The LINNEAN SOCIETY of London ZOOLOGICAL Journal of the Linnean Society

Zoological Journal of the Linnean Society, 2015, **174**, 733–759. With 13 figures

Species delimitation in the Andean grasshopper genus *Orotettix* Ronderos & Carbonell (Orthoptera: Melanoplinae): an integrative approach combining morphological, molecular and biogeographical data

MARTINA E. POCCO[1,2], CAROLINA MINUTOLO[3], PABLO A. DINGHI[3], CARLOS E. LANGE[2,4], VIVIANA A. CONFALONIERI[3] and MARÍA MARTA CIGLIANO[1,2]*

[1]*División Entomología, Museo de La Plata, Universidad Nacional de La Plata, La Plata, Argentina*
[2]*Centro de Estudios Parasitológicos y de Vectores (CEPAVE), CCT La Plata, CONICET, La Plata, Argentina*
[3]*Departamento de Ecología, Genética y Evolución, Facultad de Ciencias Exactas y Naturales, Universidad de Buenos Aires, Buenos Aires, Argentina*
[4]*Comisión de Investigaciones Científicas, Ministerio de Producción, Ciencia y Tecnología, Provincia de Buenos Aires (CICPBA), Argentina*

Received 21 November 2014; revised 19 January 2015; accepted for publication 26 January 2015

The reciprocal illumination nature of integrative taxonomy through hypothesis testing, corroboration and revision is a powerful tool for species delimitation as more than one source has to support the hypothesis of a new species. In this study, we applied an integrative taxonomy approach combining molecular and morphological data sets with distributional patterns to examine the level of differentiation between and within the grasshopper *Orotettix* species. *Orotettix* was described based on five valid species distributed in the Andes of Peru. In our study, initially a molecular-based hypothesis was postulated and tested against morphological data and geographical patterns of distribution. Results from molecular and morphological analyses showed agreement among the species delimitation in *Orotettix*, and were also consistent with the geographical distribution. The analyses allowed us to delimit five new species for the genus (***O. lunatus* sp. nov.**, ***O. astreptos* sp. nov.**, ***O. colcaensis* sp. nov.**, ***O. paucartambensis* sp. nov. and *O. dichrous* sp. nov.**) from the Eastern and Western Cordilleras of Peru. We also provide critical knowledge on the phylogenetic relationships and distribution of the genus and conduct a revision of *Orotettix*.

doi: 10.1111/zoj.12251

ADDITIONAL KEYWORDS: Acrididae – Andes – integrative taxonomy – male genitalia – mitochondrial DNA.

INTRODUCTION

As species are essential units of analysis in biology, species delimitation is arguably the most fundamental aspect of systematics. However, the delineation of species boundaries continues to be a difficult problem and therefore species criteria, definitions and delimitations occupy one of the most debatable issues in systematics (de Queiroz, 2007; Knowles & Carstens, 2007; Wiens, 2007). Species are sometimes extremely difficult to identify and the problem of species delimitation is even more critical in groups characterized by polymorphisms (Pocco *et al*., 2014) as well as in groups that exhibit little variation, mostly closely related species that are very similar and difficult to distinguish morphologically (Jaiswara *et al*., 2012). Although monophyly usually supports species separation, incongruence has been observed not only between phylogenies based on morphological versus molecular markers (Wiens &

*Corresponding author. E-mail: cigliano@fcnym.unlp.edu.ar

Penkrot, 2002; Sites & Marshall, 2004), but also between molecular markers (mitochondrial versus nuclear DNA) (Shaw, 2002). Therefore, those studies attempting to define species boundaries, particularly in cases of recent speciation events or species that are very similar and difficult to distinguish morphologically, need the consensus of numerous independent criteria (Dayrat, 2005). Lacking standardized operational criteria to delimit species, several studies have stressed the importance of integrative taxonomy, a multisource approach to separate species (Dayrat, 2005; Roe & Sperling, 2007; Schlick-Steiner *et al*., 2010; Heethoff *et al*., 2011; Fujita *et al*., 2012). Under integrative taxonomy, when naming new species, taxonomists should present different sources of evidence to support the hypothesis that a population is evolving independently. Species taxa supported by several independent and concurring kinds of characters could be considered stable hypotheses (Padial & De la Riva, 2009). Any species delimitation depends on a species concept and whichever species concept (Sites & Marshall, 2004; de Queiroz, 2007) is chosen, a delimitation criterion appropriate to that concept is required; and, obviously, the concept used will affect the choice, analysis and interpretation of data. Based on the unified species concept that equates species with separately evolving metapopulation lineages (de Queiroz, 2007), the most reliable species boundaries may be those that are concordant when produced by the analysis of the most relevant data sets, according to species biology and amount of variation, using the most pertinent methods (Jaiswara *et al*., 2012).

In this study we delimit several species of *Orotettix* Ronderos & Carbonell combining morphological and molecular data sets and considering their distributional patterns. The grasshopper genus *Orotettix* is endemic to the Andes of Peru and Bolivia, between latitudes 11° and 15°S. The majority of the species are distributed at the Eastern Cordillera of Peru, but there are also representatives in the Central Highlands and Western Cordillera (Gonzalez & Pfiffner, 2012) and one species is endemic to the Altiplano (Graham, 2009). Species are found between 1970 and 4500 m a.s.l., whereas regional species richness peaks from 2900 to 3500 m a.s.l. (Eades *et al*., 2015).

Orotettix exhibit little variation in body colour and external morphology, and reports on taxonomy and geographical distribution of the five known brachypterous species are scarce and mostly based on the original descriptions (Bruner, 1913; Ronderos & Carbonell, 1994; Franco, 2013). New collections of *Orotettix* from the Andes of Peru and Bolivia have resulted in the discovery of specimens that we were not able to assign to any of the known species of the genus. Moreover, further clarification is needed on the geographical distribution of the species because the limits of their sympatry are not clearly defined.

In the present study we use molecular and morphological characters to examine the level of differentiation between and within the *Orotettix* species. The results obtained allow us to delimit five new species of the genus, bring to light new morphological characters in *Orotettix* male genitalia and provide critical knowledge on the phylogenetic relationships and distribution of the genus. Based on results of our species delimitation analyses, we conduct a revision of *Orotettix*.

MATERIAL AND METHODS

Studied material

Most material (583 specimens) examined originates from several surveys conducted by Cigliano and Lange in the Andes of Bolivia and Peru during 2003, 2007, 2008 and 2013, collected from 42 locations. Samples representing all species of *Orotettix* were collected for analyses except *O. laevis*. For this taxon, type locality could not be detected due to vague locality records and the species was not found in the surveyed area. Legs of specimens were stored in absolute ethanol for DNA analysis. Specimens and DNA extracts were kept as vouchers in the entomological collection at the Museo de la Plata in Argentina (MLPA). In addition, type specimens and other material of the five previously described *Orotettix* species held in the Museo de La Plata collection were examined in the morphological studies.

Morphological studies

Descriptions and diagnosis of the species are based mostly on male specimens because *Orotettix* females are very difficult to separate, and thus identification can be made only by association with males collected at the same time and place. To study male genitalia, dry specimens were relaxed in a humid chamber and abdomen terminalia moistened with ammonia to speed up the process. Genitalia were then pulled off from the body using a finely hooked pin, cleared in potassium hydroxide and stored in glycerine. Terminology for external morphology and male genitalia follows Otte (1981) and Amédégnato (1976), respectively.

High-resolution photographs of the habitus were captured with a Canon EOS Rebel digital camera. Images of the distal segments of the abdomen and phallic complex were captured with a Micrometrics digital camera attached to a Nikon SMZ1000 stereomicroscope. The program Combine Z5.3 (Hadley, 2006) was used for focus stacking.

Measurements are given in millimetres. Body length was measured from the fastigium verticis to the end of the abdomen. Prozona and metazona of the pronotum and tegmina were measured along the midline from

the front to hind margin. Length of hind femur was measured from the dorso-proximal lobe to the distal extremity.

Electronic content and hyperlinks

All relevant information including digital images and geo-referenced records displayed in Google Maps has been integrated into Orthoptera Species File (OSF, http://orthoptera.speciesfile.org) for each species, following procedures described by Cigliano & Eades (2010). The OSF LSIDs (Life Science Identifiers) for each species with embedded hyperlinks to the taxon page in OSF are included in the paper. LSIDs can be resolved and the associated information viewed through any standard LSID resolver.

Phylogenetic analyses

Specimens included in the phylogenetic analyses

Fifty-six *Orotettix* specimens were included in the phylogenetic analyses, sampled across different locations indicated in Table 1 and Figure 1. Specimens representing all the surveyed localities were selected for the analyses including representatives from the already known species of the genus (except for *O. laevis*). Three specimens of Dichroplini species were also analysed and included as outgroups: *Dichroplus fuscus* (Thunberg), *Coyacris collis* Ronderos and *Coyacris saltensis* Ronderos. *Ronderosia forcipata* (Rehn) [Colombo *et al*., 2005; GenBank accession number (AN) DQ083468.1] was also included and selected to root the trees.

DNA extraction and sequencing

Genomic DNA was obtained from the 56 individuals of *Orotettix* indicated in Table 1 and from the outgroup species *Dichroplus fuscus* (Thunberg), *Coyacris collis* Ronderos and *Coyacris saltensis* Ronderos, using a Qiagen DNeasy Blood and Tissue Kit. We amplified one mitochondrial fragment of the COI gene (Cytochrome C Oxidase subunit I) using a standard PCR protocol. The following sequences were used as primer F, GGAGGATTTGGAAATTGATTAGTTCC and R, GCTAATCATCTAAAAATTTTAATTCCTGTTGG (Normark, 1996). Previous studies in Acrididae (Guzman & Confalonieri, 2010; Husemann *et al*., 2013) have demonstrated that this molecular marker is informative and useful for comparison within and between species.

PCR amplification reactions were performed as follows. In a 50-μL final volume reaction, 33.8 μL deionized H_2O, 5 μL of 10× PCR buffer, 3 μL $MgCl_2$, 5 μL dNTP mixture (0.2 μM each) and 0.2 μL Taq Polymerase (Invitrogen). Amplification was carried out in a thermal cycler (Applied Biosystems) under the following temperature profile: 94 °C for 4 min, followed by 33 cycles of 94 °C for 1 min, 52 °C for 45 s and 72 °C for 1 min, with a final step of 72 °C for 10 min.

PCR products were visualized on a 1% agarose gel stained with Gel Red (0.1×, Biotium) and purified with an Accuprep PCR Purification Kit (Bionner Corp). The purified PCR products were sequenced at the 'Unidad de Secuenciación y Genotipificado' FCEyN, UBA, Buenos Aires, Argentina. Sequences were inspected and aligned using Sequencher v 4.5 (Gene Codes). Sequences were deposited at GenBank (Table 1).

Morphological characters and data matrix

Morphological characters comprised structures from the pronotum (two), tegmina (three), male external (two) and internal genitalia (eleven) and coloration (one). The morphological characters and their states are listed in Appendix 1 and illustrated in Figures 6–13. The data matrix is presented in Table 3.

Data analyses

Phylogenetic analysis of molecular characters

Phylogenetic analyses of molecular characters were performed employing Bayesian (BA) and maximum-parsimony (MP) searching criteria. The MP analysis was performed with the program TNT (Goloboff, Farris & Nixon, 2003a) and Bayesian analyses were applied using the 'metropolis-coupled Markov chain Monte Carlo' (MC3) algorithm implemented in MRBAYES v. 3.1.2 (Huelsenbeck & Ronquist, 2001; Ronquist & Huelsenbeck, 2003). MP heuristic searches were performed through 'TBR branch swapping' applied to a series of 100 random addition sequences, retaining ten cladograms per replicate. Support for individual nodes was assessed by calculation of bootstrap values (1000 replicates). Two independent analyses were run under Bayesian searches, with a random starting tree over 1000 000 generations, with a sample frequency of 1000. The model of sequence evolution for COI data partition was the general time-reversible (GTR; Lanave *et al*., 1984; Tavaré, 1986; Rodríguez *et al*., 1990) model. Rates were assumed to vary across sites according to a gamma distribution (G; Yang, 1994) with a proportion of invariable sites (I; Gu, Fu & Li, 1995) (GTR+I+G). Tree space was explored using four chains [one cold and three incrementally heated chains, with temperature (T) set to 0.20]. Stationarity of the cold Markov chain was checked with TRACER 1.5 (Rambaut & Drummond, 2007), in addition to the standard deviation of the split frequencies. All posterior samples of a run prior to the burn-in point were discarded. The remaining trees were used to construct a 50% majority-rule consensus tree with mean branch length estimates. The frequency of all bipartitions was estimated to assess the support of each node (Huelsenbeck & Ronquist, 2001).

Table 1 List of specimens analysed including ID, geographical location, altitude, species name based on the species delimitation analyses performed in this study, and accession number

Specimen ID	Geographical coordinates and location	Altitude (m)	Species name	Accession number
C1	13°15′32.1″S, 72°15′42.6″W – Cusco (Ollantaytambo), Perú	2964	*O. carrascoi*	KP099673
C3	13°37′14.9″S, 71°42′28.1″W – Cusco (Ruinas Pikillaqta), Perú	3191	*O. andeanus*	KP099695
C4	13°18′58.3″S, 74°26′48.6″W – Ayacucho (5 km from 'Bosque de Piedra' Huaraca), Perú	3837	*O. ceballosi*	KP099678
C6	12°57′11.70″S, 74°01′09.2′W – Ayacucho (Tambo), Perú	3429	*O. ceballosi*	KP099691
C7	11°31′04.5″S, 75°38′37.2″W – Junín (17 km from Tarma to Jauja), Perú	3835	*O. ceballosi*	KP099683
C9	13°33′02.3″S, 72°44′0.3″W – Apurimac (Curahuasi), Perú	2709	***O. dichrous* sp. nov.**	KP099663
C10	13°31′06″S, 71°58′41″W – Cusco (Pisac), Perú	2900	*O. andeanus*	KP099702
C11	13°22′55.3″S, 71°54′49.4″W – Cusco (between Coya and Lamay), Perú	2950	*O. andeanus*	KP099693
C12	13°16′25.3″S, 72°10′28″W – Cusco (Yanahuara), Perú	2981	*O. andeanus*	KP099699
C13	13°19′25.8″S, 72°04′04″W – Cusco (detour Tamillopata), Perú	3223	*O. andeanus*	KP099701
C14	13°37′39.8″S, 71°43′35.2″W – Cusco (Lucre), Perú	3112	*O. andeanus*	KP099696
C15	13°05′12.6″S, 71°48′08.6″W – Cusco (Tipon), Perú	3184	*O. andeanus*	KP099700
C16	13°29′21.4″S, 72°03′41.1″W – Cusco (Cachimayo, outskirts of Poroy), Perú	3448	*O. andeanus*	KP099704
C17	13°33′50.9″S, 72°35′29.1″W – Cusco (between Cusco and Apurimac), Perú	1971	***O. astreptos* sp. nov.**	KP099655
C18	13°33′02.3″S, 72°44′0.3″W – Apurimac (Curahuasi), Perú	2709	***O. dichrous* sp. nov.**	KP099664
C19	13°20′20.1″S, 72°06′40.3′W – Cusco (Maras), Perú	3407	*O. andeanus*	KP099703
C20	12°42′47.4″S, 74°54′04.5″W – Huancavelica (24 km Huancavelica from Huancayo), Perú	4074	*O. ceballosi*	KP099692
C21	12°42′47.4″S, 74°54′04.5″W – Huancavelica ('Bosque de Piedra' Sachapite), Perú	4177	*O. ceballosi*	KP099684
C22	13°25′27″S, 71°51′28″W – Cusco (Pisac), Perú	2900	*O. andeanus*	KP099705
C24	13°16′25.3″S, 72°10′28″W – Cusco (Yanahuara), Perú	2981	*O. andeanus*	KP099707
C32	12°57′58.3″S, 74°05′39.6″W – Ayacucho (between Tambo and Ayacucho), Perú	4144	*O. ceballosi*	KP099682
C33	12°57′49.5″S, 74°05′19.5″W – Ayacucho (between Tambo and Ayacucho), Perú	4121	*O. ceballosi*	KP099680
C34	12°57′11.7″S, 74°01′09.2″W – Ayacucho (Tambo), Perú	3429	*O. ceballosi*	KP099679
C35	12°57′58.3″S, 74°05′39.6″W – Ayacucho (between Tambo and Ayacucho), Perú	4144	*O. ceballosi*	KP099677
C36	12°57′58.3″S, 74°05′39.6″W – Ayacucho (between Tambo and Ayacucho), Perú	4144	*O. ceballosi*	KP099690
C37	13°22′07.42″S, 74°11′14.9″W – Ayacucho (from Toccto to Condorcocha), Perú	4035	*O. ceballosi*	KP099685
C41	12°56′05.9″S, 74°01′29.6″W – Ayacucho (from Tambo to San Francisco), Perú	3129	*O. ceballosi*	KP099689
C46	12°57′11.7″S, 74°01′09.2″W – Ayacucho (Tambo), Perú	3429	*O. ceballosi*	KP099687
C48	12°56′05.9″S, 74°01′29.6″W – Ayacucho (from Tambo to San Francisco), Perú	3129	*O. ceballosi*	KP099688
C51	12°56′05.9″S, 74°01′29.6″W – Ayacucho (from Tambo to San Francisco), Perú	3129	*O. ceballosi*	KP099686
C52	13°26′59.6″S, 74°11′38.3″W – Ayacucho (Condorcocha), Perú	3652	*O. ceballosi*	KP099681
C56	13°37′39.8″S, 71°43′35.2″W – Cusco (Lucre), Perú	3112	*O. andeanus*	KP099694
C57	13°17′19.5″S, 71°35′57.6″W – Cusco (Parpacalla in the outskirts of Paucartambo), Perú	2943	***O. paucartambensis* sp. nov.**	KP099660
C58	13°17′19.5″S, 71°35′57.6″W – Cusco (Parpacalla in the outskirts of Paucartambo), Perú	2943	***O. paucartambensis* sp. nov.**	KP099661
C85	13°34′04.9″S, 72°49′19.9″W – Apurimac (39 km from Curahuasi to Abancay), Perú	3920	***O. dichrous* sp. nov.**	KP099666
C86	13°30′59.9″S72°31′16.7″W – Cusco (20 km from Curahuasi to Cusco), Perú	2751	***O. astreptos* sp. nov.**	KP099656
C87	13°31′08.0″S, 72°49′11″W – Apurimac (Cachora), Perú	2768	***O. dichrous* sp. nov.**	KP099672
C88	13°31′08.0″S, 72°49′11″W – Apurimac (Cachora), Perú	2768	***O. dichrous* sp. nov.**	KP099669
C90	13°31′08.0″S, 72°49′11″W – Apurimac (Cachora), Perú	2768	***O. dichrous* sp. nov.**	KP099671
C95	13°34′04.9″S, 72°49′19.9″W – Apurimac (39 km from Curahuasi to Abancay), Perú	3920	***O. dichrous* sp. nov.**	KP099665
C96	13°36′43.3″S, 72°51′51″W – Apurimac (3 km from Abancay to Cusco), Perú	2690	***O. lunatus* sp. nov.**	KP099652
C98	13°36′43.3″S, 72°51′51″W – Apurimac (3 km from Abancay to Cusco), Perú	2690	***O. lunatus* sp. nov.**	KP099653
C99	13°36′43.3″S, 72°51′51″W – Apurimac (3 km from Abancay to Cusco), Perú	2690	***O. lunatus* sp. nov.**	KP099654
C101	13°31′08.0″S, 72°49′11″W – Apurimac (Cachora), Perú	2768	***O. dichrous* sp. nov.**	KP099668
C103	13°32′57.7″S, 72°45′29.3″W – Apurimac (10 km from Curahuasi to Abancay), Perú	3084	***O. dichrous* sp. nov.**	KP099670
C104	13°11′52.8″S, 71°38′30.9″W – Cusco (20 km from Paucartambo to Pillahuata, mountain pass Ajanacu), Perú	3464	***O. paucartambensis* sp. nov.**	KP099662
C105	13°34′04.9″S, 72°49′19.9″W – Apurimac (39 km from Curahuasi to Abancay), Perú	3920	***O. dichrous* sp. nov.**	KP099667
C107	13°29′21.4″S, 72°03′41.1″W – Cusco (Cachimayo, outskirts of Poroy), Perú	3448	*O. andeanus*	KP099697
C119	13°25′44.3″S, 71°50′45.4″W – Cusco (Pisac), Perú	3078	*O. andeanus*	KP099698
C121	13°23′22.37″S, 72°02′49.67″W – Cusco (Chinchero), Perú	3700	*O. andeanus*	KP099706
C123	14°46′41″S, 70°43′16″W – Puno (Chuquibambilla, 20 km from Ayaviri), Perú	3927	*O. hortensis*	KP099675
C126	15°37′50″S, 71°39′0.00″W – Arequipa (Valle del Colca, Coporaque), Perú	3541	***O. colcaensis* sp. nov.**	KP099659
C127	15°42′20.3″S, 70°05′47.9″W – Puno (Atuncolla in road to 'Chulpas de Silustani'), Perú	3823	*O. hortensis*	KP099676
C128	13°15′32.1″S, 72°15′42.6″W – Cusco (Ollantaytambo ruins), Perú	2964	*O. carrascoi*	KP099674
C794	15°37′92″S, 71°51′14.6″W – Arequipa (Valle del Colca, Pinchollo), Perú	3707	***O. colcaensis* sp. nov.**	KP099657
C815	15°37′50″S, 71°39′00″W – Arequipa (Valle del Colca, Coporaque), Perú	3541	***O. colcaensis* sp. nov.**	KP099658
C_collis	18°03′30.9″S, 63°54′35.6″W – Santa Cruz (Samaipata, PN Amboró), Bolivia	2323	*Coyacris collis*	KP099709
C_saltensis	23°41′2.92′S, 64°53′57.79′W – Jujuy (PN Calilegua, Monolito), Argentina	1700	*Coyacris saltensis*	KP099708
Dichroplus	26°24′21.49′S, 54°26′30.94′W – Misiones (Ruta 17, 20 km E El Dorado), Argentina	230	*Dichroplus fuscus*	KP099710

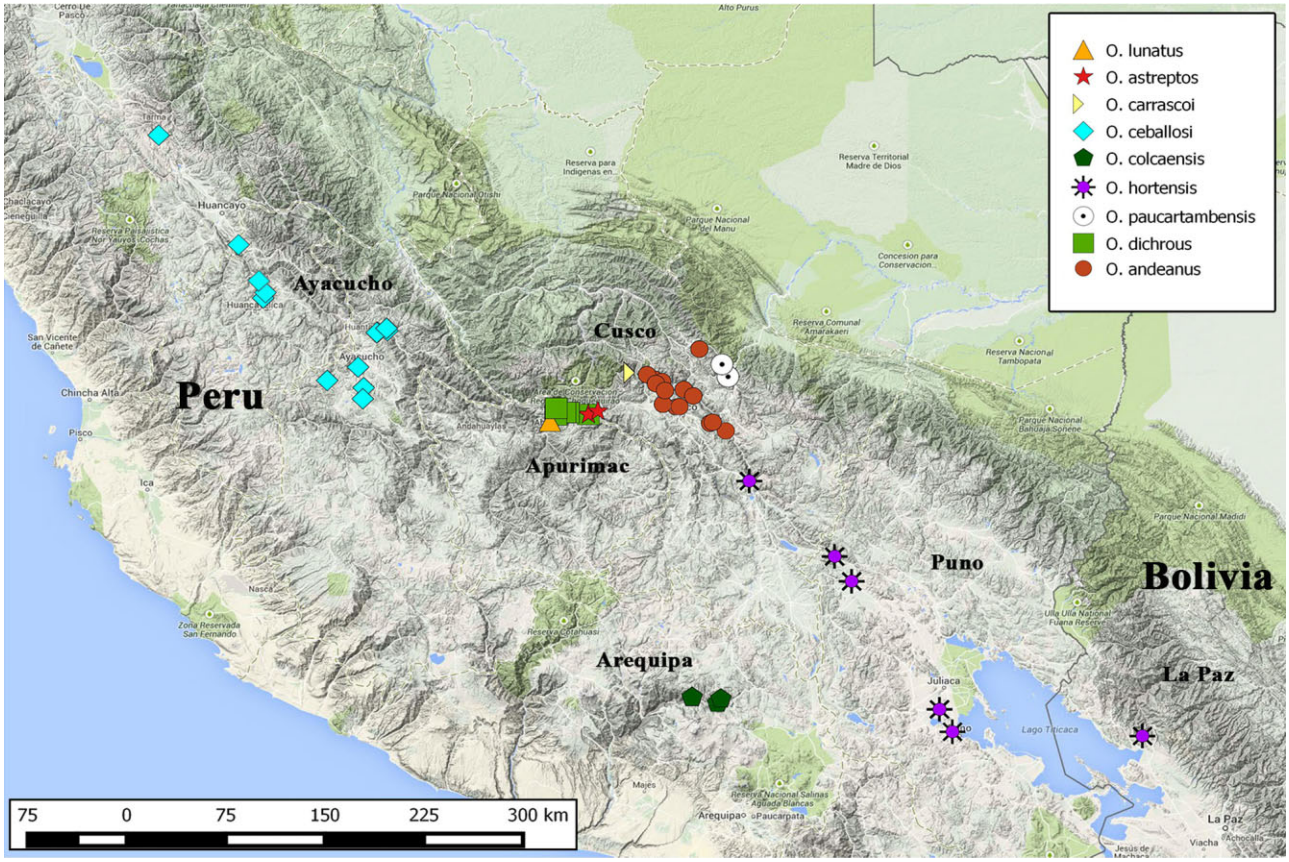

Figure 1 Geographical distribution of *Orotettix* species (except for *O. laevis*), considering all specimens examined in this study. The map was produced with QGIS 2.4 Chugiak.

General Mixed Yule coalescent (GMYC) model

We applied the GMYC method for species delimitation based on molecular characters (Pons *et al*., 2006; Fontaneto *et al*., 2007). GMYC is a likelihood method for delimiting species by fitting within- and between-species branching models to reconstruct single-locus gene trees (Fujisawa & Barraclough, 2013). It is a likelihood method based on a single-locus tree. It assumes that the branching points in the tree correspond to one of two events: divergence events between species-level taxa (modelled by a Yule process), or coalescent events between lineages sampled from within species (modelled by the coalescent). Because the rate of coalescence within species is expected to be dramatically greater than the rate of cladogenesis, the GMYC aims to find the demarcation between these two types of branching. The likelihood of the null model that all samples belong to a single species is compared with that of the alternative model that separates coalescent groups nested within the species tree through a likelihood ratio test. Confidence limits are provided which correspond to threshold values ± 2 log L units around the ML estimate. The point of highest likelihood of this mixed model (threshold) can be interpreted as the species boundary (Pons *et al*., 2006) and is the most likely position in which a shift between the two processes has occurred. For GMYC we obtained an ultrametric tree including only the non-identical haplotypes of COI sequences under a strict molecular clock using BEAST v. 1.6.1 (Drummond & Rambaut, 2007). Tree prior was set to coalescent constant size. A Markov chain Monte Carlo run with 10 million generations and sampling every 1000 generations was performed. Burn-in was determined with Tracer 1.5.0 (Rambaut & Drummond, 2007). The maximum clade credibility tree was found using TreeAnnotator (Drummond & Rambaut, 2007) with all options set to default and imported into the statistics software R 2.15.1 (http://cran.r-project.org). We used the script available within the SPLITS package for R (http://r-forge.r-project.org/projects/splits/).

Cladistic analysis of morphological characters

The dataset was analysed using two procedures: (i) equally weighted character analysis and (ii) implied weighting method (Goloboff, 1993). Under the implied weighting criterion the existing character conflicts in the dataset are resolved in favour of the characters

with lower homoplasy by searching trees with a maximum total fit. We repeated this analysis with concavity (*K*) values of 1–30. All tree searches were conducted in TNT (Goloboff *et al*., 2003a). Under the heuristic procedure which consisted of 'TBR branch swapping' applied to a series of 100 random addition sequences, retaining ten cladograms per replicate, multistate characters were treated as unordered. Support for individual nodes was assessed by calculation of absolute Bremer support (Bremer, 1994) and bootstrap support (100 replicates) for the equally weighted analysis, and symmetric resampling (change probability = 33), which is not distorted by weights (Goloboff *et al*., 2003b), was used for the implied weighting analysis, with 500 replicates (Goloboff *et al*., 2003b).

Phylogenetic analysis based on combined characters

Phylogenetic analysis among the species of *Orotettix* was performed with TNT (Goloboff *et al*., 2003a) under MP using both molecular and morphological data sets simultaneously. Morphological characters considered as autapomorphies (ch. 13–18) were excluded from this analysis. The heuristic search procedure consisted of 'TBR branch swapping' applied to a series of 100 random addition sequences, retaining ten trees per replicate. We applied an extended implied weighting strategy (Goloboff, 2014). This approach allows a better treatment of data sets because implied weighting can be applied only to morphological characters, leaving molecular characters with constant weight. Extended implied weighting was applied using 'xpiwe/4x1L' (L = molecular set). To estimate the support of each node, symmetric resampling (change probability = 33) with 100 replicates was used, which is not distorted by weights (Goloboff *et al*., 2003b).

RESULTS

Phylogenetic analysis of molecular characters

Alignment of 60 COI sequences produced a matrix of 672 molecular characters. Fifty-five different haplotypes were identified (Table 2) within *Orotettix*; all specimens represented different haplotypes except for two individuals of *O. carrascoi*. *Orotettix ceballosi* displayed the highest average value of evolutionary divergence over all sequence pairs (Table 2).

Bayesian analysis of the data matrix (Fig. 2) reveals that *Orotettix* does not resolve as monophyletic. *Orotettix andeanus*, *O. carrascoi*, *O. hortensis* and *O. ceballosi* are recovered as monophyletic groups. However, *O. ceballosi* splits into three groups with high PP, with longer branch lengths compared with other groups in the tree. The remaining 21 *Orotettix* specimens were grouped into five clades, all of them with high PP values.

Table 2 Molecular variability within *Orotettix* species

Species	*N*	*Hp*	*V*	*Pi*	*S*	*D*
O. andeanus	15	15	40	9	31	0.0112
O. ceballosi	16	16	76	41	35	0.0367
O. hortensis	2	2	11	–	–	0.0188
O. carrascoi	2	1	0	–	–	0
O. sp. 1	10	10	20	2	18	0.0072
O. sp. 2	2	2	11	–	–	0.0183
O. sp. 3	3	3	5	0	5	0.0062
O. sp. 4	3	3	9	0	8	0.0087
O. sp. 5	3	3	15	0	13	0.0169

N = number of individuals. *Hp* = number of different haplotypes. *V* = number of variable nucleotide sites. *Pi* = number of parsimony-informative sties. *S* = number of singletons. *D* = average evolutionary divergence over all sequence pairs.

One clade (hereafter *O.* sp. 5) with high support comprised specimens c57, c58 and c104 from Cusco (Paucartambo), collected between 2943 and 3464 m altitude (Fig. 1, Table 1). A second clade (hereafter *O.* sp. 3) included specimens c126, c794 and c815 from Arequipa inhabiting localities between 3500 and 3707 m (Fig. 1, Table 1). The remaining clades correspond to specimens from Cusco and Apurimac endemic to the Eastern Cordillera. Specimens c96, c98 and c99 from Apurimac, Abancay, resolve as monophyletic with maximum posterior probability (hereafter considered as *O.* sp. 4). Another clade is formed by specimens c17 and c86 (hereafter *O.* sp. 2) from Cusco collected at altitudes between 1970 and 2751 m. The remaining clade comprised individuals c9, c18, c85, c87, c88, c90, c95, c101, c103 and c105 (hereafter *O.* sp. 1) from Apurimac (Cachora, Curahuasi) collected between 2700 and 3920 m (Fig. 1, Table 1).

MP searches gave similar results to the BA. Forty most parsimonious trees were obtained of length 454 steps. The topology of the strict consensus tree shows that *O. andeanus*, *O. carrascoi*, *O. hortensis*, *O.* sp. 1, *O.* sp. 2, *O.* sp. 3, *O.* sp. 4 and *O.* sp. 5 were also recovered as monophyletic and with high bootstrap support (Fig. 2), except for *O. ceballosi*, which was also monophyletic but with very low support. The genus *Orotettix* was not recovered as monophyletic.

GYMC model

The GMYC model provided a significantly better fit to the data than the null model's hypothesis of the entire sample being derived from a single species with uniform branching (LLNull Model = 380.815, LLGMYC Model = 385.5815, Likelihood ratio = 9.531721, $P = 0.008515559^{**}$). It identified 16 clusters

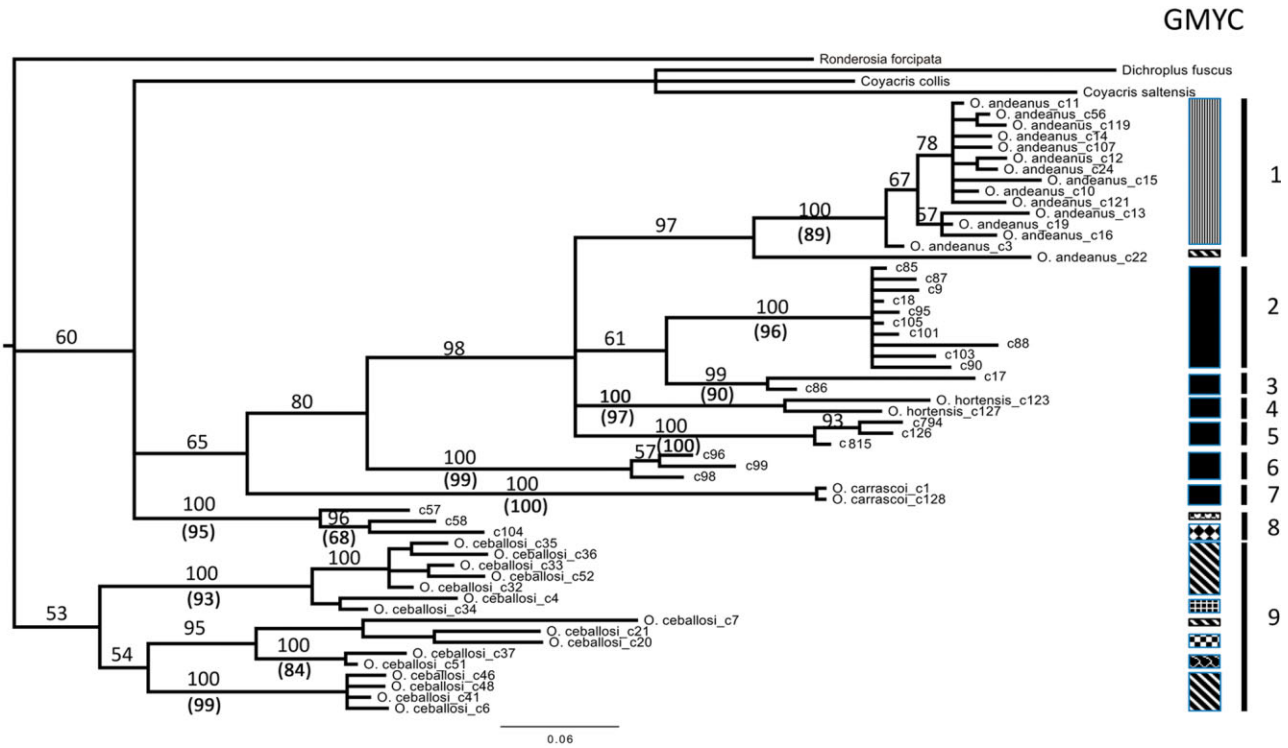

Figure 2 Bayesian phylogenetic analysis of COI characters. Acronyms of specimens according to Table 1. Numbers on branches indicate posterior probabilities. Numbers in parentheses indicate bootstrap supports of maximum-parsimony analysis. Results of General Mixed Yule-coalescent (GMYC) analysis are represented as lateral bars with different line patterns. Each bar indicates a different cluster identified by GMYC. Solid black patterns indicate clusters which coincide with species delimitation based on results from the other molecular, morphological and geographical analyses. 1, *O. andeanus*; 2, *O.* sp. 1; 3, *O.* sp. 2; 4, *O. hortensis*; 5, *O.* sp. 3; 6, *O.* sp. 4; 7, *O. carrascoi*; 8, *O.* sp. 5; 9, *O. ceballosi*.

(confidence intervals 10–16), which are represented in Figure 2. Coincidently with the previous molecular analysis, *O. hortensis*, *O. carrascoi*, *O.* sp. 1, *O.* sp. 2, *O.* sp. 4 and *O.* sp. 3 were recovered as separate evolutionary units. All specimens identified as *O. andeanus* were clustered together, except for c22 (Fig. 2). *O.* sp. 5 is recognized as a separate cluster from the remaining specimens but splits into two clusters; and finally, *O. ceballosi* is divided into six clusters, in agreement with its high level of 'interspecific' variability. Therefore, results suggest the existence of at least four new entities, which could represent different species (*O.* sp. 1, *O.* sp. 2, *O.* sp. 3 and *O.* sp. 4). According to this analysis, specimens belonging to *O.* sp. 5 would also represent a separate cluster with respect to the other specimens, although it splits into two units.

Cladistic analysis of morphological characters

Parsimony analysis under equal weights of the morphological data matrix (Table 3) resulted in one most parsimonious tree (Fig. 3) of length 55 [consistency index (CI), 0.87; retention index (RI), 0.97]. The same relationships were obtained under implied weighting (on trees with $K = 1–30$) with an increase of K from 1 to 30 yielding no change in topology. *Orotettix* was found to be monophyletic with high bootstrap support (86%). Most specimens clustered in the molecular analysis rendered monophyletic groups. The species *O. andeanus* from the Eastern Cordillera is defined by tegmina length (character 17:2 from Appendix 1); *O. hortensis* from the Altiplano is grouped by the conspicuous lateral carinae of pronotum and the colour of hind tibiae (characters 15:1 and 18:6 from Appendix 1, respectively); *O. ceballosi* from the Central Highlands is based on the development of sheath of aedeagus (character 10:1 from Appendix 1); and *O. carrascoi* from the Eastern Cordillera is defined by the hind tibiae bright red (character 18:5 from Appendix 1) (Fig. 3). The remaining 21 *Orotettix* specimens were grouped into four clades. *O.* sp. 5, from the Eastern Cordillera, is recovered and defined by tegmina length (character 17:2 from Appendix 1) and the colour of hind tibiae (character 18:7 from

Table 3 Data matrix for the 19 morphological characters

	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
Ronderosia_forcipata	0	0	0	0	0	0	0	0	0	0	0	0	0	?	0	0	?	0	0
Dichroplus_fuscus	0	0	0	1	1	5	0	1	1	0	2	0	0	?	0	0	?	0	1
Coyacris_collis	1	1	1	2	1	5	0	1	1	1	2	0	2	?	0	0	?	2	2
Coyacris_saltensis	1	1	1	2	1	5	0	1	1	1	2	0	2	?	0	0	?	2	3
O_andeanus_c11	1	2	0	3	2	1	1	2	2	2	0	0	1	?	0	0	0	1	2
O_andeanus_c56	1	2	0	3	2	1	1	2	2	2	0	0	1	?	0	0	0	1	2
O_andeanus_c3	1	2	0	3	2	1	1	2	2	2	0	0	1	?	0	0	0	1	2
O_ceballosi_c35	1	2	0	4	5	4	1	5	2	1	1	1	2	?	0	0	?	2	[23]
O_sp_1_c85	1	3	0	3	2	2	1	3	2	2	0	0	1	?	1	0	?	2	4
O_sp_5_c57	1	2	0	4	5	4	1	5	2	1	0	1	2	?	0	0	?	1	7
O_sp_1_c87	1	3	0	3	2	2	1	3	2	2	0	0	1	?	1	0	?	2	4
O_sp_5_c58	1	2	0	4	5	4	1	5	2	1	0	1	2	?	0	0	?	1	7
O_hortensis_c123	1	2	0	5	4	4	1	4	2	0	0	1	2	?	0	1	?	2	6
O_sp_3_c794	1	2	0	5	4	3	1	4	2	2	1	0	1	?	0	0	?	1	6
O_sp_3_c815	1	2	0	5	4	3	1	4	2	2	1	0	1	?	0	0	?	1	6
O_andeanus_c14	1	2	0	3	2	1	1	2	2	2	0	0	1	?	0	0	0	1	2
O_andeanus_c107	1	2	0	3	2	1	1	2	2	2	0	0	1	?	0	0	0	1	2
O_andeanus_c119	1	2	0	3	2	1	1	2	2	2	0	0	1	?	0	0	0	1	2
O_andeanus_c12	1	2	0	3	2	1	1	2	2	2	0	0	1	?	0	0	0	1	2
O_andeanus_c15	1	2	0	3	2	1	1	2	2	2	0	0	1	?	0	0	0	1	2
O_andeanus_c13	1	2	0	3	2	1	1	2	2	2	0	0	1	?	0	0	0	1	2
O_andeanus_c10	1	2	0	3	2	1	1	2	2	2	0	0	1	?	0	0	0	1	2
O_andeanus_c19	1	2	0	3	2	1	1	2	2	2	0	0	1	?	0	0	0	1	2
O_andeanus_c16	1	2	0	3	2	1	1	2	2	2	0	0	1	?	0	0	0	1	2
O_andeanus_c22	1	2	0	3	2	1	1	2	2	2	0	0	1	?	0	0	0	1	2
O_sp_1_c9	1	3	0	3	2	2	1	3	2	2	0	0	1	?	1	0	?	2	4
O_sp_1_c18	1	3	0	3	2	2	1	3	2	2	0	0	1	?	1	0	?	2	4
O_sp_1_c95	1	3	0	3	2	2	1	3	2	2	0	0	1	?	1	0	?	2	4
O_sp_1_c105	1	3	0	3	2	2	1	3	2	2	0	0	1	?	1	0	?	2	4
O_sp_1_c101	1	3	0	3	2	2	1	3	2	2	0	0	1	?	1	0	?	2	4
O_sp_1_c88	1	3	0	3	2	2	1	3	2	2	0	0	1	?	1	0	?	2	4
O_sp_1_c103	1	3	0	3	2	2	1	3	2	2	0	0	1	?	1	0	?	2	4
O_sp_1_c90	1	3	0	3	2	2	1	3	2	2	0	0	1	?	1	0	?	2	4
O_sp_2_c17	1	3	0	3	3	2	1	3	2	2	0	0	1	1	0	0	?	2	3
O_sp_2_c86	1	3	0	3	3	2	1	3	2	2	0	0	1	1	0	0	?	2	3
O_hortensis_c127	1	2	0	5	4	4	1	4	2	0	0	1	2	?	0	1	?	2	6
O_carrascoi_c1	1	2	0	5	4	1	1	2	2	2	0	0	1	?	0	0	1	2	5
O_carrascoi_c128	1	2	0	5	4	1	1	2	2	2	0	0	1	?	0	0	1	2	5
O_ceballosi_c4	1	2	0	4	5	4	1	5	2	1	1	1	2	?	0	0	?	2	[23]
O_ceballosi_c34	1	2	0	4	5	4	1	5	2	1	1	1	2	?	0	0	?	2	[23]
O_ceballosi_c33	1	2	0	4	5	4	1	5	2	1	1	1	2	?	0	0	?	2	[23]
O_ceballosi_c52	1	2	0	4	5	4	1	5	2	1	1	1	2	?	0	0	?	2	[23]
O_ceballosi_c32	1	2	0	4	5	4	1	5	2	1	1	1	2	?	0	0	?	2	[23]
O_ceballosi_c7	1	2	0	4	5	4	1	5	2	1	1	1	2	?	0	0	?	2	[23]
O_ceballosi_c21	1	2	0	4	5	4	1	5	2	1	1	1	2	?	0	0	?	2	[23]
O_ceballosi_c37	1	2	0	4	5	4	1	5	2	1	1	1	2	?	0	0	?	2	[23]
O_ceballosi_c51	1	2	0	4	5	4	1	5	2	1	1	1	2	?	0	0	?	2	[23]
O_ceballosi_c46	1	2	0	4	5	4	1	5	2	1	1	1	2	?	0	0	?	2	[23]
O_ceballosi_c48	1	2	0	4	5	4	1	5	2	1	1	1	2	?	0	0	?	2	[23]
O_ceballosi_c41	1	2	0	4	5	4	1	5	2	1	1	1	2	?	0	0	?	2	[23]
O_sp_5_c104	1	2	0	4	5	4	1	5	2	1	0	1	2	?	0	0	?	1	7
O_sp_4_c96	1	3	0	3	3	2	1	3	2	2	0	0	1	0	0	0	?	2	4
O_sp_4_c98	1	3	0	3	3	2	1	3	2	2	0	0	1	0	0	0	?	2	4
O_sp_4_c99	1	3	0	3	3	2	1	3	2	2	0	0	1	0	0	0	?	2	4
O_ceballosi_c36	1	2	0	4	5	4	1	5	2	1	1	1	2	?	0	0	?	2	[23]
O_sp_3_c126	1	2	0	5	4	3	1	4	2	2	1	0	1	?	0	0	?	1	6
O_ceballosi_6	1	2	0	4	5	4	1	5	2	1	1	1	2	?	0	0	?	2	[23]
O_andeanus_c121	1	2	0	3	2	1	1	2	2	2	0	0	1	?	0	0	0	1	2
O_andeanus_c24	1	2	0	3	2	1	1	2	2	2	0	0	1	?	0	0	0	1	2
O_ceballosi_c20	1	2	0	4	5	4	1	5	2	1	1	1	2	?	0	0	?	2	[23]

Unknown character states are denoted by '?'. Order of characters according to Appendix 1. Acronyms of specimens are according to Table 1.

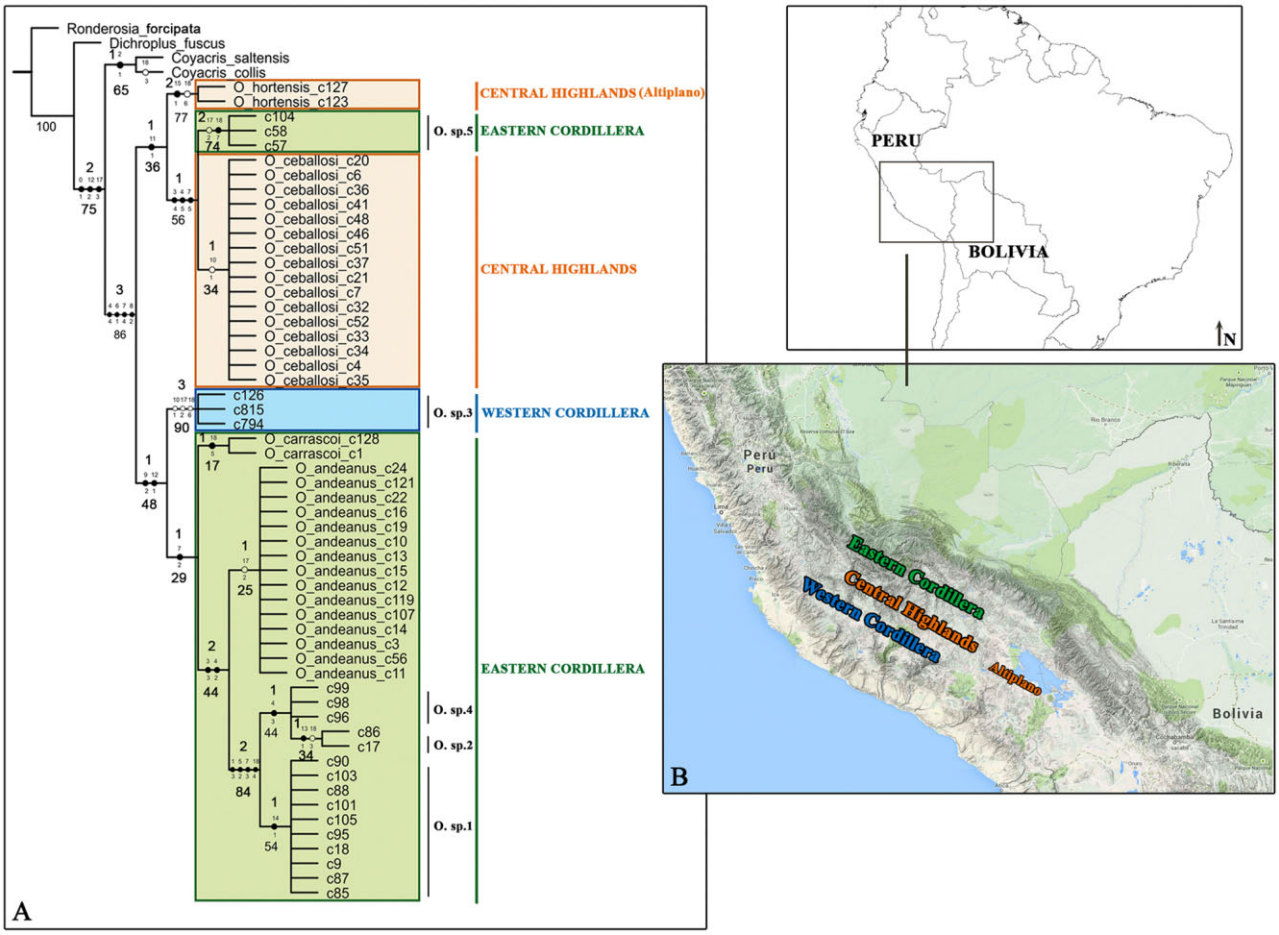

Figure 3 A, most parsimonious tree of the genus *Orotettix* (length 55, CI = 0.87, RI = 0.97) resulting from the cladistic analysis of the morphological character dataset, under equal weights. Black circles indicate unique changes and white circles indicate homoplasies. The numbers below the nodes are bootstrap support values, and those above are Bremer support values. The new species (*O.* sp. 1; *O.* sp. 2; *O.* sp. 3; *O.* sp. 4; *O.* sp. 5) delimited in this study based on molecular, morphological and geographical analyses are indicated in the tree. Lateral bars indicate the distribution of the specimens according to the geomorphic units of the Andes delimited by Gonzalez & Pfiffner (2012). B, geomorphological units of the Andes delimited by Gonzalez & Pfiffner (2012).

Appendix 1) (Fig. 3). *O.* sp. 3, from Arequipa in the Western Cordillera, is defined by the development of the sheath of aedeagus, tegmina length and hind tibiae light purple (characters 10:1, 17:2 and 18:6 from Appendix 1, respectively) (Fig. 3). The remaining clades correspond to specimens from Cusco and Apurimac endemic to the Eastern Cordillera (Fig. 3). Specimens c96, c98 and c99 resolve as a basal polytomy within a clade that includes a sister group (*O.* sp. 2). This sister group is defined by the shape of apical valves and hind tibiae green (characters 13:1 and 18:3 from Appendix 1, respectively) (Fig. 3, Table 1). Finally, the remaining clade was constituted by the specimens that belong to *O.* sp. 1 from Apurimac (Cachora, Curahuasi) (Fig. 3), defined by the shape of apical valves of aedeagus (character 14:1 from Appendix 1).

Species delimitation in *Orotettix*

Results from analyses based on molecular characters exhibited a clear delimitation of *O.* sp. 1, *O.* sp. 2, *O.* sp. 3, *O.* sp. 4 and *O.* sp. 5 with high support values, and shown to be strongly coincident with GMYC results except for *O.* sp. 5, which split into two clusters. However, specimens within these two clusters are sympatric, identical in morphology and show levels of evolutionary divergence for mitochondrial characters that are similar or even lower than other identified

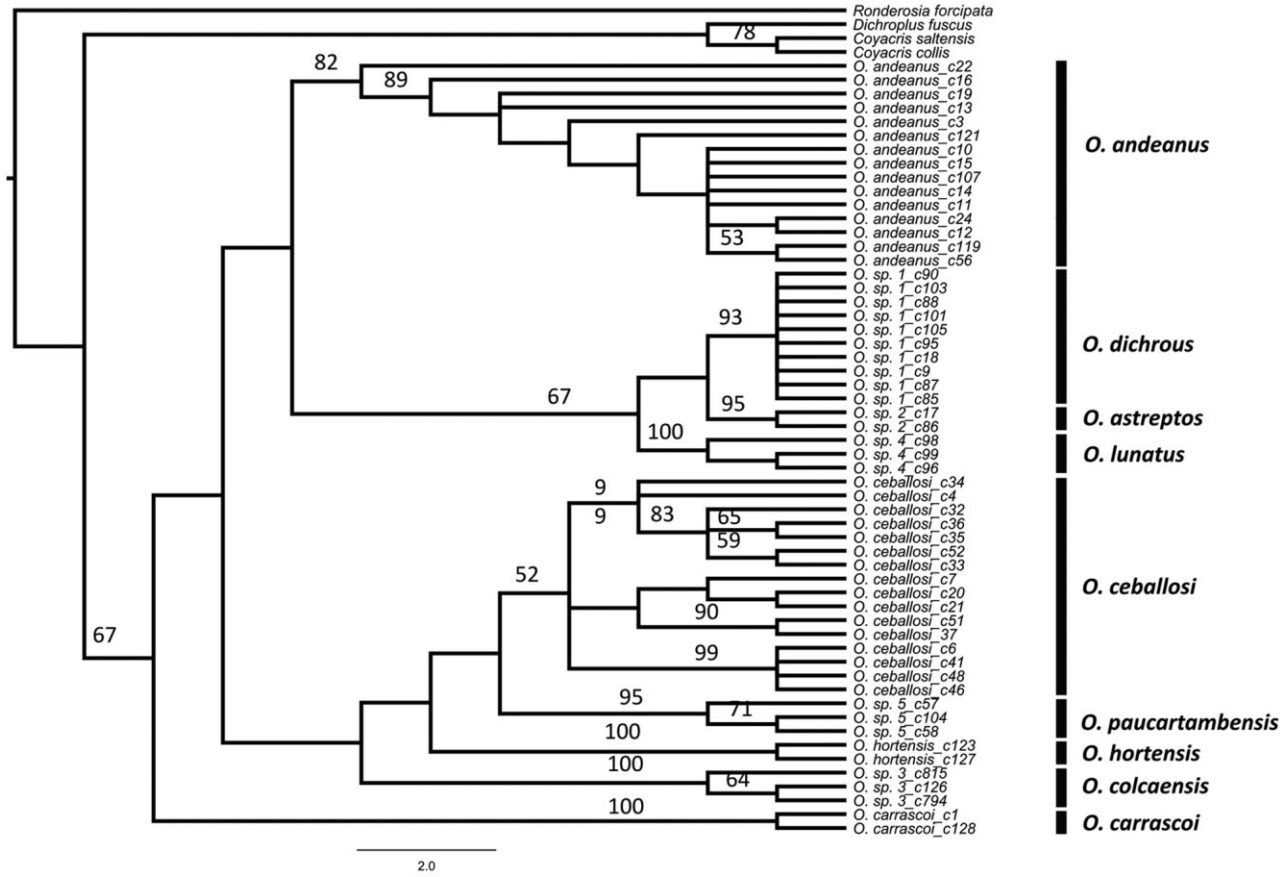

Figure 4 Combined molecular and morphological phylogenetic analysis under parsimony criteria, using an extended implied weighting strategy. Numbers indicate branch supports (symmetric resampling).

species (Table 2). Parsimony analysis of morphological characters revealed that most specimens clustered in the molecular analysis rendered monophyletic groups except for *O.* sp. 4, which resolved as a basal polytomy within a clade that included *O.* sp. 2. Yet, detailed morphological analysis indicated that *O.* sp. 4 can be separated from the remaining species of *Orotettix* by the following combination of characters: unique shape of the apical valves of the male genitalia (Fig. 9M), posterior tibiae red (Fig. 6E), tegmina lobiform and male cerci with compressed apex (Fig. 7I).

We consider the agreement between the different lines as a good reason to regard the species delimitation hypotheses as plausible and so we propose the presence of five new species for the genus *Orotettix*: *Orotettix dichrous* sp. nov. (*O.* sp. 1.), *Orotettix astreptos* sp. nov. (*O.* sp. 2), *Orotettix colcaensis* sp. nov. (*O.* sp. 3), *Orotettix lunatus* sp. nov. (*O.* sp. 4) and *Orotettix paucartambensis* sp. nov. (*O.* sp. 5). Geographical evidence also supports this distinction as there is a clear correspondence between the groups delimited and their biogeographical distribution (Fig. 1).

Phylogenetic analysis of the genus *Orotettix* based on combined data

Parsimony analysis under the extended implied weighting strategy resulted in 40 most parsimonious trees, and the consensus recovered *Orotettix* as a monophyletic group with moderate support values (67) (Fig. 4). Within *Orotettix*, all specimens belonging to the new five species delimited in the previous analyses as well as those corresponding to the already known species were recovered as monophyletic groups. *Orotettix carrascoi* was basal to the remaining species, which were recovered in two major groups: one group comprising *O. colcaensis* sp. nov. as sister to the clade ((*O. hortensis* (*O. paucartambensis* sp. nov., *O. ceballosi*)). The other group included *O. andeanus* as sister to a clade constituted by (*O. lunatus* sp. nov., (*O. astreptos* sp. nov., *O. dichrous* sp. nov.)). However, these relationships have to be taken with caution because many of them did not have good branch support.

Finally, the analysis resolves *Orotettix* as the sister group of the clade constituted by *Dichroplus fuscus* and the sister species *Coyacris saltensis* and *Coyacris collis.*

TAXONOMY OF *OROTETTIX*

OROTETTIX RONDEROS & CARBONELL, 1994

http://lsid.speciesfile.org/urn:lsid:Orthoptera.speciesfile.org:TaxonName:54963

Orotettix Ronderos & Carbonell, 1994: 88; Eades *et al.*, 2015.

Type species. *Paradichroplus andeanus* Bruner, L., by original designation.

Diagnosis. Small (males 12.8–16.7 mm; females 17.6–22.2 mm) brachypterous insects. Fastigium prominent, with median sulcus; frons slanted; pronotum with lateral borders slightly divergent at metazona; hind margin of pronotum disc straight or slightly rounded; hind femur proximally wide; tegmina not surpassing the second abdominal segment, not overlapping dorsally; male cerci robust at base, tapering towards the apex, slightly curved towards the epiproct; subgenital plate conical with acute apex; phallic complex (Fig. 5A, B): apical valves of aedeagus thin, slightly curved upwards, with the apices slightly overlapped or touching each other; sheath of aedeagus with lateral and median dorsal lobes.

Closely related to *Coyacris* Ronderos but differing as follows: smaller size, tegmina narrower and shorter, not overlapping dorsally, and if so only slightly overlapped, without raised median longitudinal vein; postocular band mostly indistinct; male cerci not compressed at distal portion; apical valves of aedeagus not strongly up-curved, without membranous expansions, with the apices if overlapped, only slightly; sheath of aedeagus with lateral lobes and without transverse flange; ectophallus less sclerotized; epiphallus with narrower lateral plates and lophi not so largely developed.

Distribution: Peru (Ayacucho, Arequipa, Apurimac, Cusco, Puno) and Bolivia (La Paz) (Fig. 1).

Key to the species of *Orotettix*: see Appendix 2.

***OROTETTIX DICHROUS* SP. NOV.** CIGLIANO, POCCO & LANGE

http://lsid.speciesfile.org/urn:lsid:Orthoptera.speciesfile.org:TaxonName:466734

(FIGS 1, 5A, B, 6C; 7E, F, 9G–I)

Diagnosis Male cerci dorsally curved in an acute angle (Fig. 7F), with conical apex (Fig. 7E). Apical valves of aedeagus widening at the forked distal portion, slightly divergent dorsally (Fig. 9H); mid-dorsal apical lobes of sheath of aedeagus moderately developed, covering 2/3 of the apical valves, edge straight (Fig. 9H); lateral lobes prominent (Fig. 9H); tegmina lobiform, touching dorsally; hind tibiae orange–red (Fig. 6C).

Description Males. Lateral carinae of pronotum obsolete (Fig. 6C). Tegmina lobiform, contiguous dorsal edges, with rounded apex (Fig. 6C); cerci sharply curved over the epiproct, with conical apex, slightly surpassing the end of epiproct (Fig. 7F). Phallic complex: apical valves of aedeagus widening at the forked distal portion, slightly divergent dorsally (Fig. 9H); mid-dorsal apical lobes of sheath of aedeagus moderately developed, covering 2/3 of the apical valves, edge straight (Fig. 9H); lateral lobes prominent (Fig. 9H); lophi of epiphallus prominent, not expanded towards the posterior process of the lateral plates (dorsal view) (Fig. 9I). Body homogenously green, abdomen ventrally yellow, hind tibiae orange–red (Fig. 6C).

Measurements (in mm) Body length: males 13.64 (12–15.2), females 19 (18–20); femur III: males 8.54 (8–9.2), females 10.5 (10–11); tegmina length: males 2.86 (2.2–3.1), females 3.94 (3–4.7).

Distribution Peru: Cusco, Apurimac (Curahuasi, Abancay, Cachora) (Fig. 1).

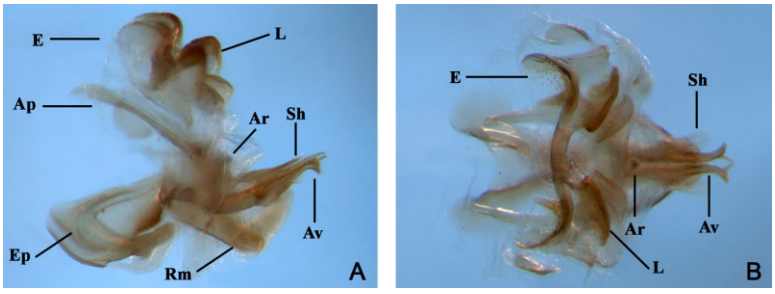

Figure 5 *Orotettix*, phallic complex, in lateral (A) and dorsal views (B). Abbreviations: Ap, apodemes of cingulum; Ar, arch of aedeagus; Av, aedeagal valves; Ep, endophallic plates; E, epiphallus; L, lophi of epiphallus; Rm, rami; Sh, sheath of aedeagus.

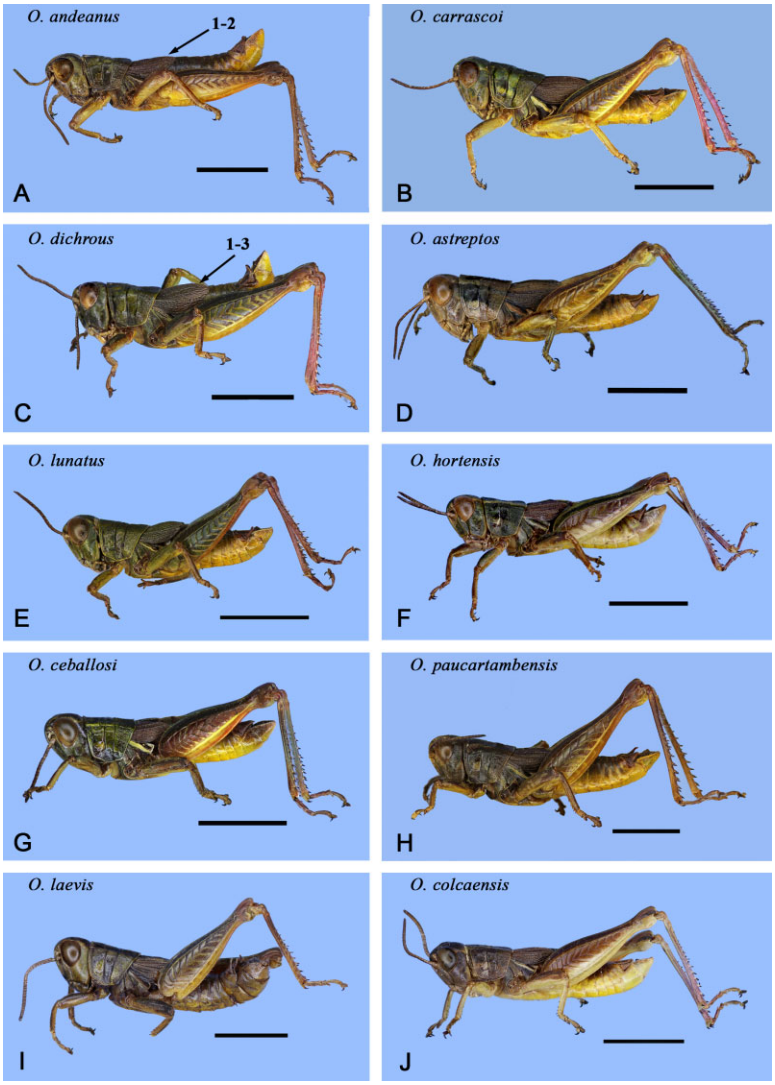

Figure 6 *Orotettix* males, species as indicated. A–J, habitus. Scale bars: 5 mm. Numbers indicate characters and states used in the phylogenetic analyses.

Etymology The name refers to the forked shape of the distal portion of aedeagal valves; *dikros* (Gr.): forked.

Material examined Peru, holotype male, allotype female, Peru, Apurimac, Cachora, 13°31′08.0″S 72°49′11.0″W, 2768 m, 19/05/2008, Cigliano & Lange, MLPA. Paratypes: 1 male, Cusco, between Cusco and Apurimac, 13°33′50.9″S 72°35′29.1″W, 1971 m, 24/04/2007, Cigliano & Lange, MLPA; 10 males, 5 females, Apurimac, Curahuasi, 13°33′02.3″S 72°44′00.3″W, 2709 m, 24/04/2007, Cigliano & Lange, MLPA; 2 males, 2 females, Apurimac, 10 km from Curahuasi to Abancay, 13°32′57.7″S 72°45′29.3″W, 3084 m., 18/05/2008, Cigliano & Lange, MLPA; 4 males, 3 females, Apurimac, 39 km from Curahuasi to Abancay, 13°34′04.9″S 72°49′19.9″W, 3920 m, 18/05/2008, Cigliano & Lange, MLPA; 6 males, 6 females, Apurimac, Cachora, 13°31′08.0″S 72°49′11.0″W, 2768 m, 19/05/2008, Cigliano & Lange, MLPA.

***OROTETTIX ASTREPTOS* SP. NOV.** CIGLIANO, POCCO & LANGE

http://lsid.speciesfile.org/urn:lsid:Orthoptera.speciesfile.org:TaxonName:466735

(FIGS 1, 6D, 7G, H, 9J–L)

Diagnosis Closely related to *O. dichrous*, from which it can be distinguished by the following features: male cerci slightly curved over the epiproct, with acute apex (Fig. 7H); apical valves of aedeagus wider; distal portion sub-triangular (Fig. 9K); body colour dull-brown; hind tibiae greenish (Fig. 6D).

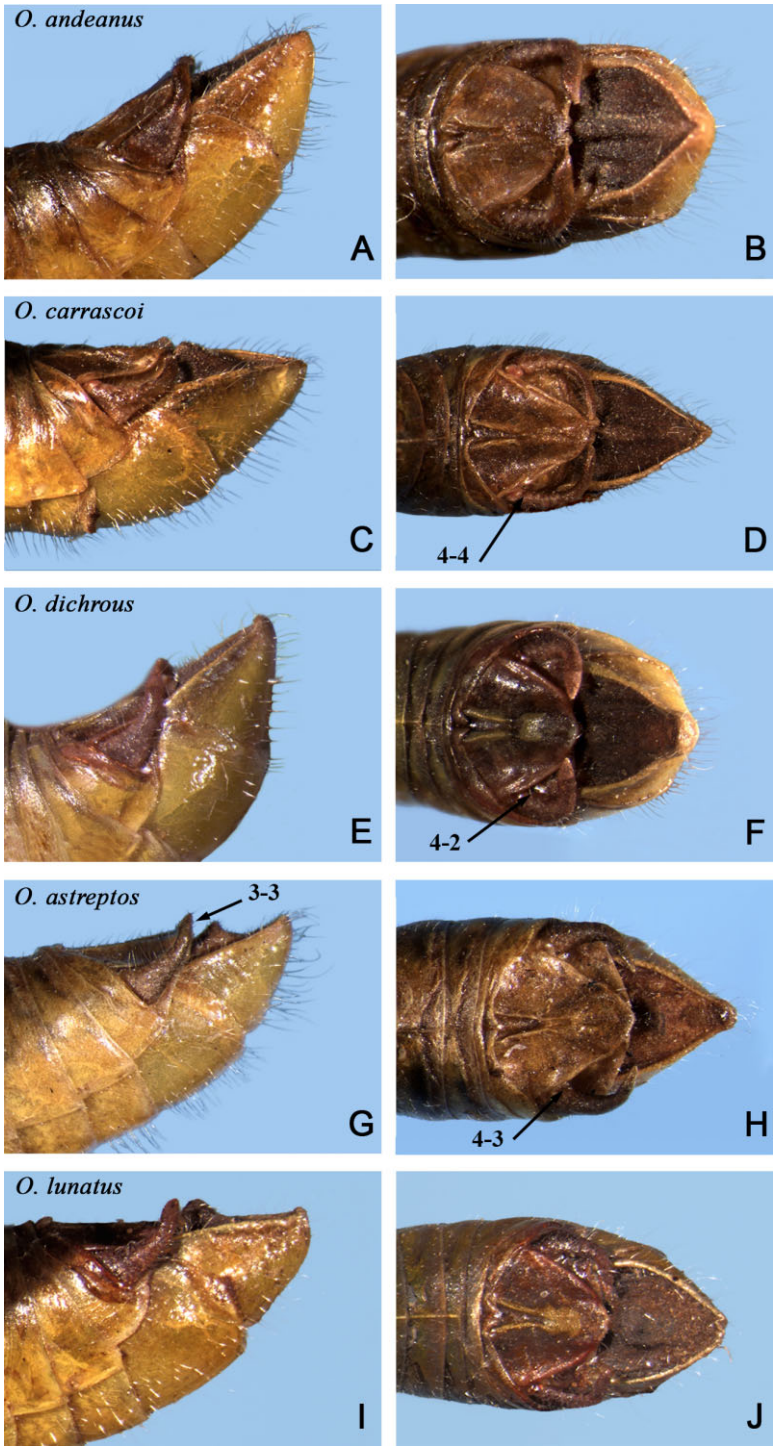

Figure 7 *Orotettix* males, species as indicated. A, C, E, G, I, distal abdominal segments, lateral view; B, D, F, H, J, distal abdominal segments, dorsal view. Numbers indicate characters and states used in the phylogenetic analyses.

Description Males. Lateral carinae of pronotum obsolete (Fig. 6D). Tegmina lobiform, contiguous dorsal edges, with rounded apex (Fig. 6D); cerci slightly curved over the epiproct, with acute apex, slightly surpassing the end of epiproct (Fig. 7H). Phallic complex: apical valves of aedeagus widening at the sub-triangular distal portion, slightly divergent (Fig. 9K); mid-dorsal apical lobes of sheath of aedeagus covering 2/3 of the apical

valves, edge straight (Fig. 9K); lateral lobes prominent (Fig. 9K); lophi of epiphallus prominent, not expanded towards the posterior process of the lateral plates (dorsal view) (Fig. 9L). Body colour dull-brown, laterally with dark brown post-ocular band, extending from behind the eyes along mid-dorsal portion of lateral lobes of pronotum; mid-ventral portion of lateral lobes of pronotum cream; abdomen ventrally yellow; hind tibiae greenish (Fig. 6D).

Measurements (in mm) Body length: males 13.74 (12.5–15), females 19.83 (19–20.5); femur III: males 8.14 (7.7–9), females 10.67 (10–11); tegmina length: males 2.92 (2.5–3.1), females 4.

Etymology The name refers to the straight apical edge of the distal portion of aedeagal valves; *astreptos* (Gr.): straight.

Distribution Peru: Cusco, (Fig. 1).

Material examined Peru, holotype male, allotype female, Cusco, between Cusco and Apurimac, 13°33′50.9″S 72°35′29.1″W, 1971 m, 24/04/2007, Cigliano & Lange, MLPA. Paratypes: 6 males, Cusco, between Cusco and Apurimac, 13°33′50.9″S 72°35′29.1″W, 1971 m, 24/04/2007, Cigliano & Lange, MLPA; 2 females, Cusco, 20 km from Curahuasi to Cusco, 13°30′59.9″S 72°31′16.7″W, 2751 m, 19/05/2008, Cigliano & Lange, MLPA.

OROTETTIX COLCAENSIS SP. NOV. CIGLIANO, POCCO & LANGE

http://lsid.speciesfile.org/urn:lsid:Orthoptera.speciesfile.org:TaxonName:466736

(FIGS 1, 6J, 8I, J, 10M–O)

Diagnosis Male cerci long, slightly curved over the epiproct, distal third slender, with acute apex (Fig. 6J); apical valves of aedeagus with distal portion wide; with distal third incurved, overlapping at apex (Fig. 10N); mid dorsal apical lobes of sheath of aedeagus subtriangular in dorsal view (Fig. 10N); hind tibiae light purple (Fig. 6J).

Description Males. Disc of pronotum straight; lateral carinae faintly indicated. Tegmina foliaceous, not touching dorsally; cerci long, slightly curved over the epiproct, distal third slender, with acute apex, surpassing the end of epiproct (Fig. 6J). Phallic complex: apical valves of aedeagus with distal portion wide; with distal third incurved, overlapping at apex (Fig. 10N); mid dorsal apical lobes of sheath of aedeagus subtriangular in dorsal view (Fig. 10N), covering most of the apical valves in lateral view (Fig. 10M); lophi of epiphallus with rounded edges, prominent and dorsally narrow (dorsal view) (Fig. 10O). Body colour dark green with brown, laterally with diffuse dark brown post-ocular band; abdomen ventrally yellow, hind femora dark reddish-brown, with green carinae; hind tibiae light purple (Fig. 6J).

Measurements (in mm) Body length: males 14.8 (14–16), females 21.1 (18–23); femur III: males 8.56 (8–9.5), females 11.4 (10.5–13); tegmina length: males 2.46 (2–3), females 3.92 (3.3–4.3).

Etymology The name refers to the distribution of the species in Valle del Colca, Arequipa, Peru.

Distribution Peru: Arequipa (Valle del Colca), (Fig. 1).

Material examined Peru: holotype male, allotype female, Arequipa, Valle del Colca, Coporaque, 15°37′50.0″S 71°39′00.0″W, 3541 m, 20/01/2013, Cigliano & Lange, MLPA. Paratypes: 7 males, 7 females, Arequipa, Valle del Colca, Pinchollo, 15°37′09.2″S 71°51′14.6″W, 3707 m, 20/01/2013, Cigliano & Lange, MLPA; 7 males, 2 females, Arequipa, Valle del Colca, Yanque, 15°39′26.6″S 71°40′42.3″ W, 3409 m, 20/01/2013, Cigliano & Lange, MLPA; 8 males, 2 females, 2 nymphs, Arequipa, Valle del Colca, Coporaque, 15°37′50.0″S 71°39′00.0″W, 3541 m, 20/01/2013, Cigliano & Lange, MLPA.

OROTETTIX LUNATUS SP. NOV. CIGLIANO, POCCO & LANGE

http://lsid.speciesfile.org/urn:lsid:Orthoptera.speciesfile.org:TaxonName:466737

(FIGS 1, 6E, 7I, J, 9M–O)

Diagnosis Closely related to *O. astreptos*, from which it can be differentiated by the male cerci with apex compressed (Fig. 6J); apical valves of aedeagus with distal portion wide, with lunate edge (Fig. 9N); lateral lobes of sheath of aedeagus less prominent (Fig. 9N); lophi of epiphallus subrectangular (Fig. 9O); hind tibiae orange–red (Fig. 6E).

Description Males. Lateral carinae of pronotum obsolete. Tegmina lobiform, contiguous dorsal edges, with rounded apex; cerci slightly curved over the epiproct, with fairly compressed apex, barely surpassing the tip of epiproct (Fig. 7J). Phallic complex: apical valves of aedeagus with distal third widening at the distal portion with lunate edge (Fig. 9N); mid-dorsal apical lobes of sheath of aedeagus covering less than 2/3 of the apical valves (Fig. 9M), with straight distal edge (Fig. 9N); lateral lobes not surpassing the dorsal lobes (Fig. 9N); lophi of epiphallus subrectangular (Fig. 9O). Body colour

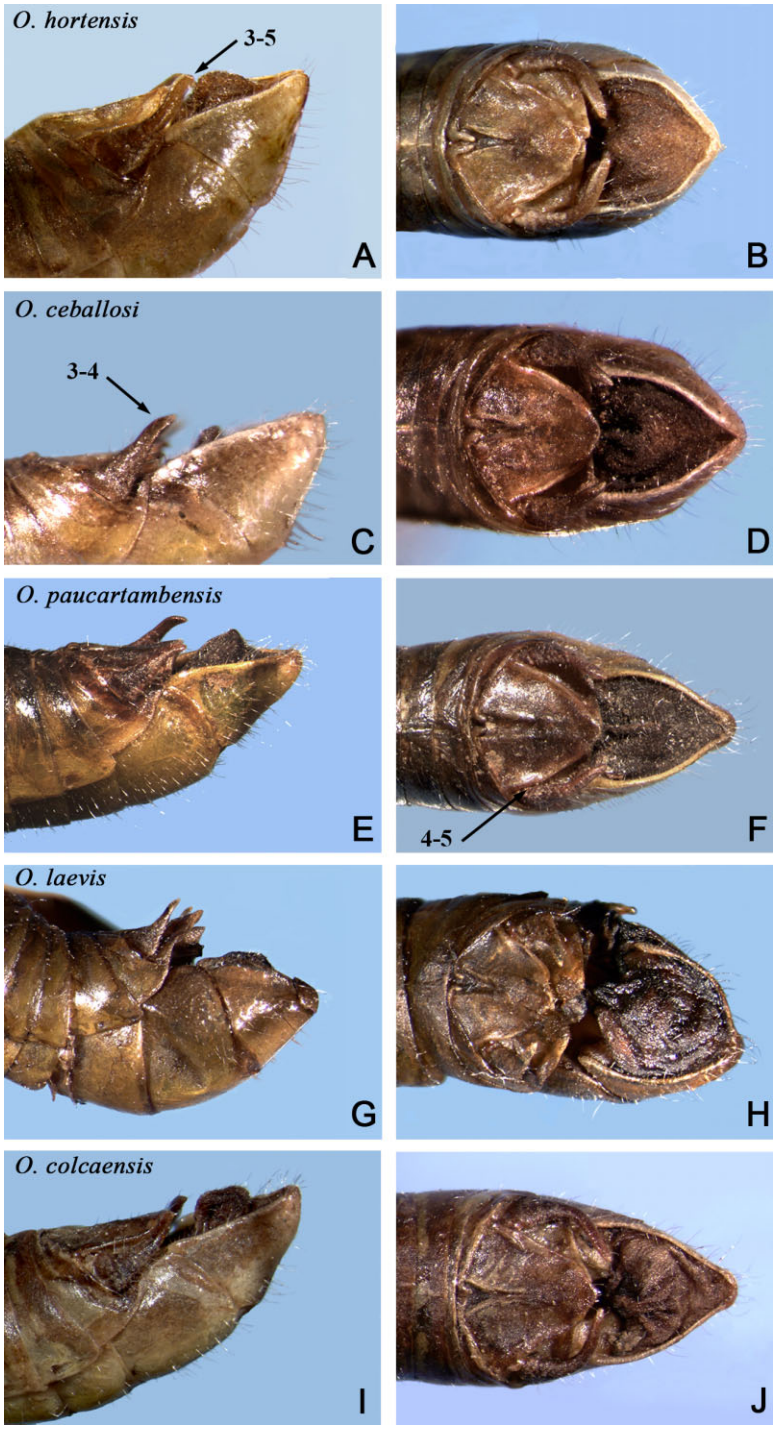

Figure 8 *Orotettix* males, species as indicated. A, C, E, G, I, distal abdominal segments, lateral view; B, D, F, H, J, distal abdominal segments, dorsal view. Numbers indicate characters and states used in the phylogenetic analyses.

brightly green; abdomen ventrally yellow; internal and ventral face of hind femora reddish; hind tibiae orange–red (Fig. 6E).

Measurements (in mm) Body length: males 12.8 (12–13.5), females 17.67 (15–19); femur III: males 7.8 (7–8), females 10.75 (10.5–11); tegmina length: males 2.9 (2.5–4), females 3.62 (3–4).

Etymology The name refers to the crescent shape of the distal portion of the aedeagal valves; *lunatus* (Latin): crescent-shaped.

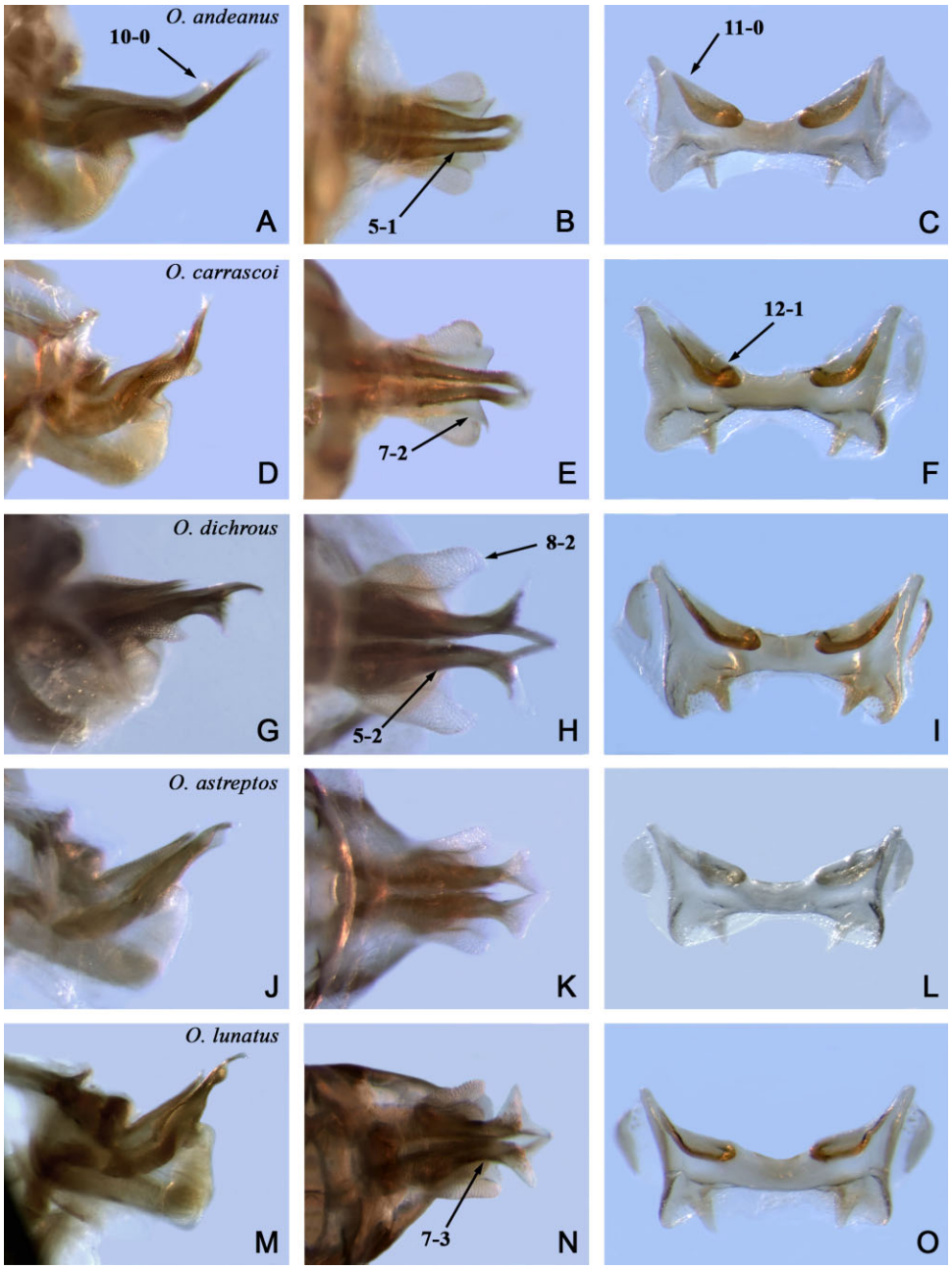

Figure 9 *Orotettix* males. Phallic complex, species as indicated. A, D, G, J, M, distal portion of aedeagal valves, lateral view; B, E, H, K, N, distal portion of aedeagal valves, dorsal view; C, F, I, L, O, epiphallus, dorsal view. Numbers indicate characters and states used in the phylogenetic analyses.

Distribution Peru: Apurimac (Abancay), (Fig. 1).

Material examined Peru: holotype male, allotype female, Apurimac, 3 km from Abancay to Cusco, 13°36′43.3″S 72°51′51.0″W, 2690 m, 19/05/2008, Cigliano & Lange, MLPA. Paratypes: 6 males, 6 females, 1 nymph, Apurimac, 3 km from Abancay to Cusco, 13°36′43.3″S 72°51′51.0″W, 2690 m, 19/05/2008, Cigliano & Lange, MLPA.

OROTETTIX PAUCARTAMBENSIS SP. NOV. CIGLIANO, POCCO & LANGE

http://lsid.speciesfile.org/urn:lsid:Orthoptera.speciesfile.org:TaxonName:466738

(FIGS 1, 6H, 8E, F, 10G–I)

Diagnosis Similar to *O. ceballosi*, from which it can be distinguished by the following features: apical valves of aedeagus slender (Fig. 10H); sheath of aedeagus

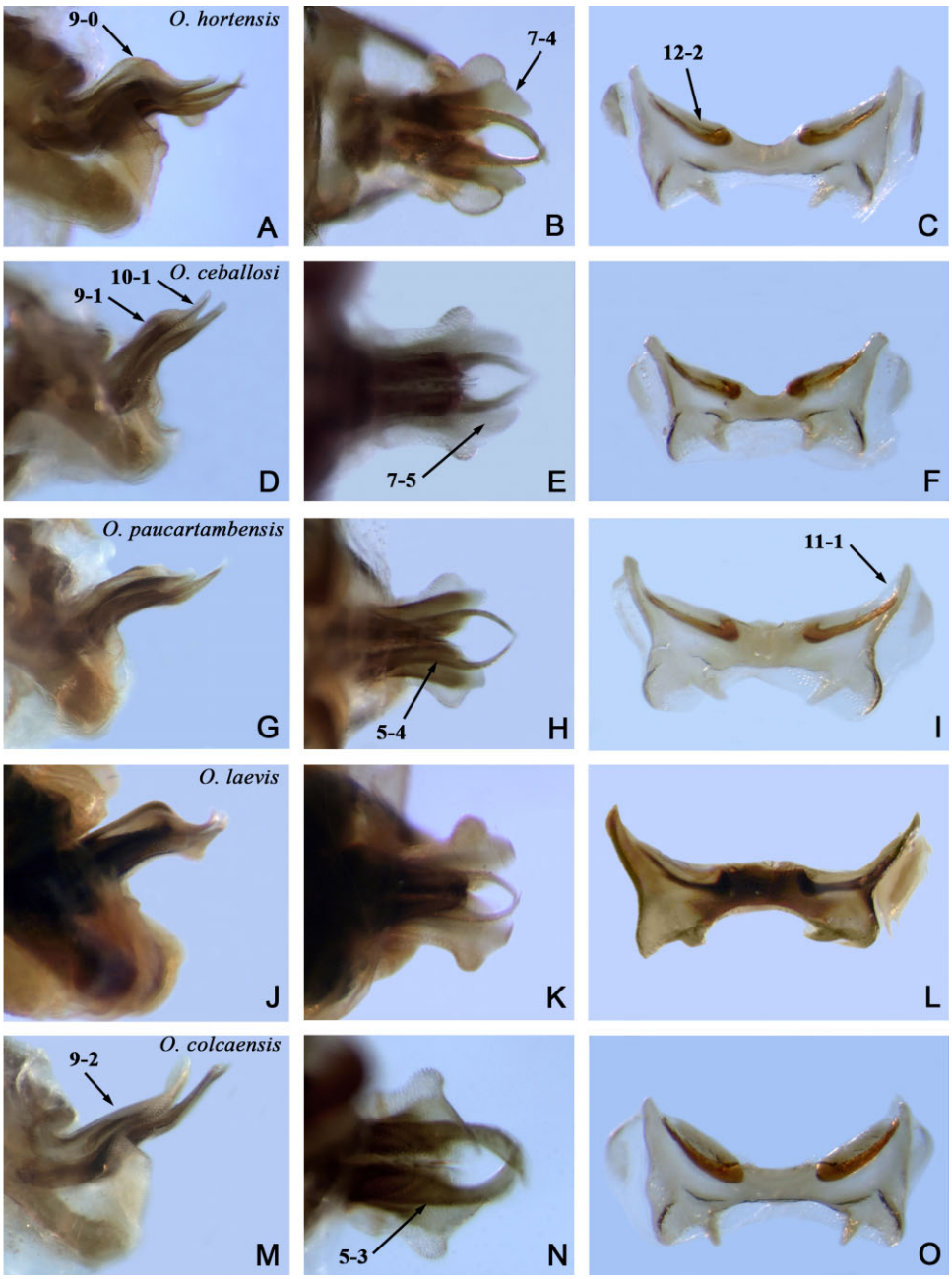

Figure 10 *Orotettix* males. Phallic complex, species as indicated. A, D, G, J, M, distal portion of aedeagal valves, lateral view; B, E, H, K, N, distal portion of aedeagal valves, dorsal view; C, F, I, L, O, epiphallus, dorsal view. Numbers indicate characters and states used in the phylogenetic analyses.

shorter, covering 2/3 of the apical valves, with mid-dorsal apical lobes less prominent (Fig. 10H); hind tibiae light reddish-brown (Fig. 6H).

Description Males. Lateral carinae of pronotum slightly indicated (Fig. 6H); tegmina foliaceous; cerci parallel to epiproct, not surpassing the end of epiproct (Fig. 8F), tapering towards the tip, compressed apex (Fig. 8E). Phallic complex: apical valves of aedeagus with distal third slender; incurved, touching each other at apex (Fig. 10H); mid-dorsal apical lobes of sheath of aedeagus divergent, covering 2/3 of the apical valves (Fig. 10H); dorsal hump slightly developed in lateral view (Fig. 10G); lateral lobes prominent (Fig. 10H); lophi of epiphallus widely expanded towards the posterior process of the lateral plates (dorsal view) (Fig. 10I).

Body colour dark reddish-brown with green; laterally with dark reddish-brown post-ocular band; mid-ventral portion of lateral lobes of pronotum dark green; epimeron with a cream longitudinal band; abdomen dorsally green and ventrally yellow; hind femora dark reddish-brown; hind tibiae light reddish-brown (Fig. 6H).

Measurements (in mm) Body length: males 16.7 (15–17.5), females 22.25 (21–24); femur III: males 10.5 (9–11), females 13.3 (11.5–15); tegmina length: males 3.34 (3–4), females 4.13 (3.5–5).

Etymology The name refers to the distribution of the species in and around Paucartambo, Peru.

Distribution Peru, Cusco (Paucartambo), (Fig. 1).

Material examined Peru, holotype male, allotype female, Cusco, 20 km from Paucartambo to Pillahuata, mountain pass Ajanacu, 13°11′52.8″S 71°38′30.9″W, 3464 m, 19/05/2008, Cigliano & Lange, MLPA. Paratypes: 2 males, 3 females, 3 nymphs, Parpacalla in the outskirts of Paucartambo, 13°17′19.5″S 71°35′57.6″W, 2943 m, 23/04/2007, Cigliano & Lange, MLPA; 3 males, 1 female, 3 nymphs, Cusco, 20 km from Paucartambo to Pillahuata, mountain pass Ajanacu, 13°11′52.8″S 71°38′30.9″W, 3464 m, 19/05/2008, Cigliano & Lange, MLPA.

Orotettix andeanus (Bruner, 1913)

http://lsid.speciesfile.org/urn:lsid:Orthoptera.speciesfile.org:TaxonName:54968

(Figs 1, 6A, 7A, B, 9A–C)

Paradichroplus andeanus Bruner, L. 1913: 185 [Holotype, male, Cusco, Peru, National Museum of Natural History, Washington D.C. (USNM)];

Pedies andeanus: Hebard, 1917: 253; 1931: 274; Liebermann, 1963: 65.

Orotettix andeanus: Ronderos & Carbonell, 1994: 88; Eades *et al*., 2015.

Diagnosis Male cerci strongly curved over the epiproct, with compressed apex (Fig. 7B); apical valves of aedeagus straight, with distal portion slightly curved inwards, overlapping at apex (Fig. 9B); sheath of aedeagus with prominent lateral lobes, and mid-dorsal apical lobes with truncated edges (Fig. 9B). Lophi of epiphallus with rounded dorsal edges not extended towards the posterior process of the lateral plates (dorsal view) (Fig. 9C). Tegmina foliaceous, not touching dorsally. Body colour green, tegmina usually burgundy, hind tibiae orange (Fig. 6A).

Measurements (in mm) Body length: males 13.88 (13–15.5), females 18.75 (16–24); femur III: males 8.38 (7.5–9), females 11.8 (11–13); tegmina length: males 2.52 (2–3), females 3.87 (3–4.8).

Distribution Peru, Cusco (Fig. 1).

Material examined Peru: 15 males, 21 females, Cusco, Urubamba, 2800 m, 15/05/1976, Carbonell, C.S., MLPA; 6 males, 3 females, 1 nymph, Cusco, Kaira, 23/08/1976, Carrasco, F., MLPA; 2 males, 3 females, Cusco, Urubamba, 01/07/1984, Ceballos B. I., MLPA; 2 males, 5 females, Cusco, San Jerónimo, 12/05/1985, Ceballos I., MLPA; 1 male, 5 females, Cusco, Lucre, 13/04/1985, Ceballos, B. I., MLPA; 4 males, 2 females, Cusco, Perayoc, 25/12/1958, Ceballos, I., MLPA; 1 male, Cusco, San Jerónimo, 11/05/1972, Bulla, MLPA; 1 male, Cusco, Urcos, 3120 m, 22/07/1962, Carrasco, F., MLPA; 6 males, 9 females, Cusco, Pisac, 35 km Cuzco, 2900, 22/04/2007, Cigliano & Lange, MLPA; 5 males, 3 females, Cusco, Cusco, between Coya and Lamay, 13°22′55.3″S, 71°54′49.4″W, 2950, 22/04/2007, Cigliano & Lange, MLPA; 7 males, 9 females, Cusco, Yanahuara, 13°16′25.3″S, 72°10′28.0″W, 2981, 22/04/2007, Cigliano & Lange, MLPA; 2 males, 6 females, 1 nymph, Cusco, detour Tamillopata, 13°19′25.8″S, 72°04′4″W, 3223, 22/04/2007, Cigliano & Lange, MLPA; 6 males, 5 females, 1 nymph, Cusco, Tipon, 13°05′12.6″S, 71°48′08.6″W, 3184, 23/04/2007, Cigliano & Lange, MLPA; 9 males, 11 females, Cusco, Lucre, 13°37′39.8″S, 71°43′35.2″W, 3112, 23/04/2007, Cigliano & Lange, MLPA; 7 males, 12 females, Cusco, Cachimayo, outskirts of Poroy, 13°29′21.4″S, 72°03′41.1″W, 24/04/2007, Cigliano & Lange, MLPA; 11 males, 13 females, Cusco, Maras, 13°20′20.1″S, 72°06′40.3″W, 3407, 24/04/2007, Cigliano & Lange, MLPA; 3 males, 3 females, Cusco, to Urcos, Ruinas Pikillaqta, 13°37′14.9″S, 71°42′28.1″W, 3191, 17/05/2008, Cigliano & Lange, MLPA; 4 males, 6 females, Cusco, Pisac (2 km from Pisac in road from Cusco), 13°25′44.3″S, 71°50′45.4″W, 3078, 13/01/2013, Cigliano & Lange, MLPA; 8 males, 6 females, Cusco, Chinchero, 13°23′22.37″S, 72°02′49.67″W, 14/01/2013, Cigliano & Lange, MLPA.

Orotettix carrascoi Ronderos & Carbonell, 1994

http://lsid.speciesfile.org/urn:lsid:Orthoptera.speciesfile.org:TaxonName:54967

(Figs 1, 6B, 7C, D, 9D–F)

Orotettix carrascoi Ronderos & Carbonell, 1994: 94 (Holotype, male, Peru, Cusco, Ollantaytambo, FCMU, Montevideo); Donato, 2000: 67; Eades *et al*., 2015.

Diagnosis Closely related to *O. andeanus*, from which it can be distinguished based on male cerci slender, widely curved inwards, with acute apex (Fig. 7D); apical valves of aedeagus longer (Fig. 9E); lophi of epiphallus slender (Fig. 9F). Tegmina foliaceous or lobiform; hind tibiae bright red (Fig. 6B).

Measurements (in mm) Body length: males 15.78 (15–16.5), females 20.5 (18.5–23); femur III: males 9 (8.5–9.5), females 10.7 (10–11); tegmina length: males 3.16 (2.5–3.5), females 4.16 (3.5–5).

Distribution Peru, Cusco (Ollantaytambo), (Fig. 1).

Material examined Peru: 1 male, 2 females, Cusco, Ollantaytambo, 2800 m, 18/03/1962, Mesa, A., MLPA (paratypes); 3 males, 3 females, Cusco, Ollantaytambo ruins, 13°15′32.1″S, 72°15′42.6″W, 2964 m, 22/04/2007, Cigliano & Lange, MLPA; 5 males, 5 females, Cusco, Ollantaytambo ruins, 13°15′32.1″S, 72°15′42.6″W, 2964 m, 13/01/2013, Cigliano & Lange, MLPA.

Orotettix ceballosi Ronderos & Carbonell, 1994

http://lsid.speciesfile.org/urn:lsid:Orthoptera.speciesfile.org:TaxonName:54966

(Figs 1, 6G, 8C, D, 10D–F)

Orotettix ceballosi Ronderos & Carbonell, 1994: 92 (Holotype, male, Peru, Ayacucho, Manzanayoc, FCMU, Montevideo); Donato, 2000: 67; Eades *et al*., 2015.

Diagnosis Male cerci short, parallel to epiproct (Fig. 8D), tapering towards the acute apex (Fig. 8C); apical valves of aedeagus with distal half slender, curved inwards, touching each other at apex (Fig. 10E). Sheath of aedeagus with well-developed mid-dorsal lobes (Fig. 10E), covering most of the apical valves (Fig. 10D); dorsal hump slightly prominent (Fig. 10D). Lophi of epiphallus widely expanded towards the posterior process of the lateral plates (dorsal view) (Fig. 10F). Lateral carinae of pronotum slightly indicated. Hind tibiae greenish or orange (Fig. 6G).

Measurements (in mm) Body length: males 13.87 (11–16), females 19.5 (18–21.5); femur III: males 7.78 (7–8.2), females 10.84 (10–12); tegmina length: males 2.83 (2.3–4.2), females 3.76 (3.2–4.1).

Distribution Peru: Ayacucho, Junín, Huancavelica, (Fig. 1).

Material examined Peru: 5 males, 3 females, Ayacucho, Manzanayoc, 3200 m, 07/03/1962, Mesa, A., MLPA (paratypes); 6 males, 6 females, Ayacucho, Parinococha, Manzanayoc, 3200 m, 07/03/1962, Mesa, MLPA; 5 males, 5 females, Ayacucho, Manzanayoc, 3200 m, 03/1962, Mesa, MLPA; 2 males, 3 females, 7 nymps, Ayacucho, between Tambo and Ayacucho, 12°57′49.5″S 74°05′19.5″W, 4121, 18/11/2007, Cigliano & Lange, MLPA; 8 males, 3 females, Ayacucho, Tambo, 12°57′11.7″S 74°01′09.2″W, 3429 18/11/2007, Cigliano & Lange, MLPA; 15 males, 14 females, 3 nymphs, Ayacucho between Tambo and Ayacucho, 12°57′58.3″S 74°05′39.6″W, 4144, 18/11/2007, Cigliano & Lange, MLPA; 13 males, 23 females, Ayacucho between Tambo and Ayacucho, 1 km from mountain pass, 4225 m, 12°58′34.7″ S 74°05′31.8″ W, 19/11/2007; 9 males, 3 females, 2 nymphs, Ayacucho, from Toccto to Condorcocha 13°13′04.1″S 74°13′37.1″W, 3268, 18/11/2007, Cigliano & Lange, MLPA; 6 males, 7 females, 5 nymphs, Ayacucho, from Toccto to Condorcocha 13°22′07.42″S, 74°11′14.9″W, 4035 m, 20/11/2007 Cigliano & Lange MLPA; 5 males, 5 females, Ayacucho, Condorcocha, 13°26′59.6″S 74°11′38.3″W, 3652, 20/11/2007, Cigliano & Lange MLPA; 13 males, 11 females, Ayacucho, from Tambo to San Francisco, 12°56′05.9″S 74°01′29.6″W, 3129 m, 21/11/2007, Cigliano & Lange MLPA; 5 males, 3 females, 4 nymphs, Ayacucho, 5 km from 'Bosque de Piedra' Huaraca, 13°18′58.3″S 74°26′48.6″W, 3837 m, 23/11/2007, Cigliano & Lange MLPA; 3 males, 2 females, Junín, 17 km from Tarma to Jauja 11°31′04.5″S 75°38′37.2″W, 3835, 28/04/2008, Cigliano & Lange, MLPA; 9 males, 5 females, Huancavelica, 24 km Huancavelica from Huancayo, 12°42′47.4″S 74°54′04.5″W, 4074 m, 28/04/2008, Cigliano & Lange, MLPA; 1 male, 1 female, Huancavelica, 'Bosque de Piedra' Sachapite, 4177 m, 29/04/2008, Cigliano & Lange. MLPA; 2 females, Junín Imperial, 35 km S Huancayo, 12°19′21.3″S 75°04′34.5″W, 3741 m, 30/04/2008, Cigliano & Lange, MLPA.

Orotettix laevis Ronderos & Carbonell, 1994

http://lsid.speciesfile.org/urn:lsid:Orthoptera.speciesfile.org:TaxonName:54964

(Figs 6I, 8G, H, 10J–L)

Orotettix laevis Ronderos & Carbonell, 1994: 95 (Holotype, male, Peru, Cusco, Ruta Abancay, FCMU, Montevideo); Donato, 2000: 70; Eades *et al*., 2015.

Diagnosis Male cerci short, parallel to epiproct, not surpassing the tip of epiproct (Fig. 8G), with basal half broad and distal half slender, apex acute (Fig. 8G). Apical valves of aedeagus slender, curved inwards, overlapping at apex (Fig. 10K). Sheath of aedeagus with well-developed mid-dorsal lobes (Fig. 10K), covering most of the apical valves (Fig. 10J); dorsal hump prominent (Fig. 10J), lateral lobes well developed (Fig. 10K);

lophi of epiphallus rectangular and widely expanded towards the posterior process of the lateral plates (dorsal view) (Fig. 10L).

Measurements (in mm) Body length: males 14.25 (13.5–15), female 17; femur III: males 8.5 (8–9); female 11; tegmina length: males 2.75 (2.5–3), female 4.

Distribution Peru, Cusco.

Material examined Peru: 2 males, 1 female, Cusco, Ruta Abancay, 17/03/1962, Mesa, A., MLPA (paratypes).

Orotettix hortensis Ronderos & Carbonell, 1994

http://lsid.speciesfile.org/urn:lsid:Orthoptera.speciesfile.org:TaxonName:54965

(Figs 1, 6F, 8A, B, 10A–C)

Orotettix hortensis Ronderos & Carbonell, 1994: 92 (Holotype lost, male, Peru, Dept. Puno, Hacienda La Huerta, near Ciudad de Puno, MLPA, La Plata); Eades *et al*., 2015. Neotype: male, Peru, Puno, Atuncolla in road to 'Chulpas de Silustani', 15°42′20.3″S, 70°05′47.9″W, 3823 m, 21/01/2013, Cigliano & Lange, MLPA.

Diagnosis Similar to *O. ceballosi*, from which it can be distinguished by the pronotum with lateral carinae clearly indicated (Fig. 6F); pronotum flat, with straight hind margin; male cerci longer, slightly curved over the epiproct, surpassing the end of epiproct (Fig. 8B); sheath of aedeagus wide, with highly developed lateral and dorsal lobes (Fig. 10B). Hind femora with raised upper carinae; hind tibiae light purple (Fig. 6F).

Measurements (in mm) Body length: males 14.5 (13–16), females 21.3 (20–23.5); femur III: males 8.34 (8–9), females 11.3 (10.5–12); tegmina length: males 3 (2.5–3.3), females 4.34 (4–5).

Distribution Peru: Puno, Cusco; Bolivia (La Paz), (Fig. 1).

Material examined Peru: 3 males, 2 females, Dpto. de Puno, 3900 m, 01/12/1947, (Weyrauch), MLPA; 6 males, 6 females, Cusco, 18 km Cuisipata, 14°02′55.3″S 71°26′51.4″W, 3458 m, 17/05/2008, Cigliano & Lange MLPA; 7 males, 5 females, Puno, from Cusco to Puno, outskirts of Santa Rosa, 14°35′49.0″S 70°50′27.5″W, 4010 m, 15/01/2013, Cigliano & Lange, MLPA; 11 males, 8 females, Puno, Chuquibambilla, 20 km from Ayaviri, 14°46′41.0″S 70°43′16.0″W, 3927 m, 15/01/2013, Cigliano & Lange, MLPA; 15 males, 5 females, 2 nymphs, Puno, Atuncolla in road to 'Chulpas de Silustani', 15°42′20.3″S 70°05′47.9″W, 3823 m, 21/01/2013, Cigliano & Lange, MLPA. Bolivia: 4 males, 1 female, La Paz, Sorata, mountain pass of Sorata, 4500 m, 24/03/2003, Cigliano & Lange, MLPA.

Observations

During the course of this study, it was not possible to find any material belonging to the type series of *Orotettix hortensis* Ronderos & Carbonell. According to the original description the holotype male and allotype female should be deposited in the Museo de La Plata (Ronderos & Carbonell, 1994). However, this collection does not hold these specimens and there is no record that these types have ever been deposited at the Museo de La Plata, and neither is there any mention of these types in the catalogue of Orthoptera types from this Museum (Donato, 2000). It was not possible to trace the private collection of F. Carrasco Z., where the two males and one female paratypes were deposited (Ronderos & Carbonell, 1994). So, we consider the types of *O. hortensis* are lost, and therefore we designate a neotype of *O. hortensis*, collected from the surrounding areas of Puno, where the type locality was situated.

DISCUSSION

In this study, results from molecular and morphological analyses showed a strong agreement among the species delimitation in *Orotettix*, which were revealed to be highly consistent with the geographical distribution. Molecular data resulted in a powerful tool for the taxonomic delimitation of species within *Orotettix* by expanding the number of characters used to distinguish them. The genetic data provided obvious evidence for the existence of five new species in the genus. The integrative taxonomy approach (Dayrat, 2005; DeSalle, Egan & Siddall, 2005; Schlick-Steiner *et al*., 2010) applied in this study, where more than one source of data has to support the hypothesis of a new species, allowed the discovery of five new entities that otherwise would have gone unnoticed under traditional taxonomy. The reciprocal illumination nature of integrative taxonomy through hypothesis testing, corroboration and revision is a powerful tool for species delimitation, as more than one of several sources (molecular, morphology and geography in our case) has to support the hypothesis of a new species. This approach is based on the assumption that with increasing support from independent data sets, the likelihood for false identifications decreases (Damm, Schierwater & Hadrys, 2010). In our study, initially a molecular-based hypothesis was postulated and tested against morphological data and geographical patterns of distribution. No immediately obvious differences in the external morphology were

found between the five new *Orotettix* species and without the inclusion of genetic data, the species would have remained undetected. However, detailed morphological re-examination of the specimens grouped into the different molecular clades showed differences in morphological characters mostly in the male internal genitalia. By contrast, our results also include an example that we interpret as an obvious over-estimation of species in coincidence with other studies where the number of GMYC entities obtained constantly exceeded the total number of morphospecies in the data set (Esselstyn *et al*., 2012; Fujisawa & Barraclough, 2013; Miralles & Vences, 2013; Talavera, Dinca & Vila, 2013). Specimens of *Orotettix paucartambensis* are split by GMYC into two distinct species but because these are sympatric and identical in morphology we consider them to be the same species.

Besides, specimens of *O. ceballosi* are split into six evolutionary units, a fact that is consistent with its high level of 'intraspecific' variability compared with the other species. Furthermore, it is the only species that displays low or moderate branch support across all (MP, BA, combined) analyses performed. Because the morphological study did not reveal any conspicuous difference among individuals, we decided to maintain its taxonomic status. Future analysis including more individuals and more genes will be necessary to resolve this enigma. Regardless, these examples illustrate how important it is to combine different data sources to determine species boundaries in taxonomy.

Results from the morphological studies showed that some characters proposed as being diagnostic at the specific level in *Orotettix* (Ronderos & Carbonell, 1994) appear ambiguous and reveal intraspecific variability (e.g. characters from head and pronotum; body coloration). External morphology in *Orotettix* is relatively conserved among the species with very little variation except for *O. hortensis*, which exhibits diagnostic differences in the external morphology, mostly in the pronotum. Despite this slight external morphological divergence, diagnostic characters are found in the male cerci (shape of apex; degree of curvature in relation to epiproct), internal male genitalia (apical valves of aedeagus; sheath of aedeagus; development of dorsal hump of the sheath of aedeagus; shape of epiphallus) and tegmina shape. The taxonomic diversity of *Orotettix* concurs with what it is known for most melanopline grasshoppers, which are characterized by the highly divergent male genitalia, usually diagnostic at the species level (see Cigliano & Lange, 2007 for a discussion on the value of using male genitalia in the Melanoplinae), and this is not the exception in *Orotettix*.

Orotettix occurs from the Departamento Junín in the Central Highlands of Peru reaching the Atiplano of Bolivia in the south. The southernmost record, registered herein, lies in the Departamento La Paz, Bolivia. Species are distributed in the Eastern Cordillera of Peru, but there are also representatives in the Central Highlands and Western Andes Cordillera (Gonzalez & Pfiffner, 2012) and one species is endemic to the Altiplano (Graham, 2009). *Orotettix* is found between 1970 and 4500 m a.s.l., whereas regional species richness peaks from 2900 to 3500 m a.s.l. (Eades *et al*., 2015). Tropical alpine-like vegetation is found in the Andes above the elevation limit of forest and below the permanent snow-line (Luteyn, 1992). In Peru, Bolivia and southward this relatively dry altitudinal zone is known as 'puna', including most of all habitats and vegetation types above 3300 m (Smith & Young, 1987). The puna extends in the Central Andes of Peru in all the departments where *Orotettix* is distributed (Huancavelica, Ayacucho, Apurímac, Puno, Junín, Arequipa, Cusco), reaching the Altiplano of Bolivia. Three species show a clear allopatric distribution (*O. ceballosi*, *O. hortensis*, *O. colcaensis* sp. nov.) while the remaining species occur in allopatry and/or parapatry with altitudinal segregation in different but similar habitats of the Eastern Cordillera. *Orotettix ceballosi* shows a broad distribution in the northern part of the Central Highlands of Peru, which corresponds to a high plateau with a mean elevation of 4000 m with low local relief. The Central Highlands are about 50 km wide and extend from Lago Junín to the south-east over a distance of more than 300 km (Gonzalez & Pfiffner, 2012). In the south-east, the Central Highlands widen into the Altiplano of Bolivia where there is only one species found, *O. hortensis*. Topographical relief of the Altiplano is moderate compared with the steep escarpments on the eastern and western slopes of the Andes. Most of the puna has a rolling topography with a wide variety of substrate types and drainage classes. *Orotettix colcaensis* is the only species of the genus endemic to the Western Cordillera, which comprises a chain of peaks reaching altitudes of 5000–7000 m. Local relief is very high owing to dissection by numerous streams, most of which flow perpendicular to the chain (Gonzalez & Pfiffner, 2012). The remaining species are distributed in the Eastern Cordillera. This is on average lower and narrower than the Western Cordillera and consequently has a smaller area of puna. Topography is relatively moderate between 3300 and 3800 m. Higher elevations are steep rocky cirques. Only in the south there are peaks high enough to have icecaps; the highest is Nevado Salcantay (6271 m). The configurations of areas of endemism of *Orotettix* species distributed in this region (*O. andeanus*, *O. carrascoi*, *O. laevis*, *O. dichrous* sp. nov., *O. astreptos* sp. nov., *O. lunatus* sp. nov., *O. paucartambensis* sp. nov.) that occur in allopatry and/or parapatry are delimited mostly by the high-altitude curves, including

transverse zones. Besides, several deep valleys cut through the puna of the Eastern Cordillera, creating biogeographical barriers (Sarmiento, 1986) that may have benefited the high diversification found in this region. Most probably, the diversification process that gave rise to the different *Orotettix* species occurred almost simultaneously as a consequence of Andean orogenesis. This fact could explain the lack of branch support of more basal relationships. However, more genes have to be analysed within a biogeographical framework to test this hypothesis.

ACKNOWLEDGEMENTS

We thank Nélida Caligaris, Hernán Pereira and Silvia Pietrokovsky for their valuable technical assistance and Marcos Mirande for suggestions on the application of the extended implied weighting strategy in TNT. We also thank Ricardo Solano Morales, SENASA, Peru, for his collaboration with the collecting permits. This work was supported in part by a grant from the National Geographic Society to M.M.C and financial support from CONICET. Financial support to V.A.C. was provided by the University of Buenos Aires, Consejo Nacional de Investigaciones Científicas y Tecnológicas (CONICET) and Agencia Nacional de Promoción Científica y Tecnológica (ANCyPT) (Argentina).

REFERENCES

Amédégnato C. 1976. Structure et evolution des genitalia chez les acrididae et familles apparentées. *Acrida* **5:** 1–15.

Bremer K. 1994. Branch support and tree stability. *Cladistics* **10:** 235–304.

Bruner L. 1913. Results from Yale Peruvian expedition of 1911. Orthoptera (Acrididae, Shorhorned locusts). *Proceedings of the United States National Museum* **44:** 177–187.

Cigliano MM, Eades D. 2010. New technologies challenge the future of taxonomy in Orthoptera. *Journal of Orthoptera Research* **19:** 15–18.

Cigliano MM, Lange CE. 2007. Systematic revision and phylogenetic analysis of the South American genus *Chlorus* (Orthoptera, Acridoidea, Melanoplinae). *Zoologica Scripta* **36:** 241–254.

Colombo P, Cigliano MM, Sequeira AS, Lange CE, Vilardi JC, Confalonieri VA. 2005. Phylogenetic relationships in *Dichroplus* Stål (Orthoptera: Acrididae: Melanoplinae) inferred from molecular and morphological data: testing karyotype diversification. *Cladistics* **21:** 375–389.

Damm S, Schierwater B, Hadrys H. 2010. An integrative approach to species discovery in odonates: from character-based DNA barcoding to ecology. *Molecular Ecology* **19:** 3881–3893.

Dayrat B. 2005. Towards integrative taxonomy. *Biological Journal of the Linnean Society* **85:** 407–415.

DeSalle R, Egan MG, Siddall M. 2005. The unholy trinity: taxonomy, species delimitation and DNA barcoding. *Philosophical Transactions of the Royal Society B-Biological Sciences* **360:** 1905–1916.

Donato M. 2000. Los ejemplares tipo de Orthoptera depositados en la colección del Museo de La Plata. *Revista de la Sociedad Entomológica Argentina* **59:** 61–84.

Drummond AJ, Rambaut A. 2007. BEAST: Bayesian evolutionary analysis by sampling trees. *BMC Evolutionary Biology* **7:** 214.

Eades DC, Otte D, Cigliano MM, Braun H. 2015. *Orthoptera Species File Online. Version 2.0/4.0* [WWW document]. Available at: http://Orthoptera.SpeciesFile.org [accessed January 2015].

Esselstyn JA, Evans BJ, Sedlock JL, Anwarali Khan FA, Heaney LR. 2012. Single-locus species delimitation: a test of the mixed Yule–coalescent model, with an empirical application to Philippine round-leaf bats. *Proceedings of the Royal Society B* **279:** 3678–3686.

Fontaneto D, Herniou EA, Boschetti C, Caprioli M, Melone G, Ricci C, Barraclough TG. 2007. Independently evolving species in asexual bdelloid rotifers. *PLoS Biology* **5:** e87.

Franco JF. 2013. Morfología fálica y cariotipo de *Orotettix* n. sp. (Orthoptera: Acrididae, Melanoplinae). *Bioma* N°13, Año 2, Noviembre 2013: 44–48.

Fujisawa T, Barraclough TG. 2013. Delimiting species using single-locus data and the generalized Mixed Yule coalescent approach: a revised method and evaluation on simulated data sets. *Systematic Biology* **62:** 707–724.

Fujita MK, Leache AD, Burbrink FT, McGuire JA, Moritz C. 2012. Coalescent-based species delimitation in an integrative taxonomy. *Trends in Ecology & Evolution* **27:** 480–488.

Goloboff P. 1993. Estimating character weights during tree search. *Cladistics* **9:** 83–91.

Goloboff P. 2014. Extended implied weighting. *Cladistics* **30:** 260–272.

Goloboff PA, Farris S, Källersjö M, Oxelman B, Ramírez MJ, Szumik CA. 2003b. Improvements to resampling measures of group support. *Cladistics* **19:** 324–332.

Goloboff PA, Farris S, Nixon K. 2003a. Tree Analysis using New Technology. Published by the authors, Tucumán [WWW document]. Available at: http://www.cladistics.com/aboutTNT.html [accessed August 2014].

Gonzalez L, Pfiffner AO. 2012. Morphologic evolution of the Central Andes of Peru. *International Journal of Earth Sciences (GR Geologische Rundschau)* **101:** 307–321.

Graham A. 2009. The Andes: a geological overview from a biological perspective. *Annals of the Missouri Botanical Garden* **96:** 371–385.

Gu X, Fu YX, Li WH. 1995. Maximum likelihood estimation of the heterogeneity of substitution rate among nucleotide sites. *Molecular Biology and Evolution* **12:** 546–557.

Guzman NV, Confalonieri VA. 2010. The evolution of South American populations of *Trimerotropis pallidipennis* (Oedipodinae: Acrididae) revisited: dispersion routes and origin of chromosomal inversion clines. *Journal of Orthoptera Research* **19:** 253–260.

Hadley A. 2006. CombineZ5. Published by the author, London [WWW document]. Available at: http://www.hadleyweb.pwp.blueyonder.co.uk [accessed 10 April 2010].

Hebard M. 1917. Notes on Mexican Melonopli. *Proceedings of the Academy of Natural Sciences, Philadelphia* **69:** 251–275.

Hebard M. 1931. Die Ausbeute der deutschen Chaco-Expedition 1925–26. Orthoptera. *Konowia, Zeitschrift für Systematische Insektenkunde* **10:** 257–285.

Heethoff M, Laumann M, Weigmann G, Raspotnig G. 2011. Integrative taxonomy: combining morphological, molecular and chemical data for species delineation in the parthenogenetic *Trhypochthonius tectorum* complex (Acari, Oribatida, Trhypochthoniidae). *Frontiers in Zoology* **8:** 1–10.

Huelsenbeck JP, Ronquist F. 2001. MRBAYES: Bayesian inference of phylogeny. *Bioinformatics* **17:** 754–755.

Husemann M, Guzman N, Canley P, Cigliano MM, Confalonieri V. 2013. Biogeography of *Trimerotropis pallidipennis* (Acrididae: Oedipodinae): deep divergence across the Americas. *Journal of Biogeography* **40:** 261–273.

Jaiswara R, Balakrishnani R, Robillard T, Rao K, Craud C, Desutter-Grandcolas L. 2012. Testing concordance in species boundaries using acoustic, morphological, and molecular data in the field cricket genus *Itaropsis* (Orthoptera: Grylloidea, Gryllidae: Gryllinae). *Zoological Journal of the Linnean Society* **164:** 285–303.

Knowles LL, Carstens B. 2007. Delimiting species without monophyletic gene trees. *Systematic Biology* **56:** 887–895.

Lanave C, Preparata G, Saccone C, Serio G. 1984. A new method for calculating evolutionary substitution rates. *Journal of Molecular Evolution* **20:** 86–93.

Liebermann J. 1963. Sinopsis bibliográfica de los acridoideos del Peru. *Revista Peruana de Entomología* **6:** 61–66.

Luteyn JL. 1992. Páramos: why study them? In: Balslev H, Luteyn JL, eds. *Páramo: an Andean ecosystem under human influence*. London: Academic Press, 1–14.

Miralles A, Vences M. 2013. New metrics for comparison of taxonomies reveal striking discrepancies among species delimitation methods in *Madascincus* lizards. *PLoS ONE* **8:** e68242.

Normark BB. 1996. Phylogeny and evolution of parthenogenetic weevils of the *Aramigus tessellatus* species complex (Coleoptera: Curculionidae: Naupactini): evidence from mitochondrial DNA sequences. *Evolution* **50:** 734–745.

Otte D. 1981. *Acrididae: Gomphocerinae and Acridinae. North American grasshoppers*. Cambridge, MA: Harvard University Press.

Padial JM, De La Riva I. 2009. Integrative taxonomy reveals cryptic Amazonian species of *Pristimantis* (Anura: Strabomantidae). *Zoological Journal of the Linnean Society* **155:** 97–122.

Pocco ME, Scattolini C, Lange CE, Cigliano MM. 2014. Taxonomic delimitation in color polymorphic species of the South American grasshopper genus *Diponthus* Stål (Orthoptera, Romaleidae, Romaleini). *Insect Systematics and Evolution* **45:** 303–350.

Pons J, Barraclough TG, Gomez-Zurita J, Cardoso A, Duran DP, Hazell S, Kamoun S, Sumlin WD, Vogler AP. 2006. Sequence based species delimitation for the DNA taxonomy of undescribed insects. *Systematic Biology* **55:** 595–609.

de Queiroz K. 2007. Species concepts and species delimitation. *Systematic Biology* **56:** 879–886.

Rambaut A, Drummond AJ. 2007. Tracer, version 1.4. Available at: http://beast.bio.ed.ac.uk/Tracer

Rodríguez F, Oliver JL, Marin A, Medina JR. 1990. The general stochastic model of nucleotide substitution. *Journal of Theoretical Biology* **142:** 485–501.

Roe AD, Sperling FAH. 2007. Population structure and species boundary delimitation of cryptic *Dioryctria* moths: an integrative approach. *Molecular Ecology* **16:** 3617–3625.

Ronderos RA, Carbonell CS. 1994. Sobre el género mexicano *Pedies* Saussure, y las especies sudamericanas atribuidas al mismo (Orthopera: Acrididae: Melanoplinae). *Revista de la Sociedad Entomológica Argentina* **53:** 83–99.

Ronquist F, Huelsenbeck JP. 2003. MRBAYES 3: Bayesian phylogenetic inference under mixed models. *Bioinformatics* **19:** 1572–1574.

Sarmiento G. 1986. Ecological features of climate in high tropical mountains. In: Vuilleumier F, Monasterio M, eds. *High altitude tropical biogeography*. New York: Oxford University Press, 11–45.

Schlick-Steiner BC, Steiner FM, Seifert B, Stauffer C, Christian E, Crozier RH. 2010. Integrative taxonomy: a multisource approach to exploring biodiversity. *Annual Review of Entomology* **55:** 421–438.

Shaw KL. 2002. Conflict between nuclear and mitochondrial DNA phylogenies of a recent species radiation: what mitochondrial DNA reveals and conceals about modes of speciation in Hawaiian crickets. *Proceedings of the National Academy of Sciences of the United States of America* **99:** 16122–16127.

Sites JW, Marshall JC. 2004. Operational criteria for delimiting species. *Annual Review of Ecology, Evolution, and Systematics* **35:** 199–227.

Smith AP, Young TP. 1987. Tropical alpine plant ecology. *Annual Review of Ecology and Systematics* **18:** 137–158.

Talavera G, Dinca V, Vila R. 2013. Factors affecting species delimitations with the GMYC model: insights from a butterfly survey. *Methods in Ecology and Evolution* **4:** 1101–1110.

Tavaré S. 1986. Some probabilistic and statistical problems in the analysis of DNA sequences. *Lecture Notes on Mathematical Modelling in the Life Sciences* **17:** 57–86.

Wiens JJ. 2007. Species delimitation: new approaches for discovering diversity. *Systematic Biology* **56:** 875–878.

Wiens JJ, Penkrot TA. 2002. Delimiting species using DNA and morphological variation and discordant species limits in spiny lizards (*Sceloporus*). *Systematic Biology* **51:** 69–91.

Yang Z. 1994. Estimating the pattern of nucleotide substitution. *Journal of Molecular Evolution* **39:** 105–111.

APPENDIX 1

List of characters

0 Pronotum: lateral borders of metazona: parallel (0); slightly divergent (1).

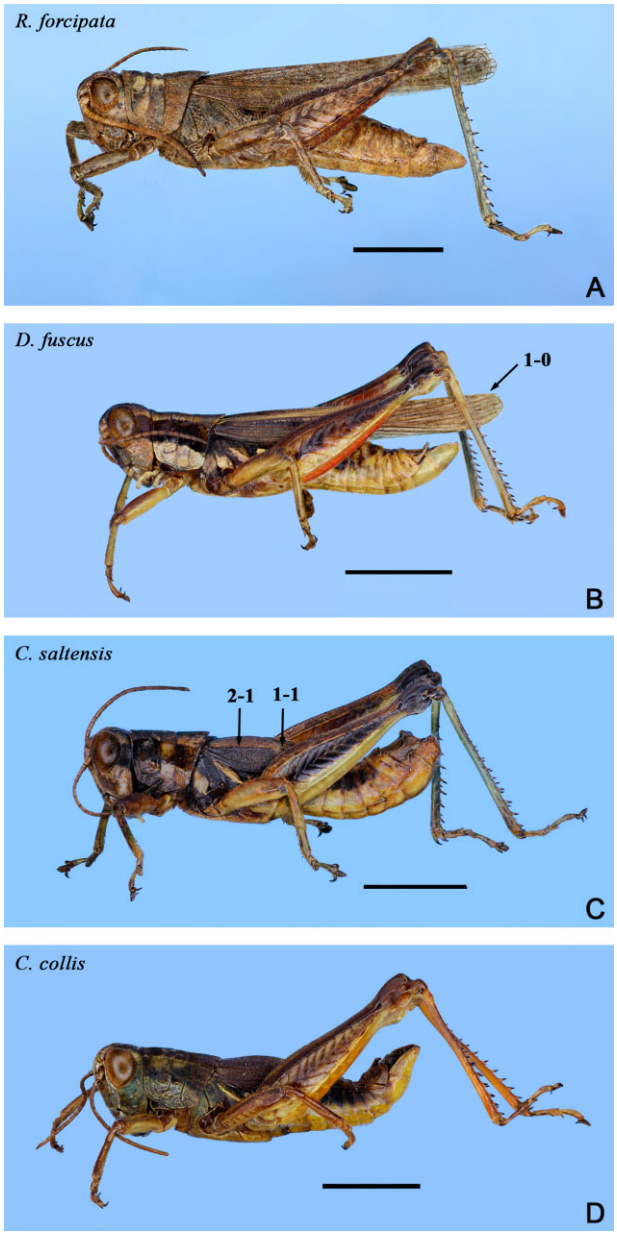

Figure 11 Outgroup taxa used in the phylogenetic analyses, species as indicated. A–D, male habitus. Scale bars: 5 mm. Numbers indicate characters and states used in the phylogenetic analyses.

1 Tegmina: macropterous (0) (Fig. 11B); braquipterous, lobiform with acute apex (1) (Fig. 11C); braquipterous, foliaceous (2) (Fig. 6A); braquipterous, lobiform with rounded apex (3) (Fig. 6C).
2 Tegmina: radial vein: not raised (0); raised (1) (Fig. 11C).
3 Male cerci: apex in lateral view: acute (0) (Fig. 12B); slightly spatulate (1) (Fig. 12D); expanded (2) (Fig. 12F); conical, up-curved (3) (Fig. 7G); conical, down-curved (4) (Fig. 8C); finger-like (5) (Fig. 8A).
4 Male cerci: curvature in dorsal view, in relation to epiproct: bent inwards (0) (Fig. 12A); in right angle (1) (Fig. 12G); in acute angle (2) (Fig. 7F); in obtuse angle (3) (Fig. 7H); widely obtuse angle (4) (Fig. 7D); parallel to epiproct, slightly incurved (5) (Fig. 8F).
5 Apical valves of aedeagus: divergent in straight angle (0) (Fig. 13B); straight with distal portion incurved (1) (Fig. 9B); slightly divergent (2) (Fig. 9H); broad with distal third incurved (3) (Fig. 10N); slender, with distal third incurved (4) (Fig. 10H); overcrossed (5) (Fig. 13E).
6 Sheath of aedeagus: with transverse flange (0) (Fig. 13E); without transverse flange (1).
7 Sheath of aedeagus: mid-dorsal apical lobes: convergent, triangular shape (0) (Fig. 13B); subrectangular (1) (Fig. 13H); divergent, sub-triangular (2) (Fig. 9E); with straight distal edge (3) (Fig. 9N); with truncated distal edge (4) (Fig. 10B); rounded (5) (Fig. 10E).
8 Sheath of aedeagus: lateral lobes: slightly developed (0) (Fig. 13B); not developed (1) (Fig. 13E); well developed (2) (Fig. 9H).
9 Sheath of aedeagus: dorsal hump: prominent (0) (Fig. 10A); slightly prominent (1) (Fig. 10D); not prominent (2) (Fig. 10M).
10 Sheath of aedeagus: covering half or 2/3 of the apical valves (0) (Fig. 9A); covering wholly or most of the apical valves (1) (Fig. 10D); less than half of the apical valves (2) (Fig. 13G).
11 Epiphallus: lophi: not extended towards the caudal tip of lateral plates (0) (Fig. 9C); extended towards the caudal tip of lateral plates (1) (Fig. 10I).
12 Epiphallus: lophi: prominent with internal conical protuberance (0) (Fig. 13F); prominent without protuberance (1) (Fig. 9F); not prominent (2) (Fig. 10C).
13 Apical valves of aedeagus slightly divergent with subtriangular apex: distal edge: concave (0); straight (1).
14 Apical valves of aedeagus: distal portion: not divided into branches (0); divided into branches (1).
15 Pronotum: lateral carinae: slightly indicated or obsolete (0); clearly indicated (1).
16 Apical valves of aedeagus straight with distal portion incurved: external margin of apex: truncate (0); rounded (1).
17 Body-tegmina length ratio: tegmina about ¾ the length of body (0); tegmina less than 1/5 the length of body (1); tegmina more than 1/5 the length of body (2)
18 Hind tibiae color: light bluish-green (0); basal half yellow and distal half light green (1); orange (2); green (3); orange-red (4); bright red (5); light purple (6); light brown (7).

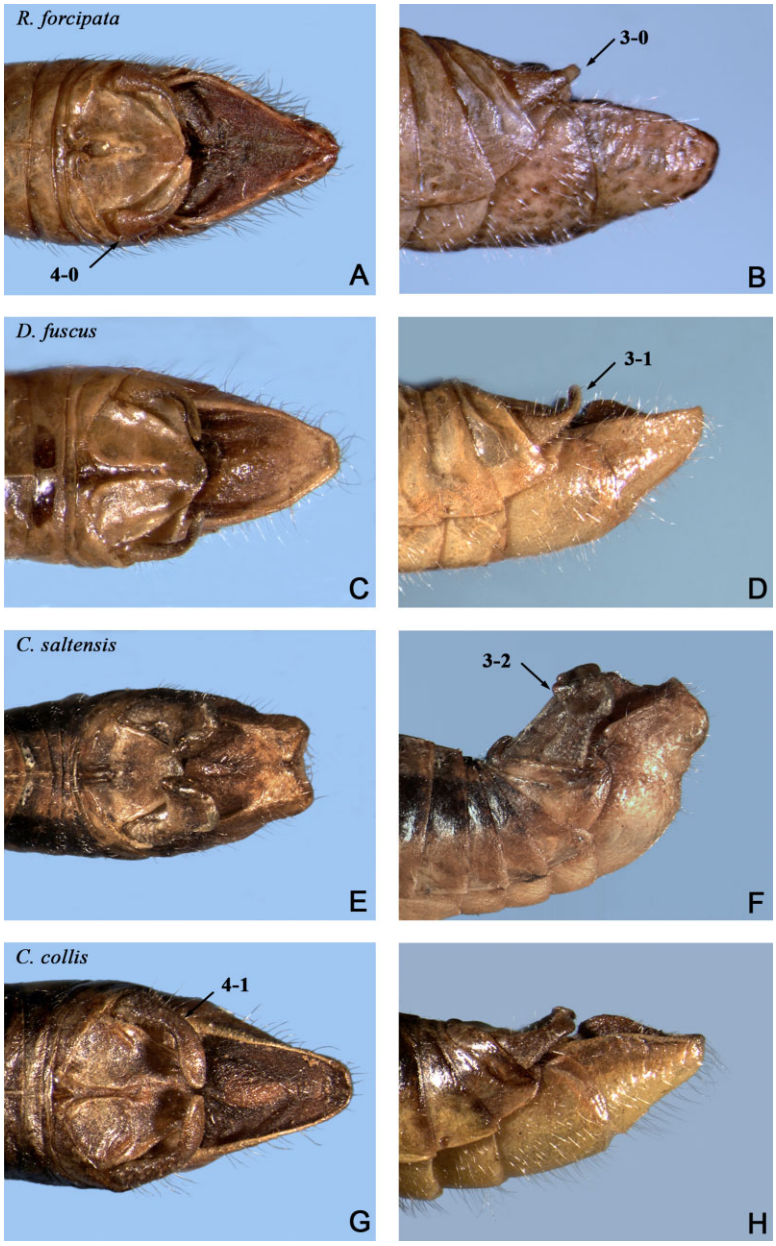

Figure 12 Outgroup taxa used in the phylogenetic analyses, species as indicated. A, C, E, G, male distal abdominal segments, dorsal view; B, D, F, H, male distal abdominal segments, lateral view. Numbers indicate characters and states used in the phylogenetic analyses.

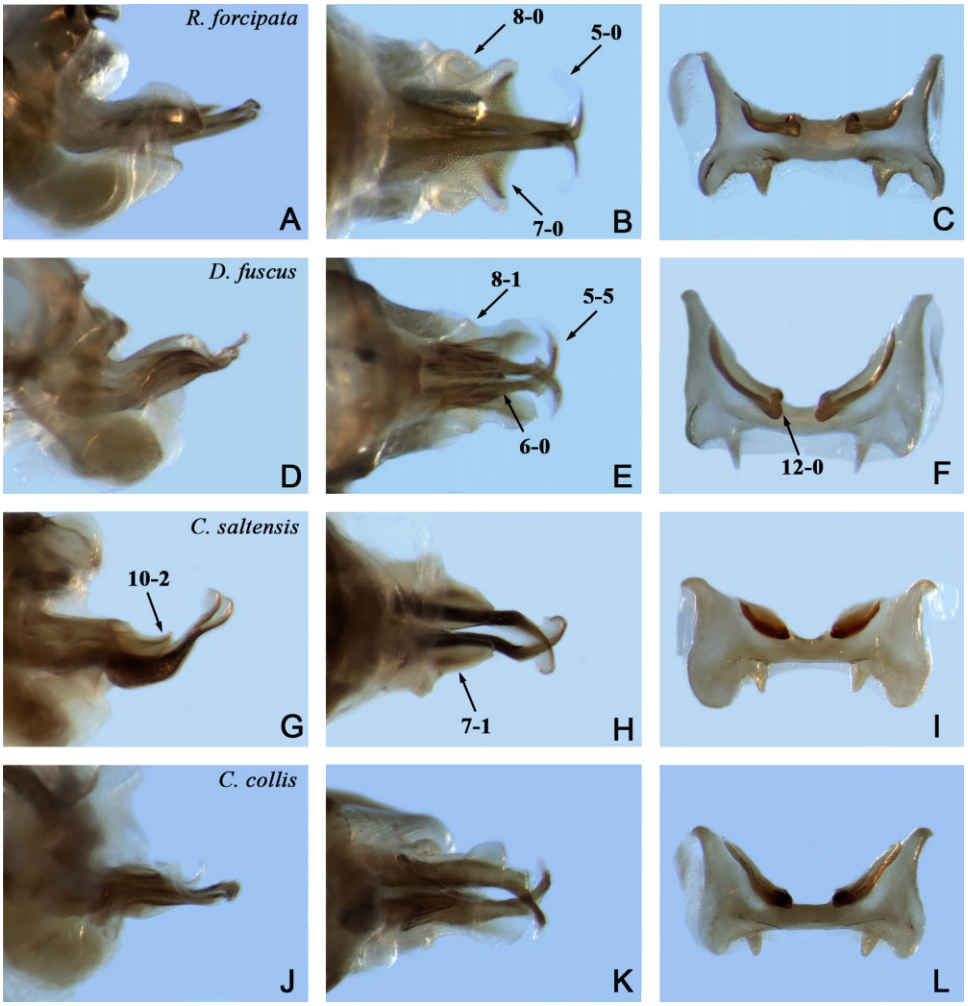

Figure 13 Outgroup taxa used in the phylogenetic analyses, species as indicated. Phallic complex. A, D, G, J, distal portion of aedeagal valves, lateral view; B, E, H, K, distal portion of aedeagal valves, dorsal view; C, F, I, L, epiphallus, dorsal view. Numbers indicate characters and states used in the phylogenetic analyses.

APPENDIX 2

KEY TO THE SPECIES OF *OROTETTIX*

1. Pronotum with lateral carinae clearly indicated (Fig. 6F)……*O. hortensis*
1′. Pronotum with lateral carinae slightly indicated or obsolete……2
2. Male cerci short, parallel to epiproct (Fig. 8F)……3
2′. Male cerci longer, sharply (Fig. 7F) or slightly curved over the epiproct (Fig. 7H)……5
3. Male cerci not surpassing the tip of epiproct (Fig. 8H); sheath of aedeagus with prominent dorsal hump (Fig. 10J). ……*O. laevis*
3′. Male cerci barely surpassing the tip of epiproct (Fig. 8F); sheath of aedeagus with slightly prominent dorsal hump (Fig. 10D)……4
4. Hind tibiae greenish or orange (Fig. 6G); apical valves of aedeagus wider; sheath of aedeagus long, covering most of the apical valves, with mid-dorsal apical lobes prominent (Fig. 10E)……*O. ceballosi*
4′. Hind tibiae reddish-brown (Fig. 6H); apical valves of aedeagus slender; sheath of aedeagus shorter, covering 2/3 of the apical valves, with mid-dorsal apical lobes less prominent (Fig. 10H)……***O. paucartambensis* sp. nov.**
5. Apical valves of aedeagus with distal half incurved (Fig. 10N); mid dorsal apical lobes of sheath of aedeagus covering most of the apical valves in lateral view (Fig. 10M)……***O. colcaensis* sp. nov.**
5′. Apical valves of aedeagus with distal half straight with incurved or divergent apex; mid-dorsal apical lobes of sheath of aedeagus covering 2/3 or less of the apical valves in lateral view……6
6. Apical valves of aedeagus strongly widening at the subtriangular distal portion, slightly divergent in dorsal view (Fig. 9K)……7
6′. Apical valves of aedeagus with distal portion as wide as proximal portion, slightly curved inwards, overlapping at apex (Fig. 9B)……9
7. Male cerci dorsally curved in an acute angle, with conical apex (Fig. 7F); apical valves of aedeagus with distal portion forked shape (Fig. 9H); hind tibiae orange-red (Fig. 6C)……***O. dichrous* sp. nov.**
7′. Male cerci slightly curved over the epiproct, with acute or compressed apex; distal portion of apical valves of aedeagus wide, sub-triangular shaped; hind tibiae orange-red or greenish……8
8. Male cerci with acute apex (Fig. 7G); sub-triangular distal portion of apical valves of aedeagus with straight apical edge (Fig. 9K); hind tibiae greenish (Fig. 6D)……***O. astreptos* sp. nov.**
8′. Male cerci with compressed apex (Fig. 7I); sub-triangular distal portion of apical valves of aedeagus with lunate apical edge (Fig. 9N); hind tibiae orange-red (Fig. 6E)……***O. lunatus* sp. nov.**
9. Male cerci narrowly curved over epiproct, with compressed apex (Fig. 7B)……*O. andeanus*
9′. Male cerci slender, widely curved inwards, with acute apex (Fig. 7D)……*O. carrascoi*