserved in Arthropoda. Using the same samples as a result of bycatch, another RAxML analysis based on all 13 mitochondrial genes (10,266 nucleotide positions) were generated. For both phylogenies, branches with bootstrap support values less than 70% were collapsed. Bootstrap values of 95% or higher are shown as black-filled circles above the nodes. Red hollow boxes indicate samples that do not form a monophyletic clade on one of the

trees. Colored shading delimits the 11 major species groups: T. *auropilosa, T. chilensis, T. distanti, T. lacertosa, T. limbata, T. opaca, T. sarcinatrix, T.* sp.1*, T.* sp.2*, T. ulnaria*, and *T. undata*. Clades have been identified with the first three letters of the species name followed by a number. For example, clades of *T. chilensis* are CHI1, CHI2, and CHI3.

Distribution Maps

Distribution maps (Figs. 2-4) were generated in R (v. 3.3.2) for field-collected specimens. Due to the difficulty of verifying species identities, the localities of museum or private collection specimens were not included. GPS coordinates were determined in the field or estimated from Google Maps. Localities and coordinates are provided in Table S2**.**

For each map, symbols were used to identify different species. For each symbol, different colors are used to identify clades.

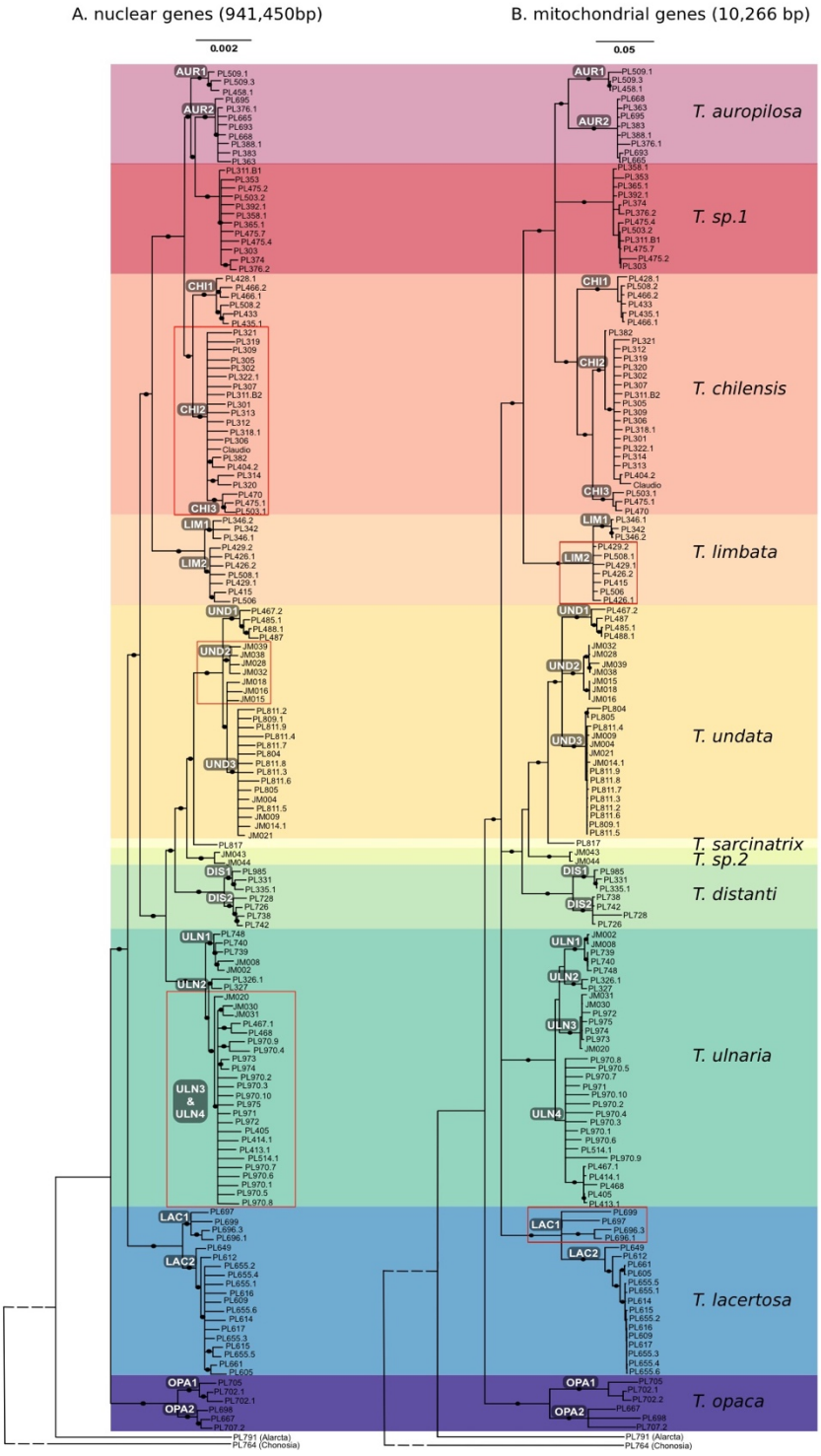


Figure 1. Maximum likelihood phylogenies of cicadas from the genus *Tettigades* based on nuclear (A) or mitochondrial (B) genes. Bootstrap values of 95% or more are shown as circles above the nodes and nodes with lower than 70% support have been collapsed. Red boxes indicate samples that do not form a monophyletic clade on one of the trees.

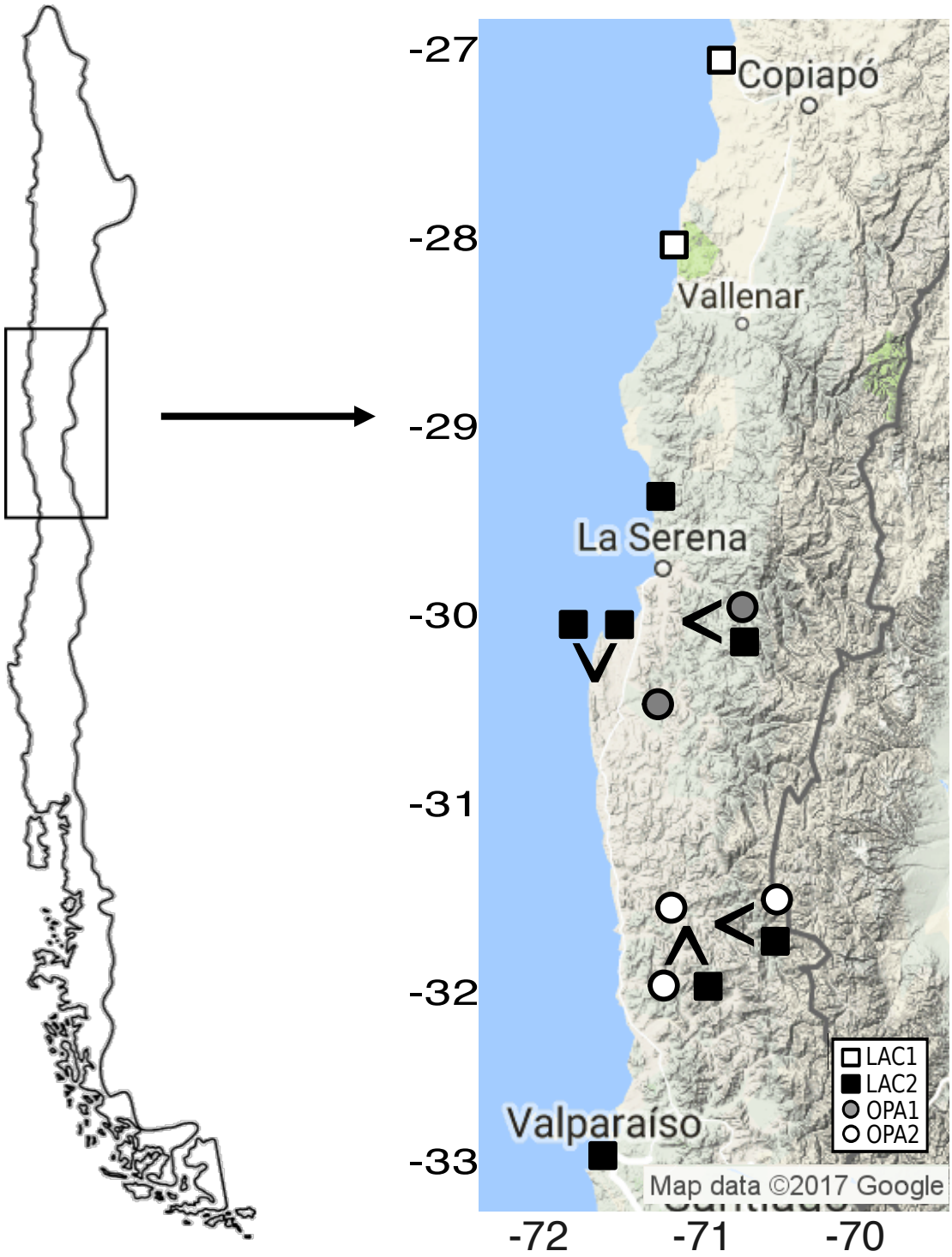


Figure 2. Localities for field-collected specimens of *Tettigades lacertosa* (squares) and *Tettigades opaca* (circles). Colored symbols correspond to clades on the phylogeny: LAC1 (white square), LAC2 (black square), OPA1 (gray circle), and OPA2 (white circle). Map of Chile reproduced from World Atlas (http://www.worldatlas.com/).

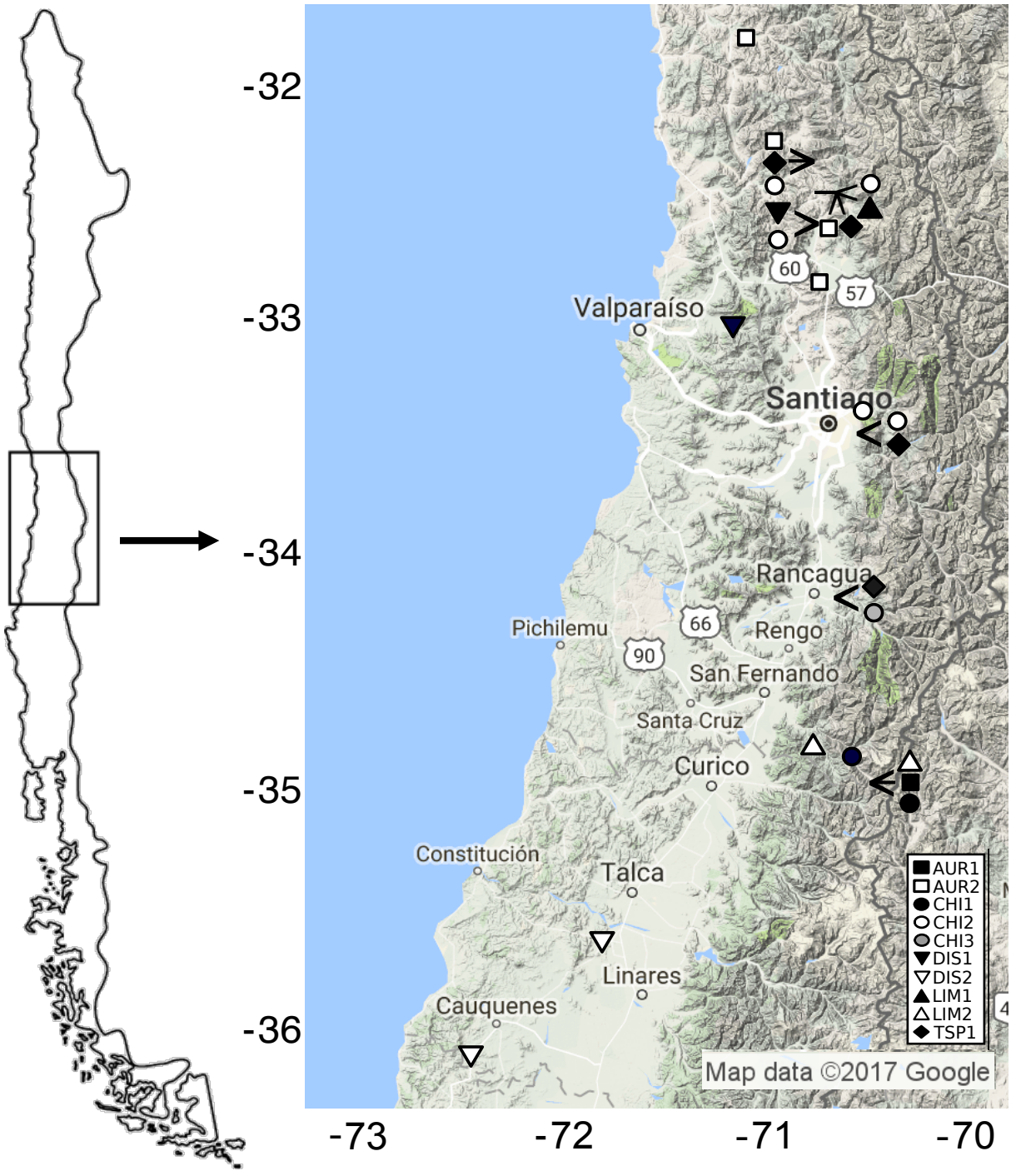


Figure 3. Localities for field-collected specimens of *Tettigades auropilosa* (squares), *Tettigades chilensis* (circles*), Tettigades distanti* (inverted triangles), *Tettigades limbata* (triangles), and *Tettigades* sp. 1 (diamonds). Colored symbols correspond to clades on the phylogeny: AUR1(black), AUR2(white), CHI1(black), CHI2(white), CHI3, (gray), DIS1(black), DIS2(white), LIM1(black), LIM2(white), and TSP1(black). Map of Chile reproduced from World Atlas (http://www.worldatlas.com/).

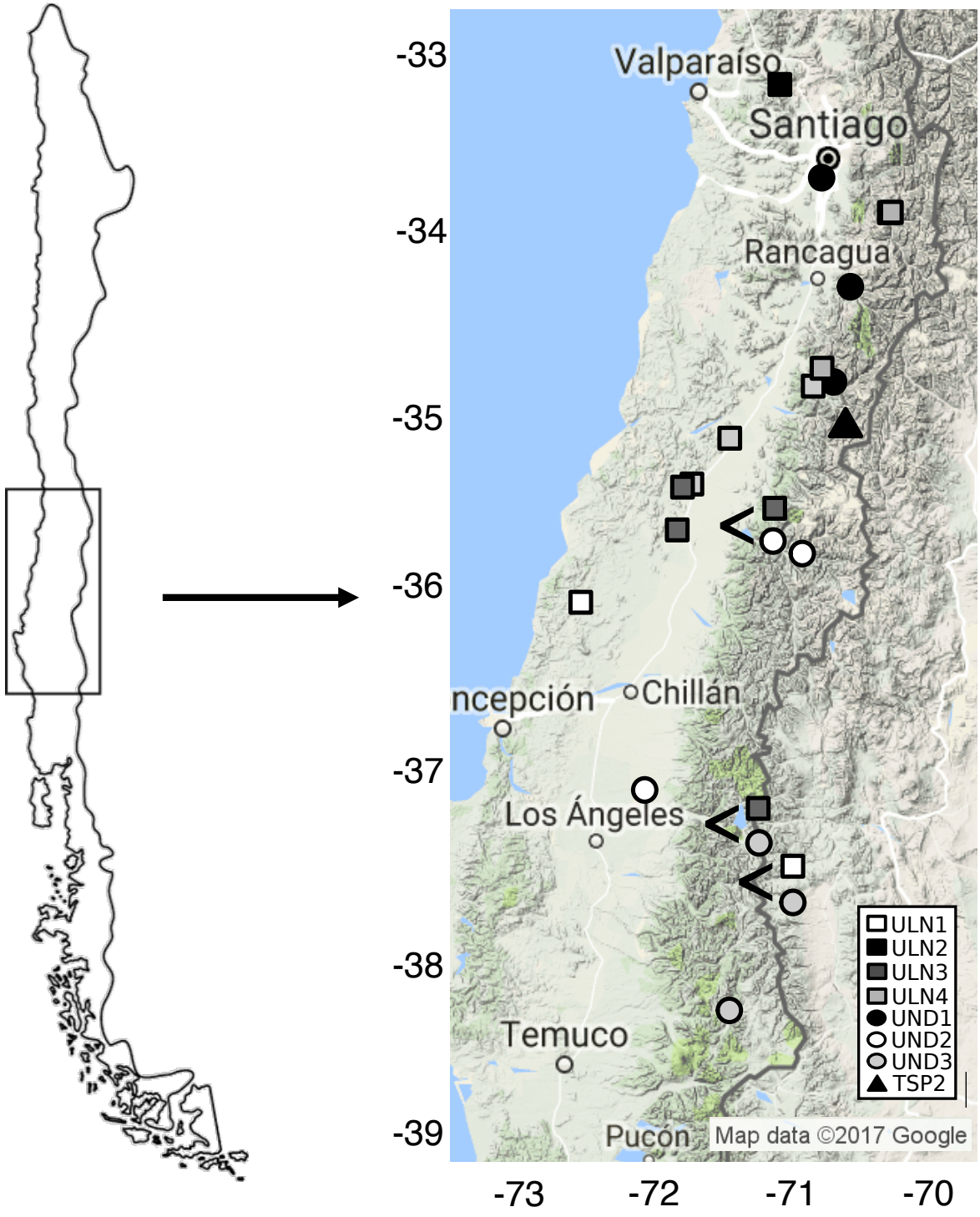


Figure 4. Localities for field-collected specimens of *Tettigades ulnaria* (squares), *Tettigades undata* (circles), and *Tettigades* sp. 2 (triangle). Colored symbols correspond to clades on the phylogeny: ULN1 (white), ULN2(black), ULN3(dark gray), ULN4(light gray), UND1(black), UND2(white), UND3(light gray), and TSP2 (black). Map of Chile reproduced from World Atlas (http://www.worldatlas.com/).

Discussion

Monophyly and relationship of the genus *Alarcta*

Due to the striking similarity between nuclear and mitochondrial phylogenies, little lineage sorting and hybridization is suggested. The low bootstrap support in the deeper nodes of both phylogenies suggests a major early radiation. *Tettigades* is estimated to have diverged about 5 to 3mya (Łukasik et al. 2017) implying that Pliocene climatic, geological, or other events were involved.

Duffels (1993) points out the monophyly of *Tettigades* and related genera is suggested by the broad head and very small eyes, but he emphasized the need for further phylogenetic analysis of this group. Our results also suggest that *Tettigades* is monophyletic; however, further studies will be required to determine the monophyly of the genus and its included species by including Argentine and other Chilean species.

Found only in Argentina and closely resembling species of *Tettigades*, several species of *Alarcta* Torres were originally described by Torres. However, in the 1940's and 1950's, Torres recognized these species as belonging to *Tettigades*. Subsequently, using characters such as the ratio of wing length to width, Torres (1958b) transferred the following species from *Tettigades* to his new genus *Alarcta*: *A. bahiensis*, *A. blanchardi*, *A. albicerata, A. macrogina*, and *A. minuta*. In that same publication, Torres described *A. quadrimacula* and *A. terrosa*. In this study, we collected a specimen representing *Alarcta quadrimacula*. As Torres concluded, this species did not belong within *Tettigades,* coming out as sister to the *Tettigades* species used in this study (Fig. 1). Further studies will be required to determine the relationship of the other species of *Alarcta* to *Tettigades.*

Species groups

A maximum likelihood analysis of both nuclear and mitochondrial genes indicates that there are nine deeply branching well-supported clades (greater than 70% bootstrap support, with only minor discrepancies between the two phylogenies (*as discussed below*). These appear to originate close together in time and correspond to previously described species: *T. auropilosa, T. chilensis, T. distanti, T. lacertosa, T. limbata, T. opaca, T. sarcinatrix, T. ulnaria*, and *T. undata*. Within these clades, there are subclades indicating variation, either recognized or unknown by the original describers of these species. Additional geographic or acoustics data will be necessary to determine in which taxa such variation is indicative of reproductive isolation, i.e., speciation. Two clades on both phylogenies contain individuals that do not match any currently recognized species of *Tettigades*. These will be treated in future work.

Morphological characters provide support for specific species groups and their relationships on both the nuclear and mitochondrial phylogenies. For clades with no obvious morphological characters, habitat and geographic localities have found to correlate with the observed variation; stated differently, the different groups correspond to different geographic areas or habitats. A morphological characterization of the various species and clades within *Tettigades* is provided in Chapter 3 of this thesis.

Tettigades chilensis

The type species of the genus, *Tettigades chilensis,* has been at the center of controversy since it was first described by Amyot & Audinet-Serville in 1843 (*see Chapter 1 and 3*). For our analysis (Fig. 1), this highly variable species was represented

by 27 individuals from eight localities, spanning the Chilean regions of Valparaíso, Metropolitan, and O'Higgins. There are three divergent clades, CHI1, CHI2, and CHI3, all highly supported as monophyletic (greater than 95%) on the mitochondrial phylogeny. In the nuclear phylogeny, CHI2, and CHI3, are not reciprocally monophyletic but instead CHI2 is a paraphyletic assemblage containing a monophyletic CHI3. Although *T. chilensis* is highly variable morphologically, none of the variability found is exclusive to any of these divergent clades. However, the three divergent clades are distinguished geographically. As seen in several of the other species within *Tettigades*, we can see a north-south pattern. CHI1 included six samples from two localities collected in the southern-most range of our *T. chilensis* field-collected samples in the region of O'Higgins (black circles – Fig.3). CHI2 included 18 samples collected in the northern-most range in the regions of Metropolitan and Valparaíso (white circles – Fig.3). CHI3 included three samples collected in one locality between CHI1 and CHI2 in the region of O'Higgins (gray circles – Fig.3). Further sampling will be necessary to fully understand the variability within *T. chilensis* and to better grasp the relationships between and among the divergent clades.

Tettigades auropilosa

Tettigades auropilosa, distinguishable by its short hairy abdomen, was represented by 11 individuals that form two clades, AUR1 and AUR2, both with high bootstrap support in the mitochondrial phylogeny. However, there are at present no identified morphological characters distinguishing these clades. Although morphological variation was present, none of the variation has yet been found to be exclusive to any of

the divergent clades. AUR1 included three individuals from one locality. AUR2 included eight individuals from four localities. Although this represents a small sample, again we can see a north-south pattern. AUR2 was found north of the city of Santiago (white squares – Fig. 3), while AUR1 was found south of Santiago (black squares – Fig. 3).

Tettigades sp.1

In comparison to the mitochondrial phylogeny, the nuclear phylogeny, identifies the same two clades of *T. auropilosa* with high support. However, together they do not form a monophyletic group but rather, AUR2 is sister to the clade labeled, *Tettigades* sp.1, which includes 12 individuals and is potentially a new species. Since mitochondrial genes evolve faster than nuclear genes and *T. auropilosa* is morphologically distinct from *Tettigades* sp. 1 (*See Chapter 3*), we suspect that this grouping in the nuclear tree is due to random error, potentially a deep hybridization or deep coalescence event. *Tettigades* sp.1 was represented by 12 individuals collected in five localities. *T. auropilosa* and *T.* sp.1 were found together at two localities: Alicahue, and Camino Putaendo-Alicahue. Although *T. auropilosa* and *T.* sp.1 share a similar S-shaped pattern of the stridulatory organ, these two groups are quite distinct morphologically, yet they are morphologically similar to *T. chilensis* (*See Chapter 3 of this thesis*). Further collecting, especially at different locations, will be necessary to fully understand the relationship within the *T. auropilosa* and *T.* sp.1 clades.

Tettigades distanti

Tettigades distanti was represented by seven individuals from four localities in the regions of Valparaíso and Maule. Both nuclear and mitochondrial phylogenies contain two divergent clades, DIS1 and DIS2, both with high bootstrap support. These two clades are morphologically and geographically distinguished. DIS1 was found in the region of Valparaíso and presents light and widely banded sternites (black inverted triangles – Fig. 3). DIS2 was found in the region of Maule and presents dark and finely banded sternites (white inverted triangles – Fig. 3). No representatives of *T. distanti* have yet to be collected around Santiago. Further collecting at different locations such as Santiago may reveal more variation within *T. distanti*.

Tettigades curvicosta described by Torres (1958a) is morphologically quite similar to *T. distanti.* The type locality of the holotype of *T. curvicosta* is unknown. Further analysis is required to delimit these two species or to determine if they are simply variants of a single biological species (*See Chapter 3 of this thesis*).

Tettigades lacertosa

Tettigades lacertosa, the largest species of *Tettigades* found in Chile, wasvrepresented in this study by 19 specimens from ten localities and is at the northern-most range of the genus in Chile. The nuclear phylogeny shows two divergent clades within *T. lacertosa*, LAC1 and LAC2; however, the mitochondrial phylogeny fails to reflect these relationships. In the mitochondrial phylogeny, LAC1 and LAC2 are not reciprocally monophyletic but instead LAC1 is a paraphyletic assemblage containing a monophyletic LAC2 clade. The two clades in the nuclear phylogeny are distinguished

both morphologically and geographically. This variation was recognized by Torres (1944) in his original species description. LAC1 was found south of the Atacama Desert (white squares – Fig. 2). It possesses a black pronotal collar and is densely covered in hair. LAC2 was found in the region of Coquimbo (black squares – Fig. 2). Samples in this population possess orange anterior and posterior borders of the pronotal collar, and they are less hairy compared to LAC1. Both clades share similar patterns of the wings and stridulatory organ.

Tettigades limbata

Tettigades limbata, characterized by the pattern and coloration of the pronotal collar, is both morphologically and geographically distinct. Unknown to Torres (1958a) at the time of his description, there are two morphotypes distinguishable by color and supported by the nuclear phylogeny. In the mitochondrial phylogeny, LIM1 and LIM2 do not form two distinct monophyletic clades. However, these two clades are morphologically distinguishable. The ten specimens representing *T. limbata* are delineated by finely colored lateral and posterior borders of the pronotal collar, which varies in color from either red or orange depending on clade. Represented by only one locality in the region of Valparaíso, samples of LIM1 possess a red pronotal collar (black triangle – Fig. 4). LIM2 was found in the region of O'Higgins, and all individuals in that clade display an orange pronotal collar (white triangle – Fig. 4).

T. limbata is morphologically similar to *T. crassa* (Torres 1958), however, during our collecting trips, none of the individuals collected appeared to be this species, but the condition of the holotype makes it difficult to tell these two species apart. Further

analysis and field work will be required to determine if these are two species, or synonymous entities (*See Chapter 3 of this thesis*).

Tettigades opaca

In this study, *Tettigades opaca* was represented by six individuals from five localities in the region of Coquimbo. Both nuclear and mitochondrial phylogenies illustrate two divergent clades, OPA1 and OPA2, both with high bootstrap support. These two clades exhibit a geographic pattern but morphological characters have not been found to distinguish the two clades. OPA1 was found in the northern region of Coquimbo (gray circles – Fig. 3) and OPA2 was found in the southern region of Coquimbo (white circles – Fig. 3).

Tettigades compacta described by Walker in 1850 is morphologically quite similar to *T. opaca.* The major difference distinguished by Torres (1958a) is the orientation of the m_{3+4} vein. In *T. opaca,* the vein is straight and in *T. compacta*, it is angled. Further analysis is required to delimit these two species, to determine if they are variants of one species or synonymous (*See Chapter 3 of this thesis*).

Tettigades ulnaria

Tettigades ulnaria described by Distant in 1906 was represented in this study by 31 individuals from 12 localities in the regions of Bío Bío, Maule, Metropolitan, and O'Higgins. The mitochondrial phylogeny shows four well-supported divergent clades, ULN1, ULN2, ULN3, ULN4, with ULN1, ULN2, and ULN3 with greater than 95% bootstrap support. The nuclear phylogeny does not illustrate ULN3 and ULN4 as distinct

monophyletic groups. The divergent clades within *T. ulnaria* do not present morphological characters they we were able to identify correlating with the clades. Once again, we observed a geographic north-south pattern. ULN1 was collected in two localities at the southernmost extent of our *T. ulnaria* collections, in the region of Bío Bío and Maule (white squares – Fig. 4). ULN2 was collected in one locality at the northern-most range of *T. ulnaria* our field-collected specimens, in the region of Metropolitan (black squares – Fig. 4). ULN3 was collected between ULN1 and ULN4, in the regions of Bío Bío and Maule (dark gray squares – Fig. 4). ULN4 was sampled from five localities between ULN2 and ULN3 in the regions of Maule and O'Higgins (light gray squares– Fig. 5).

T. ulnaria is morphologically similar to *Tettigades procera* (Torres, 1958); however, during our field work, none of the individuals that we collected appeared to represented this species. Further analysis is required to delimit these two taxa, to determine if they are variants of one species or synonymous (*See Chapter 3 of this thesis*).

Tettigades undata

Tettigades undata was represented by 26 individuals from 12 localities in the regions of Araucanía, Bío Bío, Maule, and O'Higgins in our study. The mitochondrial phylogeny shows three divergent clades, UND1, UND2, and UND3, with high support. However, in the nuclear phylogeny UND2 does not form a clade. These three clades are geographically proximate, but apparently morphologically indistinguishable from each other. Variation in *T. undata* did not appear to be exclusive to any population. *T.*

undata had the largest range of all *Tettigades* species our samples. UND1 has been collected in the northern most range of our *T. undata* field-collected samples, in the region of O'Higgins (black circles – Fig. 4). UND2 has been collected in the middle of the range of *T. undata* field-collected specimens, in the regions of Bío Bío and Maule (white circles – Fig. 4). UND3 has been collected in the southernmost range of our *T. undata* field-collected specimens, in the regions of in Araucanía and Bío Bío (light gray circles – Fig. 4).

Tettigades sp*.2*

Although morphologically similar and found in close proximity to some *T. undata* clades, *Tettigades* sp*.2,* represented by 2 individuals collected in Termas del Flaco, is the sister clade to *T. undata* + *T. sarcinatrix* (for both mtDNA and nuclear markers). Although morphologically similar, *Tettigades* sp.2 is potentially a new species. Further collections of this entity will help to distinguish it from *T. undata*.

Tettigades sarcinatrix

Field collecting in Chile suggests *T. sarcinatrix* is not restricted to Argentina, as stated by Torres (1958). We collected this species in the Chilean Andes at 887 meters (35 kilometers from the border of Argentina). Preliminary work on Argentine *T. sarcinatrix* specimens, not included in this study, indicate with high support that Chilean and Argentine specimens representing *T. sarcinatrix* belong to the same species or at least closely allied segregates.

Comparison to major biogeographic regions

Interglacial climate cycles have led to large changes in species distribution and species population genetic structure (Hewitt 1996). During these cycles, species may disperse to new locations during favorable periods, while persisting in refugia during less favorable climatic periods. In the southern hemisphere, the 'northern purity and southern richness' pattern is reversed compared to the northern hemisphere (e.g., Marshall et al. 2009) with southern clades showing little among-locality variation and northern clades showing high among-locality variation (significant population structuring). The species within *Tettigades* follow a similar pattern. Species found in southern Chile such as *T. ulnaria* appear to have little morphological variation while species found in the north such as *T. chilensis* are highly variable morphologically. Further collections will be necessary to better understand these patterns.

The uplift of the modern Andes is thought to have begun 23Mya (Woodburne et al. 2014). Geological events such as those in the Quaternary and Pre-Quaternary are known to be important forces in the evolutionary history of Patagonian lineages (Sersic et al. 2011). Climatic oscillations, volcanism, and glaciation have altered the landscape of Patagonia and have impacted the diversity and distribution of current lineages of many animals and plants (Hoorn et al. 2010; Folguera et al. 2011; Sersic et al. 2011; Turchetto-Zolet et al. 2012). Populations with high genetic diversity are known to have persisted for long periods of time or recolonized from multiple refugia (Hewitt 1996, Sersic et al. 2011, Wallis et al. 2016). In the Southern Andes, three transverse breaks (33°S, 35°S, and 38°S) (Fig. 5) have been reported in 13 terrestrial vertebrate studies (Sersic et al. 2011, Wallis et al. 2016). These breaks, particularly 35°S and 37-38°S are

shared across multiple taxa (Wallis et al. 2016). Although several causes such as tectonic, volcanic, topographic, and glacial forces have been identified as being responsible for the transverse breaks in South America, glaciation has been identified as the major cause (Wallis et al. 2016). Although we had limited localities per species collected in this study, we see consistent patterns correlating with these same previously identified phylogeographic breaks in Chile.

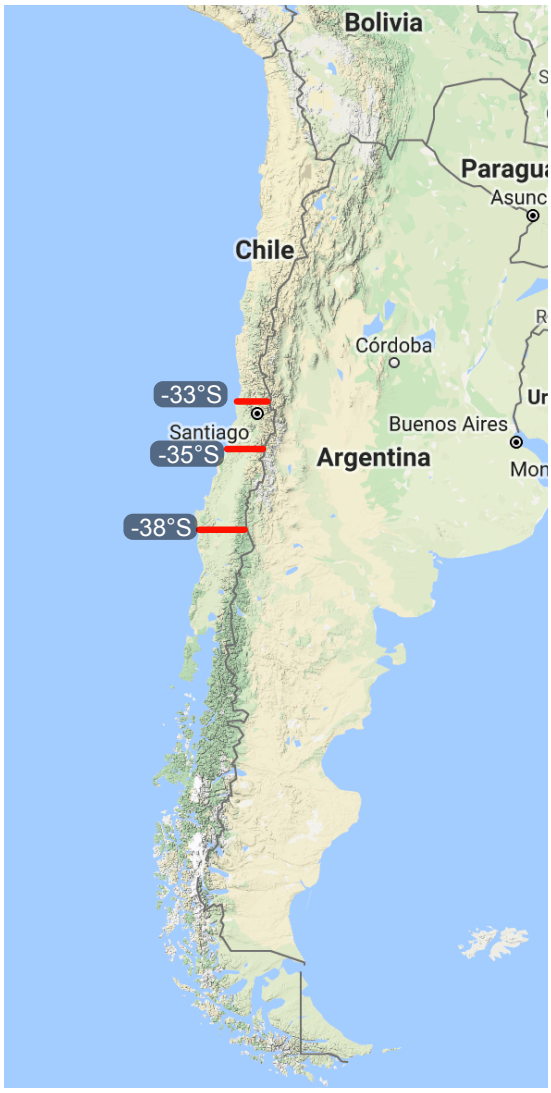


Figure 5. The three transverse breaks in Chile shown by red bars. The map of Argentina and Chile is provided by Google Maps.

Species ranges that cross the transverse break at 33°S include *T. auropilosa, T. chilensis, and T. limbata.* For *T. auropilosa*, AUR1 was found above the break at 31°S/32°S while AUR2 was found below the break at 34°S. For *T. chilensis*, CHI1 and CHI3 were found at 34°S and CHI2 was found at 32°S/33°S. *T. limbata* is the only species collected near transverse break 33°S, where its divergent clades are distinguishable by morphological characters. LIM1 was found above the transverse break at 32°S and are distinguished by the red color pattern of its pronotal collar. LIM2 was found below the transverse break at 34°S and are distinguished by the orange color pattern of its pronotal collar. Morphological variation seen in *T. auropilosa* and *T. chilensis* does not appear to be exclusive to any population and thus we did not find support for geographical structuring across this region.

The divergent clades of T. *distanti* were found at two transverse breaks, 33°S and 35°S. During our field collecting, we did not find any specimens representing *T. distanti* between 33°S and 35°S. DIS1 was found at 32°S/33°S, while DIS 2 was found at 35°S/36°S. The area between our collecting sites should be explored in greater detail to determine whether observed variation is gradual or abrupt.

Divergent clades of *T. undata* were found just below the transverse break at 33°S with UND1 found at 34°S, UND2 at 35/37°S, and UND3 at 37/38°S. The modest morphological variation seen within *T. undata* is not exclusive to any population, thus did not appear to be structured geographically.

Divergent clades of *T. ulnaria* were found between transverse breaks at 33°S, 35°S, and 37-38°S. ULN2 was found at 33°S, ULN4 between 33°S and 35°S, ULN3 at 35°S and 37°S, and ULN1 at 36°S to 37°S. The little morphological variation seen within

T. ulnaria did not appear to be exclusive to any population, thus not structured geographically.

All specimens representing *Tettigades lacertosa* and *Tettigades opaca* were found above 31°S, which does not correspond to any of the transverse breaks in the Southern Andes. The variation found within *Tettigades lacertosa* appears to be correlated with habitat. LAC1 was found in the southern part of the Atacama Desert with few shrubs--their greater pilosity may help with controlling body temperature. LAC2 was found in habitats with a higher density of shrubs, which may be used to take cover from the sun; these individuals had much less pilosity.

Tettigades sp.1 forms one monophyletic group and was found from 32°S to 34°S. *Tettigades sp.2* was found at one locality, therefore, more data must be collected before assessing this taxon.

Concluding Remarks

Future experimental plan for species delimitation using courtship song

The correct classification of a species is important for not only assessing phylogenetic relationships but also for understanding evolutionary processes, biogeography, ecology and life history, assessing conservation needs, and more. The scarcity of easy and useful morphological characters in *Tettigades* makes understanding of the species taxonomy difficult. Acoustic, morphological, and molecular analyses, separately and in combination, have been used extensively to help understand species evolution and diversity of cicadas (Alexander & Moore, 1958; Lane 1984; Moulds 2005; Marshall et al. 2011; Hertach et al. 2015; Wade et al. 2015; Hertach et al. 2016, Nunes et al. 2013; Nunes et al. 2014). Male calling songs provide a

powerful taxonomic tool for species delimitation (Alexander & Moore, 1958; Lane 1984). The analysis of species-specific songs has enhanced species circumscription and descriptions (Duffels & Trilar 2012; Popple 2013; Owen and Moulds 2016). Interestingly, the songs have been found to be strategically pitched in some species to avoid detection by predators (Sanborn et al. 2009).

Many cicadas in the subfamily Tibicininae are noted for the loud stridulatory noises made by males and sometimes also females. In addition, calling songs are produced by the timbal organs that are almost always found in male Cicadidae (Moulds 2005). These songs contribute to assortative mating and provide pre-zygotic mate recognition signals that can help identify cryptic species (Cooley & Marshall 2000, Marshall et al. 2011, Moulds 2005, Nunes et al. 2014, Hertach et al. 2015, 2016). Because *Tettigades* diverged rapidly and recently (Łukasik et al. 2017), song analysis will be an important and effective tool to improve our understanding species delimitations and to recognize species boundaries and variation.

In order to understand the diversity and complexity within *Tettigades* an in-depth analysis of other regions and species, e.g., Argentine *Tettigades* and *Alarcta*, will be vital. Not only will such provide a more rigorous test of the monophyly of the genus, but it will also clarify previous taxonomic decisions. Due to the lack of DNA evidence at the time of most *Tettigades* species descriptions, much of the variation we discovered using molecular phylogeny as a guide was missed. This new information on the variation within and among groups of *Tettigades* illustrates the importance for a taxonomic revision of the genus using multiple sources of data such as morphology, geography,

and DNA. Chapter 3 of this thesis presents a more in-depth morphological investigation of *Tettigades.*

Conclusion

The diversity and phylogenetic relationships of 152 *Tettigades* specimens and 2 outgroups (*Alarcta* and *Chonosia*) were estimated (Fig. 1). Separate maximum likelihood analyses of nuclear and mitochondrial genes revealed nine well-supported clades of previously described species: T. *auropilosa, T. chilensis, T. distanti, T. lacertosa, T. limbata, T. opaca, T. sarcinatrix, T. ulnaria*, and *T. undata*. Within these species clades there are divergent subclades, also with high support, indicating greater within-species variation than originally described. Two clades on both phylogenies do not fit any species description of *Tettigades*, indicating species yet to be described. Further geographic sampling and acoustics analyses will help determine if some of the variation, seen in this preliminary molecular and morphological study, is indicative of reproductive isolation, worthy of taxonomic recognition.

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Supplemental Material

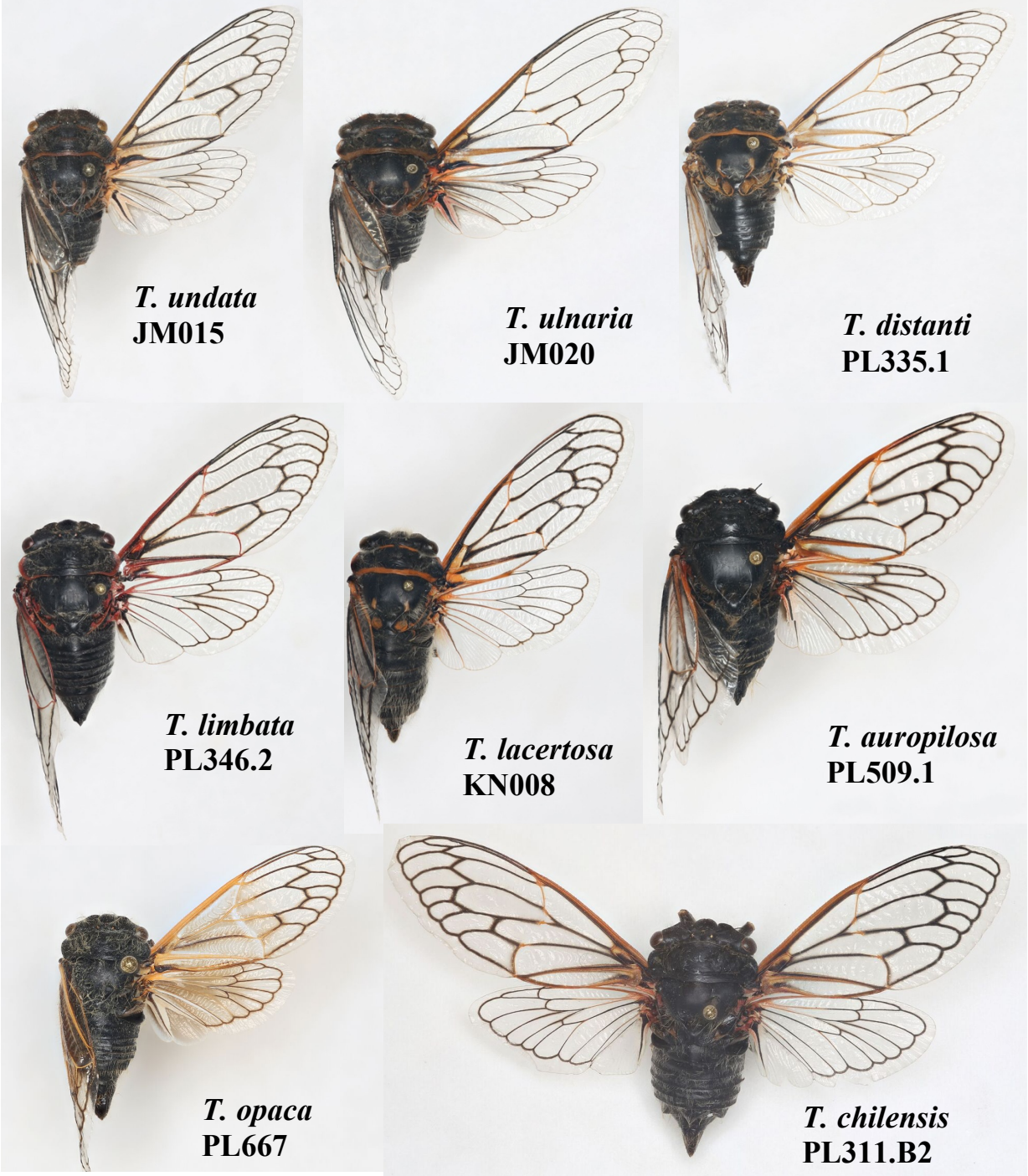

Figure S1. Representative species of the genus *Tettigades*.

pre-2014	2014	2015/6a	2015/6b	Site_ID	Full_name_of_site	Region	Lat	Lon	Elev	Comments
		22-Jan		8km de Lo Vasquez	side of road F-50, 5.5 km N from intersection with highway 68, near Lo Vasquez	Valparaiso	-33.2255333	-71.409167	306	
		12-Dec		9km W from Choapa	intersection between roads D-37-E and D-85, 9 km W from the town of Choapa	Coquimbo	-31.72216	-71.274682	300	
	11-Dec			Alicahue	Alicahue, Valparaiso	Valparaiso	-32.3327778	-71.041583	834	
		20-Dec		Alquilhe	hill near the village of Alquilhe	Maule	-35.6257167	-71.79315	94	
		13-Nov	10-Dec	Bahia Inglesa	rocky area, off road C-302, near Bahia Inglesa, Caldera	Atacama	-27.1339944	-70.859742	23	
	14-Dec			Bellavista Sitio 1 (Alto)	Sierra de Bellavista area	O'Higgins	-34.8257667	-70.741783	1455	
	14-Dec			Bellavista Sitio 2 (Bajo)	Sierra de Bellavista area	O'Higgins	-34.82615	-70.751183	1163	
	10-Dec			Caleu	Caleu, Titili area	Metropolitan	-33.01625	-70.994806	1160	
		13-Nov	12-Dec	Camino a Fray Jorge A	road towards Fray Jorge National Park, 14.2km from Highway 5	Coquimbo	-30.5609	-71.630708	500	
		13-Nov	12-Dec	Camino a Fray Jorge B	road towards Fray Jorge National Park, 18.0km from Highway 5	Coquimbo	-30.5444278	-71.639039	460	
	15-Dec			Camino a las Termas 1	side of the road towards Termas del Flaco	O'Higgins	-34.8637333	-70.548867	1236	
	15-Dec			Camino a las Termas 2	side of the road towards Termas del Flaco	O'Higgins	-34.8016333	-70.602733	945	
	15-Dec			Camino a las Termas 3	side of the road towards Termas del Flaco	O'Higgins	-34.74115	-70.7655	736	
	16-Dec	5-Dec		Camino Al Volcán	side of the road Camino Al Volcán, 3.5 km E from the village El Volcan, Cajón del Maipo	Metropolitan	-33.8233	-70.121967	1611	=Cajón del Maipo A (2014, 2015)
	15-Dec			Camino Coya-Los Cipreses, puente	area near the confluence of rivers Pangal and Cachapoal, 7 km SE from the village of Coya	O'Higgins	-34.2342667	-70.480583	855	
	16-Dec			Camino Embalse el Yeso 1	side of the road Camino Embalse el Yeso, Cajón del Maipo	Metropolitan	-33.7901	-70.19645	1415	
	16-Dec			Camino Embalse el Yeso 2	Cajón del Maipo, Camino Embalse el Yeso 2 (sitio alto)	Metropolitan	-33.78776	-70.181615	1597	
	11-Dec			Camino Putaendo-Alicahue 1	side of the road between Putaendo y Alicahue	Valparaiso	-32.58825	-70.71475	898	
	11-Dec			Camino Putaendo-Alicahue 1.5	side of the road between Putaendo and Alicahue	Valparaiso	-32.4803889	-70.709056	1214	
	11-Dec			Camino Putaendo-Alicahue 2	the high part of the road between Putaendo and Alicahue	Valparaiso	-32.46075	-70.704733	1307	
	11-Dec			Camino Putaendo-Alicahue 3	the high part of the road between Putaendo and Alicahue	Valparaiso	-32.4535833	-70.699417	1423	
	15-Dec			Camino Rancagua-Coya	hill near the road from Rancagua to Coya	O'Higgins	-34.1956167	-70.634733	703	
	6-Nov			Carrizal Bajo	Carrizal Bajo, Huasco	Atacama	-27.991785	-71.128617	119	
		20-Dec	13-Nov	Cauquenes/Quirihue	Ruta 126 between Cauquenes and Quirihue - near Estero el Cajon	Maule	-36.0992333	-72.446617	258	
		13-Nov	13-Nov	Cuesta Andacollo A	Side of road D-51 to Andacollo	Coquimbo	-30.1958167	-71.096656	792	
		13-Nov	13-Nov	Cuesta Andacollo B	Side of road D-51 to Andacollo	Coquimbo	-30.1832667	-71.099403	592	
		30-Dec		Itahue	Itahue	Maule	-35.134753	-71.359889	235	
		4-Jan		L1: Trapatrapa (Rio Queuco)	side of the road 5 km NW from the town of Trapatrapa	Bio Bio	-37.7054361	-71.274464	928	
		4-Jan		L2: Queuco-Bio-Bio Confluencia	confluence of rivers Bio Bio and Queuco	Bio Bio	-37.8285111	-71.675436	370	
		5-Jan		L3: Antuco alto	side of the road 16km E from the town of Antuco	Bio Bio	-37.3738889	-71.489722	842	
		5-Jan		L4: Rio Cholghuán - Puente la Fábrica	bridge over Rio Cholghuán, 8 km S from the town of Yungay	Bio Bio	-37.1986111	-71.980556	274	
		6-Jan		L5: Colbún	roadside 7 km N from the town of Colbún	Maule	-35.6394472	-71.394222	298	=Road to Paso Pehuenche 2
		6-Jan		L6: Central Cipreses	valley of Rio Maule	Maule	-35.8144444	-70.831389	853	
		8-Jan		L7: Termas del Flaco	Termas del Flaco	O'Higgins	-34.9594972	-70.432908	1740	
		8-Jan		L8: road to Termas	road to Termas del Flaco	O'Higgins	-34.783825	-70.646547	865	
		10-Dec		La Higuera	side of road 5, La Higuera	Coquimbo	-29.53192	-71.23807	485	
		19-Dec		La Montana	View point near the village of La Montana	Maule	-34.9690667	-70.946083	515	
		28-Jan		Lagunillas	side of the road to Lagunillas ski resort, 13 km NE from San Jose	Metropolitan	-33.618349	-70.30158	1850	
		10-Dec		Las Bandurrias	Side of the road between Auco and Las Bandurrias	Valparaiso	-32.85	-70.71	740	
		19-Dec		Las Cabras	Hill near the village of Las Cabras	O'Higgins	-34.298296	-71.263586	180	
	30-Nov			Las Condes	San Carlos de Apoquindo, Las Condes	Metropolitan	-33.4006667	-70.493917	1033	
		22-Jan		Lo Prado	side of highway 68, 3.5 km E from Los Panguiles	Maule	-33.4397333	-70.9913	320	
		3-Jan		Lonquimay	hill E from the town of Lonquimay	Araucanía	-38.4475833	-71.363183	887	
		19-Dec		Los Culenes	Hill near the village of Los Culenes	Metropolitan	-33.8928833	-71.400067	178	
27-Oct				N. Punta de Lobos	N. Punta de Lobos, PN Llanos de Challe	Atacama	-28.1555278	-71.154444	36	
		22-Jan & 15-Feb		Olmue antes de puente	side of the road F-10-G near Olmue	Valparaiso	-33.02988333	-71.141183	186	
		4-Jan		Paso Pino Hachado	Side of Ruta 181, 10km E from an border crossing	Araucanía	-38.64211	-71.001451	1469	
		22-Jan		Penablanca	hill near the exit from highway 60, near Peñablanca	Valparaiso	-33.0498333	-71.322917	197	
	9 & 16-Dec			Peñalolen	Park Quebrada de Macul, Peñalolen	Metropolitan	-33.4926389	-70.517667	900	
		18-Dec		Puente Negro	Puente Negro	O'Higgins	-34.675	-70.873409	489	
		15-Nov	15-Nov	Rio Choapa A	Santa Rosa, Salamanca	Coquimbo	-31.8164194	-70.933078	589	
		15-Nov	15-Nov	Rio Choapa B	Limahuida, Salamanca	Coquimbo	-31.7422861	-71.172483	314	
		6-Jan		Road to Paso Pehuenche 2	side of the road, valley of Rio Maule	Maule	-35.8144444	-70.831389	850	=L6: Central Cipreses (2016)
		19-Dec		Ruta 66 (state border)	Side of road 66	Coquimbo	-34.0617333	-71.377283	257	
		13-Nov	13-Nov	Salida sur Ovalle	Side of road 45 near La Chimba, south-west from Ovalle	Coquimbo	-30.6551611	-71.255883	20	
	11-Dec			Tahulico	hill south of the village of Tahulico, Salamanca	Coquimbo	-31.7943667	-71.075917	581	
		30-Dec		Talca-campus	Campus Talca de Universidad de Talca, Talca	Maule	-35.405368	-71.63597	106	
		21-Jan		Talca, Cerro La Virgen	Cerro La Virgen, Talca	O'Higgins		-71.7044	260	
	14-Dec			Termas del Flaco	the area near the village of Termas del Flaco	O'Higgins	-34.9565667	-70.43655	1763	
		8-Jan		Titili, Cuesta la Dormida	Cuesta la Dormida, Titili	O'Higgins	-33.0729667	-70.963933	765	
		22-Jan		Titili, Cuesta la Dormida - paso superior	paso superior de Cuesta la Dormida, Titili	Metropolitan	-33.0612167	-71.0099	1281	
		29-Dec		Villa Allegre	Villa Allegre	Maule	-35.675648	-71.743261	105	

Table S1. The localities of specimens included in the phylogeny.

Chapter 3

Tettigades species determinations and critique of Torres (1958)

Introduction

Although abundant and diverse, the Patagonian genus *Tettigades* was poorly documented until Torres (1958) reviewed the genus, describing and re-describing 26 species. His work provided useful insight on the variation and distribution of species in the genus. Despite Torres's efforts, some species descriptions remained vague, and at that time, of course, molecular taxonomic tools were not yet available.

The goal of this chapter is to compile and integrate previous work with updated information into a reference for future researchers. Morphological, geographical, and molecular data are combined to evaluate Chilean species of *Tettigades* and to provide insight on potential species new to science. Eight described Chilean species are examined: *T. auropilosa* Torres, 1944*, T. chilensis* Amyot & Audinet-Serville, 1843*, T. distanti* Torres, 1958, *T. lacertosa* Torres, 1944, *T. limbata* Torres, 1958*, T. opaca* Jacobi, 1907*, T. ulnaria* Distant, 1906, and *T. undata* Torres, 1958. Chilean species not included are: *T. angularis* Torres, 1958*, T. brevicauda* Torres, 1958*, T. compacta* Walker, 1850, *T. curvicosta* Torres, 1958, *T. procera* Torres, 1958*, T. pauxilla* Torres, 1958, *T. sordida* Torres, 1944*,* and *T. unipuncta* Torres, 1958, as well as additional species that likely represent previously unrecognized species. The Argentine and the remaining Chilean species will be treated in future studies.

Each species treatment, if available, contains information on type material (including images), a translated description of Torres (1944 & 1958) or original descriptions other than Torres, a diagnosis to distinguish it from closely related species,

and images of the specimens. A key to the species addressed in this chapter is also provided. Each species account is intended to be a synopsis rather than a comprehensive formal description. Note that previous descriptions are mostly based on dried specimens, which typically have faded coloration. Where possible, pictures of live specimens are included as a color reference.

Although this work does not go into detail about genitalic characters such or consider male calling songs. Cicada songs are used in sexual communication and are rich in important taxonomic information (Alexander & Moore, 1958; Lane 1984; Marshall et al. 2011, Hertach et al. 2016). Both genitalic and song characters undoubtedly will provide more evidence for species delimitations and help identify cryptic species in future studies.

Materials and Methods

Specimen Acquisition

Due to the limited amount of fresh material available for molecular and morphological analyses, two field trips were made to Chile in order to collect specimens. These trips focused on obtaining samples from a variety of habitats, both new and previously recorded. The specimens were collected in December 9-17, 2014 and December 4 2015 - January 24, 2016. Specimens were also obtained from natural history museums and private collections. Field-collected specimens were sampled from 59 locations across Chile, spanning the Atacama Desert to the Andean scrublands.

Specimens were collected during the day. Although male cicada songs signal when cicadas are present, the high summer temperatures in Patagonia can suppress calling, making *Tettigades* difficult to locate. When cicadas were present but not singing,

we used alternative methods such as clapping, tapping bushes, and searching the bases of tree trunks. Specimens were collected by hand or using an insect net.

Whole specimens, abdomens, and heads were preserved in a variety of different ways. Specimens were first separated into morphospecies with representative individuals saved for morphological and molecular analyses. Material collected in 2014 intended for use in morphological characterization was stored in 95% ethanol. However, due to specimen hardening caused by such a high concentration of ethanol, specimens collected for morphological characterization after 2014 were stored in 70% ethanol. Specimens used for DNA analysis were stored in 95% ethanol. Ethanol was replenished twice, more if necessary for large cicadas, and stored at -80C. Some individuals used for morphological work were dry pinned. RNAlater was the preservation method for dissected bacteriome tissue, while samples in Carnoy's solution were used for fluorescent microscopy. For Transmission Electron Microscopy (TEM), dissected cicada tissue was fixed in 2.5% glutaraldehyde buffer, then fully dissected and post-fixed using 1% osmium tetroxide, and embedded in epoxy resin. Although this chapter does not include any fluorescent microscopy or TEM analyses, the specimens were used for morphological and molecular characterization as well.

Specimens were collected by Katherine Nazario (U. Connecticut), Claudio Veloso (U. Chile), Piotr Łukasik (U. Montana), John McCutcheon (U. Montana), Mario Elgueta (MNHN, Chile), Francisco Urra (MNHN, Chile), and Eduardo Fuentes Contreras (U. Talca, Chile). For each species treatment, the sex of all specimens, and locality information (coordinates, region, locality description, elevation, and date of collection)

are provided. A compiled table of all specimens included in this chapter is provided in Table S1.

Representative specimens were first identified based on morphological characters provided by original descriptions and re-descriptions, as well by examining type material. The identity of several specimens used in the study was confirmed by partial sequencing of the mitochondrial cytochrome oxidase I (*COI*) gene (Łukasik et al. 2017) and by sequencing 742 nuclear and 13 mitochondrial genes (See Chapter 2 of this thesis).

The numbering system used by the University of Montana begins with the initials of one of collector ("PŁ", "KN", and "JM") followed by a sequential specimen number. An asterisk (*) following the number identifies specimens included in the phylogenetic analyses. All specimens were used for morphological analyses.

A representative from each clade in this study will be vouchered and deposited at el Museo Natural de Historia Natural (MNNC, Santiago, CL) and at the University of Connecticut Biodiversity Collections.

Museum Specimens

Type and non-type material were examined, measured (body and wings), and photographed (dorsal, ventral, stridulatory organ, and original and identification labels, if available) for morphological comparisons. Measurements were taken with a digital Titan caliper and compared to those provided by original and re-describers. Images of types were taken at the museums or received from curators (Table 1). See Fig. S1 of Chapter 1 for wing notation as described by Torres (1958a). A compiled table of museum data is included in this chapter in Table S2.

Museum abbreviations (Table 1) follow Evenhuis (2017). El Museo Natural de Historia Natural in Santiago, Chile, uses “MNHN” for their museum specimen numbers. However, “MNHN” is the abbreviation for the Museum National d’Histoire Naturelle in Paris, France. To avoid confusion, “MNNC” will be the abbreviation used for el Museo Natural de Historia Natural, Chile as used by Evenhuis (2017).

Since DNA from dried museum specimens is often degraded (Dean and Ballard 2001; Orlando and Cooper 2014), DNA material was not collected from the types. Sequences of the types may help confirm the validity of many of these species in the future.

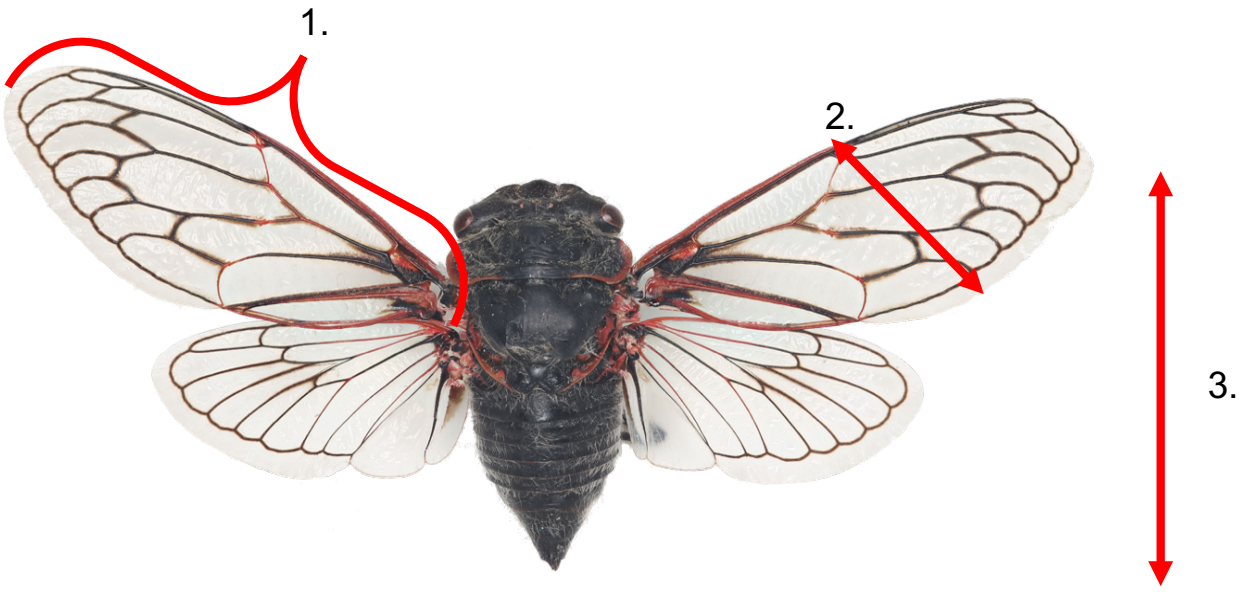

Figure 1. Characters measured when examining museum specimens. 1. wing length; 2. wing width (at broadest point); 3. Length of body. The specimen in this photo is *T. limbata* (PL346.1).

Table 1. List of collections examined and their curators. Museum acronyms are according to Evenhuis (2017).

Acronym	Collection	Location	Curator
AFSC	A.F. Sanborn Collection	Miami Shores, Florida, USA	A.F. Sanborn
NHMUK	The Natural History Museum	London, United Kingdom	M. Webb
MACN	Museo Argentino de Ciencias Naturales “Bernardino Rivadavia”	Buenos Aires, Argentina	A.R. Alsina
MLPA	Museo de La Plata	La Plata, Argentina	A.M. Marino
MNHN	Museum National d’Histoire Naturelle	Paris, France	J. Sueur
MNNC	Museo Natural de Historia Natural	Santiago, Chile	M. Elgueta
NHMW	Naturhistorisches Museum	Vienna, Austria	H. Zettel
ZMHB	Museum für Naturkunde der Humboldt-Universität,	Berlin, Germany	J. Deckert

Justification for species

The recognition of species was based upon three sources of data: DNA (targeted exome capture and mitochondrial protein coding sequences), morphology, and geographic data. Described species were identified from among the putative species recognized by comparison with type material and previous species descriptions. Some specimens were thus considered to represent new species. Genitalia were also used to delimit species; however, greater numbers of dissections and a standardization of characters will be necessary to formally delimit and describe the new, as well as previously described species. A description of the genitalia characters is provided in Fig. 2. Moulds (2005) states the subgenital plate "is more correctly termed sternite VIII.”

Torres uses the term “subgenital plate.” However, for *Additional Characters* and *Diagnoses*, sternite VIII will be used.

Listing of synonymies for previously described species has been restricted to primary entries.

Phylogenetic Analysis: Targeted Gene Capture

To determine the relationships among and between species, DNA sequences were acquired from representatives of all locations collected. The diversity and phylogenetic relationships of 152 *Tettigades* specimens and 2 outgroups (*Alarcta* Torres, 1958 and *Chonosia* Distant, 1905) were estimated (Fig. 1 of Chapter 2). Detailed phylogenetic analyses are provided in Chapter 2 of this thesis.

Distribution Maps

Distribution maps were generated in R (v. 3.3.2) for field collected specimens and for type localities, if known. GPS coordinates were determined in the field or estimated from Google Maps. Localities and coordinates are provided in tables for each species treatment.

For each map, symbols were used to identify different species. For each symbol, different colors were used to identify divergent clades.

Figure 2. Male genitalia characters used for each species treatment. (*aed*) aedeagus; (*db*) distal beak; (*end*) endotheca; (*mdl*) median lobe of uncus; (*psp*) pseudoparamere; (*pyg*) pygofer; (*stVIII*) sternite VIII; (*th*) theca. Terminology and abbreviations are from Moulds (2005). The images are the lateral and ventral view of *T. chilensis,* PL466.1.

Results

***Tettigades chilensis* Amyot & Audinet-Serville, 1843**
Synonyms: *T. chiliensis*(sic)= *Cicada rubrolineata*
Spinola, 1852=*T. rubrolineata=Fidicina crassivena*
Walker, 1858=*Cicada eremophilia* Philippi, 1866

Amyot & Audinet-Serville (1843) original description of *T. chilensis*: [text translated from French]

> "Length 0.025m. Body entirely black with fawn or gray hairs. The veins of the apical ends of the fore and hind wings shaded with intense black; veins of the wing base, blood red; Ventral surface of body, hairy. Legs the same color as the body and hairy. Female."

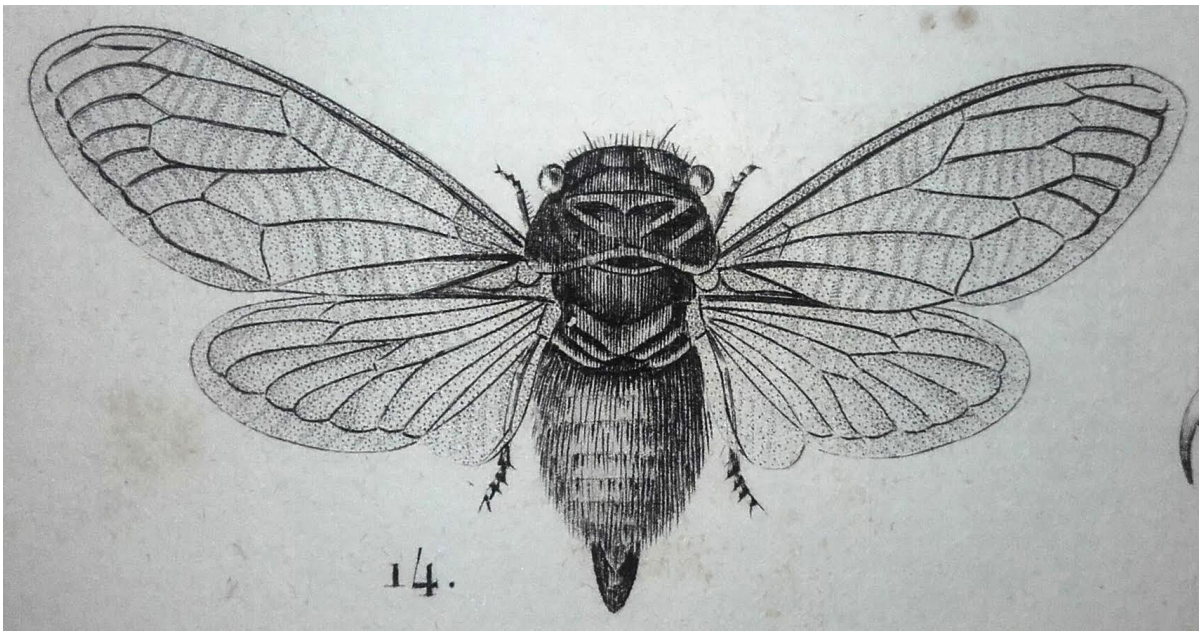

Figure 3. Drawing of the type specimen of *Tettigades chilensis* by Amyot & Audinet-Serville.

Torres (1944) re-description of *T. chilensis*: [text interpreted from Spanish]

Variations.

♂ ♂: Body length: 23.7-25mm; wing length: 28-28.5mm; wing width: 12.2-12.5mm.

♀ ♀: Body length: 22-25mm; wing length: 27-28.5mm; wing width: 11-12mm.

General Coloration. Black. Body covered in dark brown or partly grayish yellowish hairs (pilosity).

Head (male). Dorsal. Vertex and frons black with sparse pilosity. Ventral. Frons, gena, postgena, clypeus, and rostrum, black and covered in a dense and long dark brown pilosity; apex of the third segment of the rostrum passing the base of the posterior coxae.

Thorax. Pronotum black, covered in a dark brown pilosity. Height of pronotum a third greater than the distance between the forehead apex and the base of the vertex in the area of the ocelli. Mesonotum black, with some dark brown pilosity. Thorax ventrally with black segments, covered in a dense dark brown pilosity; opercula poorly developed, of the same colors as the segments of the thorax and stained ochre in their distal portions. Legs black.

Wings. Forewing and hindwings hyaline; the forewing generally with well-marked black veins. Costal membrane and costal vein, red, subcostal + radial vein, black; basal cell trapezoidal, intense dark red, almost black. The first vein of the base, red; bases of cells 1^a and 2^a medium, a spot after the origin of the fourth longitudinal vein, termination of the cubital vein, and 5^a transverse, the color reddish, cubital anal cell of the color intense dark almost black, 1^a and 2^a anal veins, clavus and articular sclerites of the same color. Second transverse vein almost double the length of the first; seventh apical area almost twice as long as the sixth and eighth apical area almost two and a half times as long as its height. Wings with the costal vein, first and second basal veins, a basal spot in between these veins, sixth apical vein and articular sclerties, all red; anal region, with a basal angular stain, pearly gray.

Abdomen. Sternites black, covered in an abundant pilosity grayish-yellowish. Sternites the same color as the first, covered in a long and dense dark brown pilosity.

Variation. A small reddish spot on either side of the anterior border of the pronotum sometimes reaches the vertex. A very small intense dark almost black spot, extremely diffuse, at the top of the pronotal expansion. A red dot in front of each previous branch of the crucial elevation. Second segment of the rostrum intense dark almost black. None of the specimens present all these variations together; these small color and size variations suggest that *Tettigades chilensis* is not present in Argentina as suggested by other authors. After studying the characters of the typical form and the extent of variation, one can clearly see the origin and development of this erroneous concept of extreme variation in the works of the various authors who treated it.

Torres (1958) re-description of *T. chilensis*: [differences from 1944 - text interpreted from Spanish]

Measurements:

Body length: 21.5-25.5mm; wing length: 27-31mm; wing width: 11-13mm; wingspan: 64-75mm.

Head. An ochre spot in front of the third ocellus. Longitudinal and transverse grooves of the postclypeus deep and well-defined.

Thorax. Stridulatory areas parallel to the posterior border of the pronotum and with 30 ridges approximately. Jugal region may demonstrate red at the base. Opercula and meracanthus ochre and stained black in its basal half.

Wings. Forewing hyaline with the veins intense dark brown in general.

Abdomen. Sternites with posterior borders slightly lightened. Pilosity denser in the ventral region especially below the head.

Museum [non-type] Material Examined: used for morphological comparisons

The original description of *T. chilensis* by Amyot & Audinet-Serville (1843) lists a female specimen from Chile. However, the original describers did not say where the type specimen was deposited. I have examined material from MACN, MLPA, and MNNC, but none of the *T. chilensis* specimens have a type label. I have also contacted MNHN, NHMW, NHMUK, and ZMHB but none of their specimens resembling *T. chilensis* have a type label.

Torres in his 1944 and 1958 monograph does not state that he examined the holotype. He provides wing and stridulatory images of *T. chilensis*; however, it is uncertain whether or not he had access to the holotype.

Dominique Pluot-Sigwald, a French entomologist and knowledgeable in old collections, suggests that if Amyot & Serville do not mention in their publication where the specimen was deposited then it was deposited in the Serville collection. The Serville collection was bought by Victor Antoine Signoret, who later sold his collection (including Serville's) to the Museum of Wien, Austria. Since the Museum of Wien does not have a *T. chilensis* specimen with a type label, it may be the case that the label was misplaced or the specimen is missing. In either case, it is critical that a new type, a neotype, be assigned. See Concluding Remarks for more information on designating new types.

Since we could not find the holotype, we examined several secondary and non-type specimens from MLPA, MNNC, and MACN, many of which were determined by Torres. Several of these specimens were old and fragile, making then difficult to use. NHMUK has *T. chilensis* specimens in very good condition (Fig. 4) for morphological

work. Images of these specimens were obtained from the museum and used to analyze coloration of characters used by Amyot & Serville and Torres; only specimens in good conditions were used in this treatment.

NHMUK Specimen Number: NHMUK № 010747796
Type Locality: Chili [Chile]
Date Collected: unknown

Figure 4. Dorsal (top) and ventral (bottom) view of a non-type specimen of *Tettigades chilensis* (NHMUK № 010747796) deposited at NHMUK (London, UK). Images courtesy of NHMUK.

Figure 5. Stridulatory organ of a non-type specimen of *Tettigades chilensis* (NHMUK № 010747796) deposited at NHMUK (London, UK). Image courtesy of NHMUK.

Field-Collected Material Examined:

A total of 27 specimens were collected in the field during 2014 and used for morphological characterization. Due to the known variation and confusion surrounding *T. chilensis*, all were selected for phylogenetic analyses (See Fig. 1 of Chapter 2 in this thesis). Due to the brief and vague description by Amyot & Audinet-Serville, a combination of the original description and the Torres re-descriptions (1944 & 1958) were used for species determinations.

During our phylogenetic analyses, three subspecific clades CHI1, CHI2, and CHI3, were distinguished which closely resemble the description of *T. chilensis*. However, no morphological characters were found to delimit the clades. I was unable to find morphological variation unique to the subclades within *T. chilensis.* In some cases, sampling was limited to one specimen per locality; therefore, diagnostic variation might have been missed. The divergent clades within *T. chilensis* can be distinguished

geographically (See the Phylogenetic Analysis later in this species treatment for more information). Locality and specimen information are provided in Table 2.

Table 2. *Tettigades chilensis* localities and specimens collected in the field. Specimen numbers with an asterisk (*) were included in phylogenetic analyses. CHI1, CHI2, and CHI3 labels are divergent clades of *T. chilensis*.

Date Collected	Specimen	Divergent Clade	Name	Region	Latitude	Longitude	Elevation	Collector(s)
XI.2014	Claudio (♀)*	CHI2	Las Condes	Metropolitan	-33.40	-70.49	1033m	CV
9.XII.2014	PL301 (♀)*	CHI2	Peñalolen	Metropolitan	-33.49	-70.52	900m	CV & PŁ
9.XII.2014	PL302 (♂)*	CHI2	Peñalolen	Metropolitan	-33.49	-70.52	900m	CV & PŁ
9.XII.2014	PL305 (♀)*	CHI2	Peñalolen	Metropolitan	-33.49	-70.52	900m	CV & PŁ
9.XII.2014	PL306 (♂)*	CHI2	Peñalolen	Metropolitan	-33.49	-70.52	900m	CV & PŁ
9.XII.2014	PL307 (♂)*	CHI2	Peñalolen	Metropolitan	-33.49	-70.52	900m	CV & PŁ
9.XII.2014	PL309 (♀)*	CHI2	Peñalolen	Metropolitan	-33.49	-70.52	900m	CV & PŁ
9.XII.2014	PL311.B2 (♂)*	CHI2	Peñalolen	Metropolitan	-33.49	-70.52	900m	CV & PŁ
9.XII.2014	PL312 (♂)*	CHI2	Peñalolen	Metropolitan	-33.49	-70.52	900m	CV & PŁ
9.XII.2014	PL313 (♂)*	CHI2	Peñalolen	Metropolitan	-33.49	-70.52	900m	CV & PŁ
9.XII.2014	PL314 (♀)*	CHI2	Peñalolen	Metropolitan	-33.49	-70.52	900m	CV & PŁ
9.XII.2014	PL318.1 (♂)*	CHI2	Peñalolen	Metropolitan	-33.49	-70.52	900m	CV & PŁ
9.XII.2014	PL319 (♂)*	CHI2	Peñalolen	Metropolitan	-33.49	-70.52	900m	CV & PŁ
9.XII.2014	PL320 (♀)*	CHI2	Peñalolen	Metropolitan	-33.49	-70.52	900m	CV & PŁ
9.XII.2014	PL321 (♂)*	CHI2	Peñalolen	Metropolitan	-33.49	-70.52	900m	CV & PŁ
10.XII.2014	PL322.1 (♀)*	CHI2	Camino Putaendo-Alicahue 1	Valparaíso	-32.59	-70.71	898m	CV & PŁ
11.XII.2014	PL382 (♂)*	CHI2	Alicahue	Valparaíso	-32.33	-70.74	834m	CV & PŁ
14.XII.2014	PL404.2 (♂)*	CHI2	Camino Putaendo-Alicahue 2	Valparaíso	-32.46	-70.70	1307m	CV & PŁ
14.XII.2014	PL428.1 (♂)*	CHI1	Termas del Flaco	O'Higgins	-34.96	-70.44	1763m	CV & PŁ
14.XII.2014	PL433 (♂)*	CHI1	Termas del Flaco	O'Higgins	-34.96	-70.44	1763m	CV & PŁ
14.XII.2014	PL435.1 (♂)*	CHI1	Termas del Flaco	O'Higgins	-34.96	-70.44	1763m	CV & PŁ
15.XII.2014	PL470 (♂)*	CHI3	Camino Rancagua Coya	O'Higgins	-34.19	-70.63	703m	CV & PŁ
15.XII.2014	PL475.1 (♀)*	CHI4	Camino Rancagua Coya	O'Higgins	-34.19	-70.63	703m	CV & PŁ
15.XII.2014	PL503.1 (♂)*	CHI5	Camino Rancagua Coya	O'Higgins	-34.19	-70.63	703m	CV & PŁ
15.XII.2014	PL466.1 (♂)*	CHI1	Camino a las Termas 1	O'Higgins	-34.86	-70.55	1236m	CV & PŁ
15.XII.2014	PL466.2 (♂)*	CHI1	Camino a las Termas 1	O'Higgins	-34.86	-70.55	1236m	CV & PŁ
15.XII.2014	PL508.2 (♀)*	CHI1	Termas del Flaco	O'Higgins	-34.96	-70.44	1763m	CV & PŁ

Distribution: Chile

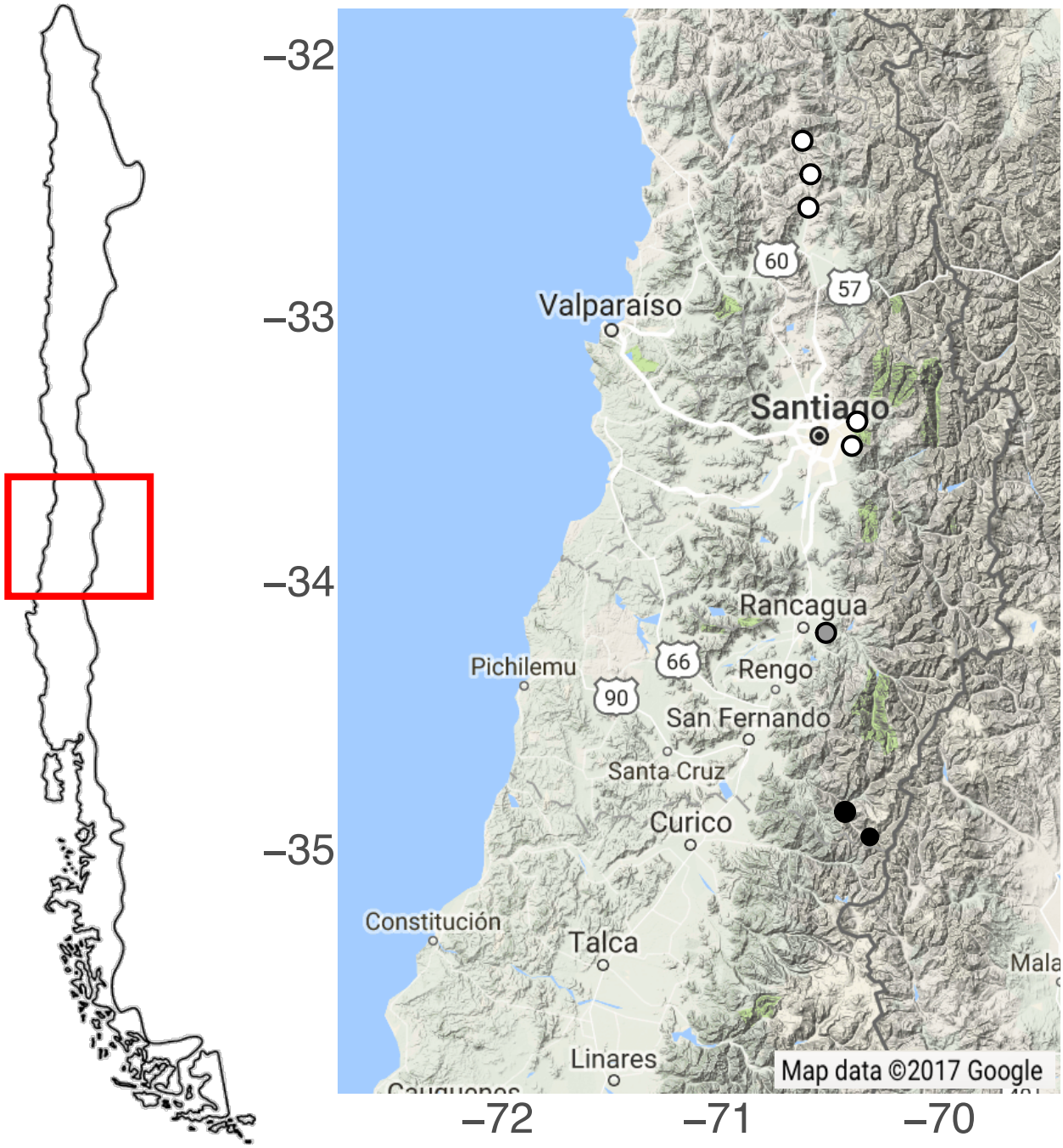

Figure 6. Map of localities for *Tettigades chilensis*. Field-collected cicada clades are identified on the map with circles. The colors indicate divergent clades: CHI1 (black), CHI2 (white), and CHI3 (gray). Map of Chile reproduced from World Atlas (http://www.worldatlas.com/).

Additional and Updated Distinguishing Features:

General. Amyot & Audinet-Serville (1843) and Torres (1944 & 1958) describe *T. chilensis* as being primarily black with red wing bases. However, our study indicates that *T. chilensis* also can be primarily black with orange wing bases. The red and orange color may vary in intensity, which is not affected by being preserved in ethanol. None of the individual specimens we collected nor any description of the species have both red and orange on the same individual.

Abdomen. In some cases, specimens have thin orange stripes bordering the dorsal abdominal sclerites (tergites). Specimens may also have thin orange stripes bordering the ventral abdominal sclerites (sternites). Few specimens have stripes on both sides of the abdomen and most have none at all. The opercula and meracanthus are black at the base with the distal half the same color as the base of the wings, either red or orange.

Thorax. Pronotal collar all black. Some museum specimens at NHMUK have red at the edges and black at the center of the posterior edge of the pronotal collar. However, the identity of those specimens has not been verified with DNA. Also, the number of localities in which we collected *T. chilensis* specimens was limited and all h

Wing groove black but in a few cases red or orange. Cruciform elevation all black and at times may have small orange spot at the ends of the anterior branches of the cruciform elevation. Coxae same color as the wing groove, either red or orange. The stridulatory organ of these specimens is perpendicular to the pronotum, with ridges evenly spaced.

Wings. Wing base is red or orange. The jugal fold is a pearly gray. Some specimens present a soft orange or red at the base of the wings which at times extends to or beyond the basal half of the jugal fold.

Genitalia (Figs. 7-9). Eighth sternite and tergite black. Dorsal beak sharply pointed and black. Pygofer black. Median lobe of uncus black on the outside, closest to the anal tube. The pseudoparamere lightly colored with few or little tooth-like structures visible on the lateral view of the aedeagus. Tooth-like structures on the outer perimeter of the pseudoparamere visible in ventral view. In ventral view, the endotheca curves back towards the specimen on the left side and curves out and downwards towards the right. [In specimen PL311.B2 and in other *Tettigades* specimens, the curvature of the endotheca to the of the aedeagus may be due to dry pinning, because all other features are similar to other members of the same clade.]

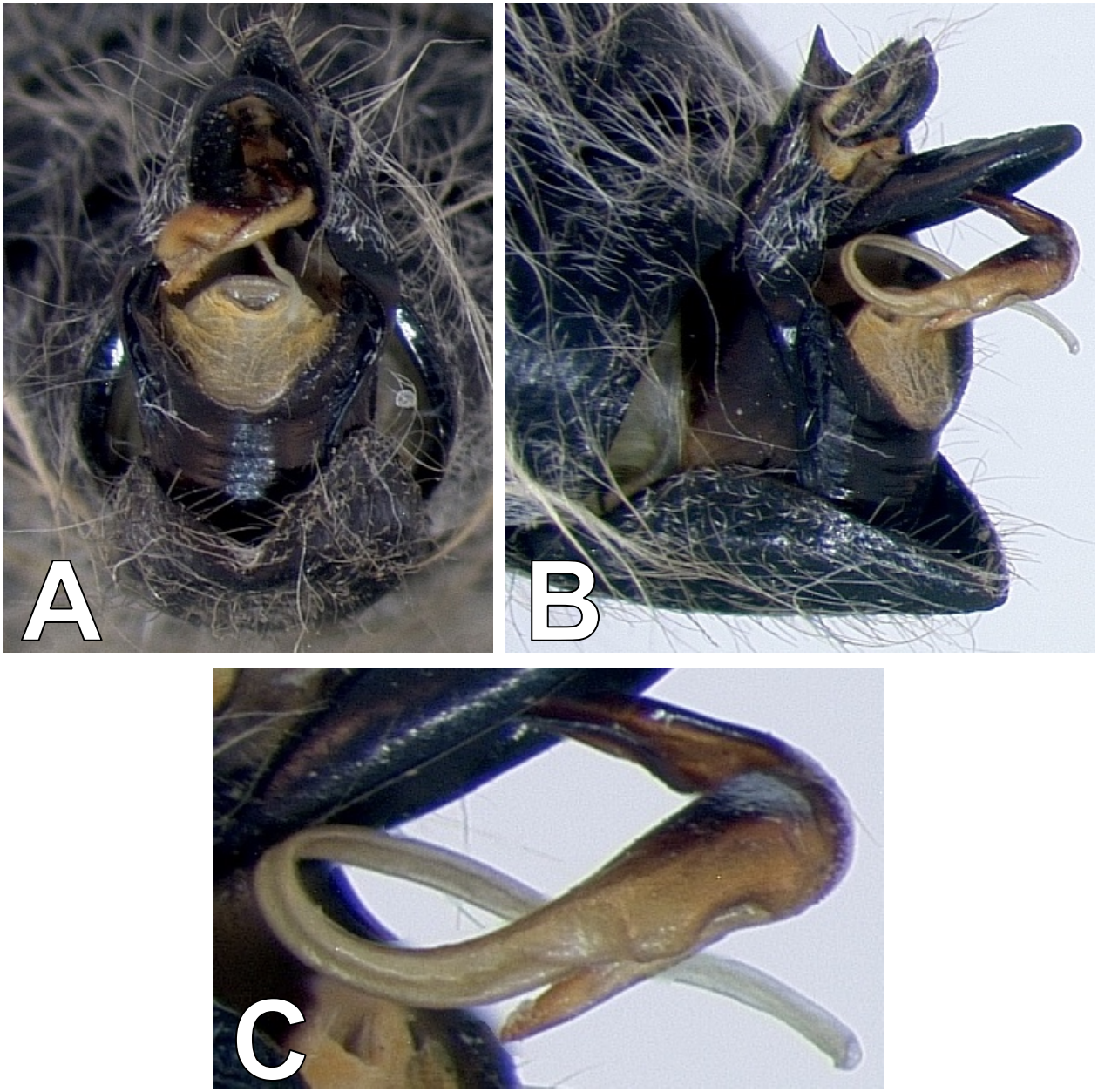

Figure 7. Male genitalia of *Tettigades chilensis*: (A) ventral, (B) lateral, and (C) lateral views of PL428.1, *T. chilensis* CHI1. Images (A) and (B) are at 2x magnification. Image (C) is at 4x magnification.

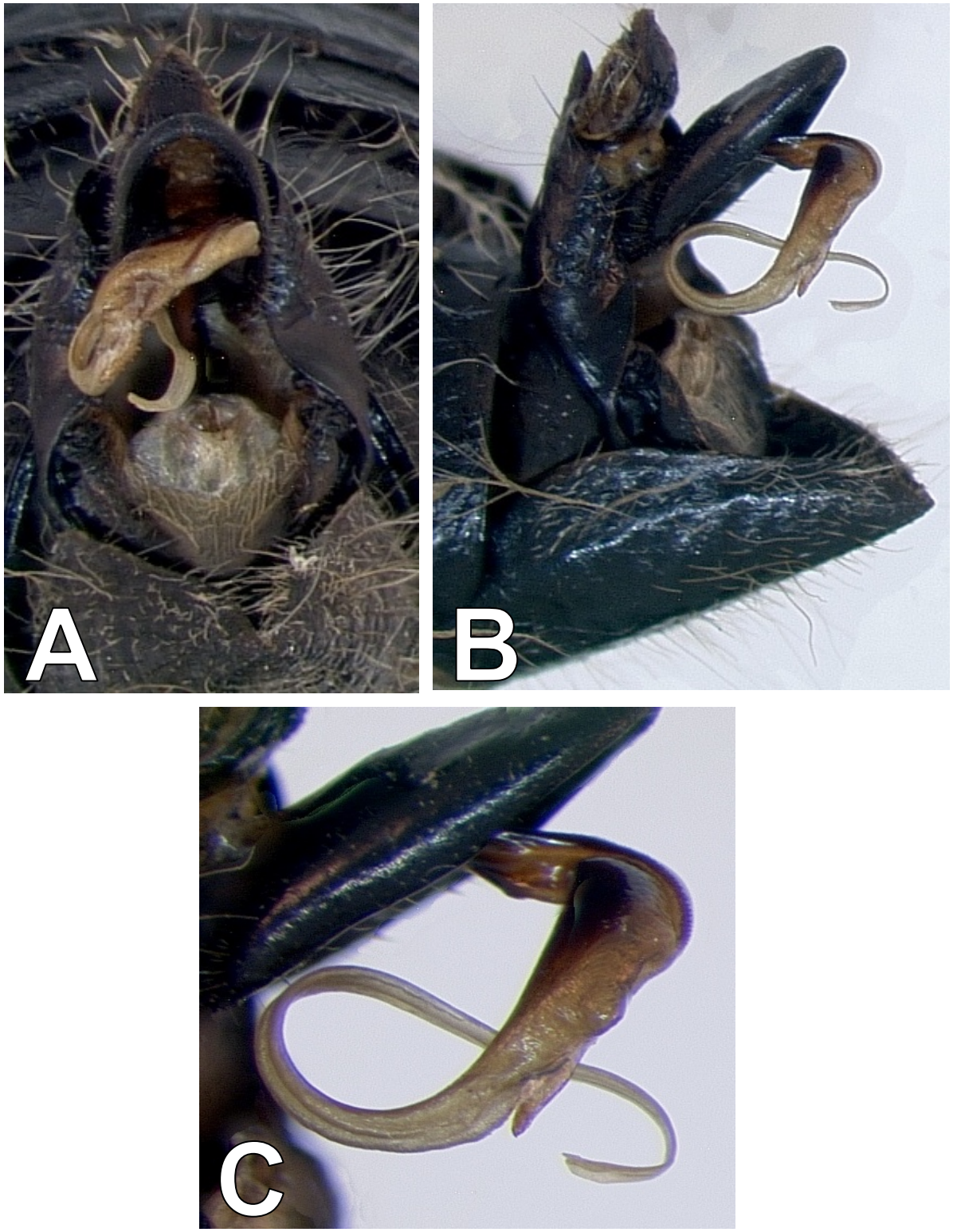

Figure 8. Male genitalia of *Tettigades chilensis*: (A) ventral, (B) lateral, and (C) lateral views of PL466.1, *T. chilensis* CHI1. Images (A) and (B) are at 2x magnification. Image (C) is at 4x magnification.

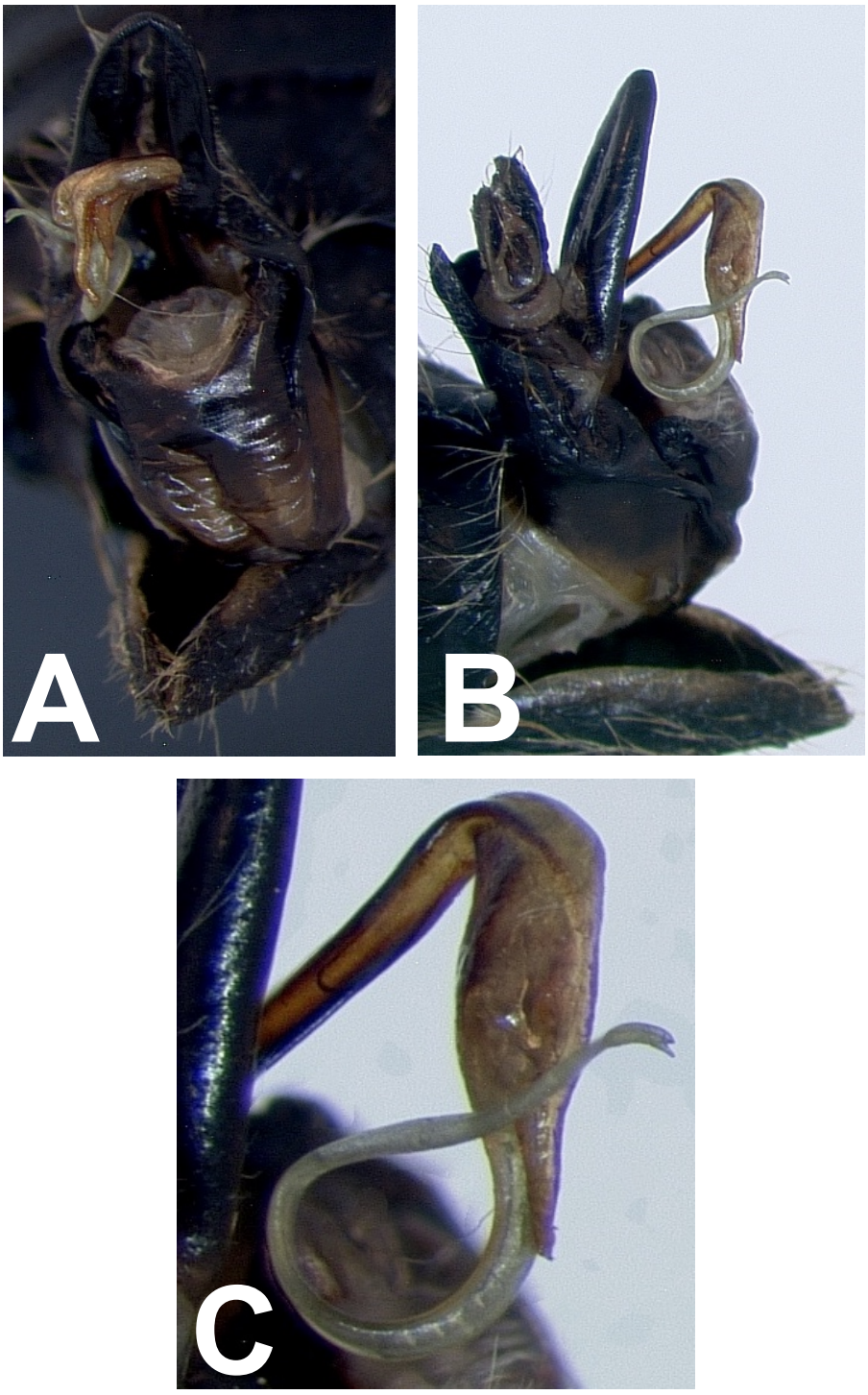

Figure 9. Male genitalia of *Tettigades chilensis*: (A) ventral, (B) lateral, and (C) lateral views of PL311.B2, *T. chilensis* CHI1. Images (A) and (B) are at 2x magnification. Image (C) is at 4x magnification.

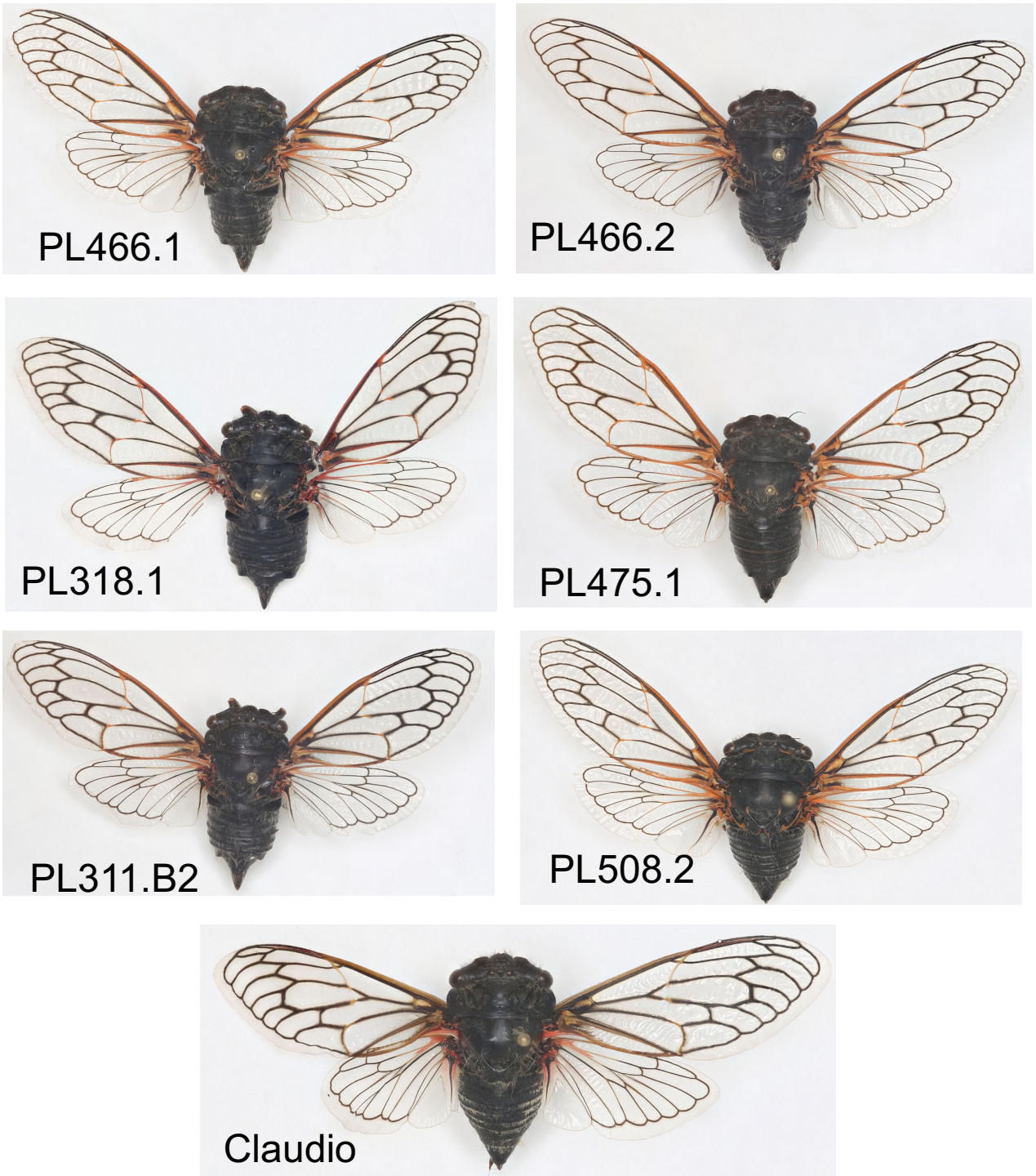

Figure 10. Dorsal views of *Tettigades chilensis* specimens collected in the field in 2014 illustrating the variation described by Torres (1944 & 1958). Specimens were collected in 95% [or 70%] ETOH and then relaxed, spread, and dried.

Diagnosis:

Tettigades chilensis is morphologically similar to *Tettigades limbata* and *Tettigades auropilosa*. It differs in the following aspects (Fig. 11):

1. *T. chilensis* and *T. limbata* have moderately dense hair. The hair of the abdomen is very dense in *T. auropilosa* compared to the other two species.
2. The stridulatory organ ridges of *T. chilensis* are parallel to the posterior border of the pronotum and evenly spaced. The stridulatory organ of *T. auropilosa* is S-shaped and the ridges are well separated. The spacing of the ridges of the stridulatory organ become narrower towards wing base in *T. limbata.* The shape of the stridulatory organ of *T. limbata* is club-shaped.
3. The jugal fold of *T. chilensis* is pearly gray with some cases of red or orange at the base. The jugal fold of *T. auropilosa* is soft orange at base with white at apex. The jugal fold of the hindwing is white in *T. limbata* with some orange or red at the base.
4. The lateral and posterior edges of body segments in *T. chilensis* and *T. auropilosa* are all black. In *T. limbata*, the pronotal collar is either red or orange on lateral and posterior edges. In a few cases, the edges *of T. chilensis* may be colored.
5. The tergites of *T. chilensis* may have fine red or orange stripes in some cases, the tergites of *T. auropilosa* and *T. limbata* are always all black.

6. In some cases, there is a spot at each end of the anterior branches of the cruciform elevation in *T. auropilosa* and *T. chilensis* but never in T. *limbata*.

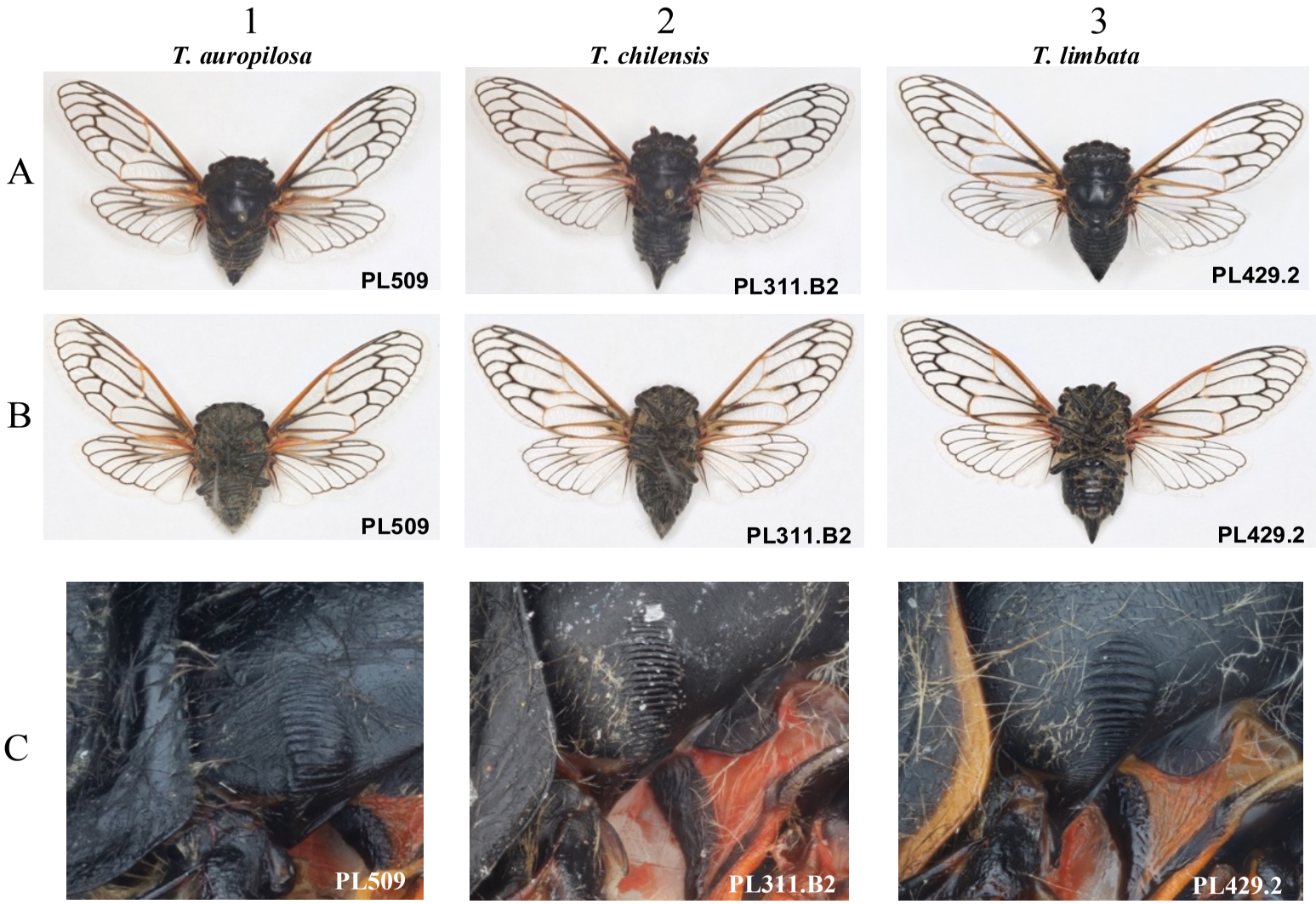

Figure 11. Dorsal, ventral, and stridulatory organ views of *T. auropilosa*, *T. chilensis*, and *T. limbata*. Columns: (1) *T. auropilosa* PL509, (2) *T. chilensis* PL311.B2, and (3) *T. limbata* PL429.2*;* (*Row*s:(A1-3) Dorsal views, (B1-B3) ventral views, and (C1-C3) stridulatory organs. Specimens were collected in 95% [or 70%] ETOH and then relaxed, spread, and dried.

Phylogenetic Assessment:

The clade, *T. chilensis,* on both the nuclear and mitochondrial phylogenies consists of 27 specimens all closely matching the description of one species, *Tettigades chilensis* (Fig. 13). The mitochondrial phylogeny resolves three divergent clades, CHI1, CHI2, and CHI3, with high support. However, in the nuclear phylogeny CHI2 and CHI3 are not distinct monophyletic clades. So far as we were able to ascertain, these three clades cannot be determined morphologically.

The divergent clades of *T. chilensis* are correlated with localities where the specimens were collected (Fig. 6). However, due to small sample sizes, greater geographic sampling will be needed to better understand the variation within *T. chilensis*. There are about 293km separating the total range of the *T. chilensis* specimens collected in the field. CHI1 specimens have been collected at the southernmost part of the range of *T. chilensis* during our field collecting trips. There is a range of about 15km separating the two localities representing CHI1. CHI2 is only represented by one locality, in the middle of CHI1 and CHI3. CHI3 was collected 131km away, at the presumed northernmost part of its range. Members of each clade have not been found together at the same locality but can be found in the same year.

The sister clades of *T. chilensis* include the species *T. auropilosa* and *T. limbata*. Although morphologically similar, these species have distinct characters which can be used to delimit the clades and divergent clades within those clades. *T. chilensis* exhibits high within-species variation compared to *T. limbata and T. auropilosa*. Similar, to *T. limbata* but unlike *T. auropilosa,* colored areas such as areas of the pronotum and wing grove can be either red or orange. This variation in color was not recognized by the original describer of *T. limbata*, Belindo A. Torres.

A synapomorphy of *T. chilensis* is the shape and pattern of the stridulatory organ. In most cases, the stridulatory organ is quite similar within closely related species, but in this case, the stridulatory organs of these its two close relatives are quite distinct. The stridulatory organ of *T. chilensis* is parallel to the posterior border of the pronotum while it is S-shaped in *T. auropilosa* and club-shaped in *T. limbata*. There may be more characters present in the genitalia or in the songs of each of these species. In the genitalia, the tooth-like projections on the aedeagus vary among all three species. An in-depth assessment of these characters will be required to understand the significance of this variation.

During our phylogenetic assessment of *Tettigades*, 12 specimens that are morphologically distinct but that did not match any *Tettigades* morphological description were also sequenced. These specimens form a monophyletic group designated *Tettigades* sp.1 and fall within the *T. auropilosa* clade in the nuclear phylogeny (See *T. auropilosa* treatment). The mitochondrial tree phylogeny shows *Tettigades* sp.1 as distinct from *Tettigades auropilosa.* Although, it looks morphologically similar to *T. chilensis*, the S-shaped pattern of the stridulatory organ of both *T. auropilosa* and *Tettigades* sp.1 helps to distinguish these species from *T. chilensis* (Fig. 12). Like *T. chilensis*, the specimens within this new clade are highly variable. Although all have a long, thin body, distinct from that of *T. chilensis*.

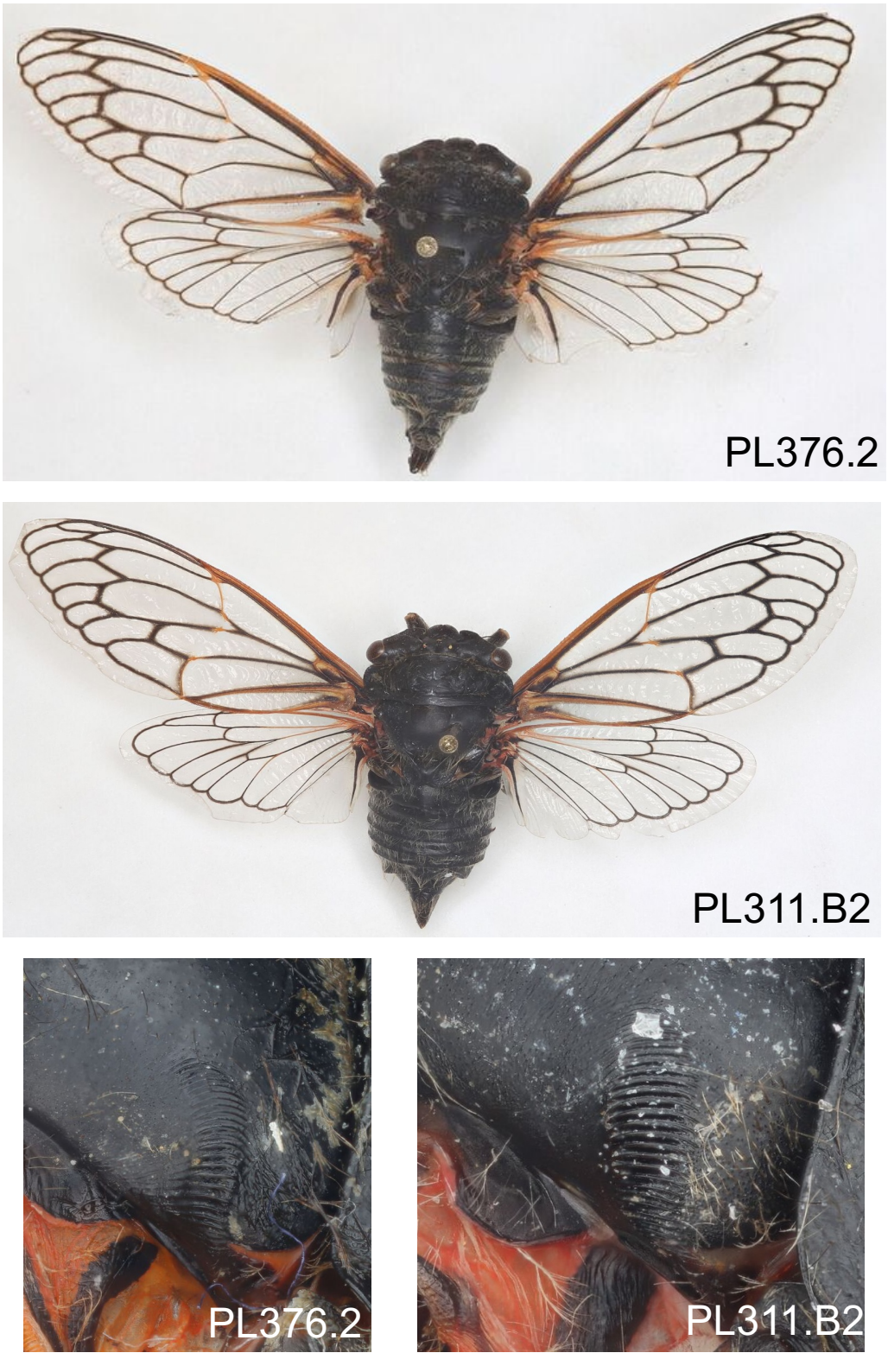

Figure 12. Dorsal and stridulatory views of *Tettigades* sp.1 (PL376.2) and *T. chilensis* (PL311.B2). Specimens were collected in 70% ETOH and then relaxed, spread, and dried

Figure 13. Dorsal and side view of *T. chilensis* specimen (PL428.1) alive in the field. Photo courtesy of Piotr Łukasik.

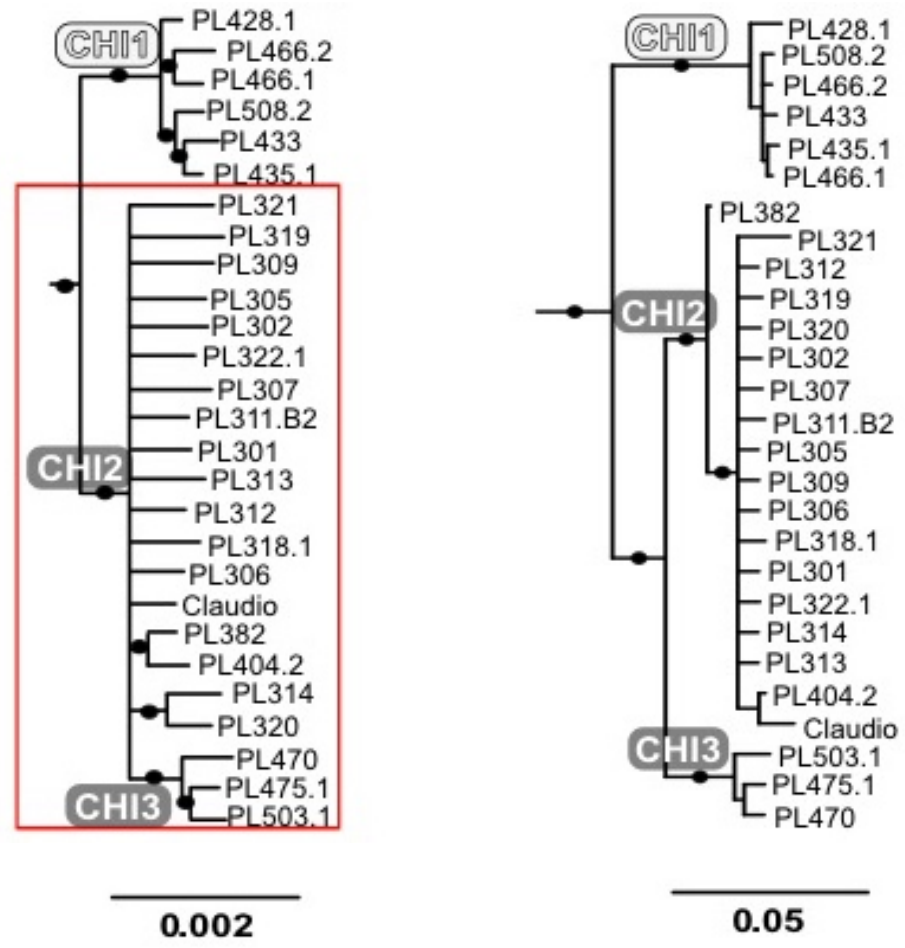

Figure 14. The *T. chilensis* subclade clipped from the maximum likelihood phylogeny (Chapter 2) based on 742 targeted-capture exons (left) and, from bycatch, 13 mitochondrial protein coding genes (right). CHI1, CHI2, and CHI3 are divergent clades. Red hollow boxes indicate samples from CHI2 and CHI3 in the nuclear phylogeny that do not form distinct monophyletic clades. Black dots represent bootstrap supports >95%; branches with <70% support are collapsed.

***Tettigades auropilosa* Torres, 1944**
No known synonyms.

Torres (1944) description of *T. auropilosa*: [text interpreted from Spanish]
Similar to *T. chilensis*

Cotypes.

♂ ♂: Body length: 21.2-22mm; wing length: 24.5-26mm; wing width: 11-11.7mm.

♀: Body length: 22mm; wing length: 26mm; wing width: 11.5mm.

Male Head. Dorsal. Vertex and frons black and covered in a dark pilosity. Ventral. Frons, clypeus, gena, postgena, and rostrum black. Apex of the rostrum reaching the posterior coxae.

Thorax. Pronotum and mesonotum black and sparsely hairy. Ventral. Segments black. Opercula black and finely outlined in ochre. Legs black.

Wings. Wings hyaline with veins black and thick. Basal area in a rectangular form, entirely intense dark, almost black, except one very small reddish-brown stripe. Base of the first and second median areas, a small spot after the origin of the fourth longitudinal vein, fifth of the transverse vein except for a small black spot in its superior portion, reddish-ochre. A dark almost black spot between the median and cubital veins. Veins $anal_1$, $anal_2$, clavus, reddish-ochre. First and second transverse veins almost equal in length; seventh apical cell longer than the sixth, and eighth apical cell almost two and a half times as long as its height. Hindwings with a reddish spot between the first and second basal veins; spot angular and basal of the anal area of the color slightly reddish, articular sclerites of the hindwing of the same color.

Abdomen. Sternites and tergites black covered in a large dark pilosity, especially the last ones.

Female. Without variation

Torres (1958) re-description: [differences from (1944) - text interpretation from Spanish]

Measurements.

Body length: 19-24mm; wing length: 24-28mm; wing width: 10.5-11.7mm; wingspan: 58-65mm.

Head. Rostrum dark brown, lighter in its second to last segment whose distal part presents a dark brown ochre.

Thorax. Mesonotum with its lateral-posterior margin a brown tint. Stridulatory areas of well-defined form, wider at base and sharp turn in the distal, approximately 16 deep ridges, separated in its proximal part and more together in the apex, sometimes stained brown.

Wings. Veins intense dark chestnut in general. Area precostal, costal vein, sc+r with the extreme or half distal of its outer face, base of cell 1^aR_3, vein r-m, a spot in the vein m_{3+4} situated a little bit distant from its origin, termination of cu_1, cu_{1b}, vein Ia, 2a, posterior tuberosity, and vein superior of the arculus, of the color brown or reddish ochre; jugal region a soft chestnut red, articular membrane of the wings reddish and sometimes dotted with white, area precostal with a linear red stain. Basal cell rectangular, internal face of sc+r, a spot situation between the origins of veins m and cu_1, area cubital-anal, distal extreme of 1a and a spot in the tuberosity posterior of the color dark brown. Veins in general finely infuscated which makes them appear thick. Wings with a spot in the base of cell 1^aR_5 and the angular spot in the vannal region, with a red tint, more intense in the jugal area whose inner core appears infuscated.

Abdomen. Pygofer and subgenital plate black. Body covered in a pilosity of golden yellowish brown hair and the thorax with pilosity of the color dark dun.

Type Material Examined: used for morphological comparisons

Cotype (1♀)

1. MNNC Specimen Number: MNNC №3275
   Type Locality: Quelteheues [Chile]
   Date Collected: XI.33

Figure 15. Dorsal (top) and ventral (bottom) view of the female cotype "MNNC №3275" of *Tettigades auropilosa* deposited at MNNC (Santiago, Chile) (originally from La Plata, MLPA.)

Torres (1944) Measurements: Body length: 22mm, wing length: 26mm, wing width: 11.5mm
Our Measurements: Body length: 22.1mm, wing length: 25.9mm, wing width:11.1mm (*wing pulled back measured*)

Cotype (3♂)

1. MNNC Specimen Number: MNNC №3276
   Type Locality: Queltehues [Chile]
   Date Collected: XI.33

Figure 16. Dorsal view of the male cotype “MNNC № 3276” of *Tettigades auropilosa* deposited at MNNC (Santiago, Chile). The specimen was fragile and on a broken pin, therefore, ventral views were not taken.

Torres (1944) Measurements: Body length: 21.2-22mm, wing length: 24.5-26mm, wing width: 11-11.7mm (*Measurements given as ranges for 2 male cotypes*)
Our Measurements: Body length: 22.3mm, wing length: 24.8mm, wing width: 11.1mm (*right wing measured*)

2. MLPA Specimen Number: MLPA № 1672/1
   Type Locality: Queltehues [Chile]
   Date Collected: XI.33

Figure 17. Dorsal (top) and ventral (bottom) view of cotype “MLPA №1672/1” of *Tettigades auropilosa* deposited at MLPA (La Plata, Argentina).

Torres (1944) Measurements: Torres mentions he has analyzed a cotype at La Plata but does not provide any measurement or the specimen number so it is uncertain which one he examined.
Our Measurements: Body length: 21.9mm, wing length: 24.4mm, wing width: 10.9mm

3. MLPA Specimen Number: MLPA №1672/2 [Specimen not photographed]
   Type Locality: Queltehues [Chile]
   Date Collected: XI.33

   Torres (1944) Measurements: Torres mentions he has analyzed a cotype at La Plata but does not provide any measurement or the specimen number so it is uncertain which one he examined.

Measurements: Body length: 21.5mm, wing length: 26mm, wing width: 12mm

Field-Collected Material Examined:

A total of 17 specimens were collected in the field between 2014 and 2015 and used for morphological characterization. Of these 17, eleven specimens were selected for phylogenetic analyses (See Fig.1 of Chapter 2 in this thesis). All specimens included in the phylogeny have been identified with an asterisk in Table 3.

During our phylogenetic analyses, two subspecific clades, AUR1 and AUR2, were distinguished which closely resembled the description of *T. auropilosa*. However, no morphological characters were identified that delimited the clades. Any morphological variation is not exclusive to either of the divergent clades. In some cases, sampling was limited to one specimen per locality therefore, variation might have been missed. The divergent clades within *T. auropilosa* can be distinguished geographically (see the phylogenetic analysis later in this species treatment). Locality and specimen information are provided in Table 3.

Table 3. *Tettigades auropilosa* localities and specimens collected in the field. Specimen numbers with an asterisk (*) were included in phylogenetic analyses. AUR1 and AUR2 labels are divergent clades of *T. auropilosa*.

Date Collected	Specimen	Divergent Clade	Name	Region	Latitude	Longitude	Elevation	Collector(s)
11.XII.2014	PL363 (♀)*	AUR1	Camino Putaendo-Alicahue 2	Valparaíso	-32.46	-70.70	1307m	CV & PŁ
11.XII.2014	PL376.1 (♂)*	AUR1	Alicahue	Maule	-32.33	-70.74	94m	CV & PŁ
11.XII.2014	PL383 (♀)*	AUR1	Alicahue	Maule	-32.33	-70.74	94m	CV & PŁ
11.XII.2014	PL388.1 (♂)*	AUR1	Alicahue	Maule	-32.33	-70.74	94m	CV & PŁ
10.XII.2015	PL695 (♂)*	AUR1	Las Bandurrias	Valparaíso	-32.85	-70.71	740m	CV & PŁ
12.XII.2015	KN011 (♂)	AUR1	Tahuilco	Coquimbo	-31.79	-71.06	581m	CV & PŁ
12.XII.2015	KN012 (♀)	AUR1	Tahuilco	Coquimbo	-31.79	-71.06	581m	CV & PŁ
12.XII.2015	KN013 (♂)	AUR1	Tahuilco	Coquimbo	-31.79	-71.06	581m	CV & PŁ
12.XII.2015	KN014 (♂)	AUR1	Tahuilco	Coquimbo	-31.79	-71.06	581m	CV & PŁ
12.XII.2015	PL665 (♂)*	AUR1	Tahuilco	Coquimbo	-31.79	-71.06	581m	CV & PŁ
12.XII.2015	PL668 (♀)*	AUR1	Tahuilco	Coquimbo	-31.79	-71.06	581m	CV & PŁ
12.XII.2015	PL693 (♂)*	AUR1	Tahuilco	Coquimbo	-31.79	-71.06	581m	CV & PŁ
15.XII.2015	PL458.1 (♀)*	AUR2	Termas del Flaco	O'Higgins	-34.95	-70.44	1763m	CV & PŁ
15.XII.2015	PL505 (♀)	AUR3	Termas del Flaco	O'Higgins	-34.95	-70.44	1763m	CV & PŁ
15.XII.2015	PL509.1 (♀)*	AUR4	Termas del Flaco	O'Higgins	-34.95	-70.44	1763m	CV & PŁ
15.XII.2015	PL509.3 (♂)*	AUR5	Termas del Flaco	O'Higgins	-34.95	-70.44	1763m	CV & PŁ
15.XII.2015	PL509.4 (♀)	AUR6	Termas del Flaco	O'Higgins	-34.95	-70.44	1763m	CV & PŁ

Distribution: Chile

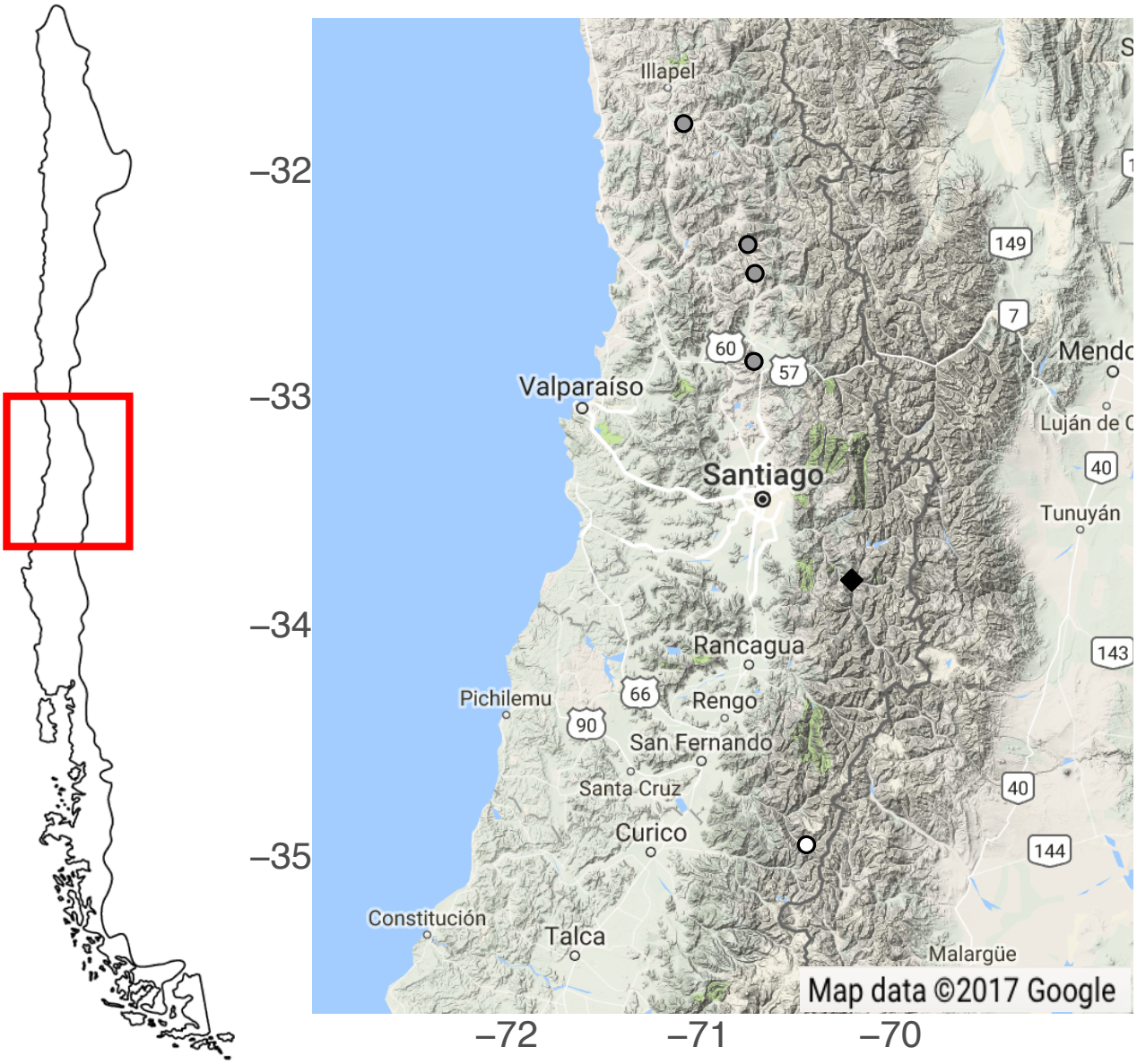

Figure 18. Map of localities for *Tettigades auropilosa*. Experimental/field collected cicada clades are identified on the map with circles. The type locality is identified with a diamond and is black since subspecific clade identity is unknown. The color indicates AUR1(gray) and AUR2 (white). The outline map of Chile is provided by WorldAtlas. Map of Chile reproduced from World Atlas (http://www.worldatlas.com/).

Additional and Updated Distinguishing Features:

Thorax. Wing groove orange. Cruciform elevation entirely black. The ends of the anterior branches of cruciform may at times have orange spots. In most cases, the ends of the anterior branches of the cruciform elevation are black. Coxae are the same color as groove, orange.

Wings. Orange at base in a few cases appears reddish-orange. Veins orange at base and black at apex. The jugal fold is soft orange in color at base and white at the apex. Veins may appear reddish-orange in some specimens.

Body. Dense hairs. The body length is short and wide compared to all other species in the genus.

Genitalia (Figs. 19 and 20). Eighth sternite and tergite black. Dorsal beak sharply pointed and black. Pygofer black. Median lobe of uncus black on outside, closest to the anal tube. The pseudoparamere lightly colored with the presence of a few tooth-like structures visible on the lateral view at the distal and proximal areas, with larger tooth-like structures on the proximal area. Tooth-like structures on the outer perimeter of the pseudoparamere visible in lateral view. In ventral view, the endotheca curves back towards the specimen on the left side and curves out and downwards towards the right.

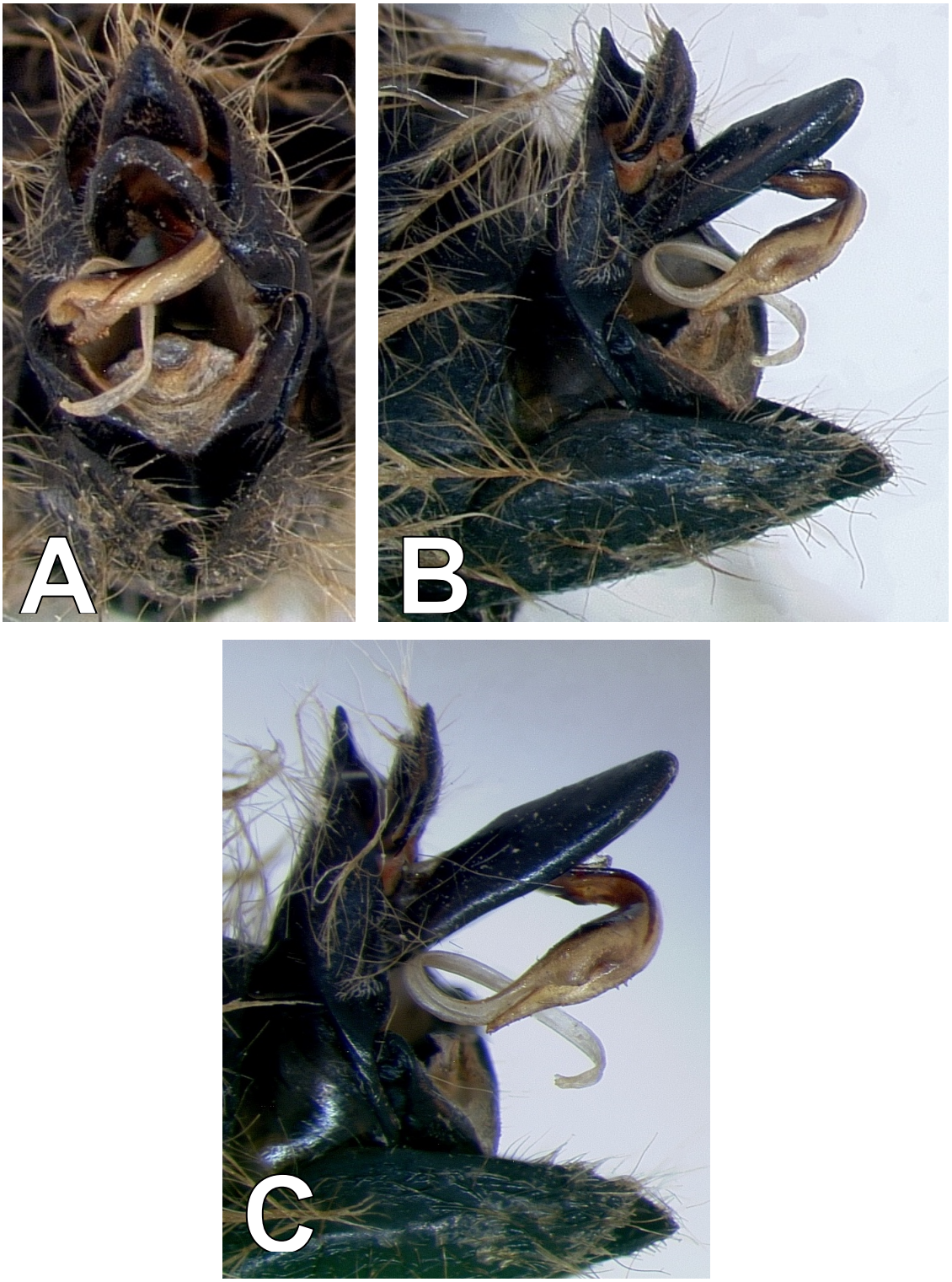

Figure 19. Male genitalia of *Tettigades auropilosa*: (A) ventral, (B) lateral, and (C) lateral views of PL388.1, *T. auropilosa* AUR2. Images (A) and (B) are at 2x magnification. Image (C) is at 4x magnification.

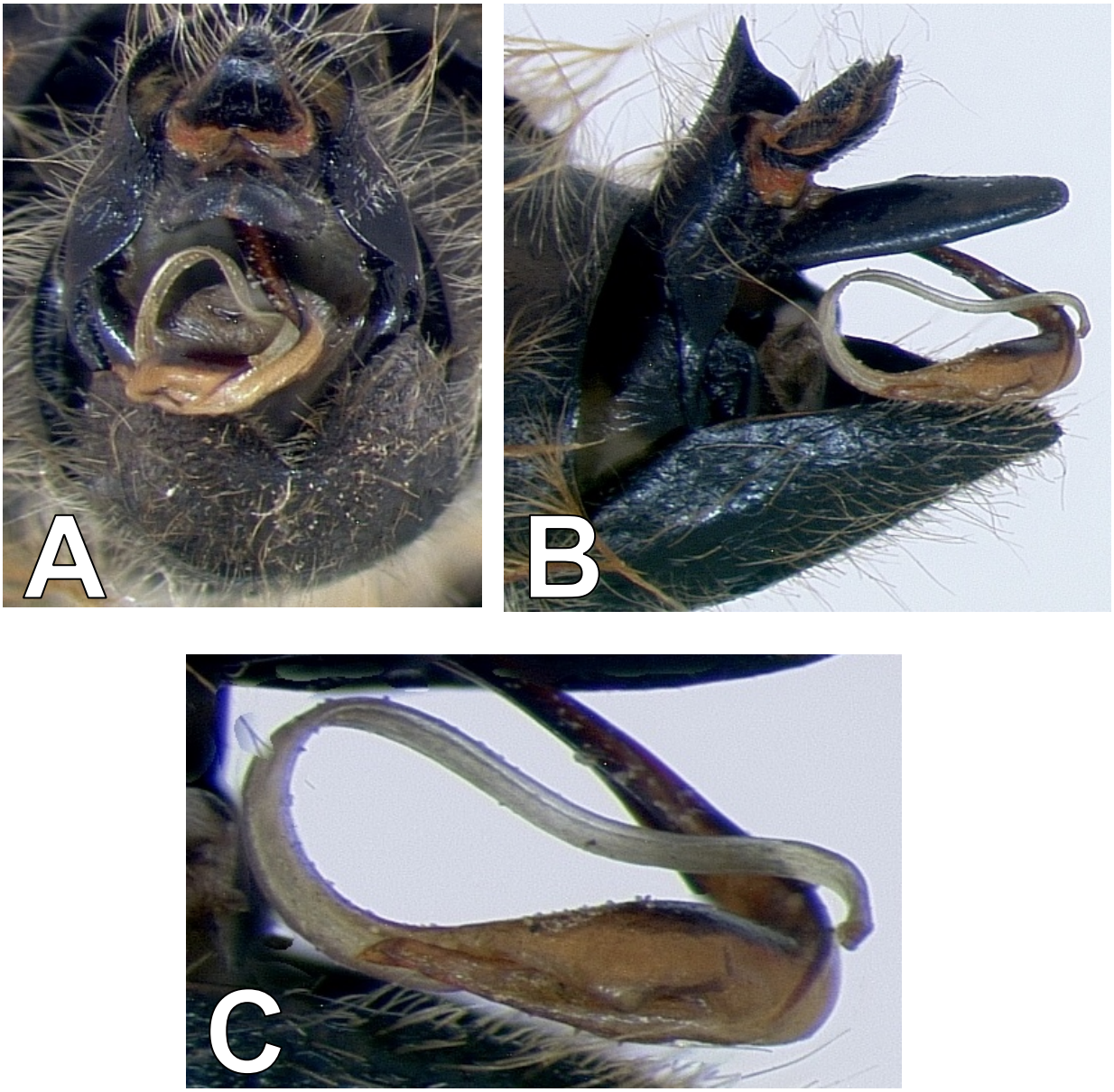

Figure 20. Male genitalia of *Tettigades auropilosa*: (A) ventral, (B) lateral, and (C) lateral views of PL694, *T. auropilosa* AUR2. Images (A) and (B) are at 2x magnification. Image (C) is at 4x magnification.

Diagnosis:

This species is morphologically similar to *Tettigades chilensis* and *Tettigades limbata*. It differs in the following aspects (Fig. 21):

1. The hair of the abdomen is very dense in *T. auropilosa* compared to the other two species. *T. chilensis* and *T. limbata* have moderately dense hair.
2. The stridulatory organ of *T. auropilosa* is S-shaped and the ridges are well separated. The stridulatory organ ridges of *T. chilensis* are parallel to the posterior border of the pronotum and evenly spaced. The spacing of the ridges of the stridulatory organ become narrower towards wing base in *T. limbata.* The stridulatory organ of *T. limbata* is club-shaped.
3. The jugal fold of *T. auropilosa* is a soft orange at base with white at apex. The jugal fold of the hindwing is white in *T. limbata with* some orange or red at the *base*. The jugal fold of *T. chilensis* is pearly gray with some cases of red or orange at the base.
4. The lateral and posterior edges of *T. chilensis* and *T. auropilosa* are all black. In some cases, the entire pronotal collar is red or orange in *T. chilensis*. In *T. limbata*, the pronotal collar is either red or orange on lateral and posterior edges.
5. The tergites of *T. auropilosa* and *T. limbata* are all black. The tergites of *T. chilensis* may have fine red or orange stripes in some cases.

6. The ends of the anterior branches of the cruciform elevation in *T. auropilosa* and *T. chilensis* may present orange or red spots but not in *T. limbata*.

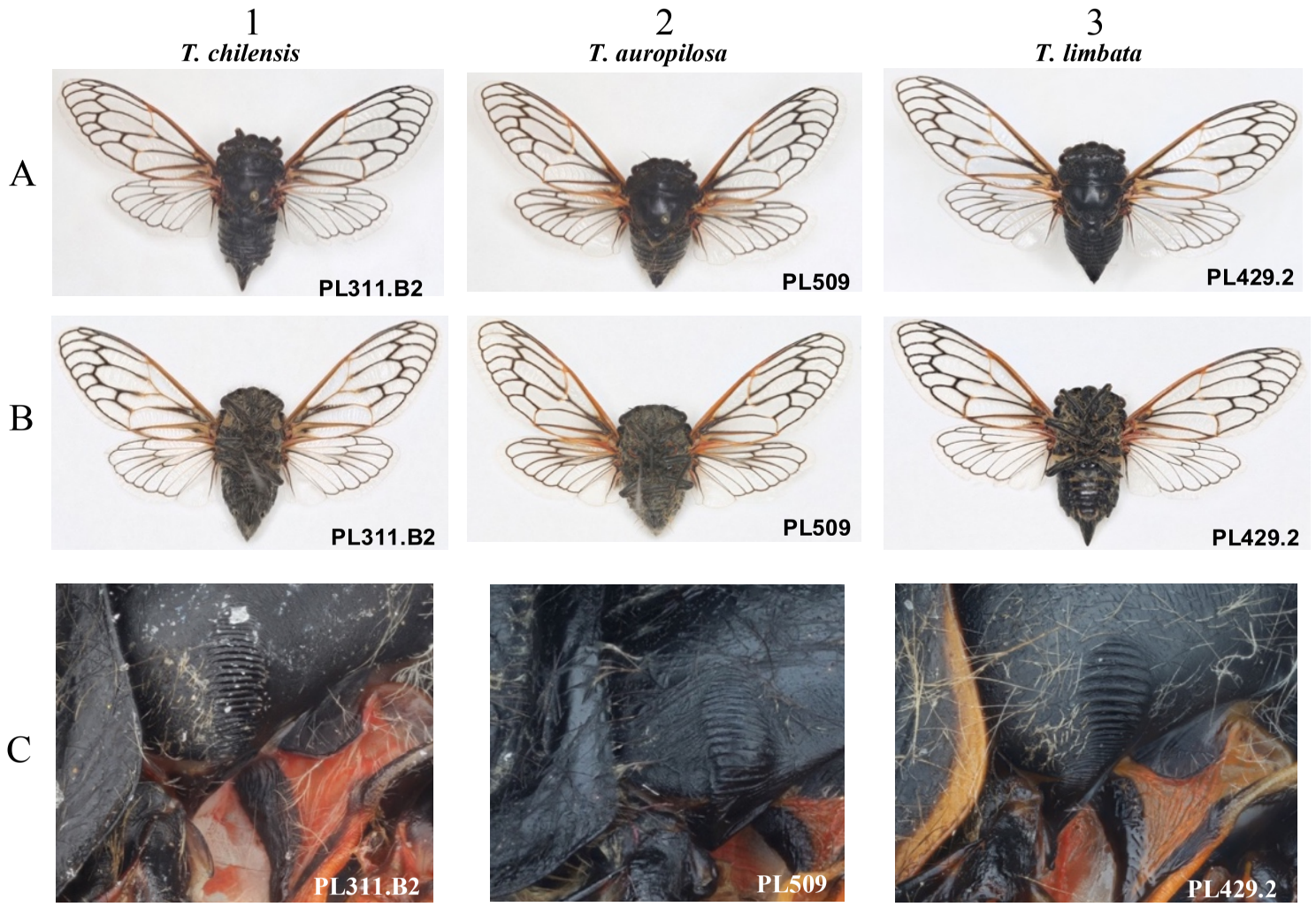

Figure 21. Dorsal, ventral, and stridulatory organ views of *T. auropilosa*, *T. chilensis*, and *T. limbata*. *Columns:* (1) T. *chilensis* PL311.B2, (2) *auropilosa* PL509, and (3) *T. T. limbata* PL429.2*;* (*Row*s:(A1-3) Dorsal views, (B1-B3) ventral views, and (C1-C3) stridulatory organs. Specimens were collected in 70% [or 95%] ETOH and then relaxed, spread, and dried.

Phylogenetic Assessment:

The clade, *T. auropilosa,* on both the nuclear and mitochondrial phylogenies consists of 11 specimens all closely matching the description of one species, *Tettigades auropilosa*. Both the 742-gene nuclear exon and the 13-gene mtDNA ML phylogenies resolve two monophyletic clades, AUR 1 and AUR2 with high support. These two clades cannot be diagnosed morphologically even though they are as divergent or more divergent in their mtDNA as well defined cicada species elsewhere (e.g., Buckley et al. 2006; Sueur et al. 2007; Marshall et al. 2008; Wade et al. 2015). The divergent clades of *T. auropilosa* correspond to the localities where they were collected (Fig. 18). However, additional geographic sampling is required to fully understand the variation within *T. auropilosa* and given the large genetic divergence it would not be surprising to find some distinguishing morphological characters. In some cases, sampling was limited to one specimen per locality; therefore, variation might have been missed. The entire range of our *T. auropilosa* specimens is 357km. At the northernmost range of our field collected *T. auropilosa* specimens, AUR1 (Fig. 22) spanned a range of about 123km. AUR2 (Fig. 23), at the southernmost range, was represented by one locality. Individuals representing both clades have never been found together at the same locality but can be found in the same year.

Figure 22. Dorsal (top) and ventral (bottom) view of *T. auropilosa* specimen, PL509.1 collected in the field representing AUR1. The specimen was collected in 95% ETOH and then relaxed, spread, and dried.

Figure 23. Dorsal (top) and ventral (bottom) view of *T. auropilosa* specimen, PL388.1 collected in the field representing AUR2. The specimen was collected in 95% ETOH and then relaxed, spread, and dried.

The sister clades of *T. auropilosa,* include the species *T. chilensis* and *T. limbata*. Although morphologically similar, these species have distinct characters which can be used to delimit the clades and divergent clades within those clades. *T. auropilosa* exhibits little within-species variation compared to *T. chilensis*. All areas that are colored such as the edges of the pronotal collar and the wing groove are never red unlike *T.*

chilensis and *T. limbata*. This variation in color was not recognized by the original describer, Belindo A. Torres.

A character that can be used to support the node delimiting *T. auropilosa* on the phylogeny and in specimens from the field, is the shape of the body, which is short and wide with a large amount of hair. Also, the S-shape of the stridulatory organ can be another morphological character distinguishing these clades from other previously described species. However, the stridulatory organ has a shape that is similar to *Tettigades* sp.1.

Tettigades sp.1.

During our phylogenetic assessment of *Tettigades*, 12 samples that did not morphologically match any *Tettigades* description were also sequenced. These specimens, *T.* sp1. form a monophyletic group that falls within the *T. auropilosa* clade, as sister to AUR2, in the nuclear phylogeny but is quite morphologically distinct. However, the mitochondrial phylogeny shows *Tettigades* sp.1 as sister to AUR1 + AUR2. The branch supporting Sp. 1 + AUR2 is quite short suggesting that this relationship may be due to systematic error, an ancient lineage sorting event, or hybridization (Fig. 28). Disagreement between the mtDNA and nuclear trees is found in only five of 23 named clades suggesting that hybridization and lineage sorting have been rare in the evolution of this genus.

In some localities, *Tettigades* "Sp. 1" overlaps with *T. auropilosa.* These include sites at Alicahue and Camino Putaendo-Alicahue 2 (Fig. 24). *T. auropilosa* and *T.* sp.1 can be told apart because although they have similar shapes of the stridulatory organs,

they differ drastically in body shape and density of hair on the body. The specimens within "Sp. 1" are highly variable. Some specimens have long thin bodies, others have orange stripes on several tergites; coloration of the wing groove also varies. Some of the specimens from this new group appear morphologically similar to *T. chilensis* (Fig. 25); however, the shape of the stridulatory organ is quite different. The genitalia of *Tettigades* sp.1 (Figs. 26 and 27) also differs from both *T. chilensis* and *T. auropilosa.* In *Tettigades* sp.1, the end of the pseudoparamere contains two finger-like projections. Also, the psudoparamere is covered by a hood-like projection of the theca in the lateral view which is quite distinctive compared to all the other Chilean *Tettigades* species examined. The theca of *Tettigades* sp.1 is mostly all dark, unlike both *T. chilensis* and *T. auropilosa.*

Distribution: Chile

Figure 24. The collection sites for *Tettigades* sp.1. The outline map of Chile is provided by WorldAtlas. Map of Chile reproduced from World Atlas (http://www.worldatlas.com/).

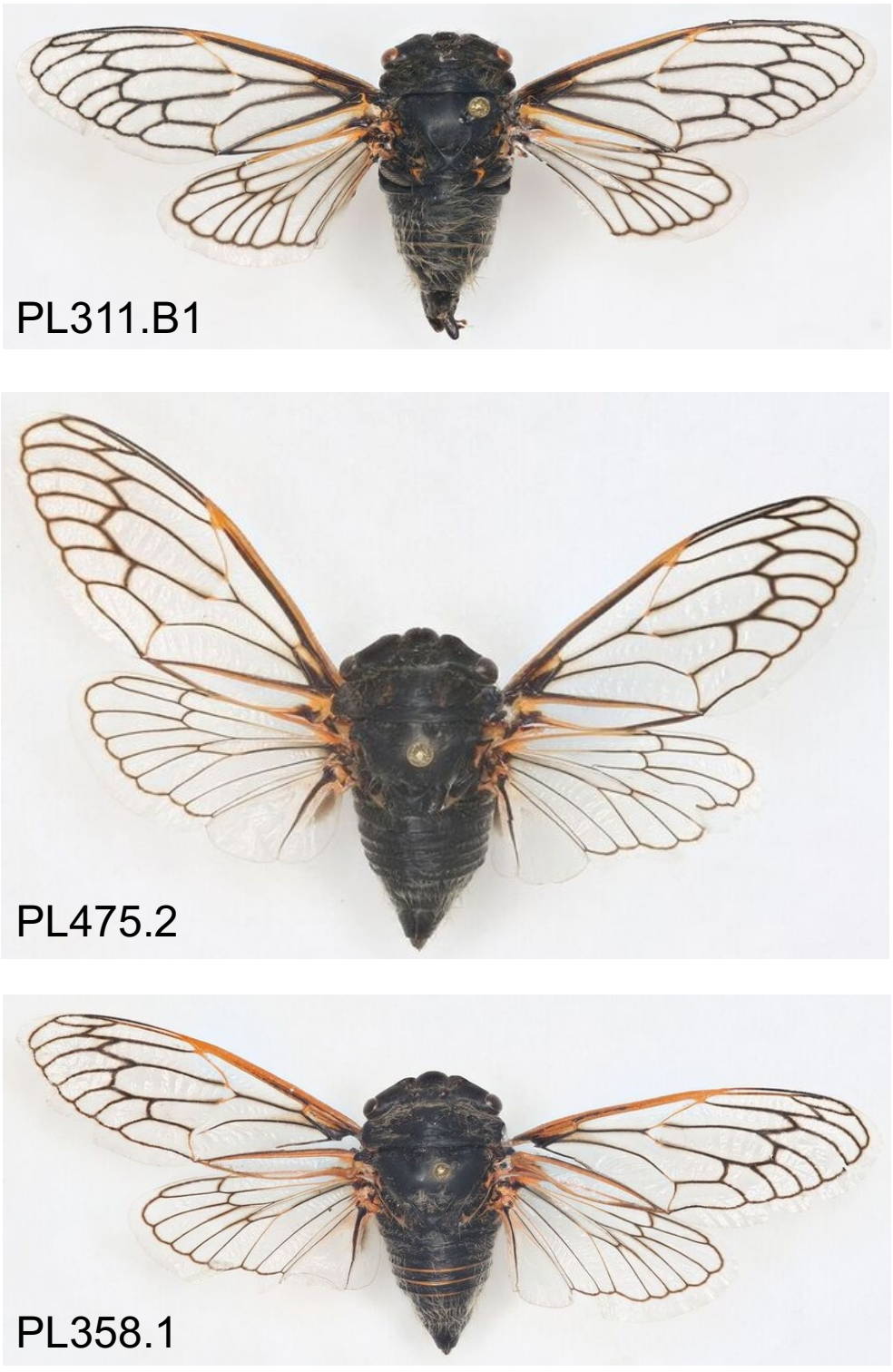

Figure 25. Doral images of the specimens that belong to *Tettigades* sp.1. Specimens were collected in 70% ETOH and then relaxed, spread, and dried.

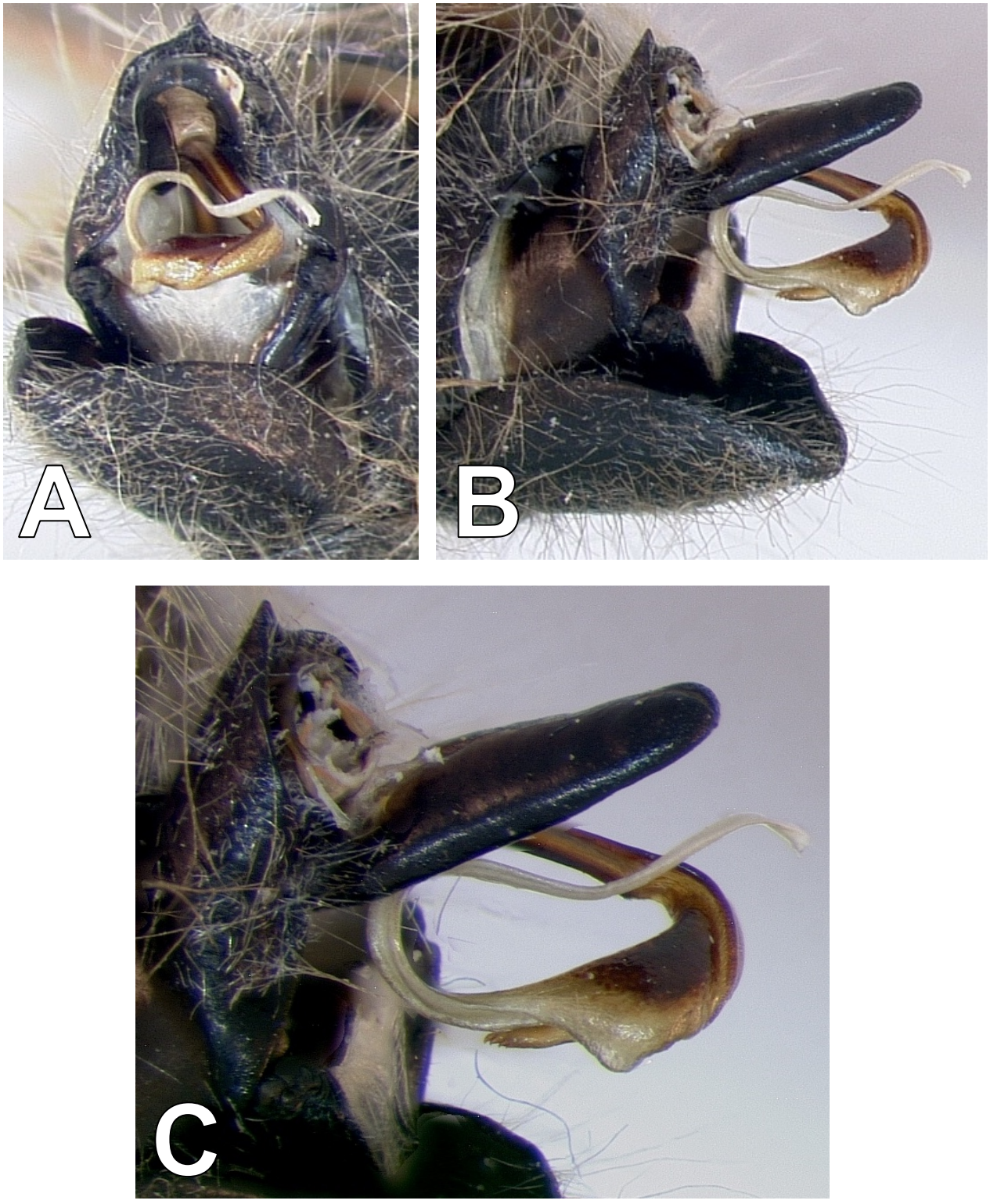

Figure 26. Male genitalia of *Tettigades sp.1*: (A) ventral, (B) lateral, and (C) lateral views of PL311.B1. Images (A) and (B) are at 2x magnification. Image (C) is at 4x magnification.

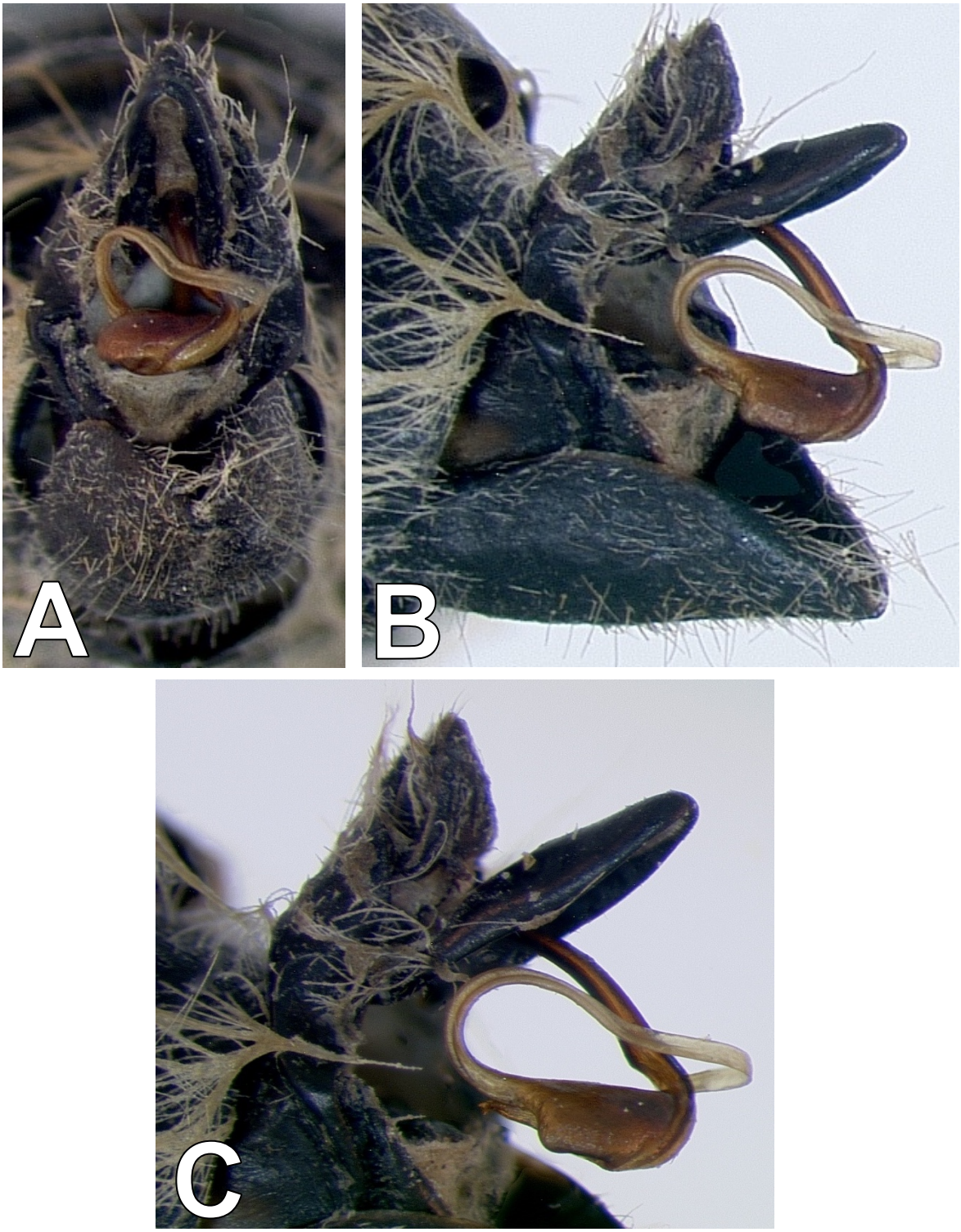

Figure 27. (Male genitalia of *Tettigades sp.1*: (A) ventral, (B) lateral, and (C) lateral views of PL376.2. Images (A) and (B) are at 2x magnification. Image (C) is at 4x magnification.

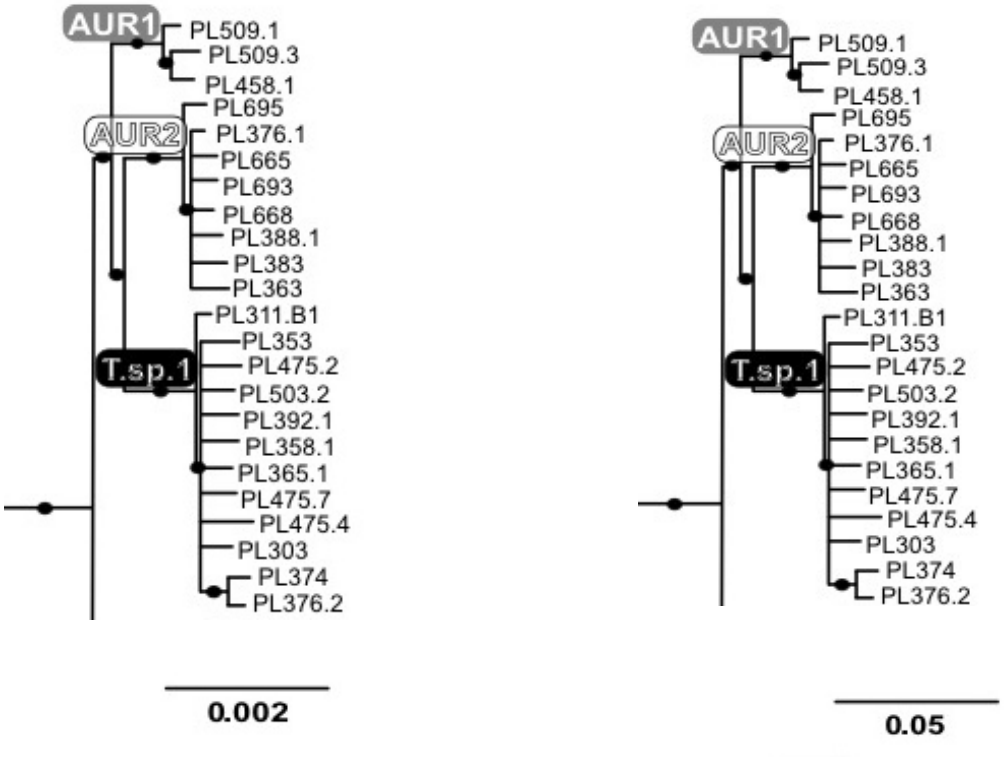

Figure 28. The *T. auropilosa* and *Tettigades* sp.1 subclades clipped from the maximum likelihood phylogenies (Chapter 2) based on 742 targeted-capture exons (left) and (from bycatch) 13 mitochondrial protein coding genes (right) with subclades CHI1, CHI2, and CHI3. Black dots represent bootstrap supports >95%; branches with <70% support were collapsed.

***Tettigades distanti* Torres, 1958**
Synonyms: none

Torres (1958) original description: [text interpreted from Spanish]

Holotype.

♂: Body length: 23mm; wing length: 28mm; wing width: 11mm; wingspan: 66mm.

Paratype.

♂: Body length: 22.2mm; wing length: 28mm; wing width: 11.3mm; wingspan: 66mm.

Head. Dorsal. Black. Frons, vertex with half of the anterior borders opposite of the eyes, edges of the one adjacent to the postclypeus, two small dips at the disk and two spots behind the eyes, brown. Ventral. Black. Apex of the anteclypeus, and second to last segment of the rostrum, ochre brown. Last segment of the rostrum intense dark brown and justly reaching the apex of the intermediate trochanters.

Thorax. Dorsal. Pronotum black in general. Anterior, lateral and posterior borders, brown. Anterior border in its middle third black, leaving a streak of brown in its central part. Mesonotum black. Distal half of anterior branches of the scutellum, a spot in front of each, a linear curved spot in each area lateral of the scutellum as well as the distal part of these and fine border posterior-lateral of the mesonotum, brown. Stridulatory area in the form of a <<S>>, with the ridges well defined and deep, approximately 27-30.

Wings. Forewing hyaline with the veins intense dark brown almost black, in general. Precostal area, costal vein, base of the area I^aR_3, vein r-m, m a little after its origin until a little before its intersection with m_{3+4}, in addition to a small extension of its distal extreme, m_{3+4} after its origin up to its basal half, final third of cu_1, cu_{1b} in all of its extension, cubital-anal area, vein 1a, half of 2a, half of the tuberosity posterior and superior vein of the arculus, ochre brown. Jugal region pale pink-ochre. Half of the

posterior tuberosity stained in black. Basal half of cu_1 and the extreme of the cubital-anal area, infuscated. Hingwings with veins intense dark brown almost black in general. Angular stain in the vannal region and a narrow internal strip in the jugal region, gray. Vein 2a black.

Abdomen. Tergites black; pygofer the same color. Sternites black with lateral and posterior borders widely lined with brown ochre. Seventh sternites bordered in this last color, presenting at the apex a notch. Subgential plate black.

Body. Body with a whitish secretion in its ventral part and generally covered in a pilosity general tint slightly darker.

Type Material Examined: used for morphological comparisons

Holotype (♂):

NHMUK Specimen Number: NHMUK № 010220841
Type Locality: Chile: Los Andes
Date Collected: 1-2.i.1927

Figure 29. Dorsal view of the holotype “NHMUK № 010220841” of *Tettigades distanti* deposited at NHMUK (London, UK). Image courtesy of NHMUK.

Figure 30. Ventral view of the holotype “NHMUK № 010220841” of *Tettigades distanti* deposited at NHMUK (London, UK). Image courtesy of NHMUK.

Torres (1958) Measurements: Body length:23mm, wing length: 28mm, wing width: 11mm, wing span: 66mm

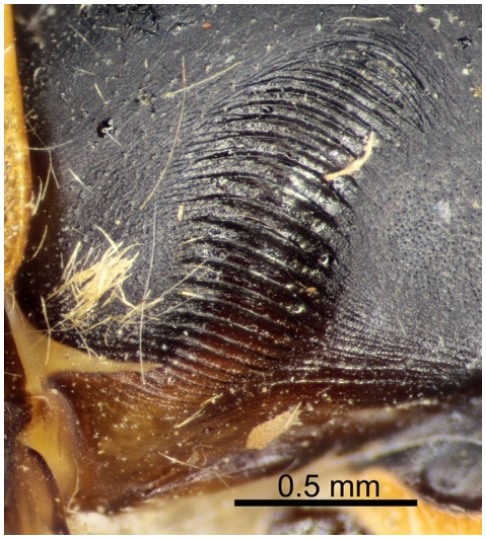

Figure 31. Stridulatory organ view of the holotype “NHMUK № 010220841” of *Tettigades distanti* deposited at NHMUK (London, UK). Image courtesy of NHMUK.

Field-Collected Material Examined:

A total of 21 specimens were collected in the field between 2014-2016 and used for morphological characterization. Of those 21, seven specimens were selected for phylogenetic analyses (*See Chapter 2*).

During our phylogenetic analyses, two divergent clades with high bootstrap support on both phylogenies, DIS1 and DIS2, closely resemble the description of *T. distanti*.

These two clades can be distinguished morphologically using the color and pattern of the sternites (*See Phylogenetic Analysis below*). Torres' (1958) description of *Tettigades distanti* lists the lateral and posterior borders of the sternites as widely lined with brown ochre. This character state is seen within DIS1. However, DIS2 has a darker fine ochre color on the lateral and posterior borders of the sternites compared to the other clade.

The two divergent clades within *T. distanti* and the characters we determined to distinguish these groups correlate with geographic localities where these groups are found. Locality and specimen information are provided in Table 4.

Table 4. *Tettigades distanti* localities and specimens collected in the field. Specimen numbers with an asterisk (*) were included in phylogenetic analyses. DIS1 and DIS2 labels are divergent clades of *T. distanti.*

Date Collected	Specimen	Divergent Clade	Name	Region	Latitude	Longitude	Elevation	Collector(s)
11.XII.2014	PL331 (♂)*	DIS1	Camino Putaendo-Alicahue 1	Valparaíso	-32.59	-70.79	94m	CV & PŁ
11.XII.2014	PL332 (♂)	DIS1	Camino Putaendo-Alicahue 1	Valparaíso	-32.59	-70.79	94m	CV & PŁ
11.XII.2014	PL334.1 (♂)	DIS1	Camino Putaendo-Alicahue 1	Valparaíso	-32.59	-70.79	94m	CV & PŁ
11.XII.2014	PL334.2 (♂)	DIS1	Camino Putaendo-Alicahue 1	Valparaíso	-32.59	-70.79	94m	CV & PŁ
11.XII.2014	PL334.3 (♂)	DIS1	Camino Putaendo-Alicahue 1	Valparaíso	-32.59	-70.79	94m	CV & PŁ
11.XII.2014	PL335.1 (♂)*	DIS1	Camino Putaendo-Alicahue 1	Valparaíso	-32.59	-70.79	94m	CV & PŁ
11.XII.2014	PL336 (♂)	DIS1	Camino Putaendo-Alicahue 1	Valparaíso	-32.59	-70.79	94m	CV & PŁ
11.XII.2014	PL337 (♂)	DIS1	Camino Putaendo-Alicahue 1	Valparaíso	-32.59	-70.79	94m	CV & PŁ
11.XII.2014	PL338 (♂)	DIS1	Camino Putaendo-Alicahue 1	Valparaíso	-32.59	-70.79	94m	CV & PŁ
20.XII.2015	PL726 (♂)*	DIS2	Alicahue	Maule	-35.63	-71.79	94m	CV & PŁ
20.XII.2015	PL728 (♂)*	DIS2	Alicahue	Maule	-35.63	-71.79	94m	CV & PŁ
20.XII.2015	PL729 (♂)	DIS2	Alicahue	Maule	-35.63	-71.79	94m	CV & PŁ
20.XII.2015	PL730 (♂)	DIS2	Alicahue	Maule	-35.63	-71.79	94m	CV & PŁ
20.XII.2015	PL736 (♂)	DIS2	Cauquenes/Quirihue	Maule	-36.10	-72.45	258m	CV & PŁ
20.XII.2015	PL737 (♂)	DIS2	Cauquenes/Quirihue	Maule	-36.10	-72.45	258m	CV & PŁ
20.XII.2015	PL738 (♀)*	DIS2	Cauquenes/Quirihue	Maule	-36.10	-72.45	258m	CV & PŁ
20.XII.2015	PL742 (♂)*	DIS2	Cauquenes/Quirihue	Maule	-36.10	-72.45	258m	CV & PŁ
20.XII.2015	PL746 (♂)	DIS2	Cauquenes/Quirihue	Maule	-36.10	-72.45	258m	CV & PŁ
20.XII.2015	PL747 (♂)	DIS2	Cauquenes/Quirihue	Maule	-36.10	-72.45	258m	CV & PŁ
20.XII.2015	PL750 (♂)	DIS2	Cauquenes/Quirihue	Maule	-36.10	-72.45	258m	CV & PŁ
20.XII.2015	PL751 (♂)	DIS2	Cauquenes/Quirihue	Maule	-36.10	-72.45	258m	CV & PŁ
22.I.2016	PL985 (♂)*	DIS1	Olmue antes de Puente	Valparaíso	-33.03	-71.14	186m	KN, CV & PŁ

Distribution: Chile

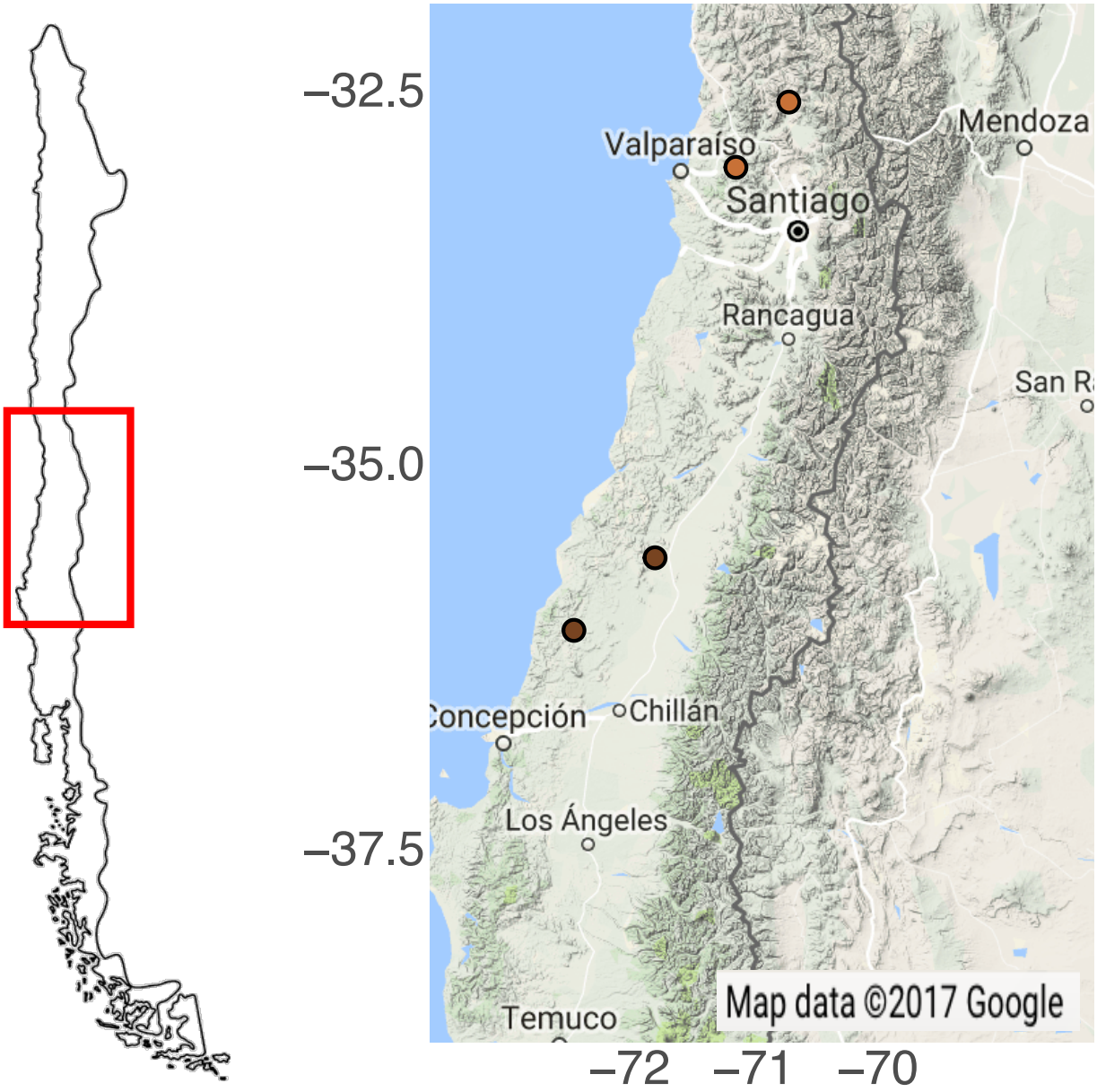

Figure 32. Map of localities for *Tettigades distanti*. Experimental/Field-collected cicada clades are identified on the map with circles. The exact location of the type locality due to its uncertainty is not included. The light and dark colors of the shapes indicate divergent clades: DIS1(light brown) and DIS2 (dark brown). The outline map of Chile is provided by WorldAtlas. Map of Chile reproduced from World Atlas (http://www.worldatlas.com/).

Additional and Updated Distinguishing Features:

General. Specimens that have been collected closely match the description by Torres for *Tettigades distanti*. Field-collected specimens have been found to have ochre color patterns throughout the body as Torres described. However, coloration appears to vary in intensity within this species.

Thorax. Areas such as the spots on and the distal half of the anterior branches of the cruciform elevation and the scutellum itself can present faded ochre to a light ochre spot. Also, the coloration of the pronotal collar can vary in thickness as well. While most specimens have thin ochre borders, some present a wider border. Even after specimens were preserved in ethanol, these colors do not change. The color of the coxae is another character Torres did not include in his description but may be useful for delimiting *T. distanti* from other species.

Again, Torres uses the stridulatory area as a character to delimit the species of *Tettigades*. While useful for delimiting species that are more distantly related, morphologically similar species such as *Tettigades curvicosta* have been found to have similar patterns. The number of ridges of the stridulatory organ are difficult to assess, because Torres describes only well-defined ridges without any indication of exactly which those are. Although the exactly number might vary, the range, shape, and pattern of the stridulatory is indeed a useful character.

Abdomen. Torres describes the sternites of the abdomen as black with lateral and posterior borders widely lined with brown ochre, and the seventh sternite as bordered in this last color (Fig. 38). Although we found field specimens with this pattern on the sternites, we also found specimens with a finely lined brown ochre color (Fig.

39). Neither DIS1 nor DIS2 show any sign of the white powdery coating mentioned by Torres on the ventral side of the abdomen.

Genitalia (Figs. 33-35). The eighth sternite and tergite are black. The dorsal beak is sharply pointed and black. The pygofer is primarily black with the outer edges a very thin ochre, which is difficult to resolve. The median lobe of uncus is black on the outside. The pseudoparamere is a light color with the presence of dark tooth-like structures visible on the lateral view down along the edges of the pesudoparamere. More tooth-like structures visible on the edge farthest from the specimen on the lateral view. Tooth-like structures on the outer perimeter of the pseudoparamere visible in ventral view. Endotheca curves tightly back towards the specimen and out towards the right almost parallel to the rest of the aedeagus. Theca primarily a light chestnut color with a small darker spot near the proximal end.

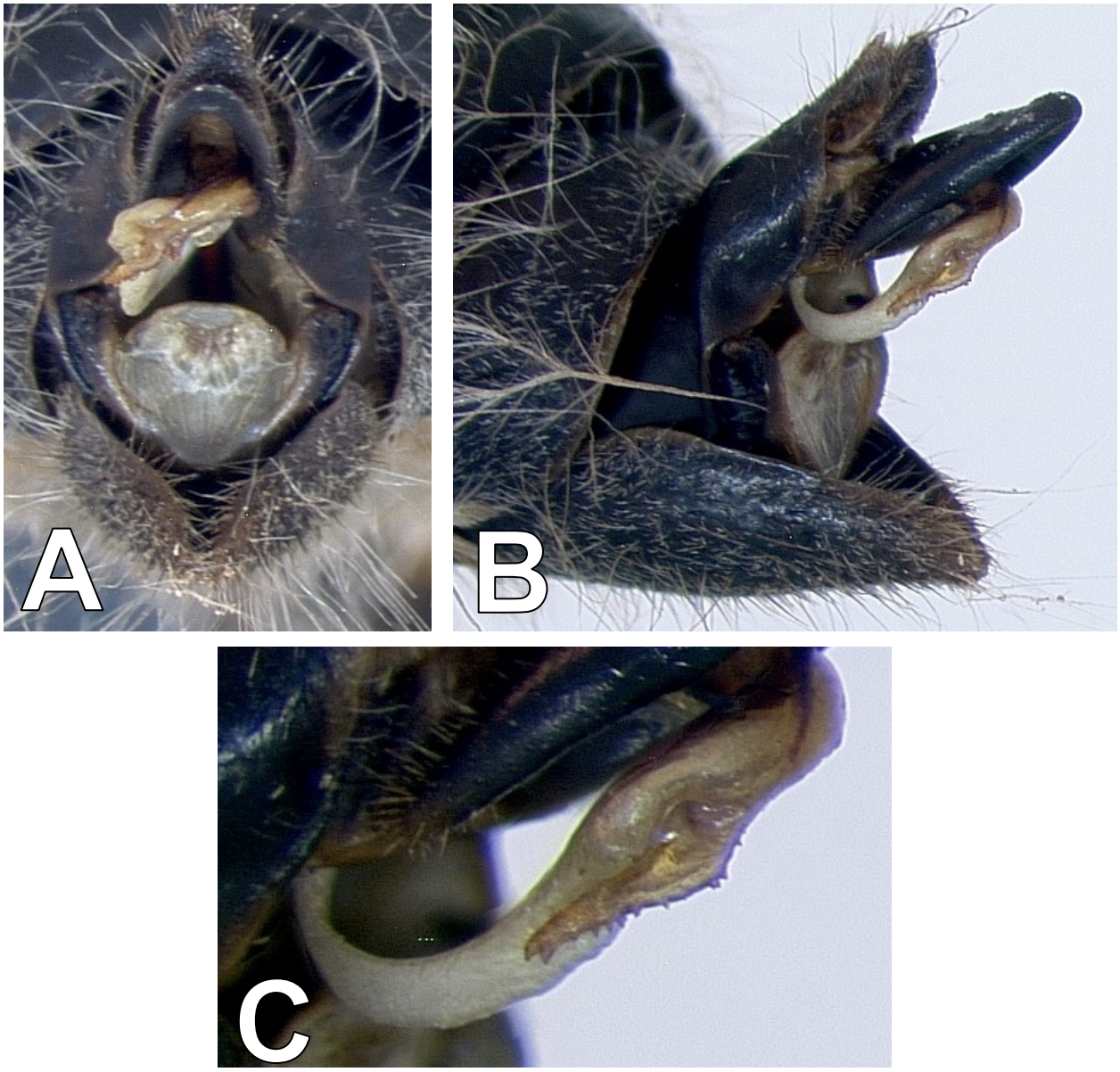

Figure 33. Male genitalia of *Tettigades distanti*: (A) ventral, (B) lateral, and (C) lateral views of PL985, *Tettigades distanti* DIS1. Images (A) and (B) are at 2x magnification. Image (C) is at 4x magnification.

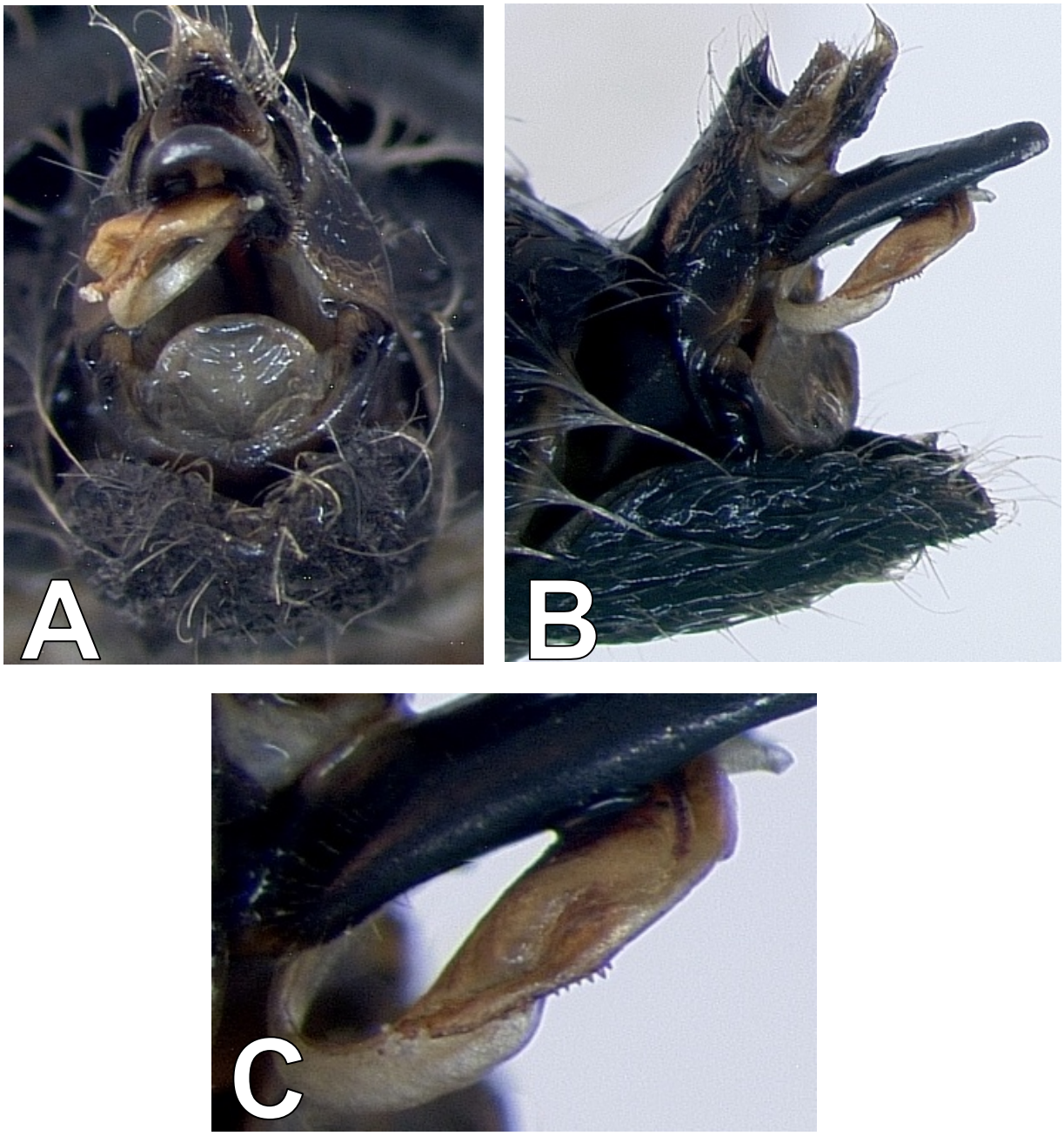

Figure 34. Male genitalia of *Tettigades distanti*: (A) ventral, (B) lateral, and (C) lateral views of PL742, *Tettigades distanti* DIS2. Images (A) and (B) are at 2x magnification. Image (C) is at 4x magnification.

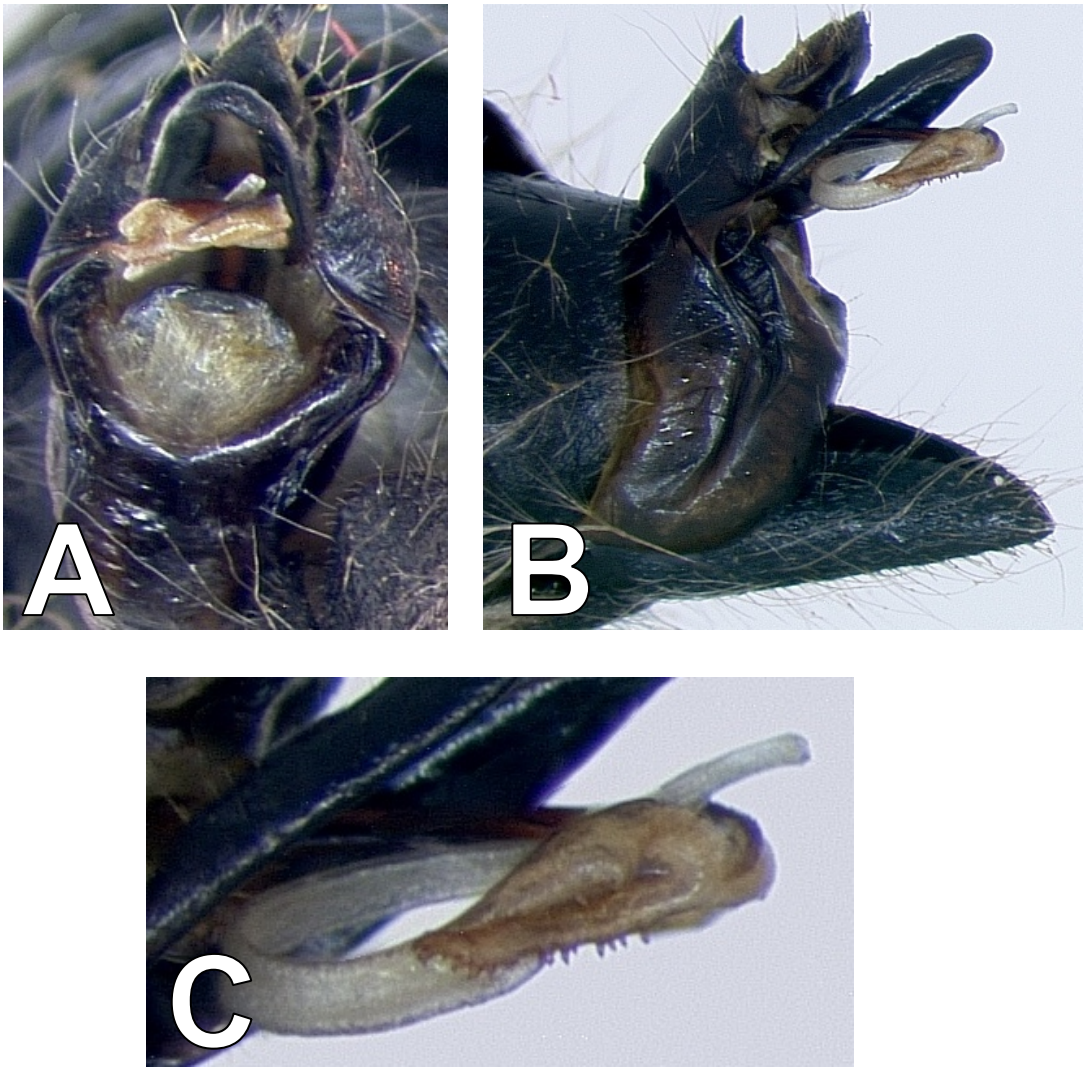

Figure 35. Male genitalia of *Tettigades distanti*: (A) ventral, (B) lateral, and (C) lateral views of PL743, *Tettigades distanti* DIS2. Images (A) and (B) are at 2x magnification. Image (C) is at 4x magnification.

Diagnosis:
Tettigades curvicosta

Tettigades distanti is morphologically similar to *Tettigades curvicosta* (Figs. 36 and 37). We have only been able to examine images of the holotype for *T. curvicosta* and none of the specimens collected in the field represent or match the description Torres (1958) uses to distinguish *T. curvicosta* from *T. distanti.* Due to the variation exhibited in other species of *Tettigades*, and the similarity of *T. curvicosta* to *T. distanti,* molecular evidence needs to be gathered in the future to determine the validity of this species and its relationship with *T. distanti.* Therefore, a complete diagnosis comparing these two species will not be provided. However, below are characters Torres (1958) provided to delimit these two species and which were used to determine the specimens collected in the field as *T. distanti*. Dorsal and ventral images of the holotype of *T. curvicosta* are also provided.

T. distanti:

1. sc vein with a minor curvature
2. Tergites VII and VIII black
3. Sternite VII with a notch

T. curvicosta:

1. sc vein with a major curvature
2. Tergites VII and VIII filed in chestnut
3. Sternite VII without a notch

Figure 36. Ventral view of holotype of *T. curvicosta* “NHMUK № 010220840” deposited at NHMUK (London, UK). Image courtesy of NHMUK.

Figure 37. Dorsal view of holotype of *T. curvicosta* “NHMUK № 010220840” deposited at NHMUK (London, UK). Image courtesy of NHMUK.

Phylogenetic Assessment:

The species clade, *T. distanti,* consists of seven specimens all closely matching the description of one species, *Tettigades distanti*. There is at least one representative specimen per locality collected. If variation was suspected or the species identification was uncertain, more than one individual per locality were examined. Within the clade representing *Tettigades distanti*, both phylogenies resolve two divergent clades with high bootstrap support, DIS1 and DIS2.

These two divergent clades can be distinguished morphologically and by geographic locality. DIS1 was found to the north of Santiago, in the region of Valparaíso, and presents a wide and light colored ventral abdomen on the lateral and posterior borders. DIS2 was found south of Santiago in the region of Maule. Specimens of DS1 can be distinguished by ochre sternites with a black central marking whereas DIS2 individuals have black sternites with just a hint of ochre color at the edges (Figs 38 and 39).

There were about 65km between the collection sites of DIS1. There were about 80km between collection sites of DIS2. Combined, there was a range of about 423km for our T. *distanti* collected in the field. No representatives of *T. distanti* have been collected around Santiago. Further collecting at different locations such as Santiago may reveal more variation within *T. distanti.*

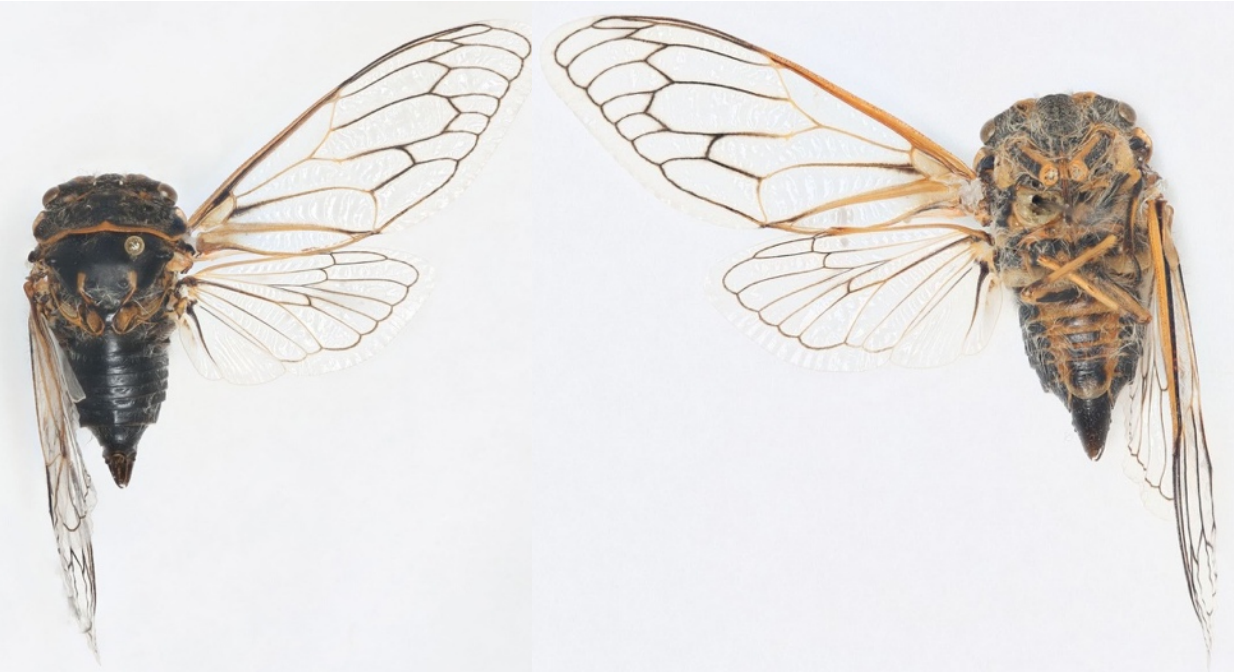

Figure 38. Dorsal and ventral view of specimen collected in the field (PL335.1) representing DIS1. Specimens were collected in 95% ETOH and then relaxed, spread, and dried.

Figure 39. Dorsal and ventral view of specimen collected in the field (PL742) representing DIS2. Specimens were collected in 95% ETOH and then relaxed, spread, and dried.

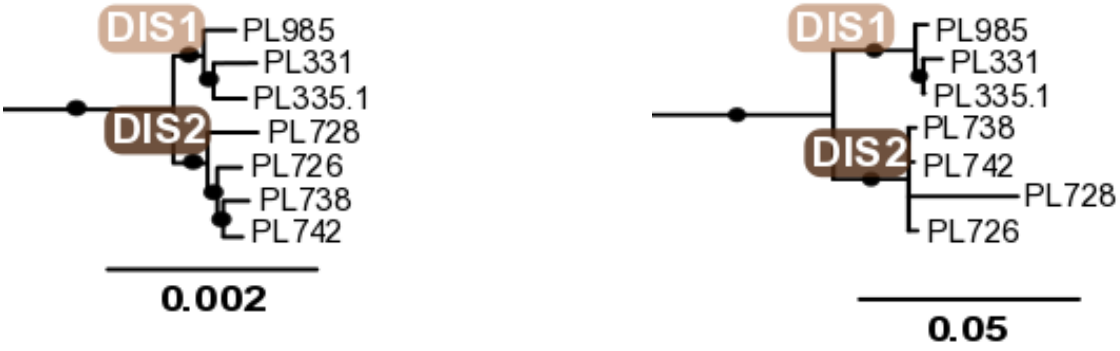

Figure 40. The *T. distanti* clades clipped from the maximum likelihood phylogenies (Chapter 2) based on nuclear targeted capture data of 742 exons (left) and (from bycatch) mitogenomes of 13 genes (right) with subclades DIS1 and DIS2. Black dots represent bootstrap supports >95%; branches with <70% support were collapsed.

***Tettigades lacertosa* Torres, 1944**
No known synonyms.

Torres (1944) original description of *T. lacertosa*: [text interpretation from Spanish]

Cotypes.

♀♀: Body length: 25-26mm; wing length: 30-31.5mm; wing width: 11.5-12.2mm.

♂: Body length: 26mm; wing length: 29.5mm; wing width: 11.2mm.

General Coloration. Black, with chestnut or dark almost black ochre spots; body covered in dense white pilosity.

Female Head. Dorsal. Black vertex and frons of same color; both covered in white, brunette hairs. Ventral. Black frons and densely covered in yellowish white hairs which covers it entirely. Gena, postgena, and clypeus black. Rostrum black except for the second segment that presents a lighter hue, apex of the third segment reaches the base of the posterior coxae.

Female Thorax. Pronotum black. Anterior margin ochre or dark almost black ochre with a small black spot in the middle. Posterior margin the same color as the anterior, lateral margins in its upper portion with a small dark almost black ochre spot. Mesonotum black with a discal spot in front of each anterior branch of the cruciform elevation. The lateral sides ochre or at times dark almost black. Ventral segments of thorax black with some ochre spots. Legs black.

Body. Covered in a large and dense amount of yellowish brown hair. Hair dark above the head.

Wings. Hyaline with thick veins and black. Basal cell ochre. First vein of the base, membrane of and costal vein, base of the first and second median areas, a spot in the fourth longitudinal vein, end of the cubital vein, and a little more than half the

interior of the fifth transverse vein, ochre. A dark almost black ochre spot in the median vein at the origin of the forth longitudinal vein, clavus ochre, cubital vein, infuscated at its origin. Cubital anal cell area with intense dark almost black in its entirety. Hindwings with the angular basal spot of the anal area a pearly gray, with some red spots in the sclerites of the forewings and hindwings.

Abdomen. Sternites black with white hair. Pygofer black. Sternites black with posterior border a little stained in dark almost black ochre up to its ends and sometimes just discolored. Seventh ventral segment black, stained with ochre at its posterior border. The whole body, ventral, is covered with a white pilosity.

Male. Small ochre opercula with black at the base. Seventh sternite black, with ochre edges. Eighth sternite dark almost black at its border.

Variation. There are examples that can present the pronotum with black borders, almost all of the median vein an ochre color.

Torres (1958) description: [differences from Torres (1944) - text interpretation from Spanish]

Measurements.

Body length: 23.5-28mm; wing length: 28-32.5mm; wing width: 10.5-13mm; wingspan: 67-77.5mm.

Head. A small post behind the eyes which can be missing sometimes, an ochre chestnut color. Rostrum with second to last segment a chestnut ochre and the last segment an intense dark.

Thorax. Chestnut ochre border of pronotum fine at anterior and wide at posterior. Lateral expansions of the disc black of a greater or lesser degree, leaving in all cases an ochre spot at the anterior angles. Anterior margin at times interrupted in the middle.

Stridulatory areas sometimes with the lower end of its ridges and its prolonged basal half, approximately reaching 14-18 well defined ridges.

Wings. Veins intense dark brown in general. Precostal area, costal vein in the middle or at its distal thirds base of cell $1^{a}R_{3}$, r-m vein, terminal part of m vein, distal half or sometimes shorter extension of m vein in part of its origin and its intersection with m_{3+4}, terminal extreme distal of cu_{1b} often appears infuscated at its base, vein superior of arculus, basal cell, vein 1a in almost all of its extension, superior part of the plectrum posterior, and vein 2a, chestnut ochre. Vein cu_{1} in its basal half, as well as some others, especially the transverses and anastomosis, infuscated. Cubital anal area in its major and minor extension and inferior part of the posterior tuberosity, intense chestnut ochre. Jugal area a reddish ochre. 2a of the hindwing black.

Abdomen. Meracanthus and opercula dark chestnut and can appear black with meracanthus stained the same color at the base. Seventh tergite sometimes with posterior border with an indefinite brown form or chestnut. Sternites with posterior borders a chestnut or ochre chestnut. Males with margin of lateral posterior of the seventh sternites stained a chestnut ochre. Pygofer black with posterior border finely colored brown. Subgential plate almost black, presenting a lighter tone in its distal half and bordered in a dark ochre. Females with posterior border of sternites seven, dark brown, sometimes all black.

Variation. Costal vein entirely, all of the median vein and four-fifths of the cubital anal cell, ochre. The jugal region properly with a slightly reddish tint.

Type Material Examined: used for morphological comparisons

Cotypus (♀):

MLPA Specimen Number: MLPA № 1678/1
Type Locality: Chile, Valparaíso, Salto
Date Collected: XI-1940

Figure 41. Dorsal and ventral view of the female cotypus "MLPA № 1678/1" of *Tettigades lacertosa* deposited at MLPA (La Plata, AR).

Torres (1944) Measurements: Body length: 26mm, wing length: 29.5mm, wing width: 11.2mm
Our Measurements: Body length: 26mm, wing length: 31mm, wing width: 12mm

Cotypus (♂):

MLPA Specimen Number: MLPA № 1678/2
Type Locality: Chile, Valparaíso, Salto
Date Collected: XI-1940

Figure 42. Dorsal and ventral view of the male cotypus “MLPA № 1678/2” of *Tettigades lacertosa* deposited at MLPA (La Plata, AR).

Torres (1944) Measurements: Body length: 25-26mm, wing length: 30-31.5mm, wing width: 11.5-12.2mm (*Torres provided range sizes for 2 male cotypes*)
Our Measurements: Body length: 26.3mm, wing length: 28.1mm, wing width: 11.4mm

Field-Collected Material Examined:

A total of 26 specimens were collected in the field during 2010 and 2015 and were used for morphological characterization and phylogenetic analyses. Of those 26, 19 specimens were selected for phylogenetic analyses (*See Chapter 2*).

During our phylogenetic analyses, two divergent clades with high bootstrap support on the nuclear phylogeny, LAC1 and LAC2, closely resemble the description with its variation of *T. lacertosa.* However, the mitochondrial phylogeny fails to resolve LAC1 as a monophyletic clade. Due to the morphological characters supporting these two clades, and due to the fact that mtDNA in *Tettigades* and in insects in general is much more variable/informative than nuclear DNA the lack of resolution of this clade is likely due to random sorting of shared ancestral polymorphisms.

These two clades can be distinguished morphologically and geographically. Torres (1944) recognized variation in *Tettigades lacertosa* that is supported in our phylogeny. LAC1 was found in the northern-most range of our *T. lacertosa* collection, in the southern part of the Atacama Dessert. Specimens in this clade exhibit very dense hair throughout the body and black pronotal expansions. LAC2 was found in the southern-most range in shrub lands. Specimens in this clade are less hairy, and have orange pronotal expansions. Locality and specimen information are provided in Table 5.

Table 5. *Tettigades lacertosa* localities and specimens collected in the field. Specimen numbers with an asterisk (*) were included in phylogenetic analyses. LAC1 and LAC2 labels are subclade identifiers.

Date Collected	Specimen	Divergent Clade	Name	Region	Latitude	Longitude	Elevation	Collector(s)
27.X.2010	PL696.1 (♂)*	LAC1	N. Punta de Lobos	Atacama	-28.16	-71.15	36m	ME
27.X.2010	PL696.3 (♂)*	LAC1	N. Punta de Lobos	Atacama	-28.16	-71.15	36m	ME
6.XI.2015	PL697 (♂)*	LAC1	Carrizal Bajo	Atacama	-27.99	-71.13	119m	FU
13.XI.2015	PL614 (♀)*	LAC2	Camino a Fray Jorge A	Coquimbo	-30.56	-71.63	500m	CV & PŁ
13.XI.2015	PL615 (♂)*	LAC2	Camino a Fray Jorge A	Coquimbo	-30.56	-71.63	500m	CV & PŁ
13.XI.2015	PL616 (♀)*	LAC2	Camino a Fray Jorge A	Coquimbo	-30.56	-71.63	500m	CV & PŁ
13.XI.2015	PL617 (♂)*	LAC2	Camino a Fray Jorge A	Coquimbo	-30.56	-71.63	500m	CV & PŁ
13.XI.2015	PL609 (♀)*	LAC2	Camino a Fray Jorge B	Coquimbo	-30.54	-71.64	460m	CV & PŁ
13.XI.2015	PL612 (♀)*	LAC2	Cuesta Andacollo A	Coquimbo	-30.20	-71.10	792m	CV & PŁ
14.XI.2015	PL699 (♂)*	LAC1	Bahia Inglesa	Atacama	-27.13	-70.86	119m	CV & PŁ
15.XI.2015	PL605 (♂)*	LAC2	Rio Choapa A	Coquimbo	-31.82	-70.93	589m	CV & PŁ
11.XII.2015	PL655.1 (♂)*	LAC2	Camino a Fray Jorge A	Coquimbo	-30.56	-71.63	500m	CV & PŁ
11.XII.2015	PL655.2 (♂)*	LAC2	Camino a Fray Jorge A	Coquimbo	-30.56	-71.63	500m	CV & PŁ
11.XII.2015	PL655.3 (♂)*	LAC2	Camino a Fray Jorge A	Coquimbo	-30.56	-71.63	500m	CV & PŁ
11.XII.2015	PL655.4 (♀)*	LAC2	Camino a Fray Jorge A	Coquimbo	-30.56	-71.63	500m	CV & PŁ
11.XII.2015	PL655.5 (♂)*	LAC2	Camino a Fray Jorge A	Coquimbo	-30.56	-71.63	500m	CV & PŁ
11.XII.2015	PL655.6 (♂)*	LAC2	Camino a Fray Jorge A	Coquimbo	-30.56	-71.63	500m	CV & PŁ
11.XII.2015	PL649 (♂)*	LAC2	La Higuera	Coquimbo	-29.53	-71.24	485m	CV & PŁ
12.XII.2015	KN004 (♂)	LAC2	Tahuilco	Coquimbo	-31.79	-71.08	581m	CV & PŁ
12.XII.2015	KN005 (♂)	LAC2	Tahuilco	Coquimbo	-31.79	-71.08	581m	CV & PŁ
12.XII.2015	KN006 (♂)	LAC2	Tahuilco	Coquimbo	-31.79	-71.08	581m	CV & PŁ
12.XII.2015	KN007 (♀)	LAC2	Tahuilco	Coquimbo	-31.79	-71.08	581m	CV & PŁ
12.XII.2015	KN008 (♂)	LAC2	Tahuilco	Coquimbo	-31.79	-71.08	581m	CV & PŁ
12.XII.2015	KN009 (♂)	LAC2	Tahuilco	Coquimbo	-31.79	-71.08	581m	CV & PŁ
12.XII.2015	KN010 (♂)	LAC2	Tahuilco	Coquimbo	-31.79	-71.08	581m	CV & PŁ
12.XII.2015	PL661 (♀)*	LAC2	Tahuilco	Coquimbo	-31.79	-71.08	581m	CV & PŁ

Distribution: Chile

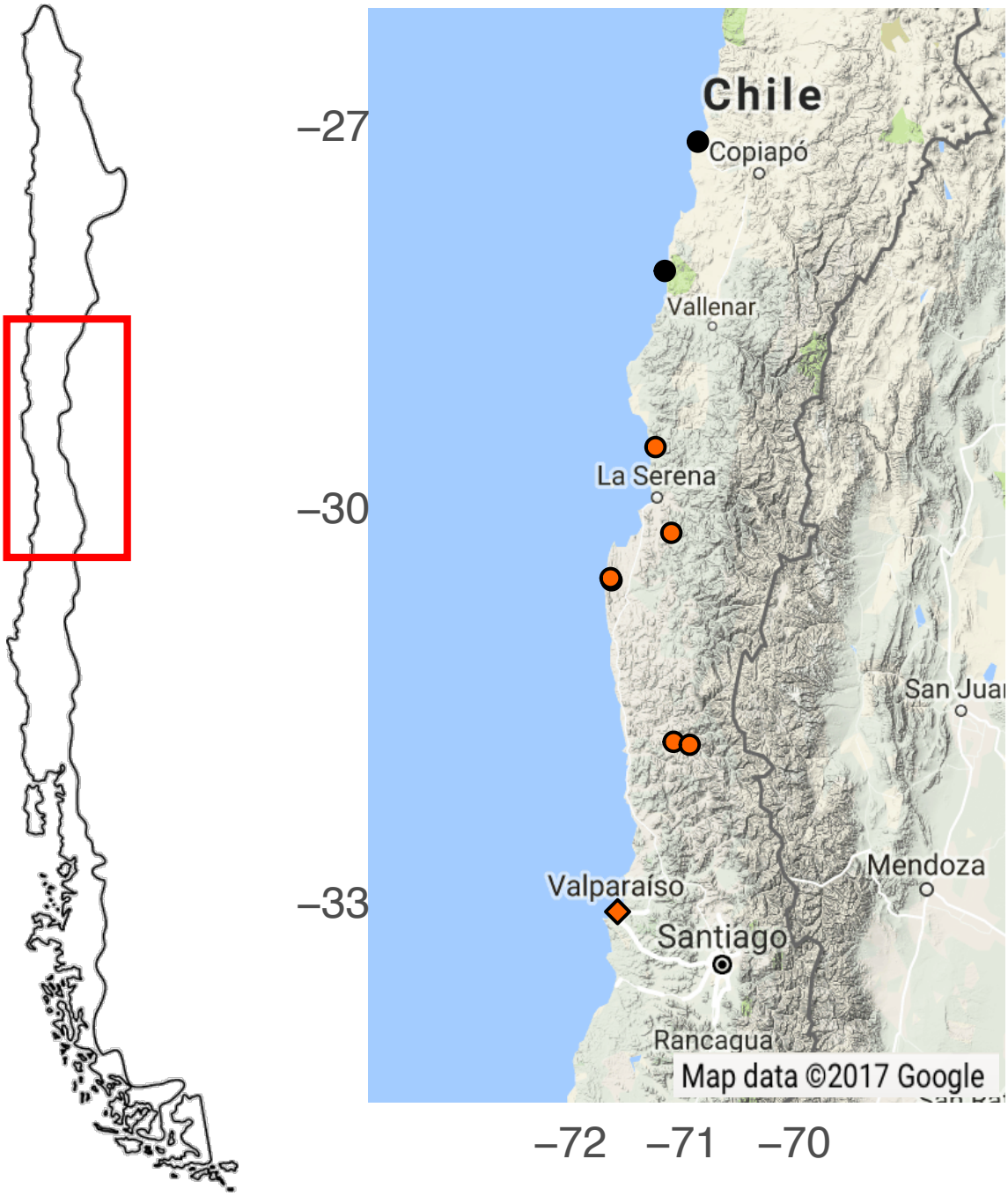

Figure 43. Map of localities for *Tettigades lacertosa*. Experimental/Field-collected cicada clades are identified on the map with circles. Type localities are identified on the map with a star. The colors of the shapes indicate divergent clades, LAC1(black) and LAC2 (orange), identified on the phylogeny or could be matched morphologically. The diamond represents the locality of the type. Map of Chile reproduced from World Atlas (http://www.worldatlas.com/).

Additional and Updated Distinguishing Features:

Abdomen. In some specimens from LAC2, orange stripes are present across the dorsal side of the abdomen.

Thorax. Specimens that have been collected in the field closely match the description by Torres for *Tettigades lacertosa*. However, the coloration described by Torres may have been influenced by the preservation of museum specimens. All *lacertosa* specimens at La Plata present the ochre color described by Torres. He described patterns such as the expansions of the pronotum, borders of the sternites, opercula, and meracanthus as an ochre color. However, field collected specimens from 2010 and 2015 indicate that it is an orange color. Field specimens collected and stored in ethanol regardless of concentration preserved the orange color. Although not mentioned by Torres, the specimens of this species we examined have orange coxae; this may turn out to be a useful distinguishing character.

Torres introduces the stridulatory area as a new character to delimit the species of *Tettigades*. While useful for delimiting species that are more distantly related, sister species have been found to have similar patterns. The number of ridges of the stridulatory organ are difficult to measure because Torres describes only well-defined ridges without any indication of which exactly those are. Although the exact number might vary, the range, shape, and pattern of the stridulatory organ is indeed a set of useful characters. For both clades, LAC1 and LAC2, the shape and pattern of the stridulatory organs are similar.

Wings. Wing veins are another character described as being chestnut ochre. However, field collected specimens indicate these veins are orange. Torres (1944) states that the angular basal spot of the anal area as a pearly gray. Although most of

this spot this indeed a pearly gray, the base is an orange color which in some specimens extends half way down this area.

Body. Hair density may appear to be less depending on preservation. Although *Tettigades limbata may* have a dense amount of hair on the body and ventral head area, this feature varies between the northern and southern groups. The northern desert group has much denser hair on the body than the southern group.

Genitalia (Figs. 44 and 45)**.** Eighth sternite and tergite black. Dorsal beak sharply pointed. Edges of distal beak black in LAC1 and orange with the color extending past the distal beak in LAC2. Median lobe of uncus black on outside facing the anal lobe. Ventral support of aedeagus dark. Pseudoparamere light with the presence of dark tooth-like structures visible on the left view along the edges pesudoparamere. More tooth-like structures visible on the edge farthest from the specimen on the left view. Tooth-like structures on the outer perimeter of the pseudoparamere visible in frontal view. Endotheca curves tightly back towards the specimen and out towards the right almost parallel to the rest of the aedeagus. Theca primarily a light chestnut color with a small darker spot near the ventral support area.

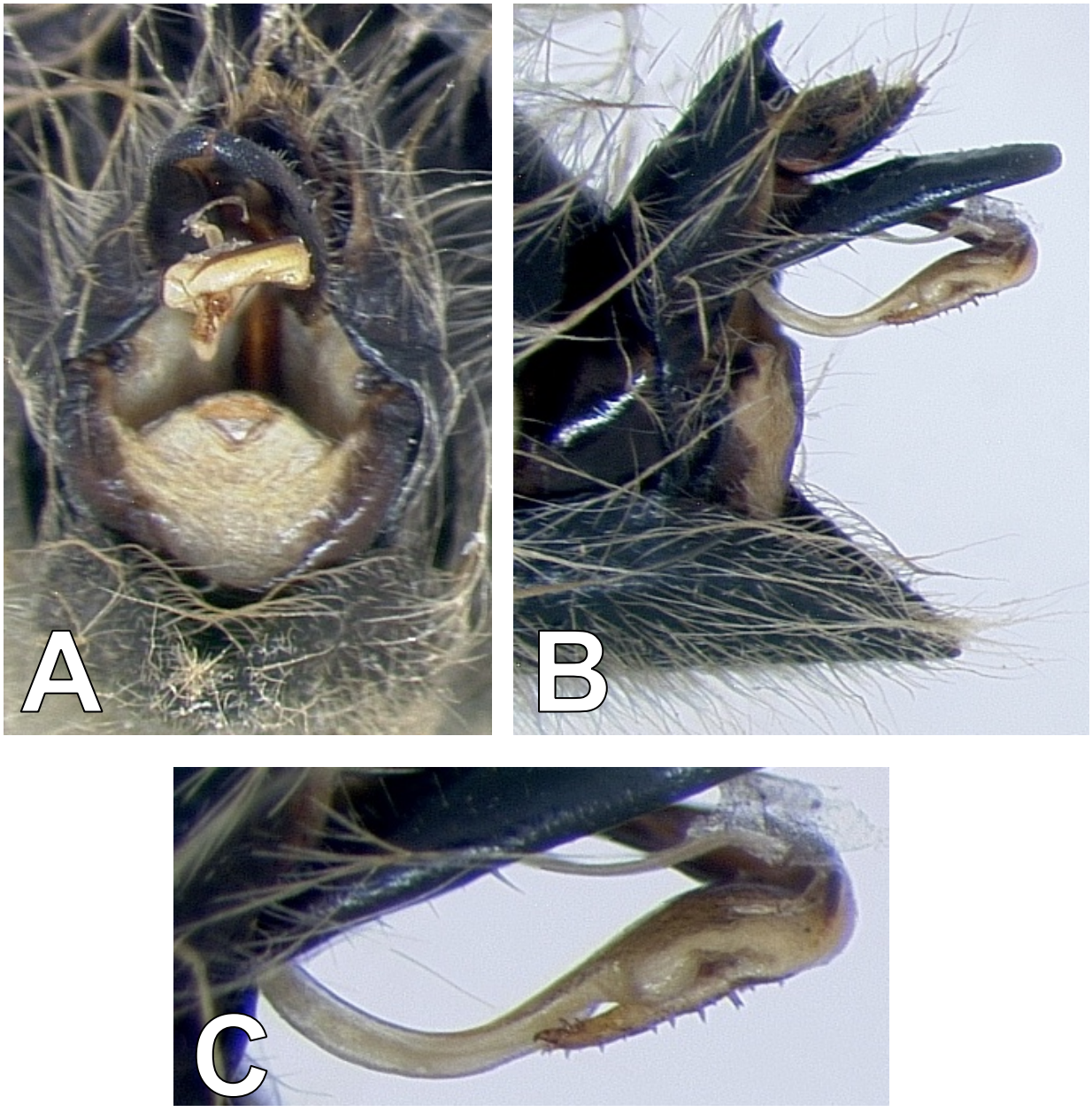

Figure 44. Male genitalia of *Tettigades lacertosa*: (A) ventral, (B) lateral, and (C) lateral views of PL696.4, *Tettigades lacertosa* LAC1. Images (A) and (B) are at 2x magnification. Image (C) is at 4x magnification.

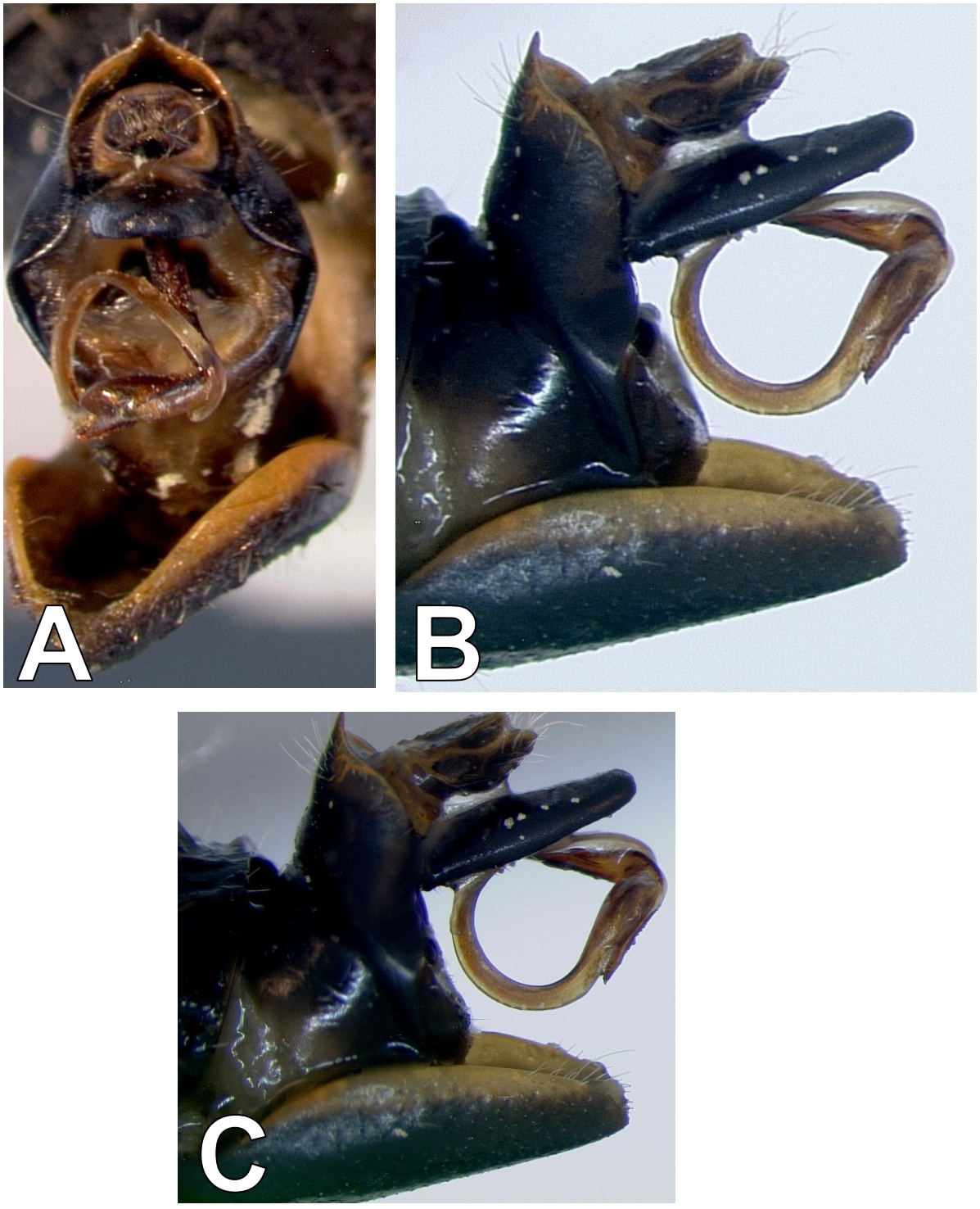

Figure 45. Male genitalia of *Tettigades lacertosa*: (A) ventral, (B) lateral, and (C) lateral views of KN004, *Tettigades lacertosa* LAC2. Images (A) and (B) are at 2x magnification. Image (C) is at 4x magnification.

Diagnosis:

This species is morphologically quite distinct from other species found in Chile. However, after examining specimens at el Museo Argentino de Ciencias Naturales (MACN) this species is quite similar to *Tettigades major* which is found in Argentina. Both species are similar in size. They differ in the following aspects (Fig. 46):

1. The locality of *T. lacertosa* is Chile while the locality of *T. major* is Argentina.
2. Jugal fold of wing pearly gray in *T. lacertosa* with some specimens showing orange stains at the base. The jugal fold of *T. major* is a creamy beige color.
3. Both divergent clades of *T. lacertosa* have orange at the anterior expansions of the pronotal collar. *T. major* does not.
4. *T. lacertosa* has orange spots at the anterior ends of the branches of the cruciform elevation. *T. major* does not present any spots at the anterior ends of the branches of the cruciform elevation.
5. Color of coxae in *T. lacertosa* orange and brown in *T. major*.
6. Color of wing veins in *T. lacertosa* black with some veins orange. Color of wing veins in *T. major* black with some veins a creamy beige.
7. High pilosity covering the postclypeus in *T. lacertosa.* Low pilosity with postclypeus visible in *T. major.*
8. The veins of *T. lacertosa* are thick while the veins of *T. major* are not.

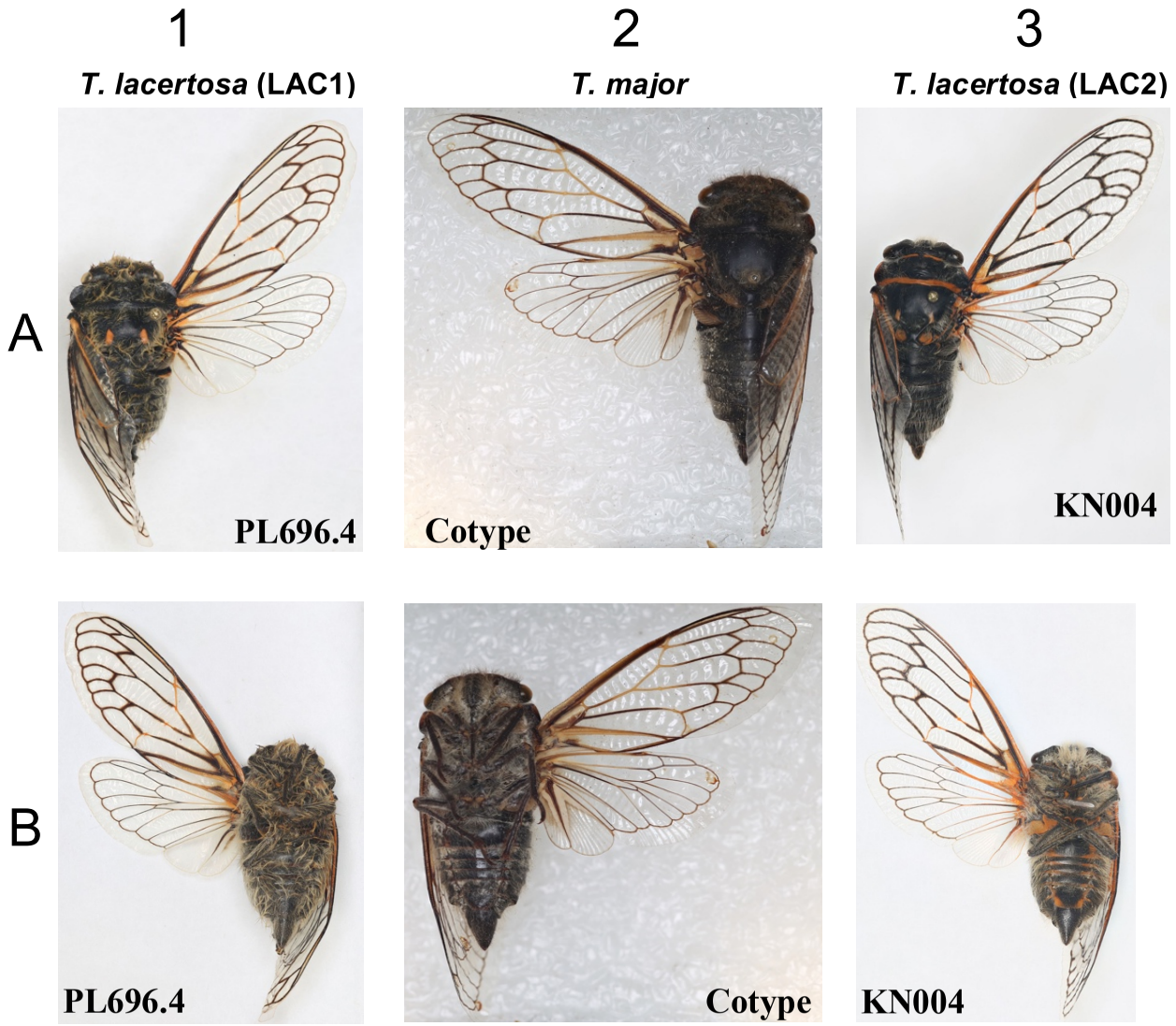

Figure 46. Dorsal and ventral views of both *T. lacertosa* clades and *T. major. Columns:* (1) *T. limbata* PL696.4 LAC1, (2) *T. major* cotype, and (3) *T. lacertosa* KN004 LAC2*; Row*s:(A1-3) Dorsal views, and (B1-B3) ventral views. *T. lacertosa* specimens were collected in 95% ETOH and then relaxed, spread, and dried..

Phylogenetic Assessment:

The clade *T. lacertosa* consists of 19 specimens all closely matching the description of one species, *Tettigades lacertosa*. Within the clade representing *Tettigades lacertosa*, the mitochondrial phylogeny resolves 2 divergent clades, LAC1 and LAC2, with high support. However, LAC1 does not form a monophyletic clade in the nuclear phylogeny. Morphological and geographic evidence supports each of the clades.

The divergent clades of *T. lacertosa* appear to be correlated with the localities at which they were collected (Table 5). LAC1 (Fig. 47) can be found in the southern part of the Atacama Desert and presents a black color pattern. LAC2 (Fig. 48) is found South of the Atacama Desert but north of Santiago in the Coquimbo region and presents an orange color pattern. Members of each clade have not been found together at the same locality but can be found in the same year. Locality and specimen information are provided in Table 5.

There are about 119km between the collection sites of LAC1 and about 258km between collection sites of LAC2. Combined, there is about 522km across the combined range of both clades. The total range is about 640km range when the type locality is included.

Due to the rapid radiation of *Tettigades*, the nuclear gene phylogeny does not have enough support to resolve the relationships between *Tettigades lacertosa* and the other species of *Tettigades*.

Figure 47. Dorsal, ventral, and stridulatory organ views of specimens collected in the field (PL696.4) representing LAC1. Specimens were collected in 95% ETOH and then relaxed, spread, and dried.

Figure 48. Dorsal, ventral, and stridulatory organ views of specimens collected in the field (KN008) representing LAC2. Specimens were collected in 95% ETOH and then relaxed, spread, and dried.

Figure 49. Dorsal, ventral, and frontal images of specimens collected in the field alive (KN008) representing LAC2. Photo courtesy of Piotr Łukasik.

Figure 50. The *T. lacertosa* clades clipped from the maximum likelihood phylogenies (Chapter 2) based on 742 targeted capture exons (left) and (from bycatch) 13 mitochondrial protein coding genes (right) with subclades LAC1 and LAC2. The red box highlights the fact that LAC1 is not a monophyletic group on the mtDNA tree. Black dots represent bootstrap supports >95%; branches with <70% support were collapsed.

***Tettigades limbata* Torres, 1958**
No known synonyms

Torres (1958) original description: [text interpretation from Spanish]
Similar to *Tettigades chilensis*

Holotype:

♂: Body length: 23.5mm; wing length: 27mm; wing width: 11.2mm; wingspan: 65mm.

Paratypes:

♂♂: Body length: 20-25mm; wing length: 24-28mm; wing width: 10-11.7mm; wingspan: 57-67.5mm.

Type material. No major differences between sexes.

Head. Dorsal. Black. Ochre spot in front of the third ocellus. Ventral. Black. Rostrum black and reaches the bases of the posterior coxae.

Thorax. Dorsal. Pronotum black. Pronotal expansions finely filed and reddish. Mesonotum the same color as the pronotum. Lateral-posterior borders with very finely colored reddish brown. Stridulatory areas curved in the form of a club with 16-20 well defined ridges with some superficial and every time more separated towards the apex. Ventral. Black with some light brown spots. Legs generally black. Opercula light chestnut or reddish chestnut and can be present to almost the distal half of this last color. Meracanthus a chestnut ochre, and black at the base.

Abdomen. Tergites and sternites black. The last ones may appear lighter at the posterior border, including the seventh. Pygofer and sternite VIII black.

Body. Covered in a long and dense amount of yellowish chestnut pilosity and dark above the head.

Wings. Hyaline with thick veins. Black in general and finely shaded somewhat more than commonly seen. Precostal area, costal vein, sc+r, base of the cell $1^{a}R_{3}$, vein r-m, two-thirds distal from m, third base of m_{3+4}, last fifth of cu_{1}, cu_{1b} entirely, vein

superior of arculus vein 1_a,2_a, half of superior plectrum red or reddish chestnut. Jugal region white with dotted with red in all of its surface. Half the base of vein m shaded dark chestnut, infused in the basal half of cu_1, originating a spot in the bases of these two veins. Branches of the m vein precisely from its bifurcation, infused. Basal cell stained dark brown almost black in much of its expansion, possibly completely cover it entirely. Cubital anal area, large spot at the base of the veins 2_a and the other in the inferior part of the plectrum, sometimes you can lack this last color. Hindwings with the vannal region stained a smoky gray. Vein 2_a black.

Type Material Examined: used for morphological comparisons

Holotype (♂)

MNNC Specimen Number: MNNC №3280

Type Locality: Los Valdes, Santiago (Elevation 1800m) [Chile]

Date Collected: 17.XII.1947

Torres (1958) Measurements: Body length: 23.5mm, wing length: 27 mm, wing width: 11.2mm, wing span: 65mm

Our Measurements: Body length: 23.4mm, wing length: 27.4mm, wing width: 11.4mm

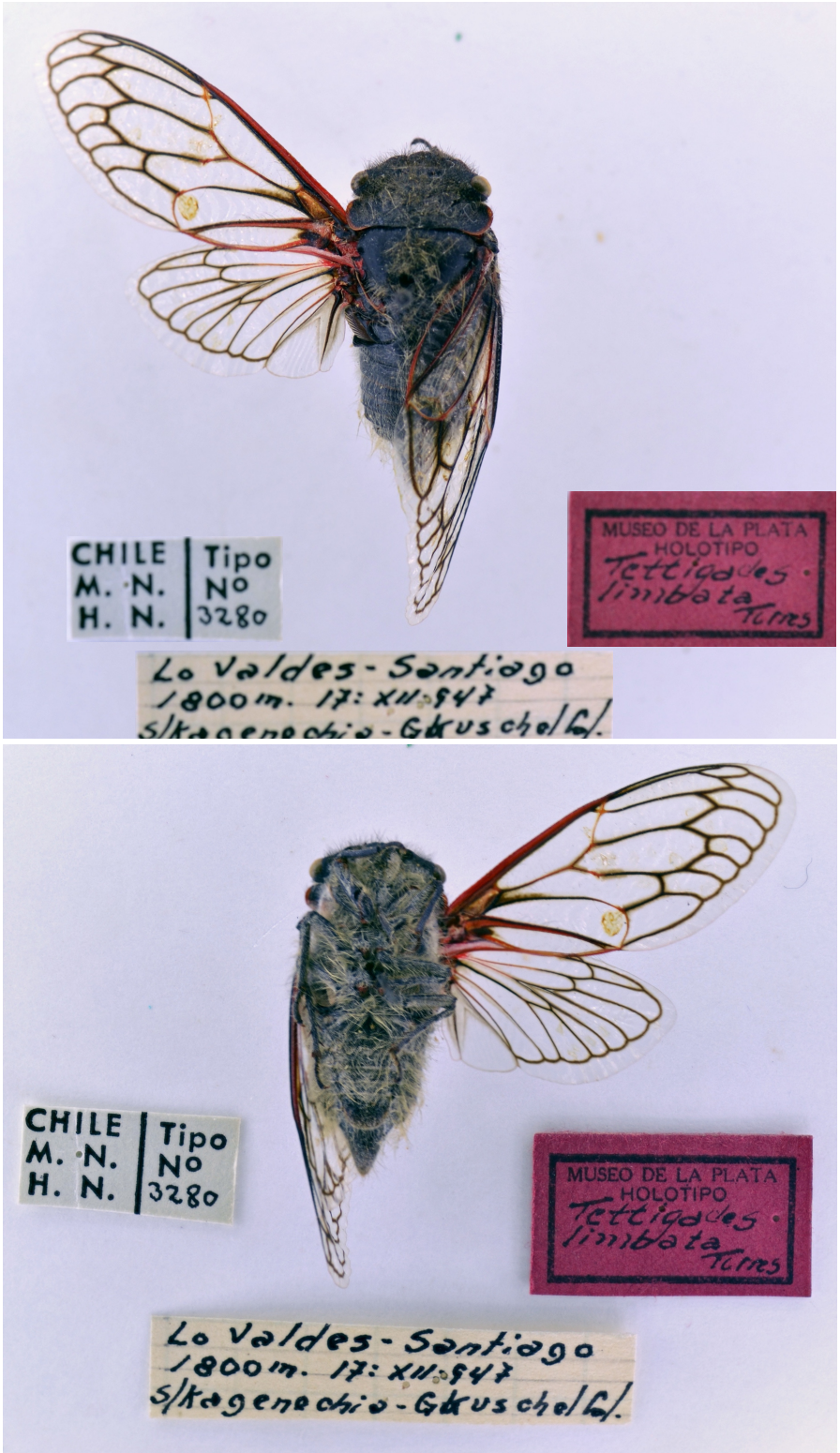

Figure 51. Dorsal and ventral view of the holotype “MNNC № 3280” of *Tettigades limbata* deposited at MNNC (Santiago, CL).

Allotype (♀)

MNNC Specimen Number: MNNC № 3281

Type Locality: Los Valdes, Santiago (Elevation 1800m) [Chile]

Date Collected: 17.XII.1947

Figure 52. Dorsal and ventral view of the allotype "MNNC № 3281" of *Tettigades limbata* deposited at MNNC (Santiago, CL).

Torres (1958) Measurements: Body length:19.3mm, wing length: 25.5mm, wing width: 10mm, wing span: 61mm

Our Measurements: Body length:19.2mm, wing length: 26.3mm, wing width: 11.5mm

Paratypes (2♂)

1. MNNC Specimen Number: MNNC № 3282

   Type Locality: Los Valdes, Santiago (Elevation 1800m) [Chile]

   Date Collected: 17.XII.1947

Figure 53. Dorsal and ventral view of the paratype “MNNC № 3282” of *Tettigades limbata* deposited at MNNC (Santiago, CL).

Torres (1958) Measurements: Body length: 20-25mm, wing length: 24-28mm, wing width: 10-11.7mm, wing span: 57-67.5mm (*Measurement ranges given for both paratypes)*
Our Measurements: Body length:21.4mm. (*Spread forewing missing)*

2. MNNC Specimen Number: MNNC № 3283
Type Locality: Los Valdes, Santiago (Elevation 1800m) [Chile]
Date Collected: 17.XII.1947

Figure 54. Dorsal and ventral view of the paratype “MNNC № 3283” of *Tettigades limbata* deposited at MNHN (Santiago, CL).

Torres (1958) Measurements: Body length: 20-25mm, wing length: 24-28mm, wing width: 10-11.7mm, wing span: 57-67.5mm (*Measurement ranges given for both paratypes)*
Our Measurements: Body length:21.3mm, wing length: 24.5mm, wing width: 9.5mm

Field-Collected Material Examined:

A total of 22 specimens were collected in the field during 2014 and used for morphological characterization. Of those 22, ten specimens were selected for phylogenetic analyses (See Fig. 1 of Chapter 2 in this thesis).

During our phylogenetic analyses, there were two subspecific clades, LIM1 and LIM2, with high bootstrap support on the nuclear phylogeny. In the mitochondrial phylogeny, LIM2 does not form a monophyletic clade. Both clades closely resemble the description of *T. limbata* and only differ in color.

These two clades are distinguishable morphologically and geographically. The color of diagnostic characters such as edges of the pronotal color, wing veins, and wing grooves vary between the two clades with specimens of LIM1 colored red and LIM2 orange. Torres described *Tettigades limbata* markings as red. He did not record any orange specimens. Museum collections examined in this thesis only contain the red morphotype which might explain why Torres did not recognize the color variation (See the phylogenetic analysis later in this species treatment for more information).

The two divergent populations within *T. limbata* correlate with the geographic localities at which these groups are found. LIM1 was found at the northern collecting localities, LIM2 was found at the southern-most part of the range. Locality and specimen information are provided in Table 6.

Table 6. *Tettigades limbata* localities and specimens collected in the field. Specimen numbers with an asterisk (*) were included in phylogenetic analyses. LIM1 and LIM2 labels are subclade identifiers.

Date Collected	Specimen	Divergent Clade	Name	Region	Latitude	Longitude	Elevation	Collector(s)
11.XII.2014	PL342 (♀)*	LIM1	Camino Putaendo-Alicahue 2	Valparaíso	-32.46	-70.70	1307m	CV & PŁ
11.XII.2014	PL344.1 (♂)	LIM1	Camino Putaendo-Alicahue 2	Valparaíso	-32.46	-70.70	1307m	CV & PŁ
11.XII.2014	PL344.2 (♀)	LIM1	Camino Putaendo-Alicahue 2	Valparaíso	-32.46	-70.70	1307m	CV & PŁ
11.XII.2014	PL344.3 (♀)	LIM1	Camino Putaendo-Alicahue 2	Valparaíso	-32.46	-70.70	1307m	CV & PŁ
11.XII.2014	PL345 (♀)	LIM1	Camino Putaendo-Alicahue 2	Valparaíso	-32.46	-70.70	1307m	CV & PŁ
11.XII.2014	PL346.1 (♂)*	LIM1	Camino Putaendo-Alicahue 2	Valparaíso	-32.46	-70.70	1307m	CV & PŁ
11.XII.2014	PL346.2 (♀)*	LIM1	Camino Putaendo-Alicahue 2	Valparaíso	-32.46	-70.70	1307m	CV & PŁ
14.XII.2014	PL415 (♂)*	LIM2	Bellavista 1	O'Higgins	-34.83	-70.74	1445m	CV & PŁ
14.XII.2014	PL416 (♂)	LIM2	Bellavista 1	O'Higgins	-34.83	-70.74	1445m	CV & PŁ
14.XII.2014	PL418 (♀)	LIM2	Bellavista 1	O'Higgins	-34.83	-70.74	1445m	CV & PŁ
14.XII.2014	PL419 (♀)	LIM2	Bellavista 1	O'Higgins	-34.83	-70.74	1445m	CV & PŁ
14.XII.2014	PL426.1 (♂)*	LIM2	Bellavista 1	O'Higgins	-34.83	-70.74	1445m	CV & PŁ
14.XII.2014	PL426.2 (♂)*	LIM2	Bellavista 1	O'Higgins	-34.83	-70.74	1445m	CV & PŁ
14.XII.2014	PL426.3 (♂)	LIM2	Bellavista 1	O'Higgins	-34.83	-70.74	1445m	CV & PŁ
14.XII.2014	PL428.2 (♀)	LIM2	Termas del Flaco	O'Higgins	-34.96	-70.44	1763m	CV & PŁ
14.XII.2014	PL429.1 (♀)*	LIM2	Termas del Flaco	O'Higgins	-34.96	-70.44	1763m	CV & PŁ
14.XII.2014	PL429.2 (♂)*	LIM2	Termas del Flaco	O'Higgins	-34.96	-70.44	1763m	CV & PŁ
14.XII.2014	PL459 (♀)	LIM2	Termas del Flaco	O'Higgins	-34.96	-70.44	1763m	CV & PŁ
14.XII.2014	PL506 (♀)*	LIM2	Termas del Flaco	O'Higgins	-34.96	-70.44	1763m	CV & PŁ
14.XII.2014	PL507.1 (♂)	LIM2	Termas del Flaco	O'Higgins	-34.96	-70.44	1763m	CV & PŁ
14.XII.2014	PL507.5 (♀)	LIM2	Termas del Flaco	O'Higgins	-34.96	-70.44	1763m	CV & PŁ
14.XII.2014	PL508.1 (♀)*	LIM2	Termas del Flaco	O'Higgins	-34.96	-70.44	1763m	CV & PŁ

Distribution: Chile

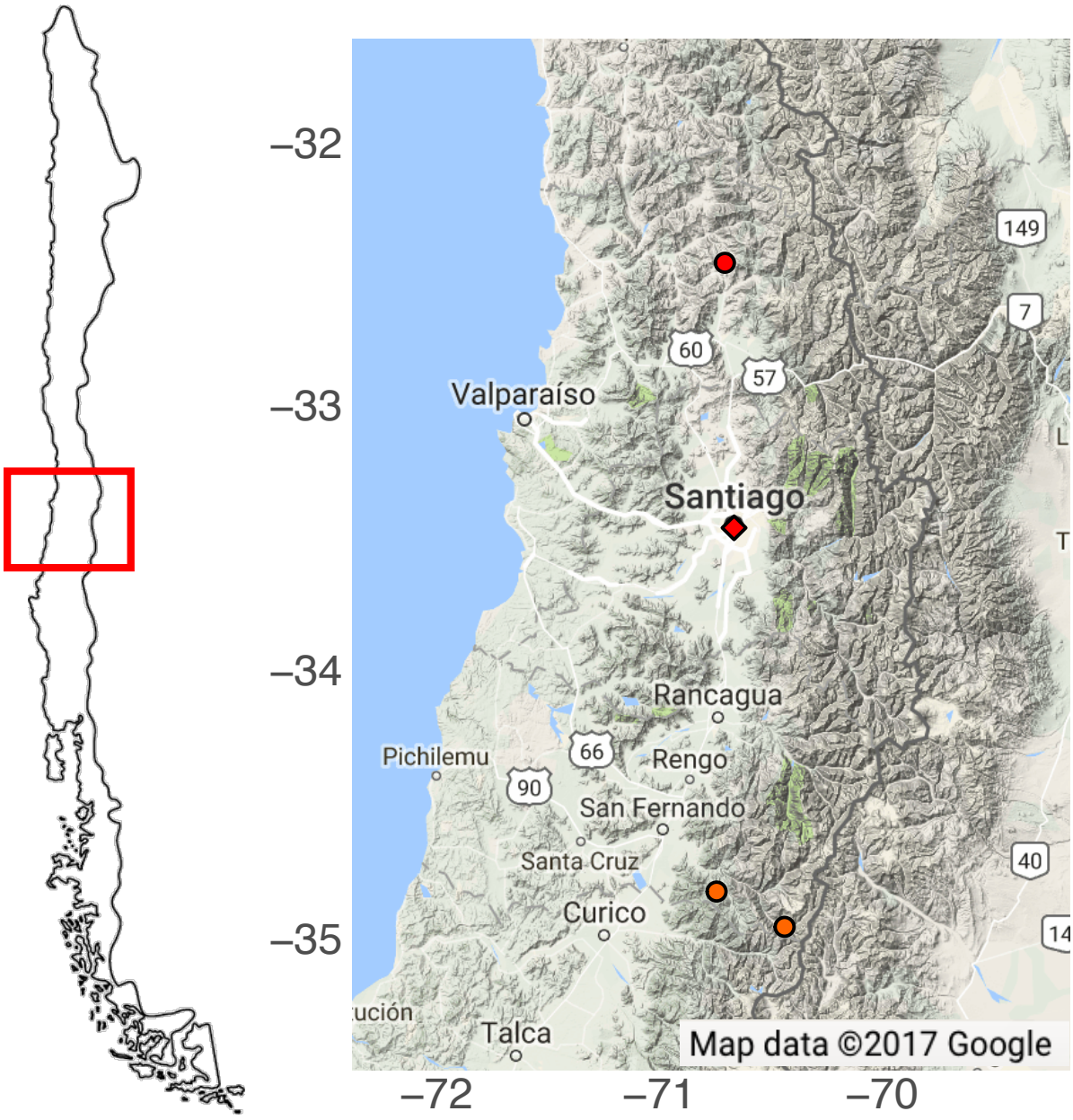

Figure 55. Map of localities for *Tettigades limbata*. Experimental/Field-collected cicada clades are identified on the map with circles. Type localities are identified on the map with a diamond. The colors of the shapes indicate distinct clades: LIM1(red) and LIM2 (orange). The outline map of Chile is provided by WorldAtlas. Map of Chile reproduced from World Atlas (http://www.worldatlas.com/).

Additional and Updated Distinguishing Features:

Thorax. Specimens have been collected that closely match the description by Torres for *Tettigades limbata* (other than the above-mentioned color variant). Field-collected specimens have been found to have either red or orange color (not both) color in the pronotal expansions, lateral-posterior borders of the mesonotum, opercula, and meracanthus. The wing grooves and coxae can also be red or orange but were not mentioned by Torres as a character. Even in high concentrations of ethanol these colors do not change.

The color of the cruciform elevation, or sometimes described by Torres as the disk of the scutellum, is used for other of his species descriptions but not for *Tettigades limbata*. However, its overall black color can be used to distinguish this species from others. The anterior branches of the cruciform are also all black.

The stridulatory organ is club-shaped and does not differ between the two clades.

Wings. Wing veins either orange (LIM2) or red (LIM1) at the base and black at the apex. The jugal fold is white with red (LIM1) or orange (LIM2) at the base.

Body. Hair density may appear to be less depending on preservation. Although *Tettigades limbata may* have a moderately dense amount of hair on the body and ventral head area, it is not as dense as species such *Tettigades auropilosa* and *Tettigades lacertosa*.

Genitalia (Figs. 56 and 57). Eighth sternite and tergite black. Dorsal beak sharply pointed and black. Pygofer all black. Median lobe of uncus black on outside. Pseudoparamere light caramel color with the presence of dark tooth-like structures

visible on the lateral view along the edges of the pesudoparamere. More tooth-like structures on the outer perimeter of the pseudoparamere are visible in frontal view. Endotheca curves tightly back towards the specimen and out. The color of the endotheca is lighter than the pseudoparamere and theca.

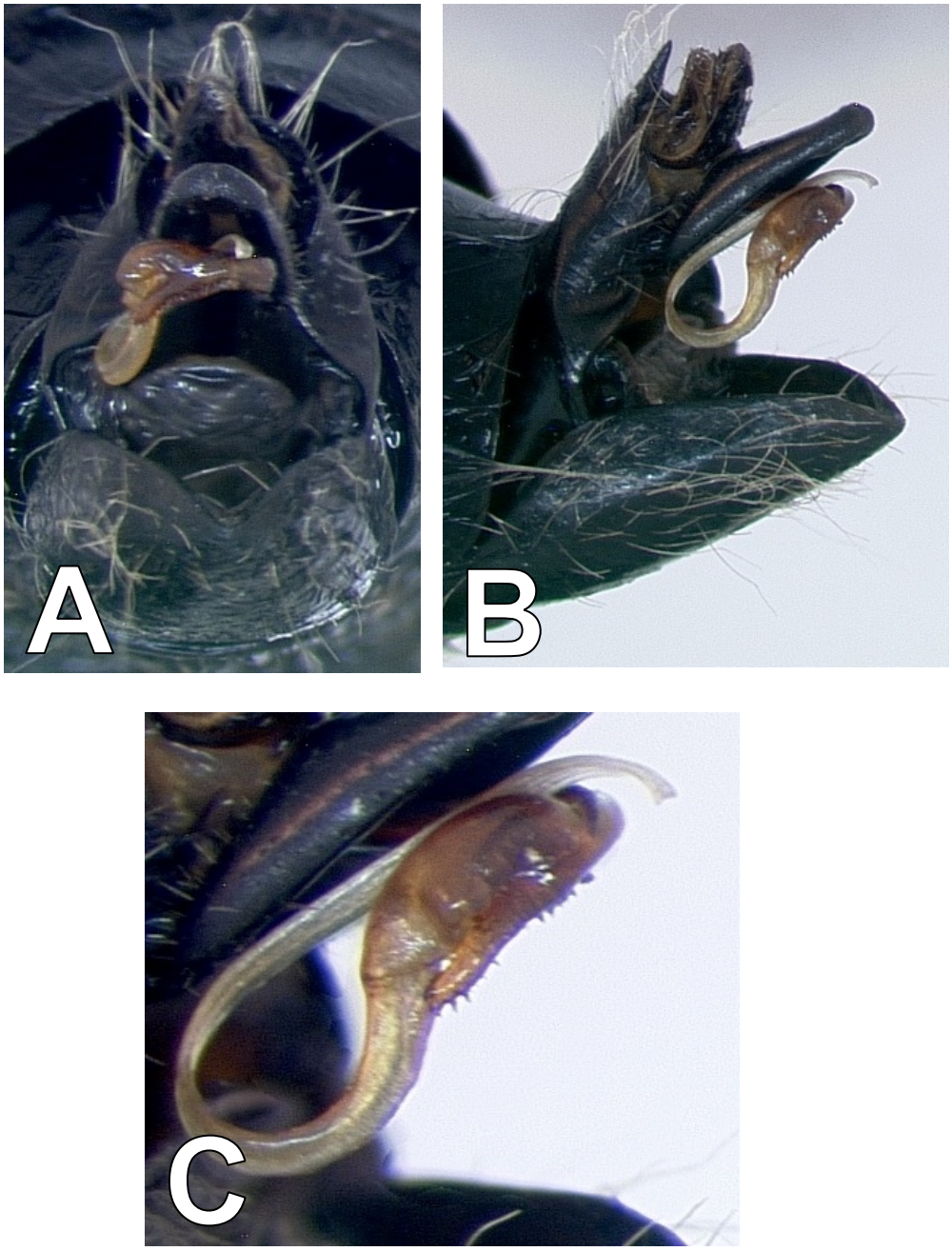

Figure 56. Male genitalia of *Tettigades limbata*: (A) ventral, (B) lateral, and (C) lateral views of PL426.2, *Tettigades limbata* LIM2. Images (A) and (B) are at 2x magnification. Image (C) is at 4x magnification.

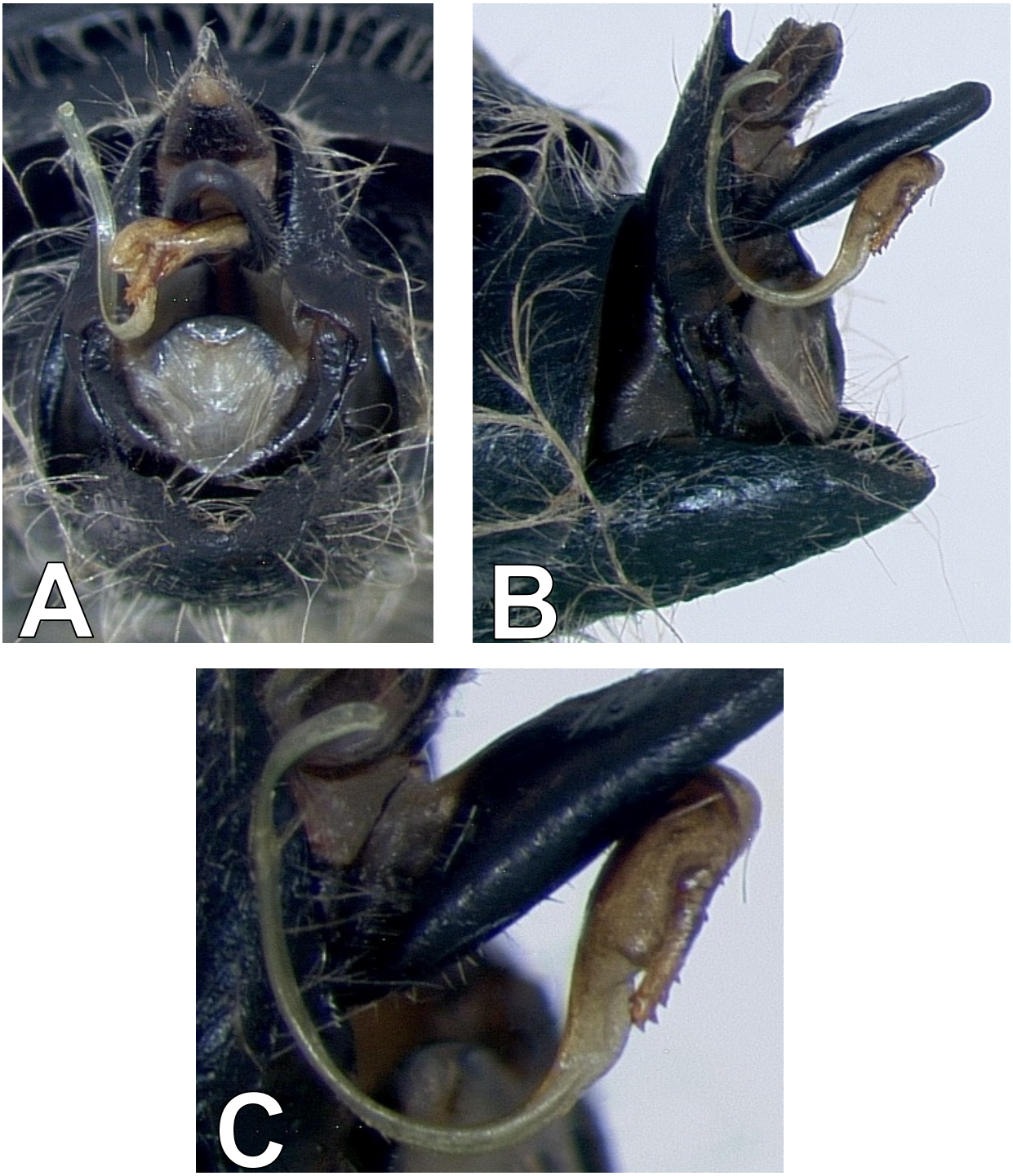

Figure 57. Male genitalia of *Tettigades limbata*: (A) ventral, (B) lateral, and (C) lateral views of PL429.2, *Tettigades limbata* LIM2. Images (A) and (B) are at 2x magnification. Image (C) is at 4x magnification.

Diagnosis:

This species is morphologically similar to *Tettigades chilensis* and *Tettigades auropilosa*. It differs in the following aspects (Fig. 58):

1. In *T. limbata*, the pronotal collar is either red or orange on lateral and posterior edges. The lateral and posterior edges of *T. chilensis* and *T. auropilosa* are all black. In some cases, *T. chilensis* may have a thin red border. However, the red extends along the entire posterior border of the pronotal collar, distinct from *T. limbata.*
2. Spacing of the ridges of the stridulatory organ become narrower towards wing base in the form of a club in *T. limbata*, while evenly spaced and parallel in *T. chilensis,* and a stretched s-shape and well-separated ridges in *T. auropilosa.*
3. The jugal fold of the hindwing is white in *T. limbata* with some orange or red at the base. The jugal fold of *T. chilensis* is a pearly gray with some cases of red or orange at the base. The jugal fold of *T. auropilosa* is a soft orange with white at base.
4. The hair of the body is moderately dense in *T. limbata* and *T. chilensis* but extremely dense in *T. auropilosa*.
5. The ends anterior branches of the cruciform elevation are always black in *T. limbata.* In *T. auropilosa* and *T. chilensis*, the anterior branches of the cruciform elevation may have red or orange spots in some cases.

6. The tergites of *T. limbata* and *T. auropilosa* are all black. The tergites of *T. chilensis* may have fine red or orange stripes in some cases.

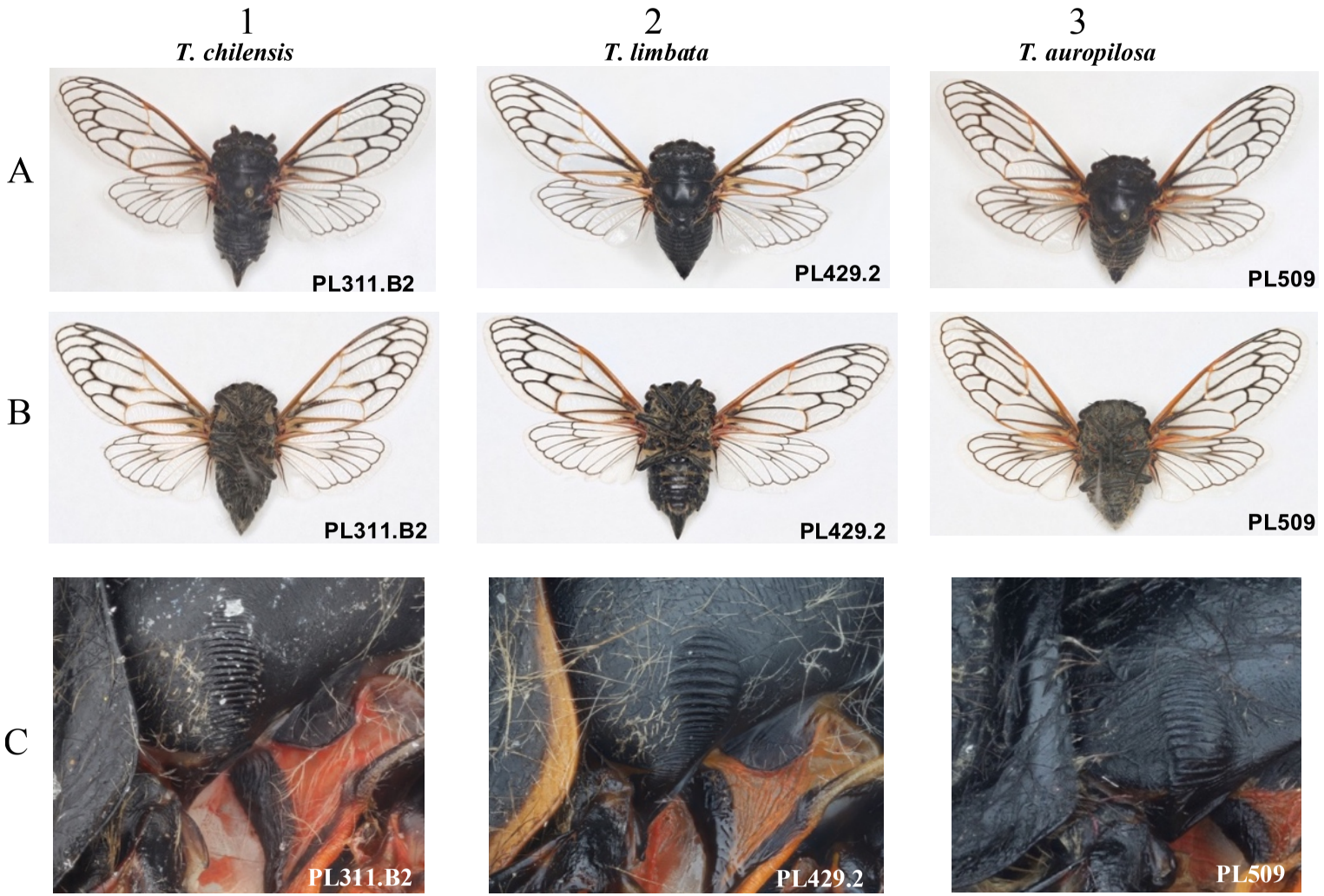

Figure 58. Dorsal, ventral, and stridulatory organ views of *T. limbata*, *T. chilensis*, and *T. auropilosa*. *Columns:* (1) *T. chilensis* PL311.B2, (2) *T. limbata* PL429.2, and (3) *T. auropilosa PL509; Row*s:(A1-3) Dorsal views, (B1-B3) ventral views, and (C1-C3) stridulatory organs. Specimens were collected in 95% [or 70%] ETOH and then relaxed, spread, and dried.

Phylogenetic Assessment:

The clade, *T. limbata,* consists of ten specimens all closely matching the description of one species, *Tettigades limbata*. Within the clade representing *Tettigades limbata*, the nuclear phylogeny resolves 2 divergent populations, LIM1 and LIM2, with high bootstrap support. However, in the mitochondrial phylogeny LIM2 forms a polytomy not a monophyletic clade. These two divergent clades are supported by morphological characters and by geographic localities. Locality and specimen information are provided in Table 6.

LIM1 (Figs. 59 and 60) was in the northern regions of Valparaíso and Metropolitan(Santiago) and presents a red color pattern. LIM1 was represented at only one of our field collected localities. LIM2 (Figs. 61, 62, and 63) was found south of Santiago in the region of O'Higgins. Specimens of this population can be distinguished by their orange color pattern. Specimens belonging to LIM2 were collected at two different localities separated by about 31km. Members of the two clades have not been found together at the same locality (i.e., they are geographically structured) but can be found in the same year. The color can also be seen in other characters including the pronotal expansions, lateral-posterior borders of the mesonotum, opercula, meracanthus, wing grooves, and coxae. The combined range of both populations is about 280km.

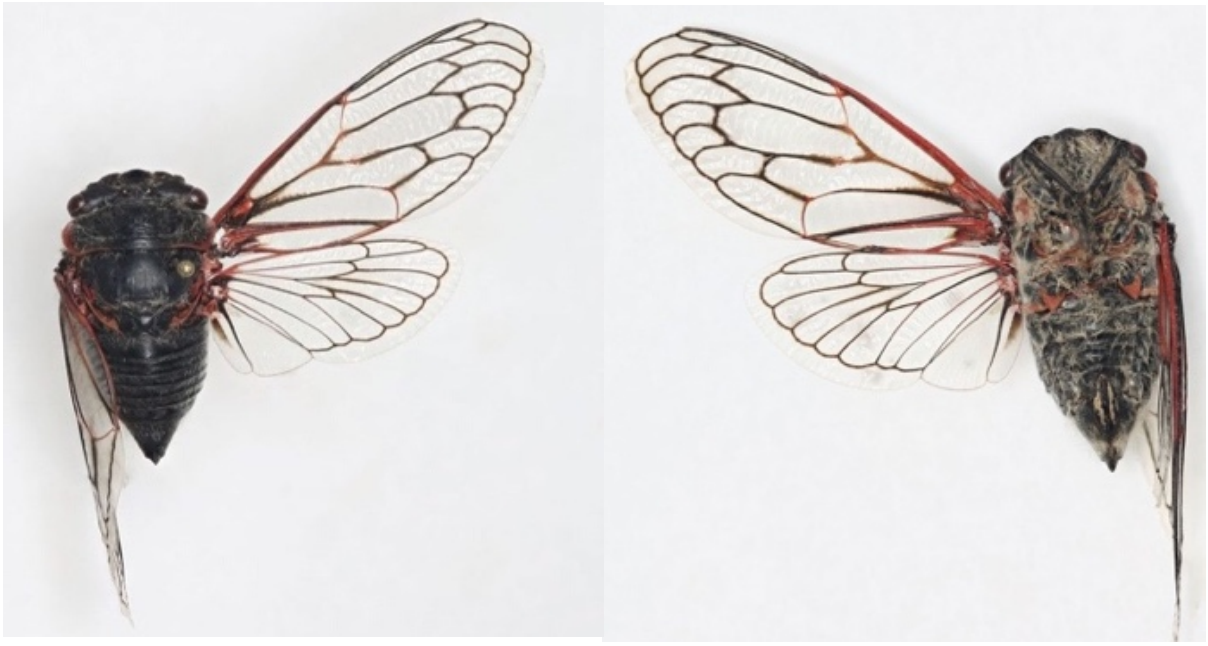

Figure 59. Field-collected specimen of *T. limbata* (PL346.2) photos taken once spread, representing LIM1. The specimen was collected in 70% ETOH and then relaxed, spread, and dried.

Figure 60. Field-collected specimen of *T. limbata* (PL346.2) photos taken alive, representing LIM1. Photo courtesy of Piotr Łukasik.

Figure 61. Field-collected specimen (PL429.2) photos taken once spread, representing LIM2. The specimen was collected in 70% ETOH and then relaxed, spread, and dried.

Figure 62. Field-collected specimen (PL429.2) photos taken alive, representing LIM2. Photo courtesy of Piotr Łukasik.

Figure 63. LIM2 field collected specimen photo taken alive from Termas del Flaco. Photo courtesy of Piotr Łukasik.

Tettigades auropilosa and *Tettigades chilensis*, although morphologically similar have distinct characters that can be used to delimit and support each of the clades. *T. limbata* exhibits little within-species variation compared to species in its sister clade such as *T. chilensis*. All areas that are colored such as the edges of the pronotal collar and the wing groove can vary between red and orange. This variation in color was not recognized by the original describer, Belindo A. Torres. Since Torres primarily worked with museum specimens, this might have led to his missing variation in his descriptions.

Tettigades crassa:

A character that can be used to support the node delimiting *T. limbata* on the phylogeny and specimens in the field is the distinct coloration of the lateral - posterior edges of the pronotal collar, which can be either red or orange. It is the only species in this study with that color pattern on the pronotal collar. Although *Tettigades crassa*

presents a color similar to this, the holotype for *T. crassa* has lost its color which might have affected Torres's description of the species (Fig. 64). Also, molecular evidence has not been gathered to indicate the validity of this species.

Figure 64. Dorsal (top) and ventral (bottom) view of the holotype “MNNC № 3274” for Tettigades crassa deposited at MNNC (Santiago, Chile).

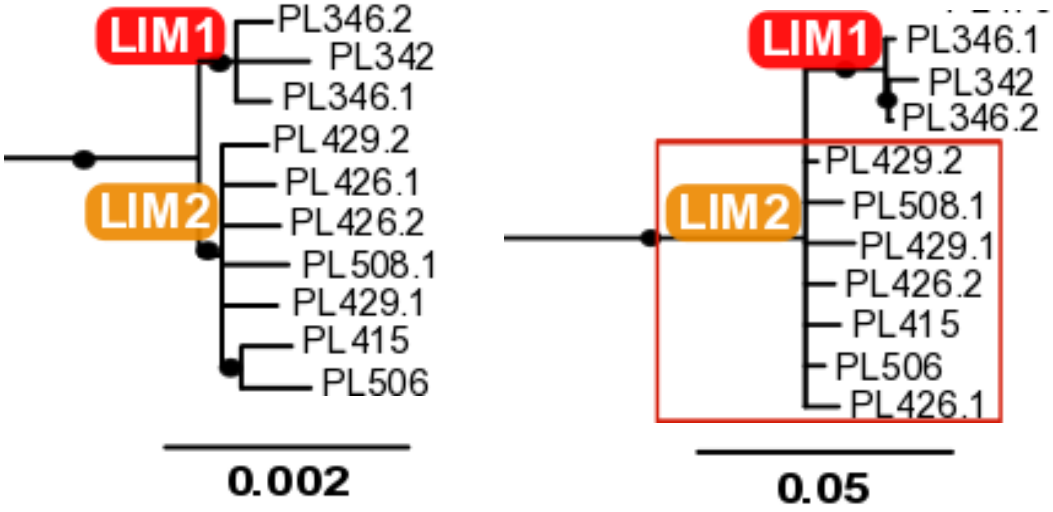

Figure 65. The *T. limbata* subclades clipped from the maximum likelihood phylogenies (Chapter 2) based on 742 targeted-capture exons (left) and (from bycatch) 13 mitochondrial protein coding genes (right) with subclades LIM1 and LIM2. The red box highlights the fact that LIM2 is not a monophyletic clade in the mtDNA tree. Black dots represent bootstrap supports >95%; branches with <70% support were collapsed

***Tettigades opaca* Jacobi, 1907**
Synonyms: *T. porteri*

Jacobi (1907) original description of *T. opaca*: [text interpreted from German]

Top pitch black, covered with yellow-brown velvety hairs; pronotal disc, on both sides, beneath the paramedian fissure, a dull chestnut-brown spot; tip of anterior arms of cruciform elevation yellowish. Venter covered with greyish hairs; rostrum and legs reddish-brown. Fore and hind wings fumigated brown, veins in basal half pale ocher-yellow, with the exception of the base of the clavus, the cubitus, and of (a line in the) jugum of the hind wing, which are blackish-brown; apical half of the veins like the latter; transverse folds of the fore wings very pronounced, and hyaline in contrast to the cloudiness of the latter.

Head width one seventh larger than the base of the mesonotum; pronotal disc evenly rounded laterally. Pronotal flanges very wide and sharp edges. Rostrum slightly surpassing the middle coxae. Subgenital plate elongated, more than 1 1/2 times as long as wide.

♂ Body length 15.5mm; Wing span 43mm.

Habitat - Chile (Mus. Wien: Coll. Signoret).

In view of the great variation of *T. chilensis* just pointed out, I have been concerned to describe the above species as a new species, because it closely resembles the abovementioned species; but the dark color of the wings has not been observed in any formerly known species, also the long subgenital plate forms a distinctive structural feature.

Figure 66. Drawing of *T. opaca* illustrated in Jacobi (1907).

Torres (1958) re-description of *T. opaca*: [text interpretation from Spanish]

Measurements.

♂: Body length: 15.5mm; wingspan 42mm.
♀: Body length: 16.5mm; wing length: 18.5mm; wing width: 7mm; wingspan: 44mm

Allotype **♀.** Dorsal. Reddish-dun. Anterior margins of the vertex and the other side of the postclypeus, slightly lightened. Border of the vertex surrounding the postclypeus and a spot in front of the third ocellus chestnut ochre. Ventral. Reddish-chestnut. Anticlypeus slightly darker. Rostrum dun, more intense at its lateral margins and passing the intermediate coxae. Grove of the postcyleus wide, something superficial at its superior and deep in the inferior.

Thorax. Dorsal. The pronotum is a dark reddish chestnut. A spot in the oblique groove a clear dun. Anterior margin of the same, beige ochre at its extreme. Mesonotum dark intense dun. Lateral and posterior lateral margins of the same and disk of the scutellum slightly clearer. A spot chestnut ochre in the extremes of each side of the anterior branches of the scutellum. Two spots linear curved, very separated and just

insinuated, in the part of the discal base of the mesonotum. Stridulatory areas with 40 ridges approximately. Ventral. Legs reddish-chestnut. Tibias of the midleg lightly clear in the superior half of its anterior face, as well as the tibias of the midleg in all of its extension; tarsi the general color of the legs. Opercula and meracanthus chestnut ochre in its distal half.

Wing. Forewing and hindwing faintly smoky, with veins a beige ochre in general. Chestnut on the transverse, anastomosis, and cu_1 of the forewing. A dark dun spot on half of the plectrum. An angular spot in the vannal region and spots of the jugal region of the hindwing a smoky white, vein 2a, chestnut ochre. Between the veins r-m and cu_{1a} and contrasting with the smokiness of the membrane appears a spot linear hyaline extending the along the abovementioned veins.

Abdomen. Abdomen with the tergites and sternites reddish-dun. Posterior border of the last and anterior and ventral basal of pygofer very slightly clear. Body covered in a pilosity of dark yellow.

Type Material Examined: used for morphological comparisons

Holotype (♂)

NHMW Specimen Number: Specimen does not have a museum number at this time.
Type Locality: San Bernando, Chile
Date collected: Dec. 1935

Figure 67. Dorsal (top) and ventral (bottom) view of the holotype of *Tettigades opaca* deposited at NHMW (Vienna, Austria). Image courtesy of NHMW. The red arrow points at the M_{3+4} vein, a diagnostic character Torres (1958) uses to distinguish this species from *T. compacta.*

Allotype (♀)

MACN Specimen Number: This specimen does not have a museum number at this time.
Type Locality: Lonquimay/Curacautin
Date collected: I.1917

Notes: Torres (1958) accepted the synonomy of *T. porteri* with *T. opaca*, and designated the holotype of *T. porteri* as the allotype of T. opaca.

Figure 68. Dorsal and ventral view of the allotype of *Tettigades opaca* deposited at MACN (Buenos Aires, AR).

Field-Collected Material Examined:

A total of 6 specimens were collected in the field in 2015 and were used for morphological characterization. Due to the high similarity to *Tettigades compacta* (see below), all specimens collected for this group were selected for phylogenetic analyses (See Fig. 1 of Chapter 2 in this thesis).

During our phylogenetic analyses, both the nuclear and mitochondrial phylogenies indicate with high support, two divergent populations, OPA1 and OPA2, which closely resemble the description of *T. opaca*. No morphological characters have been found to distinguish either clades. See *Phylogenetic Analysis*.

The two divergent populations within *T. opaca* are correlated with the geographic localities in which these groups are found. Locality and specimen information are provided in Table 7.

Table 7. *Tettigades opaca* localities and specimens collected in the field. Specimen numbers with an asterisk (*) were included in phylogenetic analyses. OPA1 and OPA2 labels are subclade identifiers.

Date Collected	Specimen	Divergent Clade	Name	Region	Latitude	Longitude	Elevation	Collector(s)
14.XI.2015	PL705 (♂)*	OPA1	Cuesta Andacollo A	Coquimbo	-30.20	-71.09	792m	CV
14.XI.2015	PL702.1 (♂)*	OPA1	Salida sur Ovalle	Coquimbo	-30.66	-71.26	20m	CV
14.XI.2015	PL702.2 (♂)*	OPA1	Salida sur Ovalle	Coquimbo	-30.66	-71.26	20m	CV
15.XI.2015	PL707.2 (♂)*	OPA2	Rio Choapa A	Coquimbo	-31.81	-70.93	589m	CV
15.XI.2015	PL698 (♂)*	OPA2	Rio Choapa B	Coquimbo	-31.74	-71.17	314m	CV
12.XII.2015	PL667 (♂)*	OPA2	Tahuilco	Coquimbo	-31.79	-71.08	581m	CV & PŁ

Distribution: Chile

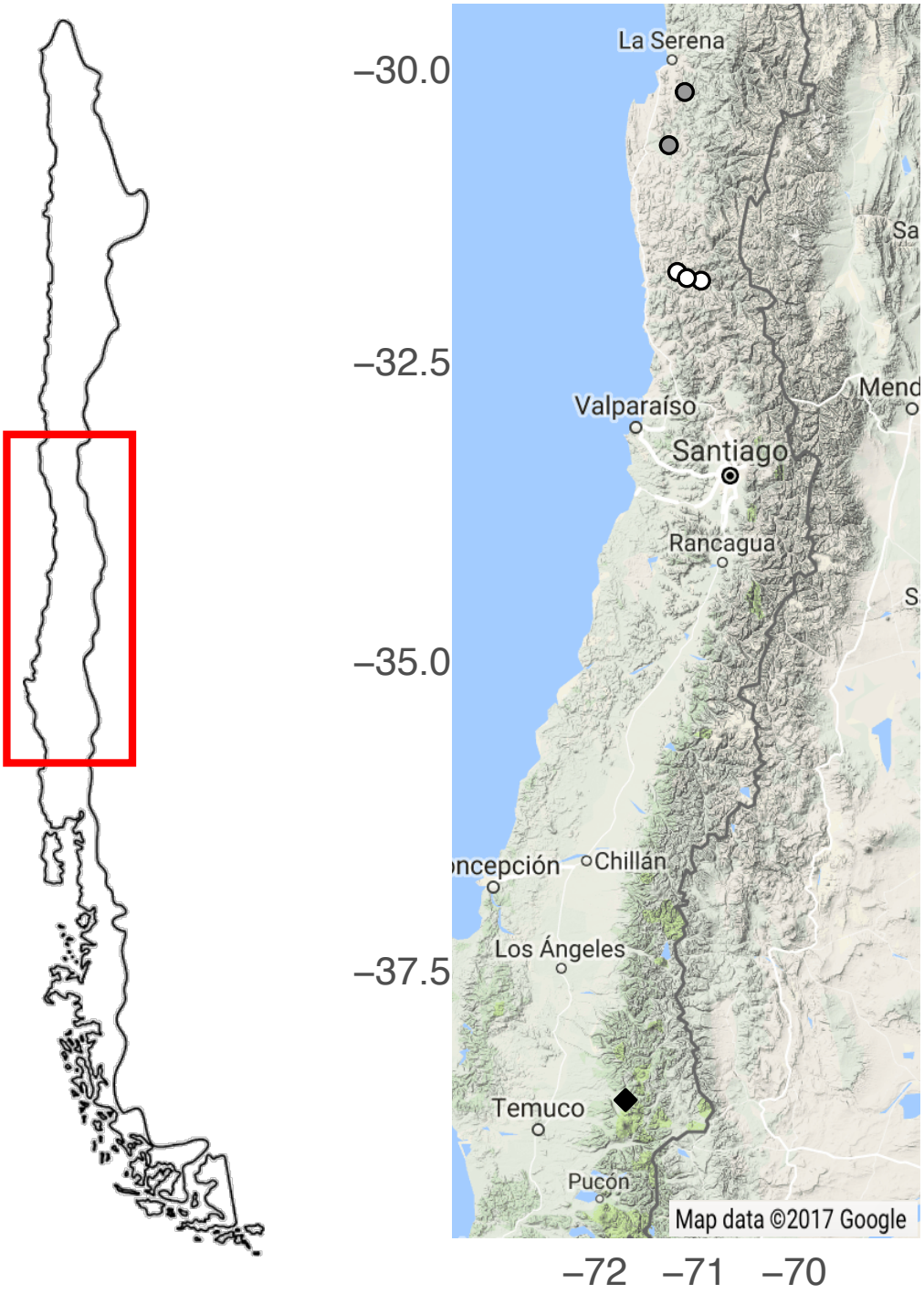

Figure 69. Map of localities for *Tettigades opaca*. Experimental/Field-collected cicada clades are identified on the map with circles. Type localities are identified on the map with a diamond. The colors of the shapes indicate distinct clades: OPA1(light gray) and OPA2 (white). The outline map of Chile is provided by WorldAtlas.

Additional and Updated Distinguishing Features:

General. The small body size is a distinguishing feature of this species (and *T. compacta*, see diagnosis). The original description by Jacobi and Torres' re-description both describe *T. opaca* has having a reddish-brown color. However, this coloration might have been due to the preservation of the specimen. After examining older specimens of different species such as those of the genus *Alarcta*, they present this similar body color. However, recently collected specimens of the same species are generally black.

Thorax. As with other species of *Tettigades*, spots at the ends of the anterior branches of the cruciform elevation may not be present. The legs are black.

Wings. Vein near the base such as described by Jacobi are yellowish. Torres has described these veins as presenting a beige color. However, after examining museum specimens of this species and *T. major*, beige would be a misleading character state.

Genitalia (Fig. 70). The eighth sternite and tergite are black. The dorsal beak is sharply pointed and black. The pygofer is all black. The median lobe of uncus is black on the outside, facing the anal lobe. The pseudoparamere is darker than rest of aedeagus and only the distal tip is visible in the left view. The pseudoparamere is small compared to other species, with tooth-like structures visible on the edges when viewing the frontal or right view. Endotheca curves tightly around twice, distinctly from other species of the genus.

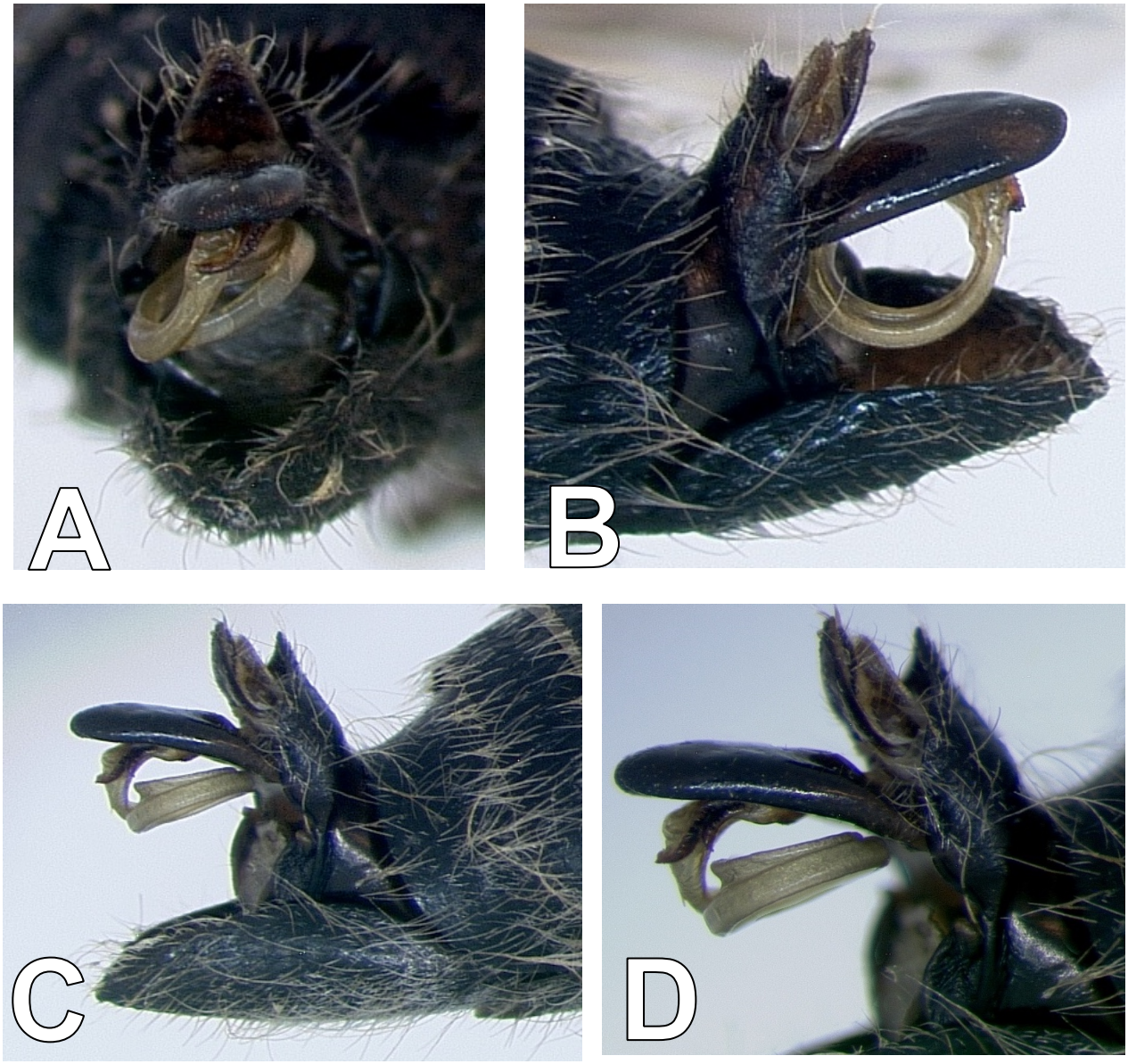

Figure 70. Male genitalia of *Tettigades opaca*: (A) ventral, (B) lateral, and (C/D) lateral views of PL667, *Tettigades opaca*. Images (A), (B), and (C) are at 2x magnification. Image (D) is at 4x magnification.

Diagnosis:
Tettigades compacta

Tettigades opaca is morphologically similar to *Tettigades compacta* (Fig. 71). These two species are distinctive in being the smallest species of Chilean *Tettigades* with wing spans between 41 and 48.5 mm. Both species have cloudy wings. We have been able to examine images the holotype for *T. opaca* as well as measured and examined the allotype for both species. Due to the variation exhibited in other species of *Tettigades* and the high similarity to *T. compacta,* molecular evidence needs to be gathered in the future validate this species and its relationship with *T. opaca.* Therefore, a complete diagnosis comparing these two species will not be provided. Torres (1958) also did not provide a diagnosis. However, in his species key, he separated both species by the shape of the M_{3+4} vein. In *T. opaca*, the vein is straight and in *T. compacta*, it is broken. Dorsal and ventral images of the holotype of *T. compacta* are also provided. A red arrow has been added to images of the holotypes to indicate the shape of the M_{3+4} vein.

Figure 71. Dorsal (top) and ventral (bottom) view of the allotype “MNNC № 3277” for *Tettigades compacta* deposited at MNNC (Santiago, Chile).

Figure 72. Dorsal and ventral view of “NHMUK № 010220843 “of *Tettigades opaca* deposited at NHMUK (London, UK). Photo courtesy of NHMUK. The red arrow points at the M_{3+4} vein, a diagnostic character Torres (1958) uses to distinguish this species from *T. opaca.*

Phylogenetic Assessment:

The clade, *T. opaca,* consists of 6 specimens all closely matching the description of one species, *Tettigades opaca*. Within the *T. opaca* clade (Fig. 75), there are two subspecific clades with high bootstrap support, OPA1 and OPA2, which cannot be distinguished morphologically. There appears to be a pattern correlating with geography; however, more sampling is required.

In some cases, sampling was limited to one specimen per locality therefore, variation might have been missed. The clades within *T. opaca* appear to correlate with the geographic localities at which these groups are found. Locality and specimen information are provided in Table 7.

OPA1 was found at the northern most part of the range where we collected *T. opaca* specimens, within 54km. OPA2 have been gathered within a range of about 23km. Members of the two clades have not been found together at the same locality (i.e., they are geographically structured) but can be found in the same year. In 2015, due to El Niño *Tettigades* emergences were low in abundance. This may explain our low sample sizes at the type locality.

Figure 73. Dorsal and ventral view of field collected specimen (PL667) when alive, representing OPA2. Photo courtesy of Piotr Łukasik.

Fi**gure 74.** Dorsal and ventral view of field-collected specimen (PL667) once pinned, representing OPA2. The specimen was collected in 70% ETOH and then relaxed, spread, and dried.

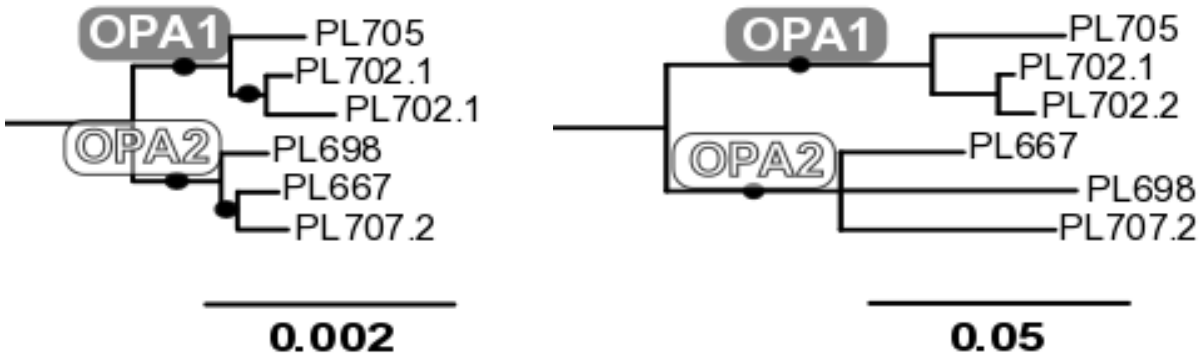

Figure 75. The *T. opaca* subclade clipped from the maximum likelihood phylogenies (Chapter 2) based on 742 targeted-capture exons (left) and, from bycatch, 13 mitochondrial protein coding genes (right) with subclades OPA1 and OPA2. Black dots represent bootstrap supports >95%; branches with <70% support were collapsed.

***Tettigades ulnaria* Distant, 1906**
Synonyms: *T. urnaria* (sic)

Distant (1906) original description of *T. ulnaria*:

> **"**Body black, brownly pilose; margins of pronotum, lateral and posterior margins of mesonotum, cruciform elevation, rostrum, and legs, ochraceous; a central fascia to base of cruciform elevation and centres of its anterior angles, anterior tibiae and tarsi, streaks to anterior femora, bases and apices of intermediate and posterior tibiae, and the tarsi, black; disk of abdomen beneath ochraceous, and in male with a central black longitudinal fascia; tegmina and wings hyaline, talc-like, transversely wrinkled, both slightly sanguineous at base; tegmina with the venation black, the costal membrane, basal cell, the ulnar veins here and there, and the claval suture, ochraceous; in some specimens the apical veins are also more or less suffused with ochraceous; wings with the venation black, more or less ochraceous at the base; tegmina elongate, about three times as long as greatest breadth, the ulnar areas long and narrow, parallel, the first, second, and third about equal in length. Long. Excl. tegm., ♂ and ♀, 19 to 22 mill.; exp. tegm. 55 to 70 mill."

Torres (1958) description of *T. ulnaria*: [text interpretation from Spanish]

Measurements.

Body length: 18.5-23mm; wing length: 25-32mm; wing width: 9.2-11.5mm; wingspan: 59-75mm.

Head. Dorsal. Black. Anterior margins of the vertex half to the eyes and continued in a dorsal spot, margins of the same contiguous to the postclypeus, a spot in front of the third ocellus, two vertex dips located at the same height of the third ocellus and a small spot behind each eye, ochre brown. Ventral. Black. Anteclypeus ochre on its distal portion. Rostrum brown with the last segment intense dark brown and passes the apex of the intermediate coxae.

Thorax. Dorsal. Pronotum black. Anterior, posterior and lateral borders of the pronotum brown. Disk of the pronotum with brown punctuated spots that can increase in size until occupying great part of it or disappear altogether. Cruciform elevation, previous branches and lateral areas of the latter and a spot in front of each previous branch, brown or ochre brown. Cruciform elevation color black with anterior branches

the same color in its middle part. Stridulatory areas with profound ridges and scaled in number between 9-11, well defined, separated, followed by other fine superficial and very together completing up to 13-18 ridges. Ventral. Ochre. Black legs with ochre brown. The first tibias, thick stripes on the first femurs, fine stripes on second and third femurs, extremes of the tibias and all other tarsi intense dark brown. Opercula and meracanthus ochre, stained with black.

Wings. Wings hyaline with the veins a dark brown in general. Precostal area, costal vein, base of the I^aR_3, vein r-m, a spot a little after the origin of m_{3+4}, final portion of cu_1, cu_{1b} almost all of its extension, superior half of the basal cell and the posterior tuberosity and vein Ia, light brown. Inferior half of the basal cell and posterior tuberosity intense dark brown or black, area cubital-anal softly infused; jugal region reddish. Males with the seventh apical area three times as long as the sixth, in females only two and a half; males with the first apical area much shorter than the second, in females this difference is less. Hindwings with an angular spot in the vannal region, spot in the jugal region reddish and vein 2a black.

Abdomen. Tergites black; sternites ochre, sometimes demonstrating black spots in females, in males presenting a median longitudinal strip of this last color in which extends up to the eight sternites. Body especially the ventral part covered in a pilosity of white or yellow white, dark pilosity on the top of the head.

Variation. The cited species offers a series of variations which can be summarized as follows: behind the third ocellus may appear a small ocher spot and the vertex dips appear black. The postclypeus in its ventral part and the anteclypeus. The lateral margins of the pronotum may appear black in the middle part of the expansions.

The scutellar disc can show only the origin of the black stripe as well as the previous branches to be stained black in its minimal extension, or to appear branches and disc of ocher and an equal color stain in the stridulating area. The vein sc+r can be irregularly brown, the basal cell almost entirely ochre and the area cubital anal with a dun ochre hue. The angular stain of the vannal region of the hindwing can present in a major or minor scale a pearly gray background on which appears reddish. The central black stripe of the abdomen in the males may sometimes appear with their boundaries not very well defined, or the sternites, including the eight appear entirely brown ochre. The seventh and eighth tergites in males as well as females may present the posterior margin stained of a light brown, just like the border of the pygofer in males.

Type Material Examined: Used for morphological comparisons.

Holotype (♂):
NHMUK Specimen Number: NHMUK № 010220838
Type Locality: Chili [Chile]
Date Collection: Not provided

Figure 76. Dorsal (top) and ventral (bottom) view of the holotype for *Tettigades ulnaria* "NHMUK № 010220838" deposited at NHMUK (London, UK). Image courtesy of NHMUK.

Distant (1906) Measurements: Body length: 19-22mm, wing span: 55-70mm (*Distant gave ranges of a male and female specimen*)
Torres (1958) Measurements: Body length: 18.5-23mm, wing length: 25-32mm, wing width: 9.2-11.5mm, wing span: 59-75mm (*Torres gave ranges of all the material examined*)

Field-Collected Material Examined:

A total of 39 specimens were collected in the field between 2014 and 2016 and were used for morphological characterization. Of those 39, 31 were selected for phylogenetic analyses (See Fig. 1 of Chapter 2 in this thesis).

During our phylogenetic analyses, four subspecific clades ULN1, ULN2, ULN3, and ULN4 were well supported on the mitochondrial phylogeny, with the first three clades having high support. In the nuclear phylogeny, ULN3 and ULN4, do not form distinct monophyletic clades. There are no morphological characters supporting these subclades. Any variation is not exclusive to any subclade.

The divergent clades of *T. ulnaria* are correlated with the localities in which the specimens were collected (Fig. 77). Locality and specimen information are provided in Table 8.

Table 8. *Tettigades ulnaria* localities and specimens collected in the field. Specimen numbers with an asterisk (*) were included in phylogenetic analyses. ULN1, ULN2, ULN3, and ULN4 labels are subclade identifiers.

Date Collected	Specimen	Divergent Clade	Name	Region	Latitude	Longitude	Elevation	Collector(s)
10.XII.2014	PL326.1 (♀)*	ULN2	Caleu	Metropolitan	-33.02	-70.99	1160m	CV & PŁ
10.XII.2014	PL327 (♀)*	ULN2	Caleu	Metropolitan	-33.02	-70.99	1160m	CV & PŁ
13.XII.2014	PL405 (♀)*	ULN4	Bellavista 2	O'Higgins	-34.83	-70.75	1163m	CV & PŁ
13.XII.2014	PL406 (♀)	ULN4	Bellavista 2	O'Higgins	-34.83	-70.75	1163m	CV & PŁ
13.XII.2014	PL413.1 (♀)*	ULN4	Bellavista 2	O'Higgins	-34.83	-70.75	1163m	CV & PŁ
13.XII.2014	PL413.2 (♂)	ULN4	Bellavista 2	O'Higgins	-34.83	-70.75	1163m	CV & PŁ
13.XII.2014	PL414.1 (♀)*	ULN4	Bellavista 2	O'Higgins	-34.83	-70.75	1163m	CV & PŁ
15.XII.2014	PL468 (♂)*	ULN4	Camino a las Termas 2	O'Higgins	-34.80	-70.60	945m	CV & PŁ
15.XII.2014	PL467.1 (♀)*	ULN4	Camino a las Termas 2	O'Higgins	-34.80	-70.60	945m	CV & PŁ
16.XII.2014	PL514.1 (♂)*	ULN4	Camino Embalse el Yeso 1	Metropolitan	-33.79	-70.20	1415m	CV & PŁ
20.XII.2015	PL739 (♂)*	ULN1	Cauquenes/Quirihue	Maule	-36.10	-72.45	258m	KN, CV, & PŁ
20.XII.2015	PL748 (♂)*	ULN1	Cauquenes/Quirihue	Maule	-36.10	-72.45	258m	KN, CV, & PŁ
20.XII.2015	PL740 (♀)*	ULN1	Cauquenes/Quirihue	Maule	-36.10	-72.45	258m	KN, CV, & PŁ
20.XII.2015	PL971 (♂)*	ULN4	Talca-campus	Maule	-35.41	-71.64	106m	EF
29.XII.2015	PL972 (♂)*	ULN3	Villa Allegre	Maule	-35.68	-71.74	105m	EF
30.XII.2015	PL970.1 (♂)*	ULN4	Itahue	Maule	-35.13	-71.36	235m	KN, CV, & PŁ
30.XII.2015	PL970.2 (♂)*	ULN4	Itahue	Maule	-35.13	-71.36	235m	KN, CV, & PŁ
30.XII.2015	PL970.3 (♂)*	ULN4	Itahue	Maule	-35.13	-71.36	235m	KN, CV, & PŁ
30.XII.2015	PL970.4 (♂)*	ULN4	Itahue	Maule	-35.13	-71.36	235m	KN, CV, & PŁ
30.XII.2015	PL970.5 (♂)*	ULN4	Itahue	Maule	-35.13	-71.36	235m	KN, CV, & PŁ
30.XII.2015	PL970.6 (♂)*	ULN4	Itahue	Maule	-35.13	-71.36	235m	KN, CV, & PŁ
30.XII.2015	PL970.7 (♂)*	ULN4	Itahue	Maule	-35.13	-71.36	235m	KN, CV, & PŁ
30.XII.2015	PL970.8 (♂)*	ULN4	Itahue	Maule	-35.13	-71.36	235m	KN, CV, & PŁ

30.XII.2015	PL970.9 (♂)*	ULN4	Itahue	Maule	-35.13	-71.36	235m	KN, CV, & PŁ
30.XII.2015	PL970.10 (♂)*	ULN4	Itahue	Maule	-35.13	-71.36	235m	KN, CV, & PŁ
4.I.2016	JM002 (♀)*	ULN1	L1: Trapatrapa (Rio Queuco)	Bío Bío	-37.71	-71.27	928m	CV & JM
4.I.2016	JM003 (♂)	ULN1	L1: Trapatrapa (Rio Queuco)	Bío Bío	-37.71	-71.27	928m	CV & JM
4.I.2016	JM006 (♂)	ULN1	L1: Trapatrapa (Rio Queuco)	Bío Bío	-37.71	-71.27	928m	CV & JM
4.I.2016	JM008 (♀)*	ULN1	L1: Trapatrapa (Rio Queuco)	Bío Bío	-37.71	-71.27	928m	CV & JM
4.I.2016	JM010 (♀)	ULN1	L1: Trapatrapa (Rio Queuco)	Bío Bío	-37.71	-71.27	928m	CV & JM
5.1.2016	JM020 (♂)*	ULN3	L3: Antuco alto	Bío Bío	-37.37	-71.49	950m	CV & JM
6.I.2016	JM030 (♂)*	ULN3	L5: Colbún	Maule	-35.64	-71.39	298m	CV & JM
6.I.2016	JM031 (♂)*	ULN3	L5: Colbún	Maule	-35.64	-71.39	298m	CV & JM
21.I.2016	PL973 (♂)*	ULN3	Talca, Cerro La Virgen	Maule	-35.42	-71.70	260m	PŁ
21.I.2016	PL974 (♂)*	ULN3	Talca, Cerro La Virgen	Maule	-35.42	-71.70	260m	PŁ
21.I.2016	PL975 (♂)*	ULN3	Talca, Cerro La Virgen	Maule	-35.42	-71.70	260m	PŁ
21.I.2016	PL976 (♂)	ULN3	Talca, Cerro La Virgen	Maule	-35.42	-71.70	260m	PŁ

Distribution: Chile

Figure 77. Map of field collection localities for *Tettigades ulnaria*. Experimental/Field-collected cicada clades are identified on the map with circles. Any coloration is used to delimit the clades on the map and are not correlated with any morphological characters. ULN1(white), ULN2(black), ULN3(dark gray), ULN4(light gray). Map of Chile reproduced from World Atlas (http://www.worldatlas.com/).

Additional and Updated Distinguishing Features:

Thorax. Specimens have been collected that closely match the description by Torres for *Tettigades ulnaria*. Ridges of stridulatory organ widely separated at the top and more densely packed at the base. The two spots on the pronotum are distinct and may vary in color intensity. None of the samples collected presents these two spots which, as noted by Torres, increase in size appear to occupying a great part of the pronotum or disappear altogether. At times the spots are faded making it appear as though they are absent.

Body. Coloration may vary between a dark brown, almost black to a light orange-brown.

Genitalia (Figs. 78 and 79)***.*** Eighth tergite primary black with thin distal edge colored in ochre. Eighth sternite black at the base with most of the sternite ochre. Dorsal beak very sharply pointed. Pygofer all black. Median lobe of uncus black on outside. Pseudoparamere light beige color with the presence of dark tooth-like structures visible along the lateral edge of the pesudoparamere. More tooth-like structures visible on the edge farthest from the specimen in lateral view. Tooth-like structures on the outer perimeter of the pseudoparamere visible in ventral view. The endotheca is lighter in color compared to the theca and pseudoaparamere. Endotheca curves tightly back towards the specimen and out towards the right almost parallel to the rest of the aedeagus.

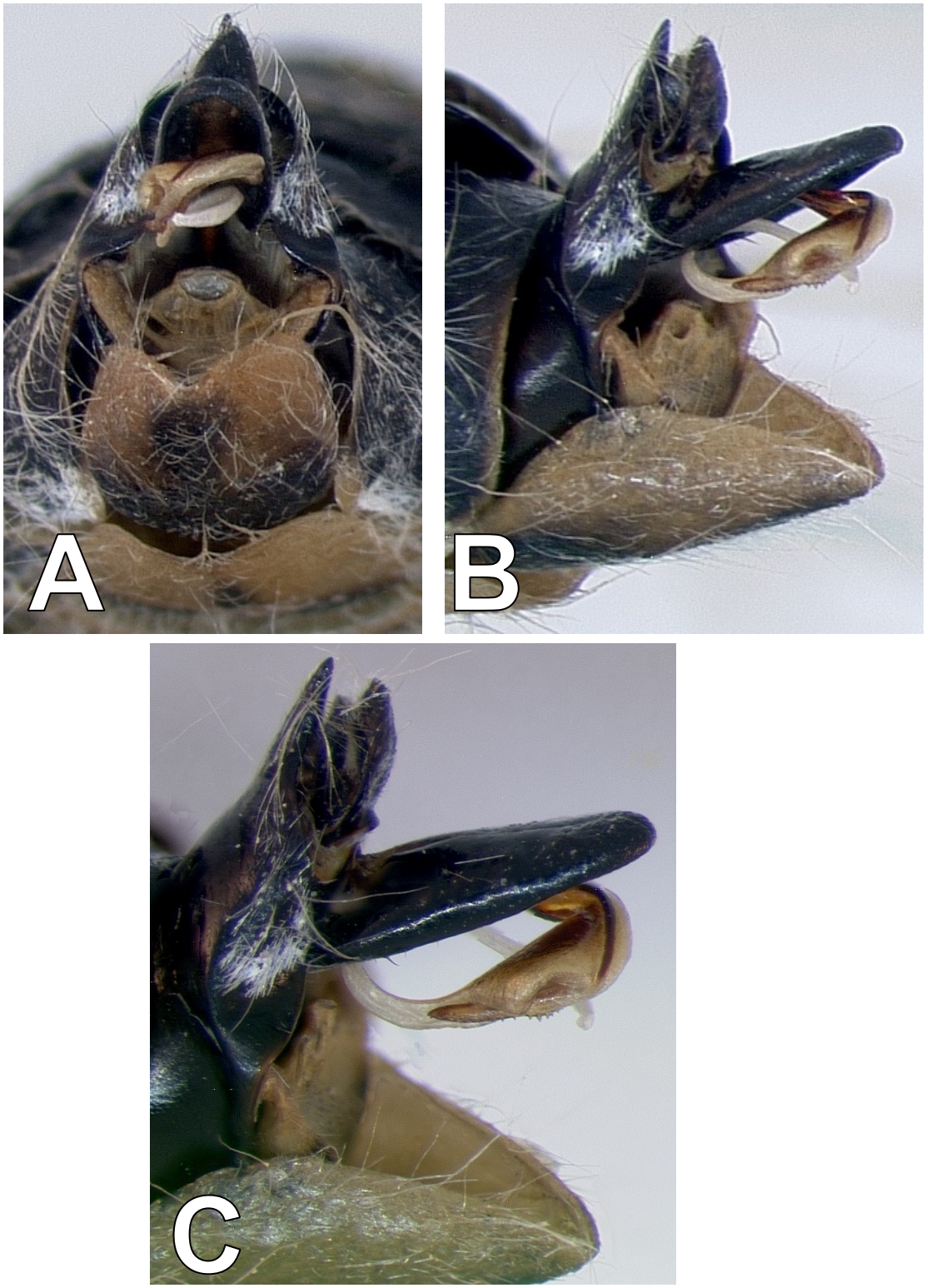

Figure 78. Male genitalia of *Tettigades ulnaria*: (A) ventral, (B) lateral, and (C) lateral views of PL514.1, *Tettigades ulnaria,* ULN4. Images (A) and (B) are at 2x magnification. Image (C) is at 4x magnification.

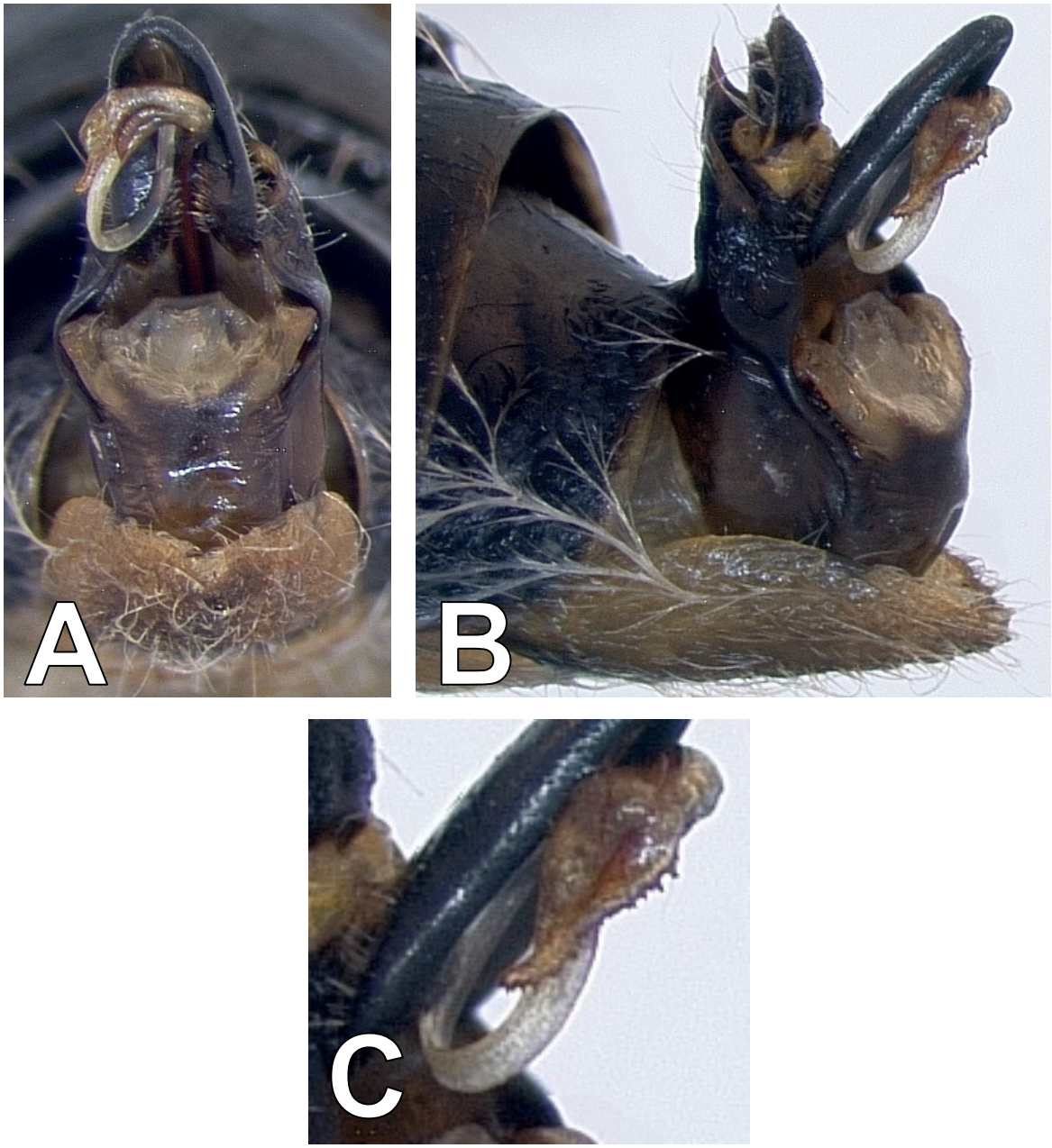

Figure 79. Male genitalia of *Tettigades ulnaria*: (A) ventral, (B) lateral, and (C) lateral views of PL974, *Tettigades ulnaria,* ULN3.. Images (A) and (B) are at 2x magnification. Image (C) is at 4x magnification.

Diagnosis:
Tettigades procera

Tettigades ulnaria is morphologically similar to *Tettigades procera* (Fig. 80). We have only been able to examine the holotype for *T. procera* and no specimens have been collected in the field representing or morphologically similar to *T. procera*. Due to the variation exhibited in other species of *Tettigades*, and the high similarity to *T. ulnaria,* molecular evidence needs to be gathered in the future to validate this species and its relationship with *T. ulnaria.* Therefore, a complete diagnosis between these two species will not be provided. Torres (1958) also did not provide a diagnosis. However, he did note that the venation of the wings is different in the two species. Dorsal and ventral images of the holotype of *T. procera* are also provided.

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Figure 80. Dorsal and ventral view of the holotype “MNNC № 3273” of Tettigades procera deposited at MNNC (Santiago, Chile).

Phylogenetic Assessment:

The clade, *T. ulnaria,* consists of 31 specimens all closely matching the description of one species, *Tettigades ulnaria* (Fig. 86). Within the clade representing *Tettigades ulnaria*, on the mitochondrial phylogeny there are four well supported subclades, ULN1, ULN2, ULN3, and ULN4. In the nuclear phylogeny, ULN3 and ULN4 do not form distinct monophyletic clades. There may be a contact zone near Talca where perhaps ULN3 and ULN4 are hybridizing. This would explain the phylogenies (Fig.85).

There are no morphological characters supporting these populations. Any variation did not appear to be exclusive to any clade. In some cases, sampling was limited to one specimen per locality therefore, variation might have been missed.

The clades within *T. ulnaria* appear to correlate with the geographic localities at which these groups are found (*See Phylogenetic Assessment*). Locality and specimen information are provided in Table 8. ULN1 was found at the southernmost part of the field-collected *T.* ulnaria range, spanning about 207km. ULN2 was only represented at one locality and was the most northern location at which we collected *T. ulnaria* in the field. The collection sites of ULN3 was separated by about 217km while the collection sites of ULN4 was separated by about 220km. There was a total range of about 523km for our field collected *T. ulnaria*. The clades are geographically structured, with only one haplotype per locality but all clades being found in the same year.

Figure 81. Field-collected specimen (JM002) photos taken once spread, representing ULN1. The specimen was collected in 70% ETOH and then relaxed, spread, and dried.

Figure 82. Dorsal (top) and ventral (bottom) view of field collected specimen (PL326.1) photos taken once spread, representing ULN2. The specimen wascollected in 70% ETOH and then relaxed, spread, and dried.

Figure 83. Dorsal (left) and ventral (right) view of field collected specimen (PL974) taken once spread, representing ULN3. The specimen was collected in 70% ETOH and then relaxed, spread, and dried.

Figure 84. Field-collected specimen PL414.1 taken alive from Bellavista 2, representing ULN4. Photo courtesy of Piotr Łukasik.

Figure 85. Field-collected specimen PL414.1 taken alive from Bellavista 2, representing ULN4. Photo courtesy of Piotr Łukasik.

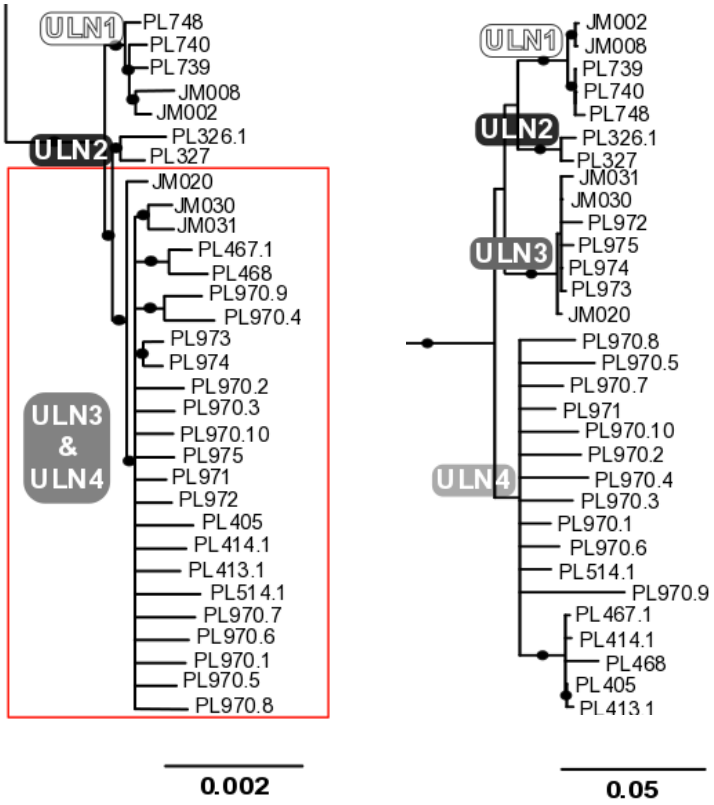

Figure 86. The *T. ulnaria* subclade clipped from the maximum likelihood phylogenies (Chapter 2) based on 742 targeted-capture exons (left) and, from bycatch, 13 mitochondrial protein coding genes (right) with subclades ULN1, ULN2, ULN3, and ULN4.The red box highlights that clades 3 and 4 do not form a monophyletic group on the nuclear tree. Black dots represent bootstrap supports >95%; branches with <70% support were collapsed.

***Tettigades undata* Torres, 1958**
Synonyms: none

Torres (1958) description of *T. undata*: [text interpretation from Spanish]

Holotype.

♂: Body length: 17.5mm; wing length: 22.55mm; wing width: 9mm; wingspan: 53.5mm.

Allotype.

♀: Body length: 18.5mm; wing length: 23mm; wing width: 9mm; wingspan: 55mm.

Paratypes.

♂♂**:** Body length: 18-19mm; wing length: 22.5-24.5mm; wing width: 9-9.5mm; wingspan: 53.5-58.5mm.

♀♀**:** Body length: 19-20mm; wing length: 25-25.5mm; wing width: 10 mm; wingspan: 60-61mm.

Head. Dorsal. Black. A spot on the anterior half of the vertex on each side of the postclypeus, two dips on the first disk, a spot in front of the third ocellus and one behind each eye, brown. Ventral. Black. Rostrum ochre brown with the apex of the last segment dark dun and passing the apex of the intermediate coxae.

Thorax. Dorsal. Pronotum black. Anterior and lateral borders very finely, and posterior, colored a light dun. Third half of the anterior border black. The pronotum may present a small lighter spot on its disks, lateral borders of this area next to the eye, in most cases, has an evident curvature up to the top, especially in lateral view. Mesonotum black. A spot in front of each anterior branch of the scutellum and extremes of these, a spot linearly curved bordering the base of the lateral areas and borders lateral-posterior of the mesonotum, ochre brown. Disk of the scutellum ochre or crossed by a thick black stripe. Stridulatory areas elliptical in shape, black, and somewhat lighter in its central part, with 18-20 ridges approximately, deep and quite vertical. Thorax below with the coxae and femur of the forelegs black with stripes of ochre brown color.

Tibia of the forelegs almost totally black and may have a frontal spot of light brown. Second and third pair of legs ochre brown in general. A spot on the midcoxae. A fine stripe and another thick on the external face and another thick on the interior femurs of the midleg, a fine stripe and another thick on the posterior-interior of the tibias of the midleg, a spot on the coxae of the hindleg, two fine stripes, parallel on the interior face an another on the interior of the femurs of the hindlegs black. Tarsi dark brown. Meracanthus ochre brown; opercula of the same color and black at the base.

Wings. Forewing presenting the same general coloration as in *Tettigades pauxilla*, however, the vein sc+r is ochre, half of the plectrum is black, jugal region and hindwings with an angular spot of the vannal region pink or pink white, vein 2a black.

Abdomen. Abdomen with tergites VII, VIII, and pygofer black with only the sides stained brown, spine of the pygofer (distal beak) black. Sternites black with posterior borders ochre brown, they might also be black only at the disk. Sternite VII and sternite VIII ochre brown, presenting on each of them two large basal spots of the color black; posterior border of the 7th low on its middle part. Body with a pilosity of yellowish-whitish and dark brown on the region of the head.

Variation. The anterior margin of the pronotum entirely ochre brown, as well as the major part of the scutellum and its anterior branches. The stridulatory areas black and in rare cases there may be more ridges. In only one example, the m vein of the wing is lighter in color. The angular spot of the vannal region of the wings may demonstrate a gray background color. The notch on sternite VII of males may be barely visible as well may disappear; the sternites may demonstrate a black disk and

interrupted in its middle part by an ochre brown spot which originates by a pair of lateral spots on each of them. Tergite VII in females broadly ochre.

Type Material Examined: used for morphological comparisons

Holotype (♂)
MLPA Specimen Number: MLPA № 1686/1
Type Locality: San Bernando, Chile
Date collected: Dec. 1935

Figure 87. Dorsal (top), ventral (middle), and stridulatory (bottom) view of the holotype MLPA № 1686/1“of *Tettigades undata* deposited at MLPA (La Plata, AR).

Torres (1958) Measurements: Body length: 17.5mm; wing length: 22.5mm; wing width: 9mm

Our Measurements: Body length: 17.71mm; wing length: 22.71mm; wing width: 9.23mm

Allotype (♀)

MNNC Type Number: MNNC № 3264
Type Locality: no data
Date Collected: no data

Figure 88. Dorsal (top) and ventral (bottom) view of the allotype "MNNC № 3264" of *Tettigades undata* deposited at MNNC (Santiago, CL).

Torres (1958) Measurements: Does not provide data about the allotype

Our Measurements: Body length: 18.181mm; wing length: 23.85mm; wing width: 9.42mm

Field-Collected Material Examined:

A total of 66 specimens were collected in the field between 2014 and 2015 and were used for morphological characterization. (*See Chapter 2*). Of those 66, 26 specimens were used for phylogenetic analyses.

Our phylogenetic analyses revealed three divergent subclades with high bootstrap support, UND1, UND2, and UND3, on the mitochondrial phylogeny all individuals of which closely resemble the description of *T. undata*. However, in the nuclear phylogeny, UND2, does not form a monophyletic clade. There are no morphological characters distinguishing these three subclades.

The three divergent clades within *T. undata* are geographically structured. Locality and specimen information are provided in Table 9.

Table 9. *Tettigades undata* localities and specimens collected in the field. Specimen numbers with an asterisk (*) were included in phylogenetic analyses. UND1, UND2, and UND3 are subclade identifiers.

Date Collected	Specimen	Divergent Clade	Name	Region	Latitude	Longitude	Elevation	Collector
15.XII.2014	PL467.2 (♂)*	UND1	Camino a las Termas 2	O'Higgins	-34.80	-70.60	945m	CV & PŁ
15.XII.2014	PL485.1 (♀)*	UND1	Camino Coya-Los Cipreses, Puente	O'Higgins	-34.23	-70.48	855m	CV & PŁ
15.XII.2014	PL487 (♂)*	UND1	Camino Coya-Los Cipreses, Puente	O'Higgins	-34.23	-70.48	855m	CV & PŁ
15.XII.2014	PL488.1 (♂)*	UND1	Camino Coya-Los Cipreses, Puente	O'Higgins	-34.23	-70.48	855m	CV & PŁ
18.XII.2015	PL467.1 (♀)	UND1	Puente Negro	O'Higgins	-34.68	-70.87	489m	CV & PŁ
3.I.2016	KN015 (♂)	UND3	Lonquimay	Araucanía	-38.45	-71.36	887m	KN, PŁ, & JM
3.I.2016	KN016 (♂)	UND3	Lonquimay	Araucanía	-38.45	-71.36	887m	KN, PŁ, & JM
3.I.2016	KN017 (♂)	UND3	Lonquimay	Araucanía	-38.45	-71.36	887m	KN, PŁ, & JM
3.I.2016	PL803 (♂)	UND3	Lonquimay	Araucanía	-38.45	-71.36	887m	KN, PŁ, & JM
3.I.2016	PL804 (♂)*	UND3	Lonquimay	Araucanía	-38.45	-71.36	887m	KN, PŁ, & JM
3.I.2016	PL805 (♂)*	UND3	Lonquimay	Araucanía	-38.45	-71.36	887m	KN, PŁ, & JM
3.I.2016	PL807 (♂)	UND3	Lonquimay	Araucanía	-38.45	-71.36	887m	KN, PŁ, & JM
3.I.2016	PL808 (♂)	UND3	Lonquimay	Araucanía	-38.45	-71.36	887m	KN, PŁ, & JM
3.I.2016	PL809.1 (♀)*	UND3	Lonquimay	Araucanía	-38.45	-71.36	887m	KN, PŁ, & JM
3.I.2016	PL809.2 (♂)	UND3	Lonquimay	Araucanía	-38.45	-71.36	887m	KN, PŁ, & JM
3.I.2016	PL810 (♀)	UND3	Lonquimay	Araucanía	-38.45	-71.36	887m	KN, PŁ, & JM
3.I.2016	PL811 (♂)	UND3	Lonquimay	Araucanía	-38.45	-71.36	887m	KN, PŁ, & JM
3.I.2016	PL811.1 (♂)	UND3	Lonquimay	Araucanía	-38.45	-71.36	887m	KN, PŁ, & JM
3.I.2016	PL811.2 (♂)*	UND3	Lonquimay	Araucanía	-38.45	-71.36	887m	KN, PŁ, & JM
3.I.2016	PL811.3 (♂)*	UND3	Lonquimay	Araucanía	-38.45	-71.36	887m	KN, PŁ, & JM
3.I.2016	PL811.4 (♂)*	UND3	Lonquimay	Araucanía	-38.45	-71.36	887m	KN, PŁ, & JM
3.I.2016	PL811.5 (♂)*	UND3	Lonquimay	Araucanía	-38.45	-71.36	887m	KN, PŁ, & JM
3.I.2016	PL811.6 (♂)*	UND3	Lonquimay	Araucanía	-38.45	-71.36	887m	KN, PŁ, & JM
3.I.2016	PL811.7 (♂)*	UND3	Lonquimay	Araucanía	-38.45	-71.36	887m	KN, PŁ, & JM
3.I.2016	PL811.8 (♂)*	UND3	Lonquimay	Araucanía	-38.45	-71.36	887m	KN, PŁ, & JM
3.I.2016	PL811.9 (♂)*	UND3	Lonquimay	Araucanía	-38.45	-71.36	887m	KN, PŁ, & JM
3.I.2016	PL811.10 (♂)	UND3	Lonquimay	Araucanía	-38.45	-71.36	887m	KN, PŁ, & JM

3.I.2016	PL811.11 (♂)	UND3	Lonquimay	Araucanía	-38.45	-71.36	887m	KN, PŁ, & JM
3.I.2016	PL811.12 (♂)	UND3	Lonquimay	Araucanía	-38.45	-71.36	887m	KN, PŁ, & JM
3.I.2016	PL811.13 (♂)	UND3	Lonquimay	Araucanía	-38.45	-71.36	887m	KN, PŁ, & JM
3.I.2016	PL811.14 (♂)	UND3	Lonquimay	Araucanía	-38.45	-71.36	887m	KN, PŁ, & JM
3.I.2016	PL811.15 (♂)	UND3	Lonquimay	Araucanía	-38.45	-71.36	887m	KN, PŁ, & JM
3.I.2016	PL812 (♂)	UND3	Lonquimay	Araucanía	-38.45	-71.36	887m	KN, PŁ, & JM
3.I.2016	PL956 (♂)	UND3	Lonquimay	Araucanía	-38.45	-71.36	887m	KN, PŁ, & JM
3.I.2016	PL957 (♀)	UND3	Lonquimay	Araucanía	-38.45	-71.36	887m	KN, PŁ, & JM
4.I.2016	JM004 (♀)*	UND3	L1: Trapatrapa (Rio Queuco)	Bío Bío	-37.71	-71.27	928m	CV & JM
4.I.2016	JM005 (♂)	UND3	L1: Trapatrapa (Rio Queuco)	Bío Bío	-37.71	-71.27	928m	CV & JM
4.I.2016	JM007 (♀)	UND3	L1: Trapatrapa (Rio Queuco)	Bío Bío	-37.71	-71.27	928m	CV & JM
4.I.2016	JM009 (♂)*	UND3	L1: Trapatrapa (Rio Queuco)	Bío Bío	-37.71	-71.27	928m	CV & JM
5.1.2016	JM014.1 (♂)*	UND3	L3: Antuco alto	Bío Bío	-37.37	-71.49	950m	CV & JM
5.1.2016	JM021 (♀) *	UND3	L3: Antuco alto	Bío Bío	-37.37	-71.49	950m	CV & JM
5.1.2016	JM022.1 (♂)	UND3	L3: Antuco alto	Bío Bío	-37.37	-71.49	950m	CV & JM
5.1.2016	JM022.2 (♂)	UND3	L3: Antuco alto	Bío Bío	-37.37	-71.49	950m	CV & JM
5.1.2016	JM023 (♂)	UND3	L3: Antuco alto	Bío Bío	-37.37	-71.49	950m	CV & JM
5.1.2016	JM024 (♂)	UND3	L3: Antuco alto	Bío Bío	-37.37	-71.49	950m	CV & JM
5.1.2016	JM025 (♂)	UND3	L3: Antuco alto	Bío Bío	-37.37	-71.49	950m	CV & JM
5.1.2016	JM026 (♂)	UND3	L3: Antuco alto	Bío Bío	-37.37	-71.49	950m	CV & JM
5.1.2016	JM027.1 (♂)	UND3	L3: Antuco alto	Bío Bío	-37.37	-71.49	950m	CV & JM
5.1.2016	JM027.2 (♂)	UND3	L3: Antuco alto	Bío Bío	-37.37	-71.49	950m	CV & JM
5.I.2016	JM015 (♂)*	UND2	L4: Rio Cholghuán - Puente la Fábrica	Bío Bío	-37.19	-71.98	274m	CV & JM
5.I.2016	JM016 (♂)*	UND2	L4: Rio Cholghuán - Puente la Fábrica	Bío Bío	-37.19	-71.98	274m	CV & JM
5.I.2016	JM017 (♂)	UND2	L4: Rio Cholghuán - Puente la Fábrica	Bío Bío	-37.19	-71.98	274m	CV & JM
5.I.2016	JM018 (♂)*	UND2	L4: Rio Cholghuán - Puente la Fábrica	Bío Bío	-37.19	-71.98	274m	CV & JM
5.I.2016	JM019 (♀)	UND2	L4: Rio Cholghuán - Puente la Fábrica	Bío Bío	-37.19	-71.98	274m	CV & JM
6.I.2016	JM028 (♂)*	UND2	L5: Colbún	Maule	-35.64	-71.39	298m	CV & JM
6.I.2016	JM029 (♂)	UND2	L5: Colbún	Maule	-35.64	-71.39	298m	CV & JM
6.I.2016	JM032 (♂)*	UND2	L5: Colbún	Maule	-35.64	-71.39	298m	CV & JM
6.I.2016	JM033 (♂)	UND2	L5: Colbún	Maule	-35.64	-71.39	298m	CV & JM

6.I.2016	JM034 (♂)	UND2	L5: Colbún	Maule	-35.64	-71.39	298m	CV & JM
6.I.2016	JM035 (♂)	UND2	L5: Colbún	Maule	-35.64	-71.39	298m	CV & JM
6.I.2016	JM036 (♂)	UND2	L5: Colbún	Maule	-35.64	-71.39	298m	CV & JM
7.I.2016	JM038 (♂)*	UND2	L6: Central Cipreses	Maule	-35.81	-70.83	853m	CV & JM
7.I.2016	JM039 (♂)*	UND2	L6: Central Cipreses	Maule	-35.81	-70.83	853m	CV & JM
7.I.2016	JM040 (♂)	UND2	L6: Central Cipreses	Maule	-35.81	-70.83	853m	CV & JM
7.I.2016	JM041 (♂)	UND2	L6: Central Cipreses	Maule	-35.81	-70.83	853m	CV & JM
7.I.2016	JM042 (♂)	UND2	L6: Central Cipreses	Maule	-35.81	-70.83	853m	CV & JM

Distribution: Chile

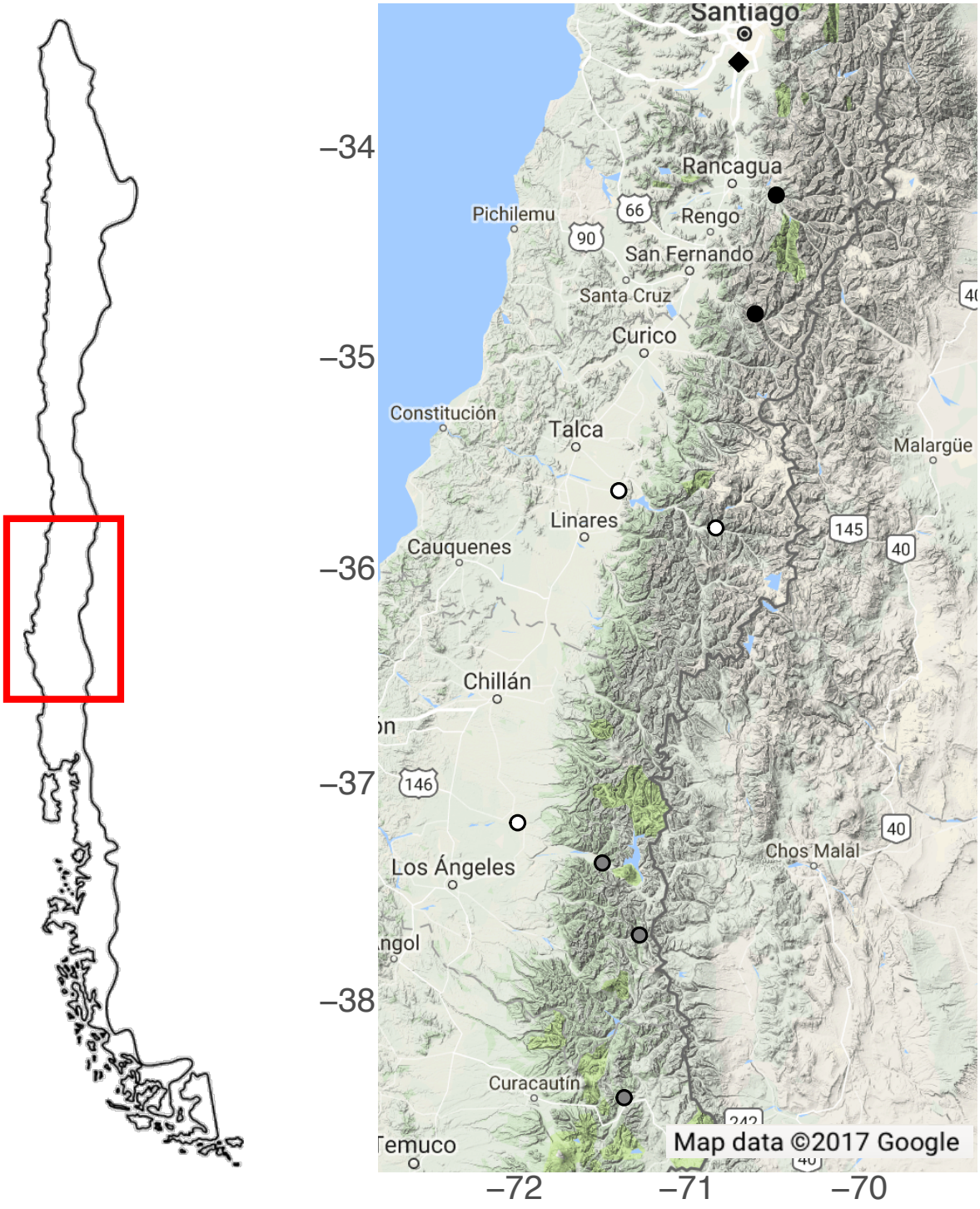

Figure 89. The collection sites for *Tettigades undata*. Collecting localities are identified on the map with circles. The type locality is indicated with a diamond. The color indicates clade UND1 (black), UND2 (white), and UND3 (gray). Map of Chile reproduced from World Atlas (http://www.worldatlas.com/).

Additional and Updated Distinguishing Features:

Thorax. As described by Torres (1958), the pronotum may present a spot in the middle which may be quite faded or absent. The borders of the pronotum may vary in thickness. The color of the border may vary from chestnut to a brown-black. Spots on the ends of the anterior branches of the cruciform elevation may vary in color intensity and size.

Wings. In live specimens, the CuP+1A vein is colored bright white (Fig. 89). This color is lost when the specimen is placed in ethanol. The coloration of the jugal fold is highly variable. Specimens may present a pale orange, or a white coloration in this area (Figs. 91 and 92). Torres recognized the color of the jugal fold as a pale pink but none of the specimens we collected have this color.

Genitalia (Figs. 93, 94, and 95***).*** Eighth sternite black at base but primarily ochre and tergite black with thin ochre border at posterior end. Dorsal beak sharply pointed and black. Pygofer primarily black with edges thinly lined in ochre. Median lobe of uncus black on outside, facing the anal lobe. The pseudoparamere and theca are a light caramel color while the endotheca is a pearly white. Pseudoparamere light with the presence of dark tooth-like structures visible on the lateral view along the edges of the pesudoparamere. Tooth-like structures on the outer perimeter of the pseudoparamere visible in ventral view. Endotheca curves tightly back towards the specimen and out towards the right parallel to the theca.

Figure 90. *T. undata*, specimen PL485.1, in the field alive, representing UND1. Photo courtesy of Piotr Łukasik.

Figure 91. Field-collected specimen (PL485) photos taken once spread with white coloration in jugal fold. The specimen was collected in 70% ETOH and then relaxed, spread, and dried.

Figure 92. Field-collected specimen (JM038) photos taken once spread with pale orange coloration in jugal fold. The specimen was collected in 70% ETOH and then relaxed, spread, and dried.

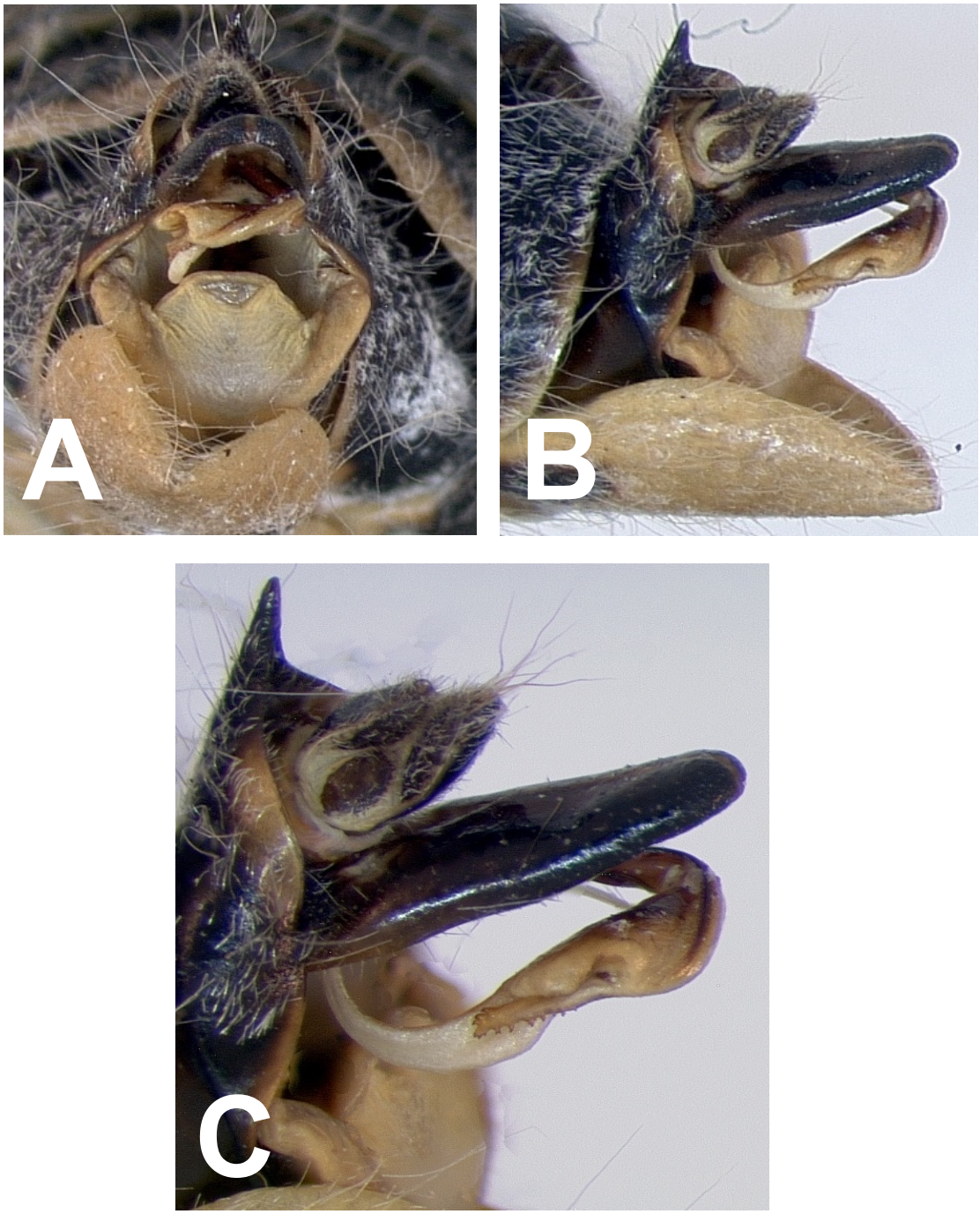

Figure 93. Male genitalia of *Tettigades undata*: (A) ventral, (B) lateral, and (C) lateral views of PL514.1, *Tettigades ulnaria,* ULN4. Images (A) and (B) are at 2x magnification. Image (C) is at 4x magnification.

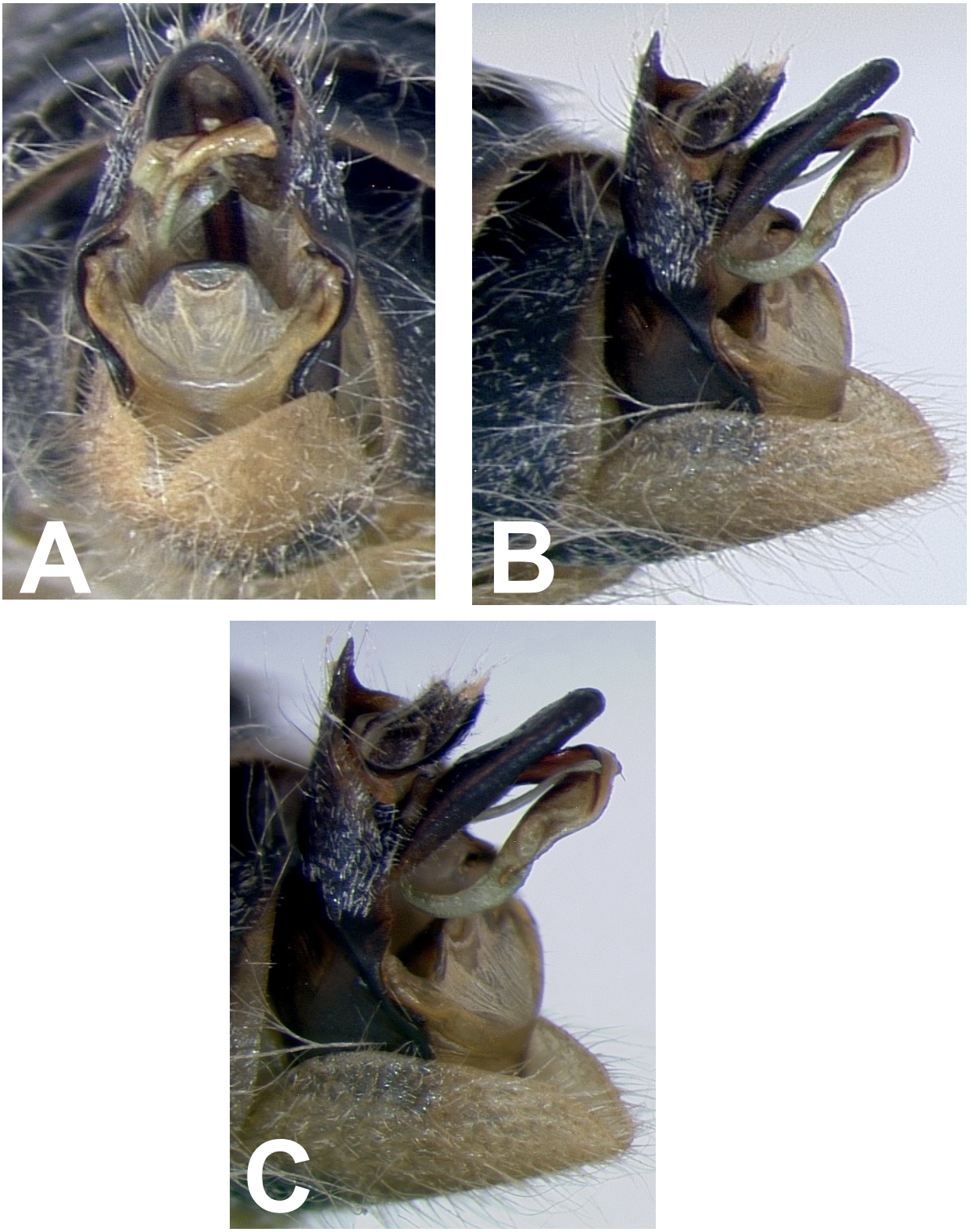

Figure 94. Male genitalia of *Tettigades undata*: (A) ventral, (B) lateral, and (C) lateral views of JM038, *Tettigades undata* UND2. Images (A) and (B) are at 2x magnification. Image (C) is at 4x magnification.

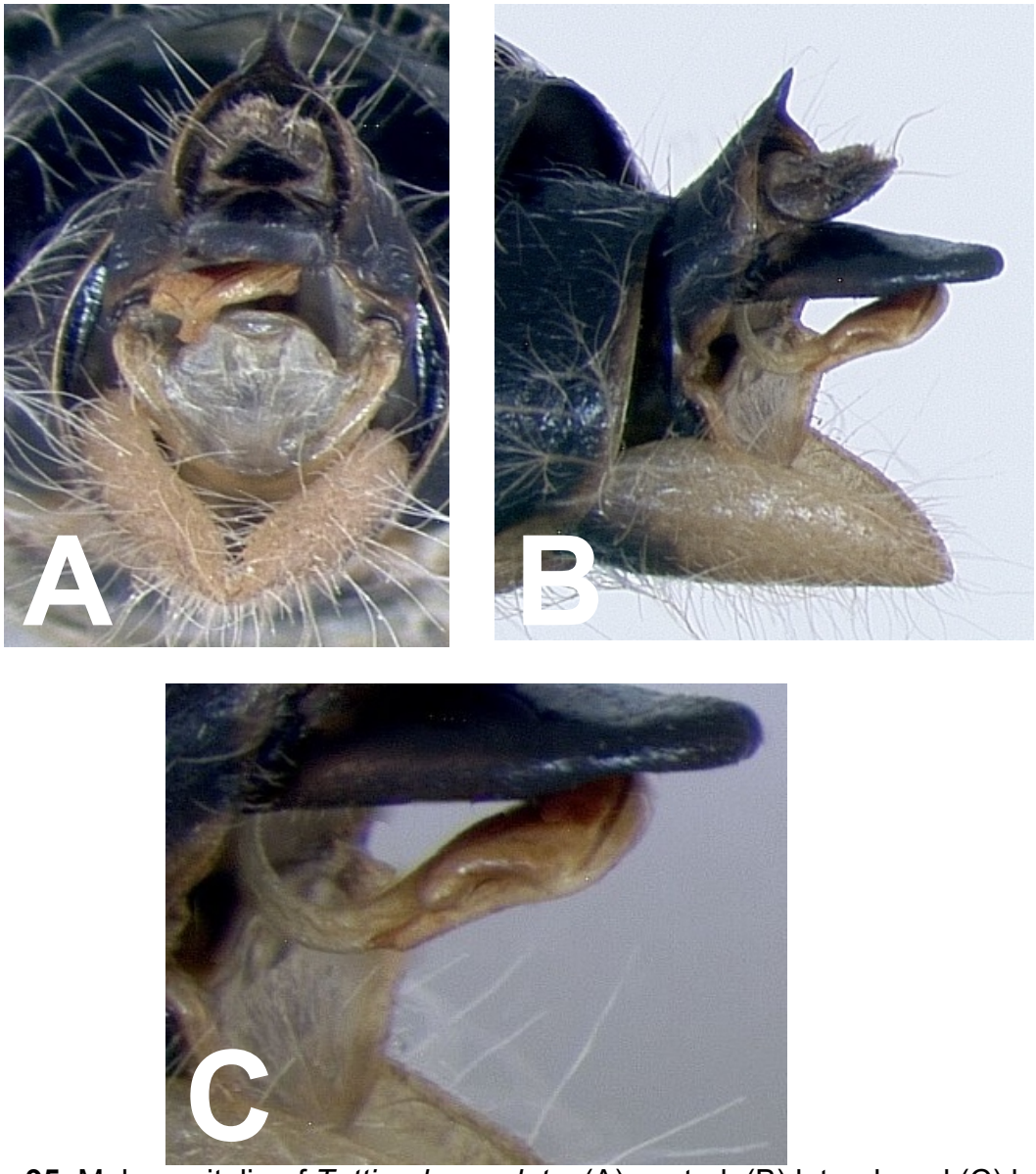

Figure 95. Male genitalia of *Tettigades undata*: (A) ventral, (B) lateral, and (C) lateral views of PL809.2, *Tettigades undata* UND3. Images (A) and (B) are at 2x magnification. Image (C) is at 4x magnification.

Diagnosis:

Tettigades pauxilla

Tettigades pauxilla is morphologically similar to *Tettigades undata* (Fig. 96). Torres (1958) in his treatment of *T. undata* did provide a diagnosis comparing *T pauxilla* and *T. undata.* However, for his treatment of *T. pauxilla,* he provided a diagnosis of *T. pauxilla* compared to *T. sordida* which are not morphologically similar. We have been able to examine the holotype for both species. During our examination of the *T.* pauxilla holotype, we were unable to study the genitalia. Torres states that the genitalia of both species are different but does not explain the differences he found. Torres did provide drawings of genitalia structures but they are difficult to interpret.

The coloration on the holotype for *T. pauxilla* is faded making a comparison difficult. We have collected *T. undata* specimens near the type locality of *T. pauxilla.* However, due to some noticeable difference between the two species, we do not believe, that we have collected *T. pauxilla* during our field collecting. For example, *T. pauxilla* has not been found to have spots at the anterior branches of the cruciform elevation. Every specimen of *T. undata* that we collected in the field or that is part of the type material examined have these spots at each end of the anterior branches of the cruciform elevation. The VII and VIII tergites *T. pauxilla* are mostly a light ochre color with a bit of black at the base. In *T. undata* these two tergites are mostly black and lightly lined in ochre.

Due to the variation exhibited in other species of *Tettigades*, and the high similarity to *T. undata,* molecular evidence and the genitalia of *T. pauxilla* need to be

examined in the future in order to validate this species and its relationship with *T. undata.*

Figure 96. Dorsal (top) and ventral (bottom) view of the holotype MLPA № 1683/1“of *Tettigades pauxilla* deposited at MLPA (La Plata, AR).

Phylogenetic Assessment:

The clade, *T. undata* consists of 26 specimens all closely matching the description of one species, *Tettigades undata*. Within the clade representing *Tettigades undata*, the mitochondrial phylogenies resolve 3 divergent populations, UND1, UND2, and UND3, with high bootstrap support. However, in the nuclear phylogeny, UND2 and UND3 do not form a monophyletic clade (Fig. 98). There are no morphological characters supporting these clades.

The divergent clades of *T. undata* are correlated with the localities at which they were collected (Fig. 89). In some cases, sampling was limited to one specimen per locality therefore, variation might have been missed. At the northernmost part of the range of the *T. undata* we collected subclade UND1; it spans a range of 63km. At the center of the range, UND2 spans 185km. At the southernmost part of the range of *T. undata* we collected, UND3; it spans a range of 122km. The entire range of all 3 divergent clades combined is 475km.

Tettigades sp.2.

There are two specimens, JM043 and JM044 (Fig 97) that treat here as *Tettigades* sp.2. They are morphologically similar to *T. undata*. However, in both the nuclear and mitochondrial phylogenies (Fig. 99), these two specimens form their own monophyletic clade and are not sister to *T. undata.* Morphologically these specimens differ from *T. undata* by the coloration of parts of the body which are orange in this new group and ochre brown in *T. undata.* Also, the segments of the pronotal collar which are colored, differ between the two groups. The color of the jugal region is a reddish-orange with white at the apex. The coloration of the sternites is also a useful character to tell these groups apart. Some of the genitalia features differ as well. For instance, the distal beak in *T.* sp.2 (Fig. 98) is orange and black in *T. undata.*

Figure 97. Dorsal (left) and ventral (right) views of *Tettigades sp.* 2 specimen, JM043. The specimen was collected in 70% ETOH and then relaxed, spread, and dried.

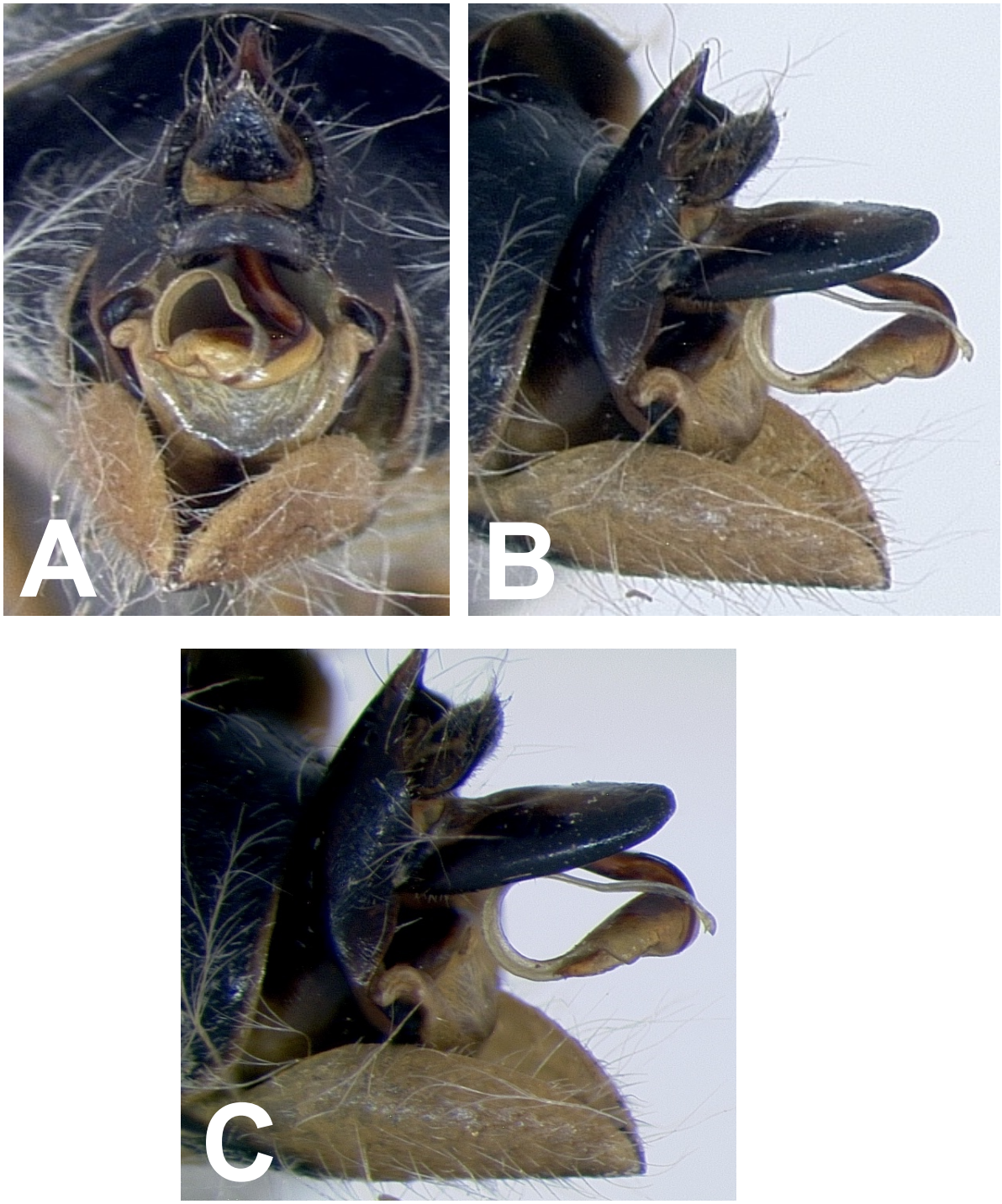

Figure 98. Male genitalia of *Tettigades ulnaria*: (A) ventral, (B) lateral, and (C) lateral views of JM043, *Tettigades* sp.2. Images (A) and (B) are at 2x magnification. Image (C) is at 4x magnification.

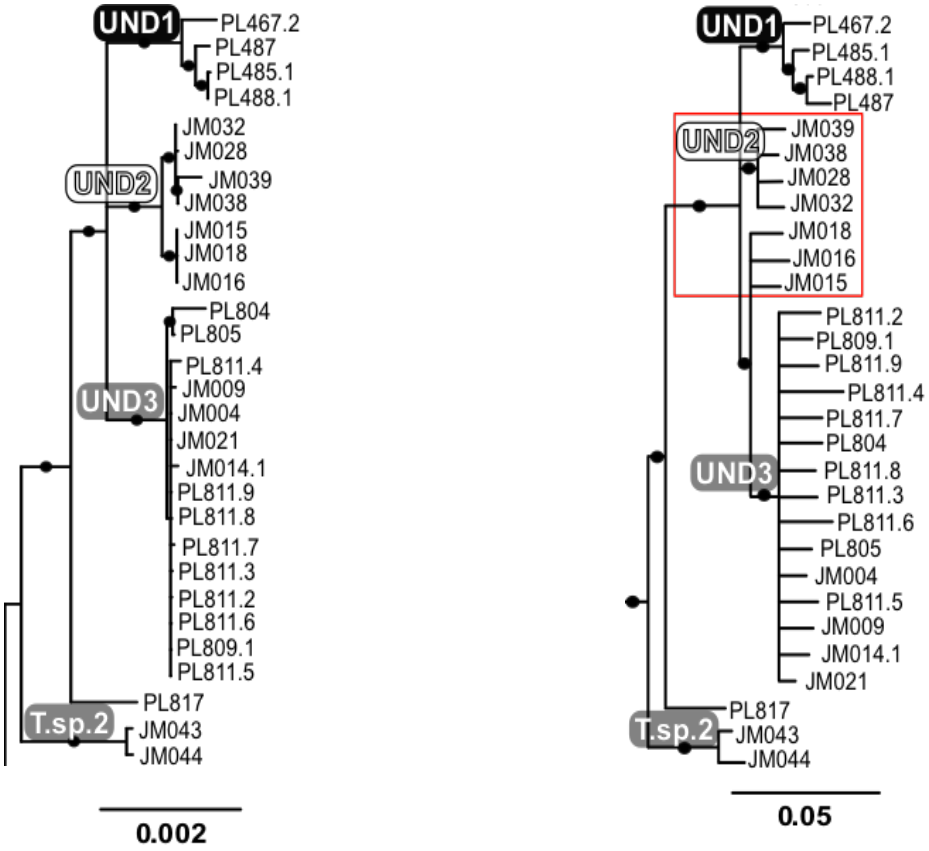

Figure 99. The *T. undata* subclade clipped from the maximum likelihood phylogenies (Chapter 2) based on 742 targeted-capture exons (left) and, from bycatch, 13 mitochondrial protein coding genes (right) with subclades UND1, UND2, and UND3. The red box highlights that clade 2 does not form a monophyletic group on the mitochondrial tree. Black dots represent bootstrap supports >95%; branches with <70% support were collapsed.

Future experimental plan for species delimitation

The correct classification of a species is important for not only assessing phylogenetic relationships, but also for understanding evolution and life histories. However, a lack of easy and useful external characters, as in *Tettigades,* makes understanding these relationships difficult. The use of acoustic, morphological, and molecular analyses, separately and in combination, has helped us understand species evolution and diversity of cicadas (Alexander & Moore, 1958; Lane 1984; Moulds 2005; Nunes et al. 2013; Nunes et al. 2014) and will also be vital for understanding the relationships within *Tettigades.*

Type material:

For many of the original descriptions of *Tettigades* species, a holotype was not selected by the author. In other cases, such as *T. chilensis*, the holotype is missing. Assigning neotypes for these species will be very important in cases where the *species* is in fact is a complex cryptic taxa with unnamed species-level taxa.

Wings:

The wing cells and veins in *Tettigades* specimens may be useful characters for delimiting species. However, many individual specimens may exhibit an extra anal or apical cell. These extra cells are quite common but most often found on one wing. With the material we examined, extra cells can occur on any wing with equal probability. For this reason, it is important to study both wings when making species determinations. The shape of the CuA vein, M_{3+4} vein, mc cell, and ulnar cells appear to be areas to examine for species delimitation because they are typically consistent within species.

Genitalia:

Torres included images of genitalia in his work; however, he did not provide detailed descriptions of these features. In fact, this is quite difficult to do. However, by examining many individuals of each of the species, a standard can be made to help identify key features. The tooth-like structures of the pseudoparamere are often different for each of the species, and appear to be a promising source of taxonomic identity. Depending on the angle at which one examines the pseudoparamere, the number and size of the tooth-like structures appears to vary. The curvature of the endotheca is another potential character. However, many samples need to be examined, preferably when the specimens are alive. The curvature of the endotheca appears to vary slightly within these divergent populations but it might be due to the way the genitalia dried when pinned.

Stridulatory Organs:

The stridulatory organ (Fig. 100) is a character Torres introduced in his 1958 monograph. However, this is another character that needs to be standardized before it can be used to the extent Torres did. Torres counted the number of “well-defined” ridges. However, it is difficult to get the same numbers he had since the particular ridges he recognizes as “well-defined” are arbitrary. The overall shape is an excellent character that Torres introduced.

The stridulatory organ has proven to be useful for delimiting species, even morphologically similar species such as *T. limbata* and *T. chilensis*. Species such as *T. curvicosta*, *T. crassa*, and *T. procera* should be examined closely and compared to other species. It might be the case that the stridulatory organ is indicating that species

such as *T. curvicosta* and *T. distanti* are the same species. However, additional study is required.

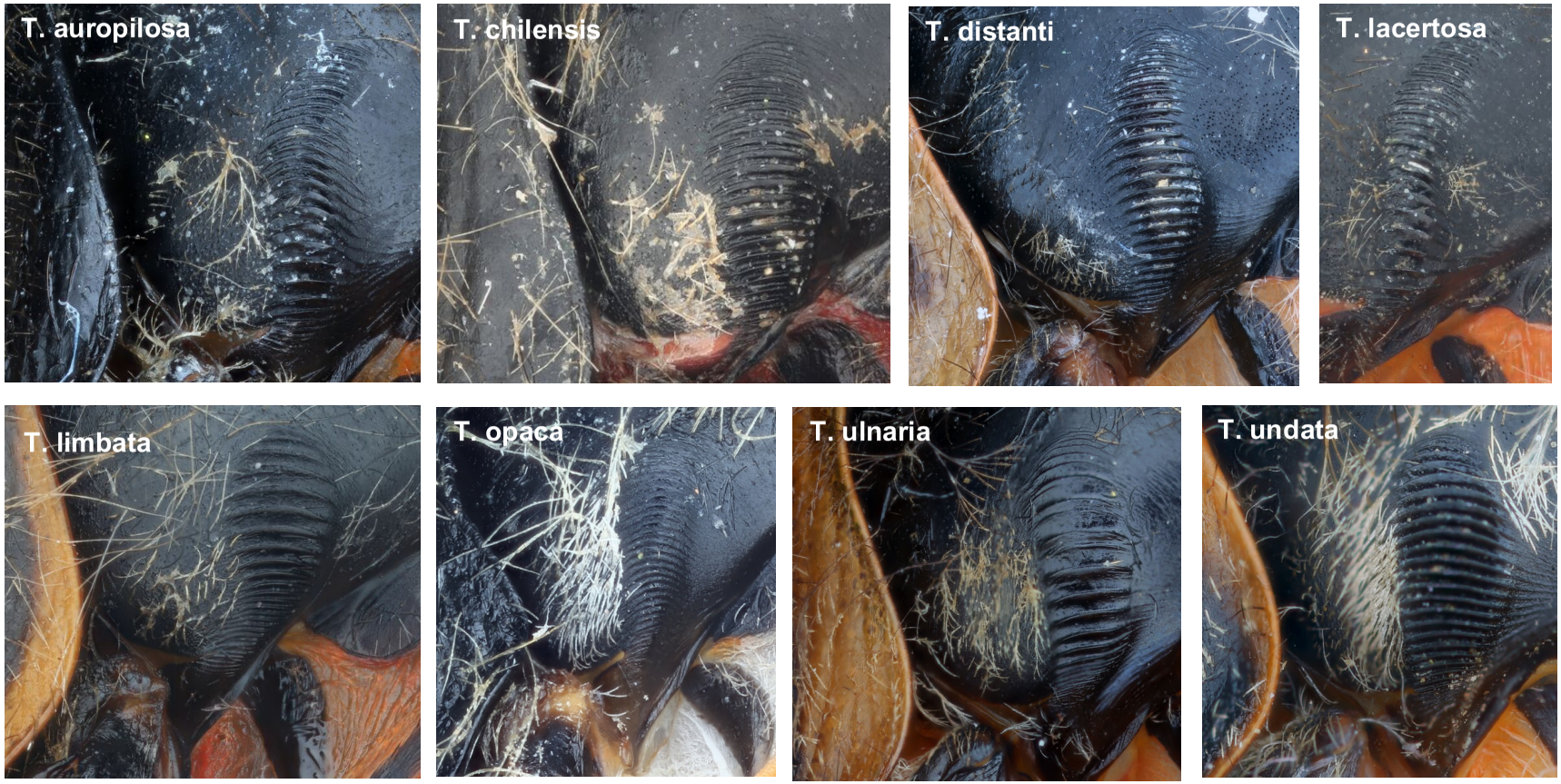

Figure 100. Stridulatory apparatuses of *T. auropilosa, T. chilensis, T. distanti, T. lacertosa, T. limbata*, *T. opaca*, *T. ulnaria*, and *T. undata*. Specimens were collected in 95% [or 70%] ETOH and then relaxed, spread, and dried.

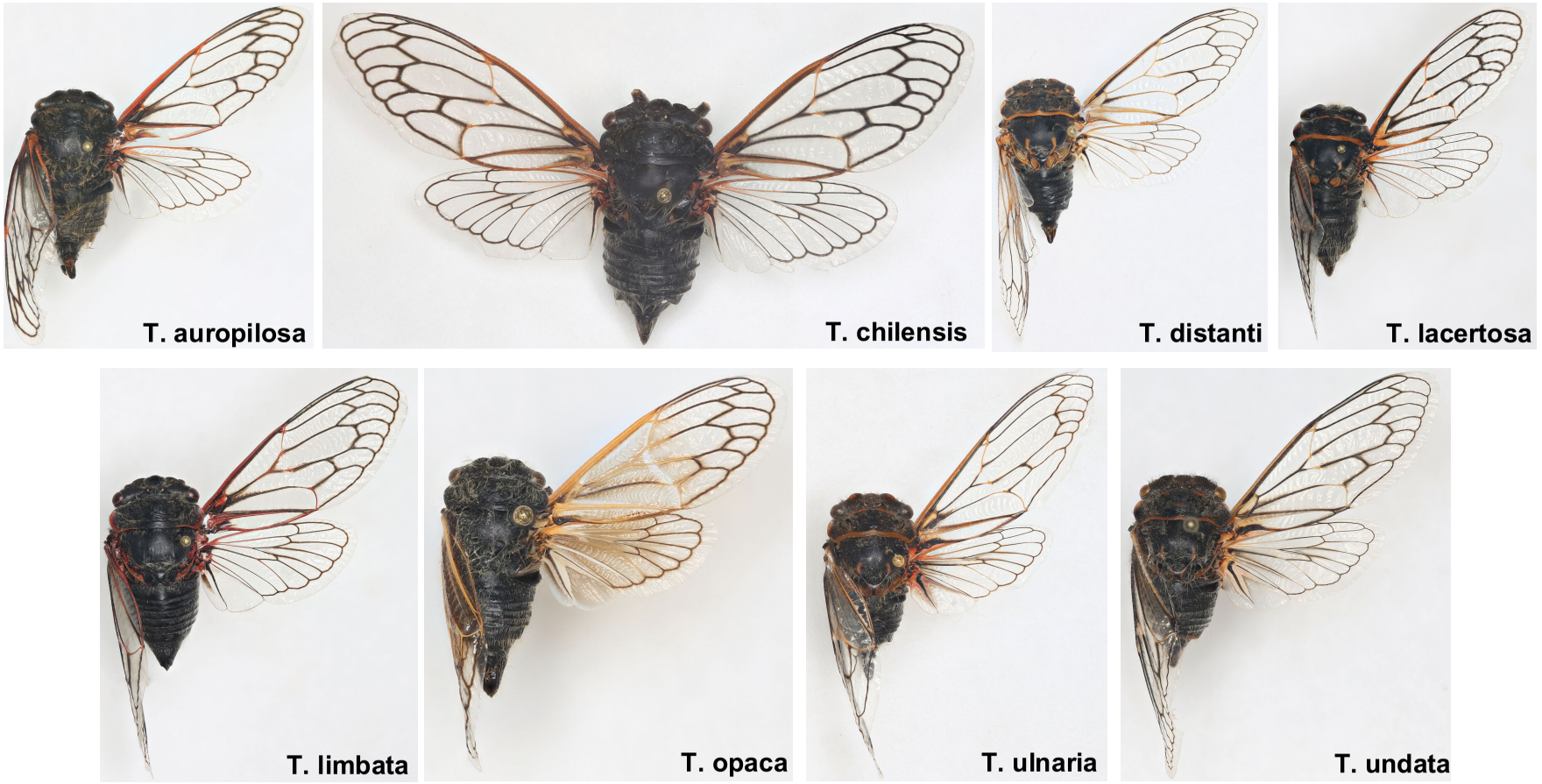

Figure 101. Dorsal views of *T. auropilosa, T. chilensis, T. distanti, T. lacertosa, T. limbata*, *T. opaca*, *T. ulnaria*, and *T. undata*. Specimens were collected in 95% [or 70%] ETOH and then relaxed, spread, and dried.

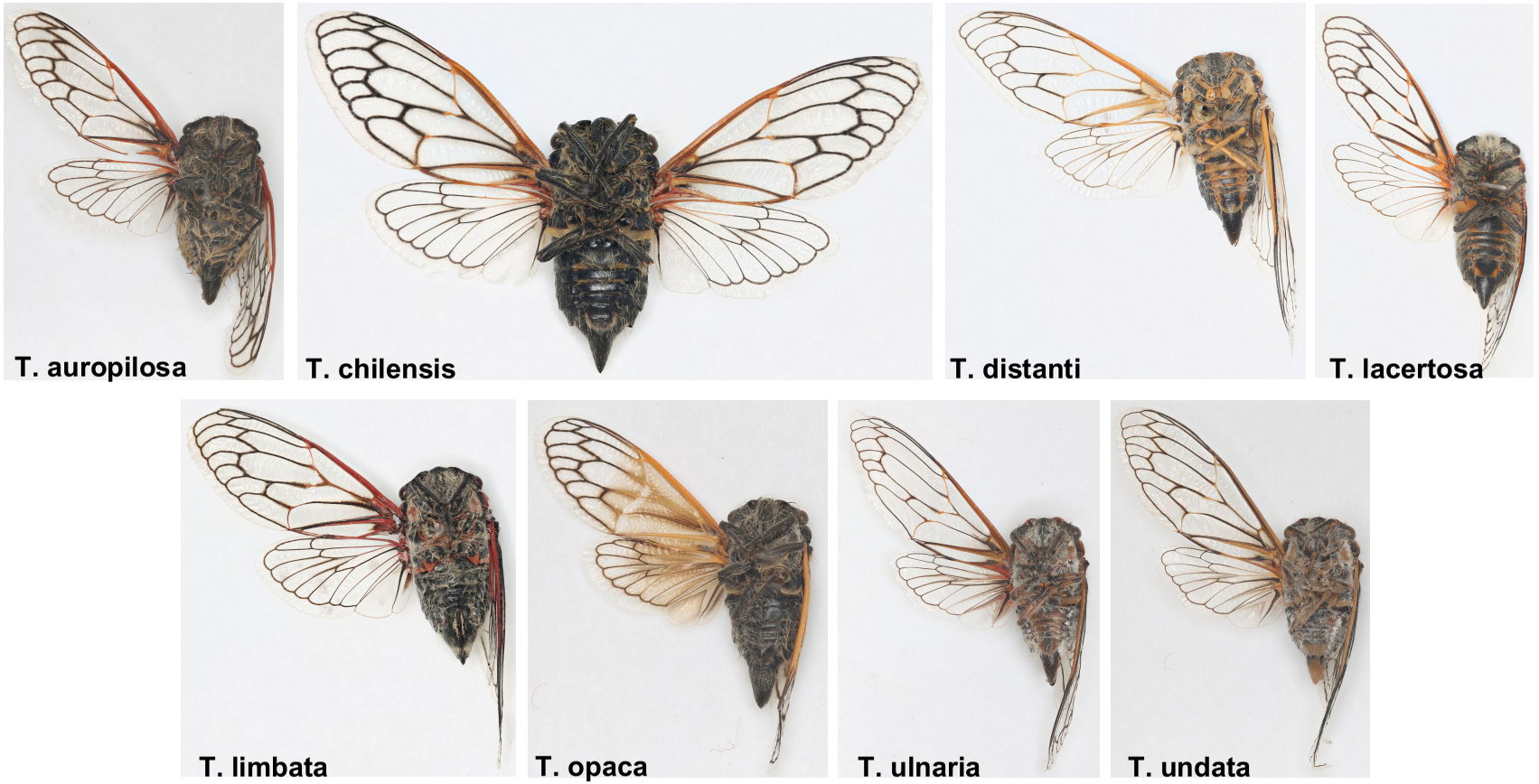

Figure 102. Ventral views of *T. auropilosa, T. chilensis, T. distanti, T. lacertosa, T. limbata*, *T. opaca*, *T. ulnaria*, and *T. undata*. Specimens were collected in 95% [or 70%] ETOH and then relaxed, spread, and dried.

. Key to Some of the Chilean *Tettigades* species

This key distinguishes the following species of *Tettigades:* T. *auropilosa, T. chilensis, T. distanti, T. lacertosa, T. limbata, T. opaca, T. ulnaria*, and *T. undata*.

Fig. K1. Wings translucent

Fig. K2. Wings hyaline

1. Wings clear hyaline (Fig.K1) ……..……..…….. 2

   Wings translucent yellow (Fig. K2) ……..……..…… T. *opaca*

Fig. K3. Pronotum with no spots

Fig. K4. Pronotum with 1 spot

Fig. K5. Pronotum with 2 spots

2. Pronotum with 1 or fewer mididorsal spots (Figs. K3- K4) ……..……..…….. 3

   Pronotum with greater than one (addorsal) spots (Fig. K5) ……..……..…… *T. ulnaria*

Fig. K6. Collar red or orange laterally

3. Lateral portions of pronotal collar not red or orange ……..…….. 4

Lateral portions of pronotal collar red or orange (Fig. K6) …….. *T. limbata*

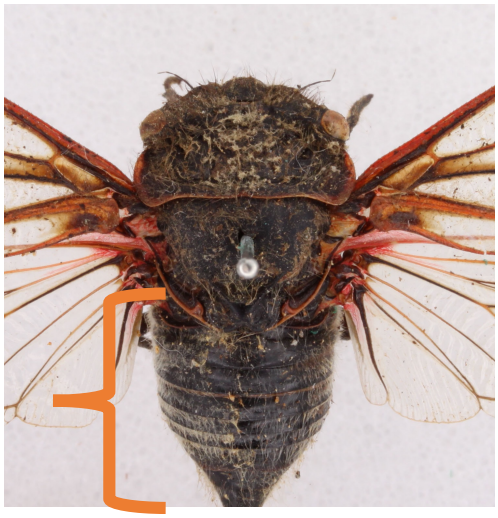

Fig. K7. Dorsal abdomen not densely hairy

Fig. K8. Dorsal abdomen densely hairy

4. Dorsal abdomen not densely hairy (Fig. K7) ……..……..…… 5

Dorsal abdomen not densely hairy (Fig. K8) ……..…… *T. auropilosa*

Fig. K9. Legs not black

Fig. K10. Legs black

5. Legs not black (Fig. K9) ……..……..…… 6

Legs black (Fig. K10) ……..……..…… 7

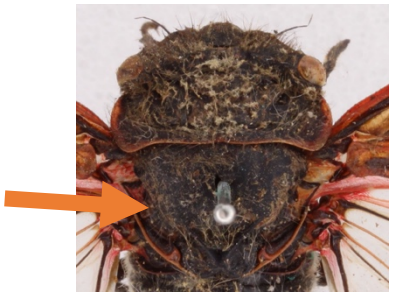

Fig.K11. Mesonotum without 2 large rectangular spots

Fig. K12. Mesonotum with 2 large rectangular spots

6. Mesonotum without large rectangular spots (Fig. K11) …… *T. chilensis*

   Mesonotum with large rectangular spots (Fig. K12) ……..……...…… *T. lacertosa*

Fig. K13. Stridulatory organ S-shaped

Fig. K14. Stridulatory organ not s-shaped

7. Stridulatory organ S-shaped (Fig. K13) …….. *T. distanti*

   Stridulatory organ not S-shaped (Fig. K14) …… T*. undata*

Conclusion

In this chapter, we compiled and integrated previous work with new morphological, geographical, and molecular data in order to treat eight of the Chilean species of *Tettigades* and to provide insight on species new to science. The following Chilean species were treated: *T. auropilosa* Torres, 1944*, T. chilensis* Amyot & Audinet-Serville, 1843*, T. distanti* Torres 1958, *T. lacertosa* Torres, 1944, *T. limbata* Torres, 1958*, T. opaca* Jacobi, 1907*, T. ulnaria* Distant, 1906, and *T. undata* Torres, 1958. Two potential new species, *T.* sp.1 and *T.* sp.2, were introduced. Future work on Argentine and other Chilean species will help enhance our knowledge of *Tettigades*. Using song phenotypes; comparative studies of genital morphology, stridulatory structures, and wings; and molecular data will serve to unravel more of the evolutionary history of *Tettigades.*

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Table S1. List of museum specimens examined.

Museum	Specimen Number	Specimen ID	Sex	Type	Date Collected	Locality	Body length (mm)	Forewing length (mm)	Forewing width (mm)
MACN	-	Tettigades opaca	Female	Allotype	I.1917	Lonquimay/Curacautin	16.6	18.75	7.59
MACN	-	Tettigades major	Female	Cotype	-	San Antonio Rio Negro	23.58	29.23	11.64
MACN	-	Tettigades major	Male	Cotype	-	San Antonio Rio Negro	27.41	28.73	11.36
MACN	-	Tettigades ulnaria	Male	Nontype	-	-	19.73	27	11.06
MACN	-	Tettigades ulnaria	Female	Nontype	-	-	21.95	27.65	11.49
MACN	-	Tettigades ulnaria	Female	Nontype	-	-	21.69	27.07	10.56
MACN	-	Tettigades pauxilla	Male	Nontype	-	-	15.96	21.46	8.8
MACN	-	Tettigades major	Female	Nontype	-	San Antonio Rio Negro	-	31.8	-
MACN	-	Tettigades major	Male	Nontype	-	San Antonio Rio Negro	27.19	30.67	11.4
MACN	-	Tettigades major	Female	Nontype	-	San Antonio Rio Negro	30.85	35.96	13.96
MACN	-	Tettigades major	Female	Nontype	-	San Antonio Rio Negro	30.65	35.92	14.22
MACN	-	Tettigades major	Male	Nontype	II.1935	Puerto Madryn	29.98	-	-
MACN	-	Tettigades sarcinatrix	Male	Nontype	I.1941	Neuquen, Hua Hum	-	-	-
MACN	-	Tettigades sarcinatrix	-	Nontype	-	Nahuel Huapi	-	-	-
MACN	-	Tettigades sarcinatrix	Male	Nontype	28.3.1945	Piedra Parada, Chubut	-	-	-
MACN	-	Tettigades pauxilla	Female	Paratype	-	-	16.55	21.8	8.79
MLPA	1672/1	Tettigades auropilosa	Male	Cotype	XI.1933	Queltehues, Santiago	21.96	24.43	10.93
MLPA	1672/2	Tettigades auropilosa	Male	Cotype	XI.1933	Queltehues, Santiago	21.47	26.03	12.02
MLPA	1678/2	Tettigades lacertosa	Male	Cotype	XI (year is smudged)	Valparaiso	26.3	28.09	11.44
MLPA	1678/1	Tettigades lacertosa	Female	Cotype	XI-1940	Valparaiso	26.71	31.02	12.01
MLPA	-	Tettigades lacertosa	-	Cotype	XI.1959	Valparaiso	-	30.61	11.08
MLPA	1681/ 1	Tettigades major	Female	Cotype	-	Mendoza	-	-	-
MLPA	1681/2	Tettigades major	Female	Cotype	-	Neuquen	-	30.94	12.2
MLPA	1681/4	Tettigades major	Male	Cotype	17.XII-1943	Bajo de Sta Rosa, Rio Negro	28.16	30.08	11.3
MLPA	1681/4	Tettigades major	Male	Cotype	17.XII-1944	Bajo de Sta Rosa, Rio Negro	-	28.26	10.93
MLPA	1684/1	Tettigades sarcinatrix	Male	Cotype	-	San Carlos de Bariloche	17.74	22.67	8.6
MLPA	1684/2	Tettigades sarcinatrix	Male	Cotype	-	San Carlos de Bariloche	17.23	22.16	8.35
MLPA	1684/3	Tettigades sarcinatrix	Male	Cotype	-	San Carlos de Bariloche	17.56	21.9	8.29
MLPA	1684/5	Tettigades sarcinatrix	Female	Cotype	-	San Carlos de Bariloche	18.63	25.81	9.52
MLPA	1684/6	Tettigades sarcinatrix	Female	Cotype	-	Nahuel Huapi	20.5	26.02	9.68
MLPA	1684/7	Tettigades sarcinatrix	Female	Cotype	-	Nahuel Huapi	-	24.32	8.89
MLPA	1684/4	Tettigades sarcinatrix	Female	Cotype	-	San Carlos de Bariloche	-	24.67	9.04
MLPA	1686/1	Tettigades undata	Male	Holotype	Dec. 1935	San Bernando	17.56	22.15	9.43
MLPA	-	Tettigades compacta	Female	Nontype	-	-	-	19.94	-
MLPA	-	Tettigades ulnaria	-	Nontype	-	Concepcion	-	-	-

MLPA	1677/3	Tettigades distanti	Male	Paratype		Limache	22.12	27.35	11.57
MLPA	1679/3	Tettigades limbata	Male	Paratype	17.XII.1947	Lo Valdez 1800m Santiago	19.37	28.13	9.88
MLPA	1679/4	Tettigades limbata	Male	Paratype	17.XII.1947	Lo Valdez 1800m Santiago	22.01	26.27	11.48
MLPA	-	Tettigades limbata	Male	Paratype	17.XII.1947	Lo Valdez 1800m Santiago	22.34	25.08	11.8
MLPA	-	Tettigades limbata	Male	Paratype	17.XII.1947	Lo Valdez 1800m Santiago	-	27.67	11.2
MLPA	1686/9	Tettigades undata	Male	Paratype	-	Concepcion	17.71	22.71	9.23
MLPA	1686/5	Tettigades undata	Female	Paratype	11.I.1948	Abanico (950m) Bio Bio	-	-	-
MLPA	1686/8	Tettigades undata	Female	Paratype	-	-	19.58	24.64	10.01
MLPA	1686/7	Tettigades undata	Female	Paratype	16.XII.1956	Bio Bio, Las Canteras	19.45	25.05	9.88
MLPA	1686/6	Tettigades undata	Male	Paratype	16.XII.1957	Bio Bio, Las Canteras	18.65	23.96	10.38
MLPA	-	Tettigades undata	Female	Paratype	16.XII.1958	Bio Bio, Las Canteras	19.07	24.38	9.98
MLPA	1676/3	Tettigades curvicosta	Male	Paratype	-	-	21.5	28.13	11.86
MLPA	-	Tettigades undata	-	Nontype	18.XII.1956	Lota	-	23.66	8.97
MLPA	1683/1	T. pauxilla Torres, 1958	Male	Holotype	-	Curacautin	16.34	21.05	8.79
MNNC	S3264	Tettigades undata	Female	Allotype	-	-	18.18	23.85	9.42
MNNC	S3269	Tettigades curvicosta	Female	Allotype	-	-	-	-	-
MNNC	S3277	Tettigades compacta	No genitalia (missing)	Allotype	-	-	16.93	-	-
MNNC	S3281	Tettigades limbata	Female	Allotype	17.XII.1947	Lo Valdes: Santiago (1800m)	19.17	16.34	11.23
MNNC	S3273	Tettigades procera	Female	Holotype	-	Valdivia	22.85	29.5	11.45
MNNC	S3274	Tettigades crassa	Female	Holotype	-	-	23.55	29.73	12.22
MNNC	S3280	Tettigades limbata	Male	Holotype	17.XII.1947	Lo Valdes: Santiago (1800m)	23.44	27.4	11.47
MNNC	Nazario, spec.36	Tettigades lacertosa large&hairy	Male	Nontype	7-Dec-91	El Higirio, prov Huasco	-	-	-
MNNC	Nazario, spec.39	Tettigades lacertosa large&hairy	-	Nontype	6-Nov-91	Bahia Inglesa, prov Copiapo	-	-	-
MNNC	Nazario, spec.37	Tettigades lacertosa_bars	Female	Nontype	25-Nov-87	Socos Limari	-	-	-
MNNC	Nazario, spec.38	Tettigades lacertosa_bars_split	Male	Nontype	9-Nov-91	Socos Limari	-	-	-
MNNC	Nazario, spec.35	Tettigades limbata?	Female	Nontype	26-Nov-61	El Salto, Santiago	-	-	-
MNNC	Nazario, spec.34	Tettigades opaca	Male	Nontype	Dec-86	Dominicos, Santiago	-	-	-
MNNC	Nazario, spec.32	Tettigades pauxilla	Male	Nontype	Oct-86	Victoria, Cautin	-	-	-
MNNC	Nazario, spec.33	Tettigades pauxilla	Male	Nontype	Oct-86	Victoria, Cautin	-	-	-
MNNC	Nazario, spec.20	Tettigades auropilosa	Male	Nontype	no data	El Canelo 33°34′ Santiago	-	-	-
MNNC	Nazario, spec.28	Tettigades chilensis	Female	Nontype	November	San Cristobal	-	-	-
MNNC	Nazario, spec.29	Tettigades chilensis	Male	Nontype	11-Dec-55	Santiago, Valle Ramon	-	-	-
MNNC	Nazario, spec.30	Tettigades chilensis	Female	Nontype	-	Illapel	-	-	-
MNNC	Nazario, spec.25	Tettigades lacertosa	Female	Nontype	no data	no data	-	-	-
MNNC	Nazario, spec.21	Tettigades limbata	Female	Nontype	no data	no data	-	-	-
MNNC	Nazario, spec.22	Tettigades limbata	Male	Nontype	17-Dec-47	Lo Valdez 1800m Santiago	-	-	-
MNNC	Nazario, spec.23	Tettigades ulnaria	Male	Nontype	Nov-1882	Rancagua	-	-	-

MNNC	Nazario, spec.24	Tettigades ulnaria	Male	Nontype	Sep-48	Chile Linares Linares	-	-	-
MNNC	Nazario, spec.26	Tettigades ulnaria	Male	Nontype	no data	Valdivia	-	-	-
MNNC	Nazario, spec.27	Tettigades ulnaria	Male	Nontype	no data	Valdivia	-	-	-
MNNC	Nazario, spec.15	Tettigades undata	Male	Nontype	no data	no data	-	-	-
MNNC	Nazario, spec.16	Tettigades undata	Male	Nontype	no data	no data	-	-	-
MNNC	Nazario, spec.18	Tettigades undata	Male	Nontype	Apr-89	San Miguel, Region Metropolitana	-	-	-
MNNC	Nazario, spec.19	Tettigades undata	Male	Nontype	5-Jan-87	Chillan, Los Lleuques	-	-	-
MNNC	S3265	Tettigades undata	Male	Paratype	18.I.1948	Albanico (800m): Bio-Bio	18.25	24.19	10.61
MNNC	S3266	Tettigades undata	Male	Paratype	-	Concepcion	15.81`	22.82	10.65
MNNC	S3267	Tettigades undata	Female	Paratype	11.I.1948	Albanico (950m): Bio-Bio	19.72	25.15	9.82
MNNC	S3268	Tettigades undata	Male	Paratype	8.1.1948	Albanico (800m): Bio-Bio	18.83	24.63	9.27
MNNC	S3270	Tettigades curvicosta	Male	Paratype	-	-	19.21	26.94	12.11
MNNC	S3282	Tettigades limbata	Male	Paratype	17.XII.1947	Lo Valdes: Santiago (1800m)	21.38	25.08	9.52 (wing end)
MNNC	S3283	Tettigades limbata	Male	Paratype	17.XII.1947	Lo Vales: Santiago (1800m)	21.34	24.51	10.63
MNNC	S3275	Tettigades auropilosa	Female	Sintype	XI.1933	Queltehues	22.13	25.85	11.11
MNNC	S3276	Tettigades auropilosa	Male	Sintype	Xi.1933	Queltehues	22.3	24.75	10.63

Table S2. List of field-collected material examined.

Sample_no	Coll_date	Location	Sex	Current Preservation	Tentative morph ID	Clade ID COI	COI ID	Gene Capture?	Capt Seq From	Capture Species ID	Capture Clade ID	Length of body	Forewing Length	Forewing depth
Claudio	end of Nov 2014	Las Condes	F	Spread	chilensis	chilensis	PL318.1	Yes	leg	Chilensis	PL301	-	-	-
JM002	1/4/16	L1: Trapatrapa (Rio Queuco)	F	Spread	ulnaria	NA	NA	Yes	leg	Ulnaria	NA	21.95mm	29.08mm	11.75mm
JM003	1/4/16	L1: Trapatrapa (Rio Queuco)	M	Spread	ulnaria	NA	NA	No	NA	NA	NA	19.65mm	27.16mm	10.74mm
JM004	1/4/16	L1: Trapatrapa (Rio Queuco)	F	Spread	undata	NA	NA	Yes	leg	Undata	Lonquimay	18.73mm	24.76mm	10.09mm
JM005	1/4/16	L1: Trapatrapa (Rio Queuco)	M	Spread	undata	NA	NA	No	NA	NA	NA	17.79mm	24.07mm	10.17mm
JM006	1/4/16	L1: Trapatrapa (Rio Queuco)	M	Head in ETH	ulnaria	NA	NA	No	NA	NA	NA	-	-	-
JM007	1/4/16	L1: Trapatrapa (Rio Queuco)	F	Whole in ETH	undata	NA	NA	No	NA	NA	NA	-	-	-
JM008	1/4/16	L1: Trapatrapa (Rio Queuco)	F	Head in ETH	ulnaria	NA	NA	Yes	bact	Ulnaria	NA	-	-	-
JM009	1/4/16	L1: Trapatrapa (Rio Queuco)	M	Head in ETH	undata	NA	NA	Yes	bact	Undata	Lonquimay	-	-	-
JM010	1/4/16	L1: Trapatrapa (Rio Queuco)	F	Head in ETH	ulnaria	NA	NA	No	NA	NA	NA	-	-	-
JM011	1/4/16	L1: Trapatrapa (Rio Queuco)	M	Head in ETH	undata	NA	NA	No	NA	NA	NA	-	-	-
JM014.1	1/5/16	L3: Antuco alto	M	Head in ETH	undata	NA	NA	Yes	leg	Undata	TETUND	-	-	-
JM015	1/5/16	L4: Rio Cholghuán - Puente la Fábrica	M	Spread	undata	NA	NA	Yes	leg	Undata	TETUND	17.51mm	23.50mm	9.16mm
JM016	1/5/16	L4: Rio Cholghuán - Puente la Fábrica	M	Head in ETH	undata	NA	NA	Yes	bact	Undata	TETUND	-	-	-
JM017	1/5/16	L4: Rio Cholghuán - Puente la Fábrica	M	Head in ETH	undata	NA	NA	No	NA	NA	NA	-	-	-
JM018	1/5/16	L4: Rio Cholghuán - Puente la Fábrica	M	Spread	undata	NA	NA	Yes	leg	Undata	Lonquimay	18.19mm	23.97mm	10.26mm
JM019	1/5/16	L4: Rio Cholghuán - Puente la Fábrica	F	Spread	undata	NA	NA	No	NA	NA	NA	17.37mm	23.07mm	9.12mm
JM020	1/5/16	L3: Antuco alto	M	Spread	ulnaria	NA	NA	Yes	leg	Ulnaria	NA	19.15mm	27.93mm	11.39mm
JM021	1/5/16	L3: Antuco alto	F	Spread	undata	NA	NA	Yes	leg	Undata	Lonquimay	16.12mm	22.58mm	8.78mm
JM022.1	1/5/16	L3: Antuco alto	M	Spread	undata	NA	NA	No	NA	NA	NA	17.40mm	23.45mm	9.22mm
JM022.2	1/5/16	L3: Antuco alto	M	Spread	undata	NA	NA	No	NA	NA	NA	17.29mm	23.60mm	10.03mm
JM023	1/5/16	L3: Antuco alto	M	Head in ETH	undata	NA	NA	No	NA	NA	NA	-	-	-
JM024	1/5/16	L3: Antuco alto	M	Head in ETH	undata	NA	NA	No	NA	NA	NA	-	-	-
JM025	1/5/16	L3: Antuco alto	M	Head in ETH	undata	NA	NA	No	NA	NA	NA	-	-	-
JM026	1/5/16	L3: Antuco alto	M	Head in ETH	undata	NA	NA	No	NA	NA	NA	-	-	-
JM027.1	1/5/16	L3: Antuco alto	M	Spread	undata	NA	NA	No	NA	NA	NA	17.02mm	22.72mm	9.30mm
JM027.2	1/5/16	L3: Antuco alto	M	Spread	undata	NA	NA	No	NA	NA	NA	15.71mm	21.34mm	8.61mm
JM028	1/6/16	L5: Colbún	M	Spread	undata	NA	NA	Yes	leg	Undata	TETUND	17.90mm	23.84mm	10.12mm
JM029	1/6/16	L5: Colbún	M	Head in ETH	undata	NA	NA	No	NA	NA	NA	-	-	-
JM030	1/6/16	L5: Colbún	M	Head in ETH	ulnaria	NA	NA	Yes	leg	Ulnaria	NA	-	-	-
JM031	1/6/16	L5: Colbún	M	Head in ETH	ulnaria	NA	NA	Yes	bact	Ulnaria	NA	-	-	-
JM032	1/6/16	L5: Colbún	M	Head in ETH	undata	NA	NA	Yes	bact	Undata	TETUND	-	-	-
JM033	1/6/16	L5: Colbún	M	Head in ETH	undata	NA	NA	No	NA	NA	NA	-	-	-
JM034	1/6/16	L5: Colbún	M	Head in ETH	undata	NA	NA	No	NA	NA	NA	-	-	-
JM035	1/6/16	L5: Colbún	M	Head in ETH	undata	NA	NA	No	NA	NA	NA	-	-	-
JM036	1/6/16	L5: Colbún	M	Head in ETH	undata	NA	NA	No	NA	NA	NA	-	-	-
JM038	1/7/16	L6: Central Cipreses	M	Spread	undata	NA	NA	Yes	leg	Undata	TETUND	19.04mm	24.52mm	10.11mm
JM039	1/6/16	L6: Central Cipreses	M	Head in ETH	undata	NA	NA	Yes	bact	Undata	TETUND	-	-	-
JM040	1/6/16	L6: Central Cipreses	M	Head in ETH	undata	NA	NA	No	NA	NA	NA	-	-	-
JM041	1/6/16	L6: Central Cipreses	M	Head in ETH	undata	NA	NA	No	NA	NA	NA	-	-	-
JM042	1/6/16	L6: Central Cipreses	M	Head in ETH	undata	NA	NA	No	NA	NA	NA	-	-	-
JM043	1/8/16	L7: Termas del Flaco	M	Spread	undata-like	NA	NA	Yes	leg	Termas	NA	17.79mm	23.48mm	9.55mm
JM044	1/8/16	L7: Termas del Flaco	M	Head in ETH	undata-like	NA	NA	Yes	bact	Termas	NA	-	-	-
KN004	12/11/15	Tahuilco	M	Dried	lacertosa	NA	NA	No	NA	NA	NA	-	-	-
KN005	12/11/15	Tahuilco	M	Dried	lacertosa	NA	NA	No	NA	NA	NA	-	-	-
KN006	12/11/15	Tahuilco	M	Dried	lacertosa	NA	NA	No	NA	NA	NA	-	-	-

KN007	12/11/15	Tahuilco	F	Dried	lacertosa	NA	NA	No	NA	NA	NA	-	-	-
KN008	12/11/15	Tahuilco	M	Dried	lacertosa	NA	NA	No	NA	NA	NA	-	-	-
KN009	12/11/15	Tahuilco	M	Dried	lacertosa	NA	NA	No	NA	NA	NA	-	-	-
KN010	12/11/15	Tahuilco	M	Dried	lacertosa	NA	NA	No	NA	NA	NA	-	-	-
KN011	12/11/15	Tahuilco	M	Dried	auropilosa	NA	NA	No	NA	NA	NA	-	-	-
KN012	12/11/15	Tahuilco	F	Dried	auropilosa	NA	NA	No	NA	NA	NA	-	-	-
KN013	12/11/15	Tahuilco	M	Dried	auropilosa	NA	NA	No	NA	NA	NA	-	-	-
KN014	12/11/15	Tahuilco	M	Dried	auropilosa	NA	NA	No	NA	NA	NA	-	-	-
KN015	1/3/16	Lonquimay	M	Dried	undata	NA	NA	No	NA	NA	NA	-	-	-
KN016	1/3/16	Lonquimay	M	Dried	undata	NA	NA	No	NA	NA	NA	-	-	-
KN017	1/3/16	Lonquimay	M	Dried	undata	NA	NA	No	NA	NA	NA	-	-	-
PL301	12/9/14	Peñalolen	F	Head in ETH	chilensis	chilensis	PL318.1	Yes	Bact	Chilensis	PL301	-	-	-
PL302	12/9/14	Peñalolen	M	Head in ETH	chilensis	chilensis	PL318.1	Yes	Bact	Chilensis	PL301	-	-	-
PL303	12/9/14	Peñalolen	F	Head in ETH	sp. 6	SP.6	311.B1	Yes	bact	Sp.6	NA	-	-	-
PL305	12/9/14	Peñalolen	F	Head in ETH	chilensis	chilensis	PL318.1	Yes	bact	Chilensis	PL301	-	-	-
PL306	12/9/14	Peñalolen	M	Head in ETH	chilensis	chilensis	PL318.1	Yes	bact	Chilensis	PL301	-	-	-
PL307	12/9/14	Peñalolen	M	Head in ETH	chilensis	chilensis	PL318.1	Yes	bact	Chilensis	PL301	-	-	-
PL309	12/9/14	Peñalolen	F	Head in ETH	chilensis	NA	NA	Yes	leg	Chilensis	PL301	-	-	-
PL311.B1	12/9/14	Peñalolen	M	Spread	sp. 6	SP.6	311.B1	Yes	leg	Sp.6	NA	-	-	-
PL311.B2	12/9/14	Peñalolen	M	Spread	chilensis	chilensis	PL318.1	Yes	leg	Chilensis	PL301	-	-	-
PL312	12/9/14	Peñalolen	M	Head in ETH	chilensis	chilensis	PL318.1	Yes	bact	Chilensis	PL301	-	-	-
PL313	12/9/14	Peñalolen	M	Head in ETH	chilensis	chilensis	PL318.1	Yes	bact	Chilensis	PL301	-	-	-
PL314	12/9/14	Peñalolen	F	Head in ETH	chilensis	chilensis	PL318.1	Yes	bact	Chilensis	PL301	-	-	-
PL318.1	12/9/14	Peñalolen	M	Spread	chilensis	chilensis	PL318.1	Yes	leg	Chilensis	PL301	22.42mm	26.46mm	11.32mm
PL319	12/9/14	Peñalolen	M	Head in ETH	chilensis	chilensis	PL318.1	Yes	bact	Chilensis	PL301	-	-	-
PL320	12/9/14	Peñalolen	F	Head in ETH	chilensis	chilensis	PL318.1	Yes	bact	Chilensis	PL301	-	-	-
PL321	12/9/14	Peñalolen	M	Head in ETH	chilensis	chilensis	PL318.1	Yes	bact	Chilensis	PL301	-	-	-
PL322.1	12/10/14	Camino Putaendo-Alicahue 1	M	Head in ETH	chilensis	chilensis	PL318.1	Yes	Leg	Chilensis	PL301	-	-	-
PL326.1	12/10/14	Caleu	F	Spread	ulnaria	ulnaria	PL326.1	Yes	leg	Ulnaria	NA	-	-	-
PL327	12/10/14	Caleu	F	Head in ETH	ulnaria	NA	NA	Yes	bac/leg	Ulnaria	NA	-	-	-
PL331	12/11/14	Camino Putaendo-Alicahue 1	M		distanti	distanti	PL335.1	Yes	bact	Distanti	Distanti	-	-	-
PL332	12/11/14	Camino Putaendo-Alicahue 1	M		distanti	distanti	PL335.1	No	NA	NA	NA	-	-	-
PL334.1	12/11/14	Camino Putaendo-Alicahue 1	M	Head in ETH	distanti	NA	NA	No	NA	NA	NA	-	-	-
PL334.2	12/11/14	Camino Putaendo-Alicahue 1	M	Head in ETH	distanti	NA	NA	No	NA	NA	NA	-	-	-
PL334.3	12/11/14	Camino Putaendo-Alicahue 1	M	Head in ETH	distanti	NA	NA	No	NA	NA	NA	-	-	-
PL335	12/11/14	Camino Putaendo-Alicahue 1	M	Whole in ETH	distanti	NA	NA	No	NA	NA	NA	-	-	-
PL335.1	12/11/14	Camino Putaendo-Alicahue 1	M	Spread (M)	distanti	distanti	PL335.1	Yes	leg	Distanti	Distanti	21.76mm	27.01mm	11.00mm
PL336	12/11/14	Camino Putaendo-Alicahue 1	M	Head in ETH	distanti	NA	NA	No	NA	NA	NA	-	-	-
PL337	12/11/14	Camino Putaendo-Alicahue 1	M	Head in ETH	distanti	NA	NA	No	NA	NA	NA	-	-	-
PL338	12/11/14	Camino Putaendo-Alicahue 1	M	Head in ETH	distanti	NA	NA	No	NA	NA	NA	-	-	-
PL342	12/11/14	Camino Putaendo-Alicahue 2	F	-	limbata	limbata	limbata	Yes	bact	Limbata	NA	-	-	-
PL344.1	12/11/14	Camino Putaendo-Alicahue 2	M	Head in ETH	limbata	NA	NA	No	NA	NA	NA	-	-	-
PL344.2	12/11/14	Camino Putaendo-Alicahue 2	F	Head in ETH	limbata	NA	NA	No	NA	NA	NA	-	-	-
PL344.3	12/11/14	Camino Putaendo-Alicahue 2	F	Head in ETH	limbata	NA	NA	No	NA	NA	NA	-	-	-
PL345	12/11/14	Camino Putaendo-Alicahue 2	F	formaldehyde (B)	limbata	NA	NA	No	NA	NA	NA	-	-	-
PL346.1	12/11/14	Camino Putaendo-Alicahue 2	M	Spread	limbata	Limbata	PL346.1	Yes	leg	Limbata	NA	22.28mm	26.66mm	12.03mm
PL346.2	12/11/14	Camino Putaendo-Alicahue 2	F	Spread	limbata	NA	limbata	Yes	Leg	Limbata	NA	22.92mm	26.71mm	11.46mm
PL353	12/11/14	Camino Putaendo-Alicahue 2	F	-	sp. 6	SP.6	311.B1	Yes	bact	Sp.6	NA	-	-	-
PL358	12/11/14	Camino Putaendo-Alicahue 2	F	Spread	sp.6	SP.6	311.B1	Yes	leg	Sp.6	NA	22.02mm	27.10mm	10.90mm
PL363	12/11/14	Camino Putaendo-Alicahue 2	F		auropilosa	Auropilosa	PL376.1	Yes	bact	Auropilosa	PL363	-	-	-
PL365.1	12/11/14	Camino Putaendo-Alicahue 2	F	Spread	Sp.6	SP.6	311.B1	Yes	leg	Sp.6	NA	-	-	-
PL374	12/11/14	Alicahue	M	-	sp. 6	SP.6	311.B1	Yes	Bact	Sp.6	NA	-	-	-

PL376.1	12/11/14	Alicahue	M	Spread	auropilosa	Auropilosa	PL376.1	Yes	Leg	Auropilosa	PL363	19.60mm	24.24mm	10.01mm
PL376.2	12/11/14	Alicahue	M	Spread	sp.6	SP.6	311.B1	Yes	Leg	Sp.6	NA	20.29mm	23.47mm	9.49mm
PL382	12/11/14	Alicahue	M	-	chilensis	Chilensis	es	Yes	Bact	Chilensis	PL301	-	-	-
PL383	12/11/14	Alicahue	F	-	auropilosa	Auropilosa	PL376.1	Yes	Bact	Auropilosa	PL363	-	-	-
PL388.1	12/11/14	Alicahue	M	Spread	auropilosa	Auropilosa	PL376.1	Yes	Leg	Auropilosa	PL363	-	-	-
PL392.1	12/11/14	Camino Putaendo-Alicahue 3	F	Whole in ETH	sp.6	SP.6	311.B1	Yes	leg	Sp.6	NA	-	-	-
PL404.2	12/11/14	Camino Putaendo-Alicahue 2	M	Head in ETH	chilensis	NA	NA	Yes	leg	Chilensis	PL301	-	-	-
PL405	12/13/14	Bellavista 2	F	Head in ETH	ulnaria	ulnaria	PL514.1	Yes	bact	Ulnaria	NA	-	-	-
PL406	12/13/14	Bellavista 2	F	head in ETH	ulnaria	NA	NA	No	NA	NA	NA	-	-	-
PL413.1	12/13/14	Bellavista 2	F	Spread	ulnaria	ulnaria	PL514.1	Yes	Leg	Ulnaria	NA	19.79mm	28.31mm	10.73mm
PL413.2	12/13/14	Bellavista 2	M	Whole in ETH	ulnaria	NA	NA	No	NA	NA	NA	-	-	-
PL414.1	12/13/14	Bellavista 2	F	Whole in ETH	ulnaria	ulnaria	PL514.1	Yes	Leg	Ulnaria	NA	-	-	-
PL415	12/14/14	Bellavista 1	M	Head in ETH	limbata	limbata	PL346.1	Yes	bact	Limbata	NA	-	-	-
PL416	12/14/14	Bellavista 1	M	Head in ETH	limbata	NA	NA	No	NA	NA	NA	-	-	-
PL418	12/14/14	Bellavista 1	F	Head in ETH	limbata	NA	NA	No	NA	NA	NA	-	-	-
PL419	12/14/14	Bellavista 1	F	Head in ETH	limbata	NA	NA	No	NA	NA	NA	-	-	-
PL426.1	12/14/14	Bellavista 1	M	Whole in ETH	limbata	NA	~limbata	Yes	Leg	Limbata	NA	-	-	-
PL426.2	12/14/14	Bellavista 1	M	Whole in ETH	limbata	Limbata	~limbata	Yes	Leg	Limbata	NA	-	-	-
PL426.3	12/14/14	Bellavista 1	M	Whole in ETH	limbata	NA	NA	No	NA	NA	NA	-	-	-
PL428.1	12/14/14	Termas del Flaco	M	Spread	chilensis	chilensis	PL508.2	No	NA	NA	NA	-	-	-
PL428.1	12/14/14	Termas del Flaco	M	Spread	chilensis	chilensis	PL508.2	Yes	leg	Chilensis	PL433	-	-	-
PL428.2	12/14/14	Termas del Flaco	F	Whole in ETH	limbata	NA	NA	No	NA	NA	NA	-	-	-
PL429.1	12/14/14	Termas del Flaco	F	Whole in ETH	limbata	NA	~limbata	Yes	Leg	Limbata	NA	-	-	-
PL429.2	12/14/14	Termas del Flaco	M	Spread	limbata	Limbata	~limbata	Yes	leg	Limbata	NA	22.07mm	27.18mm	11.59mm
PL433	12/14/14	Termas del Flaco	M	Head in ETH	chilensis	chilensis	es	Yes	bact	Chilensis	PL433	-	-	-
PL435.1	12/14/14	Termas del Flaco	M	Spread	chilensis	chilensis	PL435.1		leg	Chilensis		19.79mm	24.78mm	10.08mm
PL458.1	12/15/14	Termas del Flaco	F	Head in ETH	auropilosa	NA	NA	Yes	Leg	Auropilosa	TETAR	-	-	-
PL459	12/15/14	Termas del Flaco	F	Head in ETH	limbata	NA	NA	No	NA	NA	NA	-	-	-
PL466.1	12/15/14	Camino a las Termas 1	M	Spread	chilensis	chilensis	PL508.2	Yes	leg	Chilensis	PL433	-	-	-
PL466.2	12/15/14	Camino a las Termas 1	M	Spread	chilensis	chilensis	PL508.2	Yes	leg	Chilensis	PL433	-	-	-
PL467.1	12/15/14	Camino a las Termas 2	F	Spread	ulnaria	ulnaria	PL514.1	Yes	leg	Ulnaria	NA	-	-	-
PL467.2	12/15/14	Camino a las Termas 2	M	Spread	undata	undata	PL467.2	Yes	leg	Undata	uncta	-	-	-
PL468	12/15/14	Camino a las Termas 2	M	Whole in ETH	Ulnaria	NA	NA	No	bact	Ulnaria	N/A	-	-	-
PL470	12/15/14	Camino Rancagua-Coya	M	Head in ETH	chilensis	NA	NA	Yes	bact	Chilensis	TETCHI	-	-	-
PL475.1	12/15/14	Camino Rancagua-Coya	F	Spread	chilensis	chilensis	PL475.1	Yes	leg	Chilensis	TETCHI	-	-	-
PL475.2	12/15/14	Camino Rancagua-Coya	F	Spread	sp.6	SP.6	311.B1	Yes	leg	Sp.6	NA	-	-	-
PL475.4	12/15/14	Camino Rancagua-Coya	F	Head in ETH	sp. 6	SP.6	311.B1	Yes	bact	Sp.6	NA	-	-	-
PL475.7	12/15/14	Camino Rancagua-Coya	M	Whole in ETH	sp.6	NA	NA	Yes	leg	Sp.6	NA	-	-	-
PL485.1	12/15/14	Camino Coya-Los Cipreses, puente	F	Spread	undata	undata	PL467.2	Yes	leg	Undata	Undata	20.89mm	26.99mm	11.08mm
PL487	12/15/14	Camino Coya-Los Cipreses, puente	M	-	undata	undata	PL485.1	Yes	bact	Undata	Undata	-	-	-
PL488.1	12/15/14	Camino Coya-Los Cipreses, puente	M	Head in ETH	undata	NA	NA	Yes	Leg	Undata	Undata	-	-	-
PL503.1	12/15/14	Camino Rancagua-Coya	M	Whole in ETH	Chilensis	NA	NA	No	leg	Chilensis	TETCHI	-	-	-
PL503.2	12/15/14	Camino Rancagua-Coya	F	Head in ETH	sp.6	NA	NA	Yes	leg	Sp.6	NA	-	-	-
PL505	12/15/14	Termas del Flaco	F	-	auropilosa	NA	NA	No	NA	NA	NA	-	-	-
PL506	12/15/14	Termas del Flaco	F	Head in ETH	limbata	limbata	PL346.1	Yes	bact	Limbata	NA	-	-	-
PL507.1	12/15/14	Termas del Flaco	M	Whole in ETH	limbata	NA	NA	No	NA	NA	NA	-	-	-
PL507.5	12/15/14	Termas del Flaco	F	Whole in ETH	limbata	NA	NA	No	NA	NA	NA	-	-	-
PL508.1	12/15/14	Termas del Flaco	F	Spread	limbata	NA	NA	Yes	leg	Limbata	NA	-	-	-
PL508.2	12/15/14	Termas del Flaco	F	Spread	chilensis	chilensis	PL508.2	Yes	leg	Chilensis	PL433	-	-	-
PL509.1	12/15/14	Termas del Flaco	F	Spread	auropilosa	Auropilosa	PL509.1	Yes	leg	Auropilosa	TETAR	-	-	-
PL509.3	12/15/14	Termas del Flaco	M	Head in ETH	auropilosa	Auropilosa	PL509.1	Yes	bact	Auropilosa	TETAR	-	-	-
PL509.4	12/15/14	Termas del Flaco	F	Head in ETH	auropilosa	Auropilosa	PL509.1	No	NA	NA	NA	-	-	-

PL514	12/16/14	Camino Embalse el Yeso 1	M	Spread	ulnaria	ulnaria	PL514.1	Yes	leg	Ulnaria	NA	-	-	-
PL605	11/15/15	Rio Choapa A	M	head in ETH	lacertosa	NA	NA	Yes	bact	Lacertosa	Orange	-	-	-
PL609	11/13/15	Camino a Fray Jorge B	F	head in ETH	lacertosa	NA	NA	Yes	leg	Lacertosa	Orange	-	-	-
PL612	11/13/15	Cuesta Andacollo A	F	head in ETH	lacertosa	NA	NA	Yes	leg	Lacertosa	Orange	-	-	-
PL614	11/13/15	Camino a Fray Jorge A	F	head in ETH	lacertosa	NA	NA	Yes	bact	Lacertosa	Orange	-	-	-
PL615	11/13/15	Camino a Fray Jorge A	M	head in ETH	lacertosa	NA	NA	Yes	bact	Lacertosa	Orange	-	-	-
PL616	11/13/15	Camino a Fray Jorge A	F	head in ETH	lacertosa	NA	NA	Yes	bact	Lacertosa	Orange	-	-	-
PL617	11/13/15	Camino a Fray Jorge A	M	head in ETH	lacertosa	NA	NA	Yes	bact	Lacertosa	Orange	-	-	-
PL649	12/11/15	La Higuera	M	whole in ETH	lacertosa	NA	NA	Yes	leg	Lacertosa	Orange	-	-	-
PL655.1	12/11/15	Camino a Fray Jorge A	M	whole in ETH	lacertosa	NA	NA	Yes	bact	Lacertosa	Orange	-	-	-
PL655.2	12/11/15	Camino a Fray Jorge A	M	whole in ETH	lacertosa	NA	NA	Yes	bact	Lacertosa	Orange	-	-	-
PL655.3	12/11/15	Camino a Fray Jorge A	M	whole in ETH	lacertosa	NA	NA	Yes	bact	Lacertosa	Orange	-	-	-
PL655.4	12/11/15	Camino a Fray Jorge A	F	whole in ETH	lacertosa	NA	NA	Yes	bact	Lacertosa	Orange	-	-	-
PL655.5	12/11/15	Camino a Fray Jorge A	M	whole in ETH	lacertosa	NA	NA	Yes	bact	Lacertosa	Orange	-	-	-
PL655.6	12/11/15	Camino a Fray Jorge A	M	whole in ETH	lacertosa	NA	NA	Yes	bact	Lacertosa	Orange	-	-	-
PL661	12/12/15	Tahuilco	F	whole in ETH	lacertosa	NA	NA	Yes	leg	Lacertosa	Orange	-	-	-
PL665	12/12/15	Tahuilco	M	whole in ETH	auropilosa	NA	NA	Yes	leg	Auropilosa	PL363	-	-	-
PL667	12/12/15	Tahuilco	M	Spread	opaca	NA	NA	Yes	leg	Opaca	Opaca	15.30mm	17.00mm	7.07mm
PL668	12/12/15	Tahuilco	F	whole in ETH	auropilosa	NA	NA	Yes	leg	Auropilosa	PL363	-	-	-
PL693	12/12/15	Tahuilco	M	Spread	auropilosa	NA	NA	Yes	leg	Auropilosa	PL363	23.49mm	-	11.37mm
PL695	12/10/15	Las Bandurria	M	Spread	auropilosa	NA	NA	Yes	leg	Auropilosa	PL363	23.42mm	27.92mm	11.68mm
PL696.1	10/27/10	N. Punta de Lobos	M	Head in ETH	lacertosa	NA	lacertosa	Yes	bact	Lacertosa	desert	-	-	-
PL696.3	10/27/10	N. Punta de Lobos	M	Spread	lacertosa	NA	NA	Yes	leg	Lacertosa	desert	27.54mm	29.54mm	11.48mm
PL697	11/6/15	Carrizal Bajo	M	Spread	lacertosa	NA	NA	Yes	leg	Lacertosa	desert	24.84mm	30.73mm	12.58mm
PL698	11/15/15	Rio Choapa B	M	whole in ETH	opaca	NA	NA	Yes	leg	Opaca	Opaca	-	-	-
PL702.1	11/14/15	Salida sur Ovalle	M	whole in ETH	opaca	NA	NA	Yes	leg	Opaca	Opaca	-	-	-
PL702.2	11/14/15	Salida sur Ovalle	M	whole in ETH	opaca	NA	NA	Yes	bact	Opaca	Opaca	-	-	-
PL705	11/14/15	Cuesta Andacollo A	m	whole in ETH	opaca	NA	NA	Yes	leg	Opaca	Opaca	-	-	-
PL707.2	11/15/15	Rio Choapa A	M	whole in ETH	opaca	NA	NA	Yes	leg	Opaca	Opaca	-	-	-
PL726	12/20/15	Alquilhe	M	Spread	distanti	NA	NA	Yes	leg	Distanti	Distanti	22.36mm	29.05mm	13.07mm
PL728	12/20/15	Alquilhe	M	head in ETH	distanti	NA	NA	Yes	bact	Distanti	Distanti	-	-	-
PL729	12/20/15	Alquilhe	M	head in ETH	distanti	NA	NA	No	NA	NA	NA	-	-	-
PL730	12/20/15	Alquilhe	M	-	distanti	NA	NA	No	NA	NA	NA	-	-	-
PL732	12/20/15	Alquilhe	M	whole in ETH	distanti	NA	NA	No	NA	NA	NA	-	-	-
PL736	12/20/15	Cauquenes/Quirihue	M	head in ETH	distanti	NA	NA	No	NA	NA	NA	-	-	-
PL737	12/20/15	Cauquenes/Quirihue	M	head in ETH	distanti	NA	NA	No	NA	NA	NA	-	-	-
PL739	12/20/15	Cauquenes/Quirihue	M	Whole in ETH	Ulnaria	NA	NA	No	leg	Ulnaria	N/A	-	-	-
PL740	12/20/15	Cauquenes/Quirihue	F	Head in ETH	ulnaria	NA	NA	Yes	leg	Ulnaria	NA	-	-	-
PL742	12/20/15	Cauquenes/Quirihue	M	Spread	distanti	NA	NA	Yes	leg	Distanti	Distanti	20.75mm	27.61mm	11.58mm
PL746	12/20/15	Cauquenes/Quirihue	M	whole in ETH	distanti	NA	NA	No	NA	NA	NA	-	-	-
PL747	12/20/15	Cauquenes/Quirihue	M	whole in ETH	distanti	NA	NA	No	NA	NA	NA	-	-	-
PL748	12/20/15	Cauquenes/Quirihue	M	Whole in ETH	Ulnaria	NA	NA	No	leg	Ulnaria	N/A	-	-	-
PL750	12/20/15	Cauquenes/Quirihue	M	Head in ETH	distanti	NA	NA	No	NA	NA	NA	-	-	-
PL751	12/20/15	Cauquenes/Quirihue	M	Head in ETH	distanti	NA	NA	No	NA	NA	NA	-	-	-
PL803	1/3/16	Lonquimay	M	whole in ETH	undata	NA	NA	No	NA	NA	NA	-	-	-
PL804	1/3/16	Lonquimay	M	Head in ETH	undata	NA	undata	Yes	bact	Undata	Lonquimay	-	-	-
PL805	1/3/16	Lonquimay	M	head in ETH	undata	NA	undata	Yes	bact	Undata	Lonquimay	-	-	-
PL807	1/3/16	Lonquimay	M	head in ETH	undata	NA	NA	No	NA	NA	NA	-	-	-
PL808	1/3/16	Lonquimay	M	whole in ETH	undata	NA	NA	No	NA	NA	NA	-	-	-
PL809.1	1/3/16	Lonquimay	F	Spread	undata	NA	NA	Yes	leg	Undata	Lonquimay	17.65mm	22.31mm	9.58mm
PL809.2	1/3/16	Lonquimay	M	Spread	undata	NA	NA	No	NA	NA	NA	16.13mm	21.59mm	9.40mm
PL810	1/3/16	Lonquimay	F	head in ETH	undata	NA	NA	No	NA	NA	NA	-	-	-

PL811	1/3/16	Lonquimay	M	whole in ETH	undata	NA	NA	No	NA	NA	NA	-	-	-
PL811.1	1/3/16	Lonquimay	M	bact extracted	undata	NA	NA	No	NA	NA	NA	-	-	-
PL811.10	1/3/16	Lonquimay	M	bact extracted	undata	NA	NA	No	NA	NA	NA	-	-	-
PL811.11	1/3/16	Lonquimay	M	bact extracted	undata	NA	NA	No	NA	NA	NA	-	-	-
PL811.12	1/3/16	Lonquimay	M	bact extracted	undata	NA	NA	No	NA	NA	NA	-	-	-
PL811.13	1/3/16	Lonquimay	M	bact extracted	undata	NA	NA	No	NA	NA	NA	-	-	-
PL811.14	1/3/16	Lonquimay	M	bact extracted	undata	NA	NA	No	NA	NA	NA	-	-	-
PL811.15	1/3/16	Lonquimay	M	bact extracted	undata	NA	NA	No	NA	NA	NA	-	-	-
PL811.2	1/3/16	Lonquimay	M	bact extracted	undata	NA	NA	Yes	bact	Undata	Lonquimay	-	-	-
PL811.3	1/3/16	Lonquimay	M	bact extracted	undata	NA	NA	Yes	bact	Undata	Lonquimay	-	-	-
PL811.4	1/3/16	Lonquimay	M	bact extracted	undata	NA	NA	Yes	bact	Undata	Lonquimay	-	-	-
PL811.5	1/3/16	Lonquimay	M	bact extracted	undata	NA	NA	Yes	bact	Undata	Lonquimay	-	-	-
PL811.6	1/3/16	Lonquimay	M	bact extracted	undata	NA	NA	Yes	bact	Undata	Lonquimay	-	-	-
PL811.7	1/3/16	Lonquimay	M	bact extracted	undata	NA	NA	Yes	bact	Undata	Lonquimay	-	-	-
PL811.8	1/3/16	Lonquimay	M	bact extracted	undata	NA	NA	Yes	bact	Undata	Lonquimay	-	-	-
PL811.9	1/3/16	Lonquimay	M	bact extracted	undata	NA	NA	Yes	bact	Undata	Lonquimay	-	-	-
PL812	1/3/16	Lonquimay	M	-	undata	NA	NA	No	NA	NA	NA	-	-	-
PL817	1/4/16	Pino Hachado Pass	M	Spread	sarcinatrix	NA	NA	Yes	leg	Sarcinatrix	NA	17.47mm	23.48mm	9.79mm
PL956	1/3/16	Lonquimay	M	Spread	undata	NA	NA	No	NA	NA	NA	18.20mm	22.81mm	10.27mm
PL957	1/3/16	Lonquimay	F	Spread	undata	NA	NA	No	NA	NA	NA	18.53mm	23.48mm	10.24mm
PL970.1	12/30/15	Itahue	M	whole in ETH	ulnaria	NA	NA	Yes	leg	Ulnaria	NA	-	-	-
PL970.10	12/30/15	Itahue	M	whole in ETH	ulnaria	NA	NA	Yes	bact	Ulnaria	NA	-	-	-
PL970.2	12/30/15	Itahue	M	whole in ETH	ulnaria	NA	NA	Yes	bact	Ulnaria	NA	-	-	-
PL970.3	12/30/15	Itahue	M	whole in ETH	ulnaria	NA	NA	Yes	bact	Ulnaria	NA	-	-	-
PL970.4	12/30/15	Itahue	M	whole in ETH	ulnaria	NA	NA	Yes	bact	Ulnaria	NA	-	-	-
PL970.5	12/30/15	Itahue	M	whole in ETH	ulnaria	NA	NA	Yes	bact	Ulnaria	NA	-	-	-
PL970.6	12/30/15	Itahue	M	whole in ETH	ulnaria	NA	NA	Yes	bact	Ulnaria	NA	-	-	-
PL970.7	12/30/15	Itahue	M	whole in ETH	ulnaria	NA	NA	Yes	bact	Ulnaria	NA	-	-	-
PL970.8	12/30/15	Itahue	M	whole in ETH	ulnaria	NA	NA	Yes	bact	Ulnaria	NA	-	-	-
PL970.9	12/30/15	Itahue	M	whole in ETH	ulnaria	NA	NA	Yes	bact	Ulnaria	NA	-	-	-
PL971	12/30/15	Talca-campus	M	Whole in ETH	ulnaria	NA	NA	Yes	leg	Ulnaria	NA	-	-	-
PL972	12/29/15	Villa Allegre	M	Whole in ETH	ulnaria	NA	NA	Yes	leg	Ulnaria	NA	-	-	-
PL973	1/21/16	Talca, Cerro La Virgen	M	Spread	ulnaria	NA	NA	Yes	leg	Ulnaria	NA	17.66mm	24.62mm	9.71mm
PL974	1/21/16	Talca, Cerro La Virgen	M	Spread	ulnaria	NA	NA	Yes	leg	Ulnaria	NA	19.64mm	24.90mm	10.01mm
PL975	1/21/16	Talca, Cerro La Virgen	M	Head in ETH	ulnaria	NA	ulnaria	Yes	bact	Ulnaria	NA	-	-	-
PL976	1/21/16	Talca, Cerro La Virgen	M	in ETH	ulnaria	ulnaria	NA	NA	NA	NA	NA	-	-	-
PL976	1/21/16	Talca, Cerro La Virgen	M	Head in ETH	ulnaria	NA	ulnaria	No	NA	NA	NA	-	-	-